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REVIEW

Minimally invasive procedures on the lumbar spine

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Abstract

Degenerative disease of the lumbar spine is a common and increasingly prevalent condition that is often implicated as the primary reason for chronic low back pain and the leading cause of disability in the western world. Surgical management of lumbar degenerative disease has historically been approached by way of open surgical procedures aimed at decompressing and/or stabilizing the lumbar spine. Advances in technology and surgical instrumentation have led to minimally invasive surgical techniques being developed and increasingly used in the treatment of lumbar degenerative disease. Compared to the traditional open spine surgery, minimally invasive techniques require smaller incisions and decrease approach-related morbidity by avoiding muscle crush injury by self-retaining retractors, preventing the disruption of tendon attachment sites of important muscles at the spinous processes, using known anatomic neurovascular and muscle planes, and minimizing collateral soft-tissue injury by limiting the width of the surgical corridor. The theoretical benefits of minimally invasive surgery over traditional open surgery include reduced blood loss, decreased postoperative pain and narcotics use, shorter hospital length of stay, faster recover and quicker return to work and normal activity. This paper describes the different minimally invasive techniques that are currently available for the treatment of degenerative disease of the lumbar spine.

Key words: Minimally invasive surgery; Spine surgery; Lumbar spine; Degenerative disease; Interbody fusion; Posterolateral fusion; Decompression; Indirect decompression techniques

Core tip: Degenerative disease of the lumbar spine is a common and increasingly prevalent condition that is often implicated as the primary reason for chronic low back pain and the leading cause of disability in the western world. Compared to the traditional open spine surgery, minimally invasive techniques require smaller incisions and decrease approach-related morbidity. The benefits of minimally invasive surgery over traditional open surgery include reduced blood loss, decreased postoperative pain and narcotics use, shorter hospital length of stay, faster recovery and quicker return to work and normal activity.

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INTRODUCTION

Modern minimally invasive spine surgery (MIS) was introduced in the 1990s with the description of tubular retractors for access to the lumbar spine and the report of the first lumbar microendoscopic discectomy[1,2]. Since that time, advances in technology and surgical instrumentation have led to MIS developing into an important and rapidly growing filed of spine surgery. Today, MIS techniques and approaches are used in the treatment of a wide variety of spinal pathologies including degenerative disc disease, disc herniation, instability, deformity, fracture, infection and tumors[3]. Compared to the traditional open spine surgery, MIS was pursued as a means to reduce iatrogenic tissue trauma during surgery. The theoretical benefits of MIS over traditional open surgery include smaller incisions, less soft tissue damage, reduced estimated blood loss (EBL), decreased postoperative pain and narcotics use, shorter hospital length of stay (LOS), faster recover and quicker return to work and normal activity[4,5]. Traditional open spine surgery approaches often require extensive muscular and ligamentous disruption during the surgical approach to the spine resulting in decreased spinal stability and subsequent associated morbidities[6]. MIS minimizes approach-related morbidity by avoiding muscle crush injury by self-retaining retractors, preventing the disruption of tendon attachment sites of important muscles at the spinous processes, using known anatomic neurovascular and muscle planes, and minimizing collateral soft-tissue injury by limiting the width of the surgical corridor[7]. The decrease in the approach-related morbidity and indirect iatrogenic destabilization of the spine are important advantages of MIS over open spine surgery.

MIS approaches have been increasingly used in the treatment of degenerative diseases of the lumbar spine. As microsurgery, endoscopy and various percutaneous techniques advance and our rapidly aging population drives greater demand for spinal care, MIS will likely play an increasingly important role in the treatment of lumbar degenerative disease. This paper describes the different MIS techniques that are currently available for the treatment of degenerative disease of the lumbar spine.

MINIMALLY INVASIVE NON-FUSION PROCEDURES

MIS microdiscectomy

Lumbosacral nerve root compression or irritation secondary to an intervertebral disc herniation is a major cause of sciatica and low back pain. Patients with disc-related sciatica may be managed conservatively or *via* surgery when conservative treatment fails or symptoms worsen over time. It is estimated that over 250000 elective lumbar spine surgeries are performed in the United States each year for persistent symptoms of sciatica[8]. Of those, lumbar discectomy remains one of the most commonly performed procedures[9]. The goal of surgery is most commonly to remove intervertebral disc material and decompress the nerve root. Traditionally, the standard surgical treatment of lumbosacral disc herniation has been open microdiscectomy, however, with the rapid advances in surgical techniques and technology, there has been a growing trend towards MIS microdiscectomy.

An MIS microdiscectomy involves the use of serial tubular retractors to dilate the paraspinous musculature without stripping it off the spinous processes, and an endoscope or surgical microscope to visual the surgical field[10]. While the benefits of minimal soft tissue disruption appear to favor MIS microdiscectomy over open microdiscectomy, there is a significant learning curve associated with performing the procedure safely. Although many innovative techniques in the treatment of lumbar disc herniation have been developed, open microdiscectomy remains the standard of care at the current time[11].

A recent meta-analysis of controlled trials that compared outcomes of MIS and open microdiscectomy in patients with sciatica evaluated 29 studies (16 randomized controlled trials and 13 non-randomized studies) with a total of 4472 patients[11] (Table 1). Regarding clinical outcomes, the study found a moderate to low quality evidence of no differences between MIS and open microdiscectomy. Regarding perioperative outcomes, there was low to moderate evidence of no difference between MIS and open microdiscectomy; this was particularly notable for complications and reoperation rates. The study also found no significant difference in quality-adjusted life years (QALYs) or total costs from a societal perspective during the first year following treatment. The authors found low quality evidence that MIS took 10-15 min longer, resulted in a 52 cc reduction in EBL and reduced mean LOS by 1.5 d. The increased surgical time with MIS may be explained by the learning curve associated with MIS, variability in the techniques used and differences in how operative times were defined[11].

Currently, there is evidence from several comparative studies of MIS and open microdiscectomy suggesting that clinical outcomes between the two groups are similar. As surgeons become more proficient with MIS techniques and investigators conduct well-powered, randomized controlled trials, the indications favoring MIS microdiscectomy will be better defined.

MIS direct decompression

Lumbar spinal stenosis is the most common indication for spine surgery in patients older than 65, and its prevalence in the United States is expected to rise 59% by the year 2025[12]. Age-related degenerative changes

Table 1 Differences between outcomes of minimally invasive *vs* open surgical techniques

MIS techniques	Ref.	Differences in outcome compared to open techniques
Non-fusion techniques		
MIS microdiscectomy	Kamper *et al*[11]	Moderate to low evidence of no differences between MIS and open microdiscectomy
		No significant differences in QUALYs or total costs
		MIS took 10-15 min longer, resulted in 52 cc reduction in EBL and reduced mean LOS by 1.5 d
MIS direct decompression (Laminectomy/laminotomy)	Rahman *et al*[16]	Decreased EBL compared to open technique
		MIS procedures were 37-47 min shorter
		Decreased LOS by 2.52 d in patients undergoing decompression at ≤ 2 levels
		MIS had fewer complications (7.9% *vs* 16.1%)
	Anderson *et al*[17]	No significant differences in terms of ODI, Short-Form-12, and VAS
	Khoo *et al*[18]	Longer operative times in MIS group (109 min *vs* 88 min)
		Decreased EBL and postoperative stay in MIS group
	O'Toole *et al*[21]	0.10% surgical site infection rate
		Authors concluded that MIS technique may reduce SSI rate by 10-fold
MIS indirect decompression (Interspinous process devices)	Kuchta *et al*[26]	Statistically significant improvement in symptom severity and physical functioning throughout 2-yr follow-up period
	Bowers *et al*[27]	85% failure rate and 38% complication rate
	Brussee *et al*[28]	Poor outcome in 68.9% of patients
	Kim *et al*[29]	Cost analysis study found devices to be extremely costly and questioned cost-effectiveness
Fusion techniques		
Intertransverse onlay fusion	-	No literature available comparing MIS versus open posterolateral onlay fusion
Percutaneous pedicle screw fixation	Lehmann *et al*[34]1	EBL and muscle damage markers significantly lower in MIS group
		Compartment pressure, blood flow and EMG readings similar between both groups
		Radiation exposure greater in MIS group
MIS transforaminal lumbar interbody fusion	Seng *et al*[51]	Statistically increased fluoroscopic times (55.2 s *vs* 16.4 s) and operative times (185 min *vs* 166 min) in MIS group
		MIS had less EBL than open (127 cc *vs* 405 cc)
		Postoperative morphine use less in MIS group (8.5 mg *vs* 24.2 mg)
		Shorter LOS in MIS group (3.5 d *vs* 5.9 d)
	Parker *et al*[55]	MIS associated with reduction in mean hospital cost of $1758, indirect cost of $8474, total 2-yr social cost of $9295
		Similar 2-yr direct health care cost and QALYs gained
MIS direct lateral interbody fusion	Villavicencio *et al*[73]	Lower complication rate in MIS versus open (8.2% *vs* 16.7%)
	Rodgers *et al*[74]	Significantly lower complication rate in MIS cohort (7.5% *vs* 60%)
		Decreased EBL, lower transfusion rate and shorter LOS
	Deluzio *et al*[75]	Average LOS in MIS group 49% shorter
		Average cost savings for MIS group at 45 d of $2536/patient

[1]Animal study. MIS: Minimally invasive surgery; LOS: Length of stay; EBL: Estimated blood loss; ODI: Oswestry Disability Index; VAS: Visual analog scale; SSI: Surgical site infection; EMG: Electromyography; cc: Centimeter cubed; QALY: Quality adjusted life year.

in the lumbar spine such as hypertrophy of the facet joints with or without synovial cyst formation, foraminal stenosis due to decrease in the intervertebral disc height or osteophyte formation, ligamentum flavum thickening causing central and lateral recess compression and bulging or herniation of the intervertebral disc are all potential contributors to lumbar spinal stenosis. Surgery has been shown to decrease pain and improve functional status in patients with lumbar spinal stenosis[13]. Traditionally, an open midline approach involving a wide, bilateral laminectomy with medial facetectomy with or without foraminotomy has been the standard technique for surgical treatment of lumbar spinal stenosis. However, lumbar decompression surgery is increasingly being performed using tubular decompression, a MIS technique. This procedure utilizes a small paramedian incision and through the use of serial tubular dilators to reduce multifidus muscle injury while providing sufficient exposure of the surgical decompression site and allowing for bilateral decompression through a single incision[14]. Decompressing the contralateral side through a single unilateral paramedian incision allows the surgeon to spare the spinous process, rostral and caudal supraspinous and interspinous ligaments as well as the contralateral lamina and facet joint, thereby minimizing iatrogenic destabilization of the spine while achieving sufficient decompression for symptomatic relief. Furthermore, the use of an intraoperative endoscope or microscope for magnification and lighting allows for adequate visualization of the spinal anatomy[15].

Multiple studies have described shorter operating room times, decreased EBL, shorter LOS, lower surgical site infection rates, fewer complications and faster recovery times in MIS compared to the open lumbar decompression for spinal stenosis[16-20].

Rahman *et al*[16] retrospectively reviewed the medical records and relevant imaging of 126 patients (38 MIS *vs* 88 standard open technique) who underwent bilateral surgical decompression for lumbar stenosis to determine intraoperative EBL, length of operation, LOS, and

number and nature of complications. On average, patients undergoing open procedures had 194 cc more EBL than patients undergoing MIS procedures, with the greatest difference in patients undergoing procedures involving ⩾ 3 levels. MIS procedures were 37-47 min shorter than open procedures. Looking at the hospital LOS, the authors found that patients undergoing decompression at ⩽ 2 levels had a LOS that was 2.52 d shorter in the MIS group. In terms of overall complications, the MIS group had fewer complications than the open group (7.9% *vs* 16.1%). However, one limitation of this study was that it did not evaluate long-term outcomes of the two procedures.

Anderson *et al*[17] performed a retrospective analysis of 110 patients in two matched cohorts to compare the tubular retractor approach and traditional midline approach to decompressive surgery for unilateral lumbar radiculopathy. The two approaches were evaluated based on patient reported outcomes using the Oswestry Disability Index (ODI), Short Form-12, and visual analog scale. The authors found no significant differences between the surgical approaches with respect to patient reported outcomes.

Khoo *et al*[18] compared 25 patients undergoing microendoscopic decompressive laminotomy to 25 patients undergoing open decompression for lumbar spinal stenosis. The authors found that effective circumferential decompression was achieved in the majority of patients in both groups. Surgery was longer for the MIS procedure group compared to the open group (109 min per single level *vs* 88 min per single level). EBL was reduced by 125 cc in the MIS group and postoperative stay was decreased 48 h, from 94 h in the open group to 42 h in the MIS group.

O'Toole *et al*[21] performed a retrospective review of prospectively collected data in 1274 patients undergoing MIS decompression and found a 0.10% rate of surgical site infection. The authors concluded that MIS techniques may reduce postoperative wound infections as much as 10-fold compared to open techniques.

At the current time, favorable complication profiles and patient outcome studies with MIS tubular decompression makes this technique an acceptable treatment option in the surgical management of lumbar spinal stenosis (Table 1). Studies assessing long-term outcomes and cost-utility of MIS *vs* open lumbar decompression are needed to further establish the value of MIS decompression.

MIS indirect decompression

Lumbar interspinous process devices (IPDs) are a MIS technology intended to unload the facet joints, restore foraminal height, lower intradiscal pressure, restricts overextension and provide motion-preserving stabilization[22]. IPDs are used in the treatment of degenerative lumbar spinal stenosis and intermittent neurogenic claudication where they provide indirect decompression to the neural structures[23]. They have also been used in the treatment of discogenic low back pain, facet syndrome, disc herniation and lumbar spinal instability[24]. In 2005, the United States Food and Drug Administration (FDA) approved the first ever IPD (X-STOP, Medtronic, Memphis, TN, United States) for the treatment of patients aged 50 or older suffering from neurogenic intermittent claudication secondary to a confirmed diagnosis of lumbar spinal stenosis. The device was indicated for those patients with moderately impaired physical function who experience relief in flexion from their symptoms of leg/buttock/groin pain, with or without back pain, and have undergone a regimen of at least 6 mo of non-operative treatment. The device may be implanted at one or two lumbar levels in patients in whom operative treatment is indicated at no more than two levels[25]. Since the FDA approval of the first IPD, a growing number of devices have been introduced to the spine implant market and used for a wide range of lumbar spinal pathologies, many of them outside of the intended indications.

Outcomes with the use of IPDs have thus far been inconsistent. Kuchta *et al*[26] reported on a single-center clinical outcomes of 175 patients with symptomatic lumbar spinal stenosis treated with X-STOP implantation and reported statistically significant ($P < 0.001$) improvements in symptom severity and physical functioning throughout the 2-year follow-up period. Bowers *et al*[27] reviewed complications associated with the use of X-STOP in 13 patients and found a 85% ultimate failure rate with patients requiring additional surgery for symptomatic relief. The authors also reported a 38% complications rate, including 3 spinous process fractures and 2 instances of new onset radiculopathy. Brussee *et al*[28] review 65 patients with neurogenic claudication who underwent placement of X-STOP device and found poor outcomes in 68.9% of patients.

Multiple studies have also reported high complication and reoperation rates following implantation of IPDs. Complication rates published in the literature range from 11.6% to 38% while reported reoperation rates range from 4.6% to 85%[26-31]. Epstein *et al*[23] reported on the cost of the treatment of 16 patients with a total of 31 X-STOP devices and found a total cost of $576407 charge for the devices alone plus an added $80944 charge for the operating/recovery room. The authors concluded that IPDs appear to be extremely costly and questioned the cost-effectiveness of these devices.

At the current time, there is evidence of poor long-term outcomes as well as high complication and reoperation rates following the use of IPDs for the treatment of degenerative lumbar conditions (Table 1). Studies on the cost-effectiveness and value of IPDs are currently lacking and are needed to determine the future faith of these devices.

MIS LUMBAR FUSION PROCEDURES

MIS posterolateral intertransverse onlay fusion without instrumentation

In the traditional posterior midline approach to the

lumbar spine, access to the intertransverse region requires extensive stripping of the paraspinal musculature to the tips of the transverse processes. This process results in significant destruction, postoperative atrophy and scarring of the multifidus muscles which has been associated with significant postoperative morbidity[32]. For this reason, a MIS paraspinal muscle-splitting technique (modified Wiltse approach) using an expandable tubular dilator has become an increasingly popular technique used for exposure of the transverse processes and intertransverse space. The use of an expandable tubular retractor allows for both of the transverse processes and the fusion bed to be simultaneously exposed.

The theoretical advantages of this MIS technique are decreased paraspinal muscle destruction, decreased surgical incision size, decreased EBL, decreased postoperative narcotic use and shorter LOS. However, there are no studies in the literature comparing outcomes or cost-effectiveness of lumbar intertransverse onlay fusion utilizing the MIS muscle-splitting approach compared to the traditional open approach (Table 1).

MIS percutaneous pedicle screw fixation

Pedicle screw fixation allows for the creation of a solid and biomechanically rigid construct that allows for fusion to occur at the intended levels. Traditionally, open midline incisions were utilized for exposure of the spine in preparation for pedicle screw placement. Open procedures require extensive tissue dissection to expose the entry points and provide adequate lateral-to-medial orientation for optimal screw trajectory. This dissection in turn may result in muscular denervation, facet capsule disruption, damage to the proximal facet joints and weakening of other ligamentous structures, resulting in prolonged post-operative pain and morbidity[33]. Open lumbar fusion procedures are associated with increased operative times, increased EBL and risk of postoperative surgical site infection[34]. LOS and cost of treatment are also adversely affected by open pedicle screw fixation and spinal fusion techniques[35]. Recently, MIS percutaneous pedicle screw fixation has become an increasingly popular MIS technique for lumbar spine fixation[36-38]. Percutaneous pedicle screw placement offers several distinct advantages over the traditional open approach. It eliminates the need for a midline incision and extensive paraspinal muscle dissection. Kim *et al*[38] revealed that percutaneous *vs* open instrumentation was associated with decreased multifidus muscle atrophy, a superior postoperative trunk muscle strength, lower blood loss and less postoperative narcotic use. However, the authors did not find an improved clinical outcome in terms of patient satisfaction and pain scores. Muscle damage is also related to direct compression by muscle retractors, which result in ischemia of the compressed muscle groups and can lead to postoperative muscle necrosis. Percutaneous pedicle screw placement allows the surgeon to more easily achieve an ideal lateral-to-medial trajectory, especially advantageous in obese patients. The advantage of a minimally traumatic access to the lumbar spine carries the disadvantages of longer operative times and increased radiation exposure, both dependent on surgeon experience and comfort level.

Several authors have demonstrated reduced intraoperative EBL, perioperative risk of transfusion, improved cosmesis, decreased post-operative pain and narcotic use, decreased LOS, faster return to activity and reduced overall costs[39-43]. To date, however, there exists no high-quality literature to support the notion that MIS pedicle screw instrumentation is superior to the traditional open technique. Most of the studies evaluating open *vs* percutaneous pedicle techniques do so in context of interbody fusion techniques with no study directly evaluating the two instrumentation techniques alone. Lehmann *et al*[34] compared open *vs* percutaneous pedicle screw insertion in a sheep model and found that EBL and muscle damage markers were significantly lower in the percutaneous group while radiation time was significantly longer in the percutaneous group. In terms of compartment pressure, blood flow and electromyography measurements at different time points during the operative procedure, no significant differences were revealed.

Many technical challenges unique to the percutaneous pedicle screw placement technique and a steep learning curve exists, requiring different technical, psychomotor and cognitive skills. Mobbs *et al*[44] noted several challenges in percutaneous screw placement including changing direction of screw placement following initial pedicle cannulation, L5/S1 screw head proximity, cannulation of small pedicles, skin incision selection and insertion of rod for multi-segmental fixation, and difficult Jamshidi placement in hard pedicles. Surgeon experience plays a key role in perioperative outcomes of MIS techniques. It is recommended that surgeons have adequate experience with open techniques before attempting MIS fixation techniques and that they begin with simple MIS procedures[44].

Despite all the encouraging clinical data (Table 1), prospective outcomes studies with long-term follow up comparing percutaneous instrumented fusion to conventional open instrumented fusion are required to determine the safety, effectiveness and clinical benefit of MIS spinal fixation.

MIS transforaminal lumbar interbody fusion

The TLIF is a versatile surgical procedure modified from the posterior lumbar interbody fusion (PLIF) and pioneered by Harms and Rolinger in 1982[45]. Transforaminal lumbar interbody fusion (TLIF) utilizes the principles of load sharing to provide a circumferential fusion consisting of an anterior column support and a posterior tension band. It is designed to restore lumbar lordosis, widen neural foramina, restore disc height and indirectly relieve spinal stenosis. TLIF has been effective in the treatment of lumbar spondylosis with or without spondylolisthesis and has also been used successfully

in the treatment of lumbar degenerative disc disease, recurrent lumbar disc herniation and complex lumbar stenosis. One of the main disadvantages to TLIF is the significant postoperative morbidity due to the extensive paraspinal muscle and soft tissue dissection and retraction required in order to provide access to the vertebral column. This approach-related morbidity can potentially affect short- and long-term patient outcomes due to increased postoperative pain, delayed rehabilitation and impaired spinal function.

MIS TLIF was first described by Foley *et al*[46] in 2003 and has since become an increasingly popular MIS method to achieve lumbar arthrodesis[47-50]. MIS TLIF has been shown to have short- and long-term clinical outcomes comparable to open TLIF with the additional benefits of decreased postoperative pain, decreased EBL, faster recovery times, reduced postoperative narcotic use, faster postoperative ambulation and shorter LOS[51-54] (Table 1).

Seng *et al*[51] retrospectively analyzed 40 cases of MIS TLIF compared to 40 open TLIFs and compared fluoroscopic times, operative times, EBL, LOS, postoperative narcotic use, complication rates and patient outcomes. The authors found a statistically significant increase in fluoroscopic times in the MIS TLIF group (MIS: 55.2 s, open 16.4 s, $P < 0.001$) as well as a statistically insignificant increase in operative times for the MIS TLIF group (MIS: 185 min, open: 166 min, $P = 0.085$). MIS had less EBL than open (127 cc *vs* 405 cc, $P < 0.001$). Postoperative morphine use for the MIS group was significantly less than the open group (8.5 mg *vs* 24.2 mg, $P = 0.006$). The authors also found that patient in the MIS TLIF group ambulated on average 1.5 d earlier ($P < 0.001$) and had an overall shorter LOS (MIS: 3.6 d, open: 5.9 d, $P < 0.001$). The overall complication rate was 15% for the MIS group and 20% for the open group, however this did not reach statistical significance ($P = 0.774$). Fusion rates, assessed by the Bridwell classification, showed that grade 1 fusion was achieved in 97.5% of both groups at 5 years. Both groups showed significant improvement in ODI, neurogenic symptom score, back and leg pain and SF-36 scores at follow-ups of 6 mo up to 5 years with no significant differences between them.

Parker *et al*[55] prospectively evaluated 100 patients undergoing TLIF (50 MIS *vs* 50 open) for back-related medical resource use, missed work, and QALY. The authors found that LOS and time to return to work were less for MIS compared to open TLIF ($P = 0.006$ and $P = 0.03$, respectively). MIS and open TLIF patients demonstrated similar improvements in patient-reported outcomes assessed. MIS was associated with a reduction in mean hospital cost of $1758, indirect cost of $8474, and total 2-year social cost of $9295 ($P = 0.03$), but similar 2-year direct health care cost and QALYs gained. The authors concluded that while both MIS and open TLIF are effective treatments for degenerative spondylolisthesis, MIS TLIF may represent a valuable and cost-saving advancement from a societal and hospital perspective.

Disadvantages of the MIS TLIF are related to the decreased visualization of the surgical field and a steep learning curve for the procedure resulting in increased fluoroscopic times and operative times which may be more significant in less experienced surgeons.

MIS direct lateral approaches

The lateral transpsoas, retroperitoneal approach, also known as extreme lateral interbody fusion (XLIF) or direct lateral interbody fusion (DLIF) is a minimally invasive technique that has become an increasingly common method to achieve fusion in the lumbar spine. This MIS approach was intially described in by Pimenta[56] (DLIF) in 2001, and later by Ozgur *et al*[57] (XLIF) in 2006. The MIS direct lateral approach differs from the traditional anterior lumbar interbody fusion (ALIF) and open posterior interbody fusion techniques in several important ways. In addition to being an MIS technique, the direct lateral approach requires the patient to be in the lateral decubitus position. The technique utilizes specialized retractors allowing for direct visualization of the surgical approach corridor. Continual neurophysiologic monitoring and fluoroscopic guidance during the approach phase of the procedure is necessary as the psoas splitting technique exposes the lumbar plexus and predisposes it to injury, which can result in psoas muscle weakness and thigh numbness[58,59].

Initial studies evaluating the safety and postoperative results of the MIS transpsoas approach concluded that the MIS technique was safe and allowed for exposure of the lumbar spine without mobilization of the great vessels or sympathetic plexus[60,61].

Smith *et al*[62] compared the long term outcome of XLIF compared to ALIF in a group of 202 patients (115 XLIF *vs* 87 ALIF) and found that the overall general surgical complication rate was significantly lower for XLIF compared to ALIF (8.2% *vs* 16.7%). Rodgers *et al*[63] described the complications in a large prospective series of 600 patients who underwent XLIF. The authors found the overall incidence of perioperative complications was 6.2% (1.5% in-hospital surgery-related, 2.8% in-hospital medical events, 1.0% out-of-hospital surgery-related, and 0.8% out-of-hospital medical events). There were no wound infections, no vascular injuries, no intraperitoneal visceral injuries and a 0.7% transient postoperative neurologic deficit rate. These complication rates compared favorably to the ALIF risk of vascular injury (1.9%-3%)[64-66]. Another major risk of open ALIF approach is retrograde ejaculation, occurring in 0.6%-4.5% of men[67]. There are currently no reports of retrograde ejaculation following %g MIS lateral interbody fusion which can be attributed to the fact that the sympathetic plexus is not mobilized during the transpsoas approach. Motor deficits after MIS lateral interbody fusion have been reported to range from 0.3%-2.9% with the majority of cases resolving spontaneously within three months[63,68,69]. These rates are comparable to the

reported motor deficit rates after PLIF (1.0%-6.1%) and MIS TLIF (4.1%) procedures[70-73].

Rodgers *et al*[74] retrospectively compared lumbar fusion outcomes in geriatric patients over 80 years of age who underwent XLIF or open PLIF. The authors observed a significantly lower complication rate in the XLIF group compared to the open TLIF group (7.5% *vs* 60%) we well as less blood loss (hemoglobin change, 1.4 g *vs* 2.7 g), a lower transfusion rate (0% *vs* 70%) and shorter LOS (1.3 d *vs* 5.3 d). The authors also described a lower overall mortality rate in the lateral interbody group compared to the open PLIF group (2.5% *vs* 30%).

Deluzio *et al*[75] retrospectively reviewed 210 patients (109 MIS *vs* 101 open) who underwent 2-level lumbar spine fusion from L1-2 to L4-5. They found the average LOS in the MIS group to be 49% shorter than in the open group (1.2 d *vs* 3.2 d). The authors noted that the average cost for the entire perioperative period, including both surgical and post-surgical costs out to 45 d, showed an average savings of 9.6% or $2563/patient in the MIS group.

The MIS lateral interbody fusion appears to present a safe and effective technique for treating degenerative lumbar disorders (Table 1). Long-term outcome studies and cost-analysis and value studies are needed to further clarify the benefits of this MIS approach.

CONCLUSION

In the recent years, there has been a growing trend in MIS approaches for the treatment of degenerative diseases of the lumbar spine. Although traditional open approaches are still performed by the majority of spine surgeons, the body of evidence supporting the safety and efficacy of MIS approaches in appropriately selected patients is growing. MIS approaches for lumbar microdiscectomy, laminectomy, and lumbar interbody fusion by way of the transforaminal or direct lateral approach have shown favorable complication profiles and clinical outcomes compared to traditional open approaches. MIS interspinous process devices have shown poor long-term outcomes as well as high complication and reoperation rates.

While the disadvantages of MIS techniques are the steep learning curve, narrow operative corridor and diminished visual field, these are outweighed by the benefits of MIS techniques in many instances. Going forward, long-term outcome studies and cost-effectiveness studies will be needed to fully assess the benefits of MIS techniques for treating degenerative disease of the lumbar spine.

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REVIEW

Evaluation of chronic kidney disease in chronic heart failure: From biomarkers to arterial renal resistances

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Abstract

Chronic kidney disease and its worsening are recurring conditions in chronic heart failure (CHF) which are independently associated with poor patient outcome. The heart and kidney share many pathophysiological mechanisms which can determine dysfunction in each organ. Cardiorenal syndrome is the condition in which these two organs negatively affect each other, therefore an accurate evaluation of renal function in the clinical setting of CHF is essential. This review aims to revise the parameters currently used to evaluate renal dysfunction in CHF with particular reference to the usefulness and the limitations of biomarkers in evaluating glomerular dysfunction and tubular damage. Moreover, it is reported the possible utility of renal arterial resistance index (a parameter associated with abnormalities in renal vascular bed) for a better assesment of kidney disfunction.

Key words: Heart failure; Biomarkers; Doppler; Renal resistance index; Chronic kidney disease

Core tip: In the clinical setting of chronic heart failure the evaluation of renal dysfunction is essential. This review revises the currently available markers of renal function in chronic heart failure for a better characterization of renal function. Moreover, it is discussed the potential utility of a Doppler derived parameter, the renal resistance index, which is associated with abnormalities in the kidney vascularization.

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INTRODUCTION

Subjects with chronic heart failure (CHF) often present

chronic kidney disease (CKD) as well as worsening of renal function (WRF), which are both responsible for a poor outcome[1-3]. Over the past few years interest in this link between the kidney and heart has increased as these organs share many pathophysiological mechanisms, and are therefore able to negatively affect each other. This reciprocal influence has recently been defined as cardiorenal syndrome[4].

In this clinical setting the pathophysiological background underlying renal impairment is, in part, different when acute and chronic heart failure are considered. In acute decompensated heart failure (ADHF) acute kidney injury (AKI) can often occur[5]. Both low cardiac output and venous congestion are the principal determinants of WRF. When cardiac output decreases, renal perfusion and, consequently, glomerular filtration rate are reduced. On the other hand, venous congestion is the cause of a rise in efferent arterioles and end glomerular capillary pressure thus inducing a decrease in net filtration pressure, an increase in interstitial pressure with damage to tubules[6].

In CHF patients, abnormalities in cardiac function lead to a gradual WRF rather than AKI and a steady decrease in renal perfusion due to low cardiac output which is associated with micro and macrovascular disease[5]. However, also the presence of increased central venous pressure in CHF can favour the occurrence of WRF[7,8]. Finally, neuro-hormonal activation further enhances pathophysiological mechanisms leading to WRF[1].

On the basis of these considerations it is clear that in ADHF and CHF patients the evaluation of kidney function is extremely relevant. The aim of this review is to revise the parameters currently used to evaluate renal function in the CHF clinical setting and discuss the usefulness and limitations of biomarkers in evaluating glomerular dysfunction and tubular damage. Moreover, it is reported the possible utility of renal arterial resistance index (a parameter associated with abnormalities in renal vascular bed) for a better assesment of kidney Dysfunction.

ESTIMATION OF GLOMERULAR FUNCTION

Glomerular filtration rate and serum creatinine

Currently glomerular filtration rate (GFR) is the most used index to assess kidney function. It measures kidney filtration capacity and all the guidelines on heart failure recommend its routine use in CHF patients[9,10]. GFR can be estimated using renal clearance of an exogenous substance (inulina or I-iothalamate), however, even though this approach is more accurate, it is limited as it is expensive and time consuming. Consequently, GFR estimation is generally performed using an endogenous marker, *i.e.*, serum creatinine[11]. Creatinine is produced in the muscles from creatine phosphate and it is removed by kidneys through glomerular filtration, but also by proximal tubular secretion.

The traditional method of measuring the clearance of creatinine requires a 24 h urine sample, which can be difficult for patients to perform and is often not done correctly. Therefore GFR is generally estimated by using serum creatinine levels[9]. For this purpose, Cockroft-Gault (CG), simplified Modification of Diet in renal disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD EPI) are the most widespread equations. These equations include creatinine serum levels, age, gender, race and, in CG, weight. However, they present an imperfect performance: GFR is underestimated by MDRD and CKD EPI and overestimated by CG, particularly at lower creatine serum levels[11].

Smilde *et al*[12] were the first to validate these creatinine based equations in a large cohort of CHF patients. In particular, they showed that GFR assessed by MDRD formula is underestimated in patients with normal and near normal values and overestimated in patients with worsening renal function. MDRD has a good prognostic significance, but is lower than that of the real GFR.

More recently the CKD EPI formula has been introduced. It is currently the equation most used to evaluate GFR in CKD as it has been found to be more accurate than MDRD in patients with a preserved renal function[13,14]. Moreover, the metanalysis by Matsushita *et al*[15] showed that CKD EPI formula allowed a better risk stratification for mortality and end stage renal disease in a general population and in patients with cardiovascular diseases.

Valente *et al*[16] first evaluated the role of this equation in the setting of CHF, showing that CKD EPI classifies KDOQI stages more accurately than the MDRD equation, especially in patients with a higher GFR. Furthermore, in the metanalysis of McAlister *et al*[17] the EPI formula was more accurate in estimating the risk of patients with CHF. According to these studies the CKD EPI formula is the preferred method to estimate GFR in CHF patients, particularly in those with preserved or moderately impaired renal function.

Creatinine serum levels and GFR are also used to estimate WRF. In CHF, WRF has generally been evaluated by assessing changes in creatinine[18]. In particular, an increase of > 0.3 mg/dL and/or > 25% between two time points[3] have been shown to be related to a worse outcome, hospitalizations for heart failure and higher mortality[19-23]. However, a rise in serum creatinine could be not associated with a significant reduction in GFR. A patient presenting higher baseline values of serum creatinine will show a less marked change in GFR than a patient with lower baseline values. Also the use of changes in GFR in order to assess WRF present some limitations due to the slight fluctuations in GFR which are common. For this reason current guidelines suggest that changes of 25% or more in estimated GFR should be considered as being associated with a change in GFR category[24].

Although creatinine serum levels and estimated GFR represent the corner stone in the evaluation of renal function and its worsening, several factors influencing the creatinine value and limiting its derived measures

should also be considered. Creatinine values show a significant between-person and within-person variability, they are influenced by age, diet, gender and body mass. Moreover, when kidney filtration capacity decreases, GFR can be overestimated because of creatinine tubular active secretion[25]. Finally, in CHF patients there are several factors that can affect the validity of equations used to estimate GFR, such as the hemodynamic component of renal dysfunction, drugs interfering with renal function and the loss of muscle mass that is frequent in the end stages of the disease[12].

It is also worth noting that the kidneys use only part of their filtering capacity. A normal GFR could subtend an impairment in this increasing filtration capacity, in other words it could be observed in kidneys with a reduced renal reserve[26].

In order to avoid the limitations of creatinine, new biomarkers estimating glomerular function, such as Cystatin C, have been introduced.

Cystatin C

This is a protein of cysteine proteinase inhibitor family (13 kdal) which is secreted by all cells with nucleus. It passes freely through the glomerular basement membrane and then it is reabsorbed, but not secreted in the tubules. As a consequence, it is an ideal marker of kidney filtration capacity. Moreover age, gender, food and body mass affect less its serum levels[27]. The only clinical conditions that can influence it are inflammatory status, the use of corticosteroids and thyroid diseases[28,29]. Several studies have shown that, in patients with moderate renal impairment, Cystatin C serum levels estimate GFR better than the equations based on creatinine serum levels[30,31].

In fact the KDIGO guidelines[32] for CKD management recommend assessing Cystatin C in patients with eGFRcr of 45-60 mL/min per 1.73 m^2 , on condition that they don't present other manifestations of CKD, such as albumin-creatinine ratio > 30 mg/g.

Formulas based on Cystatin C values have also been presented in order to obtain an estimate of GFR thus facilitating the clinical use of this marker. Moreover, equations based on combined creatinine-cystatin serum levels give a more accurate estimate of GFR than those based on each single level[33,34].

Cystatin C has also been demonstrated more accurate than creatinine in stratifying the risk of mortality and cardiovascular events in old subjects[35] and people with coronary artery disease[36]. High levels are also predictors of heart failure onset[37]. It is likely that the high Cystatin C levels reflect a mild or moderate decrease in renal function that is not detected by creatinine[33].

There are limited data on the role of Cystatin C in CHF[38-40]. Arimoto *et al*[38] showed the usefulness of Cystatin C in prognostic stratification of mild or moderate CHF. Moreover, Cystatin C has been found to better estimate the increased risk of cardiovascular death in elderly affected by CHF[39] as well as in patients with preserved renal function[40].

Although Cystatin C could be an interesting marker for an early assessment of renal function and for risk stratification in CHF, at present it is not widely used to estimate GFR. This is due to the widespread and consolidated use of creatinine, as well as to the fact that the Cystatin C measurement is more expensive, even if other markers, such as NT proBNP or troponin, have a similar cost[32].

Microalbuminuria

Albumin is the protein with the highest concentration in serum but has a low concentration in urine because of its large size, the selectivity of the glomerular filtration barrier and tubular reabsorption[11]. An increased albumin urinary excretion is a recognized early marker of kidney damage and its quantification is used for monitoring patients with CKD as well as for estimating the risk of kidney disease progression[24], as shown in Table 1. The urinary excretion of albumin is currently evaluated by calculating the albumin-to-creatinine ratio (UACR) in urine samples. An UACR < 30 mg/g is defined as normoalbuminuria, a UACR between 30 and 299 mg/g as microalbuminuria, and a UACR > 300 mg/g as macroalbuminuria[41].

Microalbuminuria has been shown to be associated not only with CKD progression, but also with an increased risk of cardiovascular death in the general population as well as in subjects with diabetes and hypertension[42].

The greater prevalence of microalbuminuria in CHF subjects than in the general population was demonstrated for the first time by Van der Wall[43]. In CHF albuminuria and reduced GFR can coexist, but sometimes only one of the conditions was found because they are caused by different pathophysiological mechanisms. Albuminuria can be the consequence of damage to the glomerular basement membrane secondary to endothelial dysfunction and inflammatory cytokine activation[44]. Moreover, it can be caused by hyperfiltration due to a reduction in the number of nephrons. This condition could occur in the event of a post glomerular ischemia which leads to hypoxia, oxidative stress and tubulointerstitial injury[45]. Other mechanisms involved in the genesis of microalbuminuria could be renal congestion[46,47] and reduced tubular reabsorption of albumin as a consequence of tubular dysfunction[48].

The large substudies from CHARM and GISSI HF trials[49,50] have confirmed the high prevalence of micro and macroalbuminuria in CHF patients (microalbuminuria was more common than macroalbuminuria) and its association with a worse outcome. HF patients with higher albumin urinary levels showed a greater mortality and an increased risk of HF hospitalizations. This prognostic value was independent from serum creatinine levels, eGFR and the comorbidities that worsen renal filtration function. Another important finding of these studies was that mortality risk increased with the increase in albumin excretion, even in the normal range (< 30 mg/g) suggesting that this parameter is a continuous measure of risk.

All of these studies showed that albumin urinary excretion is not affected by treatment with angiotensin

Table 1 Risk of chronic renal disease progression according to K/DOQI clinical practice guidelines[25]

Glomerular filtration rate categories			Microalbuminuria		
			A1 normal to mildly increased < 30 mg/g	A2 moderately increased 30-299 mg/g	A3 severely increased > 300 mg/g
1	Normal or high	> 90 mL/min	Low risk	Moderately increased risk	High risk
2	Mildly decreased	60-89 mL/min			
3a	Mildly to moderately decreased	45-59 mL/min	Moderately increased risk	High risk	Very high risk
3b	Moderately to severely decreased	30-44 mL/min	High risk	Very high risk	Very high risk
4	Severely decreased	15-29 mL/min	Very high risk	Very high risk	Very high risk
5	Kidney failure	< 15 or dialysis mL/min	Very high risk	Very high risk	Very high risk

receptor blockade, contrary to that observed in patients with diabetes, hypertension and renal disease. This may be due to the different pathophysiological mechanisms underlying albuminuria in CHF[51].

MARKERS OF TUBULAR DAMAGE

Different markers reflecting tubular damage have been studied in order to obtain a more accurate evaluation of renal function.

The Neutrophil gelatinase-associated lipocalin (NGAL) is a lipocalin protein (25 kdal), which is produced by the kidney, but also by other organs (the trachea, lung, stomach and colon). After being filtered through the glomerulus, NGAL is reabsorbed in the proximal tubule. When the proximal tubule is damaged, filtered NGAL can not be totally reabsorbed and its urinary levels increase[52]. Furthermore, during tubular damage NGAL mRNA is transcribed and overexpressed in loop of Henle and collecting duct and its plasma and urinary levels rise considerably[53,54]. NGAL plasma levels can also be increased by systemic diseases such as inflammation and cancer, but urinary levels are less influenced by these conditions[54].

The N-acetyl beta glucosaminidase (NAG) is a protein produced in the proximal tubular cells which is excreted into the urine when a tubular injury occurs[55]. Its urinary levels are increased in acute and chronic kidney disease[56] but also in diabetes and hypertension.

The Kidney injury molecule (KIM1) is a transmembrane glycoprotein and its expression in proximal tubule cells increases significantly after hypoxic or nephrotoxic tubular injury[57]. Its urinary levels are high also in patients with polycystic kidney[58] and renal cancer[59].

NAG, NGAL and KIM 1 have been widely studied in the setting of AKI (Acute Kidney Injury),where they have had a diagnostic and prognostic role, and show an increase 24 h prior to creatinine[60-63]. Recently, the utility of these markers in cardiorenal syndrome[25] has been evaluated by a number of studies, mainly involving patients with ADHF[64-67].

On the other hand, there are few studies which examine these markers in patients with CHF[68]. Damman *et al*[69] have shown a significant increase in urinary NGAL levels in these patients when compared to healthy controls, suggesting that renal dysfunction in CHF is characterised not only by a decrease in GRF and an increase in urinary albumin excretion but also by tubular damage. The renal perfusion decrease that occurs in CHF produces a GRF reduction and determines hypoxia leading to tubular damage.

Another study by Damman *et al*[70] showed that NGAL urinary levels were not correlated with prognosis, while urinary NAG and KIM1 were associated to a worse outcome (death, heart failure, hospitalisations and heart transplantation) and have an additional prognostic value compared to GRF. According to the authors, one explanation for this difference could be that NAG and KIM1 are produced in two different parts of the nephron and damage to the proximal part of the tubule may be more significant for the prognosis.

Finally, in a substudy of the GISSI HF trial[71] it has been confirmed that markers of tubular damage were strongly associated with HF hospitalizations and all cause mortality. In particular, NAG was the only marker remaining significantly correlated with a worse prognosis at multivariate analysis. This association was independent from estimated GFR and urinary protein excretion.

Markers of tubular function could be also related to WRF. In another substudy of the GISSI HF trial[47] patients with WRF had higher NGAL, NAG and KIM1 levels. These outpatients with WFR had a worse outcome. A possible explanation could be that the increase in these markers is caused by renal hypoxia, leading to a progressive deterioration of renal function that makes the kidney more vulnerable. At multivariate analysis KIM1 was the strongest independent predictor of WRF among all associated variables (eGFR and albuminuria included).

Beside NAG, NGAL and KIM 1, also other markers of tubular damage have been proposed.

IL 18 is a cytokine secreted by proximal tubular cells. When AKI occurs, it has been shown to increase more quickly than serum creatinine levels. It also increases in inflammatory status, therefore it is not highly specific[72,73]. Mallat *et al*[74] showed that, in ADHF, IL 18 plasma levels increased and were higher in patients who died during follow-up.

The fatty acid-binding proteins (FABPs) are proteins able to bind free fatty acids. Among the several tissue-specific isoforms, urinary levels of liver FABP (FABP-1)

and heart FABP (FABP-3) can reflect AKI, because excreted when an ischemic tubular injury occurs[25]. Moreover, serum FABP levels have been associated to worse outcome in CHF patients[75].

RENAL CIRCULATION AND RENAL RESISTANCE INDEX

Markers of GFR and tubular damage offer information about the status of nephrons. However, kidney circulation also plays a key role in renal function. Consequently, the evaluation of renal circulation could provide useful parameters to add to the information obtained from the above mentioned biomarkers.

Renal circulation

The kidney is a highly perfused organ, receiving 22% of cardiac output at rest[76]. After entering the hilum, the renal arteries divide into the interlobar, arcuate and interlobular arteries. Afferent arterioles originate from interlobular arteries, leading to the glomerular capillaries, and then to efferent arterioles. Efferent arterioles are followed by a second capillary network, *i.e.,* peritubular capillaries, and finally by the venous system. The regulation of renal blood flow (RBF) is not dependent on oxygen demand, but on reflex and neurohormonal mechanisms underlying the control of afferent and efferent arteriole tone. These mechanisms are not discussed in this review, however it is worth noting that arteriole tone changes are the main determinants of arterial renal resistance. The arteriolar tone is determined by reflex mechanisms, such as myogenic effect and tubular-glomerular feedback, and neurohormonal mechanisms. Of the neurohormonal systems, sympathetic and renin-angiotensin-aldosterone are those which most influence efferent arteriolar tone, determining vasoconstriction and increased water and sodium reabsorption. Endothelin, nitric oxide, prostaglandins, bradykinin and natriuretic peptides are also involved in the regulation of arteriolar tone.

The self-regulation of RBF allows renal perfusion to be kept constant through a wide range of arterial pressure (70-180 mmHg). On the other hand, changes in venous renal pressure could affect RBF more than those of arterial pressure. In 1931 Winton[77] evaluated the reduction of RBF by changing arterial and venous pressures in isolated kidneys. The results demonstrated that the changes were greater when renal venous pressure had been increased. More recent studies have confirmed the association between central venous pressure and renal function worsening in acute, as well as in chronic heart failure[77-79]. The mechanisms by which central venous pressure could affect RBF are different. In particular, it can increase both intra-abdominal pressure and renal venous pressure. This leads not only to an increase in capillary pressure and a reduction in artero-venous gradient but also to greater interstitial pressures and, consequently, greater arterial renal resistances.

Functional abnormalities of RBF and of renal resistances due to neurohormonal and hemodynamic changes could also lead to structural changes. Chade[76] demonstrated that the functional increase in renal vascular resistances could lead to ischemia, endothelial dysfunction, cytokine production and finally to fibrosis. This cascade of events causes a renal vascular rarefaction which could further induce CKD worsening.

On the basis of this pathophysiological background we can conclude that an increase in arterial renal resistances could represent an altered neuro-hormonal status, an increased central venous pressure, but it could also reflect the presence of parenchymal abnormalities and vascular rarefaction which favour CKD progression.

Arterial renal resistance index

Arterial renal resistance index (RRI) is a non-invasive measurement of renal arterial resistance which is easily evaluated by Doppler technique (Figure 1). It is calculated using Pourcelot's formula[80] since the peak systolic velocity and the end-diastolic velocity are obtained. Doppler evaluation of renal arteries is generally performed at the level of one or more interlobar arteries. In healthy adult subjects the RRI mean value is around 0.60 with no significant differences between the two kidneys[81]. An RRI greater than 0.70 is generally considered abnormal[82].

RRI has been found to be associated with renal parenchymal abnormalities. Platt e coll[83] first demonstrated the relationship between an increased RRI and parenchymal and vascular abnormalities assessed by renal biopsies. Later RRI has been found to be strongly associated with arteriolosclerosis, glomerulosclerosis, interstitial renal fibrosis and tubulo-interstitial lesions[84-88].

Our study group evaluated the independent predictors of RRI values in a large group of CHF outpatients with reduced ejection fraction. In a multivariate logistic regression model including univariate predictors, only age, systolic pulmonary pressure, central venous pressure, GFR, diabetes, logarithm of NT-proBNP, pulse arterial pressure and NYHA class remained significantly correlated to RRI. These results help to confirm the pathophysiological background responsible for the modification of RRI in CHF. In particular, RRI seems to carry information about renal artery abnormalities and/or alterations of arterial stiffness, as suggested by the correlation we found with the related parameters such as age, diabetes and pulse pressure. Moreover, the relationship between RRI and central venous pressure highlights the role of intra abdominal pressure and renal venous pressure in determining an increase in renal arterial resistances[89].

In a group of patients with CHF and preserved left ventricular ejection fraction, Ennezat *et al*[90] first demonstrated the significance of RRI in predicting prognosis. In our series of outpatients affected by CHF (mainly due to a reduced left ventricular ejection fraction), we have also demonstrated the prognostic significance of RRI[89]. The combined end-point that we considered was death, urgent heart transplantation or hospitalization due to heart failure worsening. RRI was associated with events in an univariate Cox regression model (HR

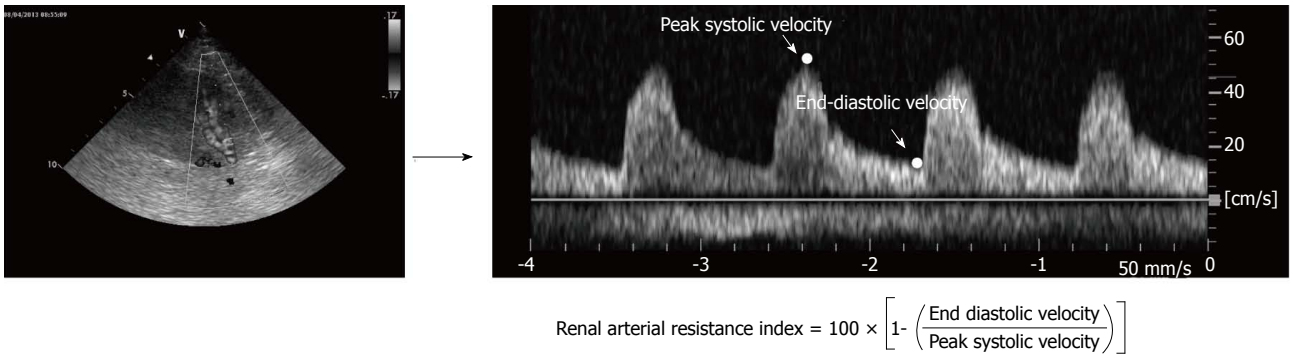

Figure 1 Calculation of Renal arterial Resistance Index. Renal arterial Doppler was performed using a 4 MHz probe, with the patient in the sitting position and using a posterior approach to visualise the kidney. The course of the right or left kidney segmental arteries is visualised by color Doppler flow and, at the middle tract level of the best one visualised, pulsed Doppler is performed. Peak systolic velocity and end diastolic velocity are used to calculate the renal arterial resistance index according to Pourcelot's formula.

= 1.14; 95%CI: 1.09-1.19; $P < 0.001$; C-index: 0.77), but also in a multivariate model (HR = 1.08; 95%CI: 1.02-1.13; $P = 0.004$; C-index: 0.86) correcting for the independent predictors, i.e. LVEF, GFR and logNT-proBNP. Moreover, with the addition of RRI, the reclassification model showed an important rise according to both category-free net reclassification improvement NRI (47%; 95%CI: 13%-80%; $P = 0.006$) and integrated discrimination improvement IDI (0.034; 95%CI: 0.006-0.061; $P = 0.016$). Likewise, in our series, RRI has been also found to be independently associated with an increased risk of death (at multivariate analysis: HR = 1.06; 95%CI: 1.01-1.12; $P = 0.023$; C-index = 0.783)[91].

The pathophysiological factors influencing RRI offer an explanation to the incremental prognostic value of this parameter when added to a model already including GFR. In addition to GFR, RRI could provide further information about renal function by reflecting not only glomerular function but also the other factors which influence the progression of kidney disease. Therefore, RRI could complete the information carried by GFR and allow a better characterization of renal dysfunction in CHF.

This is also supported by the fact that in our series RRI was able not only to predict heart failure progression but also worsening of renal function. At multivariate analysis a RRI > 70 was independently associated with an increase in creatinine > 0.3 mg/dL[92], regardless of baseline GFR values.

CONCLUSION

The evaluation of renal dysfunction is a cornerstone for CHF patients' management. GFR estimation is, at present, considered the best parameter to assess overall kidney function and it is recommended for routinely use. However, serum creatinine, traditionally used to estimate glomerular filtration rate, has several limitations. As a consequence the use of other parameters to evaluate hyperfiltration, tubular function and hemodynamic status could be useful in order to better define renal function. To this purpose RRI, an echo-Doppler derived parameter, which reflects abnormalities of the renal vascular bed, seems to be very promising as it carries an independent and incremental information to detect patients with worse prognosis and increased risk of WRF.

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REVIEW

Anthrax: A disease of biowarfare and public health importance

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Abstract

Bioterrorism has received a lot of attention in the first decade of this century. Biological agents are considered attractive weapons for bioterrorism as these are easy to obtain, comparatively inexpensive to produce and exhibit widespread fear and panic than the actual potential of physical damage. *Bacillus anthracis* (*B. anthracis*), the etiologic agent of anthrax is a Gram positive, spore forming, non-motile bacterium. This is supposed to be one of the most potent BW agents because its spores are extremely resistant to natural conditions and can survive for several decades in the environment. *B. anthracis* spores enter the body through skin lesion (cutaneous anthrax), lungs (pulmonary anthrax), or gastrointestinal route (gastrointestinal anthrax) and germinate, giving rise to the vegetative form. Anthrax is a concern of public health also in many countries where agriculture is the main source of income including India. Anthrax has been associated with human history for a very long time and regained its popularity after Sept 2001 incidence in United States. The present review article describes the history, biology, life cycle, pathogenicity, virulence, epidemiology and potential of *B. anthracis* as biological weapon.

Key words: Anthrax; *Bacillus anthracis*; Biological warfare; Epidemiology; Infection; Public health

Core tip: Anthrax is primarily a zoonotic disease which is caused by *Bacillus anthracis* (*B. anthracis*) and for human it has both, public health as well as biodefence importance. Anthrax has been known since ancient times; however it acquired attention as biological warfare disease after 2001 incidence in United States. *B. anthracis* is supposed to be the most potent BW agent because of its hardy spores, various modes of infection and high mortality rate. Understanding about the life cycle, virulence, pathogenicity and detection and diagnosis of *B. anthracis* is important to curb the disease.

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INTRODUCTION

Bacillus anthracis (*B. anthracis*), the causative organism

of anthrax is a Gram-positive spore forming bacillus commonly found in soil of endemic areas. Anthrax is a zoonotic disease which is mainly associated with herbivores and domestic animals. The disease occurs regularly in countries where widespread vaccination of animals is not practiced. Human anthrax is less common and usually spreads to human populations through close occupational proximity to infected livestock by handling infected domestic animals including cattle and goats or their products like skin, meat, hides and bones. This bacterium can infect humans by cutaneous, gastrointestinal, or respiratory routes[1]. *B. anthracis* exists in two forms, vegetative cells (inside the host) and spores for persistence in the soil or environment[2]. In the soil, *B. anthracis* is generally found in endospore form where it can remain viable for decades in this form. As *B. anthracis* forms spores that can be aerosolized and sprayed to spread disease, the potential use of this bacterium as a bioterrorism agent has long been suspected. However, the events in 2001 have confirmed that bioterrorism is no longer a threat but a reality[3]. Owing to its highly pathogenic nature and spore forming capability, *B. anthracis* is considered as one of the most important biological warfare agents[4,5].

There are two major virulence factors in *B. anthracis*, poly-γ-D-glutamic acid capsule and a tripartite toxin[6]. Pathogenic *B. anthracis* bacteria produce capsule which mimics the immune system of host by masking the bacteria from macrophages[7]. The tripartite toxin of anthrax consists of three independently secreted proteins, *i.e.,* protective antigen (PA), lethal factor (LF) and edema factor (EF)[8,9]. Anthrax toxin is a binary A-B toxin, where PA acts as the binding (B) domain and LF and EF act as active (A) domains individually to form the binary toxins lethal toxin (LTx) and edema toxin (ETx), respectively[10]. After ingestion or coming in contact with skin lesions, bacteria multiply and within a few days or weeks cause the death of human or animal host.

Anthrax is not a major issue of health in developed countries as only a few incidences are reported from such countries. However, for developing countries whose economy is mainly agriculture dependent, cutaneous anthrax is still a major concern of health. India ranks first in having the world's largest livestock population. Therefore, animal anthrax is common in several regions in India. However, only a few intermittent cases of human anthrax are reported from the Southern states[11]. Human cutaneous anthrax is a concern of public health in some states like Orissa and Andhra Pradesh[12].

HISTORY OF ANTHRAX

Anthrax, caused by *B. anthracis* is a highly contagious and fatal. Anthrax has a long association with human history and was known in Europe (1190-1491 BC) and China (3000 BC). Anthrax was described in the early literature of the Greeks, Romans and Hindus. The name anthrax was derived from the Greek word "anthrakis" which means coal because coal black skin lesions are formed in cutaneous form of anthrax. The description of fifth plague of Egypt, an epidemic of ancient Egypt in the book of Genesis (1491 BC), which exterminated the Egyptian livestock including cattle, sheep, goats, camels, horses and donkeys without affecting the Israelites livestock, may be due to anthrax. The disease described by Virgil (29 BC) in his third Georgics (selection of poems on agriculture and animal husbandry) seems to be anthrax in domestic and wild animals as it was an economically important agricultural disease in Europe during the 16th to the 18th centuries[13].

In 19th century, research on anthrax led to a lot of medical developments. In 1850, Pierre Rayer first described filiform bodies (small rods, about half the length of a red blood corpuscle) in the blood of sheep that had died due to anthrax. Casimir-Joseph Davaine in 1863 suggested that the "corpuscles" were the etiology of anthrax that could be transmitted to sheep, horses, cattle, guinea pigs, and mice by subcutaneous inoculation of infected blood[14]. Tiegel and Klebs in 1864 demonstrated that anthrax-infected blood, if filtered through a clay candle (bacterial filter), lost its infectivity, while the deposit on filter remained infective[15]. These observations in absence of culture of the organism strongly supported the concept that the causative agent of anthrax was a living organism that multiplied in the body, invaded the blood stream, and produced death by septicaemia. Robert Koch derived his three postulates for germ theory of disease considering anthrax as prototype. In 1876, he conclusively proved that *B. anthracis* was the etiological agent of anthrax by applying his postulates for the first time during his research in Wollstein, Germany[16]. Thus, anthrax was the first disease whose causative agent was established as microbial agent. After isolating the anthrax bacteria from skin lesions of sheep, he obtained the pure cultures by growing the bacilli on the aqueous humor of ox's eye, and injected the bacteria into healthy sheep. He performed another experiment by growing pure cultures of rods from the aqueous humor of an ox's eye. By studying, drawing and photographing these cultures, he recorded the multiplication of the bacilli and found that under unfavourable environmental conditions, especially under conditions of oxygen deprivation, they produced round spores within themselves. The spores return to bacilli when growth conditions are favourable, proving the spore formation as self-protective mechanism of *B. anthracis*. Thus, by now it was clear how certain pastures or agriculture areas became dangerous. When any animal dies from anthrax infection, the infected blood and body fluids comes out in soil from the natural orifices of animal. The bacteria, which are in the vegetative form in the blood, convert into spores on exposure to air. These spores are extremely resistant to natural conditions and could remain dormant in the soil for decades. These spores remain available to cause new infections among susceptible animals that graze in the field.

Pasteur *et al*[17] proved the buried cadavers of anthrax

infected animals as important origin of new infections. They further revealed that spores from buried soil could be transported to the upper surface by the activities of earthworms[18]. He also confirmed Koch's discovery of the anthrax germ. He found that chickens were immune to anthrax, and postulated that it was because of high body temperature (43 ℃-44 ℃) of chickens. On lowering the body temperature to 37 ℃, chickens became susceptible to anthrax. For vaccination, Pasteur heated the anthrax germs and inoculated 25 sheep. He used the heated anthrax bacteria to inoculate sheep and found that all sheep survived (only one pregnant sheep died due to some other complications), whereas all un-inoculated sheep died after one or two days of challenge with virulent *B. anthracis*. Pasteur proved that the weakened anthrax lost its virulence but still could confer immunity and this technique was termed as "vaccination". Thus, first live bacterial vaccine was developed for anthrax by Pasteur *et al*[17]. During 1876-1877, a devastating anthrax outbreak affected several sheep and cattle in France's livestock. By that time, rod-shaped *B. anthracis* was established as the causative agent of anthrax by Robert Koch. However, still many people believed that instead of bacterium itself, some toxic substance produced by *B. anthracis* was causing the disease. But, Pasteur finally proved that anthrax was caused by living *B. anthracis* and not by some toxic substance.

Anthrax was also known as woolsorters' disease. Prior to 1837, no specific disease had been associated with wool. However, after that a large number of cases occurred in and around Bradford, England and the name Bradford disease became synonymous with woolsorters' disease. In 1879, Bell proved that woolsorters' disease (now inhalational anthrax) was due to anthrax[19]. This led to institution of Bradford rules which in 1897 became law. Consequently, incidences of inhalation anthrax among sorters decreased significantly. In 1913, Eurich found that blood contamination was the important factor in woolsorters' disease[20]. Blood seemed to serve as a glue to bind anthrax spores to the raw product. Washing of wool removed soil, dried serum and blood but anthrax spores remained adherent. Elimination of inhalation anthrax as an industrial hazard followed passage of the Anthrax Prevention Act in 1919. This law mandated the construction of a decontamination station in Liverpool whereby all dangerous wool and hair products entering England were disinfected with formaldehyde[21].

During 1979-1980, the world's largest ever recorded outbreak of anthrax occurred in Zimbabwe during the civil war. In a two-year period, over 9400 cutaneous anthrax cases, including 182 fatalities were reported. Before the war, anthrax was endemic in Zimbabwe and only a few cases of anthrax were reported. Number of human anthrax cases increased significantly during this period because lack of food due to civil war in country forced people to handle and eat anthrax infected animals. Anthrax being a zoonotic disease, it first appeared in cattle and then spread in human population in all the affected areas of Zimbabwe.

Anthrax has been supposed to be developed for use as a bioweapon during world war-1 and world war-Ⅱ. Recently, in 2001, envelopes containing the *B. anthracis* organism were sent through the mail to different dignitaries in United States affecting 22 people. This was considered as an act of bioterrorism[3].

BIOLOGY OF *B. ANTHRACIS*

B. anthracis is a Gram positive, rod-shaped, aerobic, facultative anaerobic, sporulating, capsulated bacterium. It measures 1-1.2 μm in width and 3-5 μm in length. Under microscope, it appears as chain like structure. Though an aerobic organism, yet *B. anthracis* can survive in anaerobic environment because of its property of sporulation. In fact, it can survive for several years in soil, air and water in the form of spores. Unaffected to harsh environment, spores are resistant to high temperature, pressure, pH, chemicals, UV and deficiency of nutrients[22-24]. The capsule is composed of γ-linked poly-D-glutamic acid which gives mucoid appearance to the colony. Formation of capsule decides the virulence of bacteria. The capsule itself is non-toxic and doesn't provoke immune system of the host. However, it contributes significantly in establishing the infection, once the organism escapes phagocyte action, later phase of disease is controlled by anthrax toxin[25].

Pathogenic strains of *B. anthracis* harbour two virulent plasmids[26]. Plasmid *pXO1* carries toxins encoding genes and plasmid *pXO2* carries capsule encoding genes. Size of *pXO1* is 184.5 kb that harbours three structural genes, *pag* (coding for PA), *lef* (coding for lethal factor) *and cya,* coding edema factor[1]. Plasmid *pXO1* also encodes *atxA* gene which regulates the expression of gene encoded on *pXO1* and *pXO2*. Another plasmid pXO2 is 95.3 kb in size and carries the genes for capsule production, degradation and regulation. Genes *capB, capC and capA* code for capsule synthesis, and gene *dep* codes for its degradation[1]. A gerX operon is also present on plasmid *pXO1* and its deletion affects the germination of spores in macrophages. The operon codes for three proteins GerXA, GerXB and GerXC. These proteins are supposed to form a receptor, which specifically detects germinant within the host[27].

POTENCY OF *BACILLUS ANTHRACIS* AS BIOWARFARE AGENT

Anthrax was linked to soil contamination long before the identification of *B. anthracis* as its causative agent[14,16]. Spores can resist prolonged exposure to stress as desiccation, solvents and extreme temperature, pressure, pH, ultraviolet and ionizing radiation[28,29]. Spores of *Bacillus* genus are known to have a half life of about 100 years[30]. Spores are dormant form of the bacterium which returns into vegetative form on receiving the signals for germination. The surprisingly resistant spores have earned the status of potential bio-

terror weapon for anthrax. The possibility to create aerosol from spores makes *B. anthracis* a lethal biological weapon. All the attributes of spores: high resistance to temperature, pressure, pH, ionizing radiations and half life of 100 years make them a suitable bio-terror agent. After production and purification, anthrax spores can be stored in a dry form which remains viable for decades. Spores may survive in the water, soil and on surface for several years. Inhalation of spores causes inhalational anthrax which is the most dangerous form of disease. Inhalational anthrax is dangerous for obvious reasons as initial symptoms resemble to that of flu, making its early diagnosis difficult; by the time disease is correctly recognized it's too late.

The use of microorganisms as a means of waging war or as bioterror agents is becoming a real possibility now around the world. Any biological agent from a large gamut of human infection causing pathogens could be considered a potential biological weapon. However, only a small number of these agents fulfil the desirable criteria like ease of cultivation and dispersal or dissemination for recognition as possible biological weapons. Anthrax spores pose the biggest bioterrorism threat because it is easier to produce and preserve them. Anthrax spores have already been used in United States and in future also it is most likely preferable agent to be used for biothreat because of high case fatality rates, rapid transmission by aerosol and its stability in the environment. The release of any bio-warfare agent by a militant or miscreant would likely be silent and untraceable or nearly so. Therefore, of the recognized possible biological weapons, anthrax bacilli are rated the most lethal.

Naturally, anthrax is a zoonotic disease, which primarily occurs in animals and then spreads to human. Several animal species like cattle, goat and sheep are susceptible to this disease. A major public health preparedness challenge is increasing the importance of recognition of individual, potential sentinel cases of biothreat agent disease. According to CDC norms, *B. anthracis* is placed in high priority- Category A due to its ease of dissemination, high mortality rates, epidemic potential and special preparedness it requires. In 2001, mails deliberately contaminated with *B. anthracis* spores were used to terrorize people and subsequently research for the development of anthrax vaccine speeded up. Moreover, each category A biothreat agent has its unique clinical and diagnostic features and no single system can meet the challenges of all the agents. Besides, anthrax is still a concern of human as well as veterinary public heath in several states of country like India. Bioterrorism itself is an emerging problem for public health. Hence, it is not possible to look into bioterrorism and public health separately. Rather, it is the need of time to give more emphasis on such diseases which have both the potential.

DOSE-RESPONSE RELATIONSHIP

The information on dose-response relationship is prerequisite for assessment of risk of any biothreat agent. The LD_{50} of human inhalational anthrax is not known, but has been estimated from the animal studies and disease outbreaks. After conducting experiments on 1236 cynomolgus monkeys (*Macaca fascicularis*), Glassman estimated the median lethal dose to be 4130 spores with 95%CI range of 1980-8630[31]. Further, he suggested that LD_{25} was associated with a 10-fold decrease in dose *i.e.,* 413 spores. In 1957 in Manchester, 16 susceptible workers were exposed to *B. anthracis* in a goat hair processing mill and 4 persons were infected. Based on the 8-h inhaled dose, LD_{50} of *B. anthracis* was estimated to be 6200-22000 spores[32]. The infectious dose for inhalational anthrax in 50% of susceptible human population (ID_{50}) was estimated to be 8000-50000 spores by biodefense experts from the United States Army Institute of Infectious Diseases (USAMRIID, Fort Detrick, MD)[33]. In 1998, a panel of seven subject matter experts on anthrax calculated the ID_{10}, ID_{50} and ID_{90} as 1000-2000 spores, 8000 to 10000 spores and 50000 to 100000 spores, respectively[34]. Another group extrapolated the lethal dose (LD_{50}) values of 4100 spores[31] and 8000 spores[33] and suggested an LD_{10} of 50 or 98; an LD_5 of 14 or 28, an LD_2 of four or seven, and an LD_1 of one or three spores[35]. Although they did not establish the validity of extrapolation, yet they cautioned about the low number of spores.

Theoretically, even a single spore of *B. anthracis* can cause anthrax. However, in the low dose range, there is high uncertainty between the dose-response relationships of aerosolized *B. anthracis* for human. Recently, on the basis of experimental data on primates and epidemiological data of human anthrax, a new quantitative model known as Exposure-Infection-Symptomatic illness-Death (EISD) has been suggested for the dose-response as well as time course of pulmonary anthrax in human[36]. According to this model, the ID_{50}, ID_{10} and ID_1 of *B. anthracis* spores were 11000 (95%CI: 7200-17000), 1700 (1100-2600) and 160 (100-250), respectively. The ID_{50} (7200-17000) and ID_{10} (1100-2600) confidence ranges produced by this model were remarkably consistent with the corresponding ranges produced by an expert panel surveyed in 1998, *i.e.*, 8000-10000 and 1000-2000, respectively[34]. The confidence range of ID_1 from 100-250 spores as suggested by this model indicates that a threshold of 600 *B. anthracis* spore to human infection is underestimated and infection by even a single spore is overestimated in the literature. This model also suggested the median incubation time from exposure to onset of symptoms. For exposure with ID_{50} of *B. anthracis* spores, it was 9.9 d with 95%CI of 7.7 to 13.1 d, where as for ID_{10} and ID_1, it was 11.8 (95%CI: 9.5-15) d and 12.1 (95%CI: 9.9-15.3) d, respectively.

DIFFERENT STRAINS OF *BACILLUS ANTHRACIS*

Three well known strains of *B. anthracis* are Ames, Sterne and Vollum. Ames is a well studied, highly virulent strain

containing both plasmids, *i.e., pXO1* and *pXO2*. Originally it was isolated from a dead cow in Texas in 1981. Its geographic region is United States and United Kingdom. Another isolate of Ames strain is Florida which was first isolated from a victim of anthrax attack in 2001[37,38]. *B. anthracis* Sterne is a toxigenic but avirulent strain as it carries the anthrax toxin plasmid pXO1 but lacks the capsule forming plasmid pXO2[39]. This strain is generally used for vaccine development for animals. Its geographic region is in Canada[37,38]. In contrast to Sterne, Pasteur strain carries pXO2 plasmid but not pXO1. Vollum is low virulent strain used in research studies and is found in the United Kingdom, Spain and Zimbabwe[37]. Along with Vollum and Sterne, strain V770 is also used for toxin production and various research related studies.

B. anthracis belongs to *Bacillus cereus* sensu lato group, shared by six other species including *B. cereus*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus thuringiensis*, *Bacillus weihenstephanensis*, and *Bacillus cytotoxicus*[40]. *B. cereus* primarily causes foodborne illness. Besides, *B. cereus* is considered as an opportunistic pathogen that can cause wound infections, endocarditis and urinary tract infections in humans. Recent studies indicate that a *Bacillus* species other than *B. anthracis* can cause anthrax-like disease and a few *B. cereus* strains have been found to be associated with "anthrax like" infections in human[41,42]. In India, a *B. cereus* strain TF5 was isolated from the tissue fluid of cutaneous anthrax-like skin lesions of a human patient from an anthrax endemic area in India[43,44]. The strain harboured a PA gene, however, the pXO1 or pXO2-like plasmids were not present. Exoproteome analysis exhibiting qualitative and quantitative differences between the two strains indicated an altered regulatory mechanism and putative role of S-layer protein and sphingomyelinase in the pathogenesis of strain TF5[43].

EPIDEMIOLOGY OF ANTHRAX

B. anthracis bacteria are very fragile and susceptible to disinfectant or exposure to moderate temperature. However, *B. anthracis* vegetative cells convert into spores on exposure to air. These spores are highly resistant to heat and to most of the disinfectants. Therefore, post-mortem of anthrax infected animals is never recommended to avoid the exposure of bacteria to oxygen. A peculiar feature of anthrax infection in animals is that blood does not clot and drains from the natural orifices like nose, mouth and bowl. This results in contamination of soil and water with bacteria which ultimately transform into spores[45]. As much as 10^9 *B. anthracis* bacteria may be present in the oozing blood[46]. Even the processed parts and products like leather, hides, wool, *etc.*, of an anthrax infected animal can carry spores for year. The spores can remain viable for a prolonged period in the soil, especially when deposited 15 cm below the upper soil levels.

Environmental and climatic factors have a great influence on the ecology of anthrax[47]. Climatic factors like rainfall and temperature play a pivotal role in incidences of anthrax cases[48]. However, it is not easy to understand the anthrax occurrence and its epidemiology due to large variations in timing of different outbreaks and associated deaths of a particular species even within a single ecosystem[49]. It has been hypothesized that some soil factors like alkaline pH, high organic content, moisture, and ambient temperature (in excess of 15.5 ℃) favor the germination of *B. anthracis* spores into vegetative bacteria, which ultimately results into amplification of number of spores[22]. It has been observed that high pH and high contents of calcium in soil contribute to maintain the spores viable for a longer time. These soil spores cause new infections when come into contact of a suitable new host[22,50,51]. Therefore, alkaline pH of soil, high moisture and organic contents, precipitation and ambient temperature in excess of 15 ℃ are deciding factors for triggering a large anthrax outbreak and can be considered to predict exposure and infection risk of anthrax in a particular area[48]. During grazing, herbivores animals are most likely to be exposed to *B. anthracis* spores by inhalation or ingestion during grazing. It has been observed that *B. anthracis* bacteria need specific nutrients (animal blood, viscera) and physiological conditions and therefore it is very difficult to survive outside a viable host and convert into spores. Moreover, the vegetative cells of *B. anthracis* are poor competitor and are easily killed by other bacterial species outside the host in environment. Moreover, virulence of *B. anthracis* is reduced when grown outside the host and bacteria with reduced virulence will not lead to an outbreak[22].

According to an estimate, every year about 2000 to 20000 human anthrax cases occur globally. Apart from India and Pakistan, anthrax has also been reported from Bangladesh, Zimbabwe, United States, South Africa, Iran, Iraq and Turkey. In India, southern states are more prone to anthrax. Reports of anthrax appear almost every year from Andhra Pradesh, Tamil Nadu and Karnataka but exact figures are not available. In 1980s, there were only 2000 cases reported worldwide most of them were of cutaneous anthrax. Most of the anthrax cases recorded were from the persons involved in industrial occupations related to processing of animal parts and products like meat packing, bone meal processing, tanning of leather and sorting of hair wool[52]. Several outbreaks have been recorded in recent history. Anthrax outbreaks in animals are more prominent and common than humans. From 1991 to 1996, a total of 1612 anthrax outbreaks occurred in India. In Nepal, a total of 222 animals were affected during 19 different outbreaks in 1996[53]. In 1996, about 1570 cases of ruminant anthrax were reported in China. The death of 204 livestock in Australia was reported in 1997[54]. From 1984 to 1989, thousands of wild animals were killed in an anthrax epidemic in Namibia and South Africa[53]. In Iran, about one million sheep were killed during an anthrax outbreak in 1945. In Manchester, United States, a large anthrax epidemic occurred in 1957 in a goat hair processing plant resulting in four fatalities

and nine cases[55]. In Russia during 1979, an unusual, accidental anthrax outbreak in a Soviet military laboratory of Sverdlovsk killed 68 persons out of 79 infected[56]. In Zimbabwe, 10000 cases occurred between 1979 and 1980 leading to 182 deaths. In Tibet, 507 anthrax cases resulted in 162 deaths in 1989 and in China, 898 and 1210 anthrax cases were recorded in 1996 and 1997, respectively. Between 1991 and 1995, a relatively large number of anthrax incidences was observed in Spain[49,57], Central America[57] and Africa[53]. In most of the cases, exposure was through cutaneous route which accounts for a total of 95% cases. The inhalational route accounts for 5% anthrax cases reported, while gastrointestinal anthrax is quite rare[58,59]. In 2007, a few animal and human cases of anthrax were reported from Orissa and West Bengal, India[60]. The most recent anthrax cases were found in 2010 in Bangladesh. More than 600 peoples were killed in the outbreak due to consumption of infected cattle meat[61].

As India stands first in having the largest population of livestock in the world, therefore anthrax is endemic in several regions. Based on the epidemiological study from 1991 to 2010 by National Animal Disease Referral Expert System (NADRES) in India, anthrax was found one of the ten major diseases causing deaths in livestock[62]. During 1991-2010, anthrax was reported in eighteen states of India *viz.*, Andhra Pradesh, Assam, Bihar, Chhattisgarh, Gujrat, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Rajasthan, Tamil Nadu, and West Bengal. Although several regions are endemic for anthrax, yet seasonal fluctuation in the number of anthrax outbreaks has been observed. Most of the anthrax outbreaks are reported in post-monsoon season, *i.e.,* from July to September and November to January in different parts of India. Anthrax epidemics are generally reported between July to September and also in November and January, coinciding with the post monsoon months across the country. Several Southern states such as Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and Orissa are common endemic regions with sporadic human anthrax cases reported time to time. From the Union Territory of Pondicherry, 28 cases of anthrax were detected in 1999 and 2000[45]. Both, animal as well as human anthrax cases are reported usually from certain anthrax endemic districts like Chittoor, Cuddapah, Guntur, Prakasam and Nellore of Andhra Pradesh[63]. In 2006, some cases were noticed near Narsinghpur, Madhya Pradesh also. In 2007, 20 people were affected in two cutaneous anthrax outbreaks in Murshidabad district, West Bengal. These anthrax outbreaks were caused due to slaughtering of sick cattle and subsequently handling of meat without taking proper preventive measures[64]. An increase in number of animal and human anthrax cases has been observed in this area in recent past[65]. During a tenure of 10 years, anthrax outbreak were reported at least 61 times from Orissa affecting 750 people[65]. The anthrax outbreak is a common phenomenon in this area because tribal population mainly depends on forest for livelihood. Most of the human anthrax cases occur in agricultural workers due to handling of meat or hides of diseased animal. An anthrax outbreak was reported in Orissa, India in 2013 where several people died due to consumption of infected goat meat[66]. Recently, nine cutaneous anthrax cases were reported from the tribal population of Midnapur, West Bengal in India[67].

VIRULENCE OF *B. ANTHRACIS*

Anthrax, being a disease of mainly herbivorous is generally prevalent in those areas where animals like cattle, horse, sheep, goat, *etc.*, graze. Several animal species like pigs, dogs, cats, rats and chicken are fairly resistant to anthrax. Many scavenging birds like vultures which feed on dead animals have a natural resistance to anthrax. However, such birds may disseminate the anthrax spores from infected animals through claws, beaks or feathers.

The spores of *B. anthracis* that can remain in the environment for a prolonged time become the infectious form of anthrax. For causing anthrax, spores first germinate, *i.e.,* lose their dormancy and resistance properties, regain metabolism and start vegetative growth[68,69]. After getting favourable environmental and nutritional growth condition, spores convert into vegetative bacteria and result in further multiplication. Human skin generally does not permit spores to invade; however, spores find access through small cuts or abrasion in skin to cause cutaneous anthrax. After entry into host, *B. anthracis* remains in the capillaries of invaded organs and produce lethal and edema toxins which cause the local and fatal effects of infection.

TOXINS OF *B. ANTHRACIS*

In soil, *B. anthracis* is found in its highly resistant endospore form and therefore, can remain live for a very long period in this state. Spores of *B. anthracis* can find entry in the body through lungs, skin lesion or gastrointestinal route and germinate to yield vegetative form. In case of cutaneous infections, *B. anthracis* comes into contact with a skin lesion, or cut. In inhalational cases, herbivorous and sometimes humans are infected after inhalation of spores. After inhalation, these spores reach alveoli of lungs through air passages. Generally, herbivores get gastrointestinal anthrax infection during grazing or browsing an anthrax spore infested agricultural field having spiky or rough vegetation. Gastrointestinal tract of animals probably gets wounds due to eating of spiky vegetation which facilitates the entry of spores into tissues and resulting in gastrointestinal anthrax.

The virulence of *B. anthracis* is attributed to a tripartite anthrax toxin and a poly-D-glutamic acid capsule. After entry into the host through ingestion or skin wounds, *B. anthracis* multiply inside the tissues of animal or human host, spread in the lymphatic system and undergo rapid multiplication. This results in production of anthrax

toxin inside the body and causes death of host within a few days or weeks.

Capsule formed by the virulent *B. anthracis* vegetative cells helps the bacterium to evade the host immune system by impeding the ability of macrophages to engulf and destroy the bacteria[7]. Three non-toxic proteins namely PA, LF and EF of anthrax tripartite toxin co-assemble to produce a series of free or cell-bound toxic complexes[8,9,70]. Two of the toxins, LF and EF, are enzymes that modify substrates within the cytosolic compartments of host cells[71]. PA binds on the receptors of host cells and makes a pore for transportation of LF and EF to the cytosol[72]. Thus, anthrax toxin is an A-B type toxin, where PA acts as B subunit and it combines with the LF and EF, which act as A subunits to form the edema toxin and lethal toxin, respectively[10,17].

Anthrax PA is an 83 kDa precursor polypeptide consisting of 735 amino acids which binds to anthrax toxin receptors. There are two distinct toxin cell receptors, ANTXR1 (TEM8, Tumor endothelial marker 8) and ANTXR2 (CMG2, Capillary morphogenesis protein 2) which are widely expressed in cells[73,74]. Cleavage of PA by cellular proteases of the furin family, or by serum proteases generates a nicked 20 kDa fragment (PA20) at N-terminal and a 63 kDa fragment (PA63) at C-terminal[75,76]. The 63 kDa fragment self-associates to form a prepore which is a heptameric ring and can bind up to three copies of EF and/or LF molecules[6]. A smaller population of PA octamers (20%-30% of oligomers) is also formed, which binds up to four molecules of EF and/or LF and this structure is more stable than heptamer[77]. These hetero-oligomeric complexes are endocytosed and brought to an acidic environment, where the PA prepore makes a translocase channel after inserting into the membrane[78]. This channel is used for translocation of LF and EF into the cytosol, where by enzymatic activities they disrupt the host cell[79]. Both, LF and EF toxins reach the late endosomal compartment, where EF remains associated with the late endosomal membranes that surrounds the nucleus forming a perinuclear necklace and LF is ejected into the cytoplasm[80,81].

LF is a zinc dependent metalloprotease which inactivates the members of mitogen-activated protein kinase kinase family (MAPKK)[82-84]. Inactivation of three major MEKs *i.e.,* extracellular signal regulated kinases, c-Jun N-terminal kinases and p38 MAPKs results in impairment of various cellular processes like cell division, cell differentiation, cellular response to different types of stress and ultimately apoptosis[17].

Another protein EF is has adenylate cyclase activity. It is produced in an inactive form by the bacterium and needs calcium modulated protein (calmodulin, CaM) for its activity[71]. CaM, which acts as Ca^{2++} sensor has two Ca^{2++} binding sites on each of the C- and N-terminal domain. CaM binds with helical domain of EF using its N-terminal domain. EF is a highly active and its adenylate cyclise activity is almost equal to that of most active known cyclase. Activity of EF is also regulated by intracellular level of Ca^{++} in a biphasic manner. Resting or little high levels of Ca^{++} activate the EF *via* CaM, whereas high levels of Ca^{2++} reduce its activity due to competition between Ca^{++} and Mg^{++} ion in the EF active site[85]. Because EF is associated with the perinuclear later endosomal membrane, therefore, a cAMP gradient decreasing from the nucleus to plasma membrane is generated[80,81,86]. Contrary, endogenous host adenylate cyclises generate a cAMP gradient in opposite orientation (decreasing from plasma membrane to nucleus) because these are localized on plasma membrane[86,87]. In anthrax infection, these two toxins are responsible for immune system failure and ultimate death of host[9].

PATHOGENESIS OF *B. ANTHRACIS*

Human anthrax is mainly of two types, agriculture related anthrax that occurs in a seasonal pattern, and occupation related that can occur at any time. On the basis of route of infection, there are three clinical forms of anthrax *viz.*, cutaneous (skin), gastrointestinal (ingestion) and pulmonary (through inhalation of spores)[88]. Recently, another type of anthrax has been identified among the heroin injecting drug users Europe[89,90]. The term injectional anthrax was then coined to describe this new mode of infection. A few anthrax cases have been reported due to insect bites also, which could probably be due to feeding of insect on an anthrax infected animal[91,92]. Once inside the mammalian host, the high nutrient content of the body triggers germination of spores, although there may be host-specific germination factors as well[93]. Sporulation does not appear to occur inside the host[94]; perhaps because once the available nutrients are depleted in the dead or dying host, the oxygen tension is too low for Sporulation[95] or possibly due to the repression of sporulation by the virulence gene regulator AtxA[96]. Spores infect macrophages at the site of entry, germinate into vegetative cells and proliferate into the tissues and start producing anthrax toxin within 3 h of spore germination[93].

Cutaneous anthrax infection starts with a small itching papule resembling an insect bite at the site of infection on skin. In a day or 2, this papule enlarges and transforms into a painless ulcer with a depressed necrotic centre and a raised and round edge. Generally, such lesions are formed with 2-5 d at the site of spore entry on skin. Finally, after 7-10 d, a black eschar, surrounded by edema is formed and this leaves permanent scar after anthrax cure[97]. Regional lymph nodes draining the infected area may be swollen and enlarged. Cutaneous anthrax infection mostly remains painless and limited to dermis. However, in certain cases it can become systemic when bacteria enter into blood stream causing bacterimia. Hemorrhagic lesions can be developed on any part of body and can be fatal in bacteremic anthrax.

Gastrointestinal (GI) anthrax occurs by eating the food contaminated with anthrax spores (most often contaminated meat). After ingestion, spores germinate

and can cause lesions anywhere in the body. Based on the lesions, GI anthrax is of two types, abdominal and oropharyngeal. In abdominal GI anthrax, lesions are formed mainly in the ileum and cecum. The incubation period is generally 3-7 d. The symptoms of abdominal GI anthrax include nausea, bloody vomiting, diarrhea, abdominal pain, headache, loss of appetite and massive ascites. Another variant of intestinal anthrax is oropharyngeal anthrax where lesions are formed mainly in the oral cavity and resemble the lesions of cutaneous anthrax. Symptoms include throat pain, problem in swallowing and swelling in neck due to edema and cervical lymphadenopathy[97].

Pulmonary or inhalational anthrax occurs by inhalation of spores into lungs. This is the most severe form of anthrax. Alveolar macrophages ingest the spores and transport to lymph nodes in mediastinum. Initially, symptoms of inhalation anthrax are like cold or flu-like with mild chest discomfort, shortness of breath, nausea and finally severe respiratory collapse. Pulmonary anthrax doesn't cause pneumonia, but causes hemorrhagic mediastinitis and pulmonary edema. Historical, mortality was 92%, but, it can be reduced significantly if treated early as only 45% mortality was observed during the 2001 anthrax attack in United States.

Symptoms of anthrax caused by injection remain the same as in cutaneous anthrax, but there may be infection deep under the skin or in the muscle where the drug is injected. Sometimes there is redness at the area of injection. Injectional anthrax is difficult to diagnose because several other common bacteria can cause skin and injection site infections. Therefore, it is hard to treat injectional anthrax as it spreads throughout the body very fast.

There are two basic stages in the systemic anthrax infection, a prodromal and fulminant. The prodromal stage is mainly asymptomatic and generally lasts 2-4 d[98]. In this stage, macrophages engulf the spores and release to lymph nodes near the port of entry. Behaviour of macrophages and phagocytic cells is changed due to action of anthrax toxins resulting in the apoptosis and release and germination of spores into vegetative bacteria. In the fulminant stage, bacteria multiply and are distributed to different organs through bloodstream[99,100]. In human inhalation anthrax, treatment is started after the onset of fulminant stage because prodromal stage is largely asymptomatic. The symptoms at fulminate stage are flu-like and include labored breathing, chest pain, hypotension, headache and disorientation[55,99-102]. Bacteria secrete anthrax toxins which affect functioning of different organs like spleen, lymph nodes, liver, kidney, heart and brain. It becomes very difficult to cure the disease by antibiotic therapy at this stage and action of anthrax toxins ultimately leads to septic shock and death of host in 1-2 d.

LIFE CYCLE OF *B. ANTHRACIS*

B. anthracis is found in two forms, vegetative cells and spores. Adverse environmental conditions induce the sporulation and endospores are released from the mother vegetative cells. The endospores are dormant, well organized and highly resistant to various stress conditions. Therefore, these endospores can remain viable for a prolonged time in the environment and can germinate into vegetative bacteria after getting the suitable environmental and nutritional requirements. During both the processes, *i.e.*, sporulation and germination, a lot of metabolical as well as morphological changes are observed. For spore formation, *B. anthracis* bacterium is divided asymmetrically by a septum into forespore (smaller portion) and mother cells (larger portion). Each portion gets a single copy of DNA. After the asymmetric division, forespore is engulfed by the mother cell with a double-membrane system. The mother cell DNA material is degraded and forespore DNA material is surrounded an inner membrane. Two peptidoglycan layers known as primordial germ cell wall (inner thin layer) and the cortex (outer thick layer) are synthesized between the inner and the outer membrane of forespore[103,104]. The outer membrane of forespore gets deposited by various proteins to form the coat. Thickness of spore coat varies among different species of *Bacillus*. In *B. anthracis* and *B. cereus*, the spore coat is compact whereas it can be distinguished in *B. subtilis*[105,106]. During spore maturation, spore acquires resistance for temperature and UV radiations and becomes dormant. Thus, spore coat imparts important functions to protect cortex and DNA of spore from various adverse conditions like environmental stress, chemicals and peptidoglycan lysing enzymes.

The life cycle of *B. anthracis* has been shown in Figure 1. Animals get infected by uptake of anthrax endospores present in the agriculture fields. Inside the mammalian host, endospores find the favourable conditions like aqueous environment with sufficient nutrients and therefore, start germination[107]. During anthrax pathogenesis, transformation of spore into vegetative cell is a crucial step, because it is the vegetative form of bacterium only which forms the virulent factors, *i.e.,* capsule and tripartite toxin[27]. The poly-γ-D glutamic acid capsule of *B. anthracis* makes a complex surface of the bacterium and is surrounded by peptidoglycan layer and S-layers[108]. The capsule evades the host immune system and thus is a crucial factor for the survival of the bacteria in the host. On death, the capsulated bacteria are released with blood into the environment through natural orifices. On coming into contact with oxygen, the vegetative bacteria convert into spores and thus again infest the agriculture fields for subsequent anthrax infection in grazing animals.

DIAGNOSIS OF ANTHRAX

As various outbreaks are reported time to time from different areas, there is a great need of an early diagnosis of the disease to save human and animal life. Besides, requirement of rapid and reliable detection, identification and diagnosis systems for anthrax has been emphasized

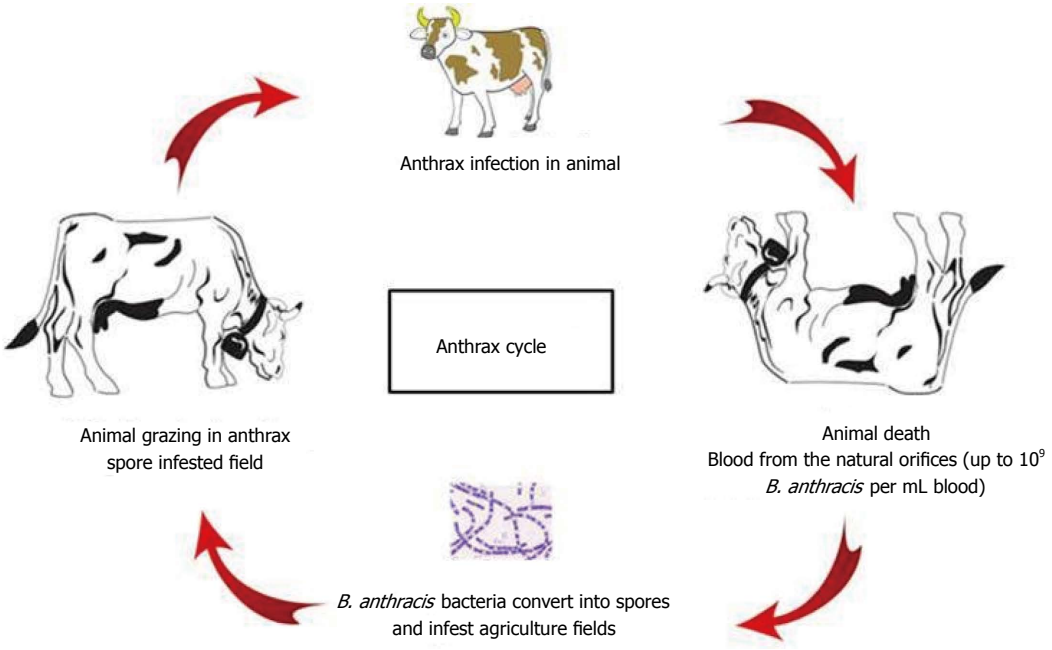

Figure 1 Life cycle of *B. anthracis*. *B. anthracis*: *Bacillus anthracis*.

by recent bioterrorism events. The early monitoring of the disease requires the detection of anthrax spores and infection both at environmental and clinical levels.

Cutaneous anthrax is diagnosed clinically employing traditional microbiological methods like gram-staining, capsule staining from the smear of the lesion or culturing of *B. anthracis*[109,110]. Several methods have been reported for isolation and identification of *B. anthracis*. However, on sheep Blood agar (5%) and other routine culture media, almost all *Bacillus* species grow well[111]. A selective media containing polymyxin-B, lysozyme, EDTA and thallous acetate was used for isolation of *B. anthracis* from contaminated and suspected samples[112]. Another media (bicarbonate agar) is used to induce capsule formation for subsequent identification of *B. anthracis*. However, there is very little utility of these selective growth media because several closely related bacteria of *B. anthracis* like *B. cereus* and *B. subtilis* also grow well on these media. Another undesirable feature is that it takes 18-24 h for *B. anthracis* to grow for characterization by various biochemical tests like catalase, oxidase, nitrate reduction, haemolysis, citrate utilization, urease[113]. Sometimes, microbiological methods like culture and Gram staining of *B. anthracis* do not hold good for patients who have already taken antibiotics before the sample[114]. Immunoflorescence has also been used for direct identification of *B. anthracis* spores[115].

Serodiagnosis is important for surveillance and confirmation of anthrax infection in animals and human. Anthrax toxin consists of PA, LF and EF. Antibodies response against these toxin components is used as a diagnostic tool for determination of past infection or vaccination.

It is well established that PA is the most important protein of anthrax tripartite toxin and it becomes the major component of anthrax vaccines including anthrax vaccine adsorbed. Therefore, antibody (IgG) levels against PA in human and animals are determined to study the host immune response to *B. anthracis* infection and anthrax vaccine[116,117]. In United States, a total of 22 individuals were identified with bioterrorism-related inhalation or cutaneous anthrax, 11 patients for each type from 4th October to 20th November 2001[118]. In 16 of 17 confirmed or suspected clinical anthrax patients, anti-PA IgG antibody could be detected after 11 d of onset of symptoms or probably 15 d after the exposure to *B. anthracis*. Antibodies against PA could be detected up to 8-16 mo in all the cases of inhalation anthrax and 7 out of 11 surviving cutaneous anthrax patients[118]. For serodiagnosis of cutaneous anthrax, an enzyme-linked immunosorbent assay was developed in India for determination of anti-PA IgGs with 99.4% specificity and 100% sensitivity[119]. A field based qualitative visual ELISA for anti-PA IgG was also developed for serodiagnosis of anthrax[120]. Results of sensitivity and specificity of visual ELISA were found compatible with the results obtained from standard ELISA measuring OD values. Likewise, a quantitative ELISA was developed for measurement of the anti-PA IgG level in human serum samples[121]. The minimum detection limits and lower limits of quantification of the assay for anti-PA IgG were 3.2 μg/mL and 4 μg/mL, respectively. The serum samples collected from the anthrax infected patients were found to have anti-PA IgG concentrations of 5.2 to 166 μg/mL[121]. CDC, United States has developed a lateral flow immunochromatographic device using colloidal gold nanoparticles for determination of anti-PA IgG in serum or whole blood[122].

However, animal studies with anthrax vaccine revealed that LF evokes higher IgG response in comparison to PA

in animals[123]. In patients of natural cutaneous anthrax, immune response to LF is higher and faster than the antibody response to EF and PA, which is lower and delayed[124]. Anti-LF IgG antibodies appeared in patients just after 4 d of onset of anthrax symptoms, whereas anti-LF and anti-PA IgG could be detected after 6 d and 13 d, respectively. In a study of human cutaneous anthrax, 11 of the 17 patients had measurable IgGs against one of the three toxin components. Anti-LF IgG was found in 65% patients, while anti-PA and anti-EF response could be found only in 18% and 24% patients. The anti-LF IgG titre in all the infected patients was higher than the titre of anti-PA or anti-EF IgG. After two weeks of infection, the mean anti-LF IgG titre in all infected patients was 69.3 μg/mL, which was twice the tire of anti-EF IgG (37.4 μg/mL) and thrice the titre of anti-PA IgG (22.6 μg/mL)[124]. It was also observed that in anthrax cases, class switching of antibody from IgM to IgG occurs faster. Anti-PA IgG could be detected just after 11 d of onset of symptoms in patients with inhalation anthrax, while no anti-PA IgG response was found till 21-34 d in patients with cutaneous anthrax[117]. Therefore, it is evident that LF evokes a faster and stronger host immune response in comparison to the other two anthrax toxins, *i.e.,* PA or EF. Therefore, detection of anti-LF IgG in human serum can be a good marker for serodiagnosis of anthrax. For detection of anti-LF antibodies, an indirect ELISA was developed for serodiagnosis of cutaneous anthrax in human[125]. The vaccinated and cases of natural anthrax infection can be differentiated by the anti-LF ELISA because PA is the principal component in anthrax vaccine.

Rapid diagnosis of anthrax at an early stage of infection *i.e.,* before the appearance of symptoms can be very useful for proper medical treatment to stop the further spread of infection and accumulation of toxins. For early diagnosis, detection of anthrax toxin in serum or plasma can be a reliable marker of infection[126]. An ultra sensitive immunoassay known as European Nanoparticle Immuno Assay (ENIA) has been developed using European nanoparticle for the detection of PA in sera, which has been found 100 times more sensitive than ELISA[85]. ENIA showed good linearity for detection of PA in the range of 10 pg/mL to 100 pg/mL, whereas range of PA detection in ELISA was 1-100 ng/mL. An engineered sandwich capture ELISA was also reported for the detection of both PA as well as LF[127]. In the sandwich ELISA for PA detection, anti-PA high affinity single chain fragment antibody or receptors for anthrax toxin (ANTXR2) were used for capturing the analyte (PA), and rabbit anti-PA polyclonal serum was used for revealing antibodies. The detection sensitivity of PA by was as low as 1 ng/mL in serum. The detection sensitivity of sandwich ELISA for LF, where PA_{63} was used for capturing of analyte was 20 ng/mL. Surface Plasmon Resonance (SPR) has also been found a very good technique for detection of PA from serum samples of human[128]. The SPR assay could detect 1 pg/mL of the purified PA and 10 pg/mL of PA in human serum[128].

Recently, a new method utilizing genetically modified phages has been developed for detection of pathogenic *B. anthracis* from clinical sources[129]. The reporter phage displays species specificity by its inability, or significantly reduced ability, to detect members of the closely related *B. cereus* group and other common bacterial pathogens.

Nucleic acid based detection methods have also been developed for detection of anthrax. These techniques make use of nucleic acid sequences unique to *B. anthracis*. The technique has gained enormous popularity for its specificity. Polymerase chain reaction (PCR) or real-time PCR amplify the specific chromosomal markers or virulence plasmids present in the *B. anthracis*. Such new rapid detection and diagnostic tests are important for clinicians for early identification of infection.

CONCLUSION

Anthrax, caused by *B. anthracis* is still an important endemic disease of public health importance in several countries of Asia, Africa and Europe. It is re-emerging in some western countries due to political unrest or changing life style (use of intravenous drugs) as evident from the recent outbreaks. In country like India, anthrax is a concern of public health as clandestinely encountering in several states like Andhra Pradesh, Kerala and Karnataka, Orissa and West Bengal. Although anthrax can be cured by prompt antibiotic therapy, yet it is fatal in several cases because of lack of proper diagnosis well in time. Among the three clinical forms of anthrax cutaneous anthrax is most frequent but can be easily cured. The other two forms, gastrointestinal and inhalational anthrax are less common but difficult to cure and have high mortality rate. Recently another form of anthrax, *i.e.,* injectional anthrax is also posing threats for early diagnosis and treatment. However, active surveillance, proper animal immunization and awareness can help to curb the disease. Rapid and accurate diagnosis of cutaneous anthrax is crucial for treatment well in time and making strategies for further spread and control of disease. Although a lot of molecular tests are available for anthrax, yet this is difficult to employ these systems keeping in mind the available resources at far off locations where anthrax is endemic. Therefore, rapid, user friendly, inexpensive serodiagnosis tests can be important tools for surveillance of anthrax and active surveillance can help to minimize the agriculture or occupation related anthrax.

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MINIREVIEWS

Enamel microabrasion: An overview of clinical and scientific considerations

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Abstract

Superficial stains and irregularities of the enamel are generally what prompt patients to seek dental intervention to improve their smile. These stains or defects may be due to hypoplasia, amelogenesis imperfecta, mineralized white spots, or fluorosis, for which enamel microabrasion is primarily indicated. Enamel microabrasion involves the use of acidic and abrasive agents, such as with 37% phosphoric acid and pumice or 6% hydrochloric acid and silica, applied to the altered enamel surface with mechanical pressure from a rubber cup coupled to a rotatory mandrel of a low-rotation micromotor. If necessary, this treatment can be safely combined with bleaching for better esthetic results. Recent studies show that microabrasion is a conservative treatment when the enamel wear is minimal and clinically imperceptible. The most important factor contributing to the success of enamel microabrasion is the depth of the defect, as deeper, opaque stains, such as those resulting from hypoplasia, cannot be resolved with microabrasion, and require a restorative approach. Surface enamel alterations that result from microabrasion, such as roughness and microhardness, are easily restored by saliva. Clinical studies support the efficacy and longevity of this safe and minimally invasive treatment. The present article presents the clinical and scientific aspects concerning the microabrasion technique, and discusses the indications for and effects of the treatment, including recent works describing microscopic and clinical evaluations.

Key words: Dental bleaching; Enamel microabrasion; Enamel surface; Esthetic treatment; Fluorosis; Hypoplasia

Core tip: Enamel microabrasion is indicated for the removal of superficial stains and irregularities of the enamel, mainly located in esthetic areas. The technique involves the mechanical rubbing of acidic and abrasive agents on the altered surface. Recent studies show that the technique

is a conservative treatment when the enamel wear is minimal and clinically imperceptible, and is effective and long lasting. The present literature review aims to discuss indications and clinical and scientific aspects of the microabrasion technique, as well as its effects on the enamel surface.

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INTRODUCTION

Several treatments have been introduced to the dental market for the restoration of dental appearance to a level that satisfies what patients seek regarding dental esthetics. These techniques are still being evaluated in order to ensure an efficient treatment with minimal chair time and low cost that is safe for professionals and patients. For superficial enamel stains or defects, enamel microabrasion is preferred, as it is considered an esthetic and conservative treatment[1-3]. Since its introduction by Croll *et al*[4] in 1986, there have been numerous reports describing various approaches[5-8], related products, and clinical successes[9-12].

The main indication for enamel microabrasion is intrinsic discoloration or texture alteration due to enamel hypoplasia, amelogenesis imperfecta, or fluorosis[13]. The technique removes the porous surface enamel layer, as well as the entrapped stains, by rubbing a gel that contains an acid and an abrasive compound in a similar way that a dental prophylaxis with pumice and water is performed. The enamel stain or defect is removed by a combination of the erosive and abrasive effects of the recommended mixture containing low acid concentrations and an abrasive agent, applied mechanically using a low-rotation micromotor[13-15]. It should be the first option for the management of teeth with intrinsic stains because it removes opaque, brown stains and smoothens surface irregularities by providing a more regular and lustrous surface[13,16]. As the technique is considered safe and minimally invasive, it can also be combined with tooth bleaching when necessary[1,9,10,13].

The success of enamel microabrasion is directly related to the correct indication of the clinical case and the proper execution of the technique. This review discusses aspects of microabrasion, such as its evolution, indications, advantages, clinical steps, and effects on the enamel structure, in order to address some concerns regarding newer trends presented in the latest research and clinical reports.

EVOLUTION OF THE TECHNIQUE

Enamel microabrasion was initially performed for the removal of fluorotic white spots using 36% hydrochloric acid, as recommended by Kane in 1926[14,17,18]. A heated metallic instrument was used to apply the acid to the altered enamel to increase its penetration[14,18] and hasten the chemical reaction between the acid and the enamel[3]. Concerned about the safety of the technique, Raper *et al*[19] suggested the use of 18% hydrochloric acid applied and rubbed with a wooden spatula wrapped with cotton for a maximum time of 10 min[19]. The authors drew attention to the thickness of the enamel, particularly at the cervical third of the tooth, which is thinner compared to the medium and incisal third. They also recommended the use of sodium bicarbonate to neutralize the effects of the hydrochloric acid.

Mechanical application with a low-rotation micromotor was first indicated in the 1970s, using a mixture of 18% hydrochloric acid, hydrogen peroxide and ether[20]. Combination with an abrasive agent was later indicated by Murrin *et al*[21] in 1982, who added pumice to 36% hydrochloric acid, resulting in a slurry that was applied using a rubber cup coupled to a micromotor[21]. Concerned about the acid concentration, Croll *et al*[4] recommended the use of the same mixture but with 18% hydrochloric acid. Croll later stated that an ideal microabrasive system should include a low acid concentration and abrasive particles in a water-soluble mixture that are applied with a low-rotation handpiece to avoid scattering the compounds, thus making the procedure safer[14]. The author again proposed the use of an extra-fine diamond bur prior to the use of the microabrasive agents to reduce the clinical time needed to perform the procedure[22].

The association of hydrochloric acid to abrasive particles resulted in the development of commercially available products. Prema Compound (Premier Dental Company, Philadelphia, PA, United States), which contains 10% hydrochloric acid, was the first to be introduced to the market. Currently, a lower concentration of hydrochloric acid is used, approximately 6.6%, under the commercial product name of Opalustre (Ultradent Products Inc., South Jordan, UT, United States). Both products use silicon carbide as an abrasive with different granulations (Table 1) dispersed in a water-soluble gel for easy removal[13]. The use of 35% phosphoric acid instead of hydrochloric acid was proposed by Kamp in 1989, and was considered advantageous as it is commonly used in clinical practice for other procedures[3,23].

INDICATIONS FOR ENAMEL MICROABRASION

The proper indications for enamel microabrasion are summarized in Table 2. Dental fluorosis is the most common indication[16], which results from demineralization of enamel caused by excessive fluoride intake. Fluorosis produces opaque white areas or yellow to dark brown discolorations with porosities on the enamel surface, depending on severity[24,25]. Fluoride-induced enamel changes range from thin, white, opaque

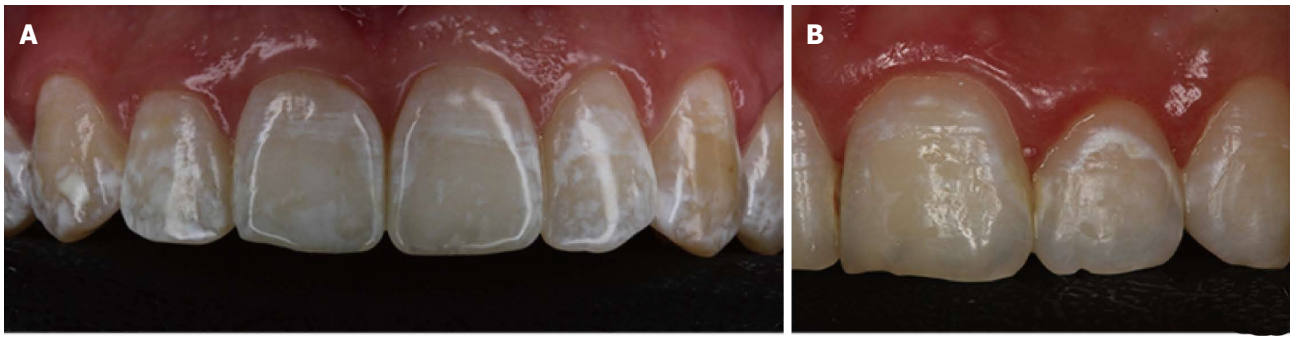

Figure 1 Indications for enamel microabrasion. Tooth staining from A: Fluorosis; B: Mineralized white spots.

Table 1 Commercial products used for microabrasion

Material	Manufacturer	Acid	Abrasive	Particle size (mm)
Prema compound	Premier Dental Company (Philadelphia, PA, United States)	10% hydrochloric acid	Silicon carbide/dioxide	30-60
Opalustre	Ultradent Products (South Jordan, UT, United States)	6.6% hydrochloric acid	Silicon carbide	20-160
Pumice	Pumex (Newcastle-under-lyme, Staffordshire, United Kingdom)	-	Pumice	30-50

Table 2 Summary of indications and advantages of the microabrasion technique

Indications	Requirements	Advantages
Stains or defects restricted only to enamel	Shallow alterations just in the enamel surface	Safe and conservative treatment
Dental fluorosis	Use of rubber dam	Minimal loss of enamel
Mineralized white stains	After completion of orthodontic treatment, if necessary	Leaves enamel surface lustrous, shiny and glass-like
Correction of surface irregularities	Supplemented with bleaching, if necessary	Roughness and microhardness alterations easily resolved by saliva
Localized enamel hypoplasia		Reduced bacterial colonization on enamel surface
Polishing of enamel and auxiliary removal of composite resin residues after orthodontic therapy		Lasting and stable esthetic results

lines corresponding to perikymata running across the tooth surface, to an entirely chalky white surface[24]. They are characterized by the presence of bilateral, diffuse, and horizontal striations[25] observed on all teeth that mineralize at the same time (Figure 1A). Enamel microabrasion usually improves esthetic appearance in cases of mild and moderate fluorosis (Thylstrup-Fejerskov Index 1-7)[16,26,27], and should always be considered the first option in the management of these cases[13,16]. Even in situations with yellow or brown discolorations, enamel microabrasion can improve the esthetic appearance of the teeth[28]. As these stains are formed by the discoloration of demineralized surfaces and from external sources, the depth of the stain is likely associated with the penetration of the staining agents[16].

Microabrasion treatment may be indicated for correction of surface irregularities on dental enamel, which may be caused by imperfect enamel formation or acquired after the removal of orthodontic appliances[13], such as the removal of residual resin composite from brackets with diamond burs, and resulting in a smooth and polished enamel surface[29]. Microabrasion is also indicated for opaque, white areas or discolorations, even with porosities, from the demineralization/remineralization process common in the enamel region adjacent to orthodontic bands or brackets (Figure 1B), or from disturbances in the mineralization process, such as hypocalcification[7,13,30]. The white spots caused by orthodontics should first be treated with mineralizing agents, such as sodium fluoride[12,31], or with an infiltration technique[29]. Infiltration of the enamel by resin was recently developed as a way to obstruct the diffusion pathways for acids and dissolved minerals[32]. The resins used have low viscosity, high surface tension, and low contact angle with the enamel, as well as a refraction index similar to enamel[33]. The infiltration technique may also be used in cases of mineralized lesions[29,34]. The technique requires pre-conditioning of the surface with 15% hydrochloric acid, which removes approximately 40 μm of enamel surface, to ensure resin penetration[32]. In

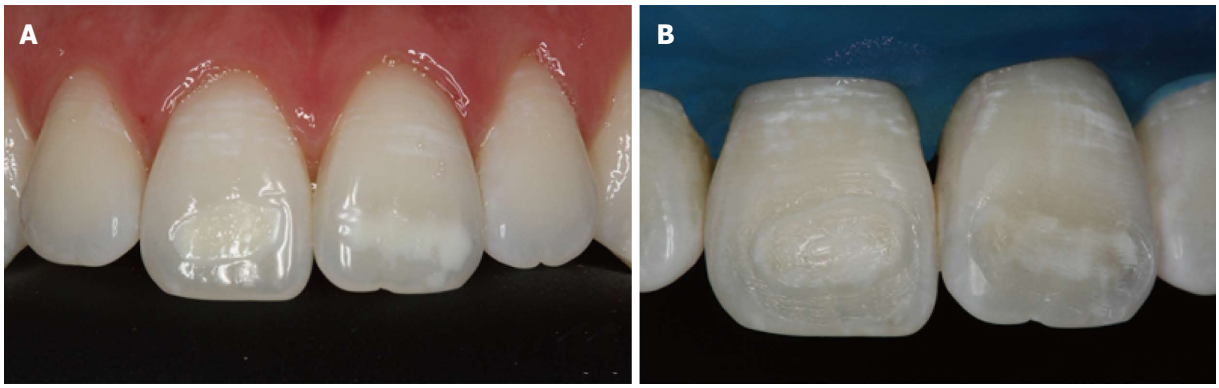

Figure 2 Deep enamel staining due to hypoplasia. A: Hypoplasia; B: Ineffective microabrasion treatment of the right central incisor.

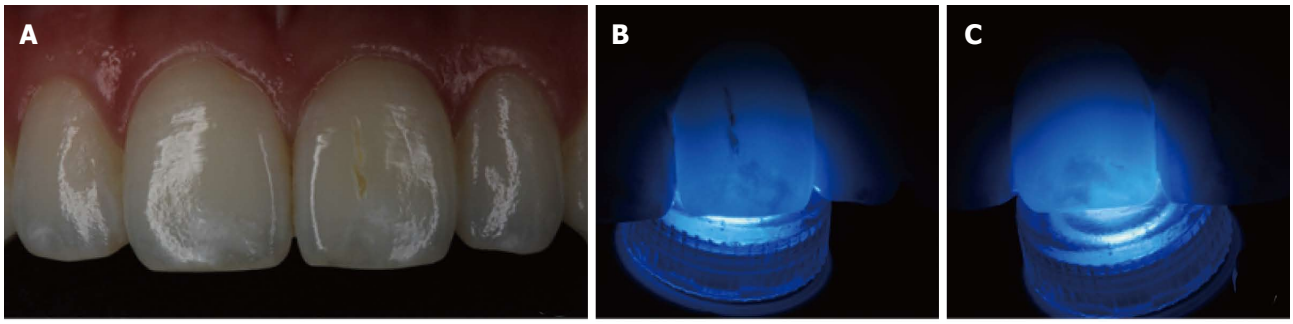

Figure 3 Transillumination to determine staining depth. A: Enamel hypoplasia in both central incisors; B, C:: Transillumination to evaluate the staining.

this way, the thickness of the enamel removed for resin infiltration is similar to microabrasion. However, there are no clinical trials evaluating the staining, abrasion wear or bacterial colonization of resin-infiltrated surfaces.

Microabrasion may be utilized in cases of localized or idiopathic enamel hypoplasia that is limited to the outer enamel layer[15,35]. Although this condition can sometimes require a restorative approach with composite resin or laminate veneer[36] (Figure 2), microabrasion should be considered as the first treatment option[16,36]. In addition to improving esthetics, it may reduce the need for enamel wear for a restorative approach, which is mainly important in young patients[36]. Otherwise, the infiltration technique may be used in cases with deeper stains not resolved by microabrasion, and may be an alternative for the invasive restorative approach[37,38]. Even if all whitish parts of a lesion do not completely disappear, the infiltration technique usually leads to considerable improvement in appearance[37] and masks the enamel stain[34,38].

Enamel microabrasion is not indicated if the patient presents deficient lip sealing, as the teeth are always exposed to air and dehydrate more easily, thus a moistened film is not formed under the enamel. With this condition, the stained appearance of the tooth is more evident, and it may characterize the failure of the microabrasion. Therefore, these patients are encouraged to first seek orthodontic treatment and/or speech therapy[1,13].

The most important factors contributing the success of enamel microabrasion are the location and depth of the enamel stain or defect[8,13,16]. The alteration must be restricted to enamel tissue, without involvement of the dentin[13]. Deeper, opaque stains, such as those resulting from hypoplasia, cannot be resolved with microabrasion, and require a restorative approach[36]. An LED/light curing unit positioned in the palatine or lingual face of the tooth can help the clinician to examine the enamel stain (Figure 3). This can be used to estimate the lesion depth, as a darker color indicates deeper staining[9]. It is also important to perform the diagnosis in wet conditions, as the difference in the refractive index between air and enamel is greater than between water and enamel[12]. Commonly, white spots are more obvious on dry teeth, thus a lesion visible on a wet tooth can be considered deeper than a lesion visible only on dry enamel.

TECHNIQUE

An ideal microabrasion technique should produce insignificant enamel loss, no damage to pulp or periodontal tissues, and satisfactory and permanent results in a short clinical time without discomfort to the patient[4]. The use of a rubber cup coupled to the rotatory mandrel enables precise application of the compound on the enamel surface, which eliminates splattering of the compound and makes the procedure safer, easier, and quicker[13]. For the safety of the patient, a rubber dam should be in place[39], though

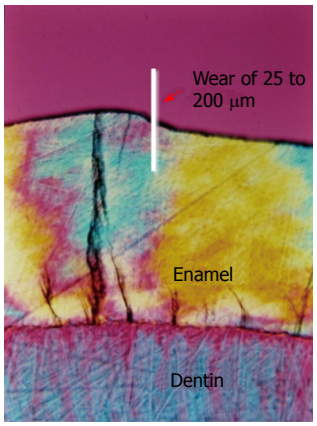

Figure 4 Depth of enamel removal. Polarized light microscopy showing the ground tooth section after enamel microabrasion with Opalustre (reprinted with permission from Sundfeld *et al*[44]).

this may be difficult when the teeth are not completely erupted[13]. It is also important that the patient, clinician, and assistants all wear eye protection during the procedure[1].

The number of applications can vary according to the severity of the enamel staining[1,9,10,12]. To reduce the clinical time, the enamel can first be "regularized" with a tapered fine-diamond bur to lightly abrade the affected area, referred to as enamel macroreduction[1,9,13,30]. With this procedure, the application of microabrasive slurry can be reduced to two or three applications to remove the remaining stains and to smooth the enamel surface ground with the diamond bur[1]. Afterwards, polishing of the microabraded surface with felt discs and polishing[26] or fluoridated[1,9,10,40] pastes is recommended. Application of sodium fluoride gel[1,9,26] is also recommended to promote the remineralization process.

Because enamel microabrasion is a noninvasive technique, it can be supplemented with bleaching procedures[1,9,13,15,41]. Often, this is necessary as microabraded teeth can acquire a darker or yellowish coloration after treatment, and the remaining enamel is thinner and more clearly reveals the dentin. Bleaching is also indicated to reduce the contrast between the remaining white-spotted lesions and the tooth surface[26,42,43]. In either situation, a low concentration of carbamide peroxide is recommended using the home-bleaching technique[1,9,10,13].

CLINICAL AND SCIENTIFIC CONSIDERATIONS

Effects of the technique

Enamel microabrasion has been shown as an effective and conservative treatment[1,9,13,15,43]. According to reports by Sundfeld *et al*[13,43,44], 5 to 10 applications of microabrasive systems (35% phosphoric acid with pumice, Opalustre) can result in the loss of 25 to 200 μm of enamel, which is acceptable for clinical conditions (Figure 4). A recent study showed that 120 s of microabrasive treatment reduces approximately 10% of the enamel thickness[5], suggesting it is a safe and conservative procedure. According to Dalzell *et al*[45], the pressure used during the microabrasion procedure is crucial for total enamel removal, such that the higher the pressure, the greater the quantity of enamel removed. In addition, enamel wear from the microabrasion technique is time-dependent[46].

In addition to the removal of discolored enamel, the microabrasion technique changes the optical characteristics of the enamel surface, called the "abrasion effect"[47,48]. The simultaneous abrasion and acid erosion of enamel prisms may compact mineralized tissue within the organic area, replacing the outer layer of prism-rich enamel with a densely compacted, prism-free region[48]. Microabrasion presents a lustrous, shiny, and glass-like surface of the enamel, which may reflect and refract light differently[13,41]. These optical properties may be able to camouflage any remaining subsurface enamel stains[48]. Tooth hydration by saliva augments these favorable optical properties[47,48]. Schimdlin *et al*[2] found that the luminescence and fluorescence of enamel after microabrasion of demineralized lesions was decreased in comparison with the untreated demineralized enamel.

Several studies have examined the effects of microabrasion on the remaining enamel surface[2,5-8,46,49-51]. The potential erosive and abrasive effects depend on several parameters, including the type, concentration and pH of the acid used, the abrasive medium, time of instrumentation, application mode, force applied, and revolutions per minute[8,50]. The microabrasion technique increases the roughness of the enamel surface, regardless of whether 18% or 35% phosphoric acid or 6.6% hydrochloric acid with abrasive was used[5,7,44,51]. Similarly, enamel microabrasion is also related to reduced enamel microhardness[6,49]. However, both effects can be reversed by the polishing procedure or saliva exposure[5,6,49,51]. Rodrigues *et al*[5] found that unlike that seen with microabrasion, the enamel surface maintained the same roughness through all the evaluated stages when mechanically treated with a silicon polisher; the authors suggested that the chemical features of enamel microabrasion are responsible for the roughness effects. Despite their concentration differences, phosphoric acid and hydrochloric acid have similar erosive effects[5,6,49], such as alterations in the enamel micromorphology with exposition of the interprismatic spaces, similar to the enamel conditioning patterns[7,46]. Although the microabrasive system causes change in the enamel surface, which can be observed by scanning electron microscopy, confocal imaging demonstrates that the subsurface is not altered (Figure 5). The smoother, dense, mineralized enamel layer created by microabrasive systems is also less favorable for bacterial colonization, particularly by *Streptococcus mutans*[52]. Additionally, Hoeppner *et al*[53] reported that the enamel surface was more resistant to demineralization four months after microabrasion with 35% phosphoric acid.

Bertoldo *et al*[49] recently reported that microabrasion with 6.6% hydrochloric acid and silica results in the incorporation of chloride ions and silica into the enamel.

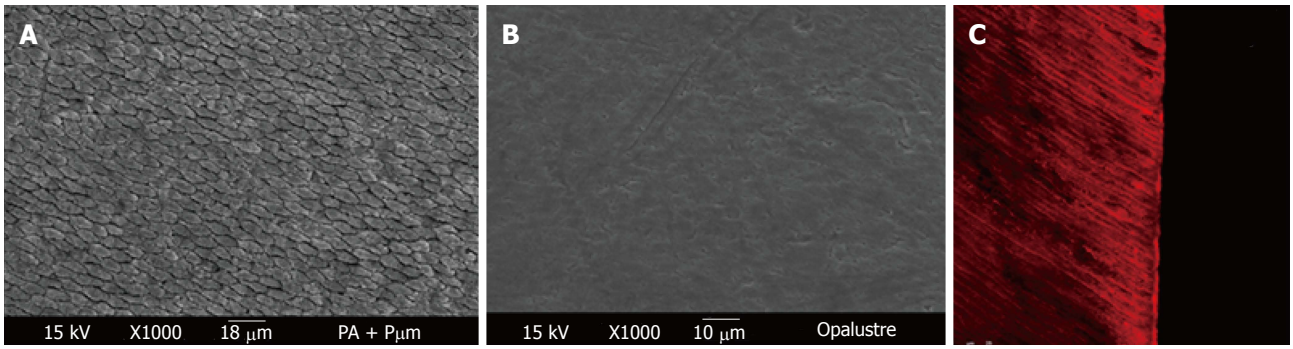

Figure 5 Enamel alterations after microabrasion. Scanning electron microscopy showing the acid conditioning pattern on the enamel surface caused by microabrasion with A: Phosphoric acid and pumice; B: Opalustre; C: Confocal laser scanning microscopy showing the minimal alteration of the enamel surface, but intact subsurface, after microabrasion with Opalustre.

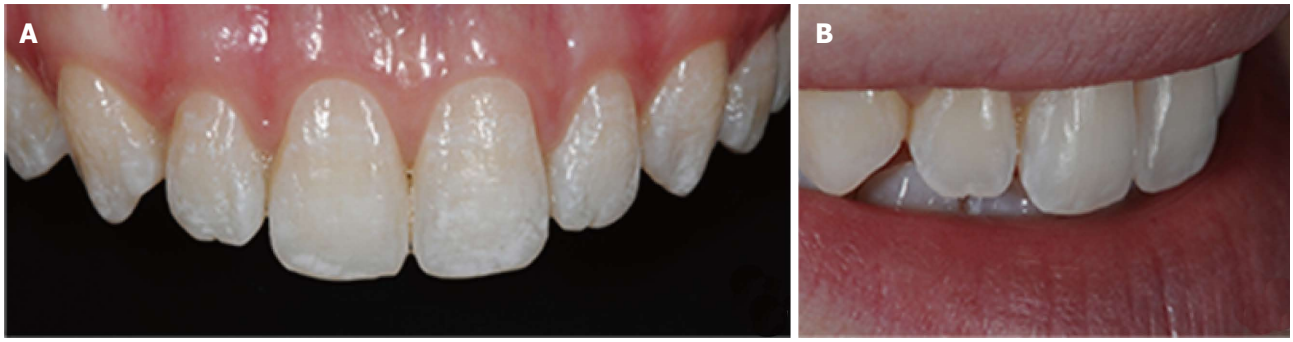

Figure 6 Resolution of fluorosis staining by microabrasion. A: Clinical case of fluorosis before treatment; B: Results after enamel microabrasion (reprinted with permission Machado *et al*[58]).

These, along with results from additional studies concerning the effects of artificial[6,49] and human[51] saliva on microabraded enamel, should encourage clinicians to consider this method. Chloride ions are strongly associated with enamel rehardening, as they account for more than 60% of the ionic strength of saliva[54,55], and the silica compound is used in a bioactive material (Ca_3SiO_5) that efficiently induces a new apatite layer on acid-etched enamel[56]. Some authors believe that these properties should be maximized and, rather than polishing the microabraded enamel, a light polishing with a feltrum disc and fluoridates or diamond toothpaste with low granulation should be applied[1,5,9].

Clinical success

Several case reports demonstrate the lasting and stable esthetic results of the microabrasion technique[1,9,10,12,35,41]. According to clinical results, enamel microabrasion produced permanent color modification of superficial enamel coloration defects because the discolored enamel was removed, rather than altered or masked[57]. Microabraded enamel surfaces achieved a brilliant luster over time[13,57]. An example clinical result of enamel microabrasion is presented in Figure 6[58].

Loguercio *et al*[59] compared two commercially available products for microabrasion for removal of fluorosis stains, and found that treatment with Opalustre was more effective than Prema Compound. This effect was possibly due to the larger size of the silica granules in the Opalustre. However, both products were efficient, and the patients were highly satisfied with the results. Similarly, Sheoran *et al*[57] compared 35% phosphoric and 18% hydrochloric acid with pumice, and found no clinical difference between them, with microabrasive compounds successful in treating enamel opacities.

Enamel microabrasion is considered effective in cases of white, yellow or brown stains located in the outer enamel layer[60]. However, it is important to recognize the severity of enamel stains when facing fluorosis. Celik *et al*[16] performed enamel microabrasion with Opalustre in mild-to-severe fluorosed teeth and found that more applications were needed when lesions were more severe. Mild staining was treated with five applications, whereas moderate to severe staining needed ten applications. Train *et al*[27] also showed that the appearance of mildly fluorosed teeth was moderately improved, but microabrasion only slightly improved the appearance of severely fluorosed teeth. However, enamel microabrasion should still be the first option for patients that seek minimally invasive treatment, even in cases with severe fluorosis. In such cases, removal of opaque white areas or brown stains may increase the success of further treatment, such as bleaching, to achieve a uniform tooth shade[16]. Castro *et al*[26] showed that enamel microabrasion combined with at-home tooth bleaching effectively reduced staining in cases of mild to severe fluorosis, improving the esthetic

appearance of the teeth and the self-perception of the patient, without incidence of side effects such as tooth sensitivity.

CONCLUSION

Accumulating evidence suggests that enamel microabrasion is efficient and effective for producing esthetic improvements. This technique involves minimal enamel loss, leaving a smooth and shiny enamel surface with permanent results. The procedure is considered a safe, conservative, atraumatic method for removing superficial enamel stains and defects. The laboratory and clinical results presented in these articles support the use of enamel microabrasion as a first treatment option for patients who prefer a less-invasive approach.

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MINIREVIEWS

Recent advances in the HER2 targeted therapy of gastric cancer

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Abstract

Recent advances in molecular targeted therapies, including targeting human epidermal growth factor receptor 2 (HER2), had a major forward step in the therapy for gastric cancer patients. Application of HER2-targeted therapies, in particular trastuzumab in combination with chemotherapy in metastatic HER2-positive gastric cancers, resulted in improvements in response rates, time to progression and overall survival. Nevertheless, as with breast cancer, many patients with gastric cancer develop resistance to trastuzumab. Several promising therapies are currently being developed in combination with chemotherapy to increase the efficacy and overcome the cancer-resistance. Here we review the current overview of clinical application of agents targeting HER2 in gastric cancer. We also discuss the ongoing trials supporting the use of HER2-targeted agents combined with cytotoxic agents or other monoclonal antibodies.

Key words: Human epidermal growth factor receptor 2; Gastric cancer; Targeting therapy; Trastuzumab

Core tip: This review summarizes the development of diagnostic and therapeutic approach for the patients with human epidermal growth factor receptor 2 (HER2)-overexpressed/amplified gastric cancer. The biology of HER2-dependent signalling is also described. The ToGA trial highlighted the importance of accurate HER2 testing to guide treatment choice of gastric cancer. Future strategies beyond the ToGA trial to address EFGR family, including HER2 pathway are discussed according to current ongoing clinical trials, as well as experimental studies.

Matsuoka T, Yashiro M. Recent advances in the HER2 targeted therapy of gastric cancer. *World J Clin Cases* 2015; 3(1): 42-51 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i1/42.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i1.42

INTRODUCTION

Gastric cancer is the fourth most common malignant

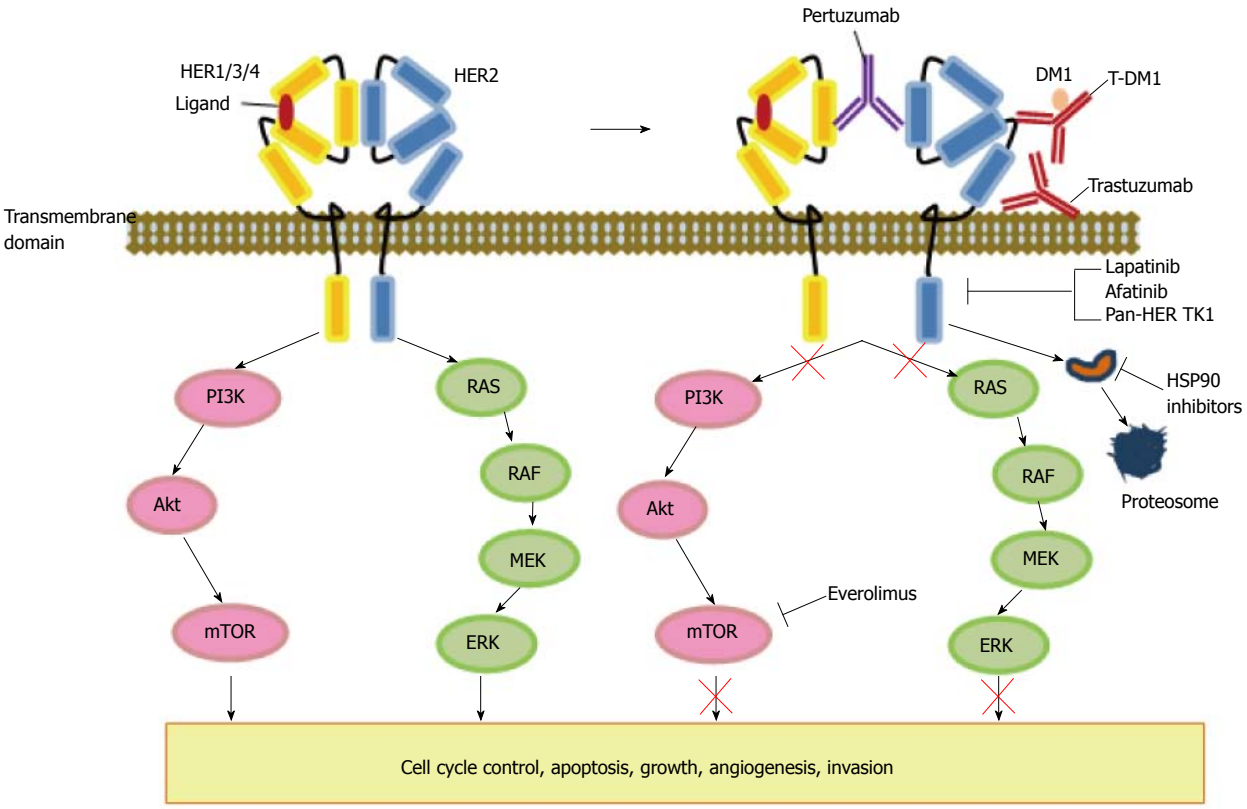

Figure 1 Human epidermal growth factor receptor 2 signaling pathway and interaction with other pathways. This demonstrates schematic representation of the HER-2 family of receptor and their interaction with downstream signalling, along the pathway which are responsible for a variety of biological processes involving cell cycle control, apoptosis, cellular growth, invasion, and angiogenesis. Examples of classes of drugs and corresponding compounds targeting the HER-2 network are also presented. HER1-4 are transmembrane proteins with associated tyrosine kinases. Heterodimerization result in tyrosine kinase activation with the subsequent signaling cascade, and subsequently activates downstream signals, including members of MAPK and PI3K/Akt/mTOR pathways. Trastuzumab and t-DM1 targtets to the extracellular domain IV of HER2. Anti-cancer activity of pertuzumab is interference with HER-receptor dimerization. Lapatinib, afatinib, and tyrosine kinase inhibitors (TKIs) compete for the binding of ATP in the intracellular domain of the receptors. HSP90 suppresses the NH2-terminal ATP binding site which leads to the degradation of client proteins by the ubiquitin proteasome pathway. HER: Human epidermal growth factor receptor; MAPK: Mitogen-activated protein kinase; MEK: Mitogen-activated protein kinase kinase; ERK: Extracellular signal-regulated kinase; PI3K: Phosphatidylinositol 3-kinase; mTOR: Mammalian target of rapamycin.

disease and the second leading cause of cancer-related death worldwide[1]. Despite the current improvements in survival of patients with gastric cancer, it is often diagnosed at an advanced stage and its prognosis is still unsatisfactory due to the high frequency of metastasis[2,3].

Recent studies have shown that several combination chemotherapies have been shown to significantly increase survival for patients with gastric cancer[4]. SPIRITS trial (S1 plus cisplatin *vs* S1 in RCT in the treatment of stomach cancer) showed that combined therapy of S1 with cisplatin significantly prolonged survival as a first-line treatment for advanced gastric cancer. Overall survival (OS) of patients treated with S1 plus cisplatin was 13.0 mo compared 11.0 mo with S1 alone[5]. Additionally, other cytotoxic agents, including docetaxel and irinotecan also prolonged survival[6,7]. Notably, capecitabine and oxaliplatin showed to be non-inferior to fluorouracil and cisplatin[8,9]. However, even with these treatments, most patients with advanced disease have a median overall survival in the range of 6-11 mo[2].

To date, with greater knowledge of the molecular basis of tumor initiation, several kinds of targeted agents have led to a better prognosis for solid tumors. One of the most important targets in human malignancy is the epidermal growth factor receptor (EGFR) family[10]. The human epidermal growth factor receptor-2 (HER2) is a receptor of tyrosine kinase and a member of the EGFR family[11]. HER2 is expressed in a significant proportion of gastric cancer[12]. Trastuzumab, a recombinant humanized monoclonal antibody that targets the extracellular domain Ⅳ of HER2, has recently been noticeably altered the treatment of gastric cancer. Trastuzumab has demonstrated a survival advantage in patients with HER2-overexpressed gastric cancer[13].

In this article, we will outline the issues concerning novel biologic agents for advanced gastric cancer, focusing on anti-HER2 therapies, such as trastuzumab, and other novel agents. We will also discuss the current clinical evidence and ongoing trials supporting the use of HER2-targeted agents combined with cytotoxic agents or other monoclonal antibodies.

MOLECULAR FEATURES OF HER2

HER2, a proto-oncogene encoded by *ERBB2* on chromosome 17, is a cell membrane surface-bound receptor

tyrosine kinase and belongs to EGFR family, including EGFR/HER1, HER2/neu, HER3, and HER4[11]. Each receptor has an extracellular domain, lipophilic transmembrane domain, and intracellular kinase domain (Figure 1). Although HER1, 3, 4 are activated by ligand binding, the specific ligand to HER2 have not been identified yet[14]. Nevertheless, aberrant HER2 activity and activation of the HER2 receptor leads to receptor dimerization (*e.g.*, HER2/HER3), and subsequently activate downstream signals, including members of the Ras/Raf/mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase/protein kinase-B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathways (Figure 1)[15,16]. Overexpression of HER2 has been found to aggressively promote these signals which are responsible for regulating a variety of tumor biology, such as cancer cell growth, differentiation, invasion and survival[17,18]. Dimerization of HER2 and HER3 is known to be the most active HER signaling dimer. With regard to gastric cancer, HER2 and HER3 are significant predictors of poor survival in multivariate study. HER3 may become another candidate for molecular-targeted therapy in gastric cancer, especially for the diffuse histological type[19,20].

HER2 EXPRESSION AND GASTRIC CANCER

The reported HER2 amplification in patients with gastric cancer ranges from 6% to 23%[13,21,22]. The rates of HER2 amplification or overexpression in gastric cancers are different depending on the primary location of the tumor, which is more frequent in cancers located in the gastroesophageal junction compared with those from elsewhere in the stomach[13,23]. Histological evaluation revealed HER2 overexpression was predominantly seen in the intestinal-type than in diffuse-type cancers (32% *vs* 6%)[22].

HER2 amplification is associated with clinicopathological features, such as age, male gender, tumor size, serosal invasion and lymph node metastasis[24,25]. HER2 expression is a biomarker for the prediction of trastuzumab response[26]. However, the prognostic significance of HER2 overexpression in gastric cancer remains controversial. A number of retrospective studies have demonstrated that HER2 positivity is a prognostic factor associated with increased risk of invasion, metastasis, and worse survival[19,27-29]. HER2 status has been reported as the second poorest prognostic variable following nodal status[30,31]. On the other hand, other studies found no association between HER2 and prognosis in both early and advanced stage cancers[13,32-38].

Several studies have investigated how differences in expression of HER2 between of primary gastric tumor and metastatic lesions. The majority of these reports has described that HER2 expression of primary and secondary sites revealed a high concordance rate, except two studies[35,39]. These data suggest that the evaluation of HER2 expression in the primary cancer is a reliable basis for determing treatment with anti-HER2 agents in patients with metastatic gastric cancer.

HER2 expression is usually determined by immunohistochemistry (IHC) or by the detection of HER2 gene amplification by fluorescence *in situ* hybridization (FISH). The evaluation of HER2 immunostained samples in gastric cancer is carried out as outlined by Hofmann *et al*[40] and optimized by Rüschoff *et al*[41]. HER2 testing is distinct from breast cancer immunohistochemistry testing. The major difference in scoring HER2 IHC staining between gastric cancer and breast cancer is that an incomplete basolateral or lateral staining alone is considered as a positive result, which lead to the frequent incidence of tumor heterogeneity[40]. This heterogeneity may represent the HER2 testing inaccuracy, resulting in the controversy of significance of HER2-expression in gastric cancer. Thus, further studies have been proposed to improve the quality of HER2 testing to make certain that patients receive the best possible therapy for their HER2-positive disease.

A recent study presented the Collaborative Enzyme Enhanced Reactive (CEER) immunoassay may be a useful technique to investigate the HER2 expression[42]. CEER-based assays showed higher sensitivity and specificity as compared to IHC-based assays. Evaluation with this high sensitivity of HER3 resulted in -20% of the IHC/FISH HER2 negative gastric cancers still expressed total HER2 although. Another study presented that the use of a quantitative variable that could be objectively measured, such as the *HER2* gene copy number or the *HER2* amplification ratio, which seems preferable to a subjective classification in accordance with IHC scores that are not regularly consistent[43]. These current development of technology may be useful for elucidating the expression of HER2 more precisely and improving prediction of clinical outcome in gastric cancer patients treated with trastuzumab.

TREATMENT FOR HER2 POSITIVE GASTRIC CANCER

Trastuzumab

Trastuzumab is the first molecular targeted agent approved as standard treatment in gastric cancer[13,44]. This agent induces antibody-dependent cellular cytotoxicity, inhibits HER2-mediated signaling and prevents cleavage of the extracellular domain of HER2 (Figure 1). Trastuzumab for Gastric Cancer (ToGA) study was an open-label phase Ⅲ, randomized controlled trial undertaken in 122 centers among 24 countries[13]. ToGA trial showed that an addition of trastuzumab to conventional cytotoxic chemotherapy demonstrated a clinical benefit compared to chemotherapy alone in terms of tumor response, which suggested that combined chemotherapy with trastuzumab can be cited as a novel accepted option for patients with HER2-positive advanced gastric cancer. Recently, a role of trastuzumab as a second-line chemotherapy got attention because of the usefulness of trastuzumab as a first-line chemotherapy. In

Table 1 Clinical trials of HER2-targeted therapy in gastric cancer (after ToGA study)

Trial	Study design	No. of patients	HER2 status	Regimen duration of treatment	Response rate	Prognosis
LOGiC	Phase Ⅲ/1st Randomized Double Blind	545	HER2 amplification	Lapatinib + XELOX XELOX No description of duration		non-significant prolongation
TyTAN	Phase Ⅲ/2nd Parallel group Randomized	1923	HER2 amplification	Lapatinib +Paclitaxel Paclitaxel 24 mo	27% *vs* 9%	OS: 11.0 mo *vs* 8.9 mo PFS: 5.4 mo *vs* 4.4 mo
HERBIS-1	Phase Ⅱ/1st Non-Randomized	56	IHC 3+ IHC 2+ FISH +	S-1/cisplatin + trastuzumab No description of duration	68%	OS: 16.0 mo PFS: 7.8 mo
PF299804	Phase Ⅱ/2nd Non-Randomized	28 (estimated)	IHC 3+ IHC 2+ FISH +	PF299804 Cycles of 28 d	7.40%	Ongoing
HIROISE (NCT01450646)	Phase Ⅲ/1st Randomized	400 (estimated)		Trastuzumab + cisplatin + capecitabine 33 wk		Ongoing
NCT01472029	Phase Ⅱ/1st Non-Randomized	53		5-FU, leucovorin, docetaxel, oxaliplatin (FLOT), trastuzumab No description of duration		Ongoing
NCT01130337	Phase Ⅱ/ preoperative Non-Randomized	36		Trastuzumab + XELOX 12 mo		Ongoing
NCT01522768	Phase Ⅱ/advanced Non-Randomized	40	IHC 3+ IHC 2+ FISH +	Afatinib + trastuzumab 24 mo		Ongoing
NCT01402401	Phase Ⅱ/2nd Non-Randomized	21	IHC 3+ IHC 2+ FISH +	AUY922 + trastuzumab Every 6 wk		Ongoing
NCT01641939	Phase Ⅱ/Ⅲ/1st Randomized	412 (estimated)	IHC 3+ IHC 2+ FISH +	Trastuzumab emtansine Taxane 12 wk		Ongoing

HER2: Human epidermal growth factor receptor; OS: Overall survival; PFS: Progression-free survival; IHC: Immunohistochemistry; FISH: Fluorescence *in situ* hybridization.

a recent retrospective analysis, gastric cancer patients with HER2 overexpression applied trastuzumab in addition to their first-line chemotherapy with or without trastuzumab maintenance therapy. The median PFS was 14.6 mo and OS was 16.4 mo with an acceptable benefit[45] (Table 1).

On the other hand, the efficacy of HER2-targeted agents have shown to be limited and unsatisfactory than that would be expected[13,46]. These insufficiencies may be overcome by the development of combined therapy with other cytotoxic agents, and strategies against primary or acquired resistance in the patients with gastric cancer. The HERBIS-1 study is the muticenter phase Ⅱ trial undertaken at 22 hospitals in Japan[47]. Patients with HER2-positive advanced gastric cancer received S1 on day 1-14, cisplatin, on 1 d, and trastuzumab on day 1 of a 21-d cycle. The RR based on RECIST was 68% (95%CI: 54%-80%; 80%CI: 58%-76%) and the disease control rate was 94% (95%CI: 84%-99%). Median OS, PFS, and TTF were estimated as 16.0, 7.8, and 5.7 mo, respectively. Trastuzumab combined with SP would be a potential new strategy for patients with HER-2 positive advanced gastric cancer. Another study presented that the level of synergistic effect on combination therapy with trastuzumab and anti-cancer drug was different depending on the expression level of HER-2. The HER-2 expression may be applied to standard for drug selection in combination of trastuzumab with known cytotoxic agents in gastric cancer[48].

Pertuzumab

Pertuzumab, one of the HER2-targeted monoclonal antibody, is distinct and complementary to trastuzumab. Pertuzumab binds the extracellular domain Ⅱ of the HER2 receptor and disrupts HER2 dimerization (Figure 1)[49]. Similar to trastuzumab, pertuzumab activates antibody-dependent cellular cytotoxicity, with equivalent efficacy, leading to cancer cell death[50]. The combination of trastuzumab plus pertuzumab was reported to be synergistic in breast cancer. This combination reduced HER2/EGFR and HER2/HER3 heterodimer formation, leading to in induction of apoptosis *in vitro*[49]. Thus the therapeutic achievement of pertuzumab in metastatic HER2 positive breast cancer provides hope for HER2 positive gastric cancer by using a similar approach. With regard to gastric cancer, a phase Ⅱa study of first-line pertuzumab in combination with trastuzumab, capecitabine and cisplatin in patients with HER2 positive advanced gastric cancer was carried out[51]. Patients were divided into two arms: pertuzumab 840 mg for cycle 1 and trastuzumab 420 mg q3w for cycle 2-6 *vs* pertuzumab 840 mg q3w for six cycles. Partial responses were achieved by 86% and 55% of patients, respectively.

Lapatinib

Lapatinib is a small tyrosine kinase inhibitor of EGFR and HER2 that interferes activation by binding to the intracellular ATP binding site of these kinases (Figure 1).

This agent inhibits HER2 and EGFR dependent activation of PI3K and Ras pathways, leading to downregulation of receptor tyrosine kinase phosphorylation in tumor cells (Figure 1). The synergistic activity was shown by lapatinib in combination with trastuzumab for HER2 positive breast cancer that presented through two lines of trastuzumab related treatments[52]. Regarding to gastric cancer, modest activity was demonstrated with single-agent lapatinib in the Southwest Oncology Group (SWOG) 0413 trial[53]. Subsequently, Tykerb with Taxol in Asian ErbB2 gastric cancer (TyTAN), an open-label randomized phase Ⅲ study comparing paclitaxel with paclitaxel plus lapatinib in patients with HER2 FISH-positive IHC3 + gastric cancer as a second-line therapy was performed. Although the median OS was prolonged by two months with lapatinib, this trial failed to achieve any OS and PFS benefit because statistically significance was not obtained[46]. Moreover, the Lapatinib Optimization study in HER2 positive Gastric Cancer (LOGiC) trial is a phase Ⅲ trial of capecitabine and oxaliplatin with or without lapatinib in first-line advanced HER2 FISH amplified gastric and GEJ cancers suggested not any signs of activity for lapatinib[54]. These negative results of TyTAN and LOGiC trials indicate that there is a presence of drug resistance or an alternative pathways from HER2-targeted therapy. On the other hand, lapatinib may be useful for treating the trastuzumab-resistant HER2-positive gastric cancer. A previous study described that lapatinib showed the antitumor efficacy for trastuzumab-resistant cell lines which was due to both G_1 cell-cycle arrest and apoptosis induction[55].

Trastuzumab-emtansine

Antibody-drug conjugates have been said to transfer cytotoxic drugs directly to tumor cells. Trastuzumab emtansine (TDM-1) is an antibody-drug conjugate of trastuzumab and, a potent microtubule inhibitor, DM1(derivative of maytansine). In preclinical gastric cancer models, TDM-1 has shown more aggressive tumor activity than trastuzumab[56]. A multicenter adaptive phase Ⅱ/Ⅲ of TDM-1 is currently underway with HER2 positive advanced gastric cancer after progression following first line treatment (NIH study trial registration number NCT01641939; ClinicalTrials. gov).

STRATEGIES TO OVERCOME TRASTUZUMAB RESISTANCE IN GASTRIC CANCER

Although HER2 targeting therapy has been advanced, most patients with gastric cancer still develop acquired resistance to trastuzumab[54]. To achieve better benefits for HER2-targeted therapy in patients with HER2-positive gastric cancer, there is an urgent need to clarify the mechanisms underlying the cancer-resistance. Some papers described that intra-tumoral heterogeneity of gastric cancer may play a role in the resistance[57,58]. In this section, we will discuss selected novel agents including those based on proposed mechanisms of resistance to HER2-targeted therapy (Figure 1).

Afatinib

Afatinib (Gilotrif, Boehringer Ingelheim), an irreversible inhibitor of EGFR, HER2, and HER4, has been shown to be effective in the elimination of cancer cells with *HER2* gene mutations[59]. This orally-bioavailable compound binds to its targets and has potential against receptors with acquired mutations that are resistant to first-generation inhibitors. The usefulness of this agent is currently examined in phase Ⅲ study in EGFR-positive non small cell lung cancer, breast cancer, and head and neck squamous cell carcinoma[59]. Phase Ⅱ study in metastatic HER2 positive trastuzumab refractory esophagogastric cancer is underway (NIH study trial registration number NCT01522768; ClinicalTrials.gov).

mTOR inhibitors

One of the most important mechanisms underlying trastuzumab resistance is dysregulation of HER2 downstream signal substrate, including the PI3K/Akt/mTOR pathway. It is well known that *PIK3CA* mutations and phosphate and tensin homolog (PTEN) inactivation result in constitutive activation of the downstream signals. HER2 overexpression is said to be significantly associated with p-Akt expression, suggesting that *PIK3CA* mutation and PTEN inactivation may affect the effectiveness of HER2-targeted therapy[60]. Inhibition of the mTOR/S6K signal by mTOR inhibitor, everolimus, enhanced fluorouracil-induced apoptosis in gastric cancer cells with HER2 amplification. Thus, it is plausible that concomitant therapy between HER2-targeted agents and mTOR inhibitor may provide substantial benefit in patients with HER2-positive gastric cancer.

HSP90 inhibitors

HSP90 is an ATP-dependent, conserved molecular chaperone that is involved in the structural folding and stability of proteins. Suppression at the NH2-terminal ATP binding site results in the degradation of client proteins by the ubiquitin proteasome pathway, which lead to the degradation of HER2 (Figure 1)[61]. Furthermore, p95-HER2, which is amino-terminal truncated form of HER2 and also a major factor of trastuzumab resistance due to the lacks of the trastuzumab binding site, shown to be degraded by HSP90 inhibitors. Several HSP90 inhibitors are undergoing early clinical evaluation. AUY922 is part of the isoxazole HSP90-inhibitor family. HSP90 inhibition by AUY922 leads to decrease the ErbB2 protein level and downstream signaling via PI3K in HER2-positive gastric cancer[62]. A recent study described that a significant synergy exists between AUY922 and trastuzumab in HER2-amplified gastric cancer. The combination of AUY922 and trastuzumab is also synergistic in HER2-amplified trastuzumb-progressed gastric cancer, which may be due to reinforce the rationale behind dual mechanisms of blockade in

HER2-amplified diseases[63]. Clinical trials on the basis of this study are currently underway in gastric cancers (NIH study trial registration number NCT01402401; ClinicalTrials.gov). Thus, the dual inhibition of HSP90 and HER2 may enhance the attenuate effects on downstream signaling, especially in trastuzumab-resistant patients.

Pan-HER TK1

Recent studies have suggested that HER3 plays a pivotal role in tumor resistance to molecular agents targeting HER2[64], and is causing the maintenance of HER2-amplified cell growth. Therefore, it is plausible that a pan-HER TK1, which targets all HER family members, may have more potent activity in HER expressed malignancies. PF00299804 (Decomitib) is an orally bioavailable, second-generation, irreversible pan-HER TKI currently under clinical development[65]. The combination of PF00299804 with chemotherapeutic agents or molecular-targeted agents including trastuzumab produced synergistic effects. These data suggested that PF00299804 may augment the antitumor efficacy of chemotherapy and/or molecular-targeted agents.

Met inhibitors

c-Met is a cell surface receptor for hepatocyte growth factor (HGF), which regulates a variety of cellular processes involved in cell growth, invasion and angiogenesis[66]. A functional crosstalk between Met and HER family members has been shown to acquire an invasive and progressive phenotype, suggesting that Met can be the dimerization partner or crosstalk with HER2[67]. Furthermore, the Met signaling has been implicated as a mediator of resistance to therapies targeting members of the HER family in several solid tumors[68]. These data indicate that HER2 targeted agents combined with Met inhibitor may be useful to facilitate the efficacy and overcome the resistance of HER2 therapy in patients with HER2-positive gastric cancer. Similarly, HGF activation of MET receptors has been described to rescue cells from lapatinib-induced growth inhibition by restimulating the downstream pathways[69]. This effect was abrogated by inhibiting MET with PHA-665752 (a highly specific MET inhibitor), suggesting that lapatinib-induced growth inhibition may be abrogated through the activation of MET. Thus, the dual application of lapatinib and MET inhibitor may be a favorite model to overcome the lapatinib-resistant gastric cancer.

FUTURE PERSPECTIVES

This review has discussed some of a large number of data showing the clinical benefits of trastuzumab for treating HER2-positive gastric cancer. Combination therapy between trastuzumab and conventional chemotherapy is now cited as a standard first-line treatment for HER2 overexpressing gastric cancer in patient with advanced stage. This treatment remains to be under investigation for more potent utilization. Abundant clinical trials are planned or currently underway to evaluate the role of anti-HER2 agents in metastatic disease in combination with cytotoxic chemotherapy as well as with targeted therapy. As previously described, some studies with anti-HER2 combination treatments indicate that the use of more than one HER2-targeted therapy was superior to one of these agents alone in HER2 positive gastric cancer[63,69-71]. With the expansion of utilizing anti-HER2 agents, identifying the right combination of these various novel agents will be urgent to benefit the clinical outcomes in patients with advanced gastric cancer.

There still are several subjects to be discussed and settled for the advancement of HER2-targeted therapy in gastric cancer.

Dose increases of trastuzumab

To date, the clinical relevance of trastuzumab's kinetic variable is not defined. It has been considered that higher dosing may be required in patients with gastric cancer (Roche, Inc. Herceptin package insert. Available at: http://www.medsafe.govt.nz/profs/datasheet/h). Pharmacokinetics data showed in the ToGA study described that the trastuzumab clearance is 0.378 L/d based on the current standard dosing, 70% higher than the rates in trastuzumab-treated patients with breast cancer. The HELOISE study: a study of trastuzumab in combination with chemotherapy in patients with HER2-positive metastatic gastric or gastro-esophageal cancer is currently under investigation (NIH study trial registration number NCT01450696; ClinicalTrials.gov.). In this phase Ⅲ study, patients are randomized to either standard dosing or higher dosing arm (trastuzumab given at 8 mg/kg loading dose followed by 10 mg/kg every 3 wk) in combination with cisplatin based chemotherapy.

Possible candidates as a new biomarker

To date, there are no predictive biomarkers of response to trastuzumab. Thus, identifying a biomarker is one of the most importance in the development of an effective targeted agent. The NeoSphere trial, examining 16 different biomarkers, including HER2 expression, *PI3KCA* mutation, p95HER2, and insulin-like growth factor receptor expression in patients with breast cancer, resulted in failure of predicting response to pertuzumab[72]. In contrast, the presence of HER2 phosphorylation and low HER3 mRNA expression were associated with improved efficacy in patients with ovarian cancer treated with pertuzumab[73]. In some clinical trials, *PIK3CA* mutation or PTEN loss has been evaluated as a possible predictive biomarker. Therefor, to clarify the association between HER2 expression and PI3K/Akt pathway alterations is necessary to develop a new therapeutic strategy.

With regard to gastric cancer, several experimental studies showed the possibility of several factors which may be useful for predicting the efficacy of trastuzumab alone or combined chemotherapy. Trastuzumab resistant cells were noted to express the decreased amount of

p27KIP1 levels and increased CDK2 activity[74]. As a result, p27Kip1 level may be utilized as a marker of trastuzumab response and potential therapeutic target in trastuzumab-resistant gastric cancer. The serum level of HER2-extracellular domain (ECD) has been said to evaluate the HER2 status of patients with gastric cancer. Moreover, the serum HER2-ECD test could enable monitoring of the dynamic changes in HER2 status over the clinical course of the disease. It can be used as an easily accessible real-time biomarker for longitudinal assessments of disease status[75].

Mechanism of resistance

Resistance to trastuzumab is currently emerging in HER2 positive gastric cancers. PI3K pathway may confer resistance to tratuzumab in preclinical studies. Enhanced signaling from HER family receptors, including overexpression of HER3 and formation of high levels of HER2-HER3 heterodimers, and insulin-like growth factor-1 receptor (IGF-1R) are also correlated with PI3K signaling activity and resistance of anti-HER2 agents[76]. Recently, several studies have presented the mechanisms of resistance in patients with gastric cancer. Cancer stem cells may be postulated mediators of the chemoresistance. The c-Met inhibitor may be a promising target molecule for irinotecan-based chemotherapy of gastric cancer[77]. Heregulin 1 can cause resistance to lapatinib in gastric cancers *in vitro* through HER3 and Akt activation[78]. Interestingly, epithelial-mesenchymal transition plays an important role in acquiring resistance to HER2-directed treatment in HER2 positive gastric cancer[79].

CONCLUSION

HER2 signaling lead to receptor dimerization such as HER2/HER3 and positively mediates its downstream signals which lead to a variety of tumor biology. Dimerization of HER2 and HER3 is known to be the most active HER signaling dimer and HER3 may be a subsequent target of therapy in gastric cancer as well as HER2, especially for the diffuse histological type. Current development of technology for identifying HER2 expression is convincing and may result in the improved clinical outcome in trastuzumab applied patients with gastric cancer. There are several molecular HER2-targeted agents applied practically and showing clinical benefits compared to chemotherapy alone in gastric cancer. However, the efficacy of these agents has shown to be modest and unsatisfactory than that would be expected which may be due to the acquisition of resistance or an unmatched combination to known cytotoxic agents. To overcome the trastuzumab resistance, several examinations have elucidated the mechanisms of resistance in gastric cancer. Clinical and experimental studies using several other molecules, which shows synergistic activity by concomitant use with trastuzumab or has potential against receptors with acquired resistance, are under investigation. There still are several subjects to be discussed for the advancement of HER2-targeted therapy in gastric cancer, such as determining the suitable dose of trastuzumab, identifying a predictive biomarker. In conclusion, HER2-targeted therapy is now acceptable for management of patients with gastric cancer and recent studies including resistance-control will provide superior strategies for treating HER2-positive gastric cancer patients. In the meanwhile, considering the low expression (6%-23%) of HER2 and modest effect of ToGA trial (only 2.7 mo prolonged survival) in gastric cancer, the development of a novel molecular targeted therapy which has a more potent activity for gastric cancer might be desirable.

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Implant biomaterials: A comprehensive review

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Abstract

Appropriate selection of the implant biomaterial is a key factor for long term success of implants. The biologic environment does not accept completely any material so to optimize biologic performance, implants should be selected to reduce the negative biologic response while maintaining adequate function. Every clinician should always gain a thorough knowledge about the different biomaterials used for the dental implants. This article makes an effort to summarize various dental biomaterials which were used in the past and as well as the latest material used now.

Key words: Biomaterials; Zirconium; Surface roughness; Ceramic; Corrosion

Core tip: This article makes an effort to review and summarize all the biomaterials used for dental implants. Materials in this article are discussed according to the era in which they were used. This review also covers the pros and cons related to these materials. Recent trends in the field of dental implants biomaterials and why these materials are superior over the previous ones. The content of the article are clinically significant and will prove to be helpful for readers to make decision while choosing implant system.

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INTRODUCTION

In attempt to replace a missing tooth many materials have been tried as an implant . With all the advancements and developments in the science and technology, the materials available for dental implants also improved[1].

Implants are traceable to early Egyptians and South Central American cultures and with all the developments in material and biological science we have come a long way. Improvements in both the quality and quantity of the implant material have made this treatment modality very promising, budding and highly practiced in today's era. The Earliest dental implants of stone and ivory were

Table 1 Implant materials can be classified based on the type of material used and the biologic response they elicit when implanted[3]

Biodynamic activity	Chemical composition		
	Metals	Ceramics	Polymers
Biotolerant	Gold		Polyethylene
	Co-Cr alloys		Polyamide
	Stainless steel		Polymethylmethacrylate
	Niobium		Polytetrafluroethylene
	Tantalum		Polyurethane
Bio inert	Commercially pure titanium	Al oxide	
	Titanium alloy (Ti-6AL-4U)	Zirconium oxide	
Bioactive		Hydroxyapatite	
		Tricalcium phosphate	
		Bio glass	
		Carbon-silicon	

reported in China and Egypt. Also Gold and Ivory dental implants were reported in the 16th and 17th centuries[2]. Metal Implants of Gold, Lead, Iridium, Tantalum, stainless steel and cobalt alloy were also mentioned in the early 20th century. Between these two periods a variety of polymers, including ultrahigh molecular weight polyurethane, polyamide, polymethylmethacrylate resin, polytetrafluoroethylene, and polyurethane, have been used as dental implant. In the present era, due to the extensive research work and advancements in the field of biomaterials available for dental implants, newer materials came into being such as zirconia, roxolid, surface modified titanium implants. These materials not only fulfill the functional requirements but are also esthetically pleasing. This article makes an effort to review various implant materials, their properties and the various pros and cons associated to those materials. To identify relevant literature an electronic search was performed of Pubmed database using the following keywords, implant biomaterials, implant material biocompatibility, recent trends in implant dentistry. The searches were limited to full text articles in English and those with associated abstracts. All the articles published from 1955 to 2012 are included in this review. All the articles in the language other than English and the articles related to surface coated implants and case reports are excluded.

Materials in this article are divided according to the era they were evolved as an implant material[3-6] (Table 1).

PROPERTIES OF AN IMPLANT BIOMATERIAL

Bulk properties[2,7]

Modulus of elasticity: Implant material with modulus of elasticity comparable to bone (18 GPa) must be selected to ensure more uniform distribution of stress at implant and to minimize the relative movement at implant bone interface.

Tensile, compressive and shear strength: An implant material should have high tensile and compressive strength to prevent fractures and improve functional stability. Improved stress transfer from the implant to bone is reported interfacial shear strength is increased, and lower stresses in the implant.

Yield strength, fatigue strength: An implant material should have high yield strength and fatigue strength to prevent brittle fracture under cyclic loading.

Ductility: According to ADA a minimum ductility of 8% is required for dental implant. Ductility in implant is necessary for contouring and shaping of an implant.

Hardness and Toughness: Increase in hardness decreases the incidence of wear of implant material and increase in toughness prevents fracture of the implants.

Surface properties

Surface tension and surface energy: It determines the wettability of implant by wetting fluid (blood) and cleanliness of implant surface. Osteoblasts show improved adhesion on implant surface. Surface energy also affects adsorption of proteins[2].

Surface roughness: Alterations in the surface roughness of implants influence the response of cells and tissue by increasing the surface area of the implant adjacent to bone and thereby improving cell attachment to the bone.

Implant surfaces have been classified on different criteria, such as roughness, texture and orientation of irregularities[8,9]: (1) Wennerberg and coworkers have divided implant surfaces according to the surface roughness as: Minimally rough (0.5-1 m), Intermediately rough (1-2 m), Rough (2-3 m); (2) The implant surface can also be classified according to their texture as: concave texture (mainly by additive treatments like hydroxyapatite (HA) coating and titanium plasma spraying),convex texture (mainly by subtractive treatment like etching and blasting); and (3) The implant surface can also be classified according to orientation of surface irregularities: Isotropic surfaces: have similar topography independent of measuring direction; Anisotropic surfaces: have clear directionality and vary considerably in roughness.

Biocompatibility

This is property of implant material to show favorable response in given biological environment in a particular function. It depends on the corrosion resistance and cytotoxicity of corrosion products.

Corrosion and corrosion resistance[9-11]: It is the loss of metallic ions from metal surface to the surrounding environment. Following types of corrosion are seen.

Crevice corrosion: It occurs in narrow region like implant screw-bone interface. When metallic ions dissolve, they can create a positively charged local environment in the crevice, which may provide opportunities for crevice corrosion.

Pitting corrosion: Pitting corrosion occurs in an implant with a small surface pit. In this the metal ions dissolve and combine with chloride ions. Pitting corrosion leads to roughening of the surface by formation of pits.

Galvanic corrosion: This occurs because of difference in the electrical gradients. Nickel and chrome ions from artificial prosthesis may pass to peri-implant tissues due to leakage of saliva between implant and superstructure .This may result in bone reabsorption and also affect the stability of the implant and eventually cause failure.

Electrochemical corrosion: In this anodic oxidation and cathodic reduction takes place resulting in metal deterioration as well as charge transfer *via* electrons. This type of corrosion can be prevented by presence of passive oxide layer on metal surface.

Clinical significance of corrosion: Implant bio-material should be corrosion resistant. Corrosion can result in roughening of the surface, weakening of the restoration, release of elements from the metal or alloy, toxic reactions. Adjacent tissues may be discolored and allergic reactions in patients may result due to release of elements.

ANCIENT ERA (through AD 1000)

Implants are traceable to ancient egyptian and south American civilization. There is a skull form pre Columbian era in which artificial tooth is carved with dark stone. Albucasis de condue Arabian surgeon, credited with a written paper of transplants as a means of replacing missing teeth[12].

Foundational period (1800-1910)

This era is the beginning of Endosseous oral implantology. Maggiolo in 1809 used gold in the shape of the tooth root. In 1887 Harris reported the use of teeth made of porcelain into which lead coated platinum posts were fitted. In 1890, Zamenski reported the implantation of teeth made of porcelain, gutta-percha, and rubber and in 1898 R.E payne places silver capsule in the tooth socket. In the early 1900's lambotte fabricated implants of aluminum, silver, brass, red copper, magnesium, gold and soft steel plated with gold and nickel[11,12].

Premodern era (1901-1930)

In 1901 a technique of capsule implantation was reported in dental cosmos which was presented by R.E payne at the clinics of third international dental congress. In 1903, Sholl in Pennsylvania, implanted porcelain tooth which was having a corrugated porcelain root. In 1913, Dr. Edward J. Greenfield introduced into the alveoli the basket of iridium and 24 carat gold. E. J Greenfield also introduced the concept of submerged implant, the healing tissue and dental implant immobility[12].

Dawn of the modern era (1935-1978)

In this era, synthetic polymers, ceramics and metal alloys started replacing the naturally derived materials because they have better performance and more predictable results than the natural ones.

Strock anchored a vitallium screw within bone and immediately mounted it with a porcelain crown. He was the first one to achieve an implant survival for 15 years[12,13].

POLYMERS

The early work with the methyl methacrylate resin implants met mostly with failures[14-18]. However, in 1969, Hodosh reported that polymers were biologically tolerable substances[16,17]. Research on polymethacrylate tooth-replica implants led to the development of the polymer dental implant concept by Milton Hodosh. In replacing a natural tooth, the polymer replica proved to be ideal for the restoration of function and appearance[18].

Polymers were selected for the following reasons[17]: (1) The physical characteristics of the polymers can be altered based on their use as their composition may be changed easily. Polymers can be changed in to more porous or softer form; (2) Polymers can be manipulated easily and allow better reproduction; (3) Polymers do not generate microwaves or electrolytic current as do metals; (4) They show fibrous connective tissue attachment; (5) They can be easily microscopically evaluated than with metals; and (6) They are more esthetically pleasing. There are some disadvantages: (1) inferior mechanical properties; (2) lack of adhesion to living tissues; and (3) adverse immunologic reactions.

Metals and metal alloys

Metals have biomechanical properties which made them suitable as an implant material. Besides these properties metals are also easy to process and have good finish. Metallic implants can be sterilized by the common sterilization procedure which makes them easy to use. But due to advancements with time and low success rates with metals (gold, stainless steel, cobalt-chromium), these materials have now become obsolete and are now replaced by newer ones. Titanium (Ti) and its alloys

(mainly Ti-6Al-4V) have become the metals of choice for dental implants. However, prosthetic components of the implants are still made from gold alloys, stainless steel, and cobalt-chromium and nickel-chromium alloys[3].

Cobalt chromium alloys

They are used in cast or cast and annealed metallurgic conditions. It allows the manufacture of customized implants, such as subperiosteal frames. The elemental composition of this alloy includes cobalt, chromium and molybdenum as the major elements. Cobalt provides continuous phase for basic properties. Chromium provides corrosion resistance through the oxide surface. Molybdenum provides strength and bulk corrosion resistance. Nickels biocorrosive product and carbon must be accurately controlled to enhance mechanical properties, such as ductility[19,20].

Iron-Chromium-Nickel Based Alloys

Stainless steel alloys are used for orthopedic and implant devices. Iron based alloys are used for ramus blade, ramus frame, stabilizer pins and some mucosal insert. The alloy is most prone to pitting corrosion and care must be taken to use and retain the passiviated (oxide) surface condition, as this alloy contains nickel as a major element. Its use in allergic patients must be avoided. They have high galvanic potentials and corrosion resistance.This can result in galvanic coupling and biocorrosion, if titanium, cobalt, zirconium or carbon implant biomaterials are used with it[8].

IMPLANTS IN 21ST CENTURY

Titanium

Titanium has a good record of being used successfully as an implant material and this success with titanium implants is credited to its excellent biocompatibility due to the formation of stable oxide layer on its surface[21,22].

The commercially pure titanium (cpTi) is classified into 4 grades which differ in their oxygen content. Grade 4 is having the most (0.4%) and grade 1 the least (0.18%) oxygen content. The mechanical differences that exist between the different grades of cpTi is primarily because of the contaminants that are present in minute quantities. Iron is added for corrosion resistance and aluminum is added for increased strength and decreased density, while vanadium acts as an aluminum scavenger to prevent corrosion. Hexagonal close-packed crystal lattice of Ti is called the α-Ti (α-phase). On heating it at 883 ℃ phase transformation occurs from hexagonal close packed to body-centered cubic lattice or β- phase. Ti is a reactive as it forms spontaneously a dense oxide film at its surface. Ti is a dimorphic metal i.e. below 882.5 ℃ it exists as α-phase and above this temperature it changes form α- phase to β phase. Because of the high passivity, controlled thickness, rapid formation, ability to repair itself instantaneously if damaged, resistance to chemical attack, catalytic activity for a number of chemical reactions, and modulus of elasticity compatible with that of bone o, Ti is the material of choice for intraosseous applications[3,22-25].

Disadvantage: There is esthetic issue due to gray color of titanium and this is more pronounced when soft tissue situation is not optimal and the dark color shines through the thin mucosa.

Titanium alloys Ti6Al4V

Titanium reacts with several other elements for eg: silver, Al, Ar, Cu, Fe, Ur, Va and Zn to form alloys. Titanium alloys exists in three forms alpha, beta and α-β. These types originate when pure titanium is heated with elements Al, Va in certain concentrations and cooled, these type originate. These added elements play like Phase- condition stabilizers. Aluminum is alpha-phase condition stabilizer and it also increases the strength and decrease the weight of the alloy. Vanadium acts as beta-phase stabilizer. The temperature at which α-to β transformation occurs changes to a range of temperatures as Al or Va is added to Ti. Both α and β forms exist in this range. Temperatures to which the desired form is present can be obtained by quenching alloy at room temperature.To increase the strength, these alloys may be heat treated. The alloys most commonly used for dental implants are of the alpha-beta variety. The most common contains 6% Al and 4% Va. (Ti 6 Al 4V)[3,26].

Ceramics

Ceramics were used for surgical implant devices because of their inert behavior and good strength and physical properties such as minimum thermal and electrical conductivity. Certain properties of ceramics like low ductility and brittleness has limited the use of ceramics[3].

Aluminum, titanium and zirconium oxides

Root form or endosteal plate form, and pin-type dental implants are generally made from High ceramics from aluminum, titanium and zirconium oxides. The compressive, tensile and bending strengths exceed the strength of compact bone by 3 to 5 times. These properties combined with high moduli of elasticity and especially with fatigue and fracture strength have resulted in specialized design requirements for this class of biomaterials[8].

MODERN ERA

Modern Implant dentistry is delineated from the period of mid 1930's to the present. Today's popularity of implants in dentistry is attributed to the developments and the research work which laid the foundation of this field. It is because of all this work in the past that we are seeing the emergence of implant concepts developing into the most refined and popularly utilized systems.

In recent years the treatment options and modalities for achieving optimal functional and aesthetic outcomes with implant restorations have clearly changed. Pure titanium is generally preferred for dental implant because

of its excellent biocompatibilty and mechanical properties. There might be aesthetic problems due to the gray color of titanium. In some situations, there may be a soft tissue recession; in such situations there is an unaesthetic display of the metal components. Therefore, implant research has focused on discovering tooth-colored implant material that improves the aesthetic appearance of dental implants and, at the same time, is highly biocompatible and able to withstand the forces present in the oral cavity and therefore zirconia came into being[27-29].

Zirconia

Zirconia was used for dental prosthetic surgery with endosseous implants in early nineties. Cranin and coworkers published first research work on Zirconia in 1975. Ceramic implants were introduced for osseointegration, less plaque accumulation resulting in improvement of the soft tissue management, and aesthetic consideration as an alternative to titanium implants[30,31].

Monoclinic (M), cubic (C), and tetragonal (T) are the three crystal forms in which polymorphic Zirconia structure is present. Zirconia,on room temperature, acquires a monoclinic structure and changes into tetragonal phase at 1170 ℃, followed by a cubic phase at 2370 ℃. At room temperature these phases are unstable and break into pieces,on cooloing. The C-phase of pure Zirconia can be stabilized by adding CaO, MgO, and Y_2O_3 (Yttrium) resulting in multiphase material called partially stabilized zirconia (PSZ) combining cubic, monoclinic, and tetragonal phases in the order of importance. Tetragonal zirconia polycrystals (TZP), containing tetragonal phase only can be obtained by adding Yttrium at room temperature. Yttria stabilized TZP possesses low porosity, high density, high bending, and compression strength and is suitable for biomedical application[32].

Titanium-zirconium alloy (Straumann Roxolid)

Titanium zirconium alloys with 13%-17% zirconium (TiZr1317) have better mechanical attributes, such as increased elongation and the fatigue strength, than pure titanium. Growth of osteoblasts, that are essential for osseointegration is not prevented by Titanium and Zirconium. Straumann developed Roxolid that fulfills requirements of dental implantologists and is 50% stronger than pure titanium.

Sandblasting and acid-etching on, TiZr1317 with a monophasic a structure results in a topographically identical surface as on pure titanium implants. Because of its superior mechanical properties.Thin implants and implant components that can be subjected to high strains can be produced using TiZr1317 due to its better mechanical properties, provided that the material shows a similar good biocompatibility as pure titanium[33].

CONCLUSION

In evaluating the present and predicting the future, one must also reconsider the past. The implant materials, their composition and properties are not talked about in most of the implant related literature. The literature also lacks the effect of the material properties on success and failure of implants and its effects on the tissues surrounding the implants.

Modern dentistry is beginning to understand, realize, and utilize the benefits of biotechnology in health care. Study of material sciences along with the biomechanical sciences provides optimization of design and material concepts for surgical implants[34].

Implants have been gaining popularity amongst the patients and frequently are being considered as a first treatment option. In the last decade implants have dominated the other treatment modalities and moved into the mainstream of dental practice. "We have come a long way but there is still more to achieve".

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MINIREVIEWS

Vasopressors in obstetric anesthesia: A current perspective

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Abstract

Vasopressors are routinely used to counteract hypotension after neuraxial anesthesia in Obstetrics. The understanding of the mechanism of hypotension and the choice of vasopressor has evolved over the years to a point where phenylephrine has become the preferred vasopressor. Due to the absence of definitive evidence showing absolute clinical benefit of one over the other, especially in emergency and high-risk Cesarean sections, our choice of phenylephrine over the other vasopressors like mephentermine, metaraminol, and ephedrine is guided by indirect evidence on fetal acid-base status. This review article evaluates the present day evidence on the various vasopressors used in obstetric anesthesia today.

Key words: Vasopressor agents; Obstetrics; Cesarean section; Hypotension; Spinal anesthesia

Core tip: Phenylephrine has emerged as the vasopressor of choice in Obstetrics. However, the present recommendations are essentially based on studies conducted in elective Cesarean sections. Further studies are needed in emergency and high risk Cesarean sections in order to clarify whether there is a benefit of phenylephrine over other vasopressors.

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INTRODUCTION

Neuraxial anesthesia remains the preferred choice for Cesarean deliveries across the world. Hypotension is the physiologic consequence of spinal anesthesia and can have a potentially deleterious maternal and fetal impact. Vasopressors, which lead to an increase in systemic vascular resistance and rise in mean arterial pressures[1], have been traditionally used for the prevention and management of hypotension after neuraxial anesthesia. However, the understanding of hypotension after neuraxial anesthesia in obstetrics, and the use of vasopressors to counteract it, continues to evolve over the years. This review article briefly explores the present understanding of the mechanism causing hypotension before discussing the current use of

the various vasopressors in obstetric anesthesia today. The authors discuss the various vasopressors used in obstetric anesthesia and put the recent evidence into perspective to guide our clinical practice today.

HYPOTENSION AFTER NEURAXIAL ANESTHESIA

The sympathectomy resulting from the neuraxial blockade is exaggerated by the physiological changes of pregnancy and puerperium, leading to hypotension in as much as 55%-90% of the mothers receiving spinal anesthesia for Cesarean section[2]. Holmes *et al*[3] and Lees *et al*[4] indicated that the compression of the vena cava by gravid uterus impeded the venous return and caused hypotension. Marx[5] postulated that the subarachnoid block resulted in venous pooling of blood in the lower legs, leading to decreased venous return and reduced cardiac output. Although our present interpretation of the mechanism causing hypotension are still based on these principles, prophylactic therapeutic interventions based upon this understanding do not definitively prevent hypotension after neuraxial anaesthesia in Cesarean sections[6].

Based on studies on pre-eclamptic women, Sharwood-Smith *et al*[6] challenged the understanding that reduced central venous pressure led to decreased cardiac output and arterial pressures. They suggested that "venous capacitance" rather than venous pressure maybe the determinant in causing hypotension after spinal anesthesia in obstetrics. The "endothelium-dependent alteration of vascular smooth muscle function" and increased presence of "vasodilator prostaglandins and nitric oxide" during pregnancy have a vasodilatory effect which is counteracted by the intrinsic sympathetic vascular tone[6]. This intrinsic vascular tone is adversely impacted after spinal anesthesia, leading to exaggerated fall in blood pressure. Studies now show that cardiac output remains nearly unchanged even after sympathetic blockade[7], challenging the concept that in parturients, spinal anesthesia results in decrease in cardiac output[8]. Despite the varied understanding of hypotension following neuraxial anesthesia in pregnancy, vasopressors remain the cornerstone in restoring the arterial pressure and mitigating the possible adverse maternal and fetal impact.

VASOPRESSORS USED IN OBSTETRICS

Vasopressors which have been used in obstetrics primarily include the directly acting selective α_1 receptors agonists, phenylephrine and methoxamine, and both directly and indirectly acting mephentermine, metaraminol and ephedrine.

METHOXAMINE

Methoxamine is an $\alpha 1$ receptor agonist which causes intense vasoconstriction following parenteral administration, raising arterial blood pressure and may result in reflex vagal inhibition of the heart rate[9]. It is devoid of any inotropic or chronotropic effect[9] and has been used to counteract the hypotension caused by spinal anesthesia[10]. Tachyphylaxis has seldom been observed with methoxamine[11]. While the peak vasopressor effect after a single intravenous dose of 2-4 mg has been observed after 0.5-2 min, its duration of action has been reported to be 10-15 min[12]. Intramuscular administration of a 10-40 mg dose has its peak onset of action at 15-20 min and its action lasts for about 1.5 h[12]. Its use in clinical obstetrics has fallen out of favor decades ago owing to concerns regarding decreased uterine blood flow and adverse impact on fetal acid-base status in animal studies[12,13].

MEPHENTERMINE

It has a mixed α and β receptor agonist action with both direct and indirect effect due to release of norepinephrine and epinephrine[14]. Its impact on the heart rate is dependent on the vagal tone. Its use in hypotension after a neuraxial blockade in obstetrics is due to its ability to increase the blood pressures by augmenting the cardiac output[14]. Tachyphylaxis to the pressor action of mephentermine develops rapidly[15]. While there is immediate onset of action peaking at 5 min and lasting 15-30 min after an intravenous dose, an intramuscular dose starts acting after 5-15 min and has a variable duration of action from 1-4 h. It is commonly used as a 3-5 mg intravenous bolus or intravenous infusion of 2-5 mg/min[16], or 25-50 mg intramuscularly[17]. There is scarce literature evidence on the fetal metabolic effect and placental transfer of mephentermine[18]. However, a few studies have shown that mephentermine is as effective as phenylephrine in preventing maternal hypotension after spinal anesthesia and has similar effect on neonatal outcome[19]. It is being widely used in developing countries like India as it is much more economical[19] than phenylephrine. Moreover, unlike phenylephrine which needs multiple dilutions from the single use 10 mg/mL (1 mL) ampoules, mephentermine offers ease of use as it does not necessitates multiple dilutions.

METARAMINOL

Although it has both mixed α and β receptor agonist action, its primary clinical use is to counteract the hypotension after spinal anesthesia in obstetrics. It has significant direct effect on vascular α adrenergic receptors along with its indirect action due to the release of norepinephrine[20,21]. Tachyphylaxis develops due to the displacement of norepinephrine from the sympathetic nerve endings by metaraminol and its action as a false neurotransmitter having inhibited vasopressor effect[17]. While an intravenous bolus dose of 0.5-5 mg has its onset of action in 1-2 min, peak action is at 10 min and duration of action is 20-60 min. An intramuscular dose of 2-10 mg has its onset by 10 min and duration of action of 1-1.5 h[22].

PHENYLEPHRINE

At clinically relevant doses, it is a selective α1 receptor agonist and β agonist action is only seen at much higher doses[20]. It is frequently used in obstetric anesthesia to counteract the hypotension after spinal anesthesia due to marked arterial vasoconstriction caused by its α1 agonist action. Potential negative chronotropic effect is due to reflex bradycardia and decreased cardiac output might not adversely influence the fetus in elective cases[23], but during emergency Cesarean sections with presence of fetal acidosis, any fall in cardiac output may further jeopardize the compromised fetus[23]. However, definitive understanding on the effect of phenylephrine in emergency situations awaits further research[21]. Tachyphylaxis with phenylephrine is possibly caused by the down-regulation of α adrenergic receptors. Its potential to be reversed by hydrocortisone has not been evaluated in an obstetric setting[24].

An intravenous dose of phenylephrine has immediate onset and duration of action of 5-10 min[17]. The optimum regimen for administration of phenylephrine has not yet been defined[25]. Prophylactic administration is associated with a higher incidence of hypertension and bradycardia[26] and treatment after onset of hypotension is associated with higher "incidence and severity of maternal predelivery hypotension"[26]. Despite some studies suggesting that to prevent spinal anesthesia induced hypotension, as an intravenous intermittent bolus dose (ED95) of phenylephrine should be at least 122-147 μg[27,28], 40-100 μg bolus dose remains the common clinical practice[25].

Prophylactic infusions have been advocated in the range of 25-100 μg/min in various studies, but claims have been made that a fixed dose of 50 μg/min minimizes the risk of higher incidence of hypotension at lower doses and reactive hypertension, bradycardia and decreased cardiac output at higher doses[23,25,26].

However, prophylactic fixed dose concept has been challenged[26], necessitating further studies to find the advantages of phenylephrine infusion.

It was even suggested in 2010 that "prophylactic fixed rate infusions may have limited application in clinical practice" and further studies into variable rate of phenylephrine infusion is needed[26]. A recent study by Siddik-Sayyid *et al*[29] has failed to demonstrate any difference in neonatal outcome with a variable rate regimen adjusted in response to changes in arterial blood pressure, as compared to prophylactic fixed rate infusion regimen. However, with respect to limiting maternal symptoms, the variable rate regimen was more effective than relying on rescue phenylephrine[29].

Despite these studies, obstetric anesthesiologists are unable to arrive at a consensus opinion on the ideal regimen for administration of phenylephrine because other studies have demonstrated that with intermittent boluses, the total dose requirement is smaller, blood pressure was better maintained in the 1st 6 min after induction, and indeed, good blood pressure control is achievable by intermittent boluses[30], which is not only simple, but also does not need the setting up of a syringe pump[31].

Although not much literature is available on the efficacy of intramuscular phenylephrine, Ayorinde *et al*[32] reported that 4 mg of intramuscular pre-emptive phenylephrine decreased the severity of hypotension and the need for rescue vasopressors in spinal anesthesia induced hypotension.

EPHEDRINE

It has both direct α and β agonist action, but indirect action is more prominent due to the "release of norepinephrine from sympathetic neurons"[20]. It increases the blood pressure by β1 receptor stimulation with increased heart rate and cardiac contractility, whereas the α agonist action causes peripheral vasoconstriction[21,33]. Prophylactic doses of 30 mg intravenous ephedrine had been suggested by Ngan Kee *et al*[34] to achieve significant reduction in the incidence of hypotension, but it was associated with the risk of reactive hypertension in as much as 45% of the patients. Subsequent studies by Kol *et al*[35] also failed to demonstrate beneficial effect of prophylactic intravenous ephedrine at 0.5 mg/kg. Even for a reduction in the need for rescue boluses of ephedrine, at least 12 mg intravenous prophylactic dose of ephedrine is needed after spinal anesthesia for Cesarean sections[36]. Ephedrine's limited ability to prevent hypotension induced by neuraxial anesthesia is probably related to its slower onset of action[34]. As a rescue vasopressor, 5-15 mg intravenous boluses are most commonly advocated for the treatment of hypotension following neuraxial anesthesia. Its clinical effect is primarily due to its indirect action of releasing norepinephrine from postganglionic nerve endings. The drug not only has delayed onset of action, it also has a longer duration of action of about 60 min. Depletion of presynaptic norepinephrine stores also lead to tachyphylaxis[35]. Due to its delayed onset of action, it should only be repeated after 5-10 min as it was observed that larger doses of ephedrine were required in the first 10 min and often caused overshoot of the desired target systolic pressures after 10 min[37]. Intravenous boluses are therefore preferred to continuous intravenous infusions as the drug exhibits delayed onset of action and tachyphylaxis.

CHOICE OF VASOPRESSOR: THE RECENT EVIDENCE

The ideal vasopressor would be one which is reliable and easy to use, has rapid onset, short duration of action, easily titrable, can potentially be used prophylactically and lack any adverse maternal and fetal impact. A Comparative analysis of the commonly used vasopressors in obstetric anesthesia is illustrated in Table 1.

Table 1 Comparative analysis of vasopressors used in obstetric anesthesia

No.	Drug	Mechanism of Action	Advantage	Disadvantage
1	Methoxamine	α1 receptor agonist	No inotropic or chronotropic effect. Tachyphylaxis has seldom been observed	Reflex bradycardia. Adverse effect on fetal acid-base status
2	Mephentermine	α and β receptor agonist. Both direct and indirectly acting	Economical and does not need multiple dilutions as compared to Phenylephrine	Tachyphylaxis. Little evidence available on placental transfer and its fetal metabolic impact
3	Metaraminol	α and β receptor agonist. Both direct and indirectly acting	No adverse effect on fetal acid-base status as compared to ephedrine	Tachyphylaxis
4	Phenylephrine	Selective α1 receptor agonist at clinical doses	Immediate onset and short duration of action. Ideal for continuous infusion. No adverse effect on fetal acid-base status as compared to ephedrine	Tachyphylaxis. Reflex bradycardia and concerns regarding decreased maternal cardiac output
5	Ephedrine	α and β receptor agonist. Both direct and indirectly acting	Economical and does not need multiple dilutions as compared to Phenylephrine. No bradycardia	Tachyphylaxis. Adverse effect on fetal acid-base status as compared to Phenylephrine

In 2002, Lee *et al*[38] challenged the "traditional idea that ephedrine is the preferred choice". for use as a vasopressor to combat hypotension after spinal anesthesia for Cesarean sections. In a quantitative systemic review they concluded that for elective Cesarean sections, phenylephrine was associated with better fetal acid-base status, although no clinical outcome difference based on the Apgar scores could be established[38].

In patients treated with ephedrine, the cause of decreased pH, base excess and oxygen content in umbilical cord arterial blood is controversial. While earlier studies indicated towards differential action of various vasopressors on uteroplacental circulation[39], studies by Ngan Kee *et al*[40] showed that depressed fetal acid base status was possibly due to ephedrine crossing the placenta and causing depression of fetal pH by its "metabolic effects secondary to stimulation of fetal β-adrenergic receptors". A recent study by Landau *et al*[41] has given a new direction to this debate. They showed that the neonatal homozygosity for Arg16 of *ADRB2* protected from neonatal acidemia in mothers treated with ephedrine[41]. The presence of this genotype in greater that 30% of the Chinese cohort and the fact that their genotype differs considerably from their North Americans indicate that clinicians should be wary of extrapolating studies of one ethnic population group on another[41].

Despite evidence in favor of phenylephrine as a superior choice, there remains widespread variation in the "choice, dosing, and method of administration of vasopressors"[25]. The United Kingdom National Institute for Health and Care Excellence Guidelines state that ephedrine and phenylephrine are equally efficacious as vasopressors in obstetric anesthesia[42]. The American Society of Anesthesiologists Task Force on Obstetric Anesthesia states that while ephedrine and phenylephrine are both acceptable, "phenylephrine may be preferable because of improved fetal acid-base status in uncomplicated pregnancies"[43]. There is much more clarity in the Canadian guidelines which state that there is "general agreement among experts to recommend the use of phenylephrine" as the first line therapy[8]. Belgian guidelines also recommend phenylephrine as the preferred vasopressor in absence of maternal bradycardia (Grade 1, A)[44].

While there is abundant literature evidence claiming superiority of phenylephrine over ephedrine in healthy parturients undergoing elective Cesarean section based on fetal acid-base status, there is dearth of evidence showing benefit in clinical outcome. Meta-analysis of 142 studies comparing phenylephrine and ephedrine failed to show the superiority of one over the other while comparing the Apgar scores[45]. However recent systematic review and meta-analysis do show that fetal acidosis defined as pH < 7.20 was associated with four- and two-fold increase in mortality and morbidity, respectively[46].

Due to a dearth of studies on the vasopressor of choice during non-elective Cesarean sections[47], it is suggested that further research is needed in high-risk pregnancies, intra uterine growth retardation, placental insufficiency, pre-eclampsia[25] and in emergency Caesareans due to fetal distress.

In one study in 2008 by Ngan Kee *et al*[48] in non-elective Cesarean sections, there was "no differences in fetal acid-base status or clinical neonatal outcome" between 100 μg phenylephrine and 10 mg ephedrine boluses to manage spinal anesthesia induced hypotension. Similarly, a retrospective study by Cooper *et al*[49] on the choice of vasopressor between phenylephrine and ephedrine in high-risk Cesarean sections, there was no statistically significant difference in the umbilical artery pH between the two groups.

There is also scarce literature available on the other vasopressors[50]. Kansal *et al*[16] concluded that mephentermine can be used as safely as ephedrine in the management of spinal anesthesia-induced hypotension in Cesarean sections. Similarly, Mohta *et al*[19] concluded that phenylephrine and mephentermine are equally effective in preventing hypotension after a spinal anesthesia for Cesarean section. Both the studies compared the Apgar scores and the neonatal acid-base status while evaluating the vasopressors[16,19]. In 2014, studies by de Aragãoa *et al*[50] compared an infusion of metaraminol with phenylephrine and ephedrine and did not find any difference in the incidence of maternal hypotension or

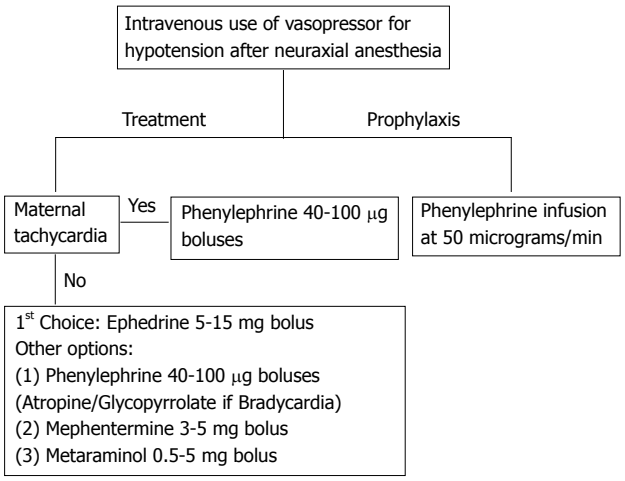

Figure 1 Suggested clinical protocol for intravenous use of vasopressor for Hypotension after Neuraxial Anaesthesia in Obstetrics.

neonatal Apgar scores. Comparison of phenylephrine, metaraminol and ephedrine showed that Ephedrine treated mothers had lower pH and base excess in their newborns[50]. However those treated with metaraminol needed fewer rescue boluses as compared to ephedrine, but not phenylephrine[50].

Combination of phenylephrine and ephedrine infusion has also demonstrated deterioration in fetal acid-base status and maternal hemodynamic control with the proportionate increase in the dose of ephedrine[51-53].

CONCLUSION

Current literature supports the use of phenylephrine as the vasopressors of choice while considering the influence on feto-maternal physiology[25,47]. However, this concept is mostly based on studies conducted in elective Cesarean sections. Therefore, this same principle cannot be extrapolated in emergency Cesarean sections and high-risk pregnancies.

Due to its potential for possible adverse impact on placental perfusion[25] when it causes bradycardia and decreased cardiac output, further studies on phenylephrine are needed, especially in presence of pre-existing fetal compromise.

Certain clinical protocols support the use of phenylephrine in the presence of maternal tachycardia (heart rate > 110/min) and ephedrine at lower heart rates (< 80/min)[54]. A suggested clinical protocol for intravenous use of vasopressor for hypotension after neuraxial anaesthesia in obstetrics is given in Figure 1.

Today, in obstetric anesthesia, both phenylephrine and ephedrine continue to be used in the "absence of relevant evidence, rather than any evidence of the absence of an effect"[55]. The same appears to be true for mephentermine and metaraminol also. Larger trials, especially in non-elective Cesarean sections, would be needed to give further direction to the obstetric anesthesiologists in choosing their preferred vasopressor.

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ORIGINAL ARTICLE

Retrospective Study

Posterior partially edentulous jaws, planning a rehabilitation with dental implants

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Author contributions: Monteiro DR and Silva EVF performed the computer search and wrote the paper; Pellizzer EP, Magro Filho O and Goiato MC designed the research, revised the paper and gave the final approval of the paper; all authors contributed equally to the work.
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Data sharing: Technical appendix, statistical code, and dataset available from the corresponding author at goiato@foa.unesp.br. Participants gave informed consent for data sharing. No additional data are available.

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Abstract

AIM: To discuss important characteristics of the use of dental implants in posterior quadrants and the rehabilitation planning.

METHODS: An electronic search of English articles was conducted on MEDLINE (PubMed) from 1990 up to the period of March 2014. The key terms were dental implants and posterior jaws, dental implants/treatment planning and posterior maxilla, and dental implants/treatment planning and posterior mandible. No exclusion criteria were used for the initial search. Clinical trials, randomized and non randomized studies, classical and comparative studies, multicenter studies, *in vitro* and *in vivo* studies, case reports, longitudinal studies and reviews of the literature were included in this review.

RESULTS: One hundred and fifty-two articles met the inclusion criteria of treatment planning of dental implants in posterior jaw and were read in their entirety. The selected articles were categorized with respect to their context on space for restoration, anatomic considerations (bone quantity and density), radiographic techniques, implant selection (number, position, diameter and surface), tilted and pterygoid implants, short implants, occlusal considerations, and success rates of implants placed in the posterior region. The results derived from the review process were described under several different topic headings to give readers a clear overview of the literature. In general, it was observed that the use of dental implants in posterior region requires a careful treatment plan. It is important that the practitioner has knowledge about the theme to evaluate the treatment parameters.

CONCLUSION: The use of implants to restore the posterior arch presents many challenges and requires a detailed treatment planning.

Key words: Dental implants; Mandible; Maxilla; Edentulous jaw; Treatment

Core tip: The treatment plan for rehabilitation with dental implants in posterior quadrants of edentulous jaws must be meticulous. The professional must cautiously evaluate the treatment parameters to guarantee predictable and long-term restorations. The treatment plan includes detailed analysis of space for restoration, bone quantity and density, radiographic techniques, selection of number, diameter, and length of the implants, and occlusion.

Monteiro DR, Silva EVF, Pellizzer EP, Magro Filho O, Goiato MC. Posterior partially edentulous jaws, planning a rehabilitation with dental implants. *World J Clin Cases* 2015; 3(1): 65-76 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i1/65.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i1.65

INTRODUCTION

Implant-borne rehabilitation is a good option of treatment for patients with partial edentulism[1-3]. The validity of osseointegrated dental implants for the rehabilitation of posterior partially edentulous jaw had been related in the literature by several studies[4-7]. These rehabilitations offers substantial benefits when compared with removable partial dentures: improved occlusion and support, simplification of the prosthesis, less invasive restorative procedures, bone maintenance and, improvement in oral health[8,9].

However, to obtain excellent results in rehabilitations with dental implants meticulous attention must be paid to details[10]. In addition, the posterior quadrants of the mouth are challenging for rehabilitation with dental implants[6,11,12] due to their anatomical and occlusal features[6,9]. Thus, this article aimed to discuss important characteristics of the use of dental implants in posterior quadrants and the rehabilitation planning.

MATERIALS AND METHODS

An electronic search of English articles was conducted on MEDLINE (PubMed) from 1990 up to the period of March 2014. The Key terms were dental implants and posterior jaws, dental implants/treatment planning and posterior maxilla, and dental implants/treatment planning and posterior mandible. No exclusion criteria were used for the initial search. Titles and abstracts of the screened articles were reviewed and the full text was assessed for an appropriate analysis. Then, the articles were analyzed through inclusion and exclusion criteria. Clinical trials, randomized and non randomized studies, classical and comparative studies, multicenter studies, *in vitro* and *in vivo* studies, case reports, longitudinal studies and reviews of the literature were included in this review. Additionally, current and previous issues of the most relevant papers were inspected, intending to obtain other articles associated to the theme. Articles that were not related to the purpose of this study were excluded from further evaluation. Finally, one textbook was included for the review.

RESULTS

One hundred and fifty-two articles met the inclusion criteria of treatment planning of dental implants in posterior jaw and were read in their entirety. The selected articles were categorized with respect to their context on space for restoration, anatomic considerations, radiographic techniques, implant selection, tilted and pterygoid implants, short implants, occlusal considerations, and success rates. The results derived from the review process were described under several different topic headings to give readers a clear overview of the literature.

DISCUSSION

Space for restoration

The discussion about the space requirements for placing an implant is very important. The mesiodistal space required is related to the type and number of teeth that will be replaced[8]. According to Misch[13], the selection of implant size is influenced by the mesiodistal distance available for implant placement. These authors indicated a guideline for this selection: (1) a distance of at least 1.5 mm must be respected between the implant and the adjacent teeth; (2) a distance of at least 3.0 mm between the implant and an adjacent implant; and (3) for the replacement of a molar teeth, a implant with a wider diameter is indicated.

If the implant-supported proshtesis is positioned with a large distance from the adjacent tooth, critical contours and cantilever forces are generated on the implant. Since the mesiodistal dimension of molar teeth is greater when compared to other teeth, a distance of at least 2.5 mm between the implant and the adjacent implant has to be respected to assure a restoration proper contours[8].

According to Gastaldo *et al*[14], a distance of 3 mm between the bone crest adjacent implants and the proximal contact point is essential, and the implant should be placed 3-5 mm away from the tooth in order to guarantee a healthy interproximal papilla.

Simşek *et al*[15] evaluated, through finite element analysis (FEA), different distances between implants that retained three unit partial prosthesis and their effects on bone stress distribution in the posterior lower jaw. Axial, horizontal and oblique forces were applied and tensile and compressive bone stresses were evaluated. The authors observed that a space of 1.0 cm was the greatest distance between the inserted two implants.

Both the mesiodistal and the buccolingual dimensions from the crestal level to the apical part of the implant site should be evaluated[16]. At least, a 6 mm of bone buccolingual extension is necessary to insert a 4 mm-wide dental implant. For diameters higher than 5 mm, a 7 mm extension is required[8]. Additionally, the intermaxillary

space is an important source. A distance of 10 mm between the residual ridge and the antagonistic arch must be respected when substituting posterior teeth[8,17].

A multidisciplinary approach is considered when planning a dental implant treatment and involves orthodontics, surgery, and restorative, so that the function and aesthetics of those patients are improved[10,16,18-20]. Generally, over-eruption of opposite teeth occurs after a long period of tooth absence, which affects the restorative space. Therefore several treatment options to creat a sufficient space for restoration are available such as enameloplasty, minimal restorative therapy, orthodontic intrusion, tooth realignment, endodontic treatment and full crown preparation, segmental osteotomy for dentoalveolar extrusion and extraction[8,10,16,18,19].

Anatomic considerations: Bone quantity and density

The low-density and quantity of bone and the presence of sinus pneumatization in maxilla are relevant anatomic characteristics in the posterior region, since they can limit the implant height[21-31]. On the other hand, the mandibular canal is an important structure that could limit the installation of dental implants in lower jaws[21,32,33]. According to Jivraj *et al*[8] and Vazquez *et al*[34], a distance of at least 2 mm between the most apical part of the implant and vascular and neurologic structures must be respect.

Additionally, the mental foramen is an important mandibular structure when placing implants in the foraminal region. The mental foramen is either oval or round and is usually placed in the apical area of the second mandibular premolar or between apices of the premolars[35,36]. Nevertheless, its location may vary from the mandibular canine to the first molar[35,37].

Guidelines to evaluate the mental foramen position and the presence of mental nerve deviations have been proposed aiming to preserve the nerve, during surgeries in the foraminal area. Previously to implant insertion, a careful observation of mental nerve and foramen, through panoramic and periapical X-rays, is essential. In case of deficiency of this technique to observe the position of the nerve, the computadorized tomography scans are necessary. After the confirmation of the secure bone height, the professional can install the implants mesially or distally placed from the mental foramen or above it[34,35,37].

The lingual mandibular bone concavity is also another important factor since it increases the risks of fenestrations or perforations during implant insertion, in case of deficient buccal-lingual angulation[20,21,38].

Nevertheless, the bone density on the implant placement region affects the primary stability and in turns determines the implant treatment success[11,39,40]. Fuh *et al*[41] determined the density of trabecular bone at potential areas for implant placement. Chinese jawbones were evaluated through computed tomography (CT) in four different regions: anterior and posterior areas of maxilla and mandible. The bone densities differed between each region, being lower in the posterior area - maxilla (332 + 136 HU) and mandible (359 + 150 HU) - and higher in the anterior area -maxilla (516 + 132 HU) and mandible (530 + 161 HU). These results were similar to those of Sogo *et al*[42], who found that the bone in the posterior maxilla was classified as type Ⅲ (350-850 HU) and type Ⅳ bone (150-350 HU). These findings illustrated the necessity of choosing a specific implant design and surface treatment for the different bone density types owing to improvement of the bone-implant contact area[21]. Furthermore, cutting torque[40,43] and the resonance frequency[40,44] can be used to determine the bone quality and implant stability, respectively, and have a major effect on the osseointegration success.

Sakka *et al*[40], in a literature review, affirmed that to classify the bone quality it is important to evaluate bone morphology and characteristics of the constitutive cells. The cortical and trabecular bone ratio, and bone quantity and density have a great effect on the implant treatment longevity. Cells associated to bone quality, as macrophages, monocytes, fibroblasts, mesenchymal progenitors, osteoclasts, and cells related with angiogenesis, could influence the osseointegration of dental implants.

The implant placement is influenced by the form and contour of the edentulous alveolar ridge[21]. Infections, trauma during dental extraction, remodeling of alveolar bone after tooth extraction create localized defects on the bone[21,25,36,37,45], affecting its height and width, and consequently, influence the dental implant placement[21,28]. Some methods have been used to overcome these complications as guided bone regeneration with resorbable and nonresorbable barriers to enhance localized ridge deformities, the utilization of short-length implants, inclined implants, zygomatic or pterygoid implants, bone grafting surgeries and sinus lifting operations[21,46-52].

Del Fabbro *et al*[46] performed a systematic review of 39 selected studies in which 2046 patients underwent sinus grafting and received 6913 implants. After an accompaniment of 12 up to 75 mo, the reported survival rate was 92.5% (range, 61.2% to 100%). Results were also divided according to type of grafting materials. Overall, the survival rate of implants was 87.7% with autogenous bone, 94.9% when autogenous bone was mixed with other grafting materials, and 95.9% with nonautogenous grafting materials. Results were also reported according to type of implant surface. Overall, the survival rate was 85.6% for implants with smooth/machined surfaces, and 95.9% for implants with rough surfaces.

Radiographic techniques

Prior to implant insertion, intraoral and panoramic radiographies should be considered. But, since those techniques just provide information in a 2-dimensional view, the bucco-lingual bone width is missed[25,34,38,45,53-57].

The localization of the mandibular canal, the submandibular fossa, and the maxillary sinuses, in addition to the angulation of the alveolar crest and the bone volume are of primary importance during implant

treatment planning in the posterior jaw area[22,31,32,34,36,57-60]. Therefore, the use of CT in all sliced images is suggested to indicate the most convenient dimensions of the implant and its optimal position and inclination[25,38,42,45,54-57,61]. Spiral/helical CT scanners provide images with higher quality, with tridimensional view, associated with lower radiation exposure, than conventional computerized tomography[54,62]. Nevertheless, the CT scan is kind of expensive and requires large equipment. The radiation dose is relatively high[63].

In general, the conventional CT liberates a higher dose of radiation than another option of image scan, the cone-beam computed tomography (CBCT), which offers realistically tridimensional sliced images[31,54,57,58]. Therefore, this method is useful during implant treatment planning for partial edentulous patient[57,58,64,65].

Implant selection: Number, position, diameter, and surface

The selection of the ideal number of implants is related to the bone volume and density. Since the posterior region of upper jaw presents a soft bone tissue, it is recommended to insert 3 implants to replace 3 missing teeth[8,65]. In case of one implant failure, the previous prosthesis may still be used. And when the anterior or posterior implant fails, a cantilevered prosthesis could be fabricated[8].

The use of either two or three implants relies on the prosthesis biomechanical function and is influenced by load application during chewing[8]. In cases when it is possible to install three implants, a different configuration with a tripod effect of their distribution can be realized[8,66], which provides greater bone support versus linear placement[66]. Additionally, when possible, multiple implants in posterior quadrants should be splinted. Guichet *et al*[67] observed that splinted implant restorations exhibited optimal stress distribution than non-splinted prosthesis. However, Clelland *et al*[68] and Vigolo *et al*[69] observed that splinted prosthesis did not differ significantly from individual restorations, regarding strain distribution data and peri-implant marginal bone loss, respectively.

Regarding the implant diameter, implants with small (from 3.0 up to 3.5 mm) or regular (from 3.75 up to 4.5 mm) diameters should be used for pre-molar teeth and are not indicated in molar region due to the high occlusion force transmission[21,70]. Prosthesis that does not respect the long axis of the implant tends to develop inappropriate biomechanical forces on the restoration/implant assembly[71,72]. In this case, screw loosening and implant or abutment fatigue may occur[71,73]. Moreover, the cantilever force may induce peri-implant stress and bone resorption[74,75].

Increased mechanical stability and bone-implant contact are achieved using implants with a large diameter (from 5.0 up to 6.0 mm)[21,76-78]. In addition, their use provide an effective counter acting occlusal force of the magnitude that may be observed in molar areas[21,79-81]. Finally, the wide-diameter implants mimic the emergence profile of the molar tooth[8,81].

Nonetheless, due to the presence of a soft bone tissue at posterior jaw, two implants can be indicated in the first molar area[82,83]. Two implants placed very close simulate an anatomical condition of the roots, which increase in two folds the anchorage surface area. Additionally, it eliminates antero-posterior cantilevers, decreases rotational forces and screw loosening. Nevertheless, the routine oral hygiene may be more difficult and insufficient mesiodistal space limits the placement of two implants[8,21].

According to Carvalho *et al*[21], different factors can influence when making a decision between one implant with a large diameter (5 mm) or two implants with a small or regular diameter. These factors are: bone volume and density, bone height between the residual rigde and important structures such as sinus and neurovascular canals and, the availableness of bone in a mesiodistal direction.

In relation to the surface of the implant, the use of rough surface implants has outnumbered machined implants[84-88], and it is supported by evidence of earlier and greater implant stability[84-87,89]. It is also argued that this fact prevents the necessity of a second surgical stage, and even encourages earlier or immediate loading in specific cases[80,90]. But, longitudinal studies comparing the two different surfaces using identical protocols in matched population groups and surgical sites have not been accessed. Therefore, the remaining question rises if the assumed improved longitudinal clinical findings are really the result of better science or the product promotion[89].

Tilted and pterygoid implants

The insertion of tilted implants may be an important alternative to bone grafting, guided bone regeneration, nerve lateralization, short implants, or height deficient atrophic posterior jaw[23,33,50,56,59,75,91-93]. Additionally, it allows for bicortical stabilization of the implants which reduces implant micromotion during osseointegration and enhances the implant success rate[93].

Krekmanov *et al*[94] and Aparicio *et al*[95] evaluated alternatives for implant insertion in severely atrophic maxillas. The authors suggested that a mesiodistal inclination of the implant, associated or not with a bucco-palatal direction, respects the maxillary sinus and are a treatment option for reabsorbed posterior upper jaws. More recently, in a report[93] comprising 196 tilted implants in 64 atrophic posterior mandible edentulous, an absence of osseointegration resulted in failure of only two implants, and the neurovascular structures were intact.

The pterygoid implant was first introduced to be placed in the bone pillar, that is formed by the three structures: pyramidal process of the palatine bone, pterygoid process of the sphenoid bone and maxillary tuberosity[96]. While the first two are formed by dense

cortical bone, the maxillary tuberosity is based on poorer bone quality[22,24,51,96-98]. The surgeon should be aware that the maxillary artery and its branches passess through the posterior and medial regions of the maxillary tuberosity[99]. In case of full-arch implant supported restorations, the use of pterygomaxillary implants gives support and retention for the restorations and eliminate the cantilever's length that may be necessary when just anterior implants are placed[47,51,98,100].

Bahat[101] reported that 7% of the 72 implants inserted with a modified technique in the tuberosity area failed after a follow-up period of 21.4 mo, while Ridell *et al*[99] did not observe failures of any of the 22 implants placed in the same area after an accompaniment of 8 years. Peñarrocha *et al*[47] evaluated 68 pterygoid implants over 1 year of loading and found a success rate of 97.05% and a peri-implant bone loss of 0.71 mm. After that period, the patients were satisfied with the functional and esthetical aspects of the oral rehabilitation.

On the other hand, Balshi *et al*[98] found a cumulative survival rate of 90.8% of 1.608 implants placed into the pterygomaxillary region. These authors compared two-stage freehand, single-stage freehand and single-stage guided protocols. They observed that single-stage protocol exhibited higher cumulative survival rate (96.45%) than two-stage protocol (85.94%) and guided surgery (93.38%). Therefore, immediate loading of those implants is beneficial to treatment.

When implants are inserted into the tuber area, normally it is necessary to tilt the implant, which is unfavorable to the biomechanical point of view, increasing the peri-implant bone resorption and reducing implant success rates. On the other hand, previous studies showed appropriated results with tilted implants *vs* straight ones[33,59,92,95]. Maybe it occurs because the tilted implants can be longer than axial ones[99].

The use of splinted implants has been indicated to reduce the stress on tilted implants[93]. This recommendation has been originated from studies that demonstrated that splinted implants showed better stress distribution when compared to non-splinted prosthesis[67]. On the other hand, Lan *et al*[12] observed, through finite element study, that tilted implants with splinted crowns exhibited greater stress concentration, specially in implants with distal tilting. Nevertheless, additional follow-up and long-term evaluations are warranted.

Short implants

Some authors[91,102-105] have defined short implants as implants no longer than 7 mm. Others[29,106-109] have considered short implants to be implants up to 10 mm long.

The length of implants is limited to the presence of some anatomical structures as the intra-alveolar canal and the maxillary sinus, and bone resorption. In these cases, the use of short implants has been recommended[3,23,29,72,97,104,105,109-112]. From a biomechanical point of view, when an implant is loaded, the peri-implant crestal bone receives the stress from the first few threads of the implant; therefore, once a minimum implant height is osseointegrated, implant diameter is more relevant when compared to an increase in length[23,28,86,108,113-116].

To Grant *et al*[117], short implants are convenient due to: (1) usually, this technique does not require a bone grafting procedure, which results in a faster and less expensive treatment and improves the patient's confort; (2) risks during the surgery, such as nerve damage, osteotomy heat and lesions on the adjacent tooth, are reduced; and (3) there is a surgical ease, in cases of insufficient interarch spaces. However, several controversies still exist to their indication owing to: (1) reduced implant surface; thus leading to less bone-to-implant contact after osseointegration; (2) reduced surface of force distribution after loading; more pressure at the crestal bone; more resorption leading to more threads exposed, decreasing the surface of osseointegrated implant; and (3) compromised crown-to-implant ratio[118].

In case of increased crown-to-implant (C/I) ratio, the crown works as a lever arm, transferring the stress to the crestal bone around the implant[115,119], which can result in peri-implant bone loss[119,120] and problems with components of the prosthesis[66,121].

Blanes[121] found that, when the C/I ratio was higher than 2, the survival rate of the implant-retained prosthesis was 94.1%. Apparently, according to these authors, the C/I ratio did not influence the marginal bone loss. Also, Rokni *et al*[122] observed that the C/I ratio did not interfere on crestal bone loss around dental implants. Similarly, Urdaneta *et al*[73] identified the same results on single-tooth implants. However, these authors noted an increase in prosthetic complications, such as implant abutment and fracture.

Crown/Implant ratios ranging from 0.5 to 1 are important to avoid stress and bone loss at a crestal bone level, which could result in implant loss[116,123,124]. Nevertheless, Tawil *et al*[125] stated that high C/I ratios are not the most relevant agent that affect load distribution and Schneider *et al*[126] added that this increase may be used successfully in implants for single-tooth replacement in posterior jaws, except for smoking patients.

Short implants are feasible solutions in case of insufficient bone height and provide favorable force orientation and distribution[111,125]. In case of full-arch fixed dental prosthesis, short implants can be an alternative in posterior jaws to give support for the cantilever, reducing lever arms and stress loading on implants[72].

Although short implants exhibited greater failure rates that longer ones[127], some studies[3,113,128] demonstrated similar outcomes for both types of implants. Probably, these divergences resulted from other variables, such as implant surface, professional ability, bone characteristics, implant primary stability and prosthodontic protocol, which also affects the implant survival[86].

Atieh *et al*[112] performed a systematic review of 33 selected studies concerning 2573 short implants inserted in posterior upper and /or lower jaws to retain fixed partial

Table 1 Cumulative success rates of short implants placed in posterior region

Ref.	Implant surface	Implant length	N implants	Period of evaluation	Success rate (%)
Bahat[127]	Machined-surface	7 mm	-	5 to 70 mo	90.50
Winkler *et al*[130]	Machined-surface	< 10 mm	181	3 yr	93.40
Friberg *et al*[103]	Machined-surface	< 10 mm	247	8 yr	93.70
Deporter *et al*[84]	Porous-surface	7 or 9 mm	48	8.2 to 50.3 mo	100.00
Tawil *et al*[107]	Machined-surface	⩽ 10 mm	269	12 to 92 mo	95.50
Griffin *et al*[113]	Hydroxyapatite-coated	8 mm	168	Up to 68 mo	100.00
Renouard *et al*[151]	Machined or oxidized surface	6 to 8.5 mm	96	2 yr	94.60
Goené *et al*[110]	Acid-etched surface	7 or 8.5 mm	311	3 yr	95.80
Misch *et al*[85]	Roughened surface	7 or 9 mm	745	6 yr	98.90
Anitua *et al*[86]	Micro-rough acid-etched surface; bioactive surface	7 to 8.5 mm	532	5 yr	99.20
Grant *et al*[117]	-	8 mm	335	up to 2 yr	99.00
Anitua *et al*[114]	-	< 8.5 mm	1.287	1 to 8 yr	99.30
De Santis *et al*[97]	Oxidized surface	< 8.5 mm	107	1 to 3 yr	98.10
Maló *et al*[104]	Oxidized surface	7 mm	217	12 mo	95.00
Pieri *et al*[111]	-	6 mm	61	2 yr	96.80
Perelli *et al*[27]	Porous-surface	5 or 7 mm	110	5 yr	90.00
Jiansheng *et al*[109]	Hydroxyapatite-coated and ankylos	5.7 to 8 mm	162	2 yr	99.40
Slotte *et al*[29]	Acid-etched surface	4 mm	100	2 yr	92.30
Deporter *et al*[88]	Porous-surface	7 or 9 mm	48	10 yr	95.50

prosthesis. A survival rate of 98% was reported, after an accompaniment period of 5 years. When comparing short and long implants, no important differences were observed. The authors affirmed that short implants represents a viable treatment option than longer ones and that the survival rate is not related to implant surface, design or width.

Morand *et al*[118] reported that the one improvement that had the most dramatic effect in improving implant treatments was the evolution of implant surfaces from machined/polished to rough-textured surfaces. Table 1 confirms this information, evidencing higher success rates for rough surfaced implants. The percentage of bone-implant contact can be modified by the surface condition of the implant. This is important because the greater the percentage of bone contact, the lesser stress is applied to the bone-implant interface[86]. Therefore, it is possible to assure that with careful case selection criteria, the longevity of short implants is greater than 90%.

Nevertheless, besides the high success rates, the most important aspect of treatment with short implants is the case selection[23,118]. Facing severe bone resorption associated with poor bone quality and overload, bone grafting techniques could prevent failure in such associations. The success rate of short implants in patients with more favorable conditions is greater which makes it the best treatment option[129].

Occlusal considerations

The excess of loading in posterior jaws associated with the functional activity of the mandible in a buccal-lingual direction and with cusp inclination can create lateral forces onto implants[9,130-132]. Thus, during implant treatment planning, a broad evaluation of the loading is essential, since a bending moment at the peri-implant bone can result in prosthesis components damages and/or crestal bone loss[20,66,115,132,133].

Various factors can overload an implant. Rangert *et al*[134] identified two principal factors that justify this excess of loading: geometric and occlusal load reasons. The first one is related with the implant number and position, and with the prosthesis configuration. The second factor includes lateral occlusal force components and parafunctional habits, which increase the loading onto implant surfaces. If forces are higher than normal, the implant can be overloaded.

Ogawa *et al*[135] affirmed that a decrease in number of supporting implants is to promote an increase in implant loading. The bending moments were higher when prosthesis were supported by three implants than four or five implants. Additionally, concerning the implant position, the smallest implant distribution increased the bending moments.

The prevention of occlusal overload should be the focus of any treatment planning[66,136]. In case of no alternative, the prosthesis should be protected from injuries with an inter-occlusal device[67,93]. Some guidelines were reported aiming to respect physiologic limits for occlusal loading: optimized passive fit, reduction of cantilevers, adequate selection of the dimensions and number of implants, presence of a correct preload in the abutment screw and a proper buccal-lingual dimension and cusp inclination of the crown[66,132,133,137-139].

Furthermore, the principles of implant occlusion are mostly based on the traditional principles of conventional restoration. Anterior guidance should be presented and during lateral excursion, a posterior disclusion is indicated for working and non working sides. Group function disocclusion is indicated when the canine is compromised[8].

Payer *et al*[140] evaluated the outcome of edentulous posterior mandible treated with implant-retained immediate provisional prosthesis. According to these authors, immediately loaded implants exhibited similar results when

Table 2 Cumulative success rates of implants placed in posterior region

Ref.	N implants	Posterior zone	Period of evaluation	Implant systems	Success rate (%)
Jemt *et al*[4]	259	Maxilla and mandible	5 yr	Nobelpharma	97.20
Zarb *et al*[144]	105	Maxilla and mandible	2.6-7.4 yr	Nobelpharma	94.30
Block *et al*[142]	443	Mandible	10 yr		79.30
Becker *et al*[143]	282	Maxilla and mandible	6 yr	Branemark	89.40
Bahat[145]	660	Maxilla	10 yr	Branemark	93.40
Attard *et al*[5]	106	Maxilla and Mandible	10 yr	Nobel biocare	94.00
Attard *et al*[152]	432	Maxilla and Mandible	15 yr	Nobel biocare	91.60
Jebreen *et al*[7]	141	Maxilla and Mandible	12-69 mo	ITI	96.45
Blanes *et al*[6]	192	Maxilla and Mandible	10 yr	ITI	97.90
Huynh-Ba *et al*[153]	273	Maxilla	8 yr	-	94.90
Maló *et al*[70]	247	Maxilla and Mandible	11 yr	Nobel biocare	95.10
Schneider *et al*[126]	100	Maxilla and Mandible	5 yr	Nobel biocare and straumann standard	95.80

compared to conventionally loaded implants. During a follow-up period of 5 years, the survival rate was 95%.

Similarly, Degidi *et al*[136] performed a randomized clinical trial that aimed to evaluate the effect of immediately loaded and immediately restored implants for edentulous posterior lower jaws. The authors found that both procedures are predictable. No differences in marginal bone loss or survival rate were observed.

Nonetheless, concerning the conditions of early-loaded implants in the posterior upper and lower jaws, Kim *et al*[141] observed that, although early loading is a predictable procedure, it is important to be careful with maxillary implants.

Success rates

Table 2 illustrates the success rates of implants inserted in the posterior jaws of patients with partial edentulism. Favorable success rates were observed when edentulous areas were replaced with implants, except for the study of Block *et al*[142], which related lower success rates for implants inserted in posterior inferior jaws (78.5% for first molars and 71.8% for second molars). Some studies showed distinct success rates for those implants placed in the posterior regions of maxilla and mandible, with lower success rates for the posterior maxilla[4,6,7,143]. However, Zarb *et al*[144] obtained a success rate of 97.6% for the 41 implants placed in the upper jaw and, of 92.2% for the 64 implants placed in the lower jaw, after a loading period of 2.6 to 7.4 years.

The non-standardization between and within studies has increased the range in success rates, *e.g.*, 79.3% to 97.9%. The differences in study design may be the driven force toward those results. Factors such as length, number, diameter and surface of the implants, bicortical fixation, and extended healing periods contribute to a good long-term success rate[4,143,145]. When the implants are placed into soft bone tissues or inserted in regions with insufficient bone height that demands grafting procedures such as sinus lifting, lack of osseointegration[11,25,146] and failure after loading[147] are prone to occur. The same problem occurs in case of smoking patients[11,148]. Additionally, the lack of oral hygiene may be another initial factor of implant loss[133,145,149], while bicortical fixation may improve osseointegration and reduce bone resorption[116,145,150].

COMMENTS

Background

The osseointegrated implants allow a functional rehabilitation for patients with partial edentulism, since they improve the occlusion and retention of the prosthesis and the bone maintenance. However, the posterior region of the maxilla and mandible requires special attention due to their anatomical and occlusal characteristics.

Research frontiers

Implant-retained prosthesis is a common procedure for posterior partially edentulous jaw rehabilitations. The knowledge regarding this topic involves maxillofacial anatomy, physiology and radiology, oral implantology, occlusion and prosthodontics, and is directly related with patient's psychological aspects.

Innovations and breakthroughs

This review of the literature presents an accurate description of the main articles that evaluated a rehabilitation with dental implants in the posterior maxilla or mandible. Different topics, such as space for restoration, anatomic considerations, radiographic techniques, selection of number, diameter, position and length of implants, occlusal considerations and success rates, were carefully discussed in this article.

Applications

The study findings suggest that professionals need to minutely evaluate the treatment parameters to guarantee the longevity and success of the rehabilitation.

Terminology

Crown-to-implant (C/I) ratio is a guideline related with the longevity and survival of the prosthesis, since a higher C/I ratio represents a lever arm of the crown over the peri-implant bone area, which can result in bone loss.

Peer review

The work is interesting,and useful to the clinicians.

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CASE REPORT

Giant xanthogranuloma of the pelvis with S1 origin: Complete removal with only posterior approach, technical note

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Abstract

Xanthogranulomas (XG) are benign proliferative disorder of histiocytes, a non-Langerhans cell histiocytosis. Whose etiology is unknown. The nature of these lesions is controversial and could be either reactive or neoplastic; the presence of monoclonal cells does, however, favor the second hypothesis. Xanthogranuloma is frequently found in young adults and children (under 20 years old), mainly in the skin. In about 5%-10% of all Juvenile XG (JXG) cases xanthogranuloma are extracutaneous. Within this group, the site most frequently involved is the eye. Other involved organs are heart, liver, adrenals, oropharynx, lung, spleen, central nervous system and subcutaneous tissue, although involvement of the spine is uncommon. Isolated lesions involving the sacral region are extremely rare. To date, this is the first reported case of a giant JXG arising from S1 with extension into the pelvic region in an adult spine.

Key words: Xanthogranulomas; Non-Langerhans cell histiocytosis; Touton giant cells; Congenital xanthoma; Neurofibromatosis

Core tip: Xanthogranulomas (XG) are benign lesions derived by histiocytes, also called histiocytosis with non-Langerhans cell. Isolated xanthogranuloma involving the sacral region are unique. We report the unique giant Juvenile XG arising from S1 with extension into the pelvic region in an adult spine. Complete surgical removal is the goal of the treatment and usually curative even if there is not a study in the literature with a long follow up able to confirm this. If total resection is not possible, the patients must be followed by strictly clinical examination or should undergo adjuvant radiotherapy.

Marotta N, Landi A, Mancarella C, Rocco P, Pietrantonio A, Galati G, Bolognese A, Delfini R. Giant xanthogranuloma of the pelvis with S1 origin: Complete removal with only posterior approach, technical note. *World J Clin Cases* 2015; 3(1): 77-80 Available

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INTRODUCTION

Xanthogranulomas (XG) are benign proliferative disorder of histiocytes, a non-Langerhans cell histiocytosis[1]. Whose etiology is unknown. The nature of these lesions is controversial and could be either reactive or neoplastic; the presence of monoclonal cells does, however, favor the second hypothesis[2]. Xanthogranuloma is frequently found in the skin of young population, especially under 20 years old. In about 5%-10% of all Juvenile XG (JXG) cases xanthogranuloma is extracutaneous. Within this group, the eye appears to be the site most frequently involved. Other involved organs include the oropharynx, heart, liver, lung, spleen, adrenals, muscles , central nervous system and the subcutaneous tissues, although involvement of the spine is uncommon[1]. Isolated xanthogranuloma of the sacral region are extremely rare[1,3]. To date, this is the first case of a giant JXG arising from S1 with extension into the pelvic region in an adult spine.

CASE REPORT

Anamnestic data and neurologic examination

A 44-year-old man was referred to our hospital with a 9 mo history of pollakiuria, stypsis, and sciatica along the S2 left dermatome, the symptoms appeared slowly worsening. The patient underwent lumbosacral magnetic resonance imaging (MRI) with gadolinium that demonstrated the presence of a presacral, giant, well-circumscribed round formation. The lesion compressed the left S4, S3 and S2 roots and dislocated the urinary bladder, rectum, and iliac artery, and it extended ventrally from the left articular mass of S1 to the pelvic region. The lesion was hypointense on T1 weighted images and isointense on T2 ones with dishomogeneous contrast enhancement after gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) (Figure 1). The computed tomography (CT) bone scanning showed the erosion of S3-S4 foramen and bone involvement of left sacral wing. On physical examination, the patient had a positive right Lasegue sign. Rectal inspection revealed the presence of a posterior hard-elastic lesion.

Treatment

The patient underwent CT-guided biopsy at the division of general surgery of the Policlinico Umberto I. A juvenile xantogranuloma was diagnosed at the histological examination. The patient was then referred to our attention and underwent surgery performing a posterior approach: the patient was placed in prone position; Intraoperative neurophysiological monitoring has been utilized. A standard electromyography monitoring of L4, L5 and S1 was conducted bilaterally. Moreover the insertion of electrodes in the urethra and anal sphincter during the surgical procedure helps to discriminate between nerve root and tumor. A U shaped skin incision was performed, followed by a complete exposure of the spinal column and partially of the sacro-iliac region with prevalent lateral extension on the left side. A complete laminectomy and partial left sacrectomy (25% of left sacrum removal) was performed. Because of the benign nature of the lesion and the space formed by tumor growth, we performed a less invasive procedure with intralesional posterior tumor removal, obtaining enough space without total sacrectomy that could be destabilizing for spine. We used the posterior approach to easily reach the lesion right after the opening of the subcutaneous layer and because it was easier to reach the origin point of the lesion and to control the S2, S3 and S4 root. Intraoperatively, the lesion appeared xanthochromic, friable, moderately bloody and presented a discontinuous capsule. The consistency was really flabby so that the cranial part of the tumor was easily mobilization and the removal was safe for structures as like as rectum and sacral plexus. The lesion was very adherent to the surrounding tissues. The lesion was macroscopically total removed. Immediately after surgery, the patient had relief of pain and had a progressive resolution of sphincter disturbances, with complete recovery after 15 d from surgery. A complete removal of the tumor was shown in the postoperative spine MRI (Figure 2). The patient was discharged on the tenth postoperative day. Clinical follow-up at 1 year showed the stability of symptoms. Radiological follow up using MRI (3T, T1, T2, STIR, T1 sequences with contrast) at 3, 6 and 12 mo showed no signs of recurrent disease.

Pathology

Gross examination showed a 13 cm × 10 cm × 13 cm round lesion, discontinuously encapsulated and adherent to surrounding tissues. The cut surface was homogeneous, yellow and of gelatinous consistency. Microscopic examination revealed the presence of typical touton giant cells. In general, a finding of such cells is sufficient for a diagnosis of xanthogranuloma. Immunohistochemical studies, in one of Dehner's series, showed an immunopositive response for CD68, factor XIIIa and Vimentin of the tumor cells, and are not reactive for S100 and CD1a (Figure 3)[4].

DISCUSSION

Proliferative disorders of histiocytes were described in 1905 by Adamson[5]. Those diseases comprehend a group of pathologies in which the main hstological patterns are dendritic cells and macrophages. The xanthogranuloma has called a non-Langerhans cell histiocytosis as soon as other entities as: Rosai-Dorfman disease (if associated with lymphadenopathy), benign cephalic histiocytosis, the papular xanthoma, and hemophagocytic histiocytosis. Xanthogranuloma is usually benign and is also known as juvenile because it occurs predominantly in young people under the age of twenty years[1]. Etiology of JXG

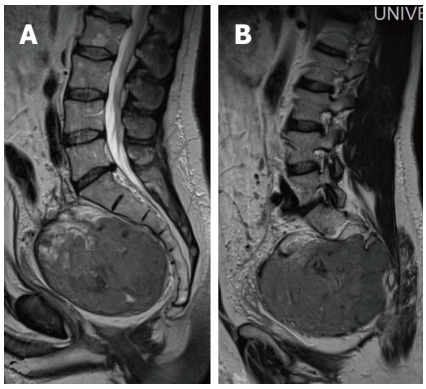

Figure 1 lumbosacral magnetic resonance imaging showing a giant, well-circumscribed round formation hypointense on T1 weighted images and isointense on T2 ones with dishomogeneous contrast enhancement after Gd-DTPA. A: A sagittal T2-wieghted midline image; B: A sagittal T2-weighted lateral image. Gd-DTPA: Gadolinium diethylenetriaminepentaacetic acid.

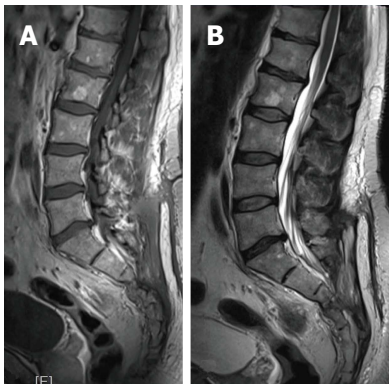

Figure 2 Postoperative spine magnetic resonance imaging showing complete removal of the tumor. A: A sagittal T1-wheighted image after Gd–DTPA; B: A sagittal T2-weighted midline image showing macroscopical total tumor removal. Gd-DTPA: Gadolinium diethylenetriaminepentaacetic acid.

is unknown, but probably is the result of alteration in macrophagic response to an aspecific injury, resulting in a granuloma.

On the other hand, the recent discovery of the monoclonal nature of the cell population of these lesions, has suggested a possible neoplastic origin[2]. The skin of neck and head are the most frequent location of the JXG 67%[3]. It can be multiple or solitary with red-to-yellowish nodules and papules. The involvement of extracutaneous tisuues occurs in about 5%-10% and mainly involves eyes (uvea). Involvement has been reported in other organs including the lung, heart, liver, oropharynx, central nervous system, spleen, adrenals, muscles and subcutaneous tissues, although spinal involvement is extremely rare[3]. The lesions are self-limiting regressing over several years and predominantly occur in childhood. In these cases, conservative treatment and clinical observation is therefore indicated. Adults do not usually experience spontaneous resolution[3] and injuries may present invasive characteristics, meaning that surgical procedures become the treatment of choice. The gold standard radiological examination is MRI, useful

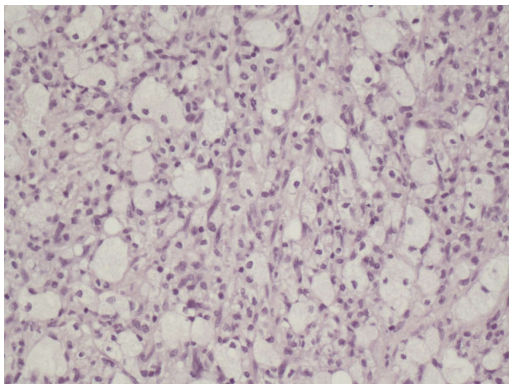

Figure 3 Histological picture: Microscopic examination revealed the presence of typical touton giant cells.

for defining the localization and relation to the other structures. Today a standard treatment for extracutaneous JXG is not defined. However, literature indicates that total removal of the tumor is the treatment of choice and it is important the excision of the origin of the tumor. According to some authors, *en-bloc* excision of the lesion to healthy margins could be a curative treatment option. If total resection is not possible, the patients should be followed under medical observation or be submitted to adjuvant radiotherapy[3]: if the symptoms recur, the patients should be re-operated[1]. On the other hand, radiation treatment, besides not being free from complications, poses the lesion at risk of malignant transformation. To date, this is the first case of giant JXG arising S1 with extension into the pelvic region in an adult spine. All spinal surgeons know and understand the risk of vascular injury because iliac arteries, aorta, and other vascular structures are strictly related to the anterior lumbar spine. We used the posterior approach to reduce the risk of vascular injury, to easily reach the lesion right after the opening of the subcutaneous layer and the origin point of the lesion and to control the S2, S3 and S4 root. Although the depth of the cable and the relationships with the adjacent vascular structures made it particularly difficult to remove the lesion, total removal was achieved by a posterior approach. The placing of neurophysiological electrodes monitor during the surgical procedure in the urethra sphincter and anal sphincter allows surgeons to distinguish between nerve roots and tumor, and to prevent urinary dysfunctions.

The follow-up at one year showed an improvement in reported preoperatively symptoms and confirmed radical removal, without any signs of disease recurrence.

A standard treatment for extracutaneous JXG is not still accepted. However, complete surgical removal is the goal of treatment although the size and the aggressive behavior of the tumor may condition the procedure. Complete surgical removal is usually curative even if there is not a study in the literature with a long follow up able to confirm this. If total resection is not possible, the patients have to be followed under close medical observation or should undergo adjuvant radiotherapy. Important is the

use of neurophysiological monitoring especially of the pelvic region to prevent any neurological dysfunction.

COMMENTS

Case characteristics

Pollakiuria, stypsis, and sciatica along the S2 left dermatome.

Clinical diagnosis

On physical examination, the patient had a positive right Lasegue sign. Rectal inspection revealed a posterior hard-elastic lesion.

Imaging diagnosis

Lumbosacral magnetic resonance imaging with gadolinium demonstrated the presence of a presacral, giant, well-circumscribed round formation. A computed tomography bone scanning showed erosion of the left sacral wing at S3-S4 foramen.

Pathological diagnosis

Microscopic examination revealed the presence of typical touton giant cells. In general, a finding of such cells is sufficient for a diagnosis of xanthogranuloma.

Treatment

Surgical treatment.

Related reports

The recent discovery of the monoclonal nature of the cell population of these lesions, has suggested a possible neoplastic origin. The lesions are self-limiting and predominantly occur in infancy and childhood, and typically regress over several years. In these cases, conservative treatment and clinical observation is therefore indicated. Adults do not usually experience spontaneous resolution and injuries may present invasive characteristics, meaning that surgical procedures become the treatment of choice.

Term explanation

Xanthogranuloma, a proliferative histiocytic disorders.

Experiences and lessons

Complete surgical removal is the gold standard for the treatment of this pathology.

Peer review

The paper describes a rare (probably unique) case of symptomatic giant xanthogranulomas of the pelvis in a 40 years old male. Authors explain clearly why they decided to manage surgically the lesion. Excision was successfully performed trough a posterior route to avoid damages to the surrounding neurovascular structures. Symptoms relief was immediate and preserved one year after surgery. The paper is really interesting.

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CASE REPORT

Conservative management of cervical pregnancy with intramuscular administration of methotrexate and KCl injection: Case report and review of the literature

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Abstract

We report the case of a cervical pregnancy successfully treated with intramuscular injection of methotrexate (MTX) and intramniotic administration of potassium chloride. A 41-year-old woman was admitted to our Department with the suspicion of ectopic pregnancy. Transvaginal ultrasound revealed empty endometrial cavity, gestational sac within the cervical canal and embryonic echo measuring crown rump length 1.5 mm. Serum beta human chorionic gonadotropine (β-HCG) was measured 28590 IU/L. No cardiac activity was detected. The diagnosis of a cervical pregnancy was made. Patient was treated with intramuscular administration of methotrexate (50 mg/m^2) in combination with ultrasound-guided intramniotic injection of KCl (2 meq/mL). Gradual decrease of β-HCG levels as well as ultrasound observation of collapsed gestational sac was observed. No curettage was necessitated. Patient was discharged on day 10th and was set in follow-up on a weekly basis. β-HCG values were measured < 10 IU/L on 56th day after MTX administration. Intramuscular administration of MTX may be effective in treatment of cervical pregnancy without additional interventional measures.

Key words: Cervical pregnancy; Methotrexate; Effectiveness; Conservative treatment; Intramuscular

Core tip: This case of cervical pregnancy is one amongst few treated successfully with intramuscular administration of methotrexate and intramniotic KCl, without demanding additional interventional treatment. Our paper also summarizes the basic conclusions about conservative treatment of cervical pregnancy, a challenging issue in which no consensus still exists.

Petousis S, Margioula-Siarkou C, Kalogiannidis I, Karavas G, Palapelas V, Prapas N, Rousso D. Conservative management of cervical pregnancy with intramuscular administration of methotrexate and KCl injection: Case report and review of the literature. *World J Clin Cases* 2015; 3(1): 81-84 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i1/81.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i1.81

INTRODUCTION

Cervical pregnancy represents < 1% of ectopic gestations with its estimated frequency ranging between 1:1000-1:18000 pregnancies[1]. It consists a rather challenging clinical condition that may even lead to life-threatening complications. Diagnosis is based on ultrasound imaging and may frequently present difficulties; however, it should be made as early as possible in order to avoid the risk of severe vaginal hemorrhage which may even necessitate emergency hysterectomy[2,3].

No consensus has yet been achieved regarding the optimal therapeutic approach of cervical pregnancy. Review of literature demonstrates lack of randomized clinical trials comparing the effectiveness of various therapeutic protocols as the rarity of cases poses reasonable scientific limitations. However, the trend of modern clinical practice is rather destinated to conservative management mainly based on the usage of methotrexate (MTX)[1,3,4]. MTX may be administrated intramuscularly (*i.m.*) or intramniotically (*i.a.*) and may also be combined with other therapeutic means, such as intramniotic administration of potassium chloride, vaginal mifepristone or uterine artery embolization (UAE)[4-11]. In any case, close follow-up is demanded in order to diagnose incompletely treated cases and perform additional interventions such as curettage, hysteroscopy or even hysterectomy[7,12,13].

We present the case of a cervical pregnancy which was successfully treated with intramuscular injection of MTX plus intramniotic administration of potassium chloride, without necessitating further treatment with curettage. Furthermore, a narrative review is also provided regarding the various therapeutic options regarding the optimal treatment of cervical pregnancy.

CASE REPORT

A 41-year-old woman was admitted to our Department with the suspicion of ectopic pregnancy. The woman was followed-up by a private physician, being on her 54th day of amenorrhea, with reported beta human chorionic gonadotropine (β-HCG) ranging within normal values, based on her reported last menstrual cycle. Conception was reported to be spontaneous. The patient had an obstetrical history of three pregnancies, of which the first one was delivered vaginally and the consequent two with caesarean section. Regarding medical-gynecological history, patient reported no severe additional pathology. During her physical and gynecological examination patient was haemodynamically stable. Pelvic examination was normal and cervix itself was closed.

Transvaginal ultrasound imaging at the time of admission revealed empty endometrial cavity, gestational sac within the cervical canal and embryonic echo measuring CRL 1.5 mm (Figure 1). Cardiac activity was detected at the time of diagnosis. Serum β-HCG was measured 28590 IU/L, while no other remarkable findings were observed from her blood test examination. The diagnosis of a cervical pregnancy was therefore made and patient was hospitalized for further treatment.

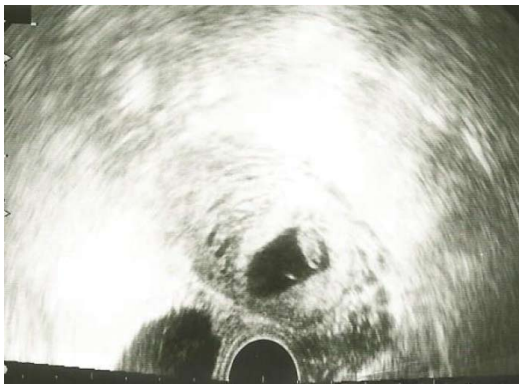

Figure 1 Ultrasound imaging of cervical pregnancy at the time of admission.

Because of patient's stable clinical condition, without signs of vaginal bleeding or pain, patient was decided to be treated with intramuscular administration of methotrexate (50 mg/m^2) in combination with ultrasound-guided intramniotic injection of KCl (2 meq/mL). Injection of KCL was well tolerated by patient without need for anesthesia administration, despite the presence of anesthesiologist during the whole procedure.

Considering that the day of medication was day 1, β-HCG was measured 25.100 IU/L on day 4, 8400 IU/L on day 7 and 1351 IU/L on day 10. Gradual decrease of β-HCG levels was also combined with ultrasound observation of collapsed gestational sac (Figure 2). No additional intervention such as curettage was decided to be performed. During hospitalization period, patient reported only minimal vaginal spotting, without reporting pain or other suspicious signs or symptoms and was therefore discharged on day 10th with the recommendation of follow-up on a weekly basis until β-HCG values are measured lower than 10 IU/L. She was also advised to use contraceptive methods of choice for the next 6 mo in order to avoid conception. Her follow-up period was totally uncomplicated, β-HCG values getting < 10 IU/L on 56th day after MTX administration. Patient was also reexamined 3 mo after cervical pregnancy diagnosis, the gynaecological examination revealing absence of residual pregnancy.

DISCUSSION

We described the case of a cervical pregnancy treated successfully with intramuscular administration of methotrexate and intramniotic injection of KCl, without necessitating additional interventional treatment.

MTX administration has been reported as an effective therapeutic option for the treatment of cervical pregnancy. However, there have been several therapeutic patterns proposed, without consensus regarding their comparative effectiveness. Ben Hamouda *et al*[14] as well as Api *et al*[15] have reported that exclusively single-dose intramuscular administration of MTX may be effective on treating cervical pregnancy without additional need for curettage[14,15]. Intramuscular MTX may also be combined

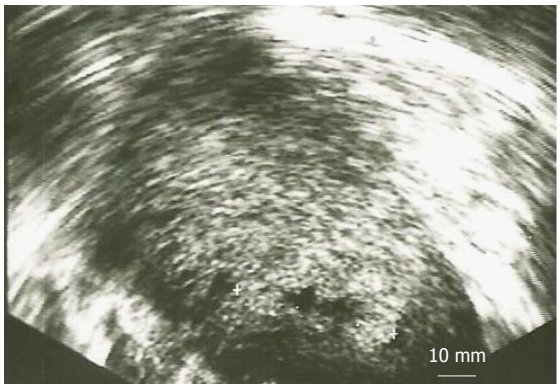

Figure 2 Ultrasound imaging of collapsed gestational sac after methotrexate treatment.

effectively with intramniotic injection of KCl[4,16], while oral mifepristone may also be co-administered effectively as reported by Shresthra *et al*[10]. Kochi *et al*[17], in another case series including 4 cervical pregnancies, have also discussed the alternative of UAE with methotrexate, a method that has been reported to be effective either as monotherapy or in combination with other medication[17]. Furthermore, apart from intramuscular injection and UAE, there is still the option of intramniotic (*i.a.*) administration of methotrexate either as mono-therapy or in combination with intramniotic injection of KCl, as characteristically described by Júnior *et al*[5] in a series of 8 successfully treated cervical pregnancies[5,18-20]. In our case, the choice of intramuscular administration of MTX was based on clinician's relative experience on this certain method of administration, given the fact of the non consensus of optimal method of treatment.

A basic endpoint of the present case report may be the fact that conservative management including *i.m.* MTX and *i.a.* KCl may be effective in treatment of cervical pregnancy without need for performing curettage. Indeed, effectiveness of exclusive administration of MTX has been reported to be as high as 81.3%, while the percentage is increased to 90% when MTX is combined with additional conservative methods[2]. However, conservative treatment with MTX may not definitely exclude the possibility of incomplete treatment, with the underlying possibility of hemorrhage still being potential[21]. Song *et al*[8], in a retrospective study including 50 cases, report that out of 30 cases being treated with *i.m.* MTX, there were only 9 cases that did not necessitate further treatment with curettage[8]. Cipullo *et al*[22], in a case series including 5 cervical pregnancies treated with im MTX + UAE reported that, because of late diagnosis, emergency hysterectomy could not be avoided in a case[22]. Furthermore, there are also reports of unsuccessful intramniotic MTX administration, such as that of Mangino *et al*[11], in which hysteroscopy was finally performed in order to effectively treat cervical pregnancy[11]. Pereira *et al*[7] have also reported the case of a residual pregnancy 3 mo after *i.m.* MTX + *i.a.* injection of KCl + UAE, therefore demonstrating that close follow-up is demanded in order to confirm definite treatment[7]. Thus, conservative treatment with MTX should definitely be combined with close follow-up, including measure of serum β-HCG levels every 3 d after the initial i.m. administration and the possibility of additional 2nd or 3rd dose or even interventional treatment should always be re-evaluated in order to avoid risk of severe hemorrhage. Besides, non-conservative treatment has also been proposed as the basic therapeutic approach by other researchers with satisfying results[23].

The most crucial point, however, regarding treatment of cervical pregnancy with MTX may be to identify patients eligible to be treated conservatively. MTX administration should be preferred in case of haemodynamically stable patients with unruptured ectopic pregnancy, without severe complaint for pelvic pain or vaginal bleeding and mainly in case the size of ectopic mass does not exceed 3-3.5 cm[24,25]. Serum β-HCG levels should always been taken into consideration as there have been implications of improved correspondence in case β-HCG levels are lower than 5000 IU/L. Compliance of patient with close follow-up is also demanded while all potential risks and side-effects should also be explained to the patient[26].

In conclusion, conservative treatment of MTX seems to be the most reasonable therapeutic approach in cases of early diagnosed cervical pregnancies. Intramuscular administration of MTX in combination with intramniotic KCL injection may be effective in the treatment of cervical pregnancy However, further multi-center observational or even randomized studies should be performed in order to assess comparative effectiveness of various therapeutic protocols. Besides, the issue of cost-effectiveness of invasive *vs* conservative management, especially taking into consideration the follow-up necessitated after MTX administration, has to be further elucidated in order to achieve definite conclusions regarding a clinical entity that still poses severe diagnostic and mainly therapeutic challenges.

COMMENTS

Case characteristics

A 41-years-old woman on her 8th gestational week with the suspicion of cervical pregnancy.

Clinical diagnosis

No specific signs or symptoms during typical gynecological examination. Cervix closed.

Differential diagnosis

Other kinds of ectopic pregnancies.

Laboratory diagnosis

Beta human chorionic gonadotropine levels measured 28590 IU/L.

Imaging diagnosis

Empty endometrial cavity, gestational sac within the cervical canal and embryonic echo measuring CRL 1.5 mm.

Treatment

The patient was treated with intramuscular administration of methotrexate and intramniotic injection of KCl. No additional interventional treatment was performed.

Related reports

Successful conservative treatment of cervical pregnancy has been reported by only a few other studies. No consensus has yet been made regarding the various therapeutic options' comparative effectiveness.

Term explanation

Cervical pregnancy accounts for < 1% of ectopic pregnancies with frequency

between 1:1000-1:18000.

Experiences and lessons

Early diagnosis and close follow-up may permit successful conservative treatment with methotrexate, potentially avoiding curettage. However, the risk of severe hemorrhage should always been taken into consideration.

Peer review

This is an interesting study. The case report is well and clearly described and the discussion is concise.

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CASE REPORT

Unusual disposition of lateral circumflex femoral artery: Anatomical description and clinical implications

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Abstract

The anatomical knowledge of arterial variations of lower limb is of utmost significance for the present day surgeons and interventional radiologists for minimizing complications during vascular reconstructive procedures, catheterization procedures and surgical intervention for embolism. Lateral Circumflex Femoral Artery (LCFA) is an important branch of Profunda Femoris artery and precise knowledge of its variations can be of great relevance during surgical and radiological procedures in femoral region. The present study reports a unique case of anomalous route taken by LCFA posterior to femoral nerve associated with a prominent muscular branch from Femoral artery mimicking the course of LCFA. Documentation of such variations is highly significant. It may serve as guideline for surgeons in reducing the incidence of postoperative complications where LCFA is used as a long vascular pedicle in anterolateral perforator thigh flap and in breast reconstruction after mastectomy. Ignorance of such variations can lead to fatal intraoperative haemorrhage and incapacitating sensory and motor deficit due to injury to femoral nerve branches which are closely related to these vessels.

Key words: Lateral circumflex femoral artery; Femoral nerve; Femoral artery; Angiography; Reconstructive surgical procedures; Surgical flaps

Core tip: The knowledge of variations in site of origin and course of the Profunda femoris artery and its circumflex branches is of utmost clinical significance during diagnostic imaging procedures and surgeries performed in the femoral triangle. The present study highlights an abnormal course of the lateral circumflex femoral artery (LCFA) posterior to the femoral nerve associated with a significant muscular branch of femoral artery which mimicked the course of LCFA. Knowledge of such variations maybe of great help to surgeons, interventional radiologists and physicians in reducing the chances of intraoperative secondary haemorrhage and postoperative complications.

Goel S, Arora J, Mehta V, Sharma M, Suri RK, Rath G. Unusual disposition of lateral circumflex femoral artery: Anatomical

description and clinical implications. *World J Clin Cases* 2015; 3(1): 85-88 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i1/85.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i1.85

INTRODUCTION

Arterial variations of lower limb have always been of utmost importance due to their involvement in vascular reconstructive surgeries, catheterization procedures and in raising myocutaneous flaps with vascular pedicles. The recent use of lateral circumflex femoral artery (LCFA) in coronary artery bypass grafting as well as anterolateral thigh cutaneous flaps for oral and oropharyngeal reconstructions has further enhanced the relevance of normal and variant anatomy of LCFA. In view of anatomical variations, preoperative angiographic evaluation of femoral arterial system becomes mandatory in surgical procedures involving the LCFA. Literature reports several variations in origin of LCFA[1,2]. However reports of variant course of LCFA as described in the present study are few.

CASE REPORT

In a unique case, during cadaveric dissection, variant course of LCFA was detected in the right lower extremity of a 53 years old adult Indian male cadaver.

The Profunda Femoris Artery (PFA) took origin as usual from the Femoral Artery (FA), at a distance of 5 cm from the mid inguinal point. At a distance of 7 cm from the same anatomical landmark, LCFA was seen to arise from PFA (Figure 1). LCFA traversed deep to the posterior division of femoral nerve unlike its usual course anterior to the latter. Coursing for 2 cm, the LCFA divided into ascending, transverse and descending branches each of which also traversed behind the posterior division of femoral nerve (Figure 2). The trifurcation of LCFA was immediately posterior to the site where the posterior division of Femoral Nerve (FN) divided into multiple muscular branches.

A prominent muscular branch was given off from FA, 3 cm distal to origin of PFA and 1.5 cm distal to LCFA. This branch traversed parallel to LCFA, mimicked the usual course of latter and passed laterally between the branches of posterior division of Femoral Nerve. The proximal part of the muscular branch was deep to the posterior division of femoral nerve while the terminal branches coursed between the saphenous nerve (SN) and never to vastus lateralis (NVL). Interestingly, this muscular branch arising from FA, appeared to take the course normally taken by LCFA, between divisions of femoral nerve before it terminated by supplying the Vastus Lateralis muscle (Figures 1 and 2).

DISCUSSION

Anatomical knowledge of LCFA including its variations has gained significant importance with the involvement of LCFA in anterolateral thigh free flap[3], aortopopliteal bypass[4] and extracranial intracranial bypass surgery[5]. With the advent of novel harvesting and reconstructive techniques, precise anatomical knowledge of LCFA becomes further important.

The LCFA, commonly a branch of PFA, traverses between divisions of FN, posterior to Sartorius and Rectus Femoris muscles. Coursing behind these structures it divides into ascending, transverse and descending branches. The LCFA contributes blood supply to head and neck of femur, greater trochanter, vastus lateralis and knee joint[6].

Literature reports the use of descending branch of LCFA as a collateral[7] and use of ascending branch in vascularised iliac transplantation[8]. Variations in the origin of LCFA have been reported in cadaveric[9] as well as angiographic studies[10]. However our study is unique as it reports an unusual route taken by LCFA, posterior to the posterior division of femoral nerve and additional presence of a prominent muscular branch of FA which mimics the normal route of LCFA, coursing between the branches of femoral nerve to terminate in vastus lateralis.

Anomalous route of LCFA is of utmost importance to surgeons while raising free rectus femoris muscle flaps with a branch of posterior division of FN, for one stage reconstruction of facial paralysis[11]. Awareness of such anatomical variations as reported in our case may prevent inadvertent injury to LCFA while handling the branches of femoral nerve.

Preoperative anatomical assessment of LCFA through arteriographic study is also essential. The LCFA is frequently explored for its use as new arterial graft for coronary artery bypass grafting[12]. During such surgical procedures, an atypical course of LCFA may lead to an unfortunate sequel of injury to branches of femoral nerve traversing in front of LCFA.

The femoral nerve block is routinely given at a site just above the origin of PFA during knee replacement surgery. Ignorance of the presence of LCFA behind the posterior division of femoral nerve may lead to misinterpretation of absence of LCFA behind the latter and hence consequential accidental injury to LCFA. Exploration of femoral nerve for anaesthetic procedures, therefore, requires awareness of variant course of LCFA in relation to femoral nerve.

Racial differences in direct origin of LCFA from FA have been reported[13]. Studies also report anatomic pattern and calibre of both LCFA and perforators nourishing the anterolateral thigh flap[1]. However, variations in course of LCFA are few. Knowledge of arterial variations is extremely important as these may be the source of intraoperative iatrogenic haemorrhage or post operative complications. Arterial variations of lower extremity as reported in the present case are also important because of their close association with repair of femoral hernias.

Literature reports cadaveric and angiographic studies involving LCFA. Angiographic studies are of utmost importance although difficulties may be encountered

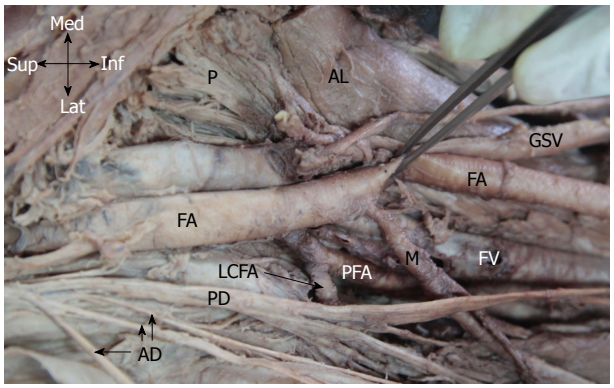

Figure 1 Right femoral region showing. Med: Medial; Lat: Lateral; Sup: Superior; Inf: Inferior; FA: Femoral Artery; FV: Femoral Vein; GSV: Great Saphenous Vein; AL: Adductor longus; P: Pectineus; PFA: Profunda femoris artery; LCFA: Lateral circumflex femoral artery; PD: Posterior division of femoral nerve; AD: Anterior division of femoral nerve; M: Muscular branch of FA.

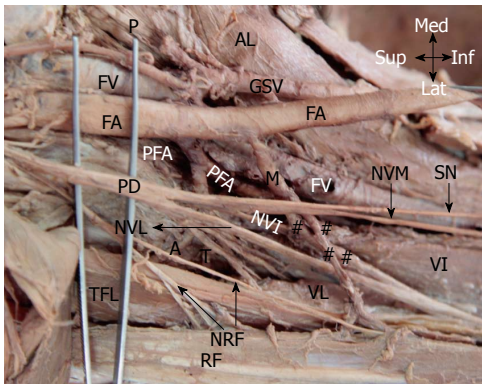

Figure 2 Right femoral region showing. Med: Medial; Lat: Lateral; Sup: Superior; Inf: Inferior; FA: Femoral Artery; FV: Femoral Vein; GSV: Great Saphenous Vein; AL: Adductor longus; P: Pectineus; PFA: Profunda femoris artery; LCFA: Lateral circumflex femoral artery; PD: Posterior division of femoral nerve; M: Muscular branch of FA; TFL: Tensor fascia lata; RF: Rectus femoris; VI: Vastus intermedialis; VL: Vastus lateralis; NVM: Nerve to vastus medialis; SN: Saphenous nerve; NVL: Nerve to vastus lateralis; NVI: Nerve to vastus intermedius; NRF: Nerve to rectus femoris; #: Terminal branches of M; A: Ascending branch of LCFA; T: transverse branch of LCFA.

while defining route and branches of LCFA on angiography. Our study describes variant course of LCFA associated with a prominent muscular branch from FA mimicking the usual route of LCFA. Reports of such cases may be of great importance in bridging the gap between cadaveric and angiographic studies of LCFA.

In aortoiliac occlusive diseases, bypass to the PFA or FA has emerged as a suitable mode of treatment. But in patients with total occlusion of femoral artery as well as profunda femoris artery, bypass to the LCFA was found to be successful[14]. Hence, knowledge of course and branching pattern of LCFA, as reported in our case is extremely important in management of patients with multilevel occlusive diseases of iliac and femoral arteries.

Anatomical knowledge of branches of LCFA is also important while using sharp ended version guidewires during hip fracture surgery[15]. Such surgical procedures, involving exploration of branches of LCFA, may lead to iatrogenic injury to the ascending branch of LCFA because of its variant course behind the femoral nerve.

In the present case, the main trunk of LCFA, as well as its ascending, transverse and descending branches coursed behind the posterior division of femoral nerve. At the same time a prominent muscular branch of FA was seen mimicking the usual course of LCFA, by coursing between the branches of posterior division femoral nerve. Knowledge of such variations is important in surgical transplantation procedures where the branches of LCFA are of utmost use. It may simplify the procedure of flap dissection involving LCFA, especially when anterolateral thigh flap is the easiest and has the least morbidity[16].

Developmental arrests at different stages may lead to anatomical variations related to branches of femoral artery. Vasculature development in the lower limb is preceded by morphological and molecular changes that occur in the limb mesenchyme, therefore variations in vascular pattern are often recorded[17].

With increasing challenges in the field of surgery and occurrence of uncommon anatomic variations, it becomes imperative for the present day surgeons, interventional radiologists and anatomists to be aware of anatomical variations of LCFA - its variant course and muscular branches mimicking the normal course of LCFA. Ignorance of such variations can not only lead to fatal intraoperative haemorrhage but also injury to the branches of femoral nerve which are in close relation to these vessels. Such avoidable femoral nerve lesions can lead to incapacitating sensory and motor deficit. Our study is a sincere effort in this field for minimizing injury to vital structures of lower limb like the femoral nerve and the lateral circumflex femoral artery. We, as anatomists, humbly submit that awareness of vascular variations as encountered in our study is of tremendous significance for successful reconstructive procedures of the region.

COMMENTS

Case characteristics

Anomalous route taken by lateral circumflex femoral artery (LCFA) posterior to femoral nerve and presence of a prominent muscular branch from Femoral artery mimicking the course of LCFA.

Clinical diagnosis

Arterial variants as reported in the present study are of utmost significance in anterolateral thigh flap surgeries, coronary artery bypass grafting and femoral nerve block in knee surgeries.

Differential diagnosis

Anatomical awareness of variant course of LCFA associated with a prominent muscular branch from Femoral Artery (FA) mimicking the course of LCFA is imperative for vascular surgeons and interventional radiologists. The muscular branch of FA can be mistaken for LCFA leading to the accidental ligation of the wrong vessel.

Related reports

Literature reports several variations in origin of LCFA. However reports of variant course of LCFA as described in the present study are few. This vessel as well as its branches are now extensively used in reconstructive and bypass surgeries.

Term explanation

LCFA normally courses in between the branches of Femoral Nerve.

Experiences and lessons

Ignorance of such variations can lead to fatal intraoperative haemorrhage and incapacitating sensory and motor deficit.

Peer review

This article highlights the importance of vascular variations of lower limb encountered in cadaveric studies and its application in surgery, anaesthesiology and interventional radiology.

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REVIEW

State-of-the-Art management of knee osteoarthritis

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Abstract

Osteoarthritis (OA) is the most common type of arthritis found in the United States' population and is also the most common disease of joints in adults throughout the world with the knee being the most frequently affected of all joints. As the United States' population ages along with the increasing trends in obesity prevalence in other parts of the world, it is expected that the burden of OA on the population, healthcare system, and overall economy will continue to increase in the future without making major improvements in managing knee OA. Numerous therapies aim to reduce symptoms of knee OA and continued research has helped to further understand the complex pathophysiology of its disease mechanism attempting to uncover new potential targets for the treatment of OA. This review article seeks to evaluate the current practices for managing knee OA and discusses emerging therapies on the horizon. These practices include non-pharmacological treatments such as providing patient education and self-management strategies, advising weight loss, strengthening programs, and addressing biomechanical issues with bracing or foot orthoses. Oral analgesics and anti-inflammatories are pharmacologicals that are commonly used and the literature overall supports that some of these medications can be helpful for managing knee OA in the short-term but are less effective for long-term management. Additionally, more prolonged use significantly increases the risk of serious associated side effects that are not too uncommon. Disease-modifying osteoarthritis drugs are being researched as a treatment modality to potentially halt or slow disease progression but data at this time is limited and continued studies are being conducted to further investigate their effectiveness. Intra-articular injectables are also implemented to manage knee OA ranging from corticosteroids to hyaluronans to more recently platelet-rich plasma and even stem cells while several other injection therapies are presently being studied. The goal of developing new treatment strategies for knee OA is to prolong the need for total knee arthroplasty which should be utilized only if other strategies have failed. High tibial osteotomy and unicompartmental knee arthroplasty are potential alternatives if only a single compartment is involved with more data supporting unicompartmental knee arthroplasty as a good treatment option in this scenario. Arthroscopy has been commonly used for many years to treat knee OA to address degenerative articular cartilage and menisci, however, several high-quality studies have shown that it is not a very effective treatment for the majority of cases and should generally not be considered when managing knee OA. Improving the management of knee OA requires a multi-faceted treatment approach along with continuing to broaden our understanding of this

complex disease so that therapeutic advancements can continue to be developed with the goal of preventing further disease progression and even potentially reversing the degenerative process.

Key words: Disease-modifying osteoarthritis drugs; Knee osteoarthritis; Disease-modifying osteoarthritis drugs; Osteoarthritis management; Non-steroidal anti-inflammatory drugs; Hyaluronic acid; Arthroscopy; Platelet-rich plasma; Corticosteroids; Stem cells

Core tip: The management of knee osteoarthritis is of growing importance in the world and especially in the United States where an aging population and increasing trends in obesity are increasing the prevalence of this disease. Treatment has traditionally focused on symptom control, however, more recently there has been a greater emphasis placed on developing new modalities that aim to slow disease progression or even reverse the process. This review aims to examine the available literature on such modalities ranging from patient education and weight loss to disease-modifying osteoarthritis drugs to platelet-rich plasma, stem cells, and other emerging injectables.

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INTRODUCTION

Osteoarthritis (OA) is the most common type of arthritis found in the United States population and is also the most common disease of joints in adults throughout the world[1,2]. The knee joint is the most frequently affected of all joints per epidemiological studies with estimates of 37% of United States' adults ⩾ 60 years of age having radiographic evidence of knee OA and 12% having symptoms related to knee OA accompanying radiographic findings[3]. Osteoarthritis risk factors include both genetic and environmental components with multivariable analysis showing significantly higher odds of symptomatic and radiographic knee OA with body mass index ⩾ 30, greater age, non-Hispanic Black race/ethnicity, and among men with manual labor occupations[2,3]. Symptomatic knee OA has also been highly associated with self-reported activity limitations, need for assistive walking devices, and increased use of prescription medications for pain relief[3]. With an aging United States' population and increasing trends in obesity prevalence, it can be expected that the burden of OA on the population, healthcare system, and overall economy will continue to increase in the future without major improvements in management of knee OA. While the synovium, bone, and cartilage are recognized as the main structures being destroyed during disease progression, further research in the field is revealing that OA is not simply a biomechanical process placing excess load on the affected joint but contributions from catabolic cytokine cascades and production of inflammatory mediators also play a significant role and should be targets for intervention[4,5]. In order to take necessary strides towards improving management of knee OA, it is crucial to recognize the complex pathophysiology of its disease mechanism in which a multi-faceted treatment strategy should be employed using both non-pharmacological and pharmacological options, along with understanding the role for surgical intervention. While numerous treatments aim to offer pain relief to better tolerate the symptoms of knee OA, other modalities are attempting to slow the disease progression, halt it, or even reverse it by trying to affect the damaged articular cartilage. Various treatment strategies, both commonly used and newer advances, for the management of knee OA will be reviewed in this present article focusing mainly on non-operative treatments.

NON-OPERATIVE MANAGEMENT

Non-pharmacological

Education and self-management: Multiple societal guidelines and expert panels recognize patient education and self-management strategies as important components of knee OA management[6]. A systematic review and meta-analysis in 2011 evaluated the effectiveness of self-management programs on pain and disability for chronic musculoskeletal pain in which small to moderate effects in improving pain and disability at the long-term level were found using self-management programs[7]. Recent randomized clinical trials have also highlighted benefits from education and self-management, specifically Ravaud *et al*[8] showed that goal-oriented visits focusing on education on OA and treatment management, information on physical exercises, and information on weight loss led to improvement in weight loss and time spent on physical activity[8,9]. These programs can play more significant roles when implemented in conjunction with weight loss and exercise programs by increasing adherence.

Weight loss and strengthening

While genetic and other endogenous risk factors can contribute to knee OA and its progression, it is important to recognize the negative effects that increased stress on the knee joint can have

in the development and progression of OA. Both weight gain and decreased strength of surrounding musculature can increase the load seen by the knee. With the average body weight of the US population increasing across all ages but more significantly in adult population and this being an issue in other parts of the world, weight loss should be addressed as part of the management of knee OA. The Framingham Study by Felson *et al*[10] demonstrated that women with an approximately 5 kg weight loss had a 50% reduction in the risk of development of symptomatic knee OA. Christensen *et al*[11] used a meta-regression analysis of randomized controlled trials to evaluate if there were changes in pain and function when overweight patients with knee OA achieve a weight loss. The study concluded that disability could be significantly improved when weight was reduced > 5.1% over a 20-wk period, or at the rate of > 0.24% reduction per week[11]. Conversely, Riddle *et al*[12] found there to be a significant dose-response relationship between the extent of percentage change in body weight and the extent of change in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) physical function and WOMAC pain scores, specifically those who gained ⩾ 10% of body weight had worse WOMAC physical function score. Not only has weight change been shown to affect pain and function, it has also been associated with MRI changes as Teichtahl *et al*[13] showed that obese individuals with OA who lost as little as 1% of their body weight were able to reduce the amount of medial femorotibial cartilage volume loss. The relationship between obesity, muscle strength, activity level, and knee OA is complex and it can be difficult to determine which factor is contributing most to the disease. While some studies have suggested that people engaging in relatively high levels of activity have an increased risk of developing knee OA compared to sedentary people, other studies have shown a protective effect. Regarding those who have already developed knee OA, a 2011 systematic review demonstrated moderate effect of strength training and exercise in reducing pain and improved physical function significantly. Furthermore, a 2013 meta-analysis including 60 trials showed that an approach combining exercises to increase strength, flexibility, and aerobic capacity was the most effective in managing lower limb OA with trials largely of patients with knee OA[14]. However, another systematic review and meta-analysis in 2014 included 48 randomized controlled trials and found similar effects in reducing pain from knee OA with aerobic, resistance, and performance exercise. In contrast to the 2013 meta-analysis, it concluded that optimal exercise programs for knee OA should have one aim and focus on improving aerobic capacity, quadriceps muscle strength, or lower extremity performance rather than combining the exercises. While both of these analyses demonstrate a positive effect of exercises on knee OA, the most beneficial regimen is still debatable[15]. The IDEA Randomized Clinical Trial included 3 groups in which participants either were involved with intensive weight loss (⩾ 10% body weight), exercise (1 h for 3 d/wk), or both. After this 18 mo randomized control trial, WOMAC pain scores were reduced to no or little pain in 20% in the weight loss only group, 22% in the exercise only group, and 40% in the weight loss and exercise group[16]. This further supports the notion that both weight loss and exercise are important in managing knee OA as they are more effective in combination than either one alone.

Biomechanical interventions (knee braces, knee sleeves, foot orthoses)

Using an appropriate specialist, assessment of biomechanics and incorporating corrective devices may be an effective intervention for knee OA. A key concept in understanding potential benefit from foot orthoses and knee bracing is in relation to the knee adduction moment (KAM) during gait in which excessive KAM has been associated with radiographic knee OA severity, radiographic knee OA progression, and pain with knee OA[17-19]. However, Zifchock *et al*[20] contended that medial joint space and peak adduction angle, not peak adduction moment, were the best predictors of knee pain. A systematic analysis on the effectiveness of knee braces and foot orthoses in conservative management of knee OA produced results suggesting that knee brace and foot orthoses are an effective means of decreasing pain, joint stiffness, and use of pain medication with minimal adverse effects[21]. However, the authors recognized that conclusions of this systematic analysis were limited due to poor quality of trials and heterogeneity of interventions. Lateral wedge insoles, also designed to reduce KAM and therefore decrease medial knee joint loading, have shown mixed results in studies with some claiming no benefit and others arguing its use as an alternative to valgus bracing for medial knee OA[22,23]. A benefit was well demonstrated in a retrospective study of 51 older adults with mild-to-severe medial knee OA in which a significant reduction in pain and improvements in function and quality of life were found with the prescription of a custom-made lateral wedge insole with arch support[24]. With regards to knee OA bracing, it is designed to create either valgus or varus force to alter the contact pressures especially with unicompartmental knee OA. A Cochrane review of orthoses for knee OA included 4 trials in which 1 investigated effectiveness of a knee brace while 3 examined foot orthoses[25]. The study on knee bracing compared a medial compartment unloader brace group, a neoprene sleeve group, and to a control

group in those that had varus deformity of the knee. Both the brace and sleeve group demonstrated significant improvement in disease specific quality of life and function compared to the control group with the brace group also demonstrating statistically significant improvement compared to the sleeve group per WOMAC pain scores[26]. The three studies on orthoses in the Cochrane review were able to conclude that there is some, though limited, evidence that a laterally wedged insole decreases non-steroidal anti-inflammatory drug intake compared with a neutral insole, patient compliance is better in the laterally wedged insole compared with a neutral insole, and a strapped insole has more adverse effects than a lateral wedge insole[25]. Haim *et al*[27] evaluated whether a biomechanical training program could effectively reduce knee adduction moments at 3 and 9 mo in which his results showed not only was there a significantly reduced knee adduction moment, there were also reduced pain and improved function in these subjects with bilateral knee OA[27]. While studies suggest the potential benefit from knee braces, knee sleeves, foot orthoses, and biomechanical training programs, they also highlight the need for more high quality studies which are currently lacking and for more effective ways to determine which subset of knee OA patients are likely to benefit from these interventions. Future research can include utilization of video gait analysis and 3D motion analysis using computer software to further assess biomechanics and individualize interventions in correcting abnormalities.

PHARMACOLOGICAL TREATMENT

Oral analgesics/anti-inflammatories

Several oral medications are prescribed for treatment of knee OA, mostly addressing the issue of pain. Many supplements are available in the United States that claim to be effective in the treatment of OA, however, few have been well studied for efficacy. Additionally, supplements are not held to the same product quality standards as FDA approved medication and thus variability in product may exist from company to company further making it difficult to determine if certain supplements are beneficial and if they should be considered in the management of knee OA. Glucosamine/chondroitin is the most extensively studied supplement for the treatment of knee OA. This oral supplement is alleged to be absorbed and incorporated into articular cartilage thus potentially allowing for the halting of disease progression and even reparative process[28]. There have been many conflicting studies showing both efficacy and lack of efficacy of glucosamine and/or chondroitin supplements which may be partially due to the difference in quality of products being such as those that are pharmaceutical grade. Fransen *et al*[29] in a double-blind randomized placebo-controlled trial showed that the combination of glucosamine-chondroitin resulted in a statistically significant reduction in joint space narrowing at 2 years of use. While there was also a reduction in knee pain over the study period, none of the groups reach a reduction of pain that statistically significant compared to placebo[29]. In a review of the available literature, many studies demonstrated OA pain relief with glucosamine and chondroitin sulfate use and given its excellent safety profile that is equal to placebo in most studies, this therapy is suggested as one that should be discussed with patients regarding potential benefits and considered as an initial treatment modality[30]. Acetaminophen has been commonly used for the treatment of knee OA and a Cochrane review in 2006 including fifteen RCTs involving 5986 participants showed acetaminophen was superior to placebo in five of the seven RCTs, however, when compared to NSAIDs the evidence suggested that NSAIDs were superior to acetaminophen for the treatment of knee OA[31]. Additionally, acetaminophen had previously been viewed as a safe medication to use as a short-term analgesic of knee OA based on a 2010 systematic review that found a low-level effect for OA pain, however, both this review and a safety review in 2012 have raised concern of its safety profile and suggest that this medication should be used more conservatively in both dosing and duration[32,33]. Many studies have demonstrated the ability for NSAIDs to provide symptoms relief for knee OA with the American Academy of Orthopaedic Surgeon's (AAOS) "Treatment of Osteoarthritis of the Knee: Evidence-Based Guideline, 2nd Edition" concluding that NSAIDs as a group should be recommended for patients with symptomatic OA of the knee and it received a strong strength of recommendation. This was determined after 19 studies were included for review with 202 favorable outcomes comparing either selective, non-selective, or topical analgesics to placebo. Out of the 202 total outcomes, 171 were statistically significant in favor of the experimental group. Fifteen outcomes were above the MCII threshold and 63 outcomes were possibly clinically significant[34]. While NSAIDs should be recognized as a good short-term treatment to manage symptomatic knee OA, it is important to acknowledge their side effect profile which makes this medication class a poor long-term treatment. A comparative effectiveness review in 2011 indicated that NSAIDs are associated with an increased risk of serious gastrointestinal (GI), cardiovascular (CV), and renal injury when compared to placebo[35]. The review also found that Celecoxib had a lower risk of ulcer complications compared to non-selective NSAIDs but had a moderately higher risk of CV complications highlighting the need to use NSAIDs conservatively by limiting dosage to lowest required to achieve pain relief and avoid prolonged use[35]. For those with a moderate comorbidity risk of GI complications,

a proton-pump inhibitor should be considered for co-prescribing with non-selective NSAIDs or this medication class should be avoided all together if there is a high risk. Topical NSAIDs can also be considered as a safer and better tolerated treatment although they have a higher risk of dermatological adverse effects. Tramadol and opioids have been evaluated as medications that may offer pain relief for symptomatic OA. Although opioids were found to have a small to moderate benefit compared to placebo in a 2009 Cochrane review, these benefits were outweighed by large increases in the risk of adverse events and therefore it was recommended they not be routinely used, even if osteoarthritic pain is severe[36]. Tramadol has been studied due to its increasing use for the treatment of OA since it does not produce GI bleeding or renal injury compared to NSAIDs. However, similarly to opioids, its benefits appear to be small in relation to pain reduction with a number of adverse events that cause participants to stop taking the medication[37]. While there are a variety of medications available to help reduce pain related to knee OA, their safety profiles need to be considered when initiating treatment and these should not be viewed as good long term treatment modalities in the management of knee OA.

Disease-modifying osteoarthritis drugs

Disease-modifying osteoarthritis drugs (DMOADs) are drugs that halt or significantly slow the progression of structural joint degeneration, specifically cartilage destruction. Several drugs have been investigated including the tetracycline antibiotic, doxycycline, as *in vitro* studies have shown that it may possess the ability to inhibit collagen degradation. Brandt *et al*[38] conducted a randomized, placebo-controlled, double-blind trial studying subjects with knee OA and measured if joint space narrowing in the medial femorotibial compartment could be reduced with doxycycline. The treatment group received 30 mo of 100 mg of doxycycline twice a day and after 30 mo, the treatment group had 33% less joint space narrowing on radiographic imaging compared to the placebo group. Doxycycline did not reduce the mean severity of joint pain and did not have any effect on either joint space narrowing or pain in the contralateral knee[38]. Additionally, when Snijders *et al*[39] evaluated doxycycline in the management of knee OA in their triple-blinded, randomized controlled trial, it was not effective in reducing symptoms over a 24-wk study period and was associated with an increased risk of adverse events[39]. Bisphosphonates have been studied after they have shown the ability to slow progression of OA in animal models and have decreased pain in states of high bone turnover[40]. When the Knee OA Structural Arthritis study tested the efficacy of risedronate in providing symptom relief and slowing disease progression in patients with knee OA, risedronate did not improve signs or symptoms of OA and did not alter progression of OA compared to placebo, however, it did show a reduction in the level of a marker of cartilage degradation[40]. Strontium ranelate is another drug that has been studied because it has been shown to be able to inhibit subchondral bone resorption and increase cartilage matrix *in vitro*. The SEKOIA trial was a 3-year randomized, double-blind, placebo-controlled trial that studied patients with moderate knee OA who received strontium ranelate 1 g/d, 2 g/d, or placebo. Treatment with strontium ranelate decreased progression of knee OA with estimates for annual difference in joint space narrowing versus placebo found to be 0.14 mm for 1 g/d and 0.10 mm for 2 g/d, with no difference between strontium ranelate groups and all values reaching statistical significance. Strontium ranelate 2 g/d also improved WOMAC total score and pain subscore with the treatment being well tolerated[41]. The SEKOIA trial has sparked more interest in strontium ranelate and has led to further studies that are currently underway which include evaluating its effect on loss of cartilage volume and bone marrow lesions using quantitative MRI. While these drugs will continue to be studied in order to more clearly understand their potential role in the management of knee OA, they will also stimulate new research into other DMOADs in hopes of providing better options to those suffering from the progressive nature of knee OA.

Intra-articular corticosteroid injections

Intra-articular (IA) corticosteroid injections for knee OA appear to be an effective way to decrease pain in the short-term and should be used when signs of inflammation arise. A 2006 Cochrane review of the current literature found that IA corticosteroids were more effective than the placebo group for pain reduction and patient global assessment at 1 wk post-injection. There was continued effect seen between 2 and 3 wk post-injection but at 4-24 wk, there was a lack of evidence of effect on pain and function. Comparing IA corticosteroids to IA hyaluronic acid injections, there was no statistically significant difference between weeks 1-4, however, between 5-13 wk post-injection, IA hyaluronic acid was more effective than IA corticosteroids for one or more of the following variables: WOMAC OA Index, Lequesne Index, pain, range of motion (flexion), and number of responders. The review concluded that IA corticosteroid injections appear to offer good short-term benefits with less evidence to support long term benefit[42]. Another review by Bannuru *et al*[43] compared the efficacy of IA hyaluronic acid with corticosteroids for knee OA. While there short-term analysis differed slightly from the Cochrane review in that the results from baseline to 4 wk showed that IA corticosteroids appear to be relatively more

effective for pain than IA hyaluronic acid, it similarly found that after 4 wk the IA hyaluronic acid continued to show superiority over IA corticosteroids further supporting the notion that IA corticosteroids should be implemented for reducing acute inflammation and relieving pain in the short-term but it is not a good treatment option for long-term management of knee OA[43].

Hyaluronic acid injections

Hyaluronans are also known as sodium hyaluronate or hyaluronic acid. Hyaluronic acid is a natural complex sugar of the glycosaminoglycan family and a normal component of synovial fluid and cartilage in the knee. Its viscosity and elasticity allow it to act as both a joint lubricant and shock absorber, respectively. Hyaluronic acid injections, often referred to as viscosupplementation, are marketed in the United States as several different formulations with some being produced from rooster comb and some from fermentation of the nonpathogenic bacterium Streptococcus zooepidemicus. The different products also vary by molecular weights, concentration of hyaluronic acid, elasticity, viscosity, and number of injections per treatment course[44]. A systematic review in 2011 showed evidence of a small but significant efficacy of IA hyaluronic acid injections for knee OA pain by week 4 post-injection with a moderate clinical significance at week 8 and continued residual benefit until 24 wk[45]. Another review, already mentioned in the previous section, compared IA corticosteroids to hyaluronic acid injections and demonstrated IA hyaluronic acid's superiority over corticosteroids after 4 wk post-injection[43]. The AAOS' "Treatment of Osteoarthritis of the Knee: Evidence-Based Guideline, 2nd Edition" gave a controversial recommendation in 2013 in which it stated they "cannot recommend" using hyaluronic acid for patients with symptomatic knee OA which was a change from their earlier 2008 recommendation that was "inconclusive" based on the available studies to recommend for or against IA hyaluronic acid injections. This update came from changes in their article selection criteria for analysis that included 14 studies, 3 of which were of high strength and 11 of moderate strength. Despite their negative recommendation, their meta-analyses of WOMAC pain, function, and stiffness subscales scores all found statistically significant treatment effects of IA hyaluronic acid compared to placebo and the WOMAC pain and WOMAC total score each were found to be clinically significant but not all of the improvements met the minimum clinically important improvement thresholds (MCII) established by the AAOS panel[34]. It should be noted that their application of the MCII has been called into question by several organizations including the Arthroscopy Association of North America who criticized the statistical analysis and inappropriate use of MCII[46]. A Cochrane review that included 40 trials comparing IA hyaluronic acid to placebo found that at the 5-13 wk post-injection period there was an improvement from baseline of 28%-54% for pain and 9%-32% for function for those receiving IA hyaluronic acid injections for knee OA. They did not find any of the different available hyaluronic acid products to be superior over another and there were very few adverse events reported in the studies. They concluded that viscosupplementation is an effective treatment for OA of the knee with benefits on pain, function, and patient global assessment. The authors also concluded that this review supports the use of the hyaluronic class of products in the treatment of knee OA and that these products provide not only statistically significant effects but also clinically important ones[47]. Some question the true efficacy of IA hyaluronic acid injections because a large placebo effect has been appreciated in several studies being as high as 30%-40%. However, reasons for this large placebo effect may include patient expectation, the Hawthorne effect of participating in a clinical trial, some "placebo" groups were actually receiving an active treatment of saline and/or arthrocentesis, and studies may not account for rescue analgesia or co-therapy being used simultaneously. The safety profile of hyaluronic acid injections is overall minimal. The most common side effects are joint effusion, arthralgia, joint warmth, and injection site erythema which all occur in less than 2.5% of patients and are clinically manageable with short-term use of ice, NSAIDs and do not have long-term sequelae[48-51]. The hylan G-F 20 product appears to have a unique side effect termed a local pseudoseptic reaction in those receiving more than one course of treatment which is hypothesized to be due to the chemical cross-linking used to increase the molecular weight and may occur in up to 21% of patients[52]. This event is not a contraindication to using other hyaluronic acid products and there is no increased risk of recurrence using other products. It should be emphasized that hyaluronic acid injection's excellent safety profile makes it a more appealing treatment for long-term use compared to NSAIDs which have risk of gastrointestinal, renal, and cardiovascular complications. Hyaluronic acid injections also have no known medication interactions making it a good option for patients on multiple medications. Overall, the body of literature appears to support the use of IA hyaluronic acid injections for the treatment of knee OA and future studies of high-quality will continue to be helpful to determine the most appropriate utilization in clinical practice.

Platelet-rich plasma

The use of platelet-rich plasma (PRP) has expanded over the past several years to not only just include the treatment of tendon and ligament injuries, but

also in the treatment of cartilage injuries such as in knee OA. PRP is derived from centrifuging whole blood in order to obtain a platelet concentration above baseline[53]. Growth factors including platelet-derived growth factor (PDGF), insulin growth factor (IGF), vascular endothelial growth factor, and transforming growth factor beta-1 are believed to be key components of PRP for structural repair. Drengk *et al*[54] showed that PRP has a proliferative effect on autologous chondrocytes and mesenchymal stem cells in an *in vitro* study. When Petrera *et al*[55] compared chondrocytes supplemented with either fetal bovine serum, PRP, or platelet-poor plasma, the presence of PRP in the culture media enhanced the *in vitro* formation of cartilage the most with increased glycosaminoglycan content, greater compressive mechanical properties, and maintained characteristics of hyaline phenotype. A randomized control trial involving dogs with documented symptomatic arthritis in a single joint was conducted by Fahie *et al*[56]. Dogs in the test group received a single injection of PRP in the affected joint and the control group dogs received a saline injection in the affected joint. After 12 wk, comfort and function improved by 55% and weight placed on the affected limb improved by 12% in the PRP group compared to the control group[56]. Further helping to understand ways in which PRP may be helpful in treating knee OA regarding anti-inflammatory effects, van Buul *et al*[57] in the Netherlands showed that PRP reduced several different effects of interleukin (IL)-1β which is involved in the catabolic process of articular cartilage in knee OA. Kon *et al*[58] did a prospective study on 115 knees with OA receiving a series of 3 PRP injections in which statistically significant improvement of all clinical scores was observed at 12 mo with maximum improvements at 6 mo[58]. Several studies have compared PRP to hyaluronic acid with each of them demonstrating positive results for these treatments of knee OA compared to placebo. PRP and hyaluronic acid have shown similar results in older patients with more advanced OA but PRP has shown better results compared with hyaluronic acid in younger patients affected by cartilage lesions or early OA[59-61]. When Cerza *et al*[61] compared PRP to hyaluronic acid, PRP was found to be more effective and there was also no statistically significant difference in the effect of PRP with regards to the severity of the knee OA. These findings counteract the argument that PRP is only helpful for milder cases of knee OA. Patel *et al*[62] compared 1 *vs* 2 PRP injections to treat knee OA and they found a single dose of PRP to be as effective as 2 injections to alleviate symptoms in early knee OA which further questions whether multiple subsequent injections are needed rather than a single injection only. A prospective cohort study following patients 1 year after PRP therapy for knee OA was conducted by Halpern *et al*[63]. Twenty-two patients with a Kellgren grade of 0-Ⅱ with knee pain were treated with PRP for early knee OA which was confirmed with a baseline MRI. Pain scores significantly decreased by 56.2% at 6 mo and 58.9% at 12 mo with 88% of patients showing improvement of at least 25% at 12 mo. Additionally, WOMAC overall score improved by 45.1% at 6 mo and 56.2% at 12 mo. In this same study by Halpern *et al*[63], qualitative MRIs demonstrated no change in the medial knee compartment in 73.3% of cases at 1 year despite the expected typical progression of knee OA and joint space narrowing. A systematic review of 59 articles (26 *in vitro*, 9 *in vivo*, 2 both *in vivo* and *in vitro*, and 22 clinical studies) analyzing the use of PRP for joint degeneration reinforced that the preclinical literature shows an overall support toward PRP with clinical studies displaying positive effects of PRP with a more significant benefit appearing to be in the younger patients with earlier stages of knee OA[64]. Cavallo *et al*[65] demonstrated that a comparison of different PRP formulations induced distinct effects on human articular chondrocytes *in vitro*, likely attributable to the differences in the concentrations of platelets, leukocytes, growth factors, and other bioactive molecules. This study highlights the fact that differences in technique and PRP composition may produce different outcomes when treating knee OA and make it difficult to compare results between various studies. However, it does appear that PRP can be a useful treatment for knee OA and certainly additional studies are needed before conclusions regarding true efficacy can be confirmed. Future studies are also needed to determine the optimal composition of PRP (*i.e.*, platelet concentration, leukocyte-rich or poor).

Stem cells

Mesenchymal stromal cells [mesenchymal stem cells (MSCs)] are multipotent cells that can be isolated from several human tissues. The immunomodulatory, reparative, and anti-inflammatory properties of MSCs have been tested in a variety of animal models and appear to have potential clinical applications which includes tissue repair[66]. One such study used scaffold-free MSCs obtained from bone marrow to directly inject intra-articularly in a rabbit model of OA. OA was induced by transecting the anterior cruciate ligament of the knee joint of rabbits and radiological assessment confirmed the development of OA after 12 wk. The rabbits then received either MSCs or medium without MSCs and at 20 wk post-operatively, the rabbits receiving the MSCs showed a lower degree of cartilage degeneration, osteophyte formation, and subchondral sclerosis compared to the control group[67]. While the exact mechanism by which MSCs are able to regenerate articular cartilage in patients with OA is not exactly clear,

these cells can induce proliferation and differentiation of resident progenitor cells and they have an innate differentiation potential to chondrocytes[68]. Orozco *et al*[69] conducted a pilot study where 12 patients with chronic knee pain unresponsive to conservative treatments and radiologic evidence of OA were treated with autologous expanded bone marrow MSCs by IA injection. They found that the patients exhibited rapid and progressive improvement in function that approached 65% to 78% by 1 year and that quantification of cartilage quality by T2 relaxation measurements demonstrated a highly significant decrease of poor cartilage areas (on average, 27%), with improvement of cartilage quality in 11 of the 12 patients[69]. This study, however, contained a small patient number and there was no control group for comparison. When Filardo *et al*[70] conducted a systematic review of the use of MSCs for the treatment of cartilage lesions, they included 72 preclinical papers and 18 clinical trials. In regards to the clinical trials focusing on cartilage degeneration, there were no randomized trials, 5 comparative studies, 6 case series, and 7 case reports. Of further note, 2 involved the use of adipose-derived MSCs, 5 the use of bone marrow concentrate, and 11 the use of bone marrow-derived MSCs. While multiple studies showed positive effects of MSCs for the treatment of OA or other cartilage defects, the authors acknowledge that these results are preliminary data on this topic due to only having available preclinical studies along with clinical studies that are of low quality due to weak methodology, small number of patients, and short-term follow-up[70]. Safety concerns have also arisen surrounding the use of MSCs which include but not limited to the neoplastic potential of MSCs due to their proliferative capacity and susceptibility to infection given their immunomodulatory effects[71]. In a systematic review by Lalu *et al*[71] to evaluate the safety of MSCs, they did not identify any significant safety issues other than a transient fever and concluded that this review should provide some assurance that MSC therapy appears to be safe. As in PRP, the use of MSCs is a therapy in that it goes beyond simply attempting to treat symptoms and instead offers the potential to stop disease progression and regenerate articular cartilage. While the possibility of such a regenerative treatment for knee OA is intriguing, before this therapy can be recommended confidently for clinical use there needs to be further studies that are of higher quality to better determine the efficacy, safety, and optimal source and preparation of cells for the treatment of knee OA.

Other injectables

Several other emerging injection therapies have been evaluated although the amount of quality studies are lacking or are still in early trial phases making it difficult to provide appropriate judgment on the efficacy of these products for the treatment of knee OA. IA botulinum toxin type A (BoNT-A) is hypothesized to have anti-nociceptive and potentially anti-inflammatory effects. Boon *et al*[72] conducted a pilot study to evaluate IA BoNT-A in painful knee osteoarthritis. Subjects were randomized to receive a single injection of corticosteroid, low-dose BoNT-A (100 units), or high-dose BoNT-A (200 units). The primary end point was pain visual analog scale score at 8 wk, which decreased in each group but only the low-dose BoNT-A group achieved statistical significance. Each of the groups did show statistically significant improvements in WOMAC Index scores (pain, stiffness, function) at 8 wk and there were no serious adverse events were noted in any group. The study overall supported a possible role for BoNT-A as a treatment option for symptomatic knee OA however it was recognized that larger double-blind randomized studies are needed[72]. Bone Morphogenic Protein-7 (BMP-7) has been studied due to its apparent strong anabolic effect on cartilage as it stimulates synthesis of cartilage matrix components, increases proteoglycan and collagen synthesis, while antagonizing catabolic mediators of cartilage such as IL-1[73]. In a rabbit model, Badlani *et al*[73] delivered BMP-7 *via* an osmotic pump to the knee 4 wk after ACL transection and when compared to a control group for the progression of knee OA, the BMP-7 group showed less cartilage degradation than the controls. In a phase I safety and tolerability study of BMP-7 for symptomatic knee OA, results showed that by week 12, all treatment groups with BMP-7 and the placebo group had improvement in pain scores with a trend toward more symptomatic improvement in the BMP-7 treatment groups although statistical significance was not achieved[74]. Fibroblast growth factor-18 (FGF-18) has also been studied for use as an IA injection to treat knee OA. Moore *et al*[75] demonstrated in animal models that there FGF-18 increased chondrogenesis and cartilage repair. Lohmander *et al*[76] conducted a proof-of-concept double-blind placebo-controlled randomized trial to evaluate the efficacy and safety of IA sprifermin, a recombinant human FGF-18, in patients with symptomatic knee OA. Their results found no statistically significant dose-response change in central medial femorotibial compartment cartilage thickness. Sprifermin though was associated with statistically significant dose-dependent reductions in loss of total and lateral femorotibial cartilage thickness and volume and in joint space width narrowing in the lateral femorotibial compartment with no association with any local or systemic safety concerns[76]. Other IA injection being studied for treatment of knee OA include IL-1 inhibitor, PDGF, IGF, amongst several others currently being studied. While trial data and preliminary studies have been

done for many of these therapies, more studies are needed to establish that they are both effective and safe.

OPERATIVE MANAGEMENT

This review has discussed many non-operative treatments that are utilized to prolong the need for total knee arthroplasty (TKA), however, there are other surgical procedures that are sometimes performed as alternatives in hopes of preventing the need for TKA. These surgical procedures include arthroscopy, high tibial osteotomy to correct abnormal alignment, and unicompartmental knee arthroplasty. High tibial osteotomy and unicompartmental knee arthroplasty are potential alternatives if only a single compartment is involved with more data supporting unicompartmental knee arthroplasty as a good treatment option in this scenario. An in depth discussion of these surgical procedures are beyond the scope of this review article, although it is important to note that arthroscopy, in the vast majority of patients, is no longer viewed as an appropriate treatment for knee OA or for meniscal degeneration in the setting of significant knee OA. Moseley *et al*[77] conducted a randomized, placebo-controlled trial in which a total of 180 patients with knee OA were randomly assigned to receive arthroscopic debridement, arthroscopic lavage, or placebo surgery consisting of skin incisions with a simulated debridement without insertion of the arthroscope. Outcomes were assessed at multiple points over a 24-mo period and they were no better after arthroscopic lavage or arthroscopic debridement than after a placebo procedure[77]. Another randomized, controlled trial was conducted by Kirkley *et al*[78] comparing surgical lavage and arthroscopic debridement together with optimized physical and medical therapy to treatment with physical and medical therapy alone. Arthroscopic surgery for knee OA was shown to provide no additional benefit to optimized physical and medical therapy and even analyses of WOMAC scores at interim visits and other secondary outcomes also failed to show superiority of surgery[78]. Arthroscopy has also been commonly used in the setting of knee OA to treat meniscal tears, although it is critical to recognize that in a study of incidental findings on knee MRI, among persons with radiographic evidence of knee OA, the prevalence of a meniscal tear was 63% in those who had knee symptoms and still remained 60% among those without symptoms[79]. When comparing surgical intervention to conservative management for meniscal degeneration in the setting of knee OA, outcomes are no better for those undergoing surgical intervention[80,81]. Based on the current literature comprised of several high-level studies, arthroscopy should not be included in the treatment algorithm for knee OA, especially without evidence of mechanical symptoms such as knee locking, as it is has not been shown to be an effective method to treat changes seen in the setting of knee OA which include degeneration of the articular cartilage and menisci.

CONCLUSION

The management of knee OA is of growing importance in the world and especially in the United States where an aging population and increasing trends in obesity are increasing the prevalence of this disease. Not only is this disease a burden on the individual patient, it is a burden on the healthcare system and overall economy. Treatment has traditionally focused on symptom control with some attention being given to prevention strategies and only more recently has there been a greater emphasis placed on trying to develop new modalities that aim to slow disease progression or even reverse the process. While there are many treatments available for knee OA, this review has attempted to provide evidence from the available literature to help guide management with the understanding that some of these modalities may be better options depending on the individual patients and clinical scenario. It is important to recognize the complex pathophysiology of this disease process and that a multi-faceted treatment approach is necessary to improve pain and function. Based on this review, education and self-management strategies should always be a part of managing knee OA as it can be used in conjunction with other treatments. Weight loss should be encouraged for patients who are overweight along with an beginning an exercise program that may involve a combination of aerobic activity, strengthening, and improving flexibility. While the optimal program regimen may be debatable, the literature demonstrates that they offer benefit to patients with knee OA and that weight loss with exercise is better than either one alone. There are several studies that have looked at the usefulness of biomechanical interventions and many of them have demonstrated potential benefit from knee braces, knee sleeves, foot orthoses, and biomechanical training programs warranting their incorporation into the management of knee OA. However, more studies are needed to better determine which patients specifically will benefit most from these various interventions. Glucosamine/chondroitin is a supplement with conflicting studies which may be partially due to the difference in quality of products being used in the studies, however, with its excellent safety profile and some studies demonstrating its superiority to placebo, it is a therapy that should be discussed with patients for potential use. Acetaminophen and NSAIDs, and to a lesser extent Tramadol and opioids, can be helpful in the short-term management of knee OA, but given their side effect profiles, they should be considered a poor long-term treatment. DMOADs were discussed in this review to

present available literature on oral medications being studied to alter the course of knee OA, however, at this time there is not enough evidence to suggest the common use of these treatments in managing knee OA. Injectables are another category of treatment for knee OA that should be considered beginning with the use of IA corticosteroids that have shown the ability to decrease pain in the short-term and should be used when signs of inflammation arise. The body of literature overall supports the use of IA hyaluronic acid injections for the treatment of knee OA and demonstrates it is a superior option for long-term management of knee OA compared to IA corticosteroids. Additionally, hyaluronic acid has an excellent safety profile making it a more suitable for being used for an extended period of time.

PRP is another injectable that when compared to hyaluronic acid has shown similar results in older patients with more advanced OA and may have better results in younger patients affected by cartilage lesions or early OA. PRP should be considered as a treatment option especially if the patient has used the other injectables mentioned without success, however, additional studies are needed before conclusions regarding true efficacy can be confirmed and these studies are also needed to help determine the optimal composition of PRP (*i.e.,* platelet concentration, leukocyte-rich or poor). The use of stem cells is emerging and while the possibility of such a regenerative treatment for knee OA is intriguing, before this therapy can be recommended confidently for clinical use there needs to be further studies that are of higher quality to better determine the efficacy, safety, and optimal source and preparation of cells for the treatment of knee OA. Several other emerging injection therapies were discussed in this review, but the amount of quality studies are lacking or are still in early trial phases making it difficult to provide appropriate judgment on the efficacy and safety profile of these products for the treatment of knee OA. While surgical interventions for knee OA were beyond the scope of this review, the current literature comprised of several high-level studies provide evidence that arthroscopy should not be included in the treatment algorithm for knee OA as it has not been shown to be an effective method to treat changes seen in the setting of knee OA with degeneration of the articular cartilage and menisci. This review hopes to provide a better understanding of treatment options available and their efficacy but it is important to highlight the need for continued research with regards to the management of knee OA. This research should focus on investigating the efficacy of new drugs such as the DMOADs or injectables as well as better understanding their safety profiles. Rather than develop treatments that target symptoms, the emphasis needs to be on developing advanced therapies that can slow or prevent further disease progression and hopefully even initiate a regenerative process. Additional research should also be directed at determining which subset of patients with knee OA may benefit from certain treatments and who are more likely to have a positive response to a given intervention so that more individualized treatment strategies can be established.

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REVIEW

Upper aerodigestive tract disorders and gastro-oesophageal reflux disease

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Abstract

A wide variety of symptoms and diseases of the upper aerodigestive tract are associated to gastro-oesophageal reflux disease (GORD). These disorders comprise a large variety of conditions such as asthma, chronic otitis media and sinusitis, chronic cough, and laryngeal disorders including paroxysmal laryngospasm. Laryngo-pharyngeal reflux disease is an extraoesophageal variant of GORD that can affect the larynx and pharynx. Despite numerous research efforts, the diagnosis of laryngopharyngeal reflux often remains elusive, unproven and controversial, and its treatment is then still empiric. Aim of this paper is to review the current literature on upper aerodigestive tract disorders in relation to pathologic gastro-oesophageal reflux, focusing in particular on the pathophysiology base and results of the surgical treatment of GORD.

Key words: Clinical management; Gastro-oesophageal reflux; Extraoesophageal disease; Upper aerodigestive tract disorders; Etiopathogenesis; Therapy

Core tip: Despite a growing number of clinical evidences, the association between gastro-oesophageal reflux disease (GORD) and extraoesophageal manifestations still derives from uncontrolled studies on small groups of patients. The evidences in the literature to support the proton pump inhibitor treatment of respiratory symptoms associated with GORD, in the absence of typical symptoms of reflux (heartburn and regurgitation), are scanty. A specific diagnostic tool, of respiratory symptoms associated with GORD, is missing even if oesophageal 24-h pH monitoring has been recommended. Large and prospective studies to assess the successful outcome of antireflux therapy, as well as surgical therapy, are still missing.

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INTRODUCTION

Gastro-oesophageal reflux disease (GORD) is a

recognized cause of oesophageal symptoms (*i.e.,* heartburn, regurgitation, chest pain, and dysphagia) and inflammatory damages such as erosive esophagitis and intestinal metaplasia (*i.e.,* Barrett's oesophagus). The association between upper airways disease and pathologic gastro-oesophageal reflux was firstly made in 1968, when laryngeal contact ulcers and granulomas were noted to occur in patients suffering from GORD[1,2], although the concept that GORD could have an impact on laryngeal and pharyngeal disorders has been fully developed in the last 30 years. The reported extraoesophageal manifestations of GORD include asthma, chronic cough, laryngeal disorders, chronic sinusitis, otitis media, and postnasal drip[1]. Laryngo-pharyngeal reflux disease (LPRD) is the term suggested by some authors to define GORD beyond the oesophagus up to the laryngeal and pharyngeal level, thus causing extraoesophageal damages[2,3]. Despite a growing number of clinical evidences to support the association between GORD and extraoesophageal disorders[1], presently the majority of reports still derive from uncontrolled studies of small groups of patients[1]. Nonetheless, in most of the published series, it is difficult to establish a firm connection between GORD and upper aerodigestive disorders (even within the laryngopharyngeal areas), since the presence of gastric content in supraoesophageal structures has been always difficult to document[1,2,4].

This article reviews the current available evidence on extra-oesophageal manifestation of GORD, devoting special attention on the pathophysiology base and results of the surgical treatment of GORD, particularly in relation to arodigestive tract disorders.

RESEARCH

The PubMed database was searched up to June 2014, for meta-analysis, systematic reviews, randomized controlled trials, and controlled trials. Full text articles were obtained when the title, abstract or keywords suggested that the study could be eligible for this review. The search was carried out independently. The search was restricted to adults, while no language restriction was applied.

The medical subject heading used included "gastroesophageal reflux", "laryngopharyngeal reflux", "gastroesophageal reflux ethiopathogenesis", "extraoesophageal gastroesophageal reflux disease symptoms", "reflux laryngitis", "posterior laryngitis".

EPIDEMIOLOGY

GORD is the most common disease of the oesophagus, possibly the most frequently faced by the gastroenterologist. Surveys in the United States have shown that it affects about 20% of the population, while about half experiences heartburn as a minimum once in a month, and 5% to 7% of the population have symptoms related to GORD every day[2,5,6].

Also because of the many clinical variables, the true prevalence of GORD among patients with suspected upper aerodigestive disorders secondary to pathologic gastro-oesophageal reflux is difficult to determine accurately, and it varies depending on the population analysed. For instance, in a study by Koufman *et al*[7], it has been estimated that about 10% of patients presenting to ears, nose and throat (ENT) clinician may have symptoms and/or findings related to GORD. In another study, it has also been reported that about two-thirds of laryngeal and voice disorders had either pathologic gastro-oesophageal reflux as primary cause or as a significant etiological co-factor[8], and that the prevalence of GORD among patients with laryngeal stenosis is 78%, reflux laryngitis 60%, globus sensation 58%, and chronic cough 52%[9,10]. In another survey, symptomatic reflux was diagnosed by pH monitoring in 30% to 60% of adults affected by asthma and chronic cough and nearly 40% of patients had endoscopic signs of esophagitis[2,11]. More recently, a systematic review has demonstrated typical symptoms of GORD in 59% of 10491 patients with asthma[12]. The proportion of asthma patients with GORD remained as high as 51% when more stringent criteria to prove GORD were adopted (*i.e.,* ambulatory 24-h pH monitoring). The average prevalence of asthma in patients with GORD was 4.6% as compared to 3.9% in controls. The overall odds ratio was 5.5 (95%CI: 1.9-15.8) for studies reporting the prevalence of GORD symptoms in patients with asthma and 2.3 (95%CI: 1.8-2.8) for those studies measuring the prevalence of asthma in patients with GORD. Interestingly, two studies that assessed whether GORD precedes asthma gave inconsistent results. The authors concluded that the analysis indicated a significant association between GORD and asthma, but the direction of causality remained undetermined[12].

PATHOPHYSIOLOGY

Upper aerodigestive tract disorders from pathologic gastro-oesophageal reflux appear to be sustained either directly or secondarily. Direct injury can be due to the result of a direct contact of gastric contents with the mucosa of the extra-oesophageal structures. Alternatively, a neurally mediated vagal reflex can be stimulated by the refluxate in the oesophageal body affecting indirectly the bronchopulmonary system, thus triggering cough, bronchial constriction or laryngospasm[6,13]. However, the evidence confirming the mechanisms of injury in LPRD is still inadequate, and in the literature very few laboratory investigations with animal models have substantiated the noxious effects of the refluxate on these anatomic sites by

both direct and indirect mechanisms[14,15].

Certainly, microaspiration of gastric contents can occur during gastro-oesophageal reflux episodes[16]. Direct contact of the refluxate with the mucosa of the pharynx and larynx could therefore represent the main mechanism of injury in LPRD. Unlike the distal oesophagus, the airways are not protected by anti-reflux clearance mechanisms or intrinsic mucosal properties. Moreover, it has been shown that a low lower oesophageal sphincter pressure as well as ineffective oesophageal motility may frequently be present in patients affected by GORD with associated respiratory disease (chronic cough, asthma, laryngitis)[17,18]. It is possible that just a single reflux episode above the oesophagus can be responsible of pharyngeal, laryngeal, and respiratory disorders.

The triggering of a vagal reflex has also been proposed as a consequence of the direct aspiration of refluxed acid into the pharynx or upper airways, besides the stimulation of the distal oesophageal mucosal receptors by the refluxed gastric content[17]. When stimulated, airway nociceptors activate protective responses such as cough and bronchospasm. It is also interesting to evidence that the pathways of some oesophageal and airway sensory nerve fibres terminate within the same regions of the central nervous system; thus, connections among oesophageal nociceptors and airway sensory nerves can exacerbate cough and the asthma-like disorders associated with GORD[19]. So, given the common vagal innervations of lungs and oesophagus, it is not surprising that many patients with asthma and chronic cough also have GORD, and that reflux often precipitates respiratory symptoms that are clinically indistinguishable from asthma[2,19].

Also the specific reflux agent or agents responsible for producing otolaryngology symptoms and pharyngo-laryngeal injury are currently debated[20]. Potential candidates include gastric contents (*i.e.*, acid and pepsin) as well as duodenal contents, including both bile acids and the pancreatic enzyme trypsin. Several animal studies suggested an injurious potential for both acid gastric contents and pepsin[2,20,21].

CLINICAL AND DIAGNOSTIC FEATURES OF AERODIGESTIVE TRACT DISORDERS RELATED TO GORD

The Montreal evidence-based global consensus, defining GORD and its constituent syndromes, recognized oesophageal syndromes and established an association between GORD and asthma, chronic cough, and laryngitis, while proposed an association with pharyngitis, sinusitis, and otitis media[22]. The Montreal consensus also acknowledged that such extra-oesophageal disorders have a multi-factorial aetiology and pathologic gastro-oesophageal reflux may well be a co-factor rather than a cause. Thus, patients with asthma, chronic cough or laryngitis should firstly be evaluated for causes not related to GORD, considering that extra-oesophageal syndromes rarely occur without concomitant typical symptoms of GORD[22]. However, the diagnosis of GORD as the cause of extra-oesophageal symptoms is very challenging and relies on the following investigations.

PPI TRIAL

Traditionally, otolaryngologists have commonly used an empiric course of double dose of proton pump inhibitors (PPIs) (*i.e.,* PPI trial) to initially diagnose and treat patients with upper aerodigestive tract disorders suspected to be related to GORD, deeming the resolution of symptoms with such a treatment as diagnostic of LPRD[3,5,6,23]. PPIs have been shown to improve asthma outcomes in terms of a significant reduction in the proportion of subjects experiencing respiratory symptoms including dyspnoea, cough, wheeze in randomized controlled trials[24,25], although a recent meta-analysis of 11 randomized trials found only small improvements of the respiratory function in adult patients with asthma, unlikely to provide a real clinical benefit[26]. Another meta-analysis of randomized trials by the Cochrane Collaboration regarding cough control with PPIs in GORD reported that PPIs were not better than placebo to resolve cough in such a patient, although they improved cough scores[27]. Finally, also for suspected GORD-related chronic laryngitis a meta-analysis of randomized trials has demonstrated no advantage of PPIs *vs* placebo[28]. Therefore, the evidence to support the treatment with PPIs in the absence of typical symptoms (*i.e.,* heartburn and regurgitation) or objective pathologic gastro-oesophageal reflux (*i.e.,* esophagitis on upper endoscopy or positive ambulatory pH monitoring) is scant[29].

ENDOSCOPY

The most frequent laryngoscopic findings that have been related to reflux are oedema and erythema: (1) of the arytenoid cartilages mucosa; (2) of the interarytenoid region; and/or (3) of the posterior third of the true vocal folds (*i.e.,* posterior laryngitis). A reflux finding score based on the presence and the severity of at least eight different grades of lesions at laryngoscopy has been proposed by Belafsky *et al*[30] to improve the diagnostic accuracy. However, laryngoscopy has revealed laryngeal irritation in more than 80% of healthy controls prospectively evaluated[31] and the concordance among ENT physicians for signs of reflux laryngitis is low when blindly evaluated[32]. Thus, a causal relationship between GORD and laryngitis should not be posed

relying on laryngoscopy findings alone.

Indeed, upper endoscopy has excellent specificity for the diagnosis of GORD in the presence of erosive esophagitis[33]; however, only one third of patients with symptoms of GORD, and even less following treatment with PPIs, have erosive esophagitis which does not establish *per se* a causal relationship between GORD and aerodigestive tract disorders[34,35].

AMBULATORY PH MONITORING

This is the only test that can objectively demonstrate the presence of abnormal oesophageal acid exposure, characterize the reflux episodes and determine their association with symptoms. The pH monitoring has high sensitivity and specificity in the presence of erosive esophagitis (both up to 100%); but its sensitivity lowers (about 70%) in patients without erosive esophagitis, although may be increased adopting impedance pH monitoring (up to 90%)[36,37].

The use of dual pH monitoring (with a probe at the level of the upper oesophageal sphincter) has been proposed by some authors to investigate for respiratory symptoms associated with GORD[23,38]. However, this procedure is not universally performed due to the significant practical problems performing pH monitoring in the pharynx[23,38].

Certainly, there is a great variability in the prevalence of abnormal pH monitoring reported in patients with asthma, chronic cough, and laryngitis[12,39-41]. However, a negative pH monitoring may address the diagnostic investigations toward aerodigestive disorders non-related to GORD, while a positive pH monitoring establishes a diagnosis of GORD, although it does not imply that the latter is the cause of the respiratory symptoms. The temporal association between reflux episodes and respiratory symptoms may be evaluated by the symptom index (SI, *i.e.,* percentage of symptoms preceded by a drop in oesophageal pH below 4.0 within a 5-min time window divided by the total number of symptoms)[42] and the symptom association probability (SAP, *i.e.,* statistical probability with which symptoms and reflux episodes are associated)[43]. The patient must promptly record the symptoms, while the machine should accurately detect the reflux episodes (*i.e.,* drop in oesophageal pH below 4.0) in order to evaluate precisely the temporal association between symptoms (*i.e.,* asthma attacks or cough events) and acid reflux episodes. A positive symptom association is declared if the SI is greater than or equal to 50% (*i.e.,* at least half of the reported symptoms are preceded within a 5-min time window by an intra-oesophageal pH below 4.0) or if the SAP is greater than 95% (*i.e.,* the probability of this association having occurred by chance is less than 5%). Unfortunately, both sensitivity and specificity of symptom association analysis tools is limited and there are no outcome studies to support treatment of extra-oesophageal GORD based on this parameter alone[29]. Recently, Smith *et al*[39] by using a microphone to record cough concurrently with the pH-impedance recording, to overcome patients not always recording timely their symptoms during pH monitoring, reported 6 to 18 times more coughing than with patient reported cough, and 2 to 3 times more than relying on manometry to suppose when cough possibly occurred. If these data will be confirmed by further studies, the evaluation of the temporal association between reflux episodes and respiratory symptoms will be improved.

ASTHMA

Some authors have reported about the possible relationship between asthma and GORD, since a percentage between 30% to 80% of asthmatic patients, have been found to have GORD and/or esophagitis[43-45]. Nonetheless, a cause effect association between GORD and asthma has not been found yet[43-45]. Most of the reports show that GORD medical therapies such as histamine H2 antagonists or PPIs can be effective on asthma outcome[45,46], even if such medications have not been reported to improve asthma symptoms or pulmonary function[43-45].

Finally, on the contrary, few reports have claimed that inhaled b2 agonists and oral corticosteroids, currently used for asthma therapy, may increase oesophageal acid refluxate[2,45-50].

CHRONIC COUGH

Chronic cough is also believed to be possibly related to GORD as proposed by several studies[51-54]. In fact Harding[44] and Pacheco-Galván *et al*[49] have reported that both, medical and surgical therapy of pathologic reflux, can improve or even resolve chronic cough in up to 51% to 100% of adult patients. Unfortunately, most of the data available in the literature come from uncontrolled studies with small sample sizes of patients, and evidences about the efficacy of therapy are still lacking. Long term follow-up studies with a large number of patients are missing.

LARYNGEAL DISORDERS

GORD is also considered to be a possible cause of laryngeal disease: it has been suggested that the aetiopathogenetic mechanism underlying laryngeal disorders, such as chronic laryngitis, could be caused by the contact of the acid refluxate with the laryngeal mucosa. Therefore, patients affected by chronic laryngitis who lack anomalies at the laryngoscopic evaluation should be addressed to a pH monitoring and gastroesophageal endoscopy in order to reveal signs of reflux[55-57]. Some authors have already

reported that they have successfully treated with PPIs some laryngeal diseases such as chronic laryngitis as well as contact granuloma and acquired subglottic stenosis[58-60]. In the study by El-Serag *et al*[61], patients treated with PPIs showed efficacious resolution of laryngeal symptoms when compared to a placebo group. Nonetheless, the small amount of patients involved, as well as the limited follow-up, represent the main drawbacks of this and of similar studies[2,49,56,61,62].

CHRONIC SINUSITIS

Some reports have advocated GORD to have a possible role in the aetiopathogenesis of chronic sinusitis and that medical anti-reflux therapy may be useful for these patients[63]. It has been speculated that GORD may cause sinonasal congestion and alteration of sinusal drainage with consequent inflammation[2,49,64].

PAROXYSMAL LARYNGOSPASM

Also paroxysmal laryngospasm episodes have been associated by some authors to GORD, and medical anti-reflux therapy with PPIs has been reported to be of benefit for these patients. According to the aetiopatogenetic mechanism indicated by these authors, paroxysmal laryngospasm could be considered as a vagally mediated reflex response of the larynx to acid refluxate, a potentially injurious stimulus[2,49,63,65-67].

OTITIS MEDIA

To date, there are only few reports indicating GORD as a possible cause of persistent middle ear problems (*i.e.,* otitis media with effusion). Unfortunately studies considering this specific issue, in adults and children, still are scant[68-70].

POSSIBLE DIAGNOSTIC ALGORITHM

The diagnosis of LPRD is still very controversial, both in clinical practice and research. Most guidelines and reviews recommend starting the diagnostic work-up and treatment of patients with upper aerodigestive symptoms of GORD with an empiric trial of PPI therapy at a double dose given for at least three months[3,5,6,23]. Traditionally, in clinical practice, otolaryngologists have considered diagnostic of LPRD the resolution of symptoms following such a PPI trial. Failure to respond, on the other hand, would indicate incorrect diagnosis of GORD or inadequate dosing or resistance to the treatment. Nonetheless, in patients who do not respond to the empirical trial, further investigations (*e.g.,* ambulatory 24-h oesophageal pH monitoring) have been recommended[2,3,5,23].

MEDICAL TREATMENT

As stated above, PPIs are the main proposed treatment for LPRD. Caution must be used, however, interpreting the available literature, as it is hampered by studies lacking strict inclusion and diagnostic criteria investigating large populations and, consequently, inconclusive meta-analyses. Nonetheless, partial improvement of both symptoms and laryngoscopic signs of laryngitis has been reported with PPI treatment and behavioural changes. Lifestyle modifications comprise the avoidance of heavy and late meals, alcohol consumption, and smoking. Also, to elevate the head of the bed and to reduce the body weight may be beneficial[6]. Most handbooks and reviews suggest a three-month treatment with PPIs at a double dose as the first step in the diagnosis and treatment of patients with upper aerodigestive symptoms attributed to GORD[3,5,6,23]. Patients who do not respond, however, are indeed particularly challenging. Several factors have been suggested to explain refractory cases, such as inadequate dosing or resistance to PPIs, sensitivity to non-acid refluxate, and incorrect diagnosis of GORD. Thus, the dosages of PPIs as well as the frequency of their administration can be increased and pro-motility agents and histamine receptor antagonists may be added. Finally, in patients with signs and symptoms of LPRD despite PPIs treatment, 24-h ambulatory pH monitoring while the patient is on medication may demonstrate persistent acid refluxate due to the lack of acid control[6].

SURGICAL TREATMENT OF AERODIGESTIVE TRACT DISORDERS RELATED TO GORD

Historical background

In 1956, Rudolph Nissen, a German surgeon, opened the era of modern anti-reflux surgery describing a complete plication of the gastric fundus around the abdominal portion of the esophagus that restored an anti-reflux barrier[71]. The Nissen operation has been modified throughout the years, new valves around the abdominal oesophagus, either complete (360°) or partial (240°-270°) have been described, but the basic principle of the operation remained unchanged: to restore a high pressure zone (HPZ) at the gastro-oesophageal junction[72-80]. In 1991, Dallemagne *et al*[81] reported the first laparoscopic anti-reflux operation. In the following decade the technique has been adopted worldwide becoming the gold standard for surgical management of GORD.

Indications and technical details

The surgical treatment of GORD is focused on restoring a HPZ at the lower oesophageal sphincter (LOS), while medication aim to modify the pH of

the refluxate. Nowadays, this purpose is achieved by a fundoplication performed laparoscopically, which offers excellent results combined with the postoperative advantage of a short hospital stay, minimal discomfort, and fast recovery time as compared to the open (*i.e.,* laparotomic) traditional operation[82-85]. According to the 2010 guidelines of the Society of American Gastrointestinal Endoscopic Surgeons, the surgical operation should be considered in the following situations: (1) failure of medical treatment because of inadequate symptom control, persistent severe regurgitation or disturbing side effects; (2) preference of the surgical treatment despite symptom control on PPIs due to quality of life, refusal of lifelong need for medication or costs of medications; (3) presence of Barrett's oesophagus or peptic stricture; and (4) extra-oesophageal symptoms such as asthma, hoarseness, cough, and chest pain or aspiration[86].

The surgical literature is full of eponyms of anti-reflux operations that quite often do not even correspond to the operation originally described by the authors[87]. Therefore, it seems very reasonable to go beyond such eponyms, and stress the surgical elements, common to most of these operations, that have been shown to guarantee long term control of reflux for the patient, namely: (1) to reduce the hiatal hernia in the abdomen; (2) to mobilize extensively the oesophagus and gastro-oesophageal junction; (3) to reduce the hiatus; (4) to interrupt the gastro-splenic ligament; (5) to construct a partial or complete fundoplication of adequate length and tightness; and (6) to fix with posterior and coronal stiches the wrap[87].

Results of anti-reflux surgery

Anti-reflux surgery (*i.e.,* fundoplication) has been shown in clinical studies to control GORD symptoms in 93% and 89% of patients after 5 years and 10 years, respectively[82]. Surgical fundoplication restores the LOS competence and improves the oesophageal peristalsis[88]. Of note, due to the restoration of a HPZ, the reflux control is equally effective when the patient is supine or upright[83]. Anti-reflux surgery has been shown to be safe and effective also in elderly patients affected by GORD[84]. In a randomized trial at a Veteran Affairs Cooperative comparing medication to anti-reflux surgery for GORD, patients on medication at 10-year follow-up in the medical therapy group as opposed to the surgical group were 92% and 62%, respectively[89]. The surgical treatment of GORD may, however, expose patients to some morbidity and increased risk of mortality. The most common complication related to anti-reflux surgery is the so called gas-bloating syndrome, affecting 15% to 20% of patients. Recently, a meta-analysis concluded that a partial (240°) fundoplication as compared to a complete (360°) wrap was associated to less postoperative dysphagia and inability to belch in patients undergoing surgical treatment of GORD[90]. A Cochrane review including more than 1200 patients from four randomized trials comparing medical to surgical therapy has demonstrated higher improvements in GORD specific quality of life after surgery, although a meta-analysis of such data was not performed[91]. Symptoms of heartburn, regurgitation, and bloating improved more after surgical fundoplication then with medication, even if small proportion of patients had persistent dysphagia after surgery. Nonetheless, the surgical operation is associated with some risk of complications and the decision to perform a surgical fundoplication needs to be thoroughly discussed with the patient.

Results on respiratory symptoms

The control of respiratory symptoms in patients with GORD undergoing anti-reflux surgery is less predictable. In the era of the open (*i.e.,* laparotomic) surgical operation, objective data regarding the ability of a fundoplication to control respiratory symptoms in patients with GORD were scarce[92,93]. For instance, Pellegrini *et al*[93] reported the successful results of a complete fundoplication performed in a small group of patients in whom GORD-induced aspiration had been diagnosed. In the past two decades, due to the advent of laparoscopic anti-reflux surgery, the number of patients undergoing fundoplication for GORD has greatly increased[87,94]. Hunter *et al*[94] reported resolution or improvement of respiratory symptoms in 76 out of 87 patients (87%) undergoing laparoscopic fundoplication. However, outcomes of patients with extra-oesophageal symptoms undergoing anti-reflux surgery are not always predictable. A Veteran Affairs Cooperative study, reported no significant improvement in pulmonary function tests one year after fundoplication, even in those patients presenting abnormal preoperative tests[95]. Analogously, a randomized trial showed that both medical and surgical therapy did not increase significantly the forced expiratory volume in 1 s after 6 mo[96]. Anti-reflux surgery can control extra-oesophageal symptoms in carefully selected patients with GORD, although these patient should be informed that the success rate is lower than in patients with typical symptoms[97]. It is particularly important to carefully evaluate the response of such patients to PPIs, as in patients who do not respond to medications, even in the presence of oesophageal acid exposure demonstrated by pH-monitoring, the results of fundoplication are not effective[98].

CONCLUSION

Upper aerodigestive tract disorders related to pathologic reflux appear to be a common but controversial disease, with conflicting data on pathophysiology, diagnosis, and treatment. Whereas trends are observed and many clinical practices are accepted widely on the basis

of experience, definitive, prospective, and controlled studies are strongly needed[2,6].

Also considering the possible implication of GORD in the development of supra-oesophageal neoplasms, as advocated by several investigators, more efforts are necessary to support the relation between extra-oesophageal disorders and GORD, in terms of prospective randomized trials[2].

A better comprehension of the physiopathological mechanisms of these conditions can help clinicians in the management of such patients. In particular, further large randomized-controlled trials in order to clarify LPRD pathophysiology, as well as to evaluate diagnostic algorithms and treatment approaches could be then particularly useful for the diagnosis and the management these disorders.

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REVIEW

Review and update on the molecular basis of Leber congenital amaurosis

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Abstract

Inherited retinal diseases are uncommon pathologies and one of the most harmful causes of childhood and adult blindness. Leber congenital amaurosis (LCA) is the most severe kind of these diseases accounting for approximately 5% of the whole retinal dystrophies and 20% of the children that study on blind schools. Clinical ophthalmologic findings including severe vision loss, nystagmus and ERG abnormalities should be suspected through the first year of life in this group of patients. Phenotypic variability is found when LCA patients have a full ophthalmologic examination. However, a correct diagnosis may be carried out; the determination of ophthalmologic clues as light sensibility, night blindness, fundus pigmentation, among other, join with electroretinographics findings, optical coherence tomography, and new technologies as molecular gene testing may help to reach to a precise diagnosis. Several retinal clinical features in LCA may suggest a genetic or gene particular defect; thus genetic-molecular tools could directly corroborate the clinical diagnosis. Currently, approximately 20 genes have been associated to LCA. In this review, historical perspective, clinical ophthalmological findings, new molecular-genetics technologies, possible phenotype-genotypes correlations, and gene therapy for some LCA genes are described.

Key words: Gene therapy; Leber congenital amaurosis; Retinal dystrophies; Childhood blindness

Core tip: Leber congenital amaurosis (LCA) is the most severe retinal dystrophy causing blindness before the age of 1 year. Clinical ophthalmological findings together with electroretinogram study, OCT imaging and retinal molecular-genetic technologies provide a precise diagnosis in these individuals. Gene-specific phenotypic features exist in LCA, and in this way is possible to predict the underlying genetic defect in some patients on the basis of ophthalmological clues. Clinical, molecular-genetics, phenotype-genotype and gene therapy aspects of LCA are described.

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INTRODUCTION

Photoreceptor and retinal pigment epithelium dystrophies are inherited retinal disorders that result in severe visual impairment in childhood and adult years. They affect more than two million individuals worldwide[1,2] and are sorted according to the inheritance trait (autosomal recessive, autosomal dominant, X-linked or mitochondrial); however, these methods of subdividing retinal disease are unsatisfactory due to the fact that they do not always discern the etio-pathogenesis of retinal degeneration[3]. Over the last decade, new molecular-genetic technologies have classified the pathogenic nucleotide variants (mutations) in a more exact way; thus, at this moment, approximately 221 genes are known to cause these diseases[4]. Retinal degeneration phenotypes have variable expressivity that can range from mild retinal dysfunction (night blindness, color blindness) to poor vision and total blindness[5].

Leber congenital amaurosis (LCA) is the most severe and earliest form of the inherited retinal diseases that causes childhood blindness[6]. This dystrophy is a genetically heterogeneous recessive disease affecting 1 in 30000[7] to 1 in 81000[8] subjects; although in consanguineous populations or isolated communities may be more frequent[9]. LCA represents almost 5% of all retinal dystrophies and 20% of children with visual impaired in special schools[10]. Although LCA have a broad expression variability, some clinical features may be specific to individual genetic abnormalities, providing a useful means of determining which gene may be responsible, thus narrowing the number of genes that may need to be tested and thereby significantly reducing the involved cost[11]. To date, eighteen genes involved in LCA have been identified, which encodes proteins important in several retinal developmental and physiologic pathways[4]. Recently, therapeutic gene replacement trials for a specific form of human LCA have started, and represent the first example for inherited blindness treatment. In the following sections, we will describe the historical perspective, the clinical characteristics, the involved genes and their functions, the genotype-phenotype correlations, and the current gene therapy treatments for LCA.

HISTORICAL CLINICAL PERSPECTIVE

In 1869, Theodor Leber described a blind child with vision loss, wandering nystagmus, amaurotic pupils, and congenital retinitis pigmentosa (RP). These characteristics were present at or close birth. This German ophthalmologist classified the disease as a new group of pigmentary retinal dystrophy, or tapetoretinal degeneration[12].

In 1957, a reduced or non-recordable ERG was identified as an essential component in the LCA diagnosis[13]. In such report, congenital keratoconus/keratoglobus and cataracts were features associated with this dystrophy, which was named LCA[13,14]. At the same year, Alstrom *et al*[15] reported that in a study from Sweden 20% of blind children had LCA (*heredoretinopathia congenitalitis monohybrid recessive autosomalis*), which had predominantly an autosomal recessive inheritance. In 1963, Waardenburg *et al*[16] described intrafamilial expression variability and association with keratoconus and cataracts.

CLINICAL CHARACTERISTICS

An appropriate clinical evaluation and ophthalmological history, as well as the determination of suggestive retinal clues make a correct diagnosis of the early-onset childhood retinal dystrophies. The utilization of newer diagnostic tools as optical coherence tomography (OCT), join to electrophysiological test (ERG) support the diagnosis. However, at present, genetic-molecular testing is necessary to obtain a definitive diagnosis of retinal dystrophies through pathogenic variants identification[10].

LCA is characterized by at least three findings: severe and early visual impairment, sluggish or near-absent pupillary responses, and severely subnormal or non-detectable ERG[12,13,17]. In LCA patients the absent of fixation or oscillations of the eyes may be seen as early as 6 wk of life. Phenotypic variability on the retina can be identified; thus, fundus appearance ranged from normal or mild retinal involvement to macular coloboma or maculopathy, bone-spicule pigment migration, marbleized fundus, among others. Refractive errors as high hypermetropia, photoaversion (photophobia), nyctalopia and the oculodigital sign, are also commonly observed[17,18].

Visual function

Visual function and visual acuity (VA) are broadly variable, generally range from 20/200 to perception of light or inclusive no perception of it[18]; thus, the prognosis in these patients are complicated. The natural history of visual impairment has been divided into three types: a stable development in most of affected subjects, visual progressive decline, and an appreciable improvement in a minority[8]. In this way, VA, fundus appearance, and systemic findings were assessed in 55 patients with LCA. Twenty-two patients were seen for follow-up examinations (5 years). Seventeen (77%) patients were found to have stable VA, four (18%) had deterioration of VA, and one patient (5%) improvement[19]. Another

longitudinal study carried out in 14 patients reported that 50% of them have invariable VA, 29% with VA deterioration, and 21% with visual improvement[20]. In a small series of nine LCA patients was showed VA stability was demonstrated in 55% of patients, while 11% and 33% demonstrated decline and improvement, respectively[21]. In summary, in all studies, 90 patients were examinated: 15%, 75%, and 10% of cases have showed deterioration, stability and improvement, respectively[8]. Patients with mutations in specific LCA genes have demonstrated distinctive VA among the different LCA subtypes. We will return to this point later on.

Ophthalmological features associated to LCA

Refraction defects are variable. Subjects with LCA most commonly are hyperopic[19,22,23]; although, they may also be highly myopic[24]. It has been proposed that an unusual emmetropic development may be caused by severe visual impairment[23]. Some children with LCA children are photophobic[24], whereas others LCA patients can have nyctalopia[25] and these symptoms may be gene-specific as we will soon see[2]. Franceschetti´s oculo digital sign, comprising eye poking, pressing, and rubbing is usual in LCA children; it is not pathognomonic for this retinal dystrophy and it can be found in other diseases[26,27]. Some LCA patients may present keratoconus and cataract, which exacerbate the poor vision of this pathology. Mutations in *AIPL1* and *CRB1* genes may be identified in these patients[28-31].

No ophthalmological features associated in LCA

Mental retardation was the most significant systemic association in LCA patients. It was reported in up to 52% on this disease[15,32-35]. As in most of these reports cerebral imaging studies were not performed, it seems that this figure is overestimated. In more recent studies where the brain was evaluated, numerous cases were found to have cerebral anomalies as cerebellar involvement (Joubert syndrome), thereby excluding LCA diagnosis[18].

Stereotypic movements and behavior (hand and rubbing movements, hair touching, facial grimaces, among others) are particularly marked in LCA[6].

Olfactory dysfunction has been described in some LCA patients (and carriers) due to mutations in the *CEP290* gene[36].

DIFFERENTIAL DIAGNOSIS OF LCA

Some inherited retinal no syndromic diseases share similarities with LCA. Achromatopsia and congenital stationary night blindness may present poor eye fixation and nystagmus, which are similar to LCA, but they show normal retinal fundus. On the other hand, ocular albinism also have nystagmus and poor fixation; however, it has albinotic retinal fundus (absent retinal pigment and choroidal vessels visible) and foveal hypoplasia[8].

Complete achromatopsia or colorblindness is an autosomal recessive pathology that presents marked photophia-photoaversion and blepharospam, decreased VA, and inability to discriminate color. At night, they have a significantly improvement. Incomplete form of achromatopsia or blue-cone monochromacy is a congenital stationary cone dysfunction that presents the same symptoms, although it is less severe. In both subtypes of cone dystrophies ERG recordings have rod photoreceptor normal function, while cone function is absent or subnormal[8,18].

CSNB is a heterogeneous group of nonprogressive retinal pathologies that present reduction of VA, myopia, strabismus, and mainly impaired night vision. ERG report absence of rod function in the complete form of CSNB, while in the incomplete form there is a subnormal rod function response[8,18].

Since the sixth week of age albinism may be confused with LCA; however, several features present in albinism as hypopigmentation of the skin, hair and eyes, and a normal ERG make a differential diagnosis[8,18].

Some syndromic inherited disorders may present similar ocular characteristics to LCA. For this reason it is important search features as mental retardation, deafness, kidney disease (nephronophthisis), skeletal anomalies, cerebral and cerebellar anomalies, among others, which can be associated to retinal photoreceptor degeneration.

Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder with a retinal degeneration that presents a rapid progress, characterized by poor vision and night blindness since the first decade of life and total blindness before the second decade. Nystagmus is extremely infrequent. Different forms of retinal dystrophy have been described, including a cone-rod dystrophy or rod-cone dystrophy, choroidal dystrophy, and so-called global severe retinal dystrophy[37]. Truncal obesity and diabetes mellitus, postaxial polydactyly, hypogonadism in males and genital anomalies in females, renal malformations, developmental delay/behavioral anomalies, ataxia, anosmia, cardiovascular anomalies, among others, can be seen in BBS[38]. Alström syndrome is an autosomal recessive disease similar as BBS that also affects severely vision since early ages; but they do not present polydactyly[39].

The neuronal-ceroid-lipofuscinosis are heterogeneous disorders characterized by intracellular storage of ceroid lipofuscin. Progressive vision loss, retinal degeneration, macular degeneration and optic atrophy cause blindness since the age of two years. Developmental delay/mental retardation, psychomotor degeneration, hypotonia, ataxia, seizures, spatiscity, and death are common characteristics seen in this syndrome. ERG shows early non-recordable res-

ponses[40,41].

Senior-Loken syndrome has 2 major features: cystic kidney known as nephronophthisis and early childhood-onset retinal degeneration (retinitis pigmentosa or LCA). Similar features join to cerebellar hypoplasia may be seen in Jourbert syndrome and Meckel syndrome, both diseases known as cerebello-oculo-renal syndromes. Anomalies in cilia proteins (which are essential for the normal development and function of a wide array of specialized tissues as retina, inner ear, kidney, and brain) have been associated in these entities. Thus, mutations in several genes related to centrosomal and ciliary function (as *CEP290*) can cause phenotypic heterogeneity, ranging from LCA to the syndromes above mentioned[42,43].

Refsum disease, neonatal adrenoleucodystrophy and Zellweger syndrome are peroxisomal disorders that have similar ocular LCA phenotype; however, the systemic cerebral, hepatic, and renal features dominate the phenotype, and the patients almost always suffers an early death[18].

MOLECULAR GENETICS OF LCA

LCA is a highly clinical and genetic heterogeneous disease that is inherited as an autosomal recessive trait in most of the affected. Recent advances in knowledge based in molecular genetics of the retina have allowed the improvement and widen of the clinical diagnosis. In this way, patients with LCA or childhood early retinal dystrophies are sooner identified, and new mutations and LCA genes are being discovered[12,15,44].

Identification of LCA genes

Mutations in least 22 genes (Table 1) have been identified in patients suffering from LCA, nonetheless in 30%-50% of LCA patients no genetic cause is confirmed (Table 1). The advent of new genotyping technologies such as DNA microarrays or next generation sequencing (NGS) offers the opportunity of discovering new LCA loci[4]. Currently, some old methods (linkage analysis and candidate gene approach) or newer methods (homozygosity/autozygosity mapping and NGS) are used for LCA genes identification.

Linkage analysis has identified some LCA genes including *AIPL1*, *GUCY2D* and *RDH12*[45-49]. On the basis of retinal expression other group of genes related to LCA have also been found (*RPE65*, *LRAT*, *CRB1*, *IMPDH1*, *CRX*, and *RPGRIP1*)[50-56].

Recently, microarrays technology is utilized to identify mutations that had already been described in other retinal dystrophy reports. This method has the advantage that is relatively cheap and fast; although it has the disadvantage that new mutations will be missed and will not identified. Thus, at least one pathogenic mutation will be found in approximately 60% of the patients when this technology is used. Another method based in microarray technology named homozygosity mapping is important in autosomal recessive diseases where the same ancestral allele is present in affected patients. Thus, this genetic-method is used for detect homozygous mutations (the same mutation in both alleles). *CEP 290*, *LCA5*, *IQCB1*, and *SPATA* are some genes that have been identified by this microarray technology[57].

New mendelian syndromes, new disease genes discovered and even new mechanism of pathogenicity have been identified[58]. A combination of homozygosity mapping and/or exome sequencing have successfully identified mutations in novel LCA genes including *KCNJ13*[59], *ALMS*[60], *CNGA3*[60], *MYO7A*[60], *BBS4*[61], *NMNAT1*[62,63] and *PRPH2*[64].

Genes, function and genotype-phenotype correlation

LCA exhibits a wide intra-and interfamilial clinical heterogeneity. However, it has been recognized that in some instances the retinal phenotype can suggest the underlying molecular defect. This phenotype-genotype correlation may help to determine rapidly a responsible gene, thus decreasing both the number of genes to be analyzed and the cost of molecular analyses. In the next sections we describe some of the most frequently mutated LCA genes.

***GUCY2D* (LCA1, OMIM #204000):** *GUCY2D* gene encodes a transmembrane protein termed guanylyl cyclase 1 which is specifically expressed in the retina and localized in the outer segment of the photoreceptors[65]. This enzyme is involved in the resynthesis of cGMP, a key step in the phototransduction recovery process. GUCY2D mutations accounts for 6%-21% (Table 1) of recessive LCA and up to 40% of dominant cone dystrophies[4,66]. Most *GUCY2D* mutations produce truncation of the protein and total loss of its function[67].

Patients with GUCY2D mutations had markedly poor vision early in life without an obvious subjective degree of progression and their visual acuity can range from 20/200 to light perception[68,69]. Patients have poor responses to visual stimuli, photophobia, preference for dim lights, hyperopia, nystagmus, and do not report night blindness[70]. Retinal fundus generally remains without abnormal findings throughout life. Patients can develop peripheral mild pigmentary changes, optic disc pallor and vascular attenuation[69]. Non-recordable ERGs are typical while OCT imaging shows a significant retinal thinning in the perifoveal area[69].

***RPE65* (LCA2, OMIM #204100):** *RPE65* encodes a retinal pigment epithelium-specific 65-Kd protein[65], which forms a complex with LRAT to act as the isomerohydrolase in the process of visual

Table 1 Leber congenital amaurosis genes and phenotype-genotype correlations

Locus name	Gene symbol	Chromosomal locus	Protein name	Protein function	% of LCA	LCA phenotype	Other retinal dystrophies	Mutations number
LCA1	GUCY2D	17p13.1	Retinal guanylyl ciclase 1	Hydrolysis cGMP	6-21	Marked poor vision, photophobia, hyperopia, nystagmus. Normal appearing fundus or mild granular pigmentary changes in periphery. OCT with significant thinning perifoveal	dCRD, dCD	155
LCA2	RPE65	1p31.3-p31.2	Retinal pigment epithelium protein 65	Isomerohydrolase in vitamin A visual cycle	3-16	Night blindness, nystagmus, poor vision on the first year, transient vision improvement and later deterioration in their third to fifth decades. OCT: thinner retina in both central and perifoveolar areas	rRP, dRP, RPci	138
LCA3	SPATA7	14q31.3	Spermatogenesis-associated protein 7	Possible vesicular transport	Approximately 3	Transient photophobia on the first year, but at three years old all patients had night blindness. Visual acuity at the end of the first decade remained stable. After, only hand motion and 20/200 can be seen. Fundus with typical appearance of RP rapidly progressive	rRP	18
LCA4	AIPL1	17p13.1	Aryl hydrocarbon interacting protein	Rod PDE chaperone	5-10	Keratoconus, cataract, and hyperopia. Variable night blindness or light sensibility. Poor vision. Fundus with bone spicules pigmentation and variable degree of maculopathy. OCT with reduced macular thickness	dCRD, rRP	52
LCA5	LCA5	6q14	Lebercilin	Ciliary functions	1-2	Severe reduced vision at, or near birth. Nystagmus and high hypermetropia. Visual acuity range between 0.20 to light perception. Extensible peripheral field loss. Fundus examination with widespread atrophy of the retina and RPE. Scattered white dots at RPE. Macula is normal most time, but in few patients may be seen macular coloboma. OCT: macular atrophy, disruption of retinal lamination and presence of hyporeflective well-circumscribed area in the outer nuclear layer, with a hyperreflective border (rossettes). Fundus autofluorescence shows hypofluorescence in the macula	No	35
LCA6	RPGRIP1	14q11	RP GTPase regulator-interacting protein 1	Connecting cilium, disc morphogenesis	4-6	Severe loss of vision early in life. Acuity visual worse than 20/200. At the beggining normal retina is seen, then it progress to pigmentary retinopathy. OCT shows remaining photoreceptor in the fovea	rCRD	82
LCA7	CRX	19q13.3	Cone-rod homeobox	Elongation of photoreceptor outer segment, photoreceptor development, phototransduction	1%-3%	Severe vision impairment is expected early in life. Nystagmus and high hyperopia. Fundus grayish with clumping or dot-pigment deposits and macular coloboma-like defect. OCT shows macular atrophy without noticeable signal of the junction between inner segments and outer segments	dCRD, dRP dLCA and rLCA	58
LCA 8	CRB1	1q 31-32.1	Crumbs homologue	Determining ad maintaining photoreceptor architectura	9-13	Nictalopia, nystagmus, keratoconus, corioretinal atrophy and nanophthalmos. Fundus with numular pigment clump, bone spicules and para-arteriolar preservation. Coloboma-like lesions and Coast like lesions	RPpa, rRP	
LCA9	NMNAT1	1p36.22	Nicotinamide nucleotide adenylyltransferase 1	Rate-limiting enzyme NAD (+) biosynthesis	----	Severe form of retinal hereditary degeneration, mainly atrophic macular lesion. Macular pseudocoloboma. Retina´s remainder with pigmentary changes. Nistagmus and severe loss of vision (only light or hand movements perception)	----	44
LCA 10	CEP290	12q21.32	Centrosomal protein Cep290	Ciliary function	20	Nystagmus, hyperopia, keratoconus and cataract. Photophobia. Light perception or no vision. Atrophic spot (dot-like) in RPE, intraretinal bone spicules in most patients. A striking tapetal reflex (specific intraretinal greyish and white marbled areas). Perifoveal thinning by OCT	Syndromes (Senior-Loken, Joubert, Meckel)	187
LCA 11	IMPDH1	7q32.1	Inosine 5´-monophosphate dehydrogensase 1	De novo synthesis de guanine nucleotide	8	Nystagmus with no fixation to light. Retina showing diffuse RPE mottling. No pigmentary deposits	dRP	18

LCA12	RD3	1q32.3	Protein RD3	Transcription and splicing. Suppress retinal membrane guanylate cyclase activity. Role in retinal maturation	< 1	Night blindness, severe nystagmus. Initial refraction was hypermetropic and changed to myopic in the disease´s course. Severe impaired visual acuity. Attenuated vessels, salt and pepper aspect, and bone spicules are seen on fundus. Macular changes as hammer beaten appearance were note on the third decade of live. OCT reveal disorganization of all retinal layers	----	9
LCA13	RDH12	14q23.3	Retinol dehydrogensase 12	Unusual dual specificity for all-trans-retinol and cis-retinols	4-5	Poor vision. Night blindness. Chorioretinopathy (reticular or fishnet pattern) with dense hyperpigmentation and bone spicules. There is little or no autofluorescence on the macula. SDOCT: severe macular thinning and loss of the foveal laminar architecture	dRP	76
LCA 14	LRAT	4q31.3	Lecithinretinol acyltransferase	Esterification essential in vitamin A visual cycle	< 1	Poor vision, nyctalopia, and visual field constriction since childhood. Peripheral RPE atrophy with little pigment migration into retina. Asteroid hyalosis occurs more frequently than RP (37% *vs* 3%). Reduced AF signal	rRP, EORD	13
LCA 15	TULP1	6p21.3	Tubby-like protein	Protein transport from the photoreceptor inner segment to the outer segment	----	Night blindness, nystagmus, moderately to severely limited visual field. Severely disturbed color vision. Fundoscopic findings are variable; pronounced maculopathy in older patients, pigmentary retinopathy in all patients. Pigmentary spicules also are variable affected	rRP	47
LCA 16	KCNJ13	2q37	Inwardly-rectifying potassium cannel subfamily J members	Maintaining resting membrane potential	----	Poor night vision, nystagmus. Cataract. Fundoscopy reveals considerable levels of pigments at RPE and a different configuration that the one seen on typical RP	dVRD	6
LCA 17	GDF6	8q22.1	Grow differentiation factor 6	Codes for a widely expressed growth factor in the TGF-b pathway specifying the dorsal-ventral retinal axis	----	Ocular and skeletal features. Limited vision to detect hand motions	Klippel Feil syndrome, dominant microphthalmia	15
	CABP4	11q13.1	Calcium binding protein 4	Modulate voltage dependent calcium channel	----	Poor vision, nystagmus, photophobia, poor visual acuity	rCSNB, rCRSD	6
	CNGA3	2q11.2	Cone photoreceptor cGMP-gated cation channel alpha subunit	Important for normal vision and olphatory signaling transduction	----	LCA phenotype is not described	Colour blindness total, achromatopsia, cone dystrophy	82
	ALMS1	2p13.1	ALMS1	ALMS protein	----	LCA phenotype is not described	Alstrom syndrome	129
	IQCB1	3q21.1			----	LCA phenotype is not described	Recessive Senior Loken syndrome	
	MYO7A	11q13.5			----	LCA phenotype is not described	Usher syndrome, congenital deafness without RP	304

dLCA: Dominant Leber congenital amaurosis; rLCA: Recessive Leber congenital amaurosis; dCRD: Dominant cone-rod dystrophy; dCD: Dominant con dystrophy; rRP: Recessive retinitis pigmentosa; dRP: Dominant retinitis pigmentosa; Rpci: Retinitis pigmentosa with choroidal involvement; PDE: Phosphodiesterase; recessive retinitis pigmentosa with para arteriolar preservation (rRPpa); EORD: Early onset retina dystrophies; AF: Autofluorescence; dVRD: Dominant viteroretinal degeneration; rCSNB: Recessive congenital stationary night blindness; CRSD: Recessive cod-rod synaptic disorder.

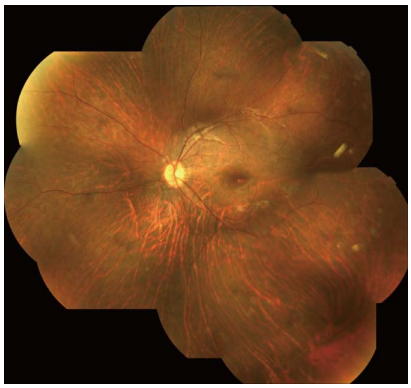

Figure 1 Patient´s fundus with *RPE65* mutations.

pigment regeneration. RPE65 is highly expressed in the RPE cells[71]. High RPE65 concentrations have demonstrated by immunocytochemistry and immunoblotting in the central area of the retina. LCA2 patients show an evident early cone loss, although residual cone preserve structure and function that may be present for many years in humans, supporting the existence of alternative pathways for cone survival[18]. RPE65 mutations account for 6%-16% of LCA cases and for approximately 2% of recessive RP cases[72]. Mutations in the *RPE65* gene are associated to severe visual loss; shortly after birth or in the first few years of life, affected child is noted to be less visually responsive than normal child[72].

In LCA2 patients, night blindness is a common characteristic and for that reason they prefer being in well-illuminated environments. Patients typically have nystagmus and poor vision since the first year of life. Improvement of visual function over the first few years of life can occur and vision may be relatively fair through teenage years, with later deterioration as patients reach their third to fifth decades[2,11]. Myopia and cataract are frequently associated[69]. The fundus appears normal at birth, but several abnormalities can progressively develop. Most patients present optic disc pallor, vascular attenuation and pigmentary retinal changes. Bull's eye maculopathy can be present at early stages while geographic atrophic lesions, diffuse hypopigmentation (dot-like changes) and pigment clumping can be observed at late stages (Figure 1). SD-OCT show 1-3 retinal layer with progressive disorganized lamellar structures. Patients with RPE65 have significantly thinner retinas in both the central and perifoveal areas[69].

***AIPL1* (LCA4, OMIM #604393):** *AIPL1* gene encodes the aryl hydrocarbon receptor protein-like 1, which is involved in nuclear transport or chaperone activity for rod phosphodiesterase (PDE)[4]. This protein is expressed early during human development in the central and peripheral retina, coinciding with rod and cone development; in contrast, *AIPL1* expression is restricted to rod photoreceptors in the adult retina[73]. Mutations in *AIPL1* resulting in blinding diseases can be classified into three categories: Class Ⅰ and class Ⅱ changes (missense and stop mutations) results in LCA (5%-10% of recessive LCA cases), while class Ⅲ mutations (small in-frame deletions) originate dominant forms of cone dystrophy (CORD5) or juvenile retinitis pigmentosa (RP13)[18,74].

The phenotype of LCA patients due to *AIPL1* mutations is relatively severe and is characterized by maculopathy and pigmentary retinopathy since young ages[31,75]. Nystagmus is observed in all patients starting at birth or at early infancy[75]. Keratoconus (26%-30%) and cataract (26%-60%) are commonly associated ocular findings[31,76] while hyperopia is the most common refraction error[2,76]. In addition, light gazing, night blindness, and photophobia can also be present[2,76]. Patients show markedly decreased visual acuities since early ages, ranging from 20/600 to no light perception associated with severe visual fields loss, and extinguished ERGs responses[76]. Fundus examination can vary from normal fundus to a salt-and-pepper retinal dystrophy and no apparent macular involvement in young patients; in contrast, older patients can exhibit typical features of retinitis pigmentosa as mild retinal vessel attenuation, bone spicule pigmentation, nummular pigmentation, and pale optic disc in combination with macular anomalies that can vary from mild foveal atrophy to macular coloboma. Autofluorescence (AF) imaging in AIPL1 young patients demonstrates mild generalized reduction in AF at the posterior pole and a relatively preserved macular AF, which demonstrate some degree of structurally intact photoreceptors and retinal pigment epithelial cells[77]. OCT imaging indicates a reduction in macular thickness in all patients (with salt-and-pepper dystrophy or RP-like lesions) with retinal lamellar structures partially retained, displaying 3 retinal layers with preservation of the outer nuclear layer and photoreceptor inner/outer segment juncture[76].

***CRB1* (LCA8, OMIM #613835):** The *CRB1* gene encodes a transmembrane protein named crumbs homolog 1 that is expressed in brain and retina and plays a central role in determining and maintaining the apico-basal cell polarity and adherens junction in embryonic epithelia[18,66]. CRB1-associated LCA is suggested to be caused by a developmental defect in the retina, since LCA patients with mutations in CRB1 have a thickened retina and lack of distinctive layering, resembling an immature retina[78]. Mutations in this gene cause multiple retinal phenotypes including LCA, retinitis pigmentosa, early onset rod-cone dystrophy, and cone-rod dystrophy[4]. The frequency of LCA8 varies considerably between populations in different geographic regions and

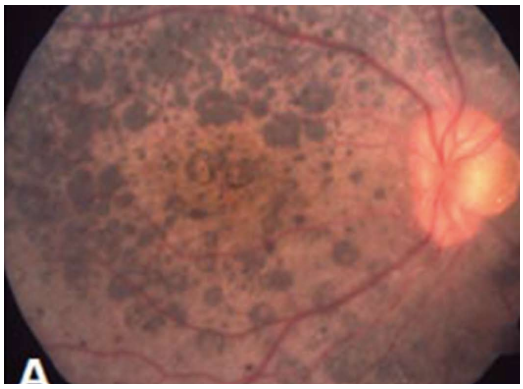

Figure 2 Patient´s fundus with *CRB1* mutations.

ranges from 17% in Spain to 0% in India[79,80].

Nystagmus is a common finding and visual acuities vary widely; thus the majority of patients retain walk-around vision in their first decade, during which median visual acuity is approximately 20/200. Later, visual acuity deteriorates with advancing age[29]. However, some reports have described VA as good as 20/40[2]. Approximately half of patients also have high hypermetropia[2,81]. Night blindness is present in the majority of affected; however, decrease of central vision and photophobia may present in some cases[81]. Keratoconus, occurring in 14%-70% of cases, contributes to visual deficiency in patients with LCA8[80]. Patients carrying CRB1 mutations have shown to be predisposed to chorioretinal atrophy[82] and nanophthalmos[83]. The fundus retinal hallmark of LCA8 is nummular pigment clumps (Figure 2), which are found in more than half of the patients. Although these feature are typical of *CRB1* mutations, it is not pathognomonic and has also been associated with mutations in other retinal dystrophy genes as *NR2E3*[82], *NRL*[84] or *TULP1*[85]. Bujakowska *et al*[81] reported two patterns of fundus pigmentation in their cohort: a typical bone spicule-shaped pigment migration within the peripheral retinal, and clumped pigmentary changes of nummular appearance at the level of the RPE[81]. Preservation of the para-arteriolar retinal pigment epithelium, retinal telangiectasia with exudation (Coat's-like vasculopathy), and macular lesions as coloboma-like lesions in macular or cystoids edema may also be seen[81,84]. SD-OCTs in patients with *CRB1* mutations show increased retinal thickness and loss of the outer limiting membrane[84,85].

***CEP290* (LCA 10, OMIM #611755):** *CEP290* gene encodes the centrosomal protein 290 which is a centrosomal protein with a probable ciliary function and expressed at high levels in the photoreceptor connecting cilium. This protein putatively interacts with the protein retinitis pigmentosa GTPase regulator and also with nephrocystin-S, which is mutated in nephronophthisis type 5[66]. *CEP290* mutations account for 6%-22% of non-syndromic LCA, depending on the population studied[18].

Although there is some inter-intrafamilial variability, patients carrying *CEP290* mutations exhibit a relatively homogeneous distinctive phenotype[86]. Most of the patients present roving eye movements or nystagmus with sluggishly papillary reactions since early childhood. Eye poking, enophthalmos, hyperopia, keratoconus and juvenile cataract are frequently observed[86]. Photophobia is present later in life, and night blindness has sometimes been described. Most patients have severe visual deficiency and visual acuity is either light perception or no vision at all from birth. In some patients visual capability may be equal to or more than hand motion and most of them are able to record progressive deterioration of visual acuity in succeeding years[86]. Fundoscopic findings are variable, from no changes to small (dot-like), well defined, atrophic spot at the level of the RPE to more pronounced RPE atrophy with intraretinal bone spicule-like pigmentation and a preserved macular region. Pasadhika *et al*[69] described LCA 10 patients with various degrees of macular changes, from a blunted foveal reflex to bull's eye maculopathy and optic disc drusen. In several patients, a striking tapetal reflex consisting of intraretinal greyish white marbled areas, more noticeable in the younger patients, is observed[86-88]. The small atrophic spots at the RPE layer and tapetal reflex-like changes seem to be specific of CEP290-associated LCA, since they have not been described in other forms of LCA. A distinct yellow scleral rim and pseudopapillary edema are also suggestive of this specific type of LCA[86]. Retinal SD-OCT analysis of patients with *CEP290* mutations shows segmentation into only three layers. ONL was notably preserved at the central macular area, but was thinner from the perifoveal area to the periphery. Such preservation tended to decline with an increase in the patient's age. Photoreceptor inner/outer segment was poorly defined in the central macula, but was invisible in the periphery. Moreover, cyst-like macular lesions can be identified in up to 43% of LCA 10 patients[69].

***RDH12* (LCA 13, OMIM #612712):** Retinol dehydrogenase 12 (*RDH12*) gene encodes a RDH12 protein that is a photoreceptor-specific enzyme involved in all-trans-and cis-retinol transformation[66]. RDH12 is expressed in the mouse and human photoreceptor inner segments and ONL[18]. Most *RDH12* mutations result in reduced expression and activity of the retinal dehydrogenase 12 enzyme, which in turns disrupts the cycle of synthesis of the visual pigment chromophore, 11-cis-retinal, from 11-trans-retinal[66]. *RDH12* mutations account for 4%-5% of recessive LCA, but may also cause other phenotypes as progressive rod-cone dystrophy, macular atrophy,

Figure 3 Patient´s fundus with *RDH12* mutations.

and early-onset RP[4].

LCA 12 patients exhibit a homogeneous clinical picture characterized by poor visual function in early life, a transition period with visual improvement, and a progressive period of decline in visual function due to rod and cone degeneration[89]. At young ages (4-6 years), patients have visual acuities in the range of 40/100 to 50/100, and visual fields are relatively well preserved; however, an age-related progressive loss of visual acuity is evident, with values from 10/100 to light perception on people at ages above 20 years. In younger subjects, residual rod and cone ERGs responses are recordable, with preferential preservation of cone function documented by use of multifocal ERG recordings; however, both rod and cone ERG are undetectable in patients older than 20 years[90]. Night blindness is the predominant symptom of this type of LCA[2]. This subjective symptom together with visual field constriction may not be frequently reported at time of presentation, but may be commonly recognized later in the disease progression[91]. Most LCA 12 patients have no photophobia while mild posterior subcapsular cataract and mild hyperopia can occur[90]. A marked pigmentary retinopathy, ranging from mild RPE atrophy and mid peripheral hyperpigmentation to severe chorioretinopahty (reticular or fishnet pattern) with dense hyperpigmentation has been associated to this form of the disease[90]. Bone spiculae are almost always present (Figure 3)[90]. There is little or no autofluorescence at macula in severe disease. SD-OCT imaging demonstrates severe macular thinning as well as excavation and loss of the foveal laminar architecture[91].

Gene therapy in LCA

Recent advances in the knowledge of the genes and the pathophysiology associated with mutations in those genes, has opened a new era of mechanism-based molecular therapeutics in ophthalmology. Immune privilege, small organ size, easy access and compartmentalization, as well as contralateral control, make the eye a perfect organ for gene therapy treatment[92,93]. Thus, to date, several independent phase I/II clinical trials for inherited retinal dystrophies including LCA2 (*RPE65*), choroideremia (*CHM*), Usher syndrome 1b (*MYO7A*), Stargardt disease (*ABCA4*), Leber hereditary optic neuropathy (*ND4*), and autosomal recessive retinitis pigmentosa (*MERTK*) are being studied[93].

Human Therapy: *RPE65*: Recently, three independent clinical trials of gene replacement therapy for LCA (specifically by *RPE65* gene) are carrying out to evaluate safety and efficacy[94-96]. Clinical assessment of these LCA2 patients analyzed ERG results, pupillary light reflex, nystagmus, and fundus abnormalities. Patients were treated in the most affected eye with a unique retinal injection of adenovirus vector (AAV2) carrying *RPE65* gene. A safety assessment has not showed the presence of serious adverse event in all trials. The reports of short-term follow-up of these trials have demonstrated an improvement in selected measures of vision, including best-corrected visual acuity, kinetic visual field, nystagmus testing, pupilarry light reflex, microperimetry, dark-adapted perimetry, and dark-adapted full-field sensitivity testing[97]. Soon, the third clinical trial phase of RPE65 replacement in these patients, will start.

Animal models in other LCA genes: Preclinical promise: In several genetic-molecular studies, *GUCY2D* is the most common *LCA* gene affected. A normal retinal fundus evaluation and relatively preserved rod-cone architecture make this group of LCA patients aspirants for gene therapy treatment. GUCYKO mouse containing recombinant AAV-*GUCY2D* gene has restored and preserved cone function during long time[98,99]. A recently study carried out in non-human primate demonstrated a persistent function in both rod and cone cells, mainly in foveal, perifoveal and parafoveal photoreceptors. These results suggested that *GUCYD2* gene therapy replacement could be a good treatment for LCA patients[100].

Other LCA genes considered for gene therapy are *AIPL1* and *CEP290*. Recently, a study developed in hypomorphic and null function mouse model shown long time rescue of rod-cone photoreceptors. Thus, these results showed that CEP290 gene therapy could be applied to patients with partial or complete loss of gene function, mainly when intervention occurs early in life[101]. Another recent study, using self-complementary viral vector has achieved functional vision rescue for this rapid retinal dystrophy[102]. CEP290 mutations may maintain cone cell structure in macular region, mainly in the fovea; in this way, this group of patients are a perfect target for a new gene therapy. Recently, Burnight *et al*[103] built a viral vector containing the *CEP290* gene and they demonstrated in cells culture that some mutated

cells developed cilia after this therapy. Thus, this gene therapy rescued the ciliogenesis anomaly, and could be effective affected subjects[103].

CONCLUSION

Current technology, such as gene testing, OCT or autofluorescence imaging studies together with the knowledge of the ocular-phenotype features of distinct LCA diseases has increased the individual diagnosis of this retinal dystrophy. This will lead to better prognosis and treatment options for LCA patients. Currently, gene therapy for RPE65-LCA2 patients is a fact, and new similar emerging therapies will be soon available. Thus, the knowledge of genotype-phenotype is necessary for a better patient management.

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REVIEW

Dengue and its effects on liver

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Abstract

Dengue has emerged as an important arboviral disease with significant impact on the disease burden in population residing in tropical countries. Dengue is spread by the bite of Aedes mosquito. The virus seems to have some hepatotoxic effects. Affliction of liver in form of derangements in the liver function tests is common and may include mild elevations in serum bilirubin, elevated transaminases and derangements in serum albumin. Although asymptomatic in most cases, clinical manifestations like jaundice, and acute liver failure (ALF) may occasionally complicate the clinical picture. Indeed, dengue has been implicated as an important cause of ALF in endemic countries. The present review focuses on the hepatic manifestations and the pathogenesis of the liver injury in dengue.

Key words: Dengue; Liver; Viral hepatitis; Acute liver failure; Transaminases; Bilirubin

Core tip: Dengue is an important cause of febrile illness in the tropical countries. It may affect the liver but the hepatic involvement is usually asymptomatic. However it is recognized as an important cause of acute hepatic failure in endemic counties. Dengue must be considered as a differential in the evaluation of acute hepatic failure and as an acute precipitant in patients presenting with acute on chronic liver failure.

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INTRODUCTION

Dengue or "break bone fever" has gradually evolved as one of the important causes of febrile illness in the tropical and subtropical region. Second only to malaria, dengue is a common mosquito-transmitted disease, and currently, it is the most common cause of arboviral disease globally. Around 2.5 billion people in 100 endemic countries are believed to be susceptible, so are the equally significant number of travelers to these tropical and subtropical regions[1,2]. Presenting with a wide range of severity, "severe" dengue (Group C) as categorized by World Health Organization (WHO) in 2009 includes the dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)[3]. Despite the inadequate surveillance of cases from the underdeveloped tropical countries, the average number being reported per year has

increased drastically[1,4]. A host of factors including the relentless urbanization with poor hygiene, dilapidated health systems to increasing international travel fuel the spread of this disease geographically and increase the disease burden of tropics significantly[1,2].

This disease has been found to have profound effect on multiple organ systems, the commonest being the liver. Starting from asymptomatic elevated transaminase levels to acute liver failure (ALF), dengue has all the properties of a hepatic illness. In this paper we review, the pathogenesis, pathology, clinicobiochemical parameters and management of the effects of dengue on liver.

DENGUE VIRUS

Dengue virus (DENV) has 4 serotypes (DEN 1-4) and is a member of the Flaviviridae family and the genus Flavivirus[5]. Though initially DEN1 and DEN2 were found around Central America and Africa, and all 4 serotypes found in Southeast Asia, currently all the serotypes have diffused in all tropical and subtropical regions of the world[1,6]. The serotypes sharing a mere 65% of the genotype among each other produce a uniformly wide array of manifestations, with most of them being asymptomatic[2].

DENVs are transmitted *via* the species Aedes aegypti, and less commonly by Aedes albopictus. The Aedes aegypti mosquito with its anthropophilic nature is well adapted for urban thriving and frequently bites several times before completing oogenesis[3,7].

If during the 5 d period of human viremia, the mosquito feeds, it gets infected and the DENV migrates from insect mid-gut to the salivary glands. After 8-12 d of life cycle of DENV inside the mosquito, with optimum high temperatures, the mosquito becomes infective, and can bite and transmit the virus to another host[1,3]. High concentrations of virus are exhibited in mosquito cell cultures with persistent infection[8].

DENV is an RNA virus with a single-stranded positive-sense RNA acting as the genome. The virus has an envelope and is icosahedral in shape. The structural proteins encoded by the DENV are capsid, precursor membrane, and envelope [E]. The virus also encodes for seven non-structural (NS) proteins one of which (NS1) has found use as a diagnostic antigen in initial phases of the disease. The E glycoprotein plays a crucial role in the biology of the DENV, starting from receptor binding to immune response[1,9].

Endocytosis of virus occurs after binding mediated by various molecules including glycoproteins like heparin sulfates as also dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin, and a carbohydrate recognizing mannose receptor[10,11]. Upon internalization, the acidic pH induces change in the classically described "herring-bone" pattern of E glycoprotein. The uncoating and release of the genome occurs once the nucleocapsid is exocytosed into the cytoplasm[12].

CASE DEFINITION

Before going into the various hepatic manifestations of dengue, the evolution of dengue case definition will be briefly discussed. Dengue has been traditionally classified into dengue fever (DF), DHF and DSS (WHO 1997 Classification)[13,14].

DF: Fever and at least two features: ocular pain, headache, muscle or joint pains, cutaneous rash, bleeding manifestations and reduced leukocyte count.

DHF: Fever, thrombocytopenia ($\leqslant 100 \times 10^9$/L), bleeding manifestations and evidence of plasma leakage.

DSS: DHF with tachycardia or low pulse pressure (< 20 mmHg) or hypotension (systolic blood pressure < 90 mmHg).

The modified categorization of WHO in 2009 includes dengue with or without warning signs or severe dengue[3].

Dengue: Fever and two of these: nausea, vomiting, skin rash, bodyache, leukopenia, or any warning sign.

Warning signs include pain in the abdominal or presence of tenderness, persistent vomiting, clinical evidence of fluid accumulation like effusions and ascites, bleeding, lassitude or restiveness, liver enlargement, or rise in hematocrit ($\geqslant$ 20%) with rapid reduction in thrombocyte count (< 50000/mm^3).

Severe dengue: Evidence of severe plasma leakage, bleeding and organ impairment. Organ impairment includes hepatic involvement in form of transaminases elevated beyond 1000 IU/L and central nervous system manifestations like alteration in sensorium or cardiac or other organ involvement.

In spite of the recent categorization, the majority of the studies widely use the more popular DF, DHF and DSS classification for case definition.

DENV INFECTION AND LIVER

With DENV infection, high level of viremia is associated with involvement of different organs (liver, brain) in the severe form of the disease[15]. The liver is the commonest organ to be involved in dengue. Hepatic manifestations are either a result of direct viral toxicity or dysregulated immunologic injury in response to the virus. The spectrum of involvement includes asymptomatic elevation of hepatic transaminases to occurrence of severe manifestation in form of ALF.

PATHOGENESIS OF LIVER INJURY

Hepatic dysfunction is a crucial feature seen in DENV

infection. Hepatocytes and Kupffer cells are prime targets for DENV infection[16-18], as confirmed in biopsies and autopsies of fatal cases[19]. For infecting cells, the major rate limiting step is the viral attachment to the receptors present on surface of host cell. The E protein has a role in the attachment of the virus[20], although the nature of the receptor used is yet to be determined[18]. Heparan Sulphate plays a pivotal role for the intrusion of the DENVs into liver (HepG2) cells[21]. A cell to be infected by a virus requires essentially viral entry and a conducive environment for the invader to grow inside the host cell and this property is influenced by viral serotype, strain and cell type. For example, the G_2 phase cells are more prone to infectivity and enhance virus replication[22]. It has been postulated that the binding of DENVs onto hepatocytes is facilitatory, one binding promotes the binding of successive particles, similar to binding of oxygen on hemoglobin. After binding of the virus, internalization is by either direct fusion or endocytosis. The entry pathway may either be mediated through the presence of receptors or even in their absence[18].

An eventual outcome of hepatocyte infection by DENV is cellular apoptosis, a phenomenon demonstrated both *in vivo* and *in vitro*[23]. After apoptosis, what stays of the cells are the Councilman Bodies[19]. The various pathways involved in this apoptotic process include viral cytopathy, hypoxic mitochondrial dysfunction, the immune response[17] and accelerated endoplasmic reticular stress. Expression of DENV-induced TRAIL[24] and TNF-α and Fas signaling[25] have also been implicated in this process. Activation of the mitochondrial cell death pathway stems from the functional and morphological defects of these mitochondria[26].

Enhancement of immune reaction due to recurrent infections is believed to be responsible for causation of severe dengue disease. DHF and DSS occur as a consequence of several factors interacting, involving the microbial and host features, with antibody-dependent enhancement which explains the phenomena of more severe disease on second infection[27]. Dengue infection induces a cytokine storm and concentrations of cytokines like interleukin (IL)-2, IL-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ reach peak levels in the initial 3 d. IL-4, IL-5 and IL-10 contribute to later in the course of disease[28]. Currently, the exact mechanism by which the host immunity damages liver is unknown, albeit a role of T cells entering the liver causing cytopathology cannot be ruled out. Thus, pathogenesis of hepatic injury in dengue is believed to be primarily a T cell mediated process involving interaction between antibodies and the endothelium and a concomitant cytokine storm often labeled as cytokine "Tsunami," and host factors like genetic polymorphisms.

PATHOLOGY

A wide spectrum of hepatic histological changes have been noted in dengue. This comprises fatty change (micro vesicular), hepatocyte necrosis, hyperplasia and destruction of Kupffer cells, Councilman Bodies and mononuclear cell infiltrates at the portal tract[29,30]. Hepatocyte injury including necrotic changes commonly involves the midzonal area followed by the centrilobular area. Probable explanation for such a finding could be that the liver cells in this area are more sensitive to the effects of anoxia or immune response or may be a preferential target zone of the DENVs. A recent autopsy series of dengue patients from Myanmar[31] showed varying degrees of damage in the liver, with majority of subjects having sinusoidal congestion of moderate to severe degree with predominant midzonal and centrilobular area cell death. Diffuse fatty change was noted within the hepatic lobules. The investigators noted no evidence of any significant fibrosis[31].

CLINICOBIOCHEMICAL PROFILE OF HEPATIC INVOLVEMENT

Clinical features suggesting dengue related hepatic involvement are the presence of liver enlargement and elevated transaminases[32].

Among the clinical features of hepatic involvement, patients have abdominal pain (18%-63%), nausea/vomiting (49%-58%) and anorexia[33,34]. Symptoms such as abdominal pain and anorexia have been found to be significantly more common in DF than DHF[35]. Hepatomegaly is present in both DF and DHF but more common in DF[35]. The frequency of hepatomegaly in the adult dengue patients ranges from 4%-52%[34-36]. Clinical jaundice has been detected in 1.7%-17% in various series[33,35,36] and hyperbilirubinemia has been found to be as high as 48%[34].

The commonest abnormality detected has been raised transaminase levels (Table 1). Raised AST levels have been seen in 63%-97% of patients, while raised ALT levels in 45%-96% of patients. In a majority of the studies, elevation in AST is more than ALT, more during the first week of infection, with a tendency to decrease to normal levels within three weeks[37]. The AST released from damaged myocytes could explain the higher levels of AST than those of ALT in patients with dengue fever at an earlier stage[38]. The increased AST/ALT ratio is useful for differential diagnosis from acute hepatitis caused by the hepatitis A, B or C viruses where it is rarely observed.

The average levels of AST ranged from 93.3 U/L[39] to 174 U/L[33], while ALT from 86 U/L[39] to 88.5 U/L[33] in various studies. More than a 10-fold rise

Table 1 Liver function abnormalities in dengue patients

Ref.	Patients	Raised AST	Raised ALT	AST > ALT	Hyper-bilirubinemia	> 10 fold rise (AST, ALT)
Kuo *et al*[37]	270	93.30%	82.20%	+	7.20%	11.1%, 7.4%
Souza *et al*[39]	1585	63.40%	45%	+	-	3.4%, 1.8%
Itha *et al*[41]	45	96%	96%	Equal	30%	-
Wong *et al*[40]	127	90.60%	71.70%	+ in 75.6%	13.4%	10.2%, 9.5%
Parkash *et al*[33]	699	95%	86%	+	-	15%
Trung *et al*[36]	644	97%	97%	+	1.7%	-
Lee *et al*[14]	690	86%	46%	-	-	1%
Karoli *et al*[34]	138	92%		+	48%	-
Saha *et al*[35]	1226				16.9%	

AST: Aspartate transaminase; ALT: Alanine transaminase.

has been seen in 3.8% cases in a large study from Brazil[39], whereas in other studies were between 1.8% and 11.1% of cases[34,40]. Severe hepatitis was present in 15% in one study[33], while in another study it was 1%[41]. The level of increase in hepatic transaminases can easily mimic acute viral hepatitis.

The median Aspartate transaminase (AST) and Alanine transaminase (ALT) values have been found to be higher for severer forms of dengue than for uncomplicated dengue fever[14,35,39,42]. This hints at a possible association between increased transaminase levels with increasing disease severity. Interestingly the values of liver enzymes were noted to be higher in the febrile and the severer phases of dengue vis-à-vis the convalescent phase[14].

AST has various sources including the heart, striated muscle, erythrocytes, *etc*., apart from the liver, whilst ALT primarily is hepatic in origin[14,43]. Acute insult to these non-hepatic tissues by the DENV can result in higher elevations of AST when compared to ALT rise. Therefore, rise in AST might not be a true reflection of hepatic involvement. Moreover, patients with high levels of enzymes may be labeled as severe disease without any effect on the final outcome.

Liver damage has been found to be more common among females in the large study from Brazil[39] (74.6% of females compared to 52.2% of males) with 4.2% of them having acute hepatitis. However, no significant difference could be elicited between males and females as far as the level of transaminase elevation was concerned.

Hypoproteinemia or hypoalbuminemia have been seen in 12.9% in one of the large studies from Kolkata, India[35], while it ranges from 16.5%-76% in various other studies[34,40,41]. The heterogeneity in the population and severity of the disease may be responsible for such a wide range observed in the various studies.

Coagulation abnormalities have been found in multiple studies. International normalized ratio (INR) > 1.5 have been found in 11% of patients in one study[35], while abnormal prothrombin time (PT), partial thromboplastin time noted in 34%-42.5% of the cases in other studies[34,40]. Increasing bleeding episodes have been found with increasing AST/ALT levels[33,37], but only a weak correlation could be demonstrated between PT and transaminase levels during the convalescent period, suggesting that liver synthetic function in terms of coagulation factor production was generally well compensated.

Dengue has a slightly different profile of hepatic involvement among children (Table 2). They have been found to have a higher percentage of liver enlargement as compared to adults.

Various factors which predict liver damage are DHF, secondary infection, thrombocytopenia, high blood concentration, female sex and children[39,40,44].

DENGUE AND LIVER FAILURE

The liver injury in dengue, as already mentioned, ranges from asymptomatic hepatic transaminase elevation to fatal ALF. Dengue related ALF has been well described in the literature, although the majority of reports are amongst children with few case reports in adults[32,49-55]. Although viral hepatitis and drugs are the predominant cause of ALF, infectious diseases such as dengue are being more and more recognized as an etiological agent.

In a study from Thailand, Poovorawan *et al*[56] found dengue to be a major cause of ALF among children, with 12 out of 35 children (34%) aged 1-15 years of age, enrolled between February 2000 to December 2001, having positive dengue serology. In a further extension, the same group enrolled 14 children of ALF from June 2002 to December 2006, in a recent study and found 2 of them to be due to dengue[57]. Jagadishkumar *et al*[46] have reported 5 (18.5%) confirmed dengue cases in a study cohort of 27 children with ALF from Northern India. The presentation can be varied, either classical presentation of dengue with hepatitis and shock syndrome or there may be less classical dengue characteristics[46]

Deepak *et al*[58] in a study from Mumbai, India, have found 5 cases of dengue associated ALF out of a total of 56 cases (8.9%) of ALF, while Tan *et al*[59] from Malaysia showed 8 out of 155 adult ALF cases (5.2%)

Table 2 Liver function abnormalities among children with dengue

Ref.	Patients (*n*)	Raised AST/ALT	Hepatomegaly	Jaundice	Hypoalbuminemia
Pires Neto Rda *et al*[45]	32	96%	37.50%	-	77%
Mohan *et al*[44]	61	87%	74%	25%	-
Jagadishkumar *et al*[46]	110	-	79%	4.50%	66%
Kulkarni *et al*[47]	948	90%	36.70%	0.95%	-
Roy *et al*[48]	120	94%	80.80%	60%	-

AST: Aspartate transaminase; ALT: Alanine transaminase.

to have dengue. Adult dengue patients developed ALF at a median of 7.5 d (5 to 13 d) after the inception of fever. Occasionally, ALF may in patients who seem to be recovering from dengue[59]. Dengue can mimic ALF and needs to be considered in differential diagnosis of acute liver failure and cerebral malaria in endemic areas[60]. Occasionally, dengue has been reported to cause ALF in patients with underlying liver disease including a HBV carrier[61].

After a period of 3-7 d incubation, the natural course runs in form of fever lasting for 2-7 d, and subsequently a critical phase may occur during defervescence starting from 3-7 d of the illness when plasma leakage dominates the clinical picture[59]. Those surviving this phase of plasma leakage would eventually recover[3,62]. More severe disease is associated with higher viral load[63].

ALF due to paracetamol (PCM) overdose may be due to either a single large overdose or cumulative, multiple overdoses. The latter has been increasingly being recognized as an important cause of ALF due to PCM overdose. Mild to moderate hepatitis is well known in dengue. However there have been ample evidences, obtained both *in vitro* and *in vivo*, that the metabolism of PCM is reduced in patients with hepatitis [64,65]. Moreover, WHO guidelines discourages the use of other nonsteroidal antiinflammatory drugs, such as ibuprofen or antipyretics, in DF[3].

Interestingly, dengue has also been implicated as the cause of worsening of chronic liver disease, *i.e.*, being the acute component of acute on chronic liver failure (ACLF)[61,66,67]. Therefore in endemic areas one should be aware of dengue as a possible cause of ACLF.

Dengue pathogenesis as outlined earlier is not fully understood and is multi factorial ranging from direct viral injury, dysregulated immune response to hypoxic/ischemic injury and even secondary to drugs such as PCM used commonly for such symptoms. Mortality data are comparable with other causes of ALF, although adults have been reported to have a slightly better prognosis as compared to children, in whom it is 50%-66%[68].

In the management of patients with dengue with ALF, besides supportive measures specific measures have also been tried with success. There have been reports of use of N-acetyl cysteine (NAC) in various case series; use of NAC by Senanayake *et al*[69] from Sri Lanka on seven children and Lim *et al*[70] from Singapore on a single child showed clinical improvement. Kumarasena *et al*[71] used NAC on 8 adult patients, 5 of which having grade I-II hepatic encephalopathy and recovered completely while the remaining 3 with higher grades of encephalopathy (grades Ⅲ and Ⅳ) died. Use of molecular adsorbent recirculating system (MARS) has also been[51] reported in dengue associated ALF. Liver transplantation becomes a difficult proposition in lieu of hemodynamic compromise, bleeding, and organ impairment seen during dengue infection.

CONCLUSION

Dengue has a wide spectrum of manifestations. The effects on liver are usually asymptomatic but can be atypical and have varied severity. From asymptomatic elevated transaminase levels to fulminant hepatic failure, the variable manifestations are a big challenge to the clinicians treating the condition. Hepatic involvement is more common and more severe in children as compared to adults. Management is primarily supportive and the outcome is usually good. Care must be taken regarding the diagnosis and use of drugs which may worsen the liver damage.

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MINIREVIEWS

Role of third molars in orthodontics

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Abstract

The role of third molars in the oral cavity has been extensively studied over the years. Literature includes numerous diagnostic and treatment alternatives regarding the third molars. However, an issue that has not been discussed at the same level is their involvement in orthodontic therapy. The aim of this study is to present a review of the contemporary literature regarding the most broadly discussed aspects of the multifactorial role of third molars in orthodontics and which are of general dental interest too.

Key words: Crowding; Extraction; Eruption; Third molar; Orthodontics; Impaction

Core tip: There are multiple factors associated with the eruption or impaction of third molars. According to the existing literature, orthodontic extraction treatment is not directly associated with the eruption of third molars, but might have a positive effect on their position and angulation. There is currently no reliable research evidence supporting the prophylactic removal of non-pathological impacted third molars for the prevention or relief of mandibular incisor crowding.

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INTRODUCTION

The development of third molars and their interaction with the rest of the dentition has been of great concern to general dentists and dental specialists for a long time. Third molar is a tooth characterized by variability in the time of its formation and calcification, its crown and root morphology, its course of eruption and final position, presence or absence in the oral cavity[1,2]. Third molars start appearing on radiographs as early as the age of 5 years and as late as the age of 16 years, usually erupting in the oral cavity between the ages of 18 and 24[3] and they present the highest rate of impaction[4,5].

Although, in the majority of cases, third molars are not directly involved in orthodontic treatment, the fact that, in some cases, they can influence the latter or be influenced it, dictates their direct involvement in treatment planning.

The main issues, concerning the third molars that are related to orthodontic treatment and have been most extensively reported throughout the literature are: the possibility of their eruption or impaction in relation to genetically predetermined factors, the possible repercussion of orthodontic treatment extractions in their position and their influence in orthodontic post-treatment mandibular incisor relapse. These are the topics that are going to be presented and discussed in the herein review.

For the purpose of this study, an extensive online search of PubMed database was conducted. The main focus of the investigation was on original research articles in English. No chronological or other limits were applied.

FACTORS INFLUENCING THE ERUPTION OR IMPACTION OF THIRD MOLARS

The possibility of eruption of third molars is of important consideration in treatment planning and in the long-term maintenance of the dentition and, therefore, of particular interest to dentists and orthodontists[6].

The presence or absence of third molars from the oral cavity has been related to genetically predetermined skeletal and/or dental factors. As early as 1936, Bowdler *et al*[7] suggested that the individual growth pattern is an important factor for the eruption of the third molar. Broadbent[8] believed that the inability of the mandible to achieve its full growth potential may be contributing to the impaction of third molar, whereas, according to Begg[9], the impaction of third molars is attributed to insufficient forward movement of the teeth of modern man due to the lack of interproximal attrition that was observed in ancient skulls.

Björk *et al*[10], in an early study, reported that in 90% of third molar impaction cases the retromolar area space was limited. A few years later, in a longitudinal cephalometric study[11] of 243 cases with the use of implants, he identified two skeletal and two dental factors that were linked with the impaction of mandibular third molars. These factors were: a vertical direction of condylar growth, a reduced mandibular length, a backward-directed eruption of the mandibular dentition and a retarded maturation of the third molars. However, in the same study, it is also stated that, depending on the case, these three factors "may either amplify or neutralize each other".

Ricketts *et al*[12], used longitudinal records in an attempt to predict the amount of mandibular growth and to estimate the amount of space for forward and upward development of the molars. He concluded in that, in order the third molar to have a 50% chance of erupting, 50% of the crown must lie ahead of the external ridge. In a previous study[13], he had also claimed that the direction of tooth eruption plays a critical role in the impaction of third molars.

Kaplan[14] also agreed that cases with impacted third molars exhibit a larger angle of mandibular growth compared with cases with erupted third molars. The distance from Xi cephalometric point to the distal surface of the second permanent molar, was used by Schulhof[15], in an attempt for a computerized prediction of third molar impaction. When this length decreased below 25 mm impaction became more likely and, conversely, less likely as the length increased towards 30 mm. However, this method of prediction presupposes impaction being solely related to available space.

Richardson[16], in a longitudinal study of a group of 95 subjects observed that skeletal Class Ⅱ cases, with a shorter in length, narrower in width and more acute angled mandible, were more prone in third molar impaction. There was also a reduced amount of mandibular growth in cases with impacted third molars, which also had a tendency, although non-significant, to be relatively larger in size. The developmental initial mesial angulation of third molars in relation to the mandibular plane was also increased in subjects with impacted third molars. Ades *et al*[17], after studying the data from cephalometric radiographs and study models from 97 patients, found no significant differences in mandibular growth between those who had impacted or fully erupted mandibular third molars.

Capelli[18], using a sample of 60 patients who had received orthodontic treatment, including the extraction of four premolars, stated that, according to his results, the impaction of third molars is associated with a vertical component of mandibular growth, high mesial inclination of the lower third molar crown in the ascending ramus and reduced mandibular length. Hattab[19], in a radiographic follow-up study of 36 students with an average age of 19.7 years, discovered that a significant proportion of mesially impacted mandibular third molars changed their angulation and became fully erupted by the time the individuals reached 24 years of age. Therefore, he concluded that the positional changes and the eruption of impacted mandibular third molars are two unpredictable phenomena.

Erdem *et al*[20] examined the data from lateral cephalograms, orthopantomograms, periapical radiographs and study models of 27 patients and came to the conclusion that impaction of lower third molar is an unpredictable event. However, they also stated as a conclusion that the chances of eruption for mandibular third molars are more increased in patients with a more vertical growth pattern in general and a vertical direction of condylar growth, with anterior rotation of the mandible. In addition, a greater mesial inclination of the impacted lower molars was also mentioned as a possibly contributing

factor in their occurrence of impaction.

Artun *et al*[21], in an attempt to identify risk factors for maxillary third molar impaction, examined the radiographs of 132 adolescent patients. According to the results of their analyses, the most predictive parameters of impaction were a mesial angulation and a distal angulation of more than 30 degrees of the maxillary third molars relative to the occlusal plane, a reduced retromolar space and a small mandibular plane/Sella-Nasion plane (MP/SN) angle. In a study published in the same year, Artun *et al*[22] retrospectively investigated the lateral cephalograms, panoramic and/or periapical radiographs and study models of 389 patients who had received orthodontic treatment with or without extractions. They concluded that mandibular third molars angulated more than 40 degrees mesially relative to the occlusal plane at the end of treatment may also be at increased risk of impaction.

Behbehani *et al*[23], in a retrospective radiographic study of 134 patients, concluded that increased mesial angulation of the third-molar buds and signs of pronounced forward mandibular growth rotation increase the risk of impaction. Eruption space and mandibular growth rotation were also indicated as the most predictive parameters of impaction. Breik *et al*[24], in the contrary, reported that subjects with horizontal facial growth pattern demonstrated two times lower incidence of third-molar impaction than subjects with vertical growth pattern. Legović *et al*[25], on the other hand, in the same year, had not found any significant differences between the position of lower third molars and type of facial growth.

Finally, Hassan[26], in a retrospective cephalometric study of 121 Saudi patients, concluded that third molar impaction is more likely to occur when the retromolar space is inadequate. The latter was attributed to different skeletal and dental features, including an increased width of the mandibular ramus and a backward rotation of the posterior teeth.

EFFECT OF PERMANENT TEETH EXTRACTIONS ON THIRD MOLARS

Orthodontic treatment, especially during the period of active growth, may significantly influence the development of the dentition. Third molars too, according to the existing literature, are affected by orthodontic therapy in various ways. One of them is the orthodontic extraction treatment, the effect of which on third molars has been broadly investigated. The teeth that are usually removed for orthodontic reasons are the premolars, the first and the second permanent molars, either bilaterally of unilaterally, depending on the individual treatment requirements.

When it comes to extraction therapy, Kaplan[14] was one of the first authors to suggest that premolar extractions increase the probability of third molar eruption. According to the same author, when eruption does not occur in extraction cases, an insignificant resorption of the anterior border of the ramus is probably responsible, which is associated with vertical growth type.

Williams *et al*[27], in a study examining the effect of different extraction sites on orthodontic incisor retraction in 260 cases of patients of the same age at (mean age 13 years), treated with the Begg technique, also investigated on the influence of extractions on third molar eruption. According to their results, the change in the rate of third molar eruption following premolar extractions was indifferent, in contrast with first molar extractions or a combination of first premolar and first molar extractions, which had a significantly positive impact.

Rindler[28], in his investigation, examined the data from the casts and lateral oblique radiographs of 78 patients between 10 and 15 years of age, with a Class II initial malocclusion and crowding in the lower arch. The patients were treated with different techniques and had both their second mandibular molars extracted at the same time with the initiation of root development of the third molars. In 21 cases no additional orthodontic treatment was involved and, in the rest of the cases, lower first molars were moved distally with the use of activators (9 cases) and fixed appliances (48 cases). As they reported in the summary of their study, the third molars successfully replaced the second molars in most cases (77%).

Haavikko *et al*[29], after the analysis of a set of 110 longitudinal orthopantomograms of patients with a mean age of 13.5 years at the start of treatment, 50 of which had had two lower premolar extractions, concluded that the possibility of lower third molar eruption increased only occasionally and that extraction treatment seamed to merely accelerate and not promote eruption. Gaumond[30] used a relatively smaller study group of 11 patients, with different types of malocclusion, which were treated with germectomy of their second molar buds, as soon as the germ of a respective third molar was visible on a radiograph. As he reported, 19 of the 22 third molars that were followed-up achieved "satisfactory of very satisfactory" positions with acceptable final angulations. Based on the outcomes of this study, the author's belief was that every case with mild or moderate mandibular crowding should be treated in this way, rather than with four premolar extractions.

Cavanaugh[31], in a clinical and radiographic evaluation of third molars after second molar extractions in 25 patients, all of which, except for 2, had had some kind of orthodontic treatment, suggested that third molars usually successfully erupt into the space provided by the removed second molars. Richardson[32] conducted a retrospective investigation

of the records (casts and sixty-degree cephalometric radiographs) of a group of 48 subjects that had had unilateral or bilateral mandibular first premolars extractions and of a control group with no mandibular extractions. She concluded that there was a significantly increased space for third molar eruption in the group of the extraction cases. She could still, though, not explain the fact that a number of third molars had become impacted in extraction cases.

Gooris *et al*[33] conducted a study using 95 sets of panoramic radiographs of patients, within an age range of 9 to 19 years, who had received orthodontic treatment involving second molar extractions and first molar distalization. Their measurements indicated that almost all erupted third molars presented with a mesial inclination. Staggers *et al*[34], in a retrospective study of panoramic radiographs from 78 orthodontically treated subjects, 33 of which had been treated with four premolar extractions, concluded that there was no relative considerable impact of the extractions on third molar angulation. All the patients used in the study had Class I skeletal and dental relationships, they were treated with straight-wire appliances and the age range was roughly 11-26 years for the extraction group and 11-17 for the non-extraction group.

Richardson *et al*[35] cooperated in a retrospective study regarding the evolution of third molars after second permanent molar extractions. In total, 63 sets of records (lateral cephalographs and study models) were selected, from patients that had bilateral or unilateral (8/63) second molar extractions. Twenty-three/sixty-three patients had acceptable initial occlusion and had not received additional orthodontic treatment. Based on the results of this study, the authors suggested that there was a tendency of mandibular third molars to autocorrect their bucco-lingual inclination subsequent to second molar extraction and that the earlier the developmental stage of a third molar is at the time of extraction the higher are the chances for their eruption.

Moffitt[36] evaluated a subgroup of 56 patients, 28 of which had unilateral maxillary second permanent molar extractions, clinically, radiographically and *via* their study models, regarding the effect of extractions in the third molar eruption and function. The results showed that after second molar extractions maxillary third molars erupted in most cases in acceptable positions and that their eruption was also accelerated in variable degrees. Orton-Gibbs *et al*[37] aimed to assess with their study the final position of third molars after the extraction of second molars, in a retrospective radiographic analysis of the records of 63 patients, with a mean age of 13 years and 3 mo and different initial types of malocclusion. According to the results of this study, both maxillary and mandibular third molars in most cases erupted in "good or acceptable" positions, based on "Richardson's score system"[35]. They also noted that the angulation of mandibular third molars improves further after the end of active treatment.

Elsey *et al*[3] conducted their own study with the objective to evaluate the influence of the extraction of mandibular premolars and subsequent orthodontic closure of the extraction spaces on the third molar development. A set of 30 consecutive patients' records with bilaterally impacted lower third molars and a history of lower premolar extraction treatment. A control group with lower premolar and third molar extractions was also used. Measurements were made on panoramic radiographs. Retrospective analysis of the collected data indicated a positive influence of lower premolar extraction on the position and inclination of impacted third molars.

Kim *et al*[38], in a retrospective study of the diagnostic records of 157 patients, 105 of which had four premolar extraction-treatment during their active growing stage of development, suggested that there was a clinically significant reduction in the impaction rate of both maxillary and mandibular third molars in these patients in comparison to the non-extraction group.

Janson *et al*[39], comparing the records of two groups of 55 patients in total, in their late adolescence, with and without maxillary premolar extractions, concluded that the number of erupted maxillary third molars was greater in the extraction group. He also claimed that the mesio-distal angulations of the un-erupted molars appeared relatively decreased and, therefore, more favorable to eruption.

De-la-Rosa-Gay *et al*[40] conducted a retrospective study based on data from panoramic radiographs, with the aim not only to assess third molar eruption after second molar extraction orthodontic treatment, but to identify the risk factors of unsuccessful eruptions too. Their sample included 48 patients, treated with fixed appliances (Ricketts or straight-wire techniques), with a median age of 14.8 years. The analysis of their results indicated that maxillary and most mandibular third molars successfully erupted and eventually obtained upright positions. Both late developmental stage of third molars and increased mesial inclination or lack of proximal contact in the beginning of treatment were identified as risk factors for an unsuccessful eruption.

Salehi *et al*[41], assessing the effect of first premolar extractions on third molar eruption, evaluated the clinical records of three groups of subjects: a group with first premolar extractions, a group with no extractions that had received orthodontic treatment and a control group with neither extractions nor orthodontic treatment. According to their results, there was a significant difference in the third molar eruption rates in the extraction (42%), non-extraction (12%) and control (20%) groups. These

findings indicate that first premolar extractions may increase the possibility of third molar eruption.

Jain *et al*[42], in a retrospective study that they conducted with the use of panoramic radiographs also investigated on the effect of first premolar extraction treatment. Their study sample consisted of 50 dental Class Ⅰ patients, between the ages of 11 and 19, half of which had received four first premolar extractions. In addition, no more than the 2/3 of the third molars had been formed in the initial radiographs and extraction spaces had been eliminated in the end of orthodontic treatment. The analysis of the collected data indicated a positive influence of first premolar extractions on the angulation of third molars.

Bayram *et al*[43] investigated the influence of orthodontic treatment involving four first molar extractions on the third molars. It was a retrospective study on panoramic radiographs from 41 patients, with a mean age of 16.6 years of age, 21 of which were treated with extraction of all four first permanent molars. No extraoral forces were used for the treatment of the above patients. According to their conclusions, first molar extractions may considerably increase the eruption space of third molars, whereas they normally have a more favorable effect on the angulation of the maxillary than of mandibular third molars. The main conclusion of this study was that the extraction of first permanent molars considerably reduces the frequency of third molar impactions.

Livas *et al*[44] used for their study lateral cephalometric radiographs from a group of 91 subjects, with a mean age of 13.2 years of age, treated with the orthodontic Begg technique and an initial Class Ⅱ Division 1 malocclusion. The subjects were divided in a group with first molar extractions and a control group with no extractions, which consisted of Class Ⅰ and Class Ⅱ cases. The findings of this study suggest that the position of third molars was significantly improved during orthodontic treatment involving the extraction of first molars.

Gohilot *et al*[45], almost two years ago, published an investigation regarding the impact of first premolar orthodontic extraction treatment on third molars. The study sample included 60 Indian patients, between 14-19 years of age, 30 of which had all first premolars extracted and the rest serving as a control non-extraction group. The root development of third molar roots did not exceed the 2/3 of its full length at the initial radiographs, all patients had been initially diagnosed with a skeletal and dental Class I malocclusion, which meant that they all had been high-anchorage cases during treatment and there were no extraction spaces in the end of treatment. Based on their results, they concluded that premolar extractions had a positive effect on maxillary third molar angulations, whereas they did not notice any difference in mandibular third molars' positions. They also suggest that borderline extraction cases with favorable third molar angulations could benefit by premolar extractions.

Türköz *et al*[46], comparing two groups of 22 non-growing patients, with and without first premolar extractions in a retrospective study, also revealed a positive influence of premolar extraction therapy in the size of retromolar space and a significantly lower impaction rate of third molars in the corresponding extraction group. Mihai *et al*[47] evaluated the panoramic radiographs of 20 initially Class I patients, who had received orthodontic treatment, with and without premolar extractions and the crowns of their third molars had already been formed in the beginning of treatment. The results of this study indicated the third molars with the most favorable positions, were the ones in the mandibles of the extraction group.

Al Kuwari *et al*[48] conducted a cross-sectional radiographic study, using 40 sets of patient records from a university clinic. Half of these patients were orthodontically treated with first premolar extractions. According to their results, orthodontic treatment premolars extraction treatment seems to have improved the angulation of impacted third molars in most cases. Finally, Halicioglu *et al*[49] recently published a large retrospective study of the panoramic radiographs of 294 patients, aged from 13 to 20 years, with at least one permanent first molar extraction. Among other conclusions, they report that the development of the third molars was significantly accelerated on the extraction sites of these patients.

THIRD MOLARS AND MANDIBULAR INCISOR CROWDING

The effect of third molar position and eruption stage on the rest of the dentition has also been the subject of many investigations and is also of great concern to the orthodontists. Especially the role of third molars in the development of a secondary mandibular incisor crowding has been an object of debate for many years.

As early as 1917 Dewey[50], examining the role of third molars in malocclusion, suggested that, in some cases, the mandibular third molars need to create space in the dental arch in order to erupt, causing crowding of the anterior teeth. Since then, numerous investigations have been conducted in an attempt to objectively identify a possible correlation between third molars and mandibular incisor crowding. Bergström *et al*[51] studied 60 subjects with unilateral molar agenesis and noted that there was greater crowding in the quadrants in where third molars were still present, than in those in which third molars they were absent. Vego[52] also found a greater percentage of dental crowding in subjects with erupting third molars in comparison to subjects with congenitally missing third molars.

Sheneman[53], in an investigation of 49 patients for a mean period of 66 mo, concluded that patients with congenitally missing third molars showed comparatively greater dental stability.

Woodside[54], on the other hand, suggested that in cases where mandibular third molars were not present, a more distal settling of the lower dentition occurred in response to growth and soft tissue pressure, implying a passive role for the third molar, which was acting as an obstacle to the settling of the dentition, rather than actively applying pressure to anteriorly positioned teeth.

Lindqvist *et al*[55], in their study, examined a group of 52 patients with bilateral third molar impactions. It was a "split mouth" design study, with extraction of the impacted molars on one side and use of the contralateral quadrant as a control side. Their data indicated the existence of less crowding on the extraction side, in 70% of the patients. Forsberg[56] conducted a study with the objective to identify the relationship between the eruption status of third molars and the relative space in the dental arches. Two groups of 75, in total, adult, non-orthodontic patients were used; one group with patients that had all third molars erupted and another group with all third molars missing due to extraction. The degree of crowding was found to be higher in the first group, although only with a small difference.

Southard *et al*[57] attempted to detect the presence of a mesial force exerted by the unerupted third molars on the rest of the dentition, by measuring and comparing proximal contact tightness before and after bilateral third molar extractions in 20 patients. The authors concluded that the removal of third molars for the relief of "interdental pressure" and, therefore, for the prevention of mandibular incisor crowding could not be supported by the results of their study. Pirttiniemi *et al*[58] examined the effect of impacted third molar extraction on the dental arches in patients with a mean age of 23.2 years. As it is stated in their conclusions, the results of this investigation could not justify the prophylactic removal of third molars, due to the absence of relative evidence regarding their association with undesirable changes in the dental arches.

van der Schoot *et al*[59] conducted an investigation with the aim to determine the relationship between dental crowding and the presence of third molars. Their sample included 99 orthodontically treated patients. According to their results the presence of third molars did not have a clinically significant relationship with the development of post-treatment crowding. Basdra *et al*[60], in an evaluation of 19 patients, who had been recalled in a university dental clinic and had had orthodontic Class Ⅱ treatment with bilateral or unilateral upper second molar extractions, reported that all third molars had erupted in very good positions.

Sidlauskas *et al*[61] investigated in their study the effect of lower third molars in lower incisor crowding, by studying the records (study models and panoramic radiographs) of 91 patients, who had not received orthodontic treatment before the collection of the records. The study group included patients with present, removed or genetically missing third molars. They concluded that the third molars in their study group were not responsible for any considerable difference in the development of lower anterior dental arch crowding. Hasegawa *et al*[62] studied a group of Mongolian subjects, with a mean age of 21 years, in order to evaluate the influence of third molars on lower anterior crowding. The analysis of their data did not reveal any significant relationship between the presence and angulation of mandibular third molars and lower incisor crowding.

Karasawa *et al*[63], in a cross sectional-study, aimed to reveal a correlation between the presence of third molars and mandibular incisor crowding in a large group of 300 subjects, with a mean age of 20.4 years. Their final results revealed no correlation between maxillary or mandibular third molars and the incisor crowding. Nevertheless, in patients that had had orthodontic treatment there was a small correlation, although this result did not reach statistical significance.

Costa *et al*[64] recently conducted a systematic review in order to investigate whether the prophylactic removal of third molars is justified as a treatment option. After filtering of the initial studies with the use of specific eligibility criteria, only 4 papers of medium (3) and low (1) quality and inadequate sample sizes eventually contributed in the analysis. As stated by the reviewers, the currently available data is inadequate for the formation of safe conclusions, which could be used in clinical treatment decisions. However, their results point towards the opinion that prophylactic third molar extraction is unjustifiable.

DISCUSSION

With regards to the possibility of third molar eruption, it seems that most investigators agree on the fact that there is a correlation between third molar impaction and certain skeletal characteristics[7-9,10,12-16,18,20-24,26], although not everyone shares the same opinion[17,19,25]. The common associated factor is the shortage of the available eruption space, due to distally directed eruption of the dentition and/or lack of adequate resorption of the anterior border of the mandibular ramus or compensatory periosteal bone apposition at the posterior outline of the maxillary tuberosity. Another factor that is linked with third molar impaction is the existence of a vertical facial growth pattern, with a vertical direction of condylar growth. Finally, increased third molar angulation is also considered to be significantly linked to third molar impactions.

Concerning the impact of extraction treatment,

the majority of investigators have reported a positive influence of the removal of teeth for orthodontic reasons on third molar position, angulation and/or eruption[3,14,27,28,30,31,33,35-48,60]. The improvement of the position and reduction of the inclination of third molars, although not directly associated with their eruption, seems to be considered as a very positive effect. Many authors also believe that orthodontic borderline extraction cases with favorable initial third molar angulations could benefit from extraction therapy[41,44,47]. In addition, even in the event of a possible future extraction of impacted third molars, a more upright position could facilitate the surgical procedure and minimize possible complications and post-operative complications[3,8,41].

However, it is important to note that there is a variety of confounding factors that could have affected the results of the above studies and were not co-evaluated in most cases, including the age of the patients, their ethnical background, the developmental stage of third molars at the time of the extractions, the initial types of malocclusion and the type of mechanics that were used for the needs of the orthodontic treatment.

Further, concerning the role of third molars in mandibular incisor crowding, it is very interesting to note the differences between the authors' beliefs and results before the after the 1990's. In the chronologically older studies[50-56] third molars seem to be more significantly associated with the occurrence of crowding in the lower arch. In contrast, more recent studies[57-59,61-64] tend to exculpate third molars and characterize their prophylactic extraction as unjustifiable. Nonetheless, both recently conducted systematic reviews on this matter, although clearly not in favor of prophylactic extractions, reported the insufficiency of high quality studies, required for the formation of secure conclusions.

The fact still is that, currently, there is no sound research evidence supporting the prophylactic removal of non-pathological impacted third molars. However, in cases where extraction is indicated, it is preferable third molars to be removed before adulthood in order to decrease the risk of complications[65].

Finally, other important clinical issues, including the impact of orthodontic first molar distalization on third molar position, the effect of third molar eruption stage on the efficiency of first molar distalization, the orthodontic force application on autotransplanted third molars, the orthodontic extraction of third molars in unfavorable positions or in close proximity to the inferior alveolar nerve and the decision of the extraction of third molars before orthognathic surgery, are also still under investigation and are, hopefully, going to be included in other reviews.

CONCLUSION

Despite the fact that the role of third molars has been a subject of research, clinical interest, discussion and dispute for so many years, there is still a lack of scientific evidence from high quality clinical studies on that matter. Several weaknesses have been pointed out, including the heterogeneity of data and the small size of the examined samples. However, the information collected from the studies presented in this review contributes to our knowledge and allows us to create a fuller picture regarding the issues that have been examined.

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Clinical outcomes for Conduits and Scaffolds in peripheral nerve repair

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Abstract

The gold standard of peripheral nerve repair is nerve autograft when tensionless repair is not possible. Use of nerve autograft has several shortcomings, however. These include limited availability of donor tissue, sacrifice of a functional nerve, and possible neuroma formation. In order to address these deficiencies, researchers have developed a variety of biomaterials available for repair of peripheral nerve gaps. We review the clinical studies published in the English literature detailing outcomes and reconstructive options. Regardless of the material used or the type of nerve repaired, outcomes are generally similar to nerve autograft in gaps less than 3 cm. New biomaterials currently under preclinical evaluation may provide improvements in outcomes.

Key words: Plastic surgery; Reconstructive surgical procedures; Nerve tissue; Conduit; Scaffold

Core tip: Nerve autograft is the gold standard for peripheral nerve reconstruction with gap. However, shortcomings of autograft have led researchers to investigate various biomaterials to improve outcomes. Clinical studies of peripheral nerve reconstruction with conduit other than autograft show similar outcomes in gaps less than 3 cm.

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INTRODUCTION

The gold standard of peripheral nerve repair is primary end-to-end coaptation of nerves. Unfortunately, this treatment is not always feasible in clinical situations. Avoidance of tension during repair is the ultimate goal to enhance potential nerve regeneration[1,2]. Prior studies have shown that injury tends to occur when nerves are stretched to greater than 10% of their original length. It may even initiate the process with stretching as little as 4%-6%[3,4]. Negative outcomes have been reported with tension greater than 25 g[5].

Most surgeons do not attempt primary closure when encountering gaps greater than 4 mm[6]. Tensionless closure is paramount to satisfactory clinical outcomes in nerve repair.

Primary treatment for repair of a nerve gap is autologous nerve grafting[7,8]. However, limited availability of donor tissue, sacrifice of a functional nerve, and possible neuroma formation make this option less than ideal[9-11]. Gluck[12] first reported use of a nerve guide in 1880, bridging with a segment of decalcified bone. Other early attempts were equally unsuccessful. In order to overcome these shortcomings, researchers and surgeons continued to improve nerve repair methods. The ideal conduit must be readily available, biocompatible, size matched to the nerve stumps, prevent axonal escape, and prevents ingress of fibroblasts and inflammatory cells. Simultaneously it should allow growth and chemotactic factors to positively influence axonal growth. Also it should prevent compression and injury to the nerve once healed. The conduit should be flexible, yet resilient enough to resist collapse[13]. Currently, such a conduit remains unavailable. Ongoing studies continue to improve the qualities of available biomaterials. Our goal is to present a survey of clinical studies published in the English literature detailing outcomes and reconstructive options.

REGENERATION BY CONDUIT

Williams *et al*[14] demonstrated the basic steps of nerve regeneration with an inert silicone conduit. In the immediate postoperative period, a fluid containing proteins, clotting factors and growth factors fills within the conduit. By 1 wk, a longitudinally oriented fibrin matrix develops. In the second week, fibroblasts, Schwann cells, macrophages, and endothelial cells enter the matrix. At the same time, axons from the proximal nerve cone extend forward. By four weeks the nerve cone has extended about 10 mm.

MATERIALS

Silicone

Silicone is a non-resorbable, nonporous, biologically inert material. Silicone in medical devices and implants are clinically ubiquitous. Since silicone is non-resorbable, presence of conduit material can lead to compression and decreased axonal conduction[15-17]. For this reason, the tubing is frequently removed[18]. With the advent of resorbable synthetic grafts and processed allografts, clinical utilization of silicone conduits have declined.

Lundborg *et al*[19] first reported in a prospective randomized study the clinical use of silicone tubes in peripheral nerve reconstruction. He reconstructed median nerve gaps of 3 to 5 mm. He then compared silicone conduit to standard repair. He found no differences in motor function. Patients experienced improved sensory recovery within the silicone group. Braga-Silva[20] reported a case series of 26 patients with median, ulnar, or median and ulnar nerve injury. Patients presented with a nerve gap ranging from 2.5 to 5.5 cm. While motor scores for each patient were not published, size of the nerve gap negatively correlated with motor function outcomes.

Expanded polytetrafluoroethylene

Expanded polytetrafluoroethylene (ePTFE) is another nonresorbable, biologically inert material. It is commercially available as Gore-Tex (W.L. Gore and Assoc., Flagstaff, AZ). Like silicone, reports of ePTFE have declined over the years.

Stanec *et al*[21] first reported clinical use of ePTFE in 43 patients exhibiting median and ulnar nerve gaps ranging from 1.5 to 6 cm. Patients with smaller gaps (up to 4 cm) had significantly improved outcomes *vs* larger gaps (78.6% *vs* 13.3% functional recovery).

Pogrel *et al*[22] utilized ePTFE conduits for reconstruction of lingual and inferior alveolar nerve (IAN) injuries in 5 patients. Patients with negative outcomes had nerve gaps greater than 1.0 cm. Pogrel *et al*[22] reported their series of 6 patients with lingual or IAN continuity defects greater than 1 cm. Mixed results were reported.

VEIN

Vein grafts are among the first non-neural biological conduits used for peripheral nerve Usually they are harvested from the dorsum of the hand during digital, median, or ulnar nerve repair. During the regeneration period, they were found to be at risk for kinking or collapse[23-25].

Wrede[26] recorded the first successful use of a vein graft. He repaired a median nerve defect with a 45 mm graft. Platt[27] (1919) also described bridging nerve grafts with autogenous vein. It failed to produce functional return of the musculospiral nerve[27]. Gibb[28] reported a single case of functional restoration using a vein conduit to reconstruct a 1 cm facial nerve gap. It was not until several animal studies demonstrated efficacy that further clinical studies were explored[29,30]. Walton *et al*[25] reported return to normal two point discrimination (2PD; less than 4 mm) in 50% of patients undergoing repair of digital nerves. Nerve gaps ranged from 1 to 3 cm. Poor outcomes were associated with larger gaps. In 1990, Chiu *et al*[24] reported a series of 15 repairs on patients receiving vein grafts for "nonessential" peripheral nerve gaps up to 3 cm. After an average follow-up of 27 mo, the cohort receiving vein graft repair had similar outcomes to autologous nerve graft. However it was inferior to direct repair cohort. Tang *et al*[23] reported 61% good or excellent outcomes in 15 patients undergoing digital nerve repair, with

gaps ranging 0.5 to 5.8 cm[23]. Patients generally had favorable outcomes when gaps were less than 3 cm, thereby corroborating the results from Chiu *et al*[29]. Two years later, Tang published outcomes in median and radial nerve vein grafts. In this study, he inserted nerve fragments from the proximal nerve stump into the vein lumen. His data suggested positive outcomes could be achieved with this technique for gaps up to 4.5 cm[31].

Pogrel *et al*[32] reported a series of 16 patients treated for lingual or IAN nerve defects, ranging from 2 to 14 mm. Using saphenous vein or facial vein, he found that negative outcomes were associated with gaps greater than 5 mm. The author discussed that nerves of trigeminal origin have had poorer outcomes versus other peripheral nerves. It is likely the cause of difficulty in repair of such small gaps (see below).

COLLAGEN

Collagen is a naturally occurring, resorbable structural protein. Purified bovine collagen, the most common source for collagen conduits, has low immunogenicity. Resorption rate can be controlled by the degree of crosslinking induced during preparation. Depending on fabrication method, degradation occurs from 1 to 48 mo[33,34]. Furthermore, preclinical studies have demonstrated that collagen conduits enhance growth and differentiation of many cell types. It is flexible yet durable. This increases its facility as a conduit material[35]. Finally, its permeability allows for diffusion of chemotactic and neurotrophic agents in the extracellular fluid. This type of conduit is commercially available under the name NeuraGen® (Integra LifeSciences, Plainsboro, NJ). Conduit sizes range from 1.5 to 7 mm diameter and are 2 or 3 cm long. Neuromatrix® and Neuroflex® (Collagen Matrix, Inc) are also Type I collagen conduits. No published studies are currently available evaluating its clinical efficacy.

In 2005, Taras *et al*[36] reported the use of commercially available type I bovine collagen in the repair of a variety of peripheral nerves[36]. A prospective series of 22 digital nerve repairs using NuraGen® achieved excellent or good sensory outcomes in 15 of 22 digits. They excluded nerve gaps greater than 20 mm[37].

Ashley *et al*[38] reported treatment of brachial plexus birth injuries with nerve gaps less than 2 cm using collagen conduits. Four of the five patients had favorable outcomes at 2 years postoperative. Lohmeyer *et al*[39] reported a case series of 6 patients undergoing repair of nerve gaps in digital and palmar nerves up to 18 mm. Two-thirds of the patients had excellent 2PD at 12 mo postoperative. They extended follow-up with nine of twelve patients achieving excellent or good sensory scores at 12 mo follow up[40]. Bushnell *et al*[41] reported a series of 12 patients undergoing digital nerve repair for gaps ranging from 1 to 2 cm. Most (88%) had good or excellent 2PD after at least 1 year. In a larger study of 126 nerve injuries in 96 patients, Wangensteen *et al*[42] reported their experience using NeuraGen®. Mean nerve gap was 12.8 (range 2.5 to 20 mm). Overall, nerve function recovery was only 43%. A variety of nerves were repaired, and seven surgeons were involved in the study. Haug *et al*[43] added 45 digital nerve repairs with type Ⅰ collagen to the body of literature. Mean defect was 12 mm (range 5 to 26 mm). All sensory measures improved over 3-, 6-, and 12-mo follow-up interval.

Farole *et al*[44] reported their experience with the NeuraGen® conduit for challenging lingual and IAN repair. In their study, all patients underwent neurolysis with or without resection of neuroma (if present) and placement of the collagen conduit as a "cuff" over coapted nerve ends. They chose this technique to prevent axonal escape, minimize scar ingrowth and nerve entrapment, and to concentrate growth factors at the repair site. Eight of nine patients had improvement after at least one year.

Kuffler *et al*[45] reported a single case of ulnar nerve reconstruction after 3 years. Nerve gap was 12 cm. Using a sheet of collagen, they fashioned a custom-sized conduit. Then they filled it with autologous platelet-rich fibrin. By three months, the patient experienced improvement in neuropathic pain. By 2 years the patient no longer required analgesics. Within 1.5 years, the patient had 4 mm 2PD. Motor function had returned by 2 years. This study showed promising results in the reconstruction of large caliber, mixed function peripheral nerves using collagen conduits. Dienstknecht *et al*[46] recently published a series of 9 patients undergoing median nerve repair. All gaps were 1 to 2 cm long and repaired within 24 h of injury. Average return to work was 8 wk (range 1 to 17). Motor, sensory, pain, and disability scores were satisfactory in 8 of the 9 enrolled patients.

DECELLULARIZED NERVE ALLOGRAFT

Nerve allograft is an alternative to nerve autograft for repair of gaps, but requires the additional administration of immunosuppression for 18 mo. Using a decellularized nerve allograft preserves the three-dimensional collagen scaffolding of a nerve while avoiding immunosuppression[47]. This scaffolding promotes cell migration, nerve fiber elongation, and diffusion of growth factors[48,49]. Laminin, also present, facilitates axonal outgrowth[50]. Human decelullarized nerve is commercially available as Avance® (AxoGen, Inc, Alachua, FL). Available grafts encompass lengths ranging 15 to 70 mm and diameters between 1 and 5 mm.

Karabekmez *et al*[51] were the first to publish clinical data on Avance®. Ten digital nerve repairs were included in the study. Gap length ranged from 0.5 to 3 cm. After an average follow-up of

nearly 9 mo, static 2PD was 5.50 mm and moving 2PD was 4.4 mm. Brooks *et al*[52] then reported a multicenter prospective study with Avance®. These authors examined repair of sensory, motor, and mixed nerves. Of the patients that met follow-up requirements, acceptable outcomes were achieved in every group. Sensory, mixed, and motor nerves recovered at 88.6%, 77%, and 85.7%, respectively. With regards to nerve gap length, short (5 to 14 mm) recovered at 100%, medium (15 to 29 mm) recovered at 76.2%, and long (30 to 50 mm) recovered at 90.9% (mean follow up 265-279 d). Meaningful recovery was defined as S3-4 or M3-5 on the Medical Research Council Classification. Guo *et al*[53] supplemented previous digital nerve repair data with their own case series. Their five patients had a mean nerve gap of 22.8 mm and a mean follow up of 13.2 mo. At the time of final follow up, static 2PD averaged 6 mm and monofilament test ranged positive for monofilaments 4.31 to 4.56. Recently, Taras *et al*[54] reported 18 digital nerve gap repairs treated with processed allograft[54]. Average gap length was 11 mm (range 5 to 30 mm). Overall, 83% of patients had good or excellent results.

Shanti *et al*[55] reported a single case using Avance® for repair of an iatrogenic IAN injury. They did not record the length of the nerve gap. However, they did report improvements in sensory testing at 5 mo postoperative.

POLYGLYCOLIC ACID

Polyglycolic acid (PGA) is a bioabsorbable substance initially used for suture material or mesh[56,57]. Mean resorption time is 90 d[58]. Typically it appears as a tight-weave mesh rolled tube. Its pores are small enough to permit nutrients while impeding invasion by fibroblasts[59]. A tube of PGA is more expensive than suture material used in standard nerve repair[59]. Additionally, PGA is at risk for extrusion prior to complete resorption[59]. PGA is commercially available as Neurotube® (Synovis Life Technologies, Inc.), which has an internal diameter of 2.3, 4, or 8 mm and 2 or 4 cm length.

Initial clinical use of PGA was by Mackinnon *et al*[60] in 1990. Repairing nerve gaps ranging from 0.5 to 3.0 cm, they were able to achieve excellent or good 2PD in 86% of the 15 patients undergoing reconstruction. Weber *et al*[59] (2000) reported his randomized prospective study of 136 nerve injuries treated with either autologous graft or PGA conduit. Although the mean gap length was greater in the PGA conduit group, there was no difference in either moving or static 2PD between the two groups. For either small (less than 4 mm) or large (greater than 8 mm) gaps, the PGA conduit group had better sensory outcomes. Kim *et al*[61] reported successful treatment of a plantar neuroma in an 11-year-old male using a PGA conduit to span a 2.0 cm defect. Pain from the neuroma resolved. Normal sensation returned by 8 mo. In 2005, Navissano *et al*[62] reported their case series of seven patients treated with PGA conduits for traumatic facial nerve terminal branch injuries. Five of seven patients had good or excellent recovery of motor function compared to contralateral side. Nerve gap ranged from 1 to 3 cm.

Battiston *et al*[63] prospectively compared Neurotube® repair of digital nerves to patients treated with vein-muscle grafts. Even though nerve gaps were larger in the Neurotube® group, there were no significant differences in sensory outcomes between the two cohorts. Most (76.9%) of the muscle-vein group had very good results, as did 76.5% of the Neurotube group. Rinker *et al*[64] performed a similar study. They prospectively compared Neurotube repair ® to vein graft repair. PGA conduit group was similar to the vein conduit cohort, including length of nerve gap (9.1 mm mean *vs* 10.3 mm, respectively). There was no significant difference between the cohorts with regards to sensory outcomes. This was true for short (less than 10 mm) or long (greater than 10 mm) gaps.

Rosson *et al*[65] reported 6 cases of PGA used to repair motor nerves. One patient had accessory nerve injury. The remainder had median or ulnar nerve injuries. Nerve gaps ranged from 1.5 to 4 cm (mean 2.8). Follow up ranged from 4 mo to 5.5 years. All patients achieved significant improvement in motor function (rated M3 or greater).

PGA-COLLAGEN

PGA-collagen conduits are composed of a PGA tube coated with 1% amorphous collagen solution. It is then filled with collagen sponge[66]. Fibers usually undergo crosslinking to prevent rapid resorption. To date, this construct is not yet commercially available. Japan was the site of clinical studies of PGA-collagen[67]. Initially it was initially used for reconstruction of intrapelvic nerves damaged during rectal cancer extirpation. Clinical improvement in the patient prompted continued use of the conduit.

In 2004, Nakamura *et al*[67] reported 2 cases using PGA-collagen conduits. The first patient had a 20 mm digital nerve gap. Following treatment, function within normal range by 4 mo. The second patient had a 65 mm superficial peroneal nerve defect with normal sensation by 3 mo. The same group later reported their experience with treatment of Complex Regional Pain Syndrome type Ⅱ[68]. In the two case reports, they described successful resolution of an otherwise challenging clinical entity. It tends to follow a vicious cycle of relapsing pain due to nerve sprouting after injury or resection. The authors theorized that placing the cut ends into the conduit would prevent nerve sprouting and guide the nerve cone to the distal stump. In 2007, Inada *et al*[69] also reported their experience with repair of a frontal branch of the

facial nerve using the same type of conduit, bridging gaps measuring 11 to 30 mm[69]. In both patients, functional recovery was noted by 5 mo. Recently, they also reported chorda tympani nerve reconstruction using their PGA-collagen construct[70]. Average nerve gap was 7 mm among the three patients studied. Electrogustometry measurements returned to normal limits by two weeks postoperative. Dysgeusia resolved between 2 wk to 3 mo.

POLY (DL-LACTIDE-ε-CAPROLACTONE)

Poly (DL-Lactide-ε-Caprolactone) (PLC) is another synthetic bioabsorbable material. Degradation occurs at 1 year. Initial constructs had thicker walls that caused swelling. This negatively impacted nerve healing. Thinner-walled tubes tended to collapse[71]. Increasing the lactide content to 65% reduced the amount of swelling, but lost mechanical strength after 10 wk of implantation[72]. Clinically available PLC may be too rigid for small needles, requiring some softening in water before use[71]. PLC is also transparent, facilitating placement of nerve stumps. It is commercially available by the name of Neurolac® (Polyganics BV, Groningen, Netherlands). They offer 1.5 to 10 mm inner diameters and a length of 3 cm.

After publishing initial clinical studies in 2003, Bertleff *et al*[73] published their follow up findings from a blinded, randomized multicenter trial comparing standard treatment to PLC in repair of peripheral nerve defects of the hand in 54 patients. In treatment of nerve gaps less than 20 mm, they found no significant difference in sensory outcomes compared to controls. Follow up was 12 mo.

FUTURE DIRECTIONS

In addition to the above clinically-tested materials, there is a multitude of materials undergoing preclinical evaluation. These include non-mammalian biodegradable polymers, artificial biodegradable polymers manufactured with electrospinning, conducting polymers, and combinations of the above with Schwann-like neural stem cells and mesenchymal stem cells. Conduits seeded with stem cells, stem-like cells, or support cells theoretically improve nerve regeneration through delivery of growth factors and neurotropic factors into the conduit lumen. Several excellent reviews documenting these advances have been published. They are beyond the scope of the current discussion[74-80].

CONCLUSION

While preclinical studies are essential to bringing new technologies to reconstructive surgeons, further in depth clinical evaluation of materials is warranted. Almost all of the published studies consist of small case series. Outcomes measures are inconsistent from study to study. Furthermore, nerve type, cause of injury, and gap size are extremely variable, making comparison of repair materials and technique difficult. Nevertheless, the above studies suggest that small gaps up to 3 cm can be repaired with available conduits with outcomes similar to nerve autograft. Efficacy of bridging longer gaps with available conduits has yet to be demonstrated. Also, several roadblocks prevent developing technologies from becoming clinically available. Feasibility of stem cell harvest and cost of cutting-edge biomaterials are problematic. These will further delay human studies for these promising therapies.

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MINIREVIEWS

Role of coronary physiology in the contemporary management of coronary artery disease

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Abstract

Coronary artery disease (CAD) remains the leading cause of death worldwide with approximately 1 in 30 patients with stable CAD experiencing death or acute myocardial infarction each year. The presence and extent of resultant myocardial ischaemia has been shown to confer an increased risk of adverse outcomes. Whilst, optimal medical therapy (OMT) forms the cornerstone of the management of patients with stable CAD, a significant number of patients present with ischaemia refractory to OMT. Historically coronary angiography alone has been used to determine coronary lesion severity in both stable and acute settings. It is increasingly clear that this approach fails to accurately identify the haemodynamic significance of lesions; especially those that are visually "intermediate" in severity. Revascularisation based upon angiographic appearances alone may not reduce coronary events above OMT. Technological advances have enabled the measurement of physiological indices including the fractional flow reserve, the index of microcirculatory resistance and the coronary flow reserve. The integration of these parameters into the routine management of patients presenting to the cardiac catheterization laboratory with CAD represents a critical adjunctive tool in the optimal management of these patients by identifying patients that would most benefit from revascularisation and importantly also highlighting patients that would not gain benefit and therefore reducing the likelihood of adverse outcomes associated with coronary revascularisation. Furthermore, these techniques are applicable to a broad range of patients including those with left main stem disease, proximal coronary disease, diabetes mellitus, previous percutaneous coronary intervention and with previous coronary artery bypass grafting. This review will discuss current concepts relevant to coronary physiology assessment, its role in the management of both stable and acute patients and future applications.

Key words: Ischaemia; Coronary physiology; Coronary flow reserve; Fractional flow reserve; Coronary artery disease

Core tip: Coronary artery disease remains the leading cause of death worldwide. There is increasing evidence to suggest that the use of invasive coronary angiography alone may not reliably identify all lesions associated with haemodynamic compromise. Technological advances have enabled the measurement of a number of coronary physiological indices which when incorporated into routine practice are associated with improved outcomes, reduced risks and greater economy. This review will

discuss current concepts relevant to coronary physiology assessment, its role in the management of both stable and acute patients and future applications.

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INTRODUCTION

Coronary artery disease (CAD) is the leading cause of death worldwide[1] with approximately 1 in 30 patients with stable CAD experiencing acute myocardial infarction (AMI) or cardiovascular death each year[2]. The presence of resultant myocardial ischaemia and its extent has been shown to confer increased risk of adverse outcomes[3-6]. With an increasing burden of atherosclerotic coronary disease and the associated high event rate, there is a need to identify both patients at highest risk with most to benefit from revascularisation strategies and also those that would be best managed by a conservative approach to improve clinical outcomes and minimise exposure to procedural risks.

Prevention by risk factor control and optimal medical therapy (OMT) including aspirin[7], beta-blockers[8], statins[9] and angiotensin converting enzyme inhibitors forms the cornerstone of the management of patients with stable CAD[10,11]. However, a significant number of patients present with myocardial ischaemia refractory to OMT and subsequently undergo coronary revascularisation by percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) with the aim of reducing ischaemic burden and therefore risk[12].

Historically, visual assessment of coronary lesions by invasive coronary angiography in isolation has been used to determine the severity of coronary lesions in both stable and acute settings. It is increasingly clear that this approach fails to accurately and consistently identify the haemodynamic significance of lesions, especially those that are "intermediate" in severity[13] and that revascularisation based upon angiographic appearances may not reduce coronary events above OMT alone[14]. Recent advances in technology and understanding of coronary physiology have resulted in its central role in the assessment of patients in the catheterization laboratory and their optimal management[15,16]. This review will discuss current concepts relevant to coronary physiology assessment, its role in the management of patients and possible future applications.

CORONARY PHYSIOLOGY

Technological advances have enabled the measurement of a number of physiological indices including the fractional flow reserve (FFR), the index of microcirculatory resistance (IMR) and the coronary flow reserve (CFR). The advantages and disadvantages of each of the coronary physiology indices is summarised in Table 1.

FFR

FFR is the ratio of myocardial blood flow in a stenosed coronary artery at maximal hyperaemia in comparison to normal (proximal) myocardial flow. It quantifies the pressure drop measured across a coronary artery stenosis[17] and therefore the physiological significance of the lesion. The pressure drop is directly proportional to stenosis length, inversely proportional to lumen cross-sectional area and related to the square of the blood velocity. FFR is thus related to both lesion morphology and the volume of viable subtended myocardium and is independent of changes in haemodynamic conditions[18].

CFR

CFR is the ratio of hyperaemic to resting coronary flow and incorporates both the epicardial and microvascular circulations[19]. A value of < 2.0 is correlated with stenosis severity[20].

IMR

IMR is a measure of true microcirculatory resistance and is calculated by measuring the distal arterial pressure at hyperaemia divided by the inverse of the transit time. The IMR is not influenced by the presence or absence of epicardial artery stenosis[21]. There is no absolute validated "normal" value but a cut-off value of 32 units has been shown to be predictive of myocardial recovery following AMI[22].

Technique

To measure the FFR, a wire with a distal pressure sensor is advanced into a guiding coronary artery catheter, the pressure is equalised, and then passed distal to the coronary stenosis of interest (Figure 1A). The aortic pressure (Pa) is measured from the guide catheter and the distal pressure (Pd) from the pressure sensor distal to the stenosis (Figure 1B). To calculate the FFR, hyperaemia is achieved by the administration of intravenous (140 mcg/kg per minute) or intracoronary (20-50 mcg) adenosine and is the ratio of hyperaemia Pd/Pa (Figure 1C). Other hyperaemic stimuli can be used but adenosine is the most widely validated.

In the measurement of IMR and CFR, the shaft of the pressure wire is used to detect changed in the temperature-dependent electrical resistance and thus acts as a proximal thermistor. The sensor at the end of the wire is used to simultaneously measure pressure and temperature at the distal end of the artery. Therefore, the transit time of room-temperature saline injected through the guiding

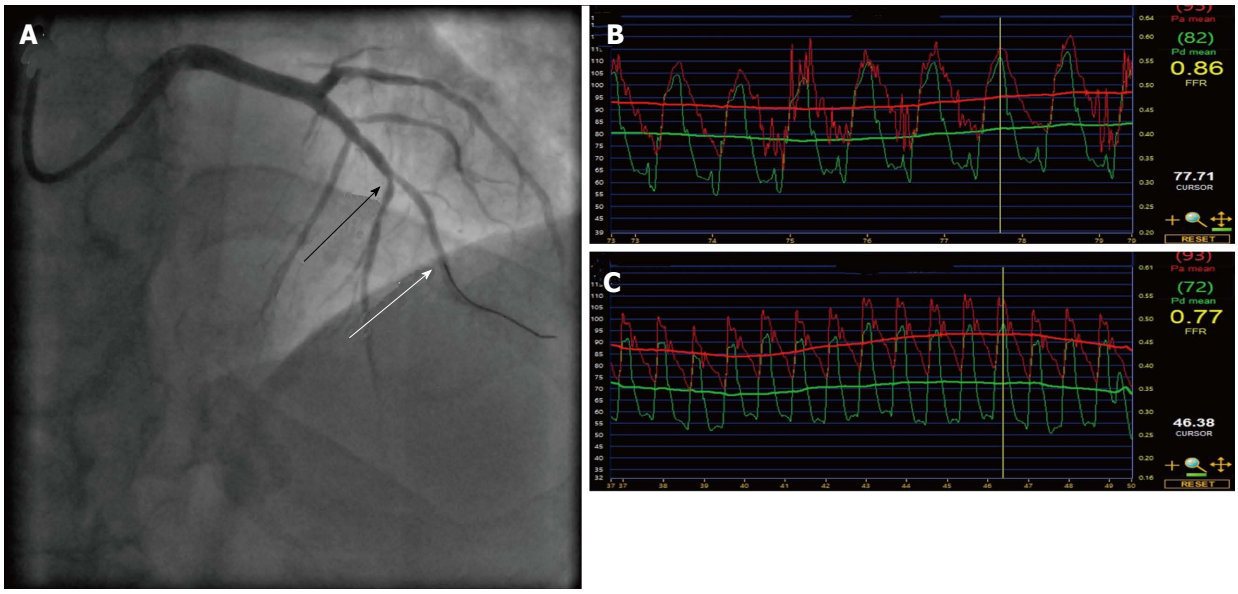

Figure 1 Fractional flow reserve of an "intermediate" lesion in the left anterior descending artery. A: Fluoroscopic image obtained in the right anterior oblique projection demonstrating an angiographically intermediate stenosis (black arrow) and a pressure wire in-situ (white arrow); B: Pressure trace demonstrating a fractional flow reserve (FFR) of 0.86; C: Pressure trace demonstrating a FFR of 0.77 at maximal hyperaemia that is positive. The patient then proceeded to successful percutaneous coronary intervention of the LAD.

coronary artery catheter can be calculated using a thermodilution technique[23]. The initial transit time is recorded (T_{mn}) following three injections of room-temperature saline. Following hyperaemia, three further injections of room-temperature saline are administered and hyperaemia T_{mn} is measured. The thermodilution CFR is calculated by dividing the resting T_{mn} by the hyperaemic T_{mn}. The IMR is calculated as the distal coronary pressure (Pd) at maximal hyperaemia divided by the inverse of the hyperaemic T_{mn}.

MYOCARDIAL ISCHAEMIA

Many studies have demonstrated that the presence and extent of myocardial ischaemia is closely related to adverse clinical events[5,6,24] with the annual rate of cardiac death and AMI positively correlated with the extent of myocardial ischaemia[24]. Coronary revascularisation has been demonstrated to be beneficial in individuals with moderate and severe ischaemia, with OMT being optimal in patients with mild or no ischaemia[25,26]. In patients with demonstrated extensive ischaemia (> 20% myocardium), an early revascularisation strategy (as opposed to OMT alone) is associated with a 30% reduction in risk of all-cause death[25].

The identification of the presence and extent of myocardial ischaemia in patients presenting with stable CAD is critical to their optimal management. Myocardial perfusion imaging is the most commonly used modality however stress echocardiography and magnetic resonance imaging are increasingly being employed.

There is some evidence that an ischaemia-guided revascularisation strategy is associated with improved prognosis and outcome[27,28]. In patients that underwent myocardial perfusion imaging prior to revascularisation (PCI or CABG) and then in the follow up period, the incidence of patients with worsening ischaemia (> 5% of total myocardium) was more common in patients treated medically in comparison to patients who underwent revascularisation (PCI or CABG) and was an independent predictor of adverse outcomes[29].

CORONARY PHYSIOLOGY AND STABLE CORONARY ARTERY DISEASE

PCI reduces mortality in patients with acute coronary syndromes[30], however, in patients presenting with stable CAD, PCI results in an improvement of angina symptoms alone without a mortality advantage in comparison to OMT alone[14]. These results may be a consequence of sub-optimal patient selection due to the sole reliance of visual assessment of coronary lesion by invasive coronary angiography to determine the severity of disease with no information as to the haemodynamic significance of these lesions[31,32]. This can result in inappropriate coronary revascularisation with little benefit and potential procedural risk.

The severity of a lesion (and resultant extent of myocardial ischaemia) is dependant on a number of factors including the severity of luminal narrowing, lesion length and extent of subtended myocardium. As discussed in the previous section, whilst non-invasive techniques can be employed to ascertain

Table 1 Advantages and disadvantages of the different coronary physiology indices

	Advantages	Disadvantages
FFR	Clear "cut-off" value	Requires administration of vasodilator
	Clinically validated	Risk of coronary artery injury
	Can be used in a wide range of patients	Relatively expensive
	Accounts for collateral circulation	
IMR	True measure of microcirculatory resistance independent of epicardial coronary disease	Requires administration of vasodilator
		The full extent of clinical utility is currently unknown
	A tool to potentially predict prognosis in acute patients	
CFR	A tool to potentially predict prognosis in acute patients	Value is affected by both epicardial disease and microvasculature
		The full extent of clinical utility is currently unknown
		Influenced by hemodynamics

FFR: Fractional flow reserve; IMR: Index of microcirculatory resistance; CFR: Coronary flow reserve.

the extent of myocardial ischaemia, many patients present to the cardiac catheterization laboratory without having undergone such assessment and indeed, in the setting of multi-vessel disease, non-invasive stress tests are often not able to definitively detect and localise ischaemia[33]. Furthermore, if a non-invasive image approach is taken, a positive test will result in repeat catheterization for PCI - thus further subjecting a patient to procedural complications, delaying revascularisation and being less economical. The use of coronary physiology provides the unique ability to gain immediate information with regards to the haemodynamic significance of specific coronary lesions in patients already in the cardiac catheterization laboratory attending for coronary angiography and identify those at highest risk who are most likely to benefit from PCI.

In stable CAD, CFR decreases as stenosis severity increases. When compared to non-invasive parameters a value of < 2.0 has been shown to correlate with significant ischaemia[20]. However, because CFR takes account of both epicardial and microvascular circulations, this measure can be influenced by exogenous factors[19] and therefore due to confounding factors is no longer used for stenosis assessment[34].

In the setting of stable CAD, the IMR has recently been shown to be independent of the severity of epicardial stenosis when collateral coronary flow is accounted for[35]. IMR may however, play a role in predicting outcome following elective PCI, with a high IMR pre-PCI predicting peri-procedural myocardial infarction following PCI[36].

FFR is a highly reproducible technique and is insensitive to external factors such as changes in haemodynamics[18]. The normal FFR is 1 with a value of $\leqslant$ 0.75 associated with ischaemia[37] and $\geqslant$ 0.8 not associated with significant ischaemia[38]. There is therefore a "grey zone" of between 0.75-0.8, however the majority of clinical studies to date have adopted a lower normal value of 0.8[12] to define significant ischaemia.

As opposed to relying solely on angiographic appearances, a FFR guided strategy has been shown to identify patients who would most benefit from coronary revascularisation. In the DEFER (deferral versus performance of PCI of non-ischaemia-producing stenoses) study[39], in patients with single-vessel coronary disease and a measured FFR $\geqslant$ 0.75, deferral of PCI was associated with similar event free survival in both OMT and PCI groups at five years[40].

In patients presenting with multi-vessel coronary disease, the clinical utility of myocardial perfusion imaging has been doubted. The technique measures relative differences (normal *vs* abnormal) in myocardial perfusion between coronary artery territories. Thus, in multi-territory ischaemia, the relative differences may be less pronounced resulting in "balanced ischaemia" even in the presence of significant ischaemia as determined by FFR. The FAME study[12] (FFR *vs* angiography for multi-vessel evaluation trial) which investigated patients with multi-vessel coronary disease, supported an FFR-guided strategy in comparison to angiography alone with an associated reduction in mortality or MI at 2 years[15]. This benefit was also found to be true when compared to contemporary OMT in the more recent FAME Ⅱ study[16] that indicated that an FFR-guided strategy resulted in a lower rate of urgent revascularisation.

FFR has been shown to be reproducible both in singe and multi-vessel coronary disease. However in certain instances, caution should be taken in interpreting coronary physiology parameters.

Left main stem disease

In patients presenting with left main stem disease, FFR has been shown to be useful in managing revascularisation strategies[41]. This is also true of left main stem disease and concomitant downstream stenosis if the pressure wire is placed in a non-stenosed downstream vessel and the other vessel does not have a critical proximal stenosis[42].

Post PCI

FFR following PCI has been shown to predict

outcome. Post PCI FFR was found to be the strongest predictor of major adverse cardiovascular events at 6 mo[43]. FFR has also been utilised following bifurcation stenting, illustrating that even in the presence of appearances in keeping with severe pinching of side branches, FFR was rarely $\leqslant$ 0.75 and therefore of no haemodynamic significance[44].

Myocardial scar

Following AMI, irreversibly injured myocardium is replaced by scar tissue that results in a reduction in the microcirculation to this territory. FFR in this context can therefore still be used to guide future management strategies, with the value representing viability of the subtended myocardium, but after an appropriate interval to allow for myocardial healing following AMI to ensure adequate hyperaemia[45].

Grafts

FFR can also safely be used in patients with previous CABG. In an observational study, patients with intermediate stenoses in both arterial and vein graft conduits that were managed by adopting a FFR-guided PCI strategy suffered significantly lower major adverse clinical endpoints as compared to an angiography guided group[46].

Diabetes mellitus

FFR depends upon the vasodilatative capacity of the coronary system and therefore achieving maximal hyperaemia. Patients with diabetes mellitus, suffer abnormalities in microvascular function with altered vasodilatative capacity and increased vascular resistance. Whilst caution should be taken in when using FFR in this patient group[47], a recent study comparing FFR in diabetic and non-diabetic patients has shown that FFR appears to be accurate and applicable in this patient group[48].

These studies highlight the critical role played by coronary physiology in identifying haemodynamically significant coronary stenoses that may benefit from revascularisation, and allow targeted vessel specific treatment beyond the angiographic appearances. The concept of the functional as opposed to the anatomical SYNTAX score appears to stratify patients appropriately to CABG or PCI or patients that would be best managed by OMT. FFR is broadly applicable to all patient groups and is associated with improved outcomes.

CORONARY PHYSIOLOGY AND ACUTE CORONARY SYNDROMES

In the setting of AMI, myocardial inflammation resulting in oedema can result in blunting of the hyperaemic response in the microcirculation resulting in falsely high FFR values, however coronary physiology parameters can potentially still be useful in guiding the management of this patient group.

The IMR when measured in the setting of primary PCI has been shown to correlate with the extent of microvascular obstruction and independently predicted left ventricular systolic function and infarct volume[22,49] and thus provides important prognostic information in this patient group. The clinical utility of this approach, however, is presently unknown.

The thermodilution CFR when measured in the first day after primary PCI also offers important prognostic information with a significant decrease in CFR in patients with impaired left ventricular systolic function. Conversely a greater increase in CFR by day 1 was associated with a higher salvage index[50].

A significant number of patients presenting with acute coronary syndromes also have visually severe "non-culprit" epicardial artery lesions. FFR of "non-culprit" lesions has been shown to be reliable[51] and has been used to guide revascularisation of these lesions. A large prospective multicentre randomised trial is currently underway to investigate the utility of this approach further[52].

LIMITATIONS

The adjunctive beneficial role that coronary physiology plays in the management of CAD has been discussed thus far. There are however, some limitations. The possibility of false negative or false positive results does exist, for example if maximal hyperaemia is not achieved or if instrumentation of the coronary artery induces coronary artery spasm. There is a risk of coronary artery injury (perforation or dissection) with instrumention of the artery to obtain measurements. Finally, there is an additional economic cost when adopting coronary physiology into routine clinical practice with the cost of pressure wires, cost of adenosine, and extra cardiac catheterization laboratory time required. These have all currently limited widespread uptake of these techniques.

FUTURE DIRECTIONS

A current limitation to invasive coronary physiology techniques is the need to induce maximal hyperaemia with agents such as adenosine. Patients may have contraindications to this agent, and additionally there is a time and cost implication. More recently an adenosine independent index of stenosis severity - the instantaneous wave-free ratio (iFR) has been developed[53]. The accuracy of this ratio in comparison to FFR has been shown to be approximately 80%[54] and outcome data from the on-going DEFINE-FLAIR (functional lesion assessment of intermediate stenosis to guide revascularisation) study[55] are awaited to ascertain if this index can be used routinely in clinical practice.

In the future, there may be non-invasive anatomical

and functional imaging surrogates for FFR. Current anatomical imaging modalities [*e.g.*, computed tomography (CT)] correlate poorly with lesion haemodynamic significance and do not capture information related to translesional energy/pressure losses[56]. Newer techniques including CT myocardial perfusion[57], the measurement of contrast gradients in conventional CT angiography[58] and the use of three-dimensional luminal anatomy are currently being evaluated to investigate if they correlate with FFR values for the evaluation of coronary stenoses[59]. Current technologies however have not been shown to equal the sensitivity and specificity of FFR[60].

DISCUSSION

The integration of invasive coronary physiology measurements into the routine management of patients presenting to the cardiac catheterization laboratory with CAD represents a critical adjunctive tool in the optimal management of these patients. The use of FFR can identify patients that would most benefit from revascularisation either by PCI or CABG and importantly also highlights patients that would not gain benefit and therefore reducing the likelihood of adverse outcomes associated with coronary revascularisation. In the setting of acute coronary syndromes, the use of IMR and CFR provides important information with regard to outcome and myocardial salvage, although the clinical value of these measures remains uncertain. The interpretation of the described coronary physiology indices is now essential in current interventional cardiology practice and is represented by current training medical curricula in this sub-specialty field.

The use of newer techniques to derive FFR– both invasively that do not depend on the administration of agents to induce hyperaemia and non-invasive functional imaging may result in coronary physiology parameters playing an even more central role in the future.

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MINIREVIEWS

Review on microbiota and effectiveness of probiotics use in older

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Abstract

The aim of the present systematic review is to summarize the existing knowledge about the human microbiota in the elderly and the effects of probiotics in elderly population. The elderly subjects, compared to adult population, show a reduction in the diversity of the microbiota, characterized by a large interindividual variability, with lower numbers of *Firmicutes*, *Bifidobacteria*, *Clostridium cluster* XIV, *Faecalibacterium Prausnitzii*, *Blautia coccoides-Eubacterium rectal* and higher presence of *Enterobacteriaceae* and *Bacteroidetes*. These differences of the intestinal microbiota of the elderly may not necessarily be caused by aging, but they could be associated with the decline of the general state of health with malnutrition and with increased need for medication, such as antibiotics and nonsteroidal anti-inflammatory drugs, situations that occur frequently in the elderly. Differences have been demonstrated in the composition of the microbiota between healthy elderly subjects and hospitalized or institutionalized elderly subjects. These findings which further indicates that the living conditions, health status, nutrition and drugs have a significant effect on the composition of the microbiota. According to the available knowledge, the use of probiotics is safe and could represent an useful intervention to prevent or treat antibiotic-associated diarrhea, in addition to reducing the severity of symptoms, other than to help the management of constipation.

Key words: Microbiota; Elderly; Probiotics; Antibiotic-associated diarrhea; Constipation

Core tip: The intestinal microbiota of elderly manifested a reduction in the diversity, characterized by a large interindividual variability, with lower numbers of *Firmicutes*, *Bifidobacteria*, *Clostridium cluster* XIV, *Faecalibacterium Prausnitzii*, *Blautia coccoides-Eubacterium rectal* and higher in *Enterobacteriaceae* and *Bacteroidetes*. These derangements may not necessarily

aging-correlated, but they can be consequent to the decline of general state of health, malnutrition and increased use of drugs. As regards probiotics, the main double-blind studies in the elderly have shown that use is safe and could represent an interesting support to reduced frequency and/or duration of antibiotic-associated diarrhea, other than to help for constipation.

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INTRODUCTION

Differences in the composition of the microbiota have been shown in the literature, when healthy elderly and adult have been compared, but differences in the composition of the microbiota have been also shown when healthy elderly and hospitalized or institutionalized elderly patients have been compared. Then, the topic concerning the changes in the composition of the microbiota with age is much debated in the literature. Another issue of great interest is whether the use of probiotics may be effective in the elderly population.

Given this background, the aim of the present review is to summarize the state of the art according to the extant literature about two topics: the changes in the microbiota associated with aging and the activity of probiotics on the microbiota in this age group evaluated with two issues: (1) the effect on the composition of the microbiota after administration of probiotics; and (2) the efficacy of intake of probiotics on symptoms of major gastrointestinal diseases, including the iatrogenic ones, that frequently affect elderly subjects, including constipation, diarrhea secondary to the intake of antibiotics, particularly when linked to the presence of *Clostridium difficile*.

RESEARCH

The present systematic review was performed following the steps by Egger *et al*[1] as follows: configuration of a working group: three operators skilled in clinical nutrition in the geriatric age, of whom one acting as a methodological operator and two participating as clinical operators. Formulation of the revision question on the basis of considerations made in the abstract: "microbiota in elderly, use of probiotics during aging". Identification of relevant studies: a research strategy was planned, on PubMed [Public MedIine run by the National Center of Biotechnology Information (NCBI) of the National Library of Medicine of Bathesda (United States)], as follows: (1) definition of the key words (microbiota, elderly, probiotics), allowing the definition of the interest field of the documents to be searched, grouped in inverted commas ("…") and used separately or in combination; (2) use of: the Boolean AND Operator, that allows the establishments of logical relations among concepts; (3) research modalities: advanced search; (4) limits: time limits: papers published in the last 20 years; humans; languages: English; and (5) manual search performed by the senior researchers experienced in clinical nutrition through the revision of reviews and individual articles on microbiota in elderly published in journals qualified in the Index Medicus. Analysis and presentation of the outcomes: the data extrapolated from the revised studies were collocated in tables; in particular, for each study we specified: the author, the name of the journal where the study was published and year of publication, study characteristics. The analysis was carried out in the form of a narrative review of the reports.

MICROBIOTA IN ELDERLY POPULATION

This research has been carried out based on the following keywords: "microbiota" AND "elderly"; 1040 articles were found. Among them, 6 case control studies, 1 Randomized Controlled Trial (RCT), 6 cross sectional researches, 5 observational studies, 6 reviews, 1 prospective and 1 population based studies have been selected and discussed.

The changes in the microbiota associated with aging are still far from being clarified with certainty, but there are numerous studies that suggest that aging has a significant effect on the microbiota.

First of all, it has to be taken into account that changes in the intestinal microbiota of the elderly may not necessarily be caused by aging: they can be influenced by the decline of the general state of health, by malnutrition and increased need for medication, such as antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs)[2,3], that occur frequently in the elderly. Differences in the composition of the microbiota have been shown when healthy elderly and hospitalized or institutionalized elderly patients have been compared[4,5], thus proving that the living conditions, health status, nutrition and drugs have a significant effect on the composition of the microbiota.

Moreover, the colonic transit has a great influence on the function of the large bowel and increased transit time is related to a reduced fecal bacterial cell mass[6]. Due to a lack of exercise and long periods of bed rest[7,8], intestinal transit times are often significantly increased in hospitalized elderly patients[7], and this may be a factor influencing the changes in the intestinal microbiota in hospitalized or institutionalized elderly compared to healthy

subjects. Moreover, modified diets, parenteral and enteral artificial nutrition and different eating habits during hospitalization[9] may contribute to these variations.

The normal intestinal microbiota provides an important natural defence mechanism against invading pathogens and prevents the overgrowth of opportunistic microorganisms (colonization resistance). Therefore, alterations in the composition of the microbiota that occur in the elderly may lead to negative consequences, such as a decreased efficacy of in the immune system and a higher incidence of gastrointestinal infections, which is more frequent in the elderly than in the young[10]. The immunological changes associated with aging involve reductions of efficiency of the responses mediated by B and T cells[11]. In addition, the increase of circulating antibodies against commensal intestinal bacteria in the elderly has been associated with age-related changes, such as the reduction of gastric acid secretion and increased mucosal permeability[12].

The elderly subjects, compared to the young adult population, show a reduction in the diversity of the microbiota[13], characterized by a large interindividual variability, with a lower number of Bifidobacteria and a higher number of Enterobacteriaceae[14,15]. In addition, the *Bacteroidetes* are more numerous, while the *Firmicutes* appear to be fewer than in the elderly compared to younger adult controls[16]. The elderly population also shows decreased levels of *Clostridium cluster* XIV and *Faecalibacterium Prausnitzii*, which are known as major producers of butyrate[17,18]. Moreover, the levels of *Blautia coccoides - Eubacterium rectal* (formerly known as *Clostridium coccoides - E. Rectale*) are lower in the elderly than in adults[19]. Finally, aging has been associated with an increase in the diversity of species not yet identified[20]. In an interesting recent study[21], a cohort of 178 (non on antibiotic treatment) elderly subjects (mean age 78, range 64-102 years) were recruited by taking into account their place of residence (a group of healthy elderly were resident in a community and a group of frail elderly institutionalized in a long-term care center) and were compared with 13 young adults (mean age 36 years, range 28-46 years), who were used as a control group. The data were acquired through the reading of more than 5 million sequences generated from 16S rRNA amplicons of the gene. The results revealed that the composition of the intestinal microbiota of the elderly considered in the study was correlated with the place where they live. The elderly community residents had a greater number of *Firmicutes* and lower incidence of *Bacteroidetes* when compared the long-term care residents. Considering the division according to the enterotype, six co-abundance groups were detected. The dominant genera were *Bacteroides, Prevotella, Ruminococcus, Oscillibacter, Alistipes* and *CAG Odoribacter*. The transition from healthy elderly living in the community to elderly and frail institutionalized in a long-term care center is accompanied by the dominance of a CAG distinctive, with a significantly greater number of *Prevotella* and *Ruminococcus CAG* in the cohort residing in the community and *Alistipes* and *CAG Oscillibacter* in the cohort of elderly subjects institutionalized in a long-term care center. This study also demonstrated a correlation between alterations of the microbiota and the state of fragility of the elderly, confirming the results of previous study[22]. In addition, the study demonstrated a correlation with increased inflammation (as assessed by the determination of C-reactive protein and interleukin 6 and 8), thus confirming the hypothesis that there is a close association between the presence of the so-called "inflammaging" and alterations of the microbiota[23]. Finally, a clear association between diet and microbiota has been outlined in this study: thus confirms the results of many other studies[24-26].

A recent study by Biagi *et al*[15] evaluated, by means of "Human Intestinal Tract Chip" and quantitative PCR of the 16S rRNA genes of Bacteria and Archaea, the microbiota of centenarians and compared these data with the microbiota of young and not centenarians elderly subjects. The results show that *Firmicutes* and *Bacteroidetes* dominate the intestinal microbiota of centenarians (representing over 93% of total bacteria). Compared to adults and centenarians, in pre centenarian elderly subjects changes were observed in the relative proportion of specific subgroups of *Firmicutes*. A decrease in the contribution of *Clostridium cluster* XIV, an increase in Bacilli, and a rearrangement of *Clostridium cluster* IV, and of *Clostridium cluster* XIVa were found. *Clostridium cluster* XIVa is one of the main bacteria that produce methane, a short chain fatty acid, which is a source of energy for the enterocytes and has been implicated in the protection against intestinal inflammatory diseases. A lower number of several producers of butyrate was observed in centenarians when compared with other age groups, including *Ruminococcus obeum et rel*, *Roseburia intestinalis et rel*, *E. ventriosum et rel*., *E. rectale et rel*., *E. hallii et rel.* (all belonging to the *Clostridium cluster* XIV), and *Papillibacter cinnamovorans et rel*., and *F. Prausnitzii et rel*. (*Clostridium cluster* IV). Conversely, other butyrate producers, such as *Anaerotruncus colihominis et rel*. (*Clostridium cluster* IV), and *Eubacterium limosum et rel*. (*Clostridium cluster* XV) were increased in centenarians. The increase of *E. limosum* is high (about 15 times), and may indicate a group of bacteria characteristic of centenarians. Also the decrease of *F. prausnitzii* in centenarians is of interest, as this species is known to affect inflammation of the intestine. Finally, the intestinal microbiota of centenarians is more

rich in proteobacteria, a group containing many of those bacteria recently defined as "patobionti". These are considered minor and opportunistic components of human intestinal ecosystem that, in some circumstances (for example in the presence of inflammation) can get out of control and cause a disease.

Many therapeutic substances frequently taken by elderly subject[27], such as NSAIDs, are associated with alterations in the microbiota[3]. Mäkivuokko, thanks to the sequencing of the 16S rDNA, demonstrated in a group of 18 elderly people taking NSAIDs, that there are changes in all the major microbial phyla, such as a lower number of *Firmicutes* and an increase number of *Bacteroidetes*. In addition, it was reported a reduction in the number of the known butyrate producers belonging to *Clostridium cluster* XIV, as *Roseburia* and *Ruminococcus*, and, in the *Actinobacteria* cluster, a lower number of *Collinsella* spp. compared to both the young adults and the elderly subjects not taking NSAIDs.

EFFECTIVENESS OF THE USE OF PROBIOTICS IN THE ELDERLY POPULATION

The studies carried out in the elderly population to test the activity of probiotics on the microbiota in this age group, has been evaluated with two topics: the effect on the composition of the microbiota after administration of probiotics; the efficacy of intake of probiotics on symptoms of major gastrointestinal diseases, including the iatrogenic ones, that frequently affect elderly subjects, including constipation, diarrhea secondary to the intake of antibiotics, particularly when linked to the presence of *Clostridium difficile*.

This research has been carried out based on the keywords: "probiotics" AND "elderly" AND "aging"; 56 articles were sourced. Among them, as far as variations in the composition of the microbiota in the elderly after use of probiotics are concerned, 3 randomized double blind clinical trials and 1 observational research have been selected and discussed. Concerning the efficacy of use of probiotics on symptoms of major diseases that affect the elderly, 2 RCT, 7 double blind studies, 3 reviews and 1 cross sectional research have been selected and discussed.

Variations in the composition of the microbiota in elderly after use of probiotics

Regarding changes in the composition of the microbiota following the intake of probiotics, in the elderly population, three double-blind studies versus placebo were selected: (1) the study of Lahtinen *et al*[28] that considered the effect of a fermented oat beverage containing 10^9 cfu/mL *Bifidobacterium longum* 46 (DSM 14583) and *B. longum* 2C (DSM 14579) given daily for 6 mo; (2) the study by Ahmed *et al*[29] that considered the effect of a drink made of reconstituted skim milk containing 3 different doses (5 x 10^9 CFU/die, 1.0 x 10^9 CFU/die and 6.5 x 10^7 CFU/die) of *Bifidobacterium lactis* HN019 (DR10TM) administered daily for 4 wk; and (3) the study of Bartosch *et al*[4] that considered the effect of *Bifidobacterium bifidum* and *B. lactis* in combination with inulin.

Table 1 shows the changes in the composition of the microbiota of the treated group when compared to the placebo group.

Efficacy of use of probiotics on symptoms of major diseases, including iatrogenic, that affect the elderly

In a large controlled study conducted in 360 subjects older than 60 years, the effect of a 3-wk intervention of a fermented milk containing cultures of yogurt and the probiotic casei DN-114001 showed that the incidence of winter infections was not different than in the placebo group, but the duration of all pathologies was significantly lower in the intervention group (7.0-3.2 d) when compared to the control group (8.7-3.7 d)[30].

One double-blind study, which involved 24 elderly patients who had undergone artificial enteral nutrition, evaluated the effects of a 12-wk administration of fermented milk containing *Lactobacillus johnsonii* LA1. The group who took the probiotic had significantly fewer days with infections at the end of the intervention: a decrease from 15.4% of days with infection to 5.7% was observed, and this reduction was significantly greater than that recorded for the control group[31].

Numerous studies conducted in the elderly population have shown that the intake of probiotics determines a reduced frequency and/or duration of episodes of antibiotic-associated diarrhea (AAD), in addition to reducing the severity of symptoms. Probiotics have been used in combination with antibiotics as therapy for *Clostridium difficile*, which represents 20% to 25% of cases of AAD, causing more than 95% of cases of pseudomembranous colitis[32]. A study of Hickson *et al*[33] evaluated 135 hospitalized patients, with an average age of 74 years, before and after 1 wk of consumption of 100 g (97 mL) of a drink containing *Lactobacillus casei*, *L bulgaricus* and *Streptococcus thermophilus* taken twice per day during a course of antibiotics. The placebo group received a sterile milkshake. As a primary outcome the appearance of antibiotic-associated diarrhea was considered, while a secondary outcome, the presence of *Clostridium difficile* toxin and diarrhea were identified. The results showed that 7/57 (12%) of those taking the probiotic drink developed diarrhea associated with

Table 1 Changes in the composition of the microbiota of the treated group when compared to the placebo group

Design of the study	Subjects	Age	Probiotics	Results in intervention *vs* placebo	Ref.
Double blinded controlled trial	*n* = 33 placebo group;	83 ± 7 yr	Oat drink fermented with Bifidobacter um longum and *B. longum*	↑*B.catenulatum* ↑*B. bifidum* ↑*B. breve*	Lahtinen *et al*[28]
	n = 33 control group	84 ± 8 yr			
Double blinded controlled trial	*n* = 20 placebo; *n* = 20 low dose of probiotics; *n* = 20 medium dose of probiotics; *n* = 20 high dose of probiotics	> 60 yr	Reconstituted skim milk containing *Bifidobacterium lactis*	↑*Bifidobatteri* ↑*Lactobacilli* ↑*Enterococci* ↓*Enterobatteri*	Ahmed *et al*[29]
Double blinded controlled trial	*n* = 9 placebo	Mean 71 yr	*Bifidobacterium bifidum* and *B. lactis*	↑*Bifidobacteria*	Bartosch *et al*[4]
	n = 9 symbionts (mixture of probiotics and prebiotics)	Mean 73 yr	together with inulin	↑*Lactobacilli* ↓*B. bifidum*	

antibiotic use, compared with 19/56 (34%) of the placebo group (P = 0.007). Logistic regression for control of other factors gave an odds ratio of 0.25 (95%CI: 0.07-0.85) for the use of probiotics. The absolute risk reduction was 21.6% (6.6%-36.6%). No one in the group that received the probiotic and 9/53 (17%) in the placebo group had diarrhea due to *Clostridium difficile* (P = 0.001). The absolute risk reduction was 17% (7%-27%).

A further study on the use of probiotics as adjuvants to antibiotic therapy for the preventing of gastrointestinal disorders was conducted by Beausoleil in 89 elderly men with a mean age of 72 years[34]. The preparation employed was a fermented milk containing at least 50 × 10^9 colony-forming units of *L acidophilus* CL1285 and *L casei*. The scheme of administration was 49 g once a day for two days, followed by 98 g once a day to cover the entire duration of antibiotic treatment. The antibiotic-associated diarrhea occurred in 7 out of 44 patients (15.9%) in the group that received *lactobacilli* and in 16 out of 45 patients (35.6%) in the placebo group (OR = 0.34, 95%CI: 0.125-0.944, P = 0.05). The median length of hospital stay was eight days in the group that received the probiotics, compared to 10 d in the placebo group (P = 0.09). The prevention of CDAD is an important result to be considered in the elderly population, because this condition has been associated with increased mortality and morbidity[35].

In the elderly, constipation is a common condition characterized by a constellation of symptoms defined by the criteria "Rome Ⅲ criteria"[36]. A review published in 2010[37], included 3 double-blind, placebo-controlled studies conducted by Koebnick *et al*[38], Möllenbrink *et al*[39], and by Yang *et al*[40]: as a whole 266 patients were evaluated and the most of them were elderly patients. This review confirmed the efficacy of treatment with *Bifidobacterium lactis* DN-173010, *Lactobacillus casei Shirota*, and *E. coli Nissle* 1917 on the frequency of defecation and stool consistency. This improvement is secondary to the decrease of the colonic pH value that follows the probiotics intake, thanks to the production of short chain fatty acids (butyric acid, propionic acid and lactic acid). A lower pH enhances peristalsis in the colon, and thereafter, it may decrease the intestinal transit time[37].

A double blind *vs* placebo study conducted by Ouwehand *et al*[41] studied the effects of a symbiotic combination of lactitol and *Lactobacillus acidophilus* NCFM (total daily dose of 10 g lactitol and 2 × 10^{10} cells probiotic bacteria) taken twice a day for 2 wk, in a group of elderly subjects. The results of the study showed a higher frequency of evacuation in the group that received the probiotic, as well as significantly higher levels of PGE2, as well as a changes of IgA level and spermidine, thus demonstrating positive effects on the function of the intestinal mucosa.

The same working group[42] recently assessed by means of a double- blind, placebo-controlled trial, the efficacy of the same combination of *Lactobacillus acidophilus* NCFM and lactitol in 51 elderly people who followed NSAIDs treatment. Before, during and after the intervention period, the amount of six stool bacterial phylogenetic groups was determined using quantitative PCR, and variations in the composition of total microbiota were assessed by percent profiling guanine-plus-cytosine. The results of the study showed an increase of lactobacilli and bifidobacteria and also a possible stabilizing effect on the levels of *B. coccoides-E.* XIVab and *Clostridium cluster*.

CONCLUSION

The changes in the microbiota associated with aging are still far from being clarified with certainty, but there are numerous studies that suggest that aging has a significant effect on the microbiota.

Alterations in the intestinal microbiota of the elderly may not necessarily be caused by aging, but they can be consequent to conditions that occur frequently in the elderly, such as the decline of the general state of health or malnutrition or increased need for medication (*i.e.*, antibiotics and nonsteroidal anti-inflammatory drugs). Differences in the composition of the microbiota have been

found when healthy elderly and hospitalized or institutionalized elderly subjects were compared. This further indicates that the living conditions, health status, nutrition and drugs have a significant effect on the composition of the microbiota.

When compared to young adult populations, elderly subjects show a reduction in the diversity of the microbiota, which is usually characterized by a large interindividual variability, with a lower number of *Firmicutes*, *Bifidobacteria*, *Clostridium cluster* XIV, *Faecalibacterium Prausnitzii*, *Blautia coccoides-Eubacterium rectal* and a higher number of *Enterobacteriaceae* and *Bacteroidetes.* In the elderly, the intake of and NSAIDs is followed by a significant change on the composition of the microbiota. A reduction in the proportion of the known butyrate producers belonging to *Clostridium cluster* XIV, as *Roseburia* and *Ruminococcus*, and, in the *Actinobacteria* group, a lower number of *Collinsella* spp. has been reported in elderly subjects taking NSAIDs when compared to both young adults and elderly subjects who are not taking NSAIDs.

The use of probiotics in elderly population is safe and could represent an interesting support to prevent or treat AAD, in addition to reducing the severity of symptoms, other than to help the management of constipation.

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MINIREVIEWS

Conservative strategy for treatment of stable coronary artery disease

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Abstract

Patients with coronary artery disease vary widely in terms of prognosis, which is mainly dependent on ventricular function. In relation to the major outcomes of death and myocardial infarction, it is not clear in the literature if an invasive strategy of myocardial revascularization is superior to a conservative strategy of optimized medical therapy. Moreover, with the exception of patients with left main coronary disease, this similarity in prognosis also occurs in different subgroups of patients.

Key words: Coronary artery disease; Angina pectoris; Myocardial revascularization; Coronary angioplasty; Myocardial infarction; Prognosis; Disease-free survival

Core tip: Despite the evolution of myocardial revascularization techniques, the mainstay of treatment of stable coronary artery disease is optimal medical therapy. With the better understanding of the mechanisms underlying atherosclerosis, medical therapy develops and shows similar results in terms of survival and freedom from myocardial infarction compared to coronary interventions. Moreover, clinical trials have also demonstrated similar results between conservative and invasive strategies in various subgroups of patients, previously found to benefit from coronary interventions. In this review article, the authors discuss the results from main trials on specific groups of coronary artery disease patients which compared conservative and invasive strategies.

Rezende PC, Scudeler TL, da Costa LMA, Hueb W. Conservative strategy for treatment of stable coronary artery disease. *World J Clin Cases* 2015; 3(2): 163-170 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i2/163.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i2.163

INTRODUCTION

The first studies on the evolution of patients with stable coronary artery disease (CAD) and preserved left systolic ventricular function demonstrated a low incidence of major cardiac events, death or myocardial infarction, in non-revascularized patients, despite their anatomical complexity. Moreover, retrospective studies that compared optimized medical therapy (OMT) alone with coronary artery

bypass surgery (CABG) demonstrated similar rates of death or myocardial infarction in patients both with single-vessel or multivessel disease, in the presence of preserved systolic ventricular function.

In clinical conditions like diabetes mellitus or in elderly patients, the literature demonstrates that the conservative strategy of OMT is as safe as invasive strategies are, for the majority of CAD patients.

Although sub analyses from important studies have suggested surgery would be a safer strategy in patients with CAD and impaired ventricular function, this information has been questioned by a recent important prospective randomized clinical trial, which demonstrated similar results of medical therapy compared to bypass surgery.

Despite the evolution of CABG and percutaneous coronary intervention (PCI) in the last 20 years, with the widespread use of arterial grafts and surgery without the use of extra-corporeal circulation, and the emergence of pharmacological stents, the improvement in medical therapy also occurred substantially with the use of antiplatelets, beta-blockers, angiotensin-converting enzyme inhibitors and statins. Currently, these are the basis of pharmacological CAD treatment, but other options with specific mechanisms of action as ranolazine and ivabradine have also emerged as potential adjunct therapy. In addition, technical problems of invasive strategies, such as graft failure and restenosis of stents, deserve attention in this matter. Moreover, clinical complications of interventions also carry risks, especially related to cerebrovascular accidents after surgery and the possible need for future interventions with PCI. On the other hand, some specific subgroups of CAD patients do benefit by invasive strategies.

Thus, a conservative OMT strategy with multi-factorial control is a safe option for the treatment of the majority of CAD patients, especially those with well-preserved ventricular function. Invasive strategies are important tools for the management of CAD patients, and should be reserved particularly for patients with refractory symptoms, for those who develop acute coronary syndromes, and possibly for select patients with ischemic heart failure.

In this review article, the authors discuss the major findings of studies, especially clinical trials, comparing medical therapy with invasive coronary interventions, in terms of major outcomes, death and MI, in different clinical settings.

EVOLUTION OF CORONARY ARTERY DISEASE PATIENTS

The natural history of patients with CAD is impossible to observe in epidemiological studies for ethical reasons. Even if patients refuse coronary interventions, they still receive medical therapy and instruction on lifestyle modifications, which result in changes in their clinical evolution. Consequently, the evolution of CAD patients may be observed in prospective studies, and especially in randomized groups that include patients receiving medical therapy alone.

The evolution of patients with chronic, stable CAD was demonstrated in an important study published in 1989[1]. In this study, from 1977 to 1983, 150 stable CAD patients, including 92% with multivessel disease and also patients with left main coronary disease or equivalent (39.3%) with a formal indication for coronary surgical revascularization refused the procedure. They were followed for two to eight years until 1985, and medically treated with beta-blockers, nitrates, calcium-channel blockers, aspirin, and dipyridamole. Differently from modern treatment, at that time, they were not treated with angiotensin-converting enzyme inhibitors or with statins, important medications of current therapy. Despite anatomic complexity, the estimated overall survival in eight years was 89%, which represents an average annual mortality rate of 1.37%. Of note, only 10% of patients had myocardial infarction and 4% requested surgical revascularization during follow-up.

One of the first randomized studies that compared medical therapy alone with coronary bypass surgery in stable CAD patients was the Coronary Artery Surgery Study (CASS) trial[2], published in 1983. In this study, 780 CAD patients were randomized to one of the two strategies and followed for 5 years. Interestingly, in this study, the average annual mortality rate for patients assigned to medical therapy was 1.6% and to surgery 1.1% (P = 0.34). Analyzing only the patients with an ejection fraction ≥ 0.50 (75% of the entire population of the trial), those assigned to medical therapy had annual mortality rates of 1.1%, 0.6%, and 1.2%, respectively, for single-, double-, and triple-vessel disease. Patients with an ejection fraction $\geqslant$ 0.50 assigned to surgery had similar mortality rates 0.8%, 0.8%, and 1.2%, respectively, for single-, double-, and triple-vessel disease. There were no statistical differences between the two treatment strategies.

Analysis of ten-year follow-up of patients from the CASS trial[3] demonstrated an overall survival of 79% and 82% in medical and surgery groups, respectively, or an average annual mortality rate of 2.1% and 1.8% (P = 0.25).

The results of these studies demonstrate that annual mortality rates for stable CAD patients with normal ejection fraction is low and range from 0.8% to 2.1%, even in those with multivessel disease. In addition, these studies were performed during a time when patients did not receive statins or angiotensin-converting enzyme inhibitors, which are medications with the potential to lower this risk. Although great numbers of patients in these studies had a low-risk profile (preserved systolic ventricular function, stable non-limiting symptoms, and young patients), their

prognostic information is essential for understanding the results of studies on invasive strategies.

SINGLE-VESSEL CAD PATIENTS

As mentioned previously, the CASS trial as well as other trials have demonstrated that single-vessel CAD patients have low annual mortality rates, especially in the presence of preserved ventricular function (1.1% and 0.8%, respectively, for medical therapy and bypass surgery). Moreover, this good prognosis is similar among different treatment strategies.

One of the studies that addressed the comparative results of different strategies in this scenario of single-vessel CAD patients was the Medical, Angioplasty or Surgery Study Ⅰ trial[4]. This study evaluated 214 CAD patients with an isolated severe lesion > 80% at the proximal portion of the left anterior descending coronary artery. Patients with stable symptoms and well-preserved ventricular function were selected and randomized to medical therapy alone (n = 72), balloon angioplasty (n = 72), or CABG (n = 70) with an internal thoracic artery. After an average of 3-year follow-up, the primary end-point of cardiac death, myocardial infarction, or refractory angina requiring revascularization was 12%, 17%, and 3%, for medical therapy, angioplasty, and CABG. This difference was mainly dependent on new revascularizations, because mortality and myocardial infarction were similar in the 3 treatment strategies.

Another important study on single-vessel CAD patients was published in 1992[5] and compared medical therapy alone with angioplasty with the use of stents. Patients with severe stenosis in one coronary artery were randomized and followed for 6 mo. In that period, myocardial infarction occurred in 5 patients who underwent angioplasty and in 3 patients in the medical therapy group. However at the end of the follow-up, a higher number of patients assigned to angioplasty were free of angina (64% × 46% in angioplasty and medical therapy, respectively, P < 0.01), and performed better on exercise treadmill tests, despite higher costs and complications in the angioplasty group.

Thus, in the subset of single-vessel CAD patients, unless limited by refractory symptoms, the strategy of optimal medical therapy poses a similar prognosis in terms of survival and myocardial infarction compared to invasive strategies. However, patients with severe symptoms, especially if refractory to medical interventions may benefit in terms of alleviation of symptoms with angioplasty.

MULTIVESSEL CAD PATIENTS

The three most important studies conducted in the 1970's and 1980's that compared the strategy of medical therapy alone with bypass surgery were the Veterans Affairs (VA) Cooperative Study[6], the European Coronary Surgery Study (ECSS)[7], and CASS[2].

Most of the patients enrolled in these trials had multivessel CAD, and the European Study only enrolled such patients.

The VA Cooperative Study included 686 CAD patients with stable angina, electrocardiographic signs of previous infarction or ischemic changes in exercise, and at least one major coronary artery with ⩾ 50% stenosis. Patients were randomized to medical therapy alone or bypass surgery and were followed for 18 years. This study demonstrated similar rates of overall survival (33% and 30% for MT and CABG, respectively, P = 0.60), and similar rates of myocardial infarction (41% and 49% for MT and CABG, respectively). Importantly, in patients with preserved ventricular function, irrespective of the number of diseased coronary arteries, patients assigned to medical therapy had similar rates of death and myocardial infarction as those assigned to bypass surgery. On the other hand, the group with left main disease or with a high angiographic risk, characterized as triple-vessel disease associated with impaired left ventricular function, had better survival associated with bypass surgery. Of note, 41% of medical therapy patients underwent surgery during the entire 18-year follow-up.

Consistent with the findings of the VA Study, the CASS trial also showed that patients with single-, double-, or triple-vessel coronary disease had similar rates of overall survival and myocardial infarction in medical therapy and bypass surgery, if they had preserved systolic ventricular function (defined as an ejection fraction ⩾ 0.50). CASS also showed that in patients with impaired ventricular function and triple-vessel disease, surgery was a better survival option. Of note, these 5-year results were confirmed by a 10-year follow-up study[8].

Another important study, the ECSS demonstrated some differences compared to the two previous studies. This trial included 767 men with normal left ventricular function and multivessel disease and randomized them to bypass surgery or medical therapy. Differently from VA and CASS, ECSS showed higher survival rates after 5- and 12-year follow-up for surgically treated patients, but the difference between treatments in the 5-year follow-up decreased in the 12-year results (70.6% ± 5.8% *vs* 66.7% ± 5.3%, P = 0.04). However, this better survival with bypass surgery only occurred in triple-vessel disease patients. Survival of double-vessel disease patients was similar in the two strategies.

After these 3 studies, the only study that compared an invasive with a conservative strategy of OMT and included three groups of treatments was the Medical, Angioplasty or Surgery Study (MASS-Ⅱ)[8]. In this study, 611 patients with multivessel proximal CAD, preserved systolic ventricular function and stable

symptoms were randomized to receive OMT alone (n = 203), CABG surgery (n = 203), or PCI (n = 205) with the use of conventional stents. After 5-year follow-up[8], the combined primary end-points of death, myocardial infarction, and additional revascularization favored the patients assigned to bypass surgery (21.2%, 32,7% and 36.0%, respectively, for CABG, PCI and MT, P = 0.0026), especially due to a significant reduction in the rates of new revascularizations (3.9%, 11.2%, and 9.4%, respectively for CABG, PCI, and MT). However, mortality and myocardial infarction rates were statistically similar between the 3 groups. After 10-year follow-up[9], overall survival was similar between the 3 treatment groups (74.9%, 75.1%, and 69%, respectively, for CABG, PCI, and MT, P = 0.089). However, the incidences of myocardial infarction and cardiac deaths favored the surgical group. Importantly, after 10 years about 40% of MASS Ⅱ trial patients assigned to medical therapy did not develop any complications. In addition, combined and isolated end-points were similar between medical therapy and angioplasty groups.

Compared to the first studies (VA, ECSS, and CASS), the MASS trial was a more contemporary study, in which medical therapy included the use of statins, angiotensin-converting enzyme inhibitors, and dual antiplatelet therapy after PCI. However, the higher annual mortality in this trial compared to the previous ones may be due to a higher-risk profile, as patients were older at study entry, had a higher proportion of diabetics, and more complex and diseased coronary arteries (higher frequency of triple-vessel disease and lesions at the proximal portion of the left anterior descending artery). Thus, this higher-risk profile of patients from the MASS trial seemed to benefit from surgery, information similar to that of the VA and ECSS trials. The high-risk profile patients from the CASS trial also demonstrated the benefits of bypass surgery, but in this study this profile included patients with impaired ventricular function.

The MASS trial showed that medical therapy had similar outcomes when medical therapy was compared to angioplasty. However, this was not the primary objective of this study.

However, this was the major finding of another important study published in 2007, the COURAGE trial[10]. This study aimed at evaluating the clinical significance of PCI in stable CAD patients. With this purpose, 2287 patients with objective evidence of myocardial ischemia and significant CAD were randomized between 1999 and 2004 to OMT alone (n = 1138) or PCI with OMT (n = 1149). After a median 4.6-year follow-up, the primary end-point of overall death and myocardial infarction occurred in 19.0% in the PCI group and 18.5% in the medical therapy group (P = 0.62). Other isolated end-points such as myocardial infarction or hospitalization for acute coronary syndromes had similar rates in both groups. On the other hand, symptoms were better controlled by PCI, but still with a modest reduction in these rates compared to OMT.

Some meta-analysis of studies comparing PCI with medical therapy alone[11,12] demonstrated similar results, even when only patients with objective myocardial ischemia were included in the analysis[13].

Thus, the information from these trials shows that even for multivessel patients, with preserved ejection fraction, stable non-limiting symptoms, medical therapy is a safe alternative. The groups of patients who benefit from bypass surgery are those with a higher anatomical or clinical risk profile, such as patients with left main disease or limiting symptoms. The option for bypass surgery should also consider peri-procedural risks and the possibility of recent graft failure, which is mainly dependent on the surgeon's technical ability but also by anatomic characteristics, especially the coronary bench that will receive the graft. The option for PCI in stable CAD patients should also be carefully evaluated because it does not protect patients from myocardial infarction, hospitalizations, and or from the risk of death. PCI could be indicated for those patients with limiting symptoms despite optimized medical therapy and with an anatomy favorable to the procedure.

IMPAIRED VENTRICULAR FUNCTION AND CAD

As already mentioned, the CASS trial[2] was one of the first randomized trials to demonstrate that bypass surgery is superior in terms of overall survival compared to medical therapy alone in patients with triple-vessel CAD and impaired ventricular function. Interestingly, in this context, only patients with a great percentage of jeopardized ischemic myocardium had the benefits of revascularization, because single- and double-vessel disease patients with impaired ventricular function had similar survival rates with bypass surgery compared to medical therapy. However, one should consider that the CASS trial enrolled only 160 patients with left ventricular dysfunction, so that it could not have power enough to demonstrate potential differences between treatment groups. On the other hand, medical therapy at that time was quite different from current medical therapy for heart failure. For the entire population of the CASS trial, 64% of the medical group received beta-blockers at 60 mo after randomization, while only 34% of the surgical group received beta-blockers at the same follow-up period. Besides, angiotensin-converting enzyme inhibitors were not disposable at that time, nor were aldosterone blockers, which are also current essential medications for the treatment of heart

failure patients. Similarly, anesthesia, cardioplegia methods, and surgical technique have also improved since CASS trial.

Interestingly, an analysis from the CASS registry[14] with patients with severe left ventricular dysfunction, manifested by an ejection fraction below 0.36, demonstrated that the group of patients assigned to surgery had an improvement in survival compared to medical therapy patients, despite a high operative mortality of 6.9%. Moreover, a higher benefit of surgery over medical therapy was observed in patients with ejection fraction below 0.26 (5-year survival of 63% *vs* 43%, respectively, for surgery and medical therapy, $P = 0.005$) and in patients with predominantly anginal symptoms. The patients in whom heart failure symptoms predominated did not receive benefits from surgery over medical therapy. Thus, surgery probably benefited heart failure patients who had some extent of viable and ischemic myocardium, and probably did not benefit those with non-viable, fibrotic myocardium.

The findings from the CASS trial and registry were the basis for cardiology guidelines recommendations and cardiology practice supporting CABG in this scenario in the following decades.

Recently, the STICH (Surgical Treatment for Ischemic Heart Failure) trial[15], published in 2011, questioned the superiority of CABG for CAD patients with impaired ventricular function. This was a multicenter, randomized, clinical trial, in which CAD patients amenable to surgery who had an ejection fraction of 0.35 or less were randomized to OMT or CABG plus OMT. During the 56-mo follow-up, the primary end-point of overall death occurred in 41% of the medical therapy group and in 36% of the CABG group ($P = 0.12$). Of note, 17% of medical therapy group patients underwent CABG during follow-up.

In this contemporary trial, the hypothesis tested in previous studies, including CASS, that bypass surgery would be superior to medical therapy in terms of survival was contradicted by the results of such a well-designed study. Some reasons might be pointed out for this interesting finding. First, medical therapy for heart failure has improved continuously during the last 20 years. The better knowledge of the physiopathology of heart failure lead to the development and use of classes of medications directed to neurohormonal cascades[16,17] related to the progression of ventricular dysfunction. In many clinical trials[18-20], these medications were proven to positively influence survival, and currently beta-blockers, angiotensin-converting enzyme inhibitors, and aldosterone blockers are the main stain of modern treatment. On the other hand, despite its higher initial risk of complications, bypass surgical treatment of ischemic heart failure may benefit patients with jeopardized ischemic myocardium, amenable to revascularization. Of note, 52% of the population of the STICH trial had Canadian Cardiac Society (CCS) angina class 0 or 1, and 37% had dyspnea New York Heart Association (NYH) class Ⅲ or Ⅳ. However, subgroup analysis did not show differences when angina or dyspnea groups were compared. Thus, a great percentage of patients might have fibrotic ischemic scars not amenable to improving its function by bypass surgery. Thus, on the one hand, medical therapy improved substantially over time and changed the outcomes of heart failure patients. On the other hand, surgery was performed in patients with a great variability of ischemic heart disease. The patients with the highest likelihood of benefitting from CABG would be those with a higher percentage of hibernating myocardium (potential to improve function with revascularization), and especially if suitable to be revascularized (good distal benches to receive an arterial or venous grafts).

DIABETES AND CAD

In stable CAD patients, diabetes mellitus confers higher rates of complications and a worse prognosis[21]. Considering that some previous trials have demonstrated that CABG was superior to medical therapy alone in high-risk groups of patients, the BARI 2D trial[22] proposed studying the comparative results of a strategy of OMT *vs* a strategy of coronary revascularization (PCI or CABG) for type-2 diabetic CAD patients. After 5 years, the primary end-point of overall survival was similar between the 2 groups (survival rates of 88.3% and 87.9%, respectively, for revascularization and medical therapy alone, $P = 0.97$). Moreover, the rates of freedom from cardiovascular events (death, myocardial infarction, or stroke) were also similar between groups (77.2% and 75.9%, respectively, for revascularization and medical therapy groups, $P = 0.70$). When patients were stratified by the choice of PCI or CABG as the appropriate intervention, in the PCI stratum, survival and composite end-points were similar between medical therapy and PCI. In CABG stratum, survival was similar between medical therapy and CABG, although the rates of cardiovascular events were higher in medical therapy than in CABG.

Contrary to results of the BARI 2D trial, a substudy of 10-year results of the MASS Ⅱ trial[23] analyzed diabetic CAD patients in terms of comparative outcomes among medical therapy, PCI, and CABG in a long-term follow-up. Among diabetic patients ($n = 232$), mortality rates were 37.5%, 31.3%, and 27.5%, respectively, for medical therapy, PCI, and CABG ($P = 0.015$ for CABG *vs* medical therapy). Cardiac mortality also favored CABG-assigned patients, as the rates were 26.1%, 18.8%, and 12.5%, respectively ($P = 0.005$ for CABG *vs* medical therapy).

The strong evidence from BARI 2D is not con-

Table 1 Main randomized clinical trials comparing medical therapy alone with coronary interventions in stable coronary artery disease patients

Clinical scenario	Clinical trial	Randomization period	*n*	Study groups	Annual mortality	Main findings
Single-vessel CAD	MASS I (3.5 yr)	1988-1991	214	MT 72 PCI 72 CABG 70	MT 0 PCI 0.4% CABG 0.4%	Similar mortality and MI among the 3 groups
Multivessel CAD (majority of trials' patients)	VA (18 yr)	1972-1974	686	MT 354 CABG 332	MT 3.7% CABG 3.9%	Similar mortality and MI rates in the 2 groups
	ECSS (12 yr)	1973-1976	767	MT 373 CABG 394	MT 2.7% CABG 2.4%	Mortality higher in MT group in 3-vessel disease patients
	CASS (10 yr)	1975-1979	780	MT 390 CABG 390	MT 2.1% CABG 1.9%	Similar mortality in 1, 2 or 3-vessel with EF ⩾ 0.50. CABG was superior in 3-vessel with EF < 0.50
	MASS II (5 yr)	1995-2000	611	MT 203 PCI 205 CABG 203	MT 2.4% PCI 2.3% CABG 1.6%	Similar mortality in the 3 groups. Similar events in MT and PCI. CABG superior in terms of reinterventions
	COURAGE (4.6 yr)	1999-2004	2287	MT 1138 PCI 1149	MT 1.8% PCI 1.65%	Similar mortality and events in the 2 groups
Impaired ventricular function	STICH (4.6 yr)	2002-2007	1212	MT 602 CABG 610	MT 8.8% CABG 7.7%	Similar mortality rates. CABG superior in terms of hospitalization for cardiac causes
Diabetes mellitus	BARI 2D (5.3 yr)	2001-2005	2368	MT 1192 CABG/ PCI 1176	MT 2.3% CABG/PCI 2.2%	Similar mortality and MI rates in the 2 strategies
Elderly	TIME (3.1 yr)	1996-2000	282	MT 142 CABG/PCI 140	MT 7.2% CABG/PCI 6.8%	Similar mortality and MI rates between the 2 strategies

CAD: Coronary artery disease; MASS: Medical, Angioplasty or Surgery Study; VA: Veterans Affairs; ECSS: European Coronary Surgery Study; STICH: Surgical Treatment for Ischemic Heart Failure; TIME: Medical Therapy in Elderly patients; MT: Medical therapy; PCI: Percutaneous coronary intervention; CABG: Coronary artery bypass surgery.

firmed by other substudies like the MASS trial. A higher risk profile in the MASS trial, as well as the intensity of treatment in BARI 2D may explain in part such differences.

ELDERLY PATIENTS AND CAD

With the aging of the world population in recent decades, CAD has become more frequent. In addition, in the elderly, the likelihood of severe and diffuse CAD is higher than in younger populations[24]. Moreover, due to higher rates of procedure-related complications[25,26] and a lack of clinical trials comparing treatments in patients older than 65 years, the treatment of this specific population becomes even more challenging.

A sub analysis from the CASS registry[27] showed that in 1985 older CAD patients surgically treated had better survival rates compared to medically treated patients, during a 6-year follow-up (adjusted 6-year survival 79% and 64%, respectively, for surgical and medical therapy groups, $P < 0.0001$). However, this study should be carefully analyzed as this was a non-randomized study, and there were important baseline differences between the two treatment groups.

Another prospective, observational study published in 2002 analyzed clinical data and outcomes of all patients who underwent catheterization and revascularization in the province of Alberta, Canada[28]. This study showed that in 3 age cohorts (< 70 years, 70-79 years, and ⩾ 80 years), CABG was superior to PCI and medical therapy alone in terms of overall survival during 4-year follow-up. However, this study has also to be analyzed carefully because its design was observational, non-randomized, and included a great range of risk profiles, such as acute coronary syndrome patients as well as patients with impaired ventricular function, which may have favored surgical results.

One of the few studies designed to compare a conservative *vs* an invasive strategy for the treatment of elderly CAD patients was the Trial of Invasive *vs* Medical therapy in Elderly patients (TIME), published in 2004[29]. In this study, patients age 75 years or older, with Canadian Cardiac Society (CCS) class Ⅱ or greater angina, despite taking at least 2 classes of anti-anginal drugs, were randomized to medical therapy alone or to angiography and appropriate coronary revascularization (PCI or CABG). Despite their high-risk profile (mean age at entry 80-year-old, 82% with CCS class Ⅲ or Ⅳ angina), survival was similar between patients in the two strategies (91.5% *vs* 95.9% after 6 mo, 89.5% *vs* 93.9% after 1 year, and 70.6% *vs* 73.0% after 4.1 years, respectively, for medical therapy and revascularization strategies, P = NS). However, late revascularizations were more frequent in the medical therapy than in the revascularization group (45% *vs* 12%, $P < 0.0001$).

Post-hoc analysis of elderly CAD patients from the COURAGE trial[30] also demonstrated similar survival rates between conservative and invasive

strategies.

A recent post-hoc analysis of patients 65 years or older from the 10-year follow-up of the MASS Ⅱ trial[31] also showed similar overall survival rates comparing the three treatment strategies, medical therapy, PCI with conventional stents or CABG (63%, 69% and 66%, P = 0.93). The rates of myocardial infarction were also similar among the three groups. However, as demonstrated in the TIME trial, the rates of additional revascularizations were lower in the CABG group (Table 1).

EXTENSION OF MYOCARDIAL ISCHEMIA

Although ESC guidelines[32] and some retrospective studies[33,34] have suggested that patients with myocardial ischemia extension greater than 10% benefit from myocardial revascularization, no prospective study have confirmed this finding. Currently, the on-going International Study of Comparative Health Effectiveness with Medical and Invasive Approaches (ISCHEMIA) trial, which aims at randomizing 8000 coronary artery disease patients with moderate or severe ischemia to an invasive or a conservative strategy, should help to bring some reliable information in this matter.

CLINICAL IMPLICATIONS AND CONCLUSION

Despite the strength of several study findings, significant developments in aggressive MT and lifestyle prescriptions with comprehensive risk factor modification have continued to occur since trials were conducted, and this may impact the outcomes of an MT-based strategy, even in the long-term follow-up. Further evidence in this long-running debate will be provided by the results of current trials of the initial MT strategy in patients with stable multivessel disease and preserved ventricular function. Moreover, results of studies on drug-eluting stents demonstrating the superiority of CABG over PCI have been questionable, and some might argue that this procedural refinement makes the present results obsolete. However, data from randomized and nonrandomized trials show that this new type of stent has no advantageous effect on death and nonfatal MI relative to bare-metal stents despite yielding striking reductions in rates of restenosis and repeat revascularization procedures. Thus, we believe that the observations reported herein with respect to death and MI remain applicable to contemporary practice.

In summary, several trials strongly show the benefits of PCI and CABG over MT in regard to some end points at long-term follow-up, although with similar rates of overall mortality. Additionally, CABG surgery is associated with higher rates of event-free survival.

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ORIGINAL ARTICLE

Observational Study

Correlation between hypertension and hyperglycemia among young adults in India

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Abstract

AIM: To assess the correlation between blood pressure levels and fasting plasma glucose levels among young adults attending Chatrapati Shahuji Maharaj University, Kanpur, India.

METHODS: The present study was cross-sectional in nature, conducted among students in the Institute of Paramedical Sciences, Chatrapati Shahuji Maharaj University, Kanpur. Study subjects included 185 young adults. Among them, 94 were males and 91 were females, in the age group 17 to 19 years.

RESULTS: Mean age among males was 18.5 ± 1.5 years and among females was 17.9 ± 1.8 years. Of the total 185 study subjects, 61 (32.9%) were classified as pre-diabetic and 20 (10.8%) as pre-hypertensive. Mean waist circumference, systolic blood pressure and serum high density lipoprotein did not vary significantly between normoglycemic and pre-diabetic subjects. However, the mean diastolic blood pressure of pre-diabetics (82 ± 5 mmHg) was significantly higher than normoglycemics (79 ± 6 mmHg). Mean

serum cholesterol, serum triglycerides, serum low density lipoprotein (LDL) and serum very low density lipoprotein was also higher among pre-diabetic subjects in comparison to normoglycemic subjects and the difference was statistically significant. Upon multiple linear regression analysis, it was observed that body mass index (BMI) (β = 0.149), diastolic blood pressure (β = 0.375) and serum LDL (β = 0.483) were significantly associated with fasting plasma glucose. Multiple linear regression with diastolic blood pressure as the outcome variable showed that BMI (β = 0.219), fasting blood glucose (β = 0.247) and systolic blood pressure (β = 0.510) were significantly associated.

CONCLUSION: A significant prevalence of pre-diabetes and pre-hypertension in young adults is a matter of concern therefore all young adults need to be targeted for screening of diabetes and hypertension and lifestyle modification.

Key words: Adolescent; Hypertension; Diabetes; Co-prevalence; India

Core tip: In the present study, 32.9% young adults were pre-diabetic whereas 10.8% were pre-hypertensive. Around 2.7% young adults had both pre-diabetes and pre-hypertension. Among the pre-hypertensives, 25% also had pre-diabetes. However among the pre-diabetics, 8.2% had pre-hypertension. The correlation between systolic blood pressure and fasting plasma glucose was not statistically significant. However, the correlation between diastolic blood pressure and fasting plasma glucose was significant. The mean diastolic blood pressure of pre-diabetics (82 ± 5 mmHg) was significantly higher than normoglycemics (79 ± 6 mmHg). Upon multiple linear regression analysis, it was observed that body mass index (β = 0.149), diastolic blood pressure (β = 0.375) and serum LDL (β = 0.483) were significantly associated with fasting plasma glucose.

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INTRODUCTION

Diabetes and hypertension is the twin epidemic, rapidly on the rise in the developing countries[1]. Diabetes mellitus or chronic hyperglycemia is a metabolic disorder which results from defects in carbohydrate, fat and protein metabolism that occur as a consequence of deranged insulin secretion or action. Long term hyperglycemia is associated with the development of cardiovascular disease, renal disease, neuropathy, retinopathy, peripheral vasculopathy, and stroke[2]. World Health Organization (WHO) has estimated that globally the number of adults with diabetes will increase from 171 million in 2000 to 366 million in the year 2030[3]. In 2004, worldwide, around 3.4 million people died as a result of hyperglycemia[4]. Of the total deaths among diabetics, around 80% occur in developing countries[5]. According to WHO, diabetes will be the 7th leading cause of global mortality in 2030[6]. India has been declared as the capital of diabetes because approximately 41 million Indians have diabetes to date and every fifth diabetic in the world is an Indian[7].

Worldwide, hypertension or high blood pressure has caused around 7.5 million deaths, which accounts for 12.8% of the global mortality. Around 57 million disability adjusted life years (DALYS) or 3.7% of total DALYS have been attributed to hypertension[8]. The global prevalence of hypertension in adults more than 25 years of age, averaged around 40% in 2008[8]. The WHO has estimated that hypertension is directly responsible for about 62% of stroke and 49% of coronary artery disease, worldwide[9]. In a meta-analysis of prevalence studies on hypertension in India, from January 2000 to June 2012, it was observed that the prevalence of hypertension in the urban population was 40.8% whereas that in the rural population was 17.9%[10]. Hypertension leads to cardiovascular disease, peripheral vasculopathy, cerebrovascular disease, and nephropathy[11].

It has been observed that diabetes and hypertension often exist together in the population. The risk of developing hypertension is 1.5-2.0 times higher in diabetics as compared to non-diabetics, whereas around one-third of the hypertensives develop diabetes[12]. These co-morbidities hasten the progress of vascular complications[13-15].

Diabetes and hypertension both can be prevented and managed by lifestyle modification and medical intervention. Moreover, screening and early management of diabetes and hypertension, through periodic surveillance, will slow down the progress of the disease and prevent complications[16]. American Diabetic Association has described a new entity of impaired glucose metabolism as prediabetes in which two categories are included-Impaired Fasting Glucose, when fasting plasma glucose is between 100 and 125 mg/dL and Impaired Glucose Tolerance, when 2-h result following oral glucose tolerance test is between 140 and 199 mg/dL[17]. Persons with prediabetes, are at greater risk for the future development of diabetes as well as cardiovascular disease[18]. According to JNC-7,

systolic blood pressure 120-139 mmHg and/or diastolic blood pressure 80-89 mmHg was classified as pre-hypertension[19]. Similarly, individuals with pre-hypertension are pre-disposed to developing hypertension in the later years of life.

Though the manifestation of cardiovascular disease occurs in middle age and later, it has now been proved that the initiation of cardiovascular disease occurs in childhood and adolescence[20]. The known risk factors of cardiovascular disease such as hypertension, raised blood glucose, raised serum cholesterol, tobacco consumption, high fat diet and obesity start early in childhood and adolescence and then continue into adulthood[20]. Screening and early identification of these risk factors and their progenitors like pre-diabetes and pre-hypertension may go a long way to prevent cardiovascular morbidity and mortality in adults.

The overall prevalence of glucose intolerance among adolescents in South India was reported to be 3.7%[21]. Prevalence of hypertension among children and adolescents in north India was observed to be 9.4%[22].

The rising prevalence of diabetes and hypertension in India, their beginning in the adolescent age group, and the co-occurrence of the two disease entities, is a cause of concern, therefore this study was planned to study the association between hypertension and hyperglycemia in Indian young adults.

MATERIALS AND METHODS

Study design and sample size

It was a cross-sectional study. The minimum sample size required (n = 89) was calculated taking a prevalence of glucose intolerance of 3.7%, as reported in the ORANGE-2 study, with a precision of 4% and a confidence level of 95%[21]. The formula used was, $n = Z_{(1-\alpha/2)}{}^2 pq/d^2$ (where $Z_{(1-\alpha/2)}$ was taken at 95%CI; P = prevalence of obesity, q = 100-p; d = absolute precision). For this study, P = 3.7%; q = 96.3%; d = 4%. Adding a 10% for incomplete answers, the total number came out to be 98. A design effect of 2 was included to minimize any error due to inherent variation in the population. The calculated sample size was multiplied by 2 to obtain the sample size of 196.

Sampling

The study was conducted among students in the Institute of Paramedical Sciences, affiliated to Chatrapati Shahuji Maharaj University, Kanpur. The Institute of Paramedical Sciences provides a course of 4 years in Paramedical Sciences and enrols around 100 students annually. A list of all the students enrolled in the Institute in the first, second and third year was obtained. Systematic random sampling technique was applied to identify the required number of study subjects. Written informed consent was taken from the students and their parents/guardians. In case a student refused to participate in the study, the next consecutive student was included. The data was analyzed for 185 subjects only whose laboratory test results were available. Among the study subjects thus selected, 94 were males and 91 were females, in the age group 17 to 19 years.

Methodology

A standard mercury sphygmomanometer, Diamond Co., Industrial electronics and Allied Products, Pune, Maharashtra, India, was used for recording blood pressure. Blood pressure (BP) was measured on the left arm, in the sitting position, using appropriate size cuffs. Before the measurement was taken, the subject was seated for at least 5 min. Care was taken that the arm muscles were relaxed and the arm was placed at heart level. The cuff was applied to the left upper arm and was inflated until the manometer reading was 30 mmHg above the level at which the radial pulse disappeared, and thereafter the cuff was slowly deflated. The Korotkoff sounds were monitored using a stethoscope applied over the brachial artery. The first (appearance) and the fifth (disappearance) Korotkoff sounds were noted as the systolic and diastolic blood pressure, respectively. Blood pressures were measured twice and their mean was recorded. Subjects were categorized into normotensive, pre-hypertensive and Stage I and Stage II hypertensive based on the blood pressure classification for adolescents for subjects of age 17 years and according to JNC-7 for subjects $\geqslant$ 18 years[19,23]. JNC-7 has classified systolic blood pressure (SBP) < 120 mmHg and a diastolic blood pressure (DBP) < 80 mmHg as normal blood pressure; SBP 120-139 mmHg and/or DBP 80-89 mmHg as pre-hypertension; SBP 140-159 mmHg and/or DBP 90-99 mmHg as Stage I hypertension and SBP $\geqslant$ 160 mmHg and/or DBP $\geqslant$ 100 mmHg as stage II hypertension[19]. For adolescents upto 17 years of age, normal BP was defined as systolic and diastolic blood pressure < 90th percentile, Prehypertension as systolic or diastolic blood pressure 90th percentile to < 95th percentile or blood pressure > 120/80 mmHg to < 95th percentile, Stage 1 Hypertension (HTN) as systolic and/or diastolic blood pressure 95th percentile to 99th percentile plus 5 mmHg and Stage 2 HTN as systolic and/or diastolic blood pressure > 99th percentile plus 5 mmHg[23].

According to American Diabetic Association, subjects were classified as normoglycemic when fasting plasma glucose was less than 100 mg/dL after 8 h fasting, prediabetic when fasting plasma glucose was between 100 and 125 mg/dL and diabetic when fasting plasma glucose was more than

Table 1 Bio-social characteristics of study subjects

Determinant	Total	Male (n = 94)		Female (n = 91)		P value[1]
		n	%	n	%	
Place of residence						
Urban	119	56	47.1	63	53.9	0.17
Rural	66	38	57.6	28	42.4	
Religion						
Hindu	164	84	51.2	80	48.8	0.084
Muslim	15	6	40	9	60	
Sikh	4	4	100	0	0	
Christian	2	0	0	2	100	
Type of family						
Nuclear	100	36	36	64	64	< 0.001[2]
Joint	85	58	68.2	27	31.8	
Physical activity						
Sedentary	69	28	40.6	41	59.4	0.056
Moderate	92	50	54.3	42	45.7	
Heavy	24	16	66.7	8	33.3	
Smoking habit						
Non-smoker	173	82	47.3	91	52.7	0.001[2]
Smoker	12	12	100	0	0	
Alcohol intake						
Non-alcoholic	183	92	50.3	91	49.7	0.162
Alcoholic	2	2	100	0	0	
Eating habit						
Vegetarian	110	52	47.3	58	52.7	0.013[2]
Mixed	75	42	56	33	44	
Diabetic status						
Normoglycemic	124	60	48.4	64	51.6	0.347
Pre-diabetic	61	34	55.7	27	44.3	
Hypertensive status						
Normotensive	165	86	52.1	79	47.9	0.306
Pre-hypertensive	20	8	40	12	60	

[1]Pearson's χ^2 test; [2]P value < 0.05 is significant.

126 mg/dL[17].

Body weight was estimated, using Krup's weighing machine, with a least count of 0.5 kg. The subject was made to stand on the weighing scale, feet around 15 cm apart, and weight distributed on both the legs. Zero setting was done before each measurement. Height was estimated, with the subject standing upright against the wall such that the roof of the external auditory meatus was in line with the lower margin of the orbit. A hard board was placed on the wall, just over the head and height was marked on the wall and measured with a measuring tape with a least count of 0.5 cm. Waist circumference was measured, at the level of the umbilicus, with the subject in the erect position, breathing silently.

Fasting blood samples were drawn on the day subsequent to the interview. A total of 10 mL blood was collected from each subject: 4 mL in EDTA tube and 6 mL in plain tube. The samples were immediately sent to the laboratory in the Department of Biochemistry, Chatrapati Shahuji Maharaj University, Kanpur. The samples were centrifuged without any delay. The samples were analyzed for glucose on the same day. Remaining plasma and serum was aliquoted and stored at -70 deg C. Lipid

Table 2 Co-prevalence of diabetes and hypertension

	Prediabetes		Normoglycemia		Total
	n	%	n	%	
Pre-hypertension	5	25	15	75	20
Normotension	56	33.9	109	66.1	165

estimations were done in batches in serum samples. Standard internal quality control procedures for laboratory were followed. Fasting plasma glucose was estimated using the Enzymatic colorimetric GOD-PAP method, Serum Cholesterol using Enzymatic Colorimetric High Performance CHOD-PAP method, Serum HDL using Enzymatic Colorimetric High Performance CHOD-PAP method, and Serum triglycerides using colorimetric method[24].

Data was compiled using Microsoft Excel and analysed using SPSS 17.0. Pearson's Chi square test was applied to study the difference between categorical variables. Student's t-test was used to analyse the difference between continuous variables. Two-tailed P-value less than 0.05 was considered significant. Pearson's correlation coefficient was applied to determine the association between fasting plasma glucose and systolic and diastolic blood pressure. Multiple linear regression analysis was done to analyse the association of various determinants with fasting plasma glucose.

Statistical analysis

The statistical methods are adequately and appropriately applied to the best of the authors' knowledge.

RESULTS

Data was analyzed for 185 subjects, 94 males and 91 females. Mean age among males was 18.5 ± 1.5 years and among females was 17.9 ± 1.8 years. Among the subjects living in urban area, 47.1% were males whereas among those living in rural areas 57.6% were males (Table 1). Subjects predominantly belonged to Hindu religion. Among those who were sedentary, 40.6% were males, whereas among heavy workers, 66.7% were males. However, there was no statistically significant association between physical activity and gender. Of all the study subjects, 12 (6.4%) were smokers and all were male (100%). The association between smoking and gender was statistically significant.

Among the total study subjects, 61 (32.9%) were pre-diabetic whereas 20 (10.8%) were pre-hypertensive. Five (2.7%) subjects had both pre-diabetes and pre-hypertension. Among the pre-hypertensives, 25% also had pre-diabetes (Table 2). However among the pre-diabetics, 8.2% had pre-hypertension.

The correlation of systolic blood pressure with fasting plasma glucose was not found to be stati-

Table 3 Correlation between fasting plasma glucose and systolic and diastolic blood pressure among study subjects

Fasting plasma glucose	Pearson's correlation coefficient	*P* value[1]
Systolic blood pressure	0.045	0.546
Diastolic blood pressure	0.301	< 0.001[2]

[1]Pearson's Correlation coefficient; [2]*P* value < 0.05 is significant.

stically significant. However, the correlation of diastolic blood pressure with fasting plasma glucose was significant ($P < 0.001$) (Table 3).

Among the normoglycemic subjects, mean BMI was 20.6 ± 42.0 kg/m^2 whereas among the pre-diabetic subjects the BMI was 21.8 ± 3.0 kg/m^2 and the association was found to be significant (Table 4). There was no significant association between the waist circumference, systolic blood pressure and serum HDL of normoglycemic and pre-diabetic subjects. However, the mean diastolic blood pressure of pre-diabetics (82 ± 5 mmHg) was significantly higher than normoglycemics (79 ± 6 mmHg). Mean serum cholesterol, serum triglycerides, and serum VLDL was also higher among pre-diabetic subjects as compared to normoglycemic subjects and the association was found to be significant. Mean serum LDL was also significantly higher in prediabetics (104.1 ± 22.7 mg/dL) than in normoglycemics (92.7 ± 23.6 mg/dL).

Multiple linear regression analysis for the determinants of fasting plasma glucose was done and the adjusted R^2 was 23.5% (Table 5). Waist circumference, systolic blood pressure, serum cholesterol, serum triglycerides, serum HDL, serum VLDL were not significantly associated with fasting plasma glucose. For every 1 mmHg increase in diastolic blood pressure, the fasting plasma glucose was expected to rise by 0.375 mg/dL ($\beta = 0.375$) and this association was found to be significant ($P < 0.05$). Similarly, BMI ($\beta = 0.149$), and serum LDL ($\beta = 0.483$) were also significantly associated with fasting plasma glucose.

Multiple linear regression analysis was done for systolic blood pressure as the outcome variable and the adjusted R^2 was 43.7% (Table 6). Diastolic blood pressure and serum LDL were observed to be significantly associated. Multiple linear regression analysis for diastolic blood pressure as the outcome variable showed an adjusted R^2 of 49.6% (Table 7). BMI ($\beta = 0.219$), fasting plasma glucose ($\beta = 0.247$) and systolic blood pressure ($\beta = 0.510$) were found to be significantly associated.

DISCUSSION

In this study, the overall prevalence of pre-diabetes was 32.9%. In another study from Dhaka, Bangladesh, around 20% subjects aged 11-18 years, with BMI $\geqslant$ 95th percentile for age and sex using CDC growth chart, were reported to have impaired glucose tolerance as detected after two hours oral glucose tolerance test[25]. A study from United States revealed that 21 percent of obese adolescents between 11 and 18 years had impaired glucose tolerance following two hours oral glucose tolerance test[26]. The difference from our study may be due to the criteria used for impaired glucose tolerance as we have considered fasting plasma glucose, whereas the other studies have considered the plasma glucose after a two hours oral glucose tolerance test which may have greater specificity in labelling impaired glucose tolerance. In a study from South India, the prevalence of impaired glucose tolerance was 3.7% in children and adolescents 6-19 years following oral glucose tolerance test[21]. The low prevalence as compared to our study may be due to the large age range of study subjects and the criteria used for impaired glucose tolerance.

In our study, the prevalence of pre-hypertension was 10.8%. The prevalence of pre-hypertension among adolescents from Wardha, in central India, was reported as 10.6%, which was very similar to our results[27]. This was also in concordance with the results of another study from Shimla, in north India wherein the prevalence of prehypertension was found to be 12.3%[28].

The present study revealed that 5 (2.7%) subjects had both pre-diabetes and pre-hypertension. However, the multi-center Screening India's Twin Epidemic (SITE) survey revealed that 20.6% of the study subjects had co-existent diabetes and hypertension[16]. In our study, among the pre-hypertensives, 25% also had pre-diabetes whereas in the SITE study, among 7212 hypertensives, 3227 (44.7%) had diabetes. The present study showed that among the pre-diabetics, 8.2% had pre-hypertension whereas in the SITE study, among 5427 diabetics, 59.5% were hypertensive.These differences may be because only adult subjects more than 18 years were studied in the SITE survey whereas our study included subjects in the 17 to 19 years age group.

Hypertension is responsible for acceleration of the vascular complications of diabetes, including coronary artery disease, renal disease, and retinopathy[29]. The pathophysiology of hypertension occurs at the cellular level in the intima of the arteries, which involves the function of the endothelial cells. Hypertension and diabetes both alter the endothelial cell structure and function. In large and medium size vessels and in the kidney, endothelial dysfunction causes proliferation of vascular smooth muscle cells and vasoconstriction of mesangial cells[29]. These alterations in the smooth muscle cells lead to atherosclerosis and glomerulosclerosis. Similarly, proliferation of retinal capillary endothelial cells causes retinopathy. Therefore, endothelial

Table 4 Determinants of impaired fasting plasma glucose

Determinant	Normoglycemic (n = 124)		Impaired fasting glucose (n = 61)		P value[1]
	Mean	SD	Mean	SD	
BMI	20.6	4.2	21.8	3	0.026[2]
Waist circumference	76	9.3	76.7	20.1	0.794
SBP	121	12	122	8	0.238
DBP	79	6	82	5	0.003[2]
S.Cholesterol	155.9	31.6	174.2	34.5	0.001[2]
S.Triglycerides	128.1	55.9	154.7	55.6	0.002[2]
S.HDL	39.3	8.6	41.4	7.3	0.095
S.LDL	92.7	23.6	104.1	22.7	0.002[2]
S.VLDL	25.6	11.2	32.8	12.3	< 0.001[2]

[1]Student's *t*-test; [2]*P* value < 0.05 is significant. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein.

Table 5 Multiple linear regression analysis of determinants of fasting plasma glucose

Determinant	Unstandardized coefficients		Standardized β	P value[1]
	β	SE		
(Constant)	24.067	17.964		0.182
Smoking	3.075	8.899	0.023	0.730
BMI	0.530	0.263	0.149	0.046[2]
Waist circumference	0.049	0.070	0.050	0.483
SBP	0.121	0.113	0.094	0.285
DBP	0.844	0.200	0.375	< 0.001[2]
Cholesterol	0.092	0.126	0.224	0.467
Triglycerides	0.008	0.038	0.033	0.830
HDL	0.060	0.154	0.036	0.696
LDL	0.279	0.141	0.483	0.040[2]
VLDL	0.151	0.146	0.132	0.301

[1]Multiple linear regression analysis. R^2 = 48.5%, adjusted R^2 = 23.5%; [2]*P* value < 0.05 is significant. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein.

Table 6 Multiple linear regression analysis of determinants of systolic blood pressure

Determinant	Unstandardized coefficients		Standardized β	P value
	β	SE		
(Constant)	65.983	10.961		< 0.001
Smoking	1.276	5.939	0.012	0.830
BMI	0.241	0.177	0.087	0.174
Waist circumference	0.057	0.047	0.074	0.225
DBP	0.997	0.118	0.571	< 0.001[2]
Cholesterol	0.018	0.084	0.056	0.831
Triglycerides	0.009	0.025	0.048	0.715
HDL	-0.135	0.103	-0.104	0.188
LDL	0.085	0.095	0.189	0.025[2]
VLDL	0.218	0.096	0.245	0.053
FPG	0.054	0.050	0.069	0.285

[1]Multiple linear regression analysis. R^2 = 66.1%, adjusted R^2 = 47.3%; [2]*P* value < 0.05 is significant. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; FPG: Fasting plasma glucose.

cell damage is responsible for the complications of diabetes and this damage is accelerated by co-existing hypertension[29].

Co-occurrence of hypertension in diabetics increases the risk of development of macrovascular and microvascular complications[30,31]. Diabetic

Table 7 Multiple linear regression analysis of determinants of diastolic blood pressure

Determinant	Unstandardized coefficients		Standardized β	*P* value[1]
	β	SE		
(Constant)	21.387	6.314		0.001
Smoking	0.682	3.214	0.012	0.832
BMI	0.347	0.093	0.219	< 0.001[2]
Waist circumference	0.013	0.025	0.030	0.602
Cholesterol	0.053	0.045	0.288	0.249
Triglycerides	0.014	0.014	0.125	0.311
HDL	-0.090	0.055	-0.121	0.105
LDL	0.064	0.051	0.250	0.212
VLDL	0.062	0.053	0.121	0.242
FPG	0.110	0.026	0.247	< 0.001[2]
SBP	0.292	0.035	0.510	< 0.001[2]

[1]Multiple linear regression analysis. R^2 = 70.4%, adjusted R^2 = 49.6%; [2]*P* value < 0.05 is significant. BMI: Body mass index; SBP: Systolic blood pressure; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; FPG: Fasting plasma glucose.

individuals with coexisting hypertension have a much higher occurrence of cerebrovascular accidents as compared to diabetics with normal blood pressure[30,32,33]. The risk of peripheral vasculopathy also increases in case of co-existence of hypertension in diabetics[33]. Both hypertension and diabetes lead to coronary artery disease[34]. It has been observed that in hypertensive diabetics, the risk of death due to cardiovascular disease is almost doubled[34]. Hypertension accelerates the progress of diabetic retinopathy and nephropathy[35,36]. Hypertension in diabetics hastens the occurrence of microalbuminuria and the progress of nephropathy after the development of proteinuria[36].

In our study, mean waist circumference, systolic blood pressure and serum HDL did not vary significantly between normoglycemic and pre-diabetic subjects. However, the mean diastolic blood pressure of pre-diabetics (82 ± 5 mmHg) was significantly higher than normoglycemics (79 ± 6 mmHg). Correlation between systolic blood pressure and fasting plasma glucose was not statistically significant. However, the correlation of diastolic blood pressure with fasting plasma glucose was significant ($P < 0.001$).

In the present study, upon multiple linear regression analysis for fasting plasma glucose, BMI (β = 0.149) diastolic blood pressure (β = 0.375) and serum LDL (β = 0.483) were found to be significantly associated. However, in the study from South India, on multiple regression analysis, only family history of diabetes (OR 4.11) and HOMA-IR (insulin resistance assessed by homeostasis model assessment) (OR 11.22) were found to be significant in girls and only HOMA-IR (OR 5.19) was associated with glucose intolerance in boys[21]. Due to financial constraints, HOMA-IR assessment was not included in our study. Upon multiple linear regression for diastolic blood pressure, it was observed that BMI (β = 0.219), fasting plasma glucose (β = 0.247) and systolic blood pressure (β = 0.510) were significantly associated.

The present study reveals that prediabetes and pre-hypertension begin to occur in young adults. It is well known that the prevalence of cardiovascular disease is increasing among Indians, occurring especially at a younger age[12]. Therefore it is imperative that policies and programs be developed for identifying and successfully managing hypertension and diabetes at an early age.

Given the risk associated with co-prevalence of diabetes and hypertension, it is important to identify young adults with pre-diabetes and pre-hypertension who are prone to develop full blown disease as adults, and it is the need of the hour that guidelines be formulated under the National Program for Prevention and Control of Cancer, Diabetes, CVD and Stroke (NPCDCS) for primordial and primary prevention efforts through evidence-based screening and health education initiatives. Health education programs among young adults regarding lifestyle modification to curb diabetes and hypertension in their incipient stage may be considered as a cost-effective public health approach in dealing with the morbidity attributed to consequent cardiovascular diseases.

COMMENTS

Background

As per World Health Organization estimates, globally the number of adults with diabetes will rise from 171 million in 2000 to 366 million in the year 2030. India has been declared as the capital of diabetes because approximately 41 million Indians have diabetes till date and every fifth diabetic in world is an Indian.The global prevalence of raised blood pressure or hypertension in adults aged 25 and over was around 40% in 2008. A meta-analysis of prevalence studies on hypertension in India, from January 2000 to June 2012, revealed a high prevalence of hypertension in the urban (40.8%) as well as rural population (17.9%). The co-prevalence of diabetes and hypertension is strongly associated with cardiovascular disease. Prevalence of cardiovascular disease

is on the rise among Indians, especially at a younger age, therefore early detection and management of hypertension and diabetes may hold the key to reducing cardiovascular mortality in India. Prevalence of glucose intolerance among adolescents in South India was reported to be 3.7%. Prevalence of hypertension among children and adolescents in north India was observed to be 9.4%. The high prevalence of diabetes and hypertension in India with their beginning in the adolescent age group, and the co-occurrence of the two disease entities leading to cardiovascular diseases, is an area of concern. Screening and health education programs regarding lifestyle modification may be considered as a cost-effective public health approach in dealing with the morbidity attributed to cardiovascular diseases. Therefore, a precise estimate of the prevalence of diabetes and hypertension among Indian young adults is required to assess the magnitude of the problem that has to be addressed and to design programs and policies for prevention and control.

Research frontiers

Pre-diabetes and pre-hypertension have a high degree of co-prevalence among Indian young adults and this knowledge will help in shaping primordial and primary level preventive programs for our country.

Innovations and breakthroughs

In India, very few studies are available on the prevalence of diabetes and hypertension among young adults and none have analysed the association between the two co-morbidities. Given the risk associated with co-prevalence of diabetes and hypertension, it is important to estimate their prevalence among Indian young adults to provide evidence-based guidelines for preventive efforts through screening and health education initiatives.

Applications

Very few studies on the prevalence of diabetes and hypertension among young adults are available in India; and this study reveals their co-prevalence in the indigenous population and emphasizes the need to develop a strategy for prevention of these co-morbidities to bring down the consequent cardiovascular morbidity and mortality in India.

Terminology

Regression analysis is a statistical process for estimating the relationships among variables. It includes many techniques for modeling and analyzing several variables, when the focus is on the relationship between a dependent variable and one or more independent variables. More specifically, regression analysis helps one understand how the typical value of the dependent variable changes when any one of the independent variables is varied, while the other independent variables are held fixed. In statistics, linear regression is an approach for modeling the relationship between a scalar dependent variable y and one or more explanatory or independent variables denoted X. The case of one explanatory variable is called simple linear regression. For more than one explanatory variable, the process is called multiple linear regression. The beta (β) regression coefficient is computed to assess the strength of the relationship between each predictor variable and the dependent variable.

Peer review

This a well written report from a useful study.

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Use of steroids for facial nerve paralysis after parotidectomy: A systematic review

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Abstract

AIM: To systematically review the literature to assess the efficacy of corticosteroids in treating post-parotidectomy facial nerve palsy (FNP).

METHODS: We searched the Cochrane library, EMBASE and MEDLINE (from inception to 2014) for studies assessing the use of corticosteroids in post-parotidectomy FNP. Studies were assessed for inclusion and quality. Data was extracted from included studies.

RESULTS: Two randomised controlled trials met the inclusion criteria. One study assessed the use of dexamethasone and the other prednisolone. None of the studies demonstrated a significant difference in the outcome of FNP post-parotidectomy with the use of corticosteroids *vs* no therapy. The majority of FNP post-parotidectomy is transient. Preoperative factors (size of tumour and malignancy), intraoperative factors (extent of parotidectomy and integrity of facial nerve at the end of the operation) are important in determining prognosis of FNP if it does occur.

CONCLUSION: Corticosteroids do not appear to improve FNP prognosis post-parotidectomy. Further studies assessing patients by cohort and with long term follow-up are required to increase scientific evidence.

Key words: Adrenal cortex hormones; Facial paralysis; Parotid diseases; Steroids

Core tip: Parotidectomy is a common operation performed to treat benign and malignant parotid lesions. Facial nerve palsy (FNP) is a well documented complication of parotidectomy that can significantly impair quality of life. Steroids have been proposed as a treatment option for post-parotidectomy FNP. In this systematic review of randomised controlled trials, we found minimal evidence to suggest steroids improve the prognosis of FNP after parotidectomy. However, more trials are required to assess the effectiveness of steroids in specific cohorts of patients.

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INTRODUCTION

Parotid surgery remains a key surgical intervention for the treatment of both benign and malignant parotid tumours. Facial nerve palsy (FNP) is a potential complication that can occur as a consequence of a parotidectomy.

When post-parotidectomy FNP occurs it is usually transient. The incidence of temporary FNP post-parotidectomy has a reported range of 12% to just over 40%[1-6]. Permanent FNP is less common with a reported incidence of 0%-6%[1-5]. Key factors linked to increased risk of postoperative FNP include: the extent of the parotidectomy[1,3,6,7], revision surgery[3,7], proximity of the tumour to the facial nerve[5,7], malignancy[6] and inflammatory conditions (*e.g.*, sialadenitis)[4,5,7].

FNP can significantly affect quality of life[8], leading to distress for the patient[9] in addition to the potential for ocular complications[10]. Reducing the risk of FNP is therefore paramount. Intraoperatively this includes the use of key anatomical landmarks[11] and a facial nerve monitor as an adjunct[12].

As most cases of post-parotidectomy FNP are temporary there is a paucity of evidence assessing interventions to improve recovery of FNP. The use of corticosteroids significantly improves chance of complete recovery of FNP in Bell's palsy, with a reduction in neural oedema of the FN as a postulated mechanism[13]. Although Bell's palsy is a distinct entity to post-parotidectomy FNP, it is thought that corticosteroids could improve FNP prognosis through a similar mechanism[14].

To date there has been no systematic review assessing the efficacy of corticosteroids in ameliorating post-parotidectomy facial nerve paralysis. We sought to assess the effectiveness of corticosteroids versus no treatment in patients with post-parotidectomy FNP.

MATERIALS AND METHODS

Inclusion Criteria for considering studies

Types of studies: Well-designed randomised control trials that compared the use of steroids with no steroids for post-parotidectomy facial nerve paralysis were included.

Types of participants: Patients undergoing parotidectomy (superficial and deep) for benign parotid lesions and malignant parotid lesions.

Types of interventions: We included trials that utilised corticosteroids of any type for post-parotidectomy facial nerve paralysis.

Outcome measures: The primary outcome measure was facial nerve function monitored at increments after parotidectomy. Objective assessment of the facial nerve function is undertaken utilising the House Brackmann scale, and further classified based upon the location of the facial muscle.

Search strategy

We developed a search strategy to identify randomised controlled trials in the following databases: MEDLINE, EMBASE, Cochrane Library and NHS Evidence (from inception until August 2014). Search terms were as follows: "steroids" AND "parotidectomy", "dexamethasone" AND parotidectomy and "prednisolone" AND "parotidectomy". Relevant articles were then selected and their references screened to identify further articles.

Data collection and analysis

Study selection: Two review authors (KV and IB) assessed abstracts for relevant articles and the full text of these was obtained. The review authors (KV and IB) independently assessed these full-text articles, and any disagreements on inclusion were resolved by discussion with a third author (ND).

Data extraction: Two review authors (KV and IB) extracted data from included studies with standardised forms. Data extracted included: authors, year of publication, participants (sample size, demographics, type and extent of parotid lesion, type of parotid surgery performed, *etc.*), intervention (type of steroid used and duration) and results (primary and secondary outcome measures, effect size, statistical significance, adverse effects).

Quality assessment: To assess the risk of bias in included studies we utilised the Cochrane risk of bias tool[15].

Statistical analysis

Due to a variation in the type of corticosteroids utilised in included studies (with regards to potency and duration of action) and variations in the protocol of administration, a meta-analysis was not appropriate and thus not carried out.

RESULTS

Description of studies

The original search produced 46 abstracts, from which 11 duplicate studies were excluded. The remaining 35 articles were screened for relevance. 33 articles were rejected as they did not meet the inclusion criteria. A total of two papers met the

eligibility criteria[14,16].

Reasons for exclusion included the study having no relevance to the research question (n = 32) and not being a randomised controlled trial (n = 1). Table 1 summarises included studies.

Interventions

Of the two included studies the interventions used to assess corticosteroid efficacy in post-parotidectomy FNP were dexamethasone[14] and prednisolone[16].

Dexamethasone was administered in two doses intravenously (stratified based on extent of parotid surgery with superficial receiving 0.51 mg/kg and deep receiving 1.41 mg/kg) administered at 8 and 16 h postoperatively[14].

Prednisolone was administered orally as a 10-d reducing course (50 mg/d for 5 d, 30 mg/d for 3 d and 10 mg/d for 2 d)[16].

The control groups received intravenous saline[14] and oral lactose[16] administered with the same protocol as their respective interventions.

Participants

The participant cohort varied slightly between both trials. One included all patients undergoing parotid surgery[14], whilst the other only included those who developed a postoperative FNP[15]. Parotid operations ranged from superficial to total (or deep) parotidectomy in both trials and both studies included only adult patients[14,16].

Outcomes and follow-up

Both trials assessed facial nerve function through clinical assessment. One assessed four facial nerve muscle groups and graded percentage function[14]. The other utilised the House Brackmann scale[17] [grading facial from 1 (normal function) to 6 (total paralysis)][16]. Duration of postoperative follow-up ranged from 6 mo[16] to 12 mo[14].

Risk of bias in included studies

Both included studies were assessed for quality focusing particularly on: randomisation methods, concealment of allocation, effectiveness of blinding, follow up and attrition rates, comparability of groups at baseline and adherence to treatment.

Neither trial described the methods of randomisation, but both had adequate allocation concealment and effective blinding from both the patients and clinicians[14,16].

Both trials had some limitations with regards to comparability of control and intervention groups at baseline. Neither trial made reference to comparability with regards to tumour factors [type of tumour (malignant or benign) or size of the tumour][14,16]. With regards to use of a single surgeon allowing prevention of technique confounding the results, one trial utilised more than one surgeon (including surgeons in training)[14], whilst the other did not specify if a single surgeon undertook the operations[16].

One trial made no reference to extent of compliance and adherence to treatment[15], whilst the other administered treatment intravenously in the immediate postoperative period allowing total compliance[14].

Effects of interventions

Dexamethasone: A variety of analyses were undertaken due to the varying doses within the treatment protocol. Overall, no therapeutic advantage was found with the use of dexamethasone[14]. A higher dose of dexamethasone conferred no functional advantage[14]. Interestingly, early postoperative facial nerve function was better in the placebo group (overall and in superficial and deep parotidectomy cohorts) although not statistically significant; median time to complete recovery of facial nerve function was shorter in the placebo group (150 d in the dexamethasone group *vs* 60 d in the control group)[14].

Prednisolone: There was minimal difference in extent of recovery from FNP in prednisolone *vs* placebo treated patients at 1, 3 and 6 mo ($P > 0.10$)[16]. Eighty-four percent of patients with FNP had full recovery at 3 mo, increasing to 98% by 6 mo[16]. One patient that had a total parotidectomy had a permanent FNP that persisted at 18 mo[16].

Adverse effects: No adverse effects from short term dexamethasone therapy were noted[14]. One patient was found to have "minor symptoms" from the use of prednisolone (although the precise symptoms were not stated)[16].

DISCUSSION

Overall, there appears to be no benefit conferred by corticosteroids for FNP recovery post-parotidectomy. However, this systematic review demonstrates that there is a paucity of evidence assessing the use of corticosteroids in treatment of FNP post-parotidectomy.

Two corticosteroid preparations have been assessed in RCTs with slightly varying mechanisms and durations of actions[14,16]. Prednisolone has mixed glucocorticoid and mineralocorticoid properties, whilst dexamethasone only has glucocorticoid properties (albeit much more potent than prednisolone) and a longer duration of action[18]. This variation in the mechanisms of action allowed different dosing regimens in the two included trials. Despite the variation in types of steroids and dosing regimens, there was no evidence to demonstrate an improved chance of full recovery nor improve recovery

Table 1 Characteristics of included studies

Roh and Park[16]	
Methods	Randomised controlled trial
Participants	Patients undergoing parotidectomy (superficial, partial, total) ± neck dissection 44 patients Exclusion: 1 Direct FN invasion of FN requiring FN sacrifice and reconstruction 2 Incidental cutting of the facial nerve
Interventions	Started day 1 or day 2 postoperatively Reducing dose of oral prednisolone (50 mg/d for 5 d, 30 mg/d for 3 d and 10 mg/d for 2 d) Placebo group received lactose with similarly formulated doses
Outcomes	House Brackmann grading of FN by two blinded experts Assessed postoperatively: immediately, 1 wk, 1 mo, 3 mo and 6 mo
Results	Overall recovery times from FNP: At 3 mo: 84% had fully recovery At 6 mo: 98% had full recovery Prednisolone *vs* placebo recovery at 1, 3 and 6 mo (minimal difference) ($P > 0.10$)
Notes	One patient was lost to follow-up and excluded from the analysis (prednisolone group)
Risk of Bias	
Method of randomisation	Not specified
Allocation concealment	Adequate
Other confounding factors	Groups comparable demographically and extent of postoperatively FNP, however tumour size, type or type of parotid surgery not compared in between intervention and placebo groups
Lee *et al*[14]	
Methods	Randomised controlled trial
Participants	Patients undergoing superficial or total parotidectomy 49 patients Exclusion criteria: Diabetes, age < 18, peptic ulcer disease, previous adverse reaction to steroids and any other contraindication to steroids Prior parotid surgery, anticipated section of FN and pre-existing FNP
Interventions	Two doses of dexamethasone (0.51 or 1.41 mg/kg) depending on type of surgery (superficial or total parotidectomy respectively) at 8 and 16 h postoperatively Placebo group received saline at the same intervals
Outcomes	Facial nerve function in the four major regions was assessed (frontal, orbital, midface, upper lip and lower lip) at a percentage 0-100 depending on extent of function Assessed postoperatively: immediately and every month for 12 mo (or until facial nerve function returned to normal)
Results	Average early postoperative facial nerve function: All patients ($n = 49$): 75.4% Overall: Dexamethasone (69.5%) *vs* placebo (81.3%) ($P = 0.239$) Dose of dexamethasone: High (63.9%) *vs* low (74.7%) ($P = 0.118$) Type of surgery: Superficial ($P = 0.637$) and deep ($P = 0.465$) Time to full recovery of facial nerve (median): Placebo (60 d) *vs* Dexamethasone (150 d) (no *P* value stated)
Notes	As intervention administered intravenously, total compliance can be ensured
Risk of Bias	
Method of randomisation	Not specified
Allocation concealment	Adequate
Other confounding factors	Initial power calculation required 120 patients, however a nationwide shortage of the intervention drug (dexamethasone) allowed only 52 patients to be enrolled in the trial No comparison of the type of parotid lesion excised within the trial groups (*i.e.*, malignant or benign and tumour size) Operations were conducted by more than one surgeon (including junior residents)

times[14,16].

The use of corticosteroids is thought to reduce neural oedema, a proposed mechanism for their excellent efficacy in treating Bell's Palsy[13]. One postulated mechanism of FNP in parotidectomy is stretch of the FN[4,19] leading to neural degeneration[20]. This may explain the lack of efficacy of corticosteroids in treating FNP post-parotidectomy.

There is compelling evidence to suggest that most cases of FNP post-parotidectomy are transient. Moreover, the risk of FNP is associated with a plethora of tumour and intraoperative factors (deeper parotidectomy[1,3,6,7] revision surgery[3,7], the facial nerve being near the tumour[5,7], malignancy[6] and inflammatory conditions[4,5,7]).

Prevention of FNP in parotidectomy is therefore largely linked to operative techniques, including the use of key anatomical landmarks to identify the FN[11]. The use of a facial nerve monitor has been suggested as an adjunct to help prevent postoperative FNP, with reasonable efficacy demonstrated[12,21]. Unfortunately, if FNP does occur its extent may dictate likelihood of full recovery, with a FNP preventing closure of the eyes being a predictor of permanent dysfunction[22].

Nonetheless, when FNP does occur it can significantly reduce quality of life[8]. It is therefore

key to ascertain interventions that can improve time to recovery. Unfortunately, both RCTs included in this trial did not account for the variety of tumour factors that can increase of postoperative FNP. The need for a high quality RCT assessing the use of corticosteroids in specific cohorts of patients is highlighted (particularly low risk patients, *e.g.*, patients with benign parotid tumours undergoing superficial parotidectomy, in whom the perceived risk of FNP should be lower).

Few adverse effects were reported by both randomised controlled trials, highlighting the relative safety of their use.

Limitations

The randomised trials included in this study had some limitations. Most importantly, statistical assessment of confounding factors in control and treatment groups did not specifically assess tumour factors[14,16]. Moreover, one trial did not achieve the power calculation sample size[14], limiting interpretation of its statistical analysis. The methods of randomisation were also unclear in both trials[14,16].

Implications for practice

Based upon current best evidence the use of corticosteroids to ameliorate postoperative FNP cannot be recommended. It is likely that preoperative and intraoperative factors play a more important role in the risk of permanent FNP. Moreover, the majority of cases of FNP are likely to recover, an important factor to consider in preoperative counselling of patients.

Implications for research

Given the extensive effect of FNP on quality of life, it is in the interest of patients to ascertain methods of improving recovery times. Future research should focus on assessing the cohort of patients in whom permanent FNP is more likely, allowing better preoperative counselling. Moreover, well-designed randomised controlled trials that assess the use of corticosteroids in more statistically comparable groups (*i.e.*, with regards to the type of parotid operation and tumour factors), that will allow assessment of specific cohorts of patients in whom corticosteroids may provide benefit.

COMMENTS

Background

Facial nerve palsy (FNP) is a potential complication that can occur after parotidectomy. FNP can be temporary or permanent, and can significantly affect quality of life. Corticosteroids have been proposed as a treatment for post-parotidectomy FNP. A systematic review of clinical trials is needed to provide scientific evidence for the efficacy of the use of corticosteroids for post-parotidectomy FNP.

Research frontiers

Parotidectomy, facial nerve palsy.

Innovations and breakthroughs

Both studies in this systematic review demonstrated no evidence that corticosteroids improve the prognosis of FNP after parotidectomy. Preoperative factors including the size of tumour and presence of malignancy, as well as intraoperative factors including the extent of parotidectomy (superficial or deep) and facial nerve integrity at the end of the operation are key in determining prognosis of FNP when it occurs.

Applications

There is no convincing evidence to propose the routine use of corticosteroids for post-parotidectomy FNP. Further clinical trials are needed to assess the efficacy of corticosteroids in ameliorating FNP in specific cohorts of patients.

Terminology

Parotidectomy is a commonly performed operation for the treatment of both benign and malignant parotid gland pathology. The facial nerve is at risk during parotidectomy.

Peer review

This study is well conducted and written.

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CASE REPORT

Complete remission of primary hepatic lymphoma in a patient with human immunodeficiency virus

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Abstract

Diffuse large B cell primary hepatic lymphoma is a rare disease with limited available information regarding treatment strategy. Although the liver contains lymphoid tissue and is an important site for lymphocytes activation, primary hepatic lymphoma is rare. Host factors make the liver a poor environment for malignant lymphoma development. Its coexistence with human immunodeficiency virus (HIV) infection increases morbidity and mortality risks. Additionally, jaundice increases chances of developing adverse effects from chemotherapy. Here, we report a case of diffuse large B cell primary hepatic lymphoma in a 32-year-old HIV positive man. Due to elevated liver enzyme levels and jaundice, the patient was initially treated with an R-DHAP regimen, which was replaced with an R-CHOP regimen. Restaging images with a positron emission tomography scan after the latest chemotherapy cycle confirmed remission. This is the first report of complete remission of primary hepatic diffuse large B cell lymphoma in an HIV positive patient in the English literature.

Key words: Primary hepatic lymphoma; Diffuse B cell lymphoma; Human immunodeficiency virus; R-DHAP; R-CHOP

Core tip: There are limited reports related to successful management of primary hepatic lymphoma in human immunodeficiency virus (HIV) patients. This case report is not only considered as the first report of complete remission of primary hepatic diffuse large B cell lymphoma in an HIV positive patient in the English literature, but also describes the use of R-DHAP as an induction regimen in the setting of significant impaired liver function and severe immunocompromised status. The use of R-DHAP as an induction regimen in management of primary hepatic lymphoma in HIV patients was never reported.

Widjaja D, AlShelleh M, Daniel M, Skaradinskiy Y. Complete remission of primary hepatic lymphoma in a patient with human immunodeficiency virus. *World J Clin Cases* 2015; 3(2): 186-190 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i2/186.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i2.186

INTRODUCTION

Primary hepatic lymphoma (PHL) is a rare disease associated with immunodeficiency diseases and chronic viral hepatitis. From 1981 to 2003, only 358 cases of primary hepatic lymphoma were reported[1]. Data surrounding disease managements in patients with human immunodeficiency virus (HIV) infection is lacking. Here, we present a case of an HIV positive man whose PHL subsided into complete remission after chemotherapy.

CASE REPORT

A 32-year-old man with no known significant chronic medical problems was admitted to the hospital due to severe right upper quadrant abdominal pain, fever, night sweats, and unintentional weight loss. There was no history of recent heavy alcohol consumption. The patient appeared jaundiced and had a tender and firm hepatomegaly with a liver span of 19 cm. The lymph node was not enlarged. An abdominal ultrasound revealed multiple small hypoechoic lesions throughout the liver, common bile duct of 3.2 mm and normal gallbladder without gallstones. A computed tomography (CT) scan of the abdomen and pelvis with intravenous contrast revealed hepatomegaly with multiple small low attenuation nodules throughout the liver parenchyma, normal common bile duct and small ascites in the pelvis (Figure 1). Magnetic resonance imaging of the abdomen with intravenous contrast confirmed CT scan findings. All imaging studies showed no extrahepatic lymphadenopathies. A liver biopsy of lesions revealed diffuse large B cell lymphoma with non-specific lobular hepatitis (Figure 2). Immunohistochemical stains of the liver specimen revealed $CD20^+$, $CD79^+$, $CD79a^+$, $CD4^-$, and $CD3^-$ cells (Figure 3). The patient also tested positive for HIV infection. Tests for hepatitis B and C were negative. Additional tests results are shown in Table 1.

The patient was diagnosed with Stage 1BE primary liver large B cell lymphoma, and started on anti retroviral therapy along with chemotherapy during in-patient care. The initial anti retroviral medications were efavirenz, emtricitabine, and tenofovir. On the 7th day of the treatment, efavirenz was changed to raltegravir due to the presence of G190A mutation on HIV genotyping testing which confers resistance to non-nucleoside reverse transcriptase inhibitor mutation. Eight cycles of chemotherapy were administered together with anti retroviral therapy. In view of elevated liver enzymes and jaundice, chemotherapy with a platinum based regimen (R-DHAP) was initiated. The regimen of this first cycle consisted of 1.5 mg/kg of oral prednisone, 375 mg/m^2 of rituximab, 100 mg/m^2 of cisplatin, and 2000 mg/m^2 of cytarabine. As the co-administration of cisplatin and tenofovir might have increased his risk of toxicity to the kidney proximal tubule, serum creatinine was monitored closely. His serum creatinine levels were always less than 1 mg/dL and the calculated creatinine clearance was maintained at the level of 77 mL/min per 1.73 m^2. Nine days after starting the first chemotherapy cycle, he developed significant thrombocytopenia (nadir of 10000 cells/uL) and neutropenia (nadir of 300/uL). When platelet count was 10000/uL, the patient had an episode of epistaxis which was controlled after platelets transfusion. He did not develop fever during the episode of neutropenia. Filgrastim was given for 3 d when neutropenia was 300/uL. Upon the completion of first cycle of chemotherapy, the patient remained afebrile and became less jaundiced. Chemotherapy normalized liver enzymes and bilirubin (Table 1). Next, a CHOP regimen containing cyclophosphamide (600 mg/m^2), adriamycin (50 mg/m^2), vincristine (1.1 mg/m^2), and prednisone was administered. Rituximab (375 mg/m^2) was added to the CHOP regimen to start a third cycle of chemotherapy administered every 3 wk. As there was no known significant drug-drug interaction between the R-CHOP regimen and the anti retroviral regimen (tenofovir, emtricitabine and raltegravir), all medications were given according to the standard doses. After the second cycle, the patient remained anicteric with a body weight improvement of 5 kg from baseline. A CT with positron emission tomography (PET) scan performed after the 4th cycle of chemotherapy showed that the liver had reduced in size from 19.5 cm (prior to treatment) to 16.5 cm without evidence of hypermetabolic foci in the neck, chest, abdomen, or pelvis. Re-staging images with CT-PET after the 6th cycle revealed normal uptake within liver, spleen, adrenal glands, renal cortices, and the collecting system. A CT-PET scan at 5 and 11 mo after the last cycle confirmed remission (Figure 4). He is still in remission 19 mo after the treatment. During the chemotherapy, the CD4 count had improved and HIV viral loads were always undetectable (Table 1).

DISCUSSION

Fifty thousand incident cases of lymphoid neoplasms and 19000 related deaths occurred in the United States in 2005[2]. Diffuse large B-cell lymphoma is the most common non-Hodgkin lymphoma subtype and accounts for approximately 23% of all cases[3]. Chronic hepatitis C and hepatitis B and autoimmune diseases increase the risk of PHL[4,5]. Although the liver contains lymphoid tissue and is an important site for lymphocytes activation[6], PHL is rare. Host factors make the liver a poor environment for malignant lymphoma development[4,7].

Table 1 Laboratory tests prior to the chemotherapy cycles and six months post chemotherapy

	Prior to the 1st	Prior to the 2nd	Prior to the 3rd	Prior to the 4th	Prior to the 5th	Prior to the 6th	Prior to the 7th	Prior to the 8th	6 mo post chemotherapy
Hgb (g/dL)	11.9	11.3	11.1	11.3	12	12.5	11.8	12.3	14.6
Platelet count (× 10^3 /uL)	107	95	107	34	169	132	60	78	70
WBC (× 10^3/uL)	5.9	10.6	1.9	11.9	3.7	4.1	3.3	3.5	5.9
Serum albumin (g/dL)	3.5	4.5	4.4	4.5	4.5	4.5	4.6	4.1	4.9
Serum AST (unit/L)	529	38	26	26	31	23	31	26	24
Serum ALT (unit/L)	270	43	37	30	28	27	39	31	30
Serum alkaline phosphatase (unit/L)	1686	442	146	210	1025	1586	1454	190	117
Serum GGT (unit/L)	872	-	-	-	-	149	-	-	-
Serum LDH (unit/L)	1838	313	-	331	326	263	-	148	124
Total bilirubin (mg/dL)	9.7	0.9	0.6	0.5	0.2	0.2	0.4	0.3	0.7
CD4 lymphocyte count (/mm^3)	155	651	-	265	-	-	173	-	729
HIV viral load (copies/mL)	485127	81	-	< 75	-	-	< 75	-	< 75

HIV: Human immunodeficiency virus.

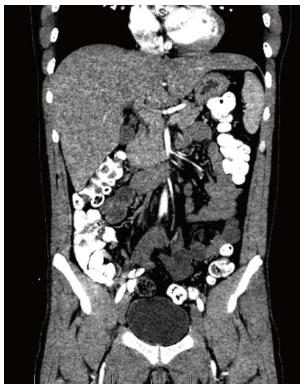

Figure 1 Computed tomography abdomen and pelvis prior to chemotherapy showing multiple small low attenuation nodules throughout the entire liver parenchyma.

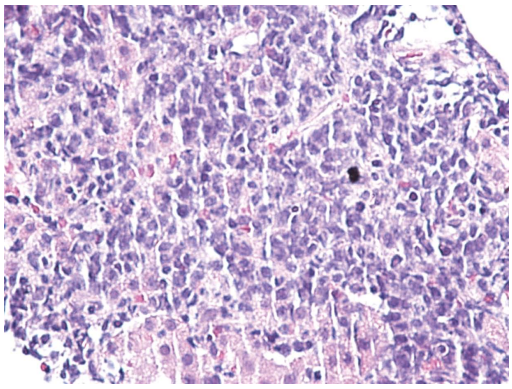

Figure 2 Liver biopsy showing diffuse large B-cell lymphomatous infiltrate.

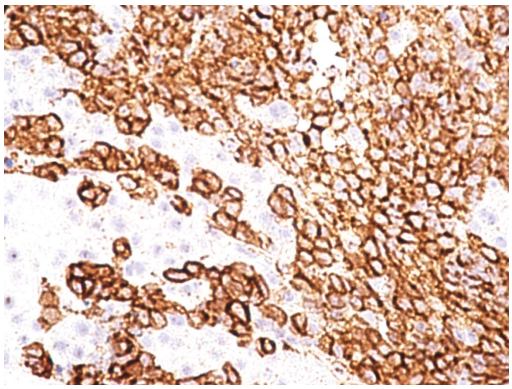

Figure 3 Immunohistochemical stain of liver specimen showing the lymphoma cells are strongly immunoreactive to CD 20.

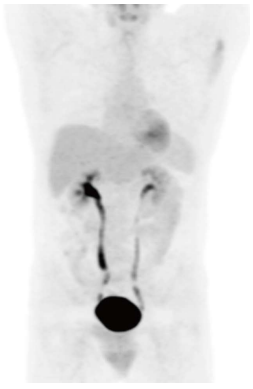

Figure 4 Maximum intensity projection of positron emission tomography/computed tomography scan at 11 mo after the last cycle of chemotherapy showing no hypermetabolic lesions in the liver.

Criteria for establishing the diagnosis of PHL include clinical, histopathological, and radiological findings. Lei *et al*[8] listed the following criteria to establish diagnosis: (1) signs and symptoms related to liver involvement at presentation including laboratory abnormalities and right upper quadrant mass or pain; (2) absence of both palpable adenopathy at presentation and radiologically evident distant lymphadenopathy; and (3) absence of leukemia on a peripheral smear.

A report from Lei[9] showed that among 90 patients with PHL, the most frequent presenting symptoms were upper abdominal pain or discomfort (56%), weight loss (40%), and fever (22%), which

occasionally mimic pyogenic liver abscess. Other symptoms included fatigue (13%), nausea and vomiting (12%), anorexia (8%), night sweats (8%), hemorrhagic diathesis (2%), dysphagia (2%), and rarely, immune thrombocytopenic purpura (1%) and hepatic encephalopathy (1%). In this particular report, 10% of patients were diagnosed incidentally without preceding symptoms. Physical examinations frequently revealed a modest hepatomegaly (82%), but jaundice infrequently presented only as a late manifestation of disease progression or related to underlying cirrhosis (13%)[9]. Lactate dehydrogenase and alkaline phosphatase are sometimes elevated[10]. Alpha-fetoprotein and carcinoembryonic antigen are often normal[4,10]. Radiologically, the presentation of multiple well-defined liver masses is more common than single lesions or diffuse hepatic involvement[11]. On ultrasound imaging, PHL is usually hypoechoic relative to a normal liver. CT scans of PHL usually show hypoattenuating lesions[4,11]. FDG-PET studies are extremely useful in evaluating treatment responses in PHL[12]. Histologically, primary diffuse large B cell lymphoma shows demarcated tumors with no intrasinusoidal invasion, atrophic reactive lymph follicles, and tests positive for the following antigens: CD10, Bcl2, Bcl6, MUM1, and CD25[5].

The United States National Comprehensive Cancer Network recommends managing diffuse large B cell lymphoma with rituximab, cyclophosphamide, adriamycin, vincristine, and prednisone (R-CHOP protocol)[13]. Other regimens include CODOX-M/IVAC (cyclophosphamide, vincristine, doxorubicin, high dose methotrexate alternating with ifosfamide, etoposide, high dose cytarabine with or without rituximab), dose adjusted EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin), dose adjusted EPOCH with rituximab, CDE (cyclophosphosphamide, doxorubicin and etoposide), CDE with rituximab, and hyperCVAD (cyclophosphamide, vincristine, doxorubicin and dexamethasone alternating with high-dose methotrexate, and cytarabine with or without rituximab)[13]. Serrano-Navarro *et al*[1] highlighted a patient with PHL who developed complete response to R-CHOP treatment regimen, remaining symptom free for more than two years. Others also reported complete response to R-CHOP in non-HIV infected patients with diffuse large B cell type primary hepatic lymphoma[14-17]. Besides the R-CHOP regimen, a regimen contains rituximab, dexamethasone, high dose cytarabine, and cisplatin (R-DHAP) has been used as a salvage therapy in patients with CD20+ diffuse large B cell lymphoma who develop first time relapse or failure with first line therapy[18]. In addition, R-DHAP, given as remission induction chemotherapy, improved progression free survival and failure free survival in patients with aggressive CD20+ non-Hodgkin lymphomas[19].

In an article in French literature, Walter *et al*[17] reported the only favorable response to an R-CHOP treatment regimen for diffuse large B cell type PHL in an HIV infected patient. The case report described a 34-year-old man whose abdomen CT scan showed multiple liver masses, the largest of which was 14 cm. After four cycles of R-CHOP treatment, the masses markedly regressed with only a residual 5 cm hypodense lesion. Our patient developed complete remission without residual hepatic lesions. To our knowledge, this is the first report of PHL treatment in an AIDS patient that resulted in a complete response. After initial treatment with an R-DHAP regimen, serum levels of liver enzymes and bilirubin, very important in preventing the prolonged half-life of cyclophosphamide and neurotoxicity of vincristine[20], decreased. This may have facilitated further remission from the R-CHOP regimen. Further studies in HIV positive patients may confirm these findings.

COMMENTS

Case characteristics

A 32-year-old man with recent history of unintentional weight loss presented with right upper quadrant pain.

Clinical diagnosis

Patient appeared jaundiced and had a tender and firm hepatomegaly with a liver span of 19 cm.

Differential diagnosis

Acute ascending cholangitis, alcoholic hepatitis and infiltrative liver disease.

Laboratory diagnosis

WBC 5.9 K/μL; ALT 270 unit/L; AST 529 unit/L; serum alkaline phosphatase 1686 unit/L; serum GGT 872 unit/L; serum total bilirubin 9.7 mg/dL; human immunodeficiency virus (HIV) viral load 485 K copies/mL.

Imaging diagnosis

Computed tomography (CT) scan of the abdomen and pelvis with intravenous contrast revealed hepatomegaly with multiple small low attenuation nodules throughout the liver parenchyma, normal common bile duct and small ascites in the pelvis. Magnetic resonance imaging confirmed CT scan findings.

Pathological diagnosis

A liver biopsy of lesions revealed diffuse large B cell lymphoma with non-specific lobular hepatitis. Immunohistochemical stains of the liver specimen revealed $CD20^+$, $CD79^+$, $CD79a^+$, $CD4^-$, and $CD3^-$ cells.

Treatment

Eight cycles of chemotherapy were administered. R-DHAP regimen was given in the first cycle. CHOP regimen was started in the second cycle. R-CHOP regimen was started in the third cycle.

Related reports

In view of elevated liver enzymes and jaundice, chemotherapy with a platinum based regimen (R-DHAP) was given in the first cycle of chemotherapy. Upon completion of the first cycle, the patient became less jaundiced.

Term explanation

Cluster of differentiation (CD) of immunohistochemical stain is referred to a group of antibodies recognizing an antigen. For example, the T-helper cell antigen is called CD4 antigen and the various antibodies reacting with this antigen are called CD4 antibodies.

Experiences and lessons

This case report is not only considered as the first report of complete remission of primary hepatic diffuse large B cell lymphoma in an HIV positive patient in the English literature, but also describes the use of R-DHAP as an induction regimen in the setting of significant impaired liver function and severe

immunocompromised status. The use of R-DHAP as an induction regimen in management of primary hepatic lymphoma in HIV patients was never been reported.

Peer review

This case report well written overall.

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CASE REPORT

Survival in unresectable sinonasal undifferentiated carcinoma treated with concurrent intra-arterial cisplatin and radiation

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Abstract

We report the successful use of RADPLAT to treat a patient with an unresectable T4N0 sinonasal undifferentiated carcinoma. This patient received 4 cycles of weekly intra-arterial cisplatin together with thiosulfate infusion with concurrent radiation therapy. Radiation therapy was given in 28 daily fractions to 54 Gy using intensity-modulated radiation therapy followed by a hypofractionated stereotactic boost of 3 fractions to 13 Gy to a total dose of 67 Gy in 31 fractions to the nasal sinus and bilateral neck. Intra-arterial cisplatin was administered using a bilateral approach due to the midline site of this tumor. Within days of the first intra-arterial cisplatin, there was an obvious decrease in tumor size. She has been followed with magnetic resonance imaging and positron emission tomography, and remains disease-free 47 mo post-treatment. Centers with expertise in intra-arterial chemotherapy could consider the RADPLAT approach for patients with unresectable sinonasal undifferentiated carcinoma.

Key words: Sinonasal undifferentiated carcinoma; Radiation; Intra-arterial cisplatin; Survival; RADPLAT

Core tip: Our patient with unresectable sinonasal undifferentiated carcinoma has enjoyed nearly 4 years disease-free survival after concurrent intra-arterial cisplatin and radiation.

Noticewala SS, Mell LK, Olson SE, Read W. Survival in unresectable sinonasal undifferentiated carcinoma treated with concurrent intra-arterial cisplatin and radiation. *World J Clin Cases* 2015; 3(2): 191-195 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i2/191.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i2.191

INTRODUCTION

Sinonasal undifferentiated carcinoma (SNUC) is a

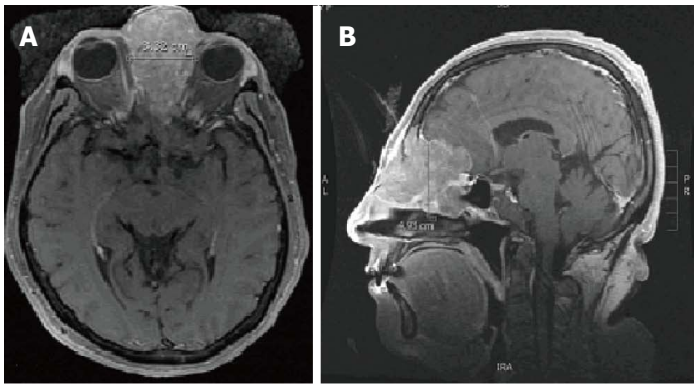

Figure 1 T1 magnetic resonance imaging of sinonasal undifferentiated carcinoma neoplasm prior to treatment. A: Axial T1 magnetic resonance imaging (MRI) and B: Sagittal T1 MRI show an avidly enhancing mass centered in the left ethmoid air cells with extension into the left frontal sinus with adjacent retained fluid and maxillary sinus with erosion of the medial orbital walls bilaterally, left greater than right. The majority of the ethmoid air cells are replaced by the neoplasm. Extension through the cribriform plate is noted with involvement of the left olfactory lobe, predominantly along the gyrus rectus. There is extensive surrounding edema in the left frontal lobe, extending back to the frontal horn of the left lateral ventricle.

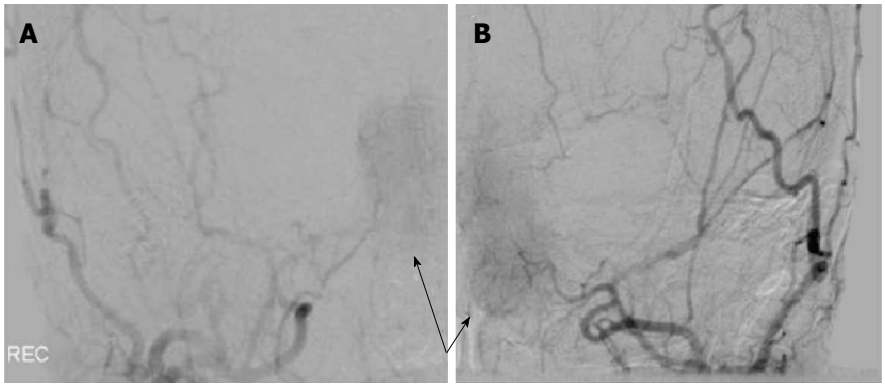

Figure 2 Right and left external carotid artery angiography injections. A: Right and B: Left external carotid angiographic injections demonstrate the tumor blush (arrows) at the first chemo treatment.

rare and highly aggressive neoplasm of the nasal cavity and paranasal sinuses[1]. Thus far, there have been less than 200 reported cases of SNUC[2]. SNUC has a poor prognosis and high mortality with one meta-analysis of 167 cases finding that the disease-free survival was only 26.3%[2]. Currently, there is not a standard treatment of care for SNUC. Treatment for SNUC typically involves a multimodal approach involving surgery (if feasible) and radiation therapy (RT) with concurrent chemotherapy[2-4]. A phase Ⅱ clinical trial in inoperable stage Ⅳ head and neck cancer previously found that the RADPLAT protocol (radiation and intra-arterial cisplatin) achieved an initial tumor response in 91% of patients with 1 and 2 years locoregional control of 82% and 69%, respectively[5]. Here, we describe the successful use of RADPLAT to treat a patient with an unresectable T4N0 SNUC.

CASE REPORT

A 60-year-old woman presented with nasal congestion and a prominence on the left side of her nose. Computed tomography (CT) revealed a tumor arising from the ethmoid sinus extending through the cribriform plate and into the anterior cranial fossa without metastasis to the chest and neck. Magnetic resonance imaging (MRI) of the face showed edema in the left frontal lobe, interpreted as suspicious for brain invasion (Figure 1). Biopsy revealed large pleomorphic tumor cells with a high nuclear to cytoplasm ratio, prominent nucleoli and focal areas of necrosis. Immunohistochemistry was positive for pancytokeratin and CD56 and weakly positive for chromogranin. She was diagnosed with a T4N0 SNUC. Since the tumor was deemed unresectable due to brain involvement, the RADPLAT protocol was chosen in hopes of maximizing her chance for local control. She received concurrent 4 cycles of weekly intra-arterial (IA) cisplatin at 150 mg/m^2, administered as a divided dose through left and right-sided feeding arteries for this midline tumor (Figure 2). With the IA cisplatin, she received intravenous (IV) thiosulfate bolus followed by thiosulfate infusion. Radiation therapy was given in 28 daily fractions to 54 Gy using intensity-modulated radiation therapy (IMRT) followed by a hypofractionated stereotactic boost of 3 fractions to 13 Gy to a total dose of 67 Gy in 31 fractions to the nasal sinus and bilateral neck. The biologically effective dose for the radiation treatment is equivalent to 82 Gy_{10} and 117 Gy_3.

Response to RADPLAT

After the first administration of intra-arterial cisplatin, there was an obvious and rapid decrease in tumor size, suggesting response to the chemotherapy. There was marked tumor size reduction after the final cycle of chemotherapy (Figure 3). She tolerated treatment well, with no toxicity from chemotherapy and expected acute sequelae including grade 2 mucositis, grade 2 dermatitis, and grade 1 conjunctivitis. Collagenase and polysporin powder with Xeroform

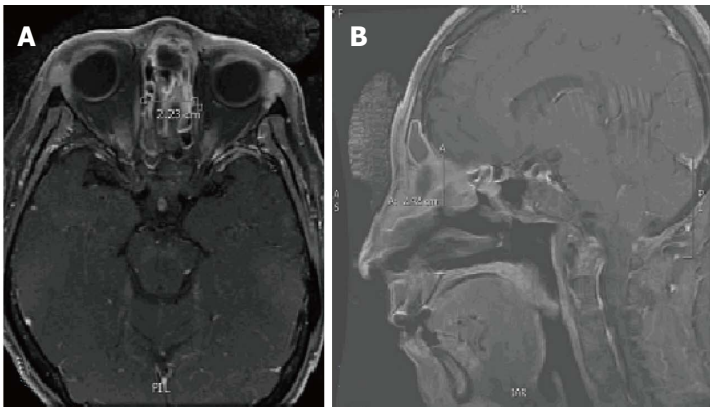

Figure 3 T1 Magnetic resonance imaging after completing fourth dose of chemotherapy. A: Axial T1 magnetic resonance imaging (MRI) and B: Sagittal T1 MRI show the mass had decreased in size as compared to the prior to treatment MRI. The mediolateral dimension is 2.23 cm which is decreased in size from the prior examination at which time it measured 3.32 cm. The AP appears to have decreased in size to 2.42 cm as compared to 4.93 cm on the prior MRI. There appears to be residual enhancing tissue in the right posterior ethmoid. The intracranial enhancement and edema within the inferior left frontal lobe is significantly decreased.

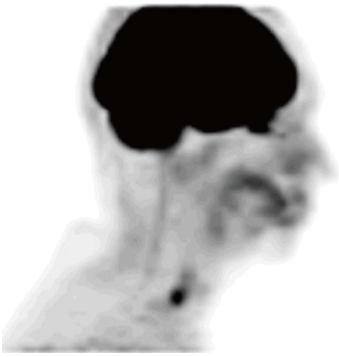

Figure 4 Positron emission tomography performed at four months post-treatment. The Positron emission tomography shows no definite or abnormal fluorodeoxyglucose (FDG) activity to suggest the presence of metabolically active tumor with special attention to the ethmoidal region adjacent to the cribriform plate. Linear FDG activity in the distal esophagus likely represents esophagitis.

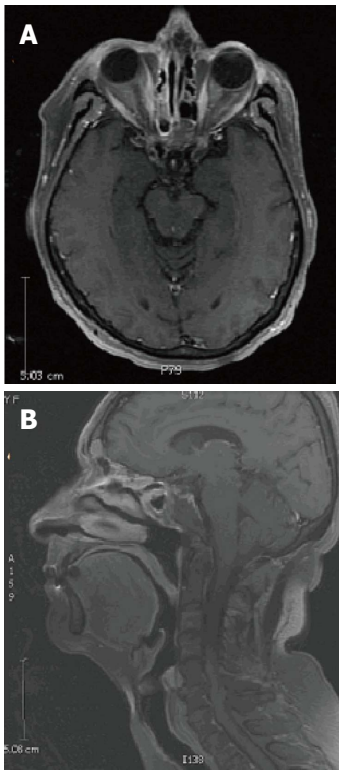

Figure 5 T1 magnetic resonance imaging 30 mo post-treatment. A: Axial T1 magnetic resonance imaging (MRI) and B: Sagittal T1 MRI show no evidence of recurrent disease. There is a decrease of the previously noted inflammatory changes in the sinuses. There is retained fluid in the bilateral frontal and left sphenoid sinuses, without bony destruction or expansion.

was used to treat radiation conjunctivitis. Positron emission tomography (PET)/CT four months post-treatment showed persistent soft tissue density in the anterior ethmoid sinuses, without fluorodeoxyglucose (FDG) uptake (Figure 4). Thirty months post treatment, MRI revealed no evidence of recurrent disease and a decrease in the previously noted inflammatory changes in the sinuses (Figure 5). The patient continues to be disease-free 47 mo post-treatment.

DISCUSSION

This case report presents long survival in a patient with an inoperable SNUC treated with concurrent intra-arterial cisplatin and radiation therapy.

Similar to our case, 84% to 92% of patients with SNUC present with T4 disease[4,6-9]. In many cases, the cancer can extend beyond the nasal and paranasal sinuses to involve the orbit and/or brain[5].

Currently there is no standard of care available for SNUC. Unresectable SNUC is generally treated with radiation or concurrent chemoradiation. Because the interventional radiologists and treating oncologists were familiar with RADPLAT, we opted to utilize this protocol in hopes of maximizing local control. In 213 patients with stage Ⅲ-Ⅳ head and neck squamous cell cancer (SCC) treated with RADPLAT, Robbins *et al*[10] reported a 5 year overall survival of 38.8% and locoregional control of 74.3%[10]. Similarly, Rabbani *et al*[11] reported locoregional control in 78% and four year overall survival in 57% in a study of 35 patients with stage Ⅲ head and neck cancer. Homma *et al*[12] evaluated the efficacy of RADPLAT for untreated advanced cancers (T3, T4a, and T4b) of the nasal and paranasal sinuses in 47 patients. During the median follow-up period of 4.6 years, the 5-year local progression-free survival rate was 78.4% for all patients[12]. Furthermore, the 5-year overall survival rate was 69.3% for all patients[12].

This study indicates that the RADPLAT protocol can not only effectively treat SCC of the head and neck, but also provide locoregional control and long-term survival in cancers specific to the paranasal and nasal sinuses.

The RADPLAT protocol involves intra-arterial infusion of cisplatin with intra-venous systemic neutralization using thiosulfate. The rapid infusion of cisplatin enables high doses of the drug to directly reach the tumor bed while the thiosulfate infusion prevents the systemic toxicity of large doses of cisplatin[10,11]. The cytotoxic effects of cisplatin are potentiated by radiation[13]. This effect was first demonstrated in murine models of tumors[14]. Studies have found that tumor resistance to cisplatin can occur within 2-4 cycles[15,16]. However, resistance can be overcome by increasing doses of cisplatin as demonstrated by *in vitro* and *in vivo* studies[17,18]. Elevated doses are not well-tolerated in patients because they can lead to undesirable side-effects such as neurotoxicity, nephrotoxicity, mucositis, and other systemic effects[19]. To circumvent the high-dose toxicity of the cisplatin, the intra-arterial infusion of cisplatin with concomitant thiosulfate enables high doses of cisplatin to reach the tumor bed without systemic toxicity. With the RADPLAT protocol, it is possible to deliver doses 10 times higher than can be delivered intravenously[17,18].

Many studies highlight the importance of surgery in improving survival in patients with SNUC[2,6,20,21]. In our case, brain involvement of the patient's SNUC made her a poor candidate for surgery, so she was treated with radical chemoradiotherapy to the primary site and bilateral neck. Elective neck irradiation for node negative SNUC is important for regional control[3,20]. Chemoradiation has previously been shown to be a viable treatment option for advanced SNUC. In one study, the 2-year progression-free survival and overall survival were 43% and 64%, respectively, with three cycles of platinum and 5-fluorouracil followed by radiation with two cycles of concurrent platinum, suggesting that induction chemotherapy followed by concurrent chemoradiation is effective[22]. This study found that among patients with SNUC treated to 50-60 Gy, all 4 patients treated with at least 60 Gy were alive without local progression at last follow-up[22]. Another study found that all patients that achieved cause-specific survival when treated with doses greater than 62.5 Gy[3]. Thus, doses of at least 60-70 Gy_2 to the primary site are recommended, if feasible.

In conclusion, our patient was effectively treated with RADPLAT with minimal toxicity and lasting disease control for nearly 4 years. Centers with expertise in intra-arterial chemotherapy could consider this modality for patients with unresectable SNUC.

COMMENTS

Case characteristics

A 60-year-old woman presented with nasal congestion and a prominence on the left side of her nose is diagnosed with unresectable T4N0 sinonasal undifferentiated carcinoma (SNUC).

Clinical diagnosis

Dullness to percussion and decrease breath sounds over the upper lobe of the right lung.

Differential diagnosis

Esthesioneuroblastoma, rhabdomyosarcoma, squamous cell carcinoma, adenocarcinoma, adenoid cystic carcinoma, lymphoma, melanoma, other soft tissue sarcoma.

Laboratory diagnosis

Labs were drawn but were found to be unremarkable.

Imaging diagnosis

Computed tomography revealed a tumor arising from the ethmoid sinus extending through the cribriform plate and into the anterior cranial fossa without metastasis to the chest and neck. Magnetic resonance imaging of the face showed edema in the left frontal lobe, extending back to the left lateral ventricle.

Pathological diagnosis

Biopsy of the mass revealed large pleomorphic tumor cells with a high nuclear to cytoplasm ratio and prominent nucleoli and focal areas of necrosis. Immunohistochemistry was positive for pancytokeratin and CD56 and weakly positive for chromogranin.

Treatment

The patient was treated with RADPLAT (concurrent intra-arterial cisplatin with simultaneous thiosulfate and radiation therapy).

Related reports

Sinonansal undifferentiated carcinoma is difficult to treat and there is currently no standard treatment protocol.

Term explanation

The RADPLAT protocol involves intra-arterial infusion of cisplatin with intra-venous systemic neutralization using thiosulfate and concurrent radiation therapy. The rapid infusion of the cisplatin enables high doses of the drug to directly reach the tumor bed while the thiosulfate infusion prevents the systemic toxicity of large doses of cisplatin.

Experiences and lessons

This case report represents long survival in a patient with an unresectable T4N0 SNUC using RADPLAT. Centers with expertise in intra-arterial chemotherapy could consider this modality for patients with unresectable SNUC.

Peer Review

These data are thorough and convincing.

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CASE REPORT

Sweet syndrome and differentiation syndrome in a patient with acute promyelocytic leukemia

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Author contributions: Solano-López G contributed to manuscript writing and patients data collection; Llamas-Velasco M contributed to main idea, patients data collection; Concha-Garzón MJ contributed to patients data collection; Daudén E contributed to supervision.

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Abstract

The differentiation syndrome is an inflammatory reaction with increased capillary permeability that occurs in up to 25% of patients with acute promyelocytic leukemia treated with all-trans retinoic acid. A 50-year-old man with acute promyelocytic leukemia underwent chemotherapy with idarubicin and all-trans retinoic acid. On day +21 the patient developed pruritic prepatelar papules as well as several 10 mm subcutaneous nodules in both thighs accompanied by persistent fever. On the day +25 the patient presented with bilateral pulmonary crackles, infiltrates in the right lower lobe and severe hypotension which required dopamine infusion. Biopsy of one of the thighs nodules was performed. A Sweet syndrome associated to a differentiation syndrome was suspected. All-trans retinoic acid therapy was discontinued and dexamethasone was administered. In 48 h the patient showed remission of the fever and the infiltrates and the skin lesions acquired a residual aspect. It is debatable whether these two syndromes are distinct entities with common mechanisms or whether they are poles of the same spectrum. Dermatologists and hematologists must be aware of these two syndromes and its pathophysiologic association.

Key words: Differentiation syndrome; Sweet syndrome; Acute promyelocytic leukemia; All-trans retinoic acid

Core tip: It is debatable whether the differentiation syndrome and the sweet syndrome are distinct syndromes with common mechanisms or whether they are poles of the same spectrum. We believe that there may be more cases of differentiation presenting with skin sweet syndrome lesions, which are underdiagnosed, overshadowed by the critical state of these patients. Dermatologists and hematologists must be aware of these two syndromes and its pathophysiologic association. It is very likely that these two specialties are staring the same phenomenon from two different points of view.

Solano-López G, Llamas-Velasco M, Concha-Garzón MJ, Daudén E. Sweet syndrome and differentiation syndrome in a patient with acute promyelocytic leukemia. *World J Clin Cases* 2015; 3(2): 196-198 Available from: URL: http://www.

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INTRODUCTION

All-trans retinoic acid (ATRA) therapy induces the differentiation of the myelogenous leukemic cells in acute promyelocytic leukemia (APL). The differentiation syndrome (DS) is an inflammatory reaction with increased capillary permeability that occurs in up to 25% of patients with APL treated with ATRA. It is characterized by respiratory distress, fever, pulmonary infiltrates, pleuropericardic effusions, renal failure and hypotension, with a mortality of up to 30% without treatment[1]. The association of sweet syndrome (SS) and DS has been exceptionally described in the literature.

CASE REPORT

A 50-year-old man presented with pancytopenia on a routinal analysis. A bone marrow (BM) aspirate showed 73% of blasts; homogeneous medium to large cells with visible nucleoli in most cases and clasmatosis. Auer rods were also seen. The red series was decreased without megakaryocytes. The BM biopsy showed that the hematopoietic parenchyma was replaced by a proliferation of myeloid cells showing a monomorphic appearance. The neoplastic cells were positive for myeloperoxidase and CD117 and negative for CD34, TdT and Glycophorin. Thirty percent of BM cells were positive for PML-RARa by fluorescence *in situ* hybridization (FISH). Diagnosed of APL the patient underwent chemotherapy with idarubicin 12 mg/m^2 and ATRA 45 mg/m^2. On day +21 of ATRA therapy, the patient developed pruritic erythematous 3-5 mm prepatelar papules as well as several 10 mm subcutaneous nodules in both thighs along with persistent fever (Figure 1). On day +25, the patient presented with bilateral pulmonary crackles, infiltrates in the right lower lobe and severe hypotension which required dopamine infusion. No features of disseminated vascular coagulation were present. The patient did not gain or lose weight and no renal or hepatic dysfunction was observed. Empirical antibiotic and antifungical treatment was started. A cutaneous biopsy of one papule showed moderate edema in the papillary dermis with perivascular infiltrates consisting predominantly of confluent neutrophils, without vasculitis or involvement of the adipose tissue (Figure 2). Blood, urine and biopsy cultures were negative. Skin lesions occurred along with the neutrophil count recovery and the disappearance of the promyelocytes in the BM smears. Based on the data, a diagnosis of SS associated with DS was made. ATRA therapy was discontinued and dexamethasone 10 mg every 12 h was administered. In 48 h the patient showed remission of the fever and the pulmonary infiltrates and skin lesions cleared. On day +29, a new BM aspiration FISH study did not show the PML-RARa translocation.

Haematologists reintroduced ATRA as maintenance therapy for the APL along with corticosteroids without new recurrences.

DISCUSSION

Cases of drug-induced SS associated with ATRA has been exceptionally described in the literature. Although systemic manifestations in SS are uncommon, there are cases of biopsy proven pulmonary involvement[2-4].

As far as we are concerned, there are only 2 cases of SS associated with DS in patients with APL (Table 1)[2,5].

It is debatable whether the DS and the SS are distinct syndromes with common mechanisms or whether they are poles of the same spectrum. They share common features such as fever, infiltration of neutrophils and improvement with steroid therapy. One of the differences between these two syndromes is that in most cases of SS, the involvement is limited to the skin while the main difference is the capillary leakage in the DS which is produced by the cytokine storm released by the promyelocytes as they mature. ATRA induces the differentiation of myelogenous leukemic cells into mature myeloid cells conferring them functional properties with modification of their migratory capability.

We know that these two syndromes are caused by ATRA therapy but we cannot rule out the possibility that they can be the sides of the same phenomenon with common mechanisms. For some authors, the SS and the DS are different inflammatory reactions with common mechanisms induced by ATRA therapy[6] while Ueno *et al*[7] thought that the SS due to ATRA therapy could represent a partial form of the DS.

We believe that there may be more cases of DS presenting with skin SS lesions which are underdiagnosed, overshadowed by the critical state of these patients. Dermatologists and hematologists must be aware of these two syndromes and its pathophysiologic association. It is very likely that these two specialties are staring the same phenomenon from two different points of view.

COMMENTS

Case characteristics

A 50-year-old man with acute promyelocytic leukemia presented with erythematous prepatelar papules as well as several subcutaneous nodules in both thighs along with persistent fever, pulmonary crackles and hypotension.

Clinical diagnosis

Sweet syndrome lesions and systemic symptoms in a patient who underwent chemotherapy with idarubicin and all-trans retinoic acid (ATRA).

Differential diagnosis

Sepsis, drug reaction.

Table 1 Cases of sweet syndrome associated with differentiation syndrome in patients with acute promyelocytic leukemia treated with all-trans retinoic acid

Ref.	Age(yr)/sex	Cutaneous location	Biopsy	Onset of skin lesions after ATRA induction therapy (d)	Onset of the DS after ATRA induction therapy (d)	DS signs and symptoms	Steroid response	Time until improvement
Takada *et al*[5]	49/F	Arms	Yes	18	28	Respiratory distress	Yes	24 h
Astudillo *et al*[2]	46/M	Trunk, arms, lower extremities	Yes	6	uk	Weight gain	Yes	Unknown
This case	50/M	Trunk lower extremities	Yes	21	25	Respiratory distress Hypotension	Yes	48 h

DS: Differentiation syndrome; ATRA: All-trans retinoic acid; M: Male; F: Female.

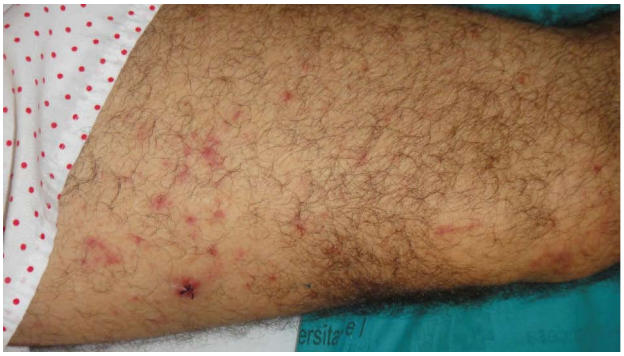

Figure 1 Papules and nodules appeared on day +21 of all-trans retinoic acid therapy.

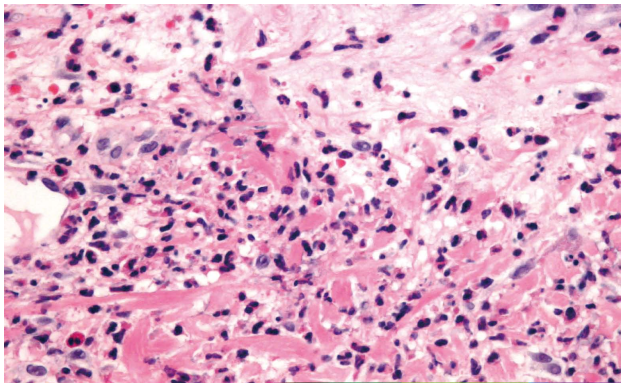

Figure 2 Infiltrates consisting predominantly of neutrophils, without vasculitis (HE, × 20).

Laboratory diagnosis

Blood, urine and biopsy cultures were negative.

Imaging diagnosis

Pulmonary infiltrates in the right lower lobe on a chest radiography.

Pathological diagnosis

A biopsy of a papule showed moderate edema in the dermis with perivascular infiltrates consisting predominantly of confluent neutrophils, without vasculitis or involvement of the adipose tissue.

Treatment

Dopamine infusión, empirical antibiotics, ATRA therapy discontinued and dexamethasone 10 mg every 12 h.

Related reports

As far as the author are concerned, there are only 2 cases of Sweet syndrome associated with differentiation syndrome in patients with acute promyelocytic leukemia.

Term explanation

Sweet syndrome is an inflammatory neutrophilic skin condition characterized by a sterile infiltrate of normal polymorphonuclear leukocytes.

Experiences and lessons

The authors believe that there may be more cases of differentiation syndrome presenting with skin sweet syndrome lesions which are underdiagnosed, overshadowed by the critical state of these patients.

Peer review

In the submitted manuscript the authors described a rare disorder–a drug-induced sweet syndrome associated with ATRA therapy for a patient with acute promyelocytic leukemia (APL). The association of sweet syndrome and ATRA-induced differentiation syndrome is rarely observed in APL. The case in this report is well-described, along with relevant lab results.

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CASE REPORT

Gingival unit transfer using in the Miller Ⅲ recession defect treatment

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Author contributions: Kuru B designed the report; Kuru B performed the surgery; Yıldırım S collected the patient's clinical data and wrote the paper.

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Abstract

The most significant factor for the success in soft tissue grafts is the synergistic relation between vascular configuration and involved tissues. In the soft tissue graft procedures, site specific donor tissue is assumed to have improved potential for function and aesthetic survive at recipient sites. On a clinical level, using site specific gingival unit graft that placed on traditionally prepared recipient site, results in predictable root coverage. In this case report the clinical effectiveness of gingival unit transfer (GUT) technique performed on Miller Ⅲ recession was presented and a similar recession case treated with free gingival graft (FGG) technique for comparison. Probing depth, recession depth, keratinized tissue width and clinical attachment level clinical parameters were measured at baseline and postoperative 8 mo. Percentage of defect coverage was evaluated at postoperative 8 mo. Creeping attachment was assessed at postoperative 1, 3, 6 and 8 mo. The GUT revealed better defect coverage and creeping attachment results than the FGG in the treatment of Miller Ⅲ defects.

Key words: Autografting; Gingiva; Gingival recession; Tooth root; Transplants

Core tip: On a clinical level, using site specific vascular configuration gingival unit graft for donor tissue that placed on traditionally prepared recipient site, results in predictable defect coverage. This report was to evaluate effectiveness of gingival unit transfer technique in comparison with free gingival graft technique on clinical parameters in the Miller Ⅲ recessions treatment.

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INTRODUCTION

Gingival recession is the denudation of root surfaces as a result of the relocation of the gingival margin apical to the cement-enamel junction (CEJ)[1] that causes root hypersensitivity and aesthetic problems[2].

Recession defects can be treated with numerous surgical procedures such as free gingival grafts[3], connective tissue grafts[4], acellular dermal matrix grafts[5], various pedicle flaps[6,7], combinations of

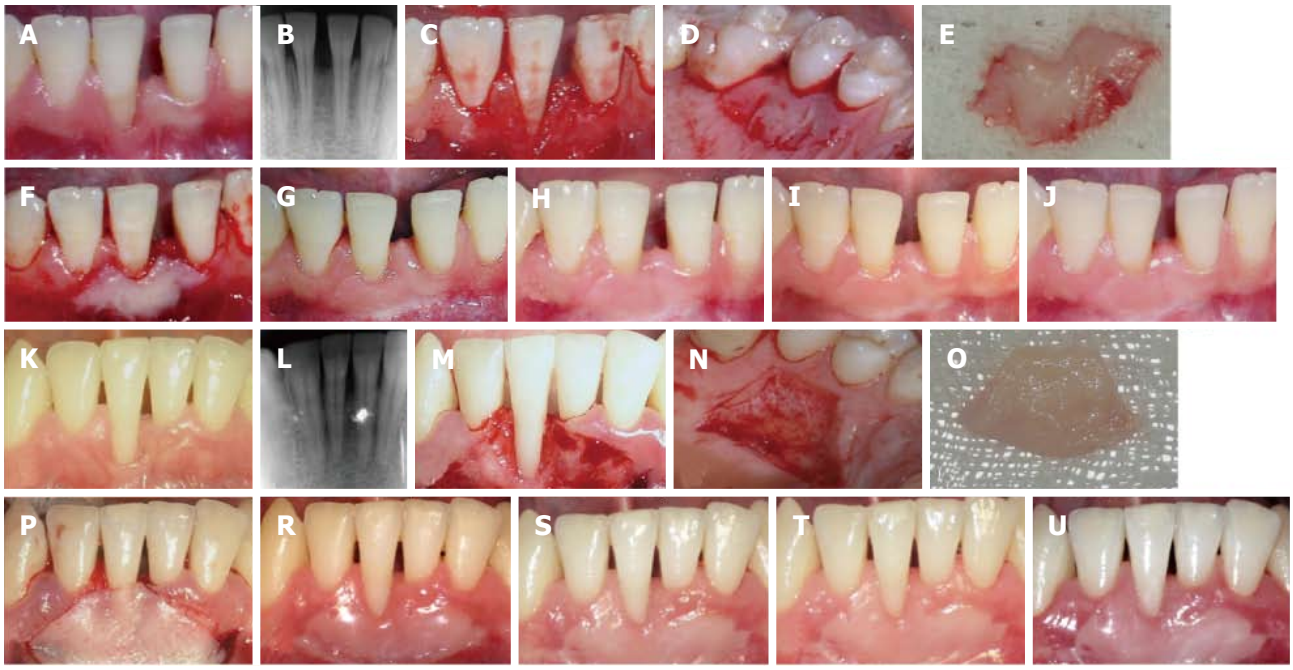

Figure 1 Surgical procedures and follow-ups in treatment with gingival unit transfer and free gingival graft. Surgical procedures and follow-ups in treatment with gingival unit transfer: (A: Initial clinical appearance; B: Radiographic appearance; C: Recipient site; D: Donor site; E: Gingival unit graft; F: Gingival unit graft in place; G: 1 mo after surgery; H: 3 mo after surgery; I: 6 mo after surgery; J: 8 mo after surgery); surgical procedures and follow-ups in treatment with free gingival graft: (K: Initial clinical appearance; L: Radiographic appearance; M: Recipient site; N: Donor site; O: Free gingival graft; P: Free gingival graft in place; R: 1 mo after surgery; S: 3 mo after surgery; T: 6 mo after surgery; U: 8 mo after surgery).

these pedicle flaps and graft techniques[8,9] and guided tissue regeneration[10]. The literature review presents different rates of success and predictability with these surgical procedures[11-13]. Nevertheless, additional clinical studies are needed to define the issues that are in a relation with the predictable and successful results[12].

The synergistic relation between vascular configuration and related tissues is one of the major factors for the success in soft tissue grafts[14,15]. Gingival tissue has complex and unique vascularity[16]. Supracrestal part of gingiva, as well as the donor tissue, is naturally created and specifically designed to function and survive above avascular denude root surfaces[14] in the soft tissue grafts procedures[17]. Gingival unit (GU) graft with site specific vascular supply placed on traditionally prepared recipient area may have capacity for survival on root surfaces and results in predictable root coverage[18].

Most clinical studies about root surface coverage have focalized on Miller Ⅰ-Ⅱ recession treatment[19]. Defect coverage by using gingival unit transfer (GUT) on Miller Ⅰ-Ⅱ recession defects revealed successful results in a previous clinical study[18]. However, there is a lack of success and ability to provide root coverage in Miller Ⅲ recession defects, because of interproximal bone and soft tissue loss[20]. There are different anatomical characteristics when compared with Miller Ⅰ-Ⅱ recession defects, as if prominent and avascular root surfaces, decreased periosteal bed and occasionally deep periodontal pocket depths[21].

The purpose of this case report is to present the clinical results of two cases of Miller Ⅲ localized recessions treated by using GUT and free gingival graft (FGG).

CASE REPORT

In April 2009, a 25-year-old woman (case Ⅰ) and 21-year-old man (case Ⅱ) with single Miller Ⅲ recession defects on mandibular right central incisor were applied to the Periodontology Department of Marmara University (Figure 1A, B, K and L). Case Ⅰ had complaints about aesthetics and tooth loss whereas case Ⅱ about hypersensitivity. Patients were non-smokers, did not have any medical problems and there were no contraindications for periodontal surgery. After clinical examination, oral hygiene motivation and mechanical periodontal treatment were performed.

Recession depth (RD) was recorded from CEJ to margin of the gingiva, probing depth (PD) was recorded from margin of the gingiva to the bottom of the pocket, clinical attachment level (CAL) was recorded from CEJ to bottom of the pocket, keratinized tissue width (KTW) was recorded from the margin of the gingiva to mucogingival junction, at baseline and postoperative 8 mo with a manual probe (PCP UNC-15, Hu-Friedy, Chicago, IL.). Only RD parameter was measured at 1, 3 and 6 mo for the evaluation of soft tissue creeping coronally.

One clinician (BK) performed surgical procedures

Table 1 Clinical parameters at baseline (0 d) and 8 mo

Parameters	Case Ⅰ gingival unit graft technique			Case Ⅱ free gingival graft technique		
	0 d	8 mo	Gain	0 d	8 mo	Gain
Recession depth (mm)	3	0.5	2.5	4	2	2
Probing depth (midbuccal) (mm)	1	1	0	2	1.5	0.5
Clinical attachment level (mm)	4	1.5	2.5	6	3.5	2.5
Keratinized tissue width (mm)	2	7	5	1.5	6	4.5
Defect closure (%)		83			50	

and another clinician (SY) evaluated clinical measurements. Local anesthesia was made and then in both cases, the recipient site was prepared by two vertical beveled incisions that extending apically to adjacent teeth, 3 to 4 mm across to the mucogingival line, and the surfaces of interdental papillae was removed (Figure 1A, C, K and M)[14]. The incisions were divergent therefore the recipient site was trapezoidal. At the mucogingival line, vertical incisions were connected by a horizontal incision. A partial thickness dissection was made apical to the alveolar mucosa. The epithelial surfaces within these incisions were deepithelized. The base of the recipient site was ⩾ 5 mm apical to the apical part of the exposed portion of the root surface. The root planning was made in the exposed portion of the root surface with hand instruments. Then irrigated with saline[18].

In case Ⅰ, the GU graft was harvested from the palatal part of the premolar area (Figure 1D) including the marginal gingival tissue and the papillae. In case Ⅱ, the donor FGG was conventionally dissected from the palate aspect of the premolar area, but ⩾ 2 mm apical from the margin of the gingiva (Figure 1N)[18]. In both cases, thickness of grafts were about 1 mm[22]. Then the grafts were sutured at the level to the CEJ (Figure 1E, F, O and P), and compressed for 2 min[18]. The periodontal dressing was applied to the donor sites for closing the wound. After 1 wk, the dressing and sutures were removed.

At the postsurgical care for infection control, the patients were advised rinse twice daily with 0.2% chlorhexidine solution for 3 wk, avoid brushing and hard chewing. After that, a gentle coronally directed brushing in the surgical area was recommended. During the first 2 mo recall appointments were scheduled every second week, and then patients were called once a month for the postoperative following period[18].

At postoperative period in both patients clinical healing in both the recipient and donor sites was complete and no complications were observed. Pre (0 d) and postsurgical (8 mo) clinical parameters are shown in Table 1. At 8 mo, 2.5 mm defect coverage with a PD of 1 mm, CAL gain of 2.5 mm and KTW gain of 5 mm was observed in case Ⅰ grafted with a GUT. Two millimetre defect coverage with a PD of 1.5 mm, CAL gain of 2.5 mm and KTW gain of 4.5 mm was observed in case Ⅱ grafted with FGG (Table 1). Percentage of defect closure were 83% and 50% in cases Ⅰ and Ⅱ, respectively. The creeping attachment level in case Ⅰ was 1.5 mm between 1 and 8 mo period (Table 2). The margin of the GU graft was moved coronally, and an acceptable colour and configuration harmony with adjacent gingival tissues was seen (Figure 1G, H, I and J). In case Ⅱ, there was no color harmony with the adjacent tissue and 1 mm of creeping was detected at the same follow-up period (Figure 1R, S, T and U) (Table 2).

DISCUSSION

GUT technique, using GU graft as a donor tissue with site specific vascular supply, was evaluated in treatment of a single Miller Ⅲ gingival recession case. GUT is a modification of FGG with the difference of including marginal gingiva and papillae in the conventional palatal tissue graft that vascular supply matches intimately with the recipient site[14,18]. After 8 mo in this case, RD reduction and defect coverage were found in favor of GU graft compared to FGG.

This is the first case reporting the use of GUT technique in the Miller Ⅲ localized gingival recession treatment. There are no clinical studies or case reports with which to compare our clinical outcomes. There is one case report in which gingival unit was used as a FGG[14] and a randomized clinical trial evaluating GUT in comparison with FGG in the Miller Ⅰ-Ⅱ recession defects treatment[18]. In this case report, the GU graft performed in the Miller Ⅲ recession defect treatment, 2.5 mm of RD reduction; 83% defect coverage together with gains in CAL and keratinized tissue (KT) were reported. The reduction in recession was in accordance with the attachment gain. The mean defect coverage was 50% in the FGG case, presenting an obvious difference from the GUT case.

According to our clinical outcomes, GUT resulted in almost indistinguishable texture and colour with neighbouring soft tissues. Creeping defines the postoperative movement of marginal gingiva coronally[23]. Allen[14] presented equivalent results in his case report that the marginal position of the

Table 2 Recession reduction in 1-3, 3-6, 6-8, and 1-8 mo periods

Recession reduction (mm)	Case I	Case II
1-3 mo	0	0.5
3-6 mo	0.5	0
6-8 mo	1	0.5
1-8 mo	1.5	1

GU graft is more coronal than the neighbouring gingival tissue at 3 mo. Creeping has been detected in several clinical studies[24-27]. With an average of 1 mm, creeping can be seen within 1-12 mo after FGG in narrow recessions[25,26]. However, after 8 mo, the coronal ascent of gingival margins in case Ⅰ, treated with a GUT (1.5 mm) was higher than in case Ⅱ (1 mm). The unique vascular supply of GU graft is believed to be of importance for this difference[18]. The present outcomes support the usefulness of GUT for suitable root coverage in aesthetic areas. Although FGG has lost its popularity for aesthetic area[28,29], it may be still the gold standard surgical technique to increased KT[30] especially when it is modified with the inclusion of marginal and papillary gingival tissue.

The GU donor site healed uneventfully. No unacceptable attachment loss or recession were detected at the premolar site where the GU graft was harvested from in case Ⅰ after postoperative 8 mo. Inevitable recession at donor site were reported in laterally positioned flap procedure[6]. This does not possible for GUT procedure. Harvesting donor graft with marginal gingiva is easy, less invasive. Any harmful results can be prevented with cautious manipulation. Before harvesting the GU graft, the depth of gingival sulcus at palatal donor premolar area was measured. Donor tissue was harvested carefully not to cause any attachment loss. If some injury had happened at the attachment, new attachment apparatus would have been developed quickly[31].

In conclusion, the GUT technique performed on case Ⅰ can be successfully used for the Miller Ⅲ recession defect treatment.

COMMENTS

Case characteristics

Twenty-five (female) and 20-year-old (male) patients with Miller Class Ⅲ localized gingival recession defects on mandibular anterior teeth.

Clinical diagnosis

Miller Class Ⅲ recession defect on mandibular anterior teeth.

Treatment

One patient was treated with gingival unit transfer whereas the other with free gingival graft technique.

Term explanation

Gingival unit graft is masticatory palatal tissue involving marginal gingival and papillary tissue.

Experiences and lessons

Surgical treatment of Miller Ⅲ gingival recessions are more challenging, due to loss of interproximal bone and soft tissues. This case report represents the effectiveness of gingival unit transfer technique in comparison with free gingival graft technique on clinical parameters in the treatment of Miller Ⅲ gingival recession.

Peer review

This is an interesting case report.

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Is Takotsubo syndrome in patients receiving chemotherapy drug-specific?

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Abstract

In commenting on a case report of a 55-year-old man who suffered Takotsubo syndrome (TTS), in the setting of receiving chemotherapy with cytarabine and daunorubicin for acute myeloid leukemia, the author expresses his views that TTS in the setting of chemotherapy for malignancies may not be chemotherapeutic drug-specific (like in the chemotherapeutic drug induced-cardiomyopathy), but may be due to the emotional and physical stresses resulting from the realization of having diagnosed with a malignancy, and the diagnostic testing, and therapeutic management which follows.

Key words: Daunorubicin; Radiotherapy; Cardiotoxicity; Takotsubo syndrome; Malignancies; Chemotherapy; Cytarabine; Anthracyclines; Cardiomyopathy; Autonomic sympathetic Nervous system.

Core tip: Is Takotsubo syndrome, in time proximity to chemotherapy, due to the specific chemotherapeutic agent?

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TO THE EDITOR

The interesting report by Goel *et al*[1], published in the October, 2014 issue of the Journal, about the 55-year-old man who suffered Takotsubo syndrome (TTS), in the setting of receiving chemotherapy with cytarabine and daunorubicin for acute myeloid leukemia, is well documented and discussed; however it makes one wonder whether we are on the right track in terms of attributing causation of TTS to specific chemotherapeutic agents. A number of cases of patients receiving a variety of chemotherapeutic drugs[2], and radiotherapy[3], have been reported, and their authors, like in the present paper, delved in the issue of cardiotoxicity of the particular drug administered, akin with what is done for cases of drug-specific (*e.g.*, anthracyclines) chemotherapy-induced cardiomyopathy, which certainly should be

differentiated from TTS. The intimate association of TTS with malignancies is intriguing[4-7], and has made some to recommend that patients with TTS should undergo evaluation for an underlying malignancy[4,5]. In terms of mechanisms many have attributed TTS, in the setting of malignancies, to paraneoplastic manifestations[4-6], a heightened autonomic sympathetic nervous system tone, emanating from the emotional stress of patients with a recently made diagnosis of malignancy, and non-specific physical stresses, related to diagnostic procedures, and administered chemotherapy and radiotherapy, without of course discarding the possible cardiotoxic role of the implemented therapies[6,7]. Incidentally, any reader of the present report will be interested in the details of further management of this patient with non M3 acute myeloid leukemia, whether he received more therapy, the specific chemotherapeutic regimen implemented, and the eventual outcome.

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EDITORIAL

Minimizing right ventricular pacing in sinus node disease: Sometimes the cure is worse than the disease

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Abstract

Traditional right ventricular (RV) apical pacing has been associated with heart failure, atrial fibrillation and increased mortality. To avoid the negative consequences of RV apical pacing different strategies have been developed, among these a series of pacing algorithms designed to minimize RV pacing. These functions are particularly useful when there is not the need for continuous RV pacing: intermittent atrio-ventricular blocks and, mainly, sinus node disease. However, in order to avoid RV pacing, the operational features of these algorithms may lead to adverse (often under-appreciated) consequences in some patients. We describe a case of a patient with sinus node disease, in whom right atrial only pacing involved long atrio-ventricular delay to allow intrinsic ventricular conduction, which led to symptomatic hypotension that could be overcome only by "forcing" also right ventricular apical pacing. We subsequently discuss this case in the context of current available literature.

Key words: Right ventricular apical pacing; Pacemaker algorithms; Dyssynchrony; Pacemaker syndrome; Right atrial pacing

Core tip: Right ventricular apical pacing has been associated with worse outcome so a series of pacing algorithms have been designed to minimize it. However the operational features of these algorithms may lead to adverse consequences in some patients. We describe a case of a patient with sinus node disease, in whom right atrial only pacing involved long atrio-ventricular delay to allow intrinsic ventricular conduction, which led to symptomatic hypotension that could be overcome only by "forcing" right ventricular apical pacing. We subsequently discuss this case in the context of current available literature.

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INTRODUCTION

Traditional right ventricular (RV) apical pacing has been associated with heart failure, atrial fibrillation

and increased mortality[1]. Dyssynchronous electrical activation of the heart from pacing creates deleterious myocardial fiber strain, mechanically inefficient contraction and adverse left ventricular (LV) remodeling with a progressive, dose-related, decline in pump function that is more evident in patients with an already compromised cardiac function at baseline[2]. To avoid the negative consequences of RV apical pacing different strategies have been developed. Pacing from alternative RV sites (septum, outflow tract, His bundle) is a promising option but, up to now, studies comparing apical to non-apical pacing with regard to hemodynamic, echocardiographic and long-term LV systolic function have been conflicting, failing to demonstrate a clear benefit[3,4]. Biventricular pacing can be considered in selected cases, *i.e.,* patients with atrio-ventricular (AV) block and LV ejection fraction < 35%, but it is not a first choice in the majority of patients candidates to receive a pacemaker. Contemporary pacemakers, from different manufacturers, include sophisticated algorithms designed to minimize ventricular pacing with the aim to reduce the incidence of atrial fibrillation and heart failure[5]. These functions are particularly useful when there is not the need for continuous RV pacing, that is in patients with intermittent AV blocks and, mainly, sinus node disease (SND). However, in order to avoid RV pacing, the operational features of these algorithms involve long AV delay to allow intrinsic conduction, which may lead to adverse (often under-appreciated) consequences in some patients[5,6].

We describe the case of a 85-year-old man implanted with a dual chamber pacemaker (Adapta DR Medtronic, leads positioned in right atrial appendage and RV apex) because of sinus node disease/bradi-tachi syndrome. He did not take any drug. Baseline ECG showed sinus bradycardia (35-40 beats per minute), normal *P* wave morphology, PR interval 180 msec, QRS 115 msec. Echocardiogram revealed moderate LV hypertrophy, ejection fraction 50%; diastolic pattern (pulsed wave mitral Doppler) showed abnormal relaxation with an adequate filling time in sinus rhythm. Before pacemaker implant he was symptomatic for palpitations, easy fatiguability, dizziness, vertigo, but no syncopal episode was described. Pacemaker was programmed DDDR 60-120 bm, Managed Ventricular Pacing (MVP)™ turned ON to avoid unnecessary RV pacing; ECG after implant showed atrial-based pacing with spontaneous ventricular activation (AP-VS) (Figure 1). Few days after hospital discharge the patient returned to our attention for episodes of near-syncope and falls occurring shortly after the passage from supine to upright position. We documented a symptomatic orthostatic hypotension with a sudden drop in systolic and diastolic blood pressure (SBP drop 30-40 mmHg and DBP drop 15-20 mmHg); during the episodes heart rate increased to about 80-90 beats per minute due to sensor-driven atrial pacing (with spontaneous ventricular activation). There was no obvious cause to justify these episodes, in particular different etiologies of orthostatic hypotension (neurogenic, non-neurogenic, drug/toxins effect) had been excluded; pacemaker did not show any malfunction. So we repeated an echocardiogram, recording diastolic filling during the episodes of symptomatic orthostatic hypotension: we found E/A wave fusion with a particularly short diastolic filling time (about 280 msec at 80 beats per minute) (Figure 2A). We also noticed that at ECG, during atrial-based pacing, AP-VS interval was about 280-300 msec and right atrial stimulus artifact was followed by a first deflection corresponding to right atrial depolarization (white arrow in Figure 1) and then a second deflection corresponding to left atrial depolarization (black arrow in Figure 1). We also tried to program the pacemaker in AAI mode with a fixed rate but the results were the same compared to MVP™. All these features suggested us that atrial-based pacing was responsible of an abnormal prolongation of AV interval (with E/A fusion), likely associated with intraatrial and interatrial conduction delay, with symptoms (near-syncope) and orthostatic exacerbation similar to "pacemaker syndrome". However, by definition pacemaker syndrome occurs when there is atrial systole during ventricular systole while E/A fusion seen in our case is a diastolic filling issue. So MVP™ was turned OFF and we optimized the programmed AV interval to 90 msec ("forcing" RV pacing) in order to ensure an adequate echocardiographic diastolic filling time, with a good separation of E and A waves at pulsed wave mitral Doppler (diastolic filling time 547 msec at 70 beats per minute) (Figure 2B). Since the first day after reprogramming the device, the episodes of orthostatic hypotension did not occur anymore; we tested the patient with an "orthostatic stress test" (a sudden change from supine to upright position while monitoring blood pressure, heart rate and diastolic filling pattern) and neither hypotension nor symptoms occurred during AP-VP paced rhythm.

Diastole begins soon after the end of systolic ejection (aortic valve closure) and includes LV pressure fall, rapid filling, diastasis and atrial contraction. Diastolic filling and cardiac output are strictly linked and the optimal performance of the LV depends on the alternation between a compliant chamber in diastole (LV filling from a low atrial pressure) and a stiff chamber in systole (ejection of the stroke volume at arterial pressures). When passing from supine to upright position there is a venous pooling in the lower extremities and splanchnic circulation as a result of the gravitational change. The consequent decrease of venous return to the heart leads to a transient reduction of ventricular filling, cardiac output and blood pressure. As compensatory mechanisms sympathetic tone increases and parasympathetic activity decreases: venous return, heart rate and vascular resistance they all increase with the aim of maintaining cardiac

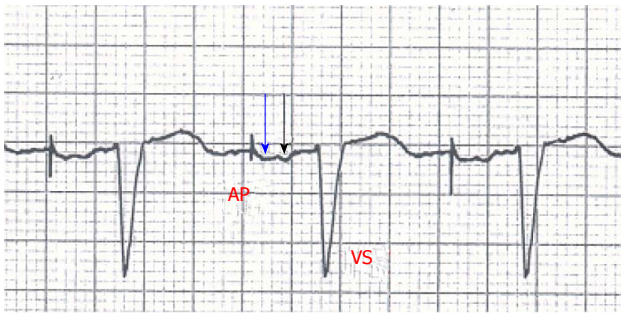

Figure 1 ECG after implant showing atrial-based pacing with spontaneous ventricular activation. AP-VS interval: 280-300 msec. Right atrial stimulus artifact is followed by a first deflection corresponding to right atrial depolarization (blue arrow) and then a second corresponding to left atrial depolarization (black arrow). AP: Atrial pacing; VS: Ventricular sensing.

output and blood pressure. When one or more of these compensatory mechanisms fail orthostatic hypotension can occur.

Several factors contribute to the clinical, ECG and echo findings in our patient. First of all pacing from right atrial appendage led to a delay in interatrial and intraatrial conduction as manifested by a "wide" P wave following stimulus artifact, with two distinct deflections corresponding to right and left atrial depolarization (Figure 1), while P wave morphology in spontaneous sinus rhythm was completely normal. As a consequence, a long AV delay occurred (AP-VS 300 msec) that was likely and mainly the consequence of inter/intra atrial conduction delay (rather than a true nodal/hisian delay, PR interval being normal during non-paced rhythm). This kind of "pseudo first degree AV block" pushed the A wave toward E wave: E/A fusion occurred so diastolic filling time was abnormally short. During orthostatic challenge the inability to fill the ventricle, because of this diastolic impairment, finally led to symptomatic hypotension with near-syncope. The only way to restore an adequate filling time was to optimize AV delay, but this involved to "force" RV pacing.

MVP™ provides an atrial-based pacing (AAI/R) with ventricular backup at an AV delay of 80 msec in absence of a ventricular sensed event following an atrial sensed or paced event. When loss of AV conduction persists (two out four non-refractory AA intervals without ventricular sensed events) the pacemaker switches to DDD/R mode at the programmed lower rate and AV delay. The algorithm, then, performs regular checks of AV conduction and switches back to AAI/R mode if possible[5]. The MVP™ tolerates markedly prolonged AV delay which can adversely affect cardiovascular hemodynamics, reducing atrial contribution to ventricular filling and favoring diastolic mitral regurgitation[5,6]. In general algorithms designed to minimize ventricular pacing operate by prolonging the AV interval with hysteresis or by switching between DDD and AAI modes; the operative features differ between manufacturers but all of them carry the risk

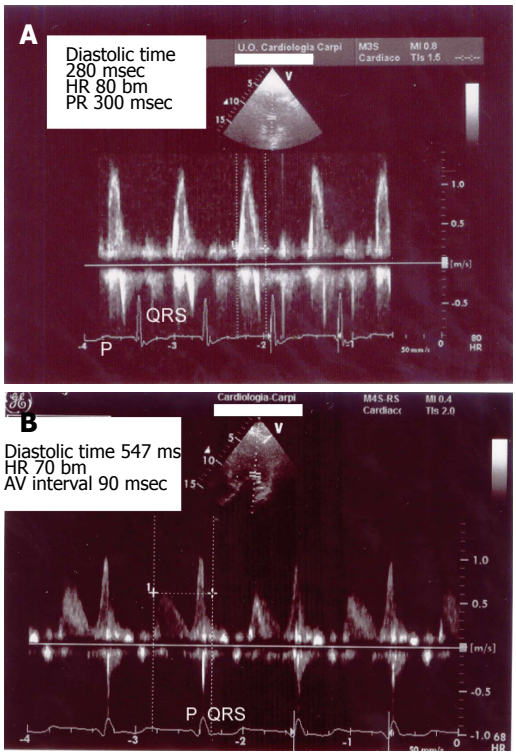

Figure 2 Pulsed wave mitral Doppler recording. A: Pulsed wave mitral Doppler recording during an episode of symptomatic orthostatic hypotension with atrial-based pacing: E/A wave fusion with a particularly short diastolic filling time (280 msec at 80 beats per minute); B: Diastolic filling after AV delay optimization at 90 msec ("forcing" RV pacing): Good separation of E and A waves at pulsed wave mitral Doppler (filling time 547 msec at 70 beats per minute).

of AV decoupling (defined as > 40% of AV intervals over 300 msec) even when baseline PR interval is normal. To prevent this adverse effect some manufactures have incorporated in their algorithms a maximum tolerated AV delay (350 msec in Ventricular Intrinsic Preference™ by St Jude Medical; 350 msec atrial sensed and 450 msec atrial paced in AAISafeR2™ by Sorin Group): if AV delay exceeds these limits, the device switches to DDD mode.

Atrial pacing "*per se*" increases AV delay: pacing from right atrial appendage can provoke marked alterations in interatrial and intraatrial impulse propagation that impairs coordinated activation and can also favor atrial fibrillation[7]. In the DANPACE trial[8], that compared AAI and DDD pacing in SND, atrial-based pacing significantly increased the risk of paroxysmal atrial fibrillation [28.4% in AAI group *vs* 23% in DDD group; hazard ratio (HR) 1.27; P = 0.024]. In a study AAI-R based pacing, in patients with SND and normal baseline PR interval, induced a clinically significant lengthening of AV conduction time, with a paradoxical increase of AV conduction during exercise in 66% of cases (that was predicted by use of antiarrhythmic class Ⅰc/Ⅲ drugs)[9]. Moreover in 23%-58% of SND patients AV conduction is already impaired at baseline, two thirds of these patients having a first degree AV block; the optimal pacing mode in these subgroup is not determined. In a

comparison study between conventional dual chamber pacing and minimal ventricular pacing mode there was no significant difference in terms of functional capacity assessed by cardiopulmonary test, quality of life and echocardiographic parameters of systolic/diastolic function; it was concluded that sequential AV pacing may be a reasonable choice for patients with SND and prolonged PR interval[10].

Alternative atrial pacing sites have also been studied: high and low interatrial septum, Bachmann bundle, lateral free wall and combinations of these sites; the concept was to improve atrial hemodynamics by reducing total atrial activation time. Although several small studies indicated that some alternative sites could help to prevent atrial fibrillation, randomized trials did not show benefit in the long term[11].

The attempt to minimize RV pacing, at expense of AV synchrony, can be particularly deleterious in patient with heart failure. In the INTRINSIC RV trial[12] patients indicated for ICD implant were randomized to dual chamber pacing with AV Search Hysteresis™ or single chamber VVI pacing 40 bpm. Patients with 10%-19% RV pacing had the most favorable outcome, while the risk of clinical events (mainly heart failure decompensation) in the 0%-9% RV pacing group was as high as the 40%-49% RV pacing group. So some ventricular pacing may be necessary, even if the optimal balance between AV synchrony and intraventricular dyssynchrony induced by RV pacing varies between patients and is not simple to define.

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This statement is to certify that all authors have seen and approved the manuscript being submitted, have contributed significantly to the work, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to the Journal. We attest that the article is the Authors' original work, has not received prior publication and is not under consideration for publication elsewhere. On behalf of all co-authors, the corresponding Author shall bear full responsibility for the submission. Any changes to the list of authors, including changes in order, additions or removals will require the submission of a new author agreement form approved and signed by all the original and added submitting authors. Patient's consent was obtained. The authors report no relationships that could be construed as a conflict of interest.

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REVIEW

Current status and emerging challenges in the treatment of hepatitis C virus genotypes 4 to 6

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Abstract

Hepatitis C virus (HCV) genotypes 4, 5 and 6 are mainly present in Africa, the Middle East and Asia and they have been less extensively studied with respect to epidemiology, natural disease history and therapeutic endpoints. Response rates to a 48-wk combined peginterferon/ribavirin treatment range to 40%-69% for HCV 4, 55%-60% for HCV 5 and 60%-90% for HCV 6. Response-guided schedules are recommended to optimize the outcomes of peginterferon/ribavirin treatment in HCV 4 and, in form of preliminary data, for HCV 6, but no data are yet available to support such an individualization of therapy for HCV 5. Recently, the direct-acting antivirals (DAAs) with pan-genotypic activities simeprevir, sofosbuvir and daclatasvir have been recommended in triple regimens with peginterferon/ribavirin for the treatment of HCV genotypes 4 to 6 infections. In the future, DAA-based interferon-free therapies are awaited to drastically improve treatment outcomes in HCV. However, efforts to improve treatment outcomes with peginterferon/ribavirin should continue, as the HCV 4-6 infected population is mainly based in resource-limited settings with restricted access to the costly DAAs.

Key words: Hepatitis C virus; Genotype 4; Genotype 5; Genotype 6; Pegylated interferon; Ribavirin; Direct-acting antivirals

Core tip: Hepatitis C virus (HCV) 4, 5 and 6 are lesser known genotypes mainly encountered in Africa, the Middle East and Asia. Studies, mostly retrospective, have reported response rates to a 48-wk peginterferon/ribavirin combination ranging to 40%-69% for HCV-4, 55%-60% for HCV-5 and 60%-90% for HCV-6. Increasing evidence has supported a response-guided approach for HCV-4, whereas no robust data are yet available concerning tailoring of treatment duration for HCV-5 and HCV-6. Direct-acting antivirals may significantly improve treatment outcomes in HCV, but use of these agents in countries endemic for HCV 4-6 is currently precluded by the very high costs.

Papastergiou V, Karatapanis S. Current status and emerging challenges in the treatment of hepatitis C virus genotypes 4 to 6. *World J Clin Cases* 2015; 3(3): 210-220 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i3/210.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i3.210

INTRODUCTION

Hepatitis C virus (HCV) remains a major health problem worldwide with over 170 million persons chronically infected and a burden of 300000 deaths annually[1,2]. Phylogenetic analyses of viral genomic sequences have identified at least 6 major HCV genotypes (and more than 70 subtypes), each with a distinct geographical distribution and sensitivity to antiviral treatment[3,4]. HCV 1, 2 and 3 are widely disseminated genotypes and have been thoroughly assessed with regard to epidemiology, natural disease history and treatment outcomes. Conversely, HCV 4, 5 and 6 have a restricted geographical distribution, mainly based in countries with limited resources and research facilities, thus epidemiological reports and treatment advances for these genotypes have been generally deficient. Prevalence of HCV genotypes 4 to 6 across different countries is summarized in Table 1. Genotype 4 is encountered throughout Middle East and Africa[5-12], whereas a spread of the infection has been described in other countries[13-15], particularly in Southern Europe[16-20]. HCV 5 is rare outside South Africa[21-23], but its sporadic presence has been reported in different parts of the world[11,15,24-28], including a pocket of the infection in Southeast Greece[29]. Lastly, HCV 6 and its subtypes are found mainly in Asia[30-35]. Crucially, due to the phenomenon of globalization, the prevalence of the HCV 4 to 6 genotypes outside of these "typical" areas is awaited to increase in forthcoming years.

During the past decade, a dual combination of pegylated interferon (PegIFN) and ribavirin (RBV) has represented the standard of care (SOC) for treating chronic hepatitis C (CHC). In 2011, introduction of first generation direct-acting antivirals (DAAs), the NS3/4A protease inhibitors (PIs) boceprevir and telaprevir, has boosted rates of sustained viral response (SVR; *i.e.*, negative HCV-RNA at 6 mo or more after cessation of treatment) in both naïve and treatment-experienced patients, although this only regarded the most difficult-to-treat CHC genotype 1[36]. Latter, in 2013, approval of the second generation DAA, NS5B polymerase inhibitor (PI) sofosbuvir, has been a further step forward due to its pangenotypic effect on HCV, better pharmacokinetics and improved resistance profiles[37]. In the light of the rapidly changing paradigm of treating CHC, new drugs were recently approved or await approval. However, in the era of DAAs, optimal treatment of HCV genotypes 4 to 6 remains, more than ever before, to be defined. Indeed, most treatment data rely on retrospective studies, extrapolations using other HCV genotypes as reference, and expert opinions.

Herein, we aimed to a concise overview on the treatment of HCV 4 to 6, including recent proposals for a response-guided treatment approach as well as the available data and future perspectives on the use of DAAs with respect to these lesser known HCV genotypes.

TREATMENT OF HEPATITIS C GENOTYPES 4 TO 6

Combination therapy with PegIFN and RBV

Hepatitis C virus genotype 4: HCV 4 has been traditionally considered a difficult to treat genotype, mainly because of the disappointing SVR rates (5%-25%) obtained in the early clinical trials using conventional interferon monotherapy[38,39]. Later introduction of RBV, used in conjunction with PegIFN, has significantly increased the efficacy of treatment, although response rates were still lower as compared to genotype 2 and 3 patients. Figure 1 summarizes results of prospective studies evaluating a fixed 48-wk treatment using standard-dose PEGIFN and RBV (PegIFNα-2a 180 μg or PegIFNα-2b 1.5 mg/kg and RBV 1-1.2 g/d) in HCV 4[40-46]. Overall, SVR rates ranged between 40% and 69%. However, a significant discrepancy could be noted between the SVR rates reported in highly endemic countries (SVR 60%-69% in studies conducted in Egypt and the Middle East)[40,42,43,47-50] and those (generally < 60%) reported in European populations infected with HCV 4; including 55% in Spain[51], 40.3% in France[52] and 43.5% in a cohort from Greece[53]. This difference has prompted the hypothesis of an impact of ethnicity on antiviral response, with 2 French analyses suggesting Egyptian (*vs* European) origin as a favorable prognostic indicator for SVR[52,54]. However, no solid pathogenetic basis has been provided for this phenomenon, although genetic or immunological ethnic-specific differences have been put forward[55,56]. Unlike the French observations, we could not identify any influence of Greek (n = 101) *vs* Egyptian (n = 76) origin on treatment outcomes[57], and only age ⩾ 45 years [odds ratio (OR) = 0.42, P = 0.01), presence of diabetes (OR = 0.23, P = 0.007), advanced liver fibrosis (Metavir F3-F4; OR = 0.39, P = 0.01) and treatment suspension (OR = 0.17, P = 0.007) were independent negative associations, in line with previous studies assessing predictors of response in HCV 4[40,42,43,52,54,58-63] (Table 2). The importance of metabolic factors has been highlighted by the observation of a beneficial effect of using an insulin-sensitizing agent, such as pioglitazone, in conjunction with antiviral treatment in patients with insulin resistance (homeostasis model assessment index > 2)[64]. Congruently, presence of hepatic steatosis, known to be a poor predictor of treatment in CHC, has been linked to host metabolic factors in HCV 4 rather than to a direct viral steatogenic effect as in the case of HCV 3 infection[65,66]. Other host factors, including the IL-28B TT genotype and high values of the interferon-c inducible protein 10 (IP-10) have been associated with a poor therapeutic outcome[59]. Interestingly, Boglione *et al*[67] have recently proposed use of the IL-28B polymorphisms upstream as a genuine basis for the identification of patients

Table 1 Indicative reports of hepatitis C virus genotype 4 to 6 prevalence in different continental areas

	Genotype 4		Genotype 5		Genotype 6	
	Country	Prevalence	Country	Prevalence	Country	Prevalence
Africa	Egypt[10]	91%	South Africa[22,23]	40%		
	Gabon[12]	71%				
	Cameroon[7]	76%				
	Nigeria[8]	60%				
Middle East	Saudi Arabia[11]	60%	Syria[25]	10%		
	Lebanon[9]	30%	Saudi Arabia[11]	1%		
	Syria[5]	30%				
	Iraq[6]	35.40%				
Asia	China[33]	0-1.7%			Hong Kong[33-35]	10%-30%
					Vietnam[30]	14%
					South Korea[31]	1.40%
					China[32,35]	0-50%
Europe	France[15]	4%-10%	France[15,26]	3%-14.2%		
	Spain[17,20]	1.4%-14%	Belgium[28]	1%-5%		
	Italy[16,19]	1.4%-3.1%	Spain[27]	0-10.3%		
	Greece[18]	13.2%-15.2%	Italy[24]	0-0.1%		
			Greece[18,29]	0.4%-1.9%		
America	United States[13,14]	0-2%				

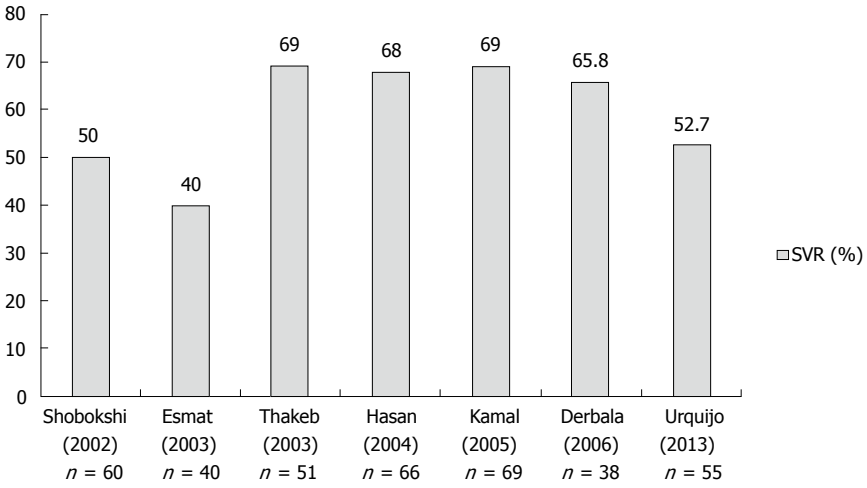

Figure 1 Prospective studies evaluating a fixed 48-wk treatment using a standard dose of pegylated interferon and ribavirin in patients with hepatitis C genotype 4. SVR: Sustained viral response.

Table 2 Predictors of response to antiviral treatment in patients with hepatitis C virus genotype 4 infection

Predictor	Ref.
Age	[57]
Liver histopathology (advanced fibrosis/severe steatosis)	[40,52,54,60]
Baseline viral load[1]	[42,43]
Ethnicity	[52,54]
Diabetes/Insulin resistance	[49,54,57]
IL28B polymorphisms	[58,63]
Plasma levels of IP-10[2]	[59]
HCV 4 subtypes	[52]
Co-infections (HIV, Schistosomiasis)	[38,61]

[1]Most studies used a cut-off value of 400.000 IU/mL; [2]Interferon-c inducible protein 10. HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; IP-10: Inducible protein 10.

unlikely to respond to standard dual therapy, and thus candidates for a DAA regimen. This individualized approach merit further robust assessment.

Definition of the optimal treatment duration is paramount to reduce costs and improve treatment tolerability without compromising the therapeutic efficacy. Due to the consistently lower response rates with 24 wk of therapy, a 48-wk treatment duration has been recommended as the SOC for genotype 4, similar to genotype 1[43,48,68]. A further refinement has been use of early viral responses to allow for shorter treatment durations in highly responsive patients (*i.e.*, response-guided approach). In a double-blind randomized study, Kamal *et al*[43] showed that in patients achieving a complete early viral response (EVR; defined as a negative HCV-RNA at week 12 of treatment), the SVR rate was 86% with a 36-wk therapy and 92% with 48 wk of therapy (P = 0.8), whereas PegIFN dose reductions were significantly more common in the 48-wk group. Two randomized controlled trials, one including exclusively genotype 4[69] and one including a mixture of both genotype 1 or 4 patients[70] have assessed the utility of a

Table 3 Studies reporting rates of sustained virological response to 48-wk interferon-based combination therapy in patients with hepatitis C genotype 5

Author/Country/Year	No. patients	Regimen	SVR
Legrand-Abravanel/France/2004[75]	12	Standard/Pegylated IFN plus RBV	63.60%
Delwaide/Belgium/2006[74]	6	Standard/Pegylated IFN plus RBV	83%
Bonny/France/2006[78]	87	Standard/Pegylated IFN plus RBV	60%
[1]Antaki/Syria/2008[77]	26	Standard/Pegylated IFN plus RBV	54%
D'Heygere/Belgium/2011[79]	38	Standard/Pegylated IFN plus RBV	55.30%
Karatapanis/Greece/2012[29]	10	Pegylated IFN plus RBV	60%
Antaki/Syria/2012[76]	49	Standard/Pegylated IFN plus RBV	49%
Mauss/Germany/2012	24	Pegylated IFN plus RBV	58%
Papastergiou/Greece/2014[81]	27	Pegylated IFN plus RBV	63%

[1]Thirteen out of 26 patients received 24 wk of treatment due to personal, financial or medical reasons. IFN: Interferon; RBV: Ribavirin; SVR: Sustained virological response.

response-guided tailoring of treatment. Based on the results of these studies, a 24- and 36-wk treatment duration have been established as sufficient in patients with a rapid viral response (RVR, defined as negative viral load at week 4 of treatment) and EVR respectively. Contrarily, Ferenci *et al*[71] showed that a 72-wk extended-duration therapy may benefit slow responders; *i.e.*, those non achieving RVR but attaining at least a partial (*i.e.*, a $\geqslant$ 2log10 drop in serum HCV-RNA) EVR and a negative HCV-RNA at week 24. Critically, a suboptimal PegIFN alpha-2a dose (135 μg/wk after week 48) may have compromised SVR rates in this study. Patients with detectable viral load at the end of week 24 are unlike to respond to treatment, and therefore assessment based on serum HCV-RNA at this time-point may serve as a futility rule, as indicated by its 92.8% negative predictive value on SVR[53]. To date, no robust conclusions can be drawn regarding efficacy of PegIFN alpha-2a *vs* alpha-2b in patients infected with HCV 4[72].

Hepatitis C virus genotype 5: Mainly due to its low worldwide prevalence, HCV 5 probably represents the less studied HCV genotype with respect to therapeutic endpoints. Epidemiological reports from France, Belgium, Canada, Syria and Greece argue that patients infected with HCV 5 have specific epidemiological characteristics: they are predominantly females of advanced age and they are characterized by high baseline viremia and advanced hepatic fibrosis[25,29,73-75]. Despite presence of these classical negative predictors of treatment response, SVR rates have been reported to 55%-60%[29,74-81], although most of the therapeutic studies on HCV 5 (Table 3) have had inherent limitations, including the retrospective design, small sample and extreme heterogeneity with respect to treatment modalities[74-76,78] and patient sampling[79]. Currently, a fixed 48-wk course of combined PegIFN and RBV is recommended for patients with HCV 5. However, the intrinsic sensitivity of HCV to combined antiviral therapy, and thus the ideal treatment duration, remains controversial. In a retrospective study by Antaki *et al*[77], 13/26 patients were treated for 24 wk due to personal, financial or medical reasons, and no impact of treatment duration (24 wk *vs* 48 wk) was found on SVR. In support of a 24-wk treatment, some retrospective data[75,78] suggested that response of HCV is similar to that observed for genotypes 2 and 3, although this was disputed in more recent studies[76,79]. Clearly, extrapolations using other HCV genotypes as reference are not an appropriate basis for treatment standardization. In a prospective, open label, single-arm trial we have evaluated 27 patients with HCV 5 and the SVR was 63%, whereas non-response was mainly due to relapse (26.1%)[81]. To our knowledge this is the only prospective therapeutic trial using exclusively a combination of PegIFN and RBV and including only treatment-naïve patients. The most striking finding of our study was the excellent predictive value of early viral responses on SVR: the positive predictive value (PPV) of RVR was 93.8%, whereas the negative predictive value when not achieving EVR was 100%. Based on these data, a response-guided schedule may be a viable option for patients with HCV 5[82]. Thus, it merits appropriate consideration in future trials, possibly conducted on a multi-center basis.

Hepatitis C virus genotype 6: Small studies, using a combination of either standard interferon[83,84] or PegIFN[85,86] and RBV have examined treatment outcomes in HCV 6. Overall, SVR rates have ranged to 60%-90%, indicating a more favorable response in comparison to HCV 1 and comparable to that of HCV 2 and 3. Crucially, a favorable IL28B status among Asians patients may have contributed significantly to these good results[87]. Apart from baseline viral load and the degree of hepatic fibrosis, which represent classical predictors of treatment response in CHC, other host factors such as age, BMI and adherence to treatment schedule have been identified as relevant in cohorts of patients with HCV 6[88]. To date, optimal treatment duration for HCV 6 has been a matter of controversy, with studies comparing a 48- *vs* a 24-wk regimen providing equivocal results (Figure 2)[86,89-91]. Most studies investigating therapeutic outcomes in HCV 6 have applied 48-wk treatment duration[83-85].

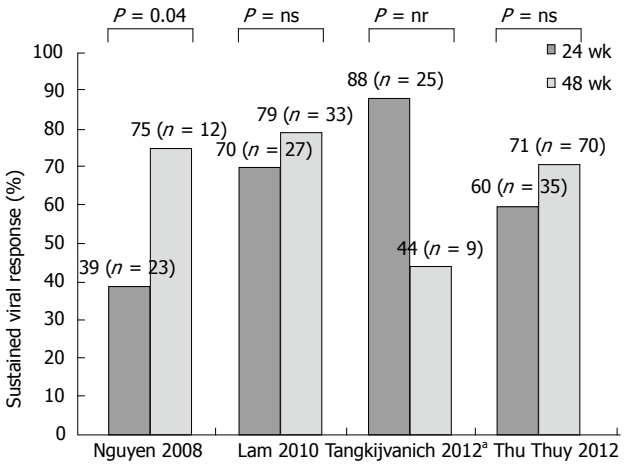

Figure 2 Studies comparing outcomes of 24 *vs* 48 wk of therapy with pegylated interferon and ribavirin in patients with hepatitis C virus genotype 6. [a]Treatment duration was tailored according to viral response at week 4: Patients with negative hepatitis C virus-RNA received 24 wk of treatment; the remainder received 48 wk. ns: Non-significant; nr: Not reported.

Moreover, in a retrospective analysis by Nguyen *et al*[86], the SVR rates were significantly lower with a 24-wk treatment schedule (39% *vs* 75%, $P = 0.044$). However, two randomized controlled trials including 60 and 105 patients have shown non-inferiority with a 24-wk (*vs* a 48-wk) regimen of PegIFN alpha/2a and RBV[89,91]. As in case of other HCV genotypes, individualization of treatment duration based on early viral dynamics may allow for reduction to drug exposure and in treatment costs. In an open-label randomized study by Thu Thuy *et al*[91], RVR occurred in about 80% of patients and had a 75%-86% PPV on SVR irrespective of treatment duration; nonetheless, among those with no RVR, even a 48-wk treatment achieved low SVR rates (8%). Efficacy of a response-guided approach based on RVR has been evaluated by Tangkijvanich *et al*[90]: in a pilot study including 34 patients with CHC genotype 6, the SVR rate in patients with RVR who underwent a 24-wk treatment was 88%. Based on these data, patients with a RVR may benefit with 24 wk of therapy. On the other hand, interruption of treatment may be the most reasonable option in patients with a detectable viral load by week 12 of therapy (non-EVR), as these patients are unlikely to respond to a 48-wk course[89,91,92] whereas there is no data to support they might benefit from longer treatment duration. Additionally to the use of early viral responses, evaluation of baseline parameters such as age, the degree of liver fibrosis, viral load and BMI may further rationalized choice of the optimum treatment duration[88]. Larger randomized trials are awaited to optimize treatment schedules for HCV 6.

Direct-acting antivirals

First generation protease inhibitors: Telaprevir and boceprevir are first generation NS3/4 PIs firstly approved in 2011 for patients infected with HCV 1. Although both drugs are not approved for HCV 4, telaprevir has shown a modest activity against this genotype. A phase Ⅱa trial (study C210) has assessed the activity of telaprevir on early viral dynamics[21]. Twenty four patients with HCV 4 were randomized to three groups: telaprevir alone; PegIFN plus RBV; and a triple regimen comprising telaprevir plus PegIFN/RBV. By day 15 of therapy telaprevir monotherapy induced only a 0.77 log10 decline in HCV-RNA levels (*vs* 4.77 log10 for HCV 1). The viral decline was more pronounced (4.32 log10) when telaprevir was administered together with PegIFN/RBV indicating a synergic effect. A descriptive subanalysis of the C210 study showed that the most frequent mutation accounting for the limited antiviral efficacy of telaprevir monotherapy was the T54A/T previously described for HCV 1[93]. Interestingly, this mutation has limited or no impact on the efficacy of subsequent treatment with dual PegIFN/RBV.

Indeed, emergence of resistant variants has generally precluded monotherapy with first generation PIs. However, use of these agents in conjunction with PegIFN/RBV still depends on interferon sensitivity, requires a high pill burden and a complex treatment algorithm. Moreover, both drugs may enhance or induce a set of considerable side effects. Anemia, neutropenia and dysgeusia are the most common side effects with boceprevir, whereas anemia, skin rash and anorectal symptoms are more frequently associated with telaprevir. Anemia, occurring in about 40%-50% of cases, may lead to discontinuation of treatment despite management with ribavirin dose adaptations, use of erythropoietin alpha or blood transfusions. Skin rush specifically related to telaprevir is generally mild and manageable using emollients and topical corticosteroids, although, in about 5% of cases, a severe life-threating cutaneous reaction may lead to treatment discontinuation[94].

Better-tolerated new generation DAAs with improved pharmacokinetics (allowing once-daily administration) and favorable resistance profiles (allowing interferon-free, all-oral regimens) were recently approved or await approval. These agents, with activities against HCV 4 to 6, will be discussed below.

Simeprevir (TMC435): It is a second generation NS3/4A PI, active against genotypes 1, 2, 4, 5 and 6. It is administered as a once-daily tablet orally and has demonstrated a favorable safety profile and limited drug-drug interactions[95]. It was approved in November 2013 by the United States Food and Drug Administration (FDA) and in Japan in September 2013.

RESTORE, a phase Ⅲ, multicenter, single-arm, open-label study, conducted in France and Belgium, evaluated simeprevir (150 mg once-daily for 12 wk in combination with PegIFN/RBV, followed by 12-36 wk of PegIFN/RBV only) in 107 patients with HCV 4, either naïve or treatment-experienced[96]. Overall, SVR

at week 12 was observed in 65.4% of patients, with rates being particularly high among treatment-naïve (82.9%) and prior-relapsers (86.4%). Both these patient groups were eligible to a shorter (totally 24 wk) treatment duration if they achieved a HCV-RNA < 25 IU/mL at week 4 and undetectable at week 12. These response-guide criteria were met by 88.6% of treatment-naïve and 90.9% of prior relapsers; among them 93.5% and 95% respectively achieved a SVR at week 12. Notably, none patient had a detectable Q80K substitution in the NS3 protease sequence at baseline, associated with decreased efficacy in patients with HCV 1a.

Overall, simeprevir has demonstrated favorable safety profile. By pooling data form phase Ⅲ clinical trials (QUEST-1, QUEST -2 and PROMISE), discontinuation of treatment due to severe adverse events occurred in 2% of patients receiving a simeprevir plus PegIFN/RBV combination[97-99]. Rates of adverse events, most commonly fatigue, influenza-like illness, headache, nausea and pruritus, were generally similar between simeprevir/PegIFN/RBV and placebo/PegIFN/RBV groups. Incidence of photosensitivity and rush has been slightly higher with simeprevir (*vs* placebo), although the vast majority of cases were graded 1/2 in severity. Transient moderate bilirubin increases were noted; however, no clinically relevant cases of hepatotoxicity were recorded.

Recent European guidelines have included a 24-48 wk simeprevir plus PegIFN/RBV combination as an option for HCV 4-related compensated liver disease (including cirrhotics), suggesting interruption of treatment if HCV-RNA levels are ⩾ 25 IU/mL at week 4, 12 or 24[100].

Sofosbuvir: This is a nucleotide inhibitor of NS5B, with a pan-genotypic effect activity and a high barrier to resistance. It is administered as an oral 400 mg tablet/day with no food effect, whereas it has been proven safe and well-tolerated in phase Ⅱ and Ⅲ clinical trial including > 2000 patients. It was approved by FDA for HCV 1 in combination with PegIFN/RBV, and in HCV 2 and 3 in interferon-free regimens in December 2013 and in Europe in January 2014.

NEUTRINO, an open-label, single-arm, phase Ⅲ trial, evaluated a 12-wk regimen, comprising sofosbuvir plus PegIFN/RBV, in 327 treatment-naïve patients with genotypes 1, 4, 5 and 6[101]. However, the vast majority (89%) of the patient population had HCV 1. Overall, 27 out of the 28 (96%) patients with HCV 4, all 6 patients with HCV 6 and the single patient with HCV 5 achieved an SVR, 12 wk after the end of treatment. Currently, a sofusbuvir-based triple combination for 12 wk appears as the most efficacious and easy-to-use interferon containing option for the treatment of HCV genotypes 4 to 6, without the risk for selecting resistant variants in case of treatment failure[100]. Critically, rates of SVR were relatively lower in cirrhotics in the NEUTRINO trial (80% *vs* 92% in patients without cirrhosis), whereas no data with this regimen has been presented in treatment-experienced patients. Thus, it remains unknown whether longer treatment duration may be required for these more difficult-to-treat patient populations.

More recently, promising data have emerged on the efficacy of an interferon-free combination of sofosbuvir plus ribavirin in patients with HCV 4. Ruane *et al*[102] randomized (1:1) 60 patients of Egyptian ancestry (treatment-naïve: 28, treatment-experienced: 32; 23% cirrhotics; 17% with the IL28B CC genotype), stratified by prior treatment status and cirrhosis, to receive 12 or 24 wk of sofosbuvir (400 mg/d) plus RBV *(*1200 mg/d). After 12 wk of treatment, SVR rates were 11/14 (79%) in treatment-naïve and 10/17 (59%) in treatment-experience patients. However, extending the duration of treatment to 24 wk resulted in higher SVR rates in both treatment-naïve (14/14; 100%) and -experienced (13/15; 87%) groups. Thus, a dual sofosbuvir/ribavirin combination given for 24 wk is currently recommended for HCV 4 patients who are interferon-intolerant or -ineligible[100].

As evident in phase Ⅲ clinical trials, sofosbuvir in combination with RBV represents a well-tolerated option with rates of treatment discontinuation as low as 1%-2%[103]. Drug-related adverse events attributable to RBV such as fatigue, insomnia and anemia were the most common, and headache was also frequent. Unsurprisingly, incidence of adverse effects commonly associated with interferon, such as influenza-like illness and depression, was significantly lower and hematological abnormalities were less prominent among patients who receive sofosbuvir/RBV than among those who receive the standard PegIFN/RBV combination[101]. Consistently, health-related quality of life and health utilities of patients have been shown to be only minimally affected by sofosbuvir/RBV irrespectively to treatment duration[104,105].

Daclatasvir: This is an HCV NS5A oral PI with a pan-genotypic activity, but a lower barrier to resistance in genotype 1a[106]. A recent phase Ⅱb double-blind, placebo-controlled study evaluated a triple combination comprising daclatasvir plus PegIFN/RBV including treatment-naïve patients with HCV 1 (n = 365) or 4 (n = 30)[107]. Patients were randomly assigned (2:2:1) to daclatasvir 20 mg or 60 mg, or placebo once daily plus PegIFN/RBV. Overall, SVR rates (week 24 post-treatment) were 8/12 (66.7%) in HCV 4 patients receiving 20 mg, 12/12 (100%) in those receiving 60 mg and 3/6 (50%) in patients receiving placebo. Patients on daclatasvir did not have adverse events beyond those typical of PegIFN/RBV.

Based on this preliminary data, a daclatasvir (dose: 60 mg/d) plus PegIFN/RBV regimen has been included as an option for the treatment of genotype 1b and 4 patients[100]. The triple combination should be administered for 12 wk. In those who do not achieve an HCV-RNA level < 25 IU/mL at week 4

and undetectable at week 10, all three drugs should be continued for an additional 12 seeks. Conversely, PegIFN/RBV should be continued alone between week 12 and 24 in those who achieve such response[100].

Other DAAs evaluated in HCV 4: Phase Ⅱ trials have evaluated other triple or quadruple drug combinations in patients infected with HCV 4. In the DAUPHINE trial, different dosing schedules of danoprevir boosted with ritonavir plus PegIFN/RBV for 12-24 wk achieved up to 100% of SVR in treatment-naïve patients with HCV 4[108]. In yet another phase Ⅱb study, 25 HCV-4 patients were assigned to asunaprevir 200 mg or placebo twice daily plus PegIFN/RBV; the SVR rates were 89% in those receiving asunaprevir *vs* 43% in the placebo group[109]. Lastly, two randomized placebo-controlled trials have evaluated use of mericitabine in combination with PegIFN/RBV including patients infected with HCV 4[110,111]. In the JUMP-C trial, a 24-wk response-guided combination of mericitabine 1000 mg twice daily plus PegIFN/RBV was well-tolerated and more effective than a standard 48-wk PegIFN/RBV combination[110].

EMERGING CHALLENGES

While standardization of dual PegIFN/RBV regimens for HCV 4 to 6 is still pending, interferon-based treatment of HCV has been superseded by the introduction of oral DAAs with pan-genotypic activities. These agents are characterized by improved antiviral efficacy and offer the perspective for short-course, all-oral and interferon-free therapies. However, as it is reasonable, most trials evaluating DAAs have focused on the more prevalent and difficult to treat HCV genotype 1. Given obvious difficulties in patient sampling, multi-centric efforts may be necessary to assess therapeutic sensitivity, optimize DAA schedules and establish cost-effective response-guided approaches for HCV 4 to 6. Crucially, the very high cost of HCV DAAs is a central barrier to their widespread use, thus interferon-based treatments are likely to continue to have a role as cost-containing options in low- or lower-middle income countries. This is particularly relevant in the case of genotypes 4 to 6 which are mainly based in resource-limited countries; but with large HCV epidemics, hence these genotypes represent > 20% of the global HCV burden. Therefore, unless low-cost DAAs become available, a large population of untreated patients will continue to spread HCV in these countries and worldwide. Furthermore, treatment with DAAs of special patient population (*e.g.*, patients with kidney disease, HIV coinfection, patients undergoing solid organ transplantation) remains a challenge, as few or no data are available.

In conclusion, it seems we need to wait for a while until arrangement of both practical and logistic issues will allow for low-cost, all-oral and interferon-free regimens, at a level to dramatically change the global epidemics of HCV 4 to 6. Until then, efforts to further rationalize the use of the traditional PegIFN/RBV treatment should continue.

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REVIEW

Highlights in pathogenesis of vitiligo

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Abstract

Vitiligo is a common pigmentary disorder. Many studies across decades and all over the world have attempted to illustrate the pathogenesis behind it; however, the pathogenesis of vitiligo remains elusive. This review article, we present the findings behind the most and updated theories behind this psychologically debilitating and disfiguring disease. The discussion begun with the role of genetic predisposition followed by neural theory first proposed in the 1950s. We highlight the autoimmune hypothesis, followed by the reactive oxygen species model, zinc-α2-glycoprotein deficiency hypothesis, viral theory, intrinsic theory and biochemical, molecular and cellular alterations accounting for loss of functioning melanocytes in vitiligo. Many theories were elaborated to clarify vitiligo pathogenesis. It is a multifactorial disease involving the interplay of several factors. Future research is needed to clarify the interaction of these factors for better understanding of vitiligo pathogenesis and subsequent successful treatment.

Key words: Etiopathogenesis; Pigmentary disorder; Non-segmental vitiligo; Segmental vitiligo; Vitiligo

Core tip: The pathogenesis of vitiligo elaborated by several theory. Future research needed to clarify the interaction of these factors for better understanding of vitiligo pathogenesis and subsequent successful treatment.

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INTRODUCTION

The pathogenesis is complex and involves the interplay of multiple factors; however, the exact pathogenesis is not well known. Lerner *et al*[1] in the 1950s firstly proposed the neural theory, and after that, model of reactive oxygen species (ROS), the autoimmune hypothesis and the melanocytorrhagy hypothesis have appeared.

DIFFERENT PATTERNS AND PHYSICAL DISTRIBUTION OF VITILIGO

Pruritus, elevated lesions, and erythematous margins present in inflammatory vitiligo. There are 2 main types: generalized vitiligo (GV) (widespread macules with a symmetrical distribution), whereas focal vitiligo (FV) (1 or few depigmented not elevated areas at a single site). After coalescing of vitiliginous areas in GV, or becomes extensive in the body with remaining of few normal areas, thus called vitiligo universalis. Non-segmental vitiligo enrolled FV and GV, but segmental vitiligo (SV) restricted to one unilateral region[2], (Figure 1). The lesions of vitiligo are asymptomatic except in inflammatory vitiligo, which is associated with pruritus and characterized by elevated lesions, and erythematous margins.

INHERITANCE OF VITILIGO

The inheritance is polygenic[3]. Family history exists in 6.25%-38% of patients with vitiligo[4]. Those who have recessive homozygosity at 3 epistatically interacting autosomal diallelic loci will be affected by vitiligo[5].

Molecular genetics-based studies

Spritz *et al*[6] (2004) revealed different loci or alleles for GV. Autoimmune susceptibility (AIS)-1, -2 (chromosome 7) and systemic lupus erythematosus vitiligo-related gene (SLEV1) (chromosome 17) both associated loci for GV and concomitant autoimmune diseases. Of 33 tested loci, only (XBP1, TSLP, and FOXP3) were primarily concomitant with GV. FOXP3 associated with X linked recessive multiple autoimmune disease syndrome. In addition, CTLA4 had association secondarily with GV, and the autoimmune diseases[7]. However, patients with GV also linked to AIS3 locus (chromosome 8)[6].

Methylation of deoxyribunucleic acid (DNA) conducted by DNA methyltransferases (DNMT1, -3a, -3b)[8]. Monocytes in vitiliginous patients and normal volunteers showed sensitivity to alterations in methylation and revealed association between IL-10 and reactivity of autoimmune system[9,10]. In comparison with controls, methylation was increased and hypermethylation of the methylation-sensitive region in IL-10 that could alter genes expression in autoimmunity[9]. In a similar way, the role of transforming growth factor beta-receptor Ⅱ (TGFBR2), which inhibits the inflammatory pathways and lymphocyte activation was revealed[11,12].

The ultraviolet radiation resistance-associated gene (UVRAG), resists photo-damage, and plays a role in autophagy[13]. In 439 controls and 225 NSV patients, UVRAG has 2 SNPs which were significantly different[14].

Both diabetes mellitus type 1 and rheumatoid arthritis with SNPs found beside the insulin-dependent diabetes mellitus 8 locus (IDDM8)[15]. This region contains SMOC2, which enrolled in growth and development[16] and cell matrix interactions[17].

Also, melanocyte proliferating gene 1 (MYG1), is elevated in skin of both vitiliginous patients with activity, and without activity[18].

Human leukocyte antigen

Studies revealed that vitiligo associated with HLA-DRB1*07, HLA-A2, 11, 28, 31, 33, HLA-B17, 35, 40 and 44[19,20].

Susceptibility loci of vitiligo are on chromosome 6 and in the MHC[21]. A study genotyped 6623 patients with vitiligo and 10740 controls for 34 SNPs. *At 6q27, 2 SNPS* found with 3 unlinked genes. These gene include RNASET2, which responsible for ribonuclease (RNAse)[22]. The other two genes are the chemokine receptor 6 gene (*CCR6*)[21], and FGFR10P are imperative to progressing of the cycle of the cell that produce receptor of (FGF)[23]. Genes encode discoidin domain receptor 1 (DDR1)[24], and tyrosine kinase receptor play role in cell's progression and function[25], both were involved in vitiligo.

THEORIES FOR VITILIGO PATHOGENESIS

The neural theory

Early theories: The "neural theory" supposed by Lerner's (1959) was based on the fact that SV follows the course of the dermatome with exhibiting hyperhidrosis and emotional upset[1,26,27].

The sympathetic nervous system's role: Dysfunction of sympathetic nervous system's role (SNS) activity affect melanin production and lead to depigmentation. With iontophoresis and laser Doppler flowmetry level of microcirculation in lesions with vitiligo assessed to reveal SNS activity[28]. 10 subjects had facial SV, and 2 groups of controls were examined. 1 control group had 10 healthy, unaffected individuals, and the 2nd control group contained 10 non-segmental-type stable vitiligo patients. Patients were matched for gender and age. Approximately, the cutaneous blood flow was higher three times on the lesions *vs* normal skin in SV. The differences was not revealed in the non-SV.

Neuropeptide and neuronal markers: Neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), and polyclonal general neuronal marker (PGP) tested for their immuno-reactivity in 12 patients with vitiligo and 7 unaffected control subjects[29]. NPY increased in the marginal areas of lesions in half of the patients *vs* normal, and associated with noradrenaline with exerting a local autonomic effect[29]. Lazarova *et al*[30] (2000) confirmed this finding; however, they found that CGRP was also non-significantly increased in vitiligo. Precipitating factor, as, stress, produce significant level of neuropeptides such as NPY that induct the disease[30,31]. A cohort study revealed

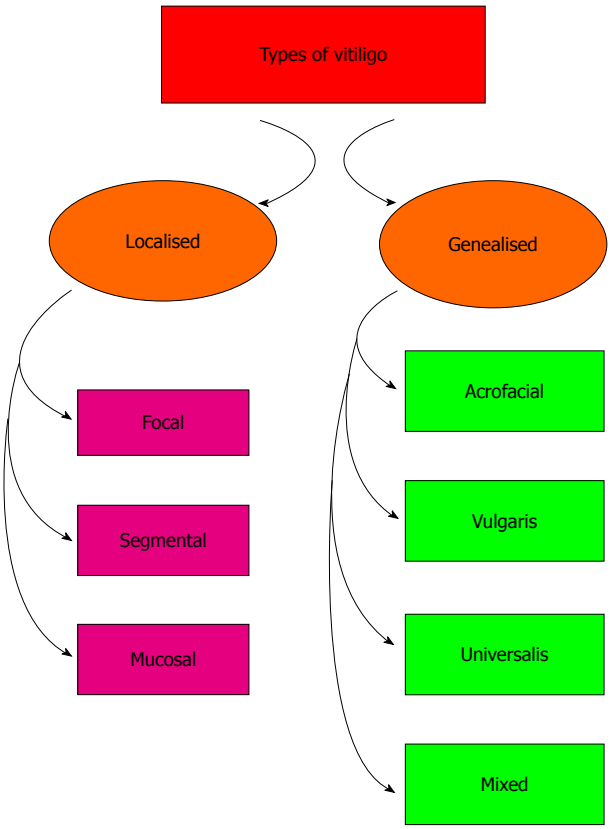

Figure 1 Types of vitiligo.

increased levels of nerve growth factor (NGF) significantly in vitiligo[32]. Stress up regulates NGF expression in hair follicles, decreases the high affinity TrkA receptor, increases production of p75NTR NGF-receptor, and increases in dorsal root ganglia the substance P neurons[33].

Catecholamine metabolite levels [homovanillic acid (HVA), vanilmandelic acid (VMA), 3-methoxytyramine (MT), normetanephrine (NMN), metanephrine (MN), 3,4-dihydroxy mandelic acid (DOMAC), and 3,4-dihydroxy phenylacetic acid (DOPAC)] were measured in 1-d urinary samples of 150 vitiliginous subjects and 50 normal subjects. HVA and VMA levels corresponded to the activity of the disease[34]. Stressors result in catecholamines discharge, which bind α-R in the mucosa and skin arteriolar wall leading to vasoconstriction, hypoxia, and overproduction of oxygen radicals that destroy melanocytes[34]. Mental stress could stimulate the hypothalamic-pituitary-adrenal axis and then secretion of catecholamines[34,35].

The autoimmune hypothesis

The etio-pathogenesis of "generalized" or non-segmental vitiligo is better explained by autoimmune mechanisms as vitiligo often has autoimmune comorbidities and it often responds to immunosuppressive treatments[36]. The reaction of immunity are cell-mediated, humoral (antibody-mediated), or through the cytokines.

The role of humoral immunity: In 2010, tyrosine hydroxylase antibodies checked with radioimmunoassay (RIA) in sera were obtained from 79 non-SV patients, 8 patients with SV, 91 subjects with other autoimmune diseases and 28 healthy subjects. TH antibodies revealed significantly in non-SV. Also, in non-SV, antibodies against MCHR1 (melanin-concentrating hormone receptor 1), tyrosinase[37] and pigment cell-surface antigens[38] were noted.

In 80% of active vitiligo patients, immunoglobulin G (IgG) and immunoglobulin M (IgM) against melanocytes were found. Low levels IgA also found in the inactive and control groups[38].

Furthermore, anti-thyroglobulin antibodies, antithyroid antibodies, anti-thyroperoxidase, and antismooth muscle antibody are present. Those are typically related to thyroid disease and other autoimmune diseases[39,40].

Melanin concentrating hormone (MCH) binds MCHR1 thus increase calcium influx and acting as an antagonist of α-melanocyte-stimulating hormone (α-MSH)[41-43].

The role of cell-mediated immunity: Immunohisto-chemical examination of the inflammatory infiltrates in perilesional vitiligo skin using single and double immunostaining for melanocytes, Langerhans cells, T-cells, and macrophages revealed higher densities of melanocytes in normal skin, *vs* non-affected skin in subjects with vitiligo. These T cell had dramatic production of (IL-2R), and increased CD8:CD4 ratio. Thus, melanocytes destruction may be cytotoxic CD8 T-cell mediated. Perilesional HLA-DR production (MHC class Ⅱ receptor) exhibited in all of the patients with vitiligo, especially along suprabasal and basal keratinocytes, due to local T cell reactivity. In addition, macrophages were numerous in vitiligo *vs* controls, whereas the CD36 subset of macrophages were higher in the later[44].

The role of cytokines in vitiligo: Beyond lymphocytes and antibodies, the immune system has a complex interplay of many cytokines. There are significantly increased expression of tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and IL-10[45]. As IFN-γ and TNF-α are T helper cell-1 (Th1) cytokines, so vitiligo is mediated by the Th1 response[46].

IL-17 plays role with macrophages, keratinocytes, and fibroblasts. In addition, it activates the expression of others, as IL-1 and IL-6, and TNF-α[47,48]. Examination of sera and tissue of 30 vitiliginous subjects and 20 normal subjects showed significant higher levels of IL-17 toward vitiliginous subjects and disease duration[47].

The biochemical Theory- reactive oxygen species model

Oxidative stress hypothesis suggests that imbalanced redox (reduction-oxidation) state of the vitiliginous skin. This results in the dramatic production of reactive oxygen species (ROS), as H_2O_2. ROS oxidize components of the cell leading to melanocytes destruction and creating the depigmented macules[49].

The redox status of vitiligo patients: Sera of

thirty-six patients with vitiligo (18 with inactive and 18 with unstable disease), and 40 normal subjects were examined for main factors of redox status involving selenium, malondialdehyde (MDA), vitamins A and E, and glutathione peroxidase (GPx) in the erythrocyte activities, catalase (CAT) and superoxide dismutase (SOD). Superoxide radicals are scavenged and their toxicity is reduced with SOD which transforms O_2- to H_2O_2 and O_2, and catalase transforms (H_2O_2) to (H_2O) and (O_2)[50]. As MDA results from lipid peroxidation, it is a marker of oxidative stress[51,52]. Selenium is required for GPx activity and vitamins A and E are important in antioxidant activity. Serum selenium, SOD and MDA are prominent in both unstable and inactive types. By enhancing SOD activity, the H_2O_2 accumulates. In addition, GPx detoxifies H_2O_2 (downstream enzyme). Therefore, GPx levels decreased in patients with vitiligo[50,53].

Increased SOD activity in patients with vitiligo is a response to oxidative stress; thus, H_2O_2 elevate as could not be eradicated by low level of CAT[53,54].

The role of tetrahydrobiopterin recycling in vitiligo: Another cellular pathway affected by accumulating H_2O_2 involves tetrahydrobiopterin. Tyrosinase is an imperative enzyme in formation of melanin[55]. L-tyrosine synthased from L-phenylalanine by the phenylalanine hydroxylase (PAH). The 5, 6, 7, 8-tetrahydrobiopterin or 6BH4 is essential cofactor for this process is. Defective recycling of 6BH4 lead to excess 7BH4 that is an inhibitor of PAH. Uncoupled PAH and 7BH4 found in suction blister material from the skin of vitiligo patients[56]. Kowlessur *et al*[57] (1996) also found that 7BH4 production yields H_2O_2. Haase *et al*[58] (2004) studied the enzyme dihydropteridine reductase (DHPR) (imperative to end the normal 6BH4 recycling). They assessed whole blood samples from 27 untreated vitiligo subjects and 8 normal subjects. The results showed that DHPR activity decrease with high concentrations of H_2O_2 and *vice versa*.

Effect of H_2O_2 on acetylcholinesterase (AchE) decrease in patients with vitiligo *vs* healthy controls[59,60]. Thus, AchE dependent on H_2O_2 concentration levels, *i.e.*, low H_2O_2 concentrations (approximately 10-6M or mol/L) activate AchE whereas high concentrations (10-3M or mol/L) deactivate AchE[60]. Butyrylcholinesterase (BchE) mediates the hydrolysis of acetylcholine. The hydrolysis reaction is one of the rate-limiting steps in cholinergic signal transduction[61,62].

In 2008, showed that xanthine oxidase (XO) is a source of H_2O_2. High concentrations of H_2O_2 inhibit the activity of XO, and *vice versa*[63].

Briefly, there are at least 5 important pathways enrolled in H_2O_2 overproduction in vitiligo: (1) Defective recycling of 6BH4[56,64,65]; (2) Catecholamine formation increased as levels of monoamine oxidase A (MAO) increased[34,66,67]; (3) Inhibition of thioredoxin/thioredoxin reductase by calcium[68-70]; (4) NADPH oxidase activities increased by the cellular infiltrate[71]; and (5) Nitric oxide synthase (NOS) activities increased[71].

Oxidative stress affects calcium homeostasis at the cellular level[72] in melanocytes and keratinocytes in vitiliginous patients[69].

Zinc-α2-Glycoprotein deficiency hypothesis

For the first time, Bagherani *et al*[73] and Yaghoobi *et al*[74] pointed the probable association which might be present between ZAG and vitiligo[73,74]. It was suggested that the pathogenesis of vitiligo could be attributed to decrease in ZAG as follows: (1) Studies have demonstrated that ZAG is acting as a keratinocyte-derived factor influencing melanocyte proliferation and dendricity[75,76]. So, ZAG could be considered as a marker of cells differentiation and maturation[76]; (2) A chronic detachment of melanocytes is an imperative pillar in the pathogenesis of vitiligo[74,77,78]. Thus, melanocyte adhesions to the other cells in epidermis will be impaired in the lack of ZAG; (3) Topical steroids are the most safeand effective forms of treatment for vitiligo, especially for the localized one[74,79], because of their ability to increase ZAG expression[80,81]; (4) Some studies have shown that zinc can precipitate ZAG[74,82]. Thus, the effectivity of zinc in treating this disease is related to its ability to precipitate circulating ZAG at the site of vitiligo[73]; and (5) The linkage signals on chromosome 7 in patients with GV and associated autoimmune diseases have been reported[73,83]. Surprisingly, ZAG gene is located on the chromosome 7[76].

Viral theory

There is a strong association between vitiligo and chronic hepatitis C virus (HCV) infection and autoimmune hepatitis[84]. Akcan *et al*[85] in 2006 reported a low hepatitis B virus (HBV) sero-positivity in vitiliginous patients. Previous or concurrent cytomegalovirus (CMV) infections may induce the etio-pathogenesis or deterioration of vitiligo[85,86].

Furthermore, other viruses as Epstein-Barr virus, hepatitis E virus, herpes virus and the human immuno-deficiency virus (HIV) also have suspicious association with vitiligo[86,87].

Intrinsic theory

Melanocytes in vitiligo have an intrinsic defect leading to their death. They demonstrate different abnormalities, including abnormal rough endoplasmic reticulum or deficiency of unidentified melanocyte growth factors such as basic fibroblast growth factor (bFGF) and decrease in the number of melanocytes expressing the c-kit receptor in lesional skin[88,89].

Melanocytes require a constant keratinocyte-derived c-Kit stimulation for their maintenance[90], thus weak expression of keratinocyte-derived factors, as stem cell factor (SCF), may lead to passive melanocyte death and might explain the Koebner phenomenon[91].

Cellular, molecular and biochemical alterations and melanocytes loss of in vitiligo

Recently, malfunctioning melanocytes found in vitiliginous lesions[92]. Electron microscopy, reverse transcription PCR (polymerase chain reaction) and southern blotting experiments revealed sporadic survival of melanocyte in vitiliginous lesions[93,94]. Thus, this points to presence of immature melanocyte precursors/stem cells[95,96]. Now, 2 pathways have been supposed for melanocytes loss: highly programmed death by apoptosis[77,91,97,98] and accelerated cell senescence[99].

Apoptosis and accelerated cell senescence: Melanocytes from non-lesional skin of vitiligo patients have abnormalities as cytoplasm vacuolization, rough endoplasmic reticulum dilatation, DNA marginalization in the nucleus, loss of dendrites and detachment[88,99,100].

Regarding keratinocytes, apoptosis occurs at least in the traumatized vitiligo skin[101]. Thus, basal and suprabasal epidermal cells in the depigmented and normally pigmented skin show degeneration due to swelling of the membrane-bound organelles, formation of vacuoles and cytoplasm condensation[102].

As vitiligo could inducted by trauma (Koebner's phenomenon). Lee *et al*[97] revealed the lower expression levels of the antiapoptotic Bcl-2 and FLIP proteins in vitiliginous skin *vs* the normally pigmented skin. On the other hand, there was dramatic levels of the proapoptoticBax and p53 proteins and of the active forms of caspase-3, 8 and 9[97].

Apoptosis triggered by normal developmental program, UV light, H_2O_2, staurosporine and other stimuli[91,103]. *NALP1* gene that stimulates cellular apoptosis[102,104], is associated with vitiligo susceptibility[105,106].

Epidermal melanocytes from epidermal melanin unit produce growth factors (GF) for melanocytes[107]. Therefore, its damage have imperative effects on melanocyte survival[91]. Thus, low levels of GF as SCF, endothelin-1 (ET-1) or high levels of melanocyte inhibiting cytokines, as TNF-α and IL-6 may lead to keratinocyte apoptosis, and then apoptosis of melanocytes[108].

Life span of lesional keratinocytes is greatly shortened when compared to the life span of normal and non-lesional vitiligo keratinocytes. It also shows modification of proliferation and senescence marker expression (p16, p53, p21), when compared to keratinocytes from clinically noninvolved skin[98].

Although apoptosis and senescence of epidermal keratinocytes is a response to various stimuli, they also share some cellular mechanisms and controlled by similar molecular regulators[109]. Both apoptosis and aging induced by stress signals as ROS accumulation and DNA damage[110]. The paradigmatic proapoptotic factor p53[111], is also a guard keeper of DNA integrity which triggers cell cycle arrest in DNA damaged cells[109].

Melanocytorrhagy theory: Gauthier *et al*[78] in 2003 mentioned that NSV occurs due to "melanocytorrhagy", or a chronic melanocytes detachment and loss caused by trauma and other stressors include catecholamines, ROS, or autoimmune elements. This theory combined the concepts from other theories mentioned before to elaborate a single integrated explanation of vitiligo pathogenesis[78].

A study done by Tobin *et al*[92] in 2000 proposed loss of melanocytes in vitiligo. They explained these findings because of oxidative stress caused by H_2O_2. Gauthier *et al*[78] (2003) also reported that impaired cell adhesion plays a role in vitiligo pathogenesis as the synthesis of extracellular matrix components by keratinocytes may be defective, the presence of focal gaps in the basement membrane and impaired formation of basement membrane. These abnormalities weaken the basal attachment of melanocytes. Trauma could aggravate this susceptibility with subsequent chronic melanocyte loss, known as melanocytorrhagy.

Le Poole *et al*[112] mentioned that the protein tenascin may play a role in decreasing melanocytes adhesion in vitiligo. This protein was highly expressed in patients with vitiligo than the controls[112]. This can explain the development of vitiligo by Koebner phenomenon, which represent "transepidermal migration"[78,113].

Integrated theory (Conversion theory)

Despite all the mentioned theories are attractive, it is likely that vitiligo is a result of the convergence of these pathological pathways. Most experts agree that vitiligo may be a syndrome with a multi-factorial etiology rather than a single entity[114] (Figure 2).

TREATMENT

The disfigurement associated with vitiligo could cause serious emotional stress for the patient and affect his quality of life[115]. Sun protection of vitiliginous areas with sun blocks is imperative[115,116] to prevent sunburn, photodamage and occurrence of Koebner phenomenon. In addition, sun blocks decrease tanning of the uninvolved skin and thus lessen the contrast with vitiliginous lesions[117].

There is no treatment ensures complete cure of vitiligo. Therefore, there is a plethora of modalities, such as topical corticosteroids, vitamin-D derivatives, calcineurin inhibitors, photochemotherapy [psoralen plus UV-A (PUVA), psoralen with sunlight (PUVAsol)], phototherapy (UV-A, narrowband UV-B), surgical techniques[117-122], excimer laser[115,117,118-123], topical prostaglandin E (PGE2)[118], and combinations of topical therapies and light treatment[79]. Complementary and integrative therapies are also used, as ginkgo biloba[79], and levamisole[124], because of their immune-modulating properties[117].

Pseudocatalase cream with Dead Sea climatotherapy can also promote repigmentation[117]. Topical flu-

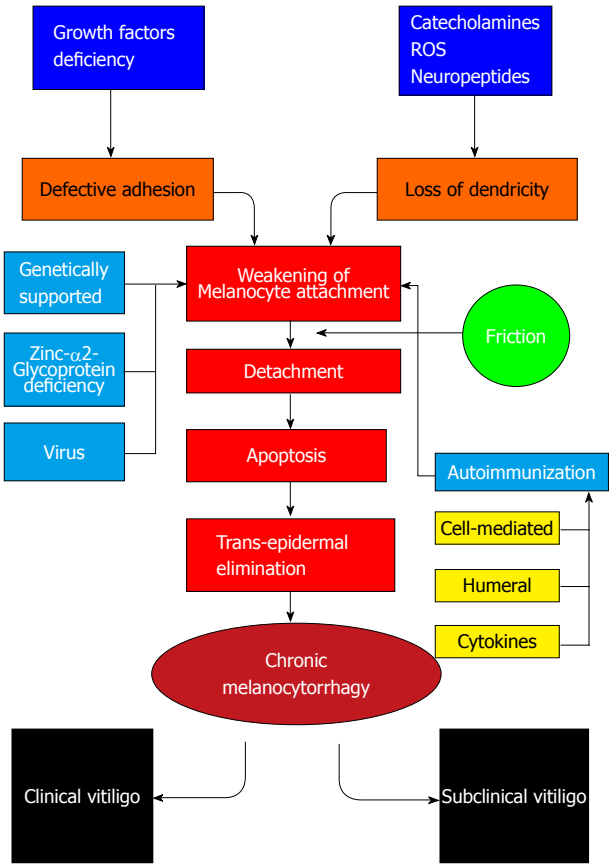

Figure 2 Pathogenesis of vitiligo.

orouracil[125], topical melagenina Ⅰ and Ⅱ, minoxidil[118], oral L-phenylalanine[117,126-129], homeopathy, ayurvedic medicine, climtologic and balneologic therapies are other therapeutic options for vitiligo[118]. Patients with widespread disease (affecting more than half of body) seeking stable matching of skin color but for whom repigmentation is not expected will be more satisfied if normal pigmented areas are depigmented with 20% monobenzyl ether of hydroquinone, twice per day for almost one year. Another helpful modality for vitiligo universalis, is combined both the topical application of 4-methoxyphenol and the Q-switched (QS) ruby laser[115]. Q-Switched ruby laser destroy the melanosomes present in melanocytes and keratinocytes by selective photothermolysis[130].

According to the impact of oxidative stress on vitiligo, α-tochopherol can be used alone or with topical corticosteroids in combinedation with psoralen plus ultraviolet A (PUVA)[131,132]. Antioxidant pool (tochopherol acetate, ubiquinone, selenomethionine, methionine) could be used in vitiligo aiming to enhance the enzymatic and the non-enzymatic antioxidant activity[131,132]. Food additives, contaminants, preservatives and cosmetic products could exacerbate vitiligo due to oxidative stress in melanocytes[133].

High consumption of omega-6 and decreased omega-3 intake produce free radicals and pro-inflammatory cytokines. On the other hand, omega-3 intake exert protection by enhancing TGF-β mRNA levels and antioxidant enzymes[134] and inhibiting pro-inflammatory cytokines as TNF-α[135].

Cell membranes enriched with omega-3 polyunsaturated fatty acids have elevated activity of glutathione peroxidase (GSH)[136]. In addition, omega-3 fatty acids contains indole-3-carbinol which activates CYP1A1 that is responsible for hydroxylation of estrogens to 2-hydroxyestrone[137]. Furthermore, omega-3 fatty acids have a vital role in the function of the central nervous system and affect the susceptibility and prognosis of depression[135]. Twenty percent of patients with vitiligo are found to have depression. This highlights the benefits of these lipids in vitiligo[137].

In conclusion, many theories were elaborated to clarify vitiligo pathogenesis. It is a multifactorial disease involving the interplay of several factors. Future research is needed to clarify the interaction of these factors for better understanding of vitiligo pathogenesis and subsequent successful treatment.

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REVIEW

Gall bladder carcinoma: Aggressive malignancy with protean loco-regional and distant spread

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Abstract

The most common malignancy of biliary tract is gallbladder cancer (GBC) which is the third most common cancer in gastrointestinal tract. It is a lethal disease for most patients in spite of growing awareness and improved diagnostic techniques. GBC has a very poor prognosis and the 5 year survival rate is < 10%. Although etiology of the carcinoma of the gallbladder is still obscure, various factors have been implicated, cholelithiasis being the most frequent. The incidence of GBC worldwide is based on the gender, geography and ethnicity which suggest that both genetic and environmental factors can cause GBC. The major route of spread of gallbladder cancer (GC) is loco-regional rather than distant. It spreads by lymphatic, vascular, neural, intraperitoneal, and intraductal routes. Sonography is usually the most common imaging test to evaluate symptoms of biliary tract disease including suspected GC. With recent advances in imaging modalities like multi-detector computed tomography (CT) scanners, magnetic resonance imaging-positron emission tomography/CT diagnosis of gallbladder cancer has improved. Studies have also targeted molecular and genetic pathways. Treatment options have included extended and radical surgeries and adjuvant chemotherapy. This review article deals in detail with important aspects of carcinoma gallbladder and its manifestations and challenges. Role of various imaging modalities in characterization and accurate staging has been discussed. The loco-regional spread of this aggressive malignancy is dealt explicitly.

Key words: Gallbladder cancer; Loco-regional and distant spread; Cholelithiasis; Imaging; Adenocarcinoma

Core tip: Gallbladder cancer is one of the most prevalent and lethal cancer of biliary tract with multi-factorial etiology. Cholelithiasis is the most common etiological factor. Adenocarcinoma is the most common histological type with loco-regional spread in majority of cases. Sonography is used widely as an initial screening tool and primary characterization of the tumor but it has a limited role in the diagnosis of early lesions. Thus, computed tomography and magnetic

resonance imaging are used for complete morphologic characterization and staging of malignant gallbladder lesions and metastatic survey.

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INTRODUCTION

Gallbladder cancer (GBC) is the most prevalent malignancy in biliary tract and third in gastrointestinal (GI) tract[1,2]. It was first described in detail in the seventies[3]. It is a lethal disease for most patients in spite of growing awareness and improved diagnostic techniques[4]. Although rare, it has high incidence in certain world populations[5]. GBC has poor prognosis and the survival rate in 5 years is less than 10%[5,6]. It affects predominantly women, four times more as compared to men[6,7]. GBC is asymptomatic in nature which makes it difficult in diagnosis and treatment. The symptoms of GBC are alike to other GI tract problems *viz.* abdominal pain, abdominal lump, anorexia, nausea, jaundice and vomiting[2]. GBC is a neoplasm which is widely known for its distinct ethnic, gender and geographical variations[8]. Precise etiology of GBC is still not known but various factors have been incriminated cholelithiasis[8], external carcinogens[9], free radicals, lipid peroxidation products, inflammatory bowel disease and secondary bile acids[10]. Congenital malformations of biliary tract (more common in Japan, China) are also observed as the risk factor for GBC. Chronic inflammatory conditions, heavy metals exposure, a high carbohydrate diet, obesity, alcohol abuse and smoking are also known as possible risk factor for GBC[11,12]. Adenocarcinoma is most prevalent histo-pathological type of GBC[8].

BACKGROUND AND EPIDEMIOLOGY

The incidence of GBC worldwide is based on the gender, geography and ethnicity which suggest that both genetic and environmental factors can cause GBC. Various epidemiological studies suggested that geographical and racial difference also affects the frequency of GBC. A high geographical variability in occurrence of GBC also correlated with the ubiquity of cholelithiasis[12]. Incidence rate of GBC is higher in South American Countries like Chile, Bolivia and Ecuador and some Asian countries like some areas of India, Pakistan, Japan, and South Korea[12]. Intermediate rate of incidence have been observed in European countries. Its rate is lower in United States but Native Americans observed to have a high incidence. Further, urban areas have less risk compared to rural region. Incidence in Chile where mortality is 5.2% is highest in the world. There, it is the most prevalent cancer affecting women and the fourth important reason of cancer deaths[5]. Marked geographical differences seen in frequency of gallbladder carcinoma suggest a possible environmental cause besides race or ethnicity. The exact etiology of GBC has not been properly known till date. It is yet to be established. But several other factors like chronic cholecystitis, gallstones, choledochal cyst, female gender, age and exposure of carcinogens have been observed to be implicated in gallbladder carcinogenesis[13,14]. Randi *et al*[5] and other studies carried out where age adjusted incidence rates of GBC in various populations based on cancer registry data were considered concluded that maximum incidence rate was found in women in Delhi, India (21.5/100000), South Karachi, Pakistan (13.8/100000) and Quito, Ecuador (12.9/100000)[5,15,16]. Another study also described a very high incidence of cancer in Northern India (1.5/100000) and Native American Indian females (14.5/100000)[17]. Therefore, there is an urgent need of early detection of GBC[18]. Gastric cancer was the main cause of death in Japan since 1999. It was reported to be the sixth leading cause of cancer related death in Japan in 2007[19]. The incidence of GBC rises with age and reaches the peak during the seventh and eighth decades of life[6]. Age-standardized incidence rates (ASIR) has been found to be high above age of 45. Delhi has been listed as having the highest ASIR with 22.08 males and 35.67 females per 10^5 persons after the age of 65 years. Studies revealed a very distinct age-related pattern among both genders[20]. Female-to-male incidence ratio was generally around 3, but ranged from 1 in Far East Asia to over 5 in Spain and Columbia[5]. The incidence was observed to be higher from the Gangetic belt, but, due to lack of cancer registries in these areas, the precise incidence cannot be known accurately. The estimated occurrence of GBC in Varanasi is around 4.4% of all types cancers and around 16% of all GI cancers[2].

PATHOLOGY

Approximately 80% GBCs follow progression from dysplastic mucosa to carcinoma in situ and invasive carcinoma[21]. Morphological and molecular changes also suggest the same[22]. Approximately 60%, 30% and 10% tumors originate in the fundus, body and neck of the GB respectively[23]. Among all the GBC, adenocarcinoma constitute about 85% in comparison to others like epidermoid carcinomas (6.5%) and adenocanthomas (4.5%). Besides these, other histologic types of GBC are small (oat) cell carcinomas[24], carcinoid tumors and anaplastic carcinomas[8]. Glandular, medullary, scirrhous, papillary, and colloid type are the parts of adenocarcinoma with incidence rate of 35.3%, 23.2%, 15.7% 14.5% and 11.3%

respectively[25]. Infiltrative, papillary and combined papillary-infiltrative forms are the different forms of GC[26]. Thickening and induration of GB wall occurred in infiltrating tumors which invades the subserosal plane and gallbladder wall into liver and neighboring structures. Papillary carcinoma shows a polypoid cauliflower like appearance which fills the GB lumen with very low wall invasion as it has the best prognosis. Recent researches also divide GC into metaplastic and non-metaplastic types[27]. Pseudopyloric and intestinal are two types of metaplastic variety. They are associated with chronic inflammation and cholelithiasis. Intestinal variety is associated with increasing age[28].

MOLECULAR AND GENETICS RELATED TO GBC

Different epidemiological studies and diversity in incidence of GBC studies also suggest an association of gene in its etiopathogenesis. Few facts are available about genetic changes in GBC. *K-ras* gene mutations have been found in 39%-59% of GBC, whereas *p53* mutations have been reported in 35%-92% of patients having GBC[6,29]. Shukla *et al*[30] reported that telomerase activity was significantly raised in gall bladder cancer tissue. Telomerase activity was mainly concentrated in poorly-differentiated adenocarcinomas (83.33%) and increased expression was present in advanced stages. The presence of telomerase may serve as a molecular marker for the diagnosis of gall bladder carcinoma and may have prognostic and therapeutic implications in the treatment of patients[30]. It has been reported in various studies that genetic alterations in k-ras, p53 and p16 take a significant part in gallbladder carcinogenesis[31-36]. Inactivation of tumor suppressor genes involves the different genetic mutational events which lead to the one allele and allelic loss of the other allele. This is the main and complex mechanism associated with it. This allelic loss is known as loss of heterozygosity (LOH) which can be detected by using microsatellite markers. Recurrent LOH has also been detected in GC at different chromosomal locations like 1p, 3p, 5q, 8p, 9q, 13q and 17p[37]. Other reports also suggested in their study that many chromosomal regions have important tumor suppressor genes which are also detected in this neoplasm. 3p (20% to 52%); 5q21 (*APCMCC* gene, 6% to 66 %); 8p22-24 (22% to 44%); 13q14 (*RB* gene, 20% to 30%); and 18q (*DCC* gene, 18% to 31%) are the some gene locations other that the *TP53* and *CDKN* gene[37]. In all the cholecystitis and adenoma patients Retinoblastoma (*Rb*) gene have been detected; however, *Rb* gene is deleted in 18%-67% of carcinoma of the gallbladder patients[38,39]. The precise molecular abnormality which causes neoplastic transformation in the gallbladder epithelium still unclear. An accurate pathway related to molecular changes which further result in neoplastic transformation in gallbladder epithelium has not fully understood. This more understanding of molecular changes events will give better tools to detect GBC in early stage.

ROUTES OF SPREAD: LOCO-REGIONAL AND DISTANT

Vascular, lymphatic, intraperitoneal, neural and intraductal routes are the leading routes of spread. Intraductal spread in GBC has a better prognosis[40]. Invasion of liver and lymph nodes has been reported in 69% and 45% of patients respectively in 984 patients from nine series[8]. Loco regional spread in GC is more common than distant metastasis. Metastases usually occur in liver (Figures 1A-B), lymph nodes, adjacent organs and peritoneum. Lymph nodes are usually found in about 60% of cases while metastasis in liver is about 76%-86% cases. Intraperitoneal spread is common with ascites, omental nodules and peritoneal implants[41-43]. 76.8%, 71% and 24.6% cases had liver, lymph node involvement and peritoneal deposits respectively in a study consists of 69 patients which undergo exploratory laparotomy[1]. Lymphatic drainage from the gallbladder occurs in a predictive fashion and correlates with the pattern of lymph node metastasis seen in GBC. Initially, cystic duct and pericholedochal nodes are involved, followed by more distant metastasis to posterior nodes to the head of the pancreas and then to interaortocaval lymph nodes. This primary route is called cholecysto-retropancreatic pathway. Secondary route of lymphatic drainage includes the retroportal and right celiac lymph nodes through the gastrohepatic ligament, called cholecysto-celiac pathway. The third one is called cholecysto-mesenteric route consisting of a pathway from the posterior of gallbladder to the aortocaval lymph nodes *via* pancreas[44]. It is common for GBC spread directly into the liver and porta hepatis which lead to narrowing or obstruction of the common hepatic or right hepatic duct. Surgical specimens from 48 patients who had undergone radical/extended cholecystectomies were examined in 5-mm stepwise tissue sections in one large surgical series[45]. Zero to three score was given to nodal spread (0: no metastases; 1: cystic, paradochal, hilar; 2: peripancreatic head, portal, hepatic artery; 3: celiac, periduodenal, perimesenteric). Out of 48 patients, 8 (17%), 7 (14.5%) and 9 (18.8%) were found to have group 1 and 3 nodes respectively. Out of 16 patients with group 2 or 3 nodes, only 3 were considered for curative resection. Such studies recommend a possible niche for regional radiation therapy where more distant nodal groups are commonly comprised for pancreatic cancer and cholangiocarcinoma (CC). Some studies also described that GBC have a greater risk for synchronous distant metastatic spread than in CC as GBC usually have a regional pattern of spread.

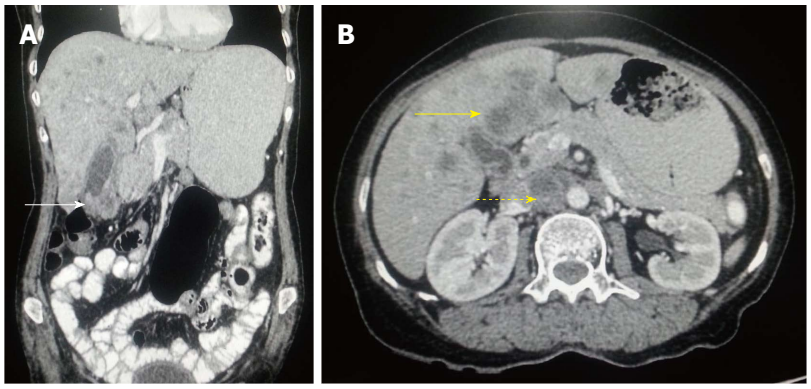

Figure 1 Carcinoma gallbladder: Nodal and hepatic metastasis. A: Coronal contrast-enhanced computed tomography (CECT) abdominal section shows relatively defined heterogenous mass involving fundus of gall bladder (arrow) with loss of fat plane with adjacent hepatic segment; B: Axial CECT abdominal section shows multiple hepatic metastasis (arrow) along with interaortocaval lymph nodal metastasis (dashed arrow).

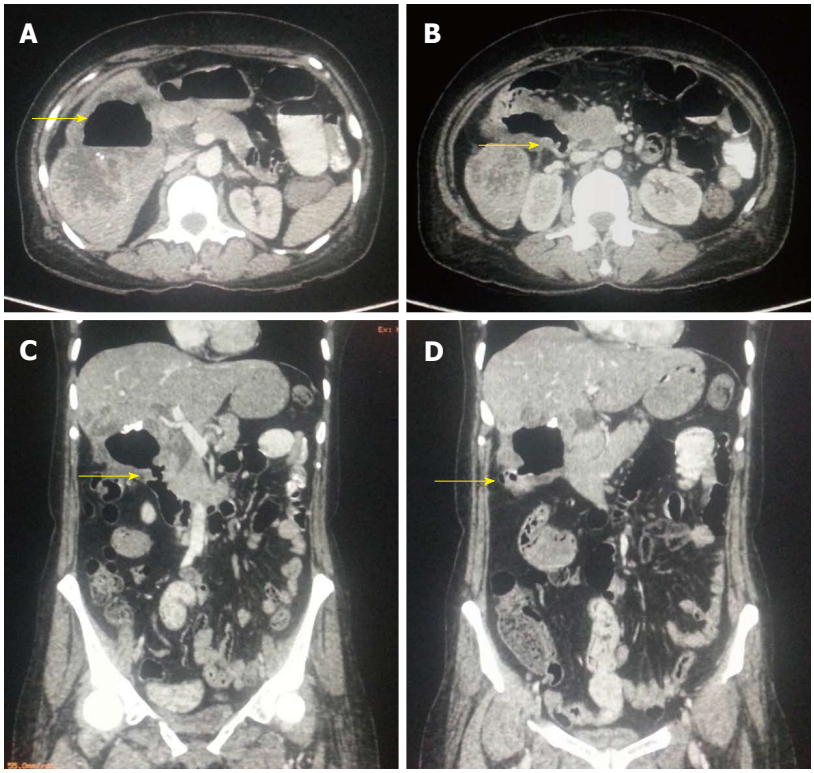

Figure 2 Aggressive gallbladder cancer and entero-biliary fistula. A: Axial contrast-enhanced computed tomography (CECT) abdominal section shows predominantly hypodense mass lesion replacing gall bladder fossa with presence of air (arrow) raising suspicion of fistula formation. Adjacent hepatic segments are also infiltrated by the mass; B and C: Axial and Coronal CECT abdominal sections clearly reveal the fistulous communication with D2 segment of duodenum (arrow); D: Coronal CECT abdominal section also show coexisting cholecysto-colonic fistula with hepatic flexure of colon (arrow).

Ninety-seven patients of GBC and 76 patients of hilar CC were included in a study who were subjected to potentially curative resection and analyzed for patterns of initial disease recurrence[46]. GBC patients had a significantly lesser time to recurrence (11.5 mo for GBC, *vs* 20.3 mo; *P* = 007) than hilar CC patients. In addition, GBC patients were far more likely to have a distant site involved at the initial period of recurrence (85% for GBC, *vs* 41%; *P* = 001). They summarized in their study that patients of GBC were having less chances to benefit from locoregional therapy given the high rate of synchronous distant disease. Such conflicting results have made difficult to recommend chemo radiation. Spontaneous biliary-enteric fistulas are developed by gallstones (90%), peptic ulcer disease (6%) and malignancy or trauma (4%). Cholecysto-duodenal type (61% to 77%) is the most common communication, followed by cholecysto-colonic (14% to 17%) and cholecysto-gastric (6%)[47] (Figures 2-5). Contrast cholangiographic studies were used in the diagnosis of gallbladder and biliary tract diseases before the emergence of USG and computed tomography (CT). Involvement of hepatic flexure and mesocolon has been observed in 33.3% of cases which was demonstrated by eccentric or circumferential wall thickening. A gallbladder mass lesion closely abutting hepatic flexure with no obvious eccentric wall thickening was observed in 2.3% cases[48]. According to Arminski[49] metastases occur to every organ including liver, lymph nodes, adrenal, kidney, spleen, brain, breast, thyroid, heart and uterus, those to the skeletal system are least frequent. Adrenal metastases (Figures 6 and 7) and venous occlusion due to tumor thrombus are unusual in newly diagnosed gallbladder carcinoma patients. Incidence of vascular metastasis is rare, but can occur. Portal vein invasion or tumor thrombus can be seen in aggressive or late cases. Vascular invasion leads to the localized involvement of

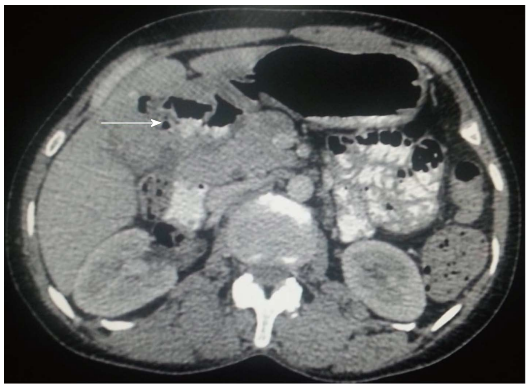

Figure 3 Axial contrast-enhanced computed tomography abdominal section shows ill-defined heterogenous mass replacing gall bladder fossa with loss of fat plane with adjacent hepatic segments. Cholecystoduodenal and cholecysto-gastric fistulas are seen with D1 segment of duodenum and antropyloric region of stomach (arrow).

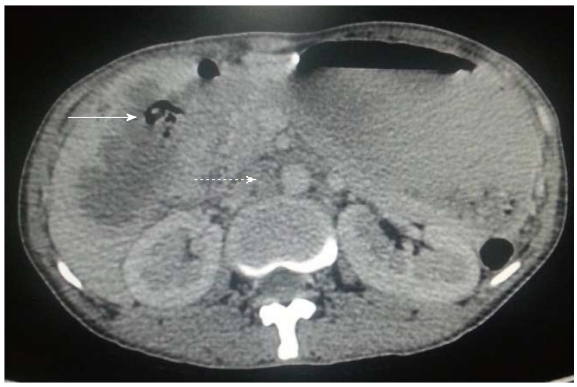

Figure 4 Axial contrast-enhanced computed tomography abdominal section shows ill-defined mass replacing gall bladder fossa with direct infiltration of hepatic segment V. Cholecystoduodenal fistula (arrow) is noted along with retroperitoneal lymph nodal metastasis (dashed arrow).

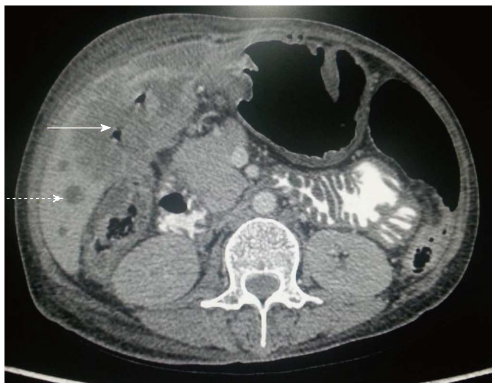

Figure 5 Axial contrast-enhanced computed tomography abdominal section shows ill-defined heterogenous mass replacing gall bladder fossa with direct infiltration of hepatic segments. Cholecystoduodenal fistula (arrow) is noted along with multiple hepatic metastasis (dashed arrow).

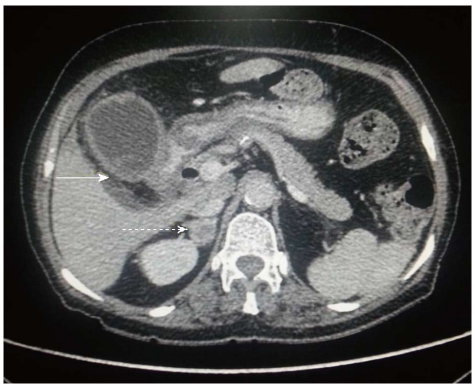

Figure 6 Axial contrast-enhanced computed tomography abdominal section shows diffuse irregular nodular enhancing wall thickening of gall bladder (arrow) along with circumferential wall thickening of distal stomach and proximal duodenum. Associated right adrenal metastasis is also seen (dashed arrow).

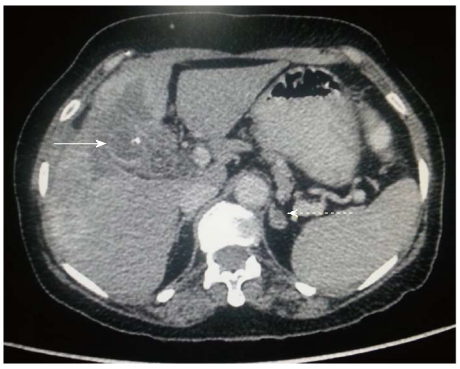

Figure 7 Axial contrast-enhanced computed tomography abdominal section shows ill-defined heterogenous mass replacing gall bladder fossa with direct infiltration of adjacent hepatic segments. Associated left adrenal lesion is also seen (dashed arrow) raising suspicion of adrenal metastasis.

the liver in the neighboring primary lesion preferably than disseminated multiple nodules[50]. Disseminated metastases appear in the late stage of the disease and caused due to retroperitoneal veins invasion. Median survival rate in patients of carcinoma gallbladder with distant metastases is only 3 to 4 mo and these patients may not be offered any intervention[42] Indian studies suggest that cases from this geographic belt are more aggressive[51]. Accuracy rate of preoperative staging by using multislice CT has an overall range from 83% to 86%[52,53]. GBC can have myriad of manifestations and spread[54]. The rarity of bony metastases from primary carcinoma has been documented by other authors[49,55-57]. In cases of skeletal metastases of the carcinoma of the gall bladder, 90% are purely osteolytic, 10% are mixed lytic and blastic type with purely osteoblastic lesions being unknown[58] (Figures 8). GC have non-specific laboratory finding. Most common laboratory finding are liver function abnormality. Serum alkaline phosphatase (ALP), direct bilirubin (conjugated bilirubin), and aspartate aminotransferase (AST) concentrations are usually deranged in more than 50% of cases. The patient is mildly hypoalbuminemic and hemoglobin level is

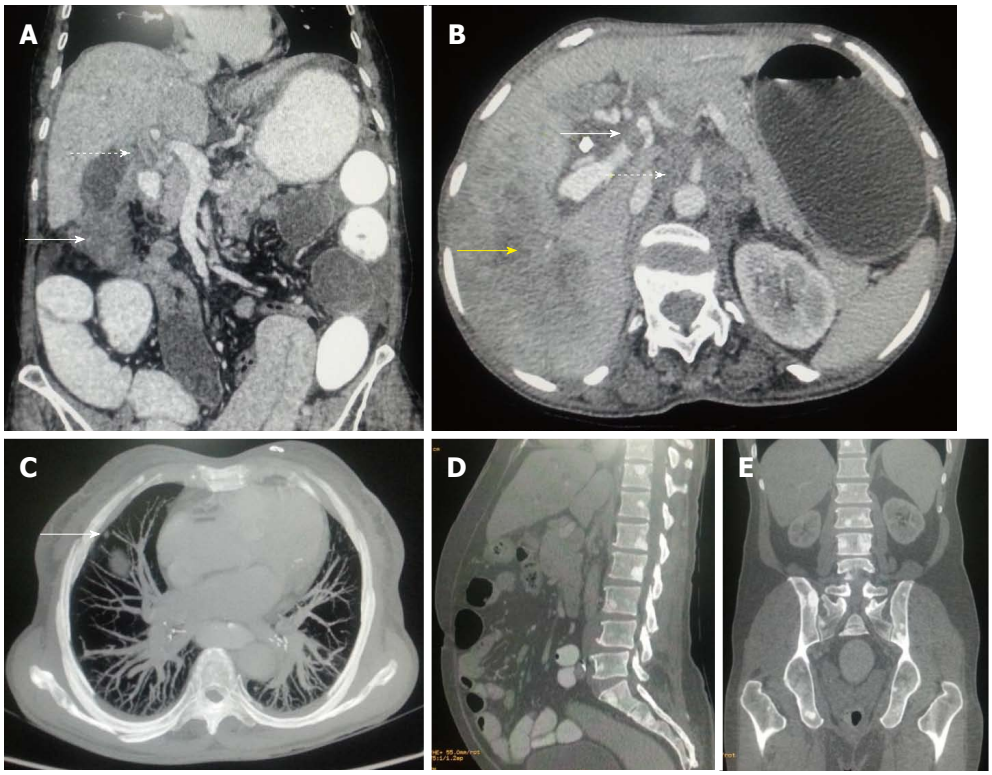

Figure 8 Gallbladder carcinoma: regional and distant metastasis. A: Axial contrast-enhanced computed tomography abdominal section shows heterogenous mass lesion involving fundus of gall bladder fossa (arrow) with associated circumferential enhancing wall thickening of CBD (dashed arrow) indicating cholangitis or intraductal spread of malignancy; B: Axial CECT abdominal section shows hypodense filling defect in left branch of portal vein indicating thrombus formation (arrow). Hepatic infiltration is seen in segment VII (yellow arrow) with retroperitoneal lymph nodal metastasis (dashed arrow); C: MIP axial CT imaging shows soft tissue nodule in anterior segment of right lung indicating pulmonary metastasis (arrow); D and E: Sagittal and coronal CECT abdominal sections (bone window) show multiple osteoblastic skeletal metastatic lesions. CBD: Corticobasal degeneration; CECT: Contrast-enhanced computed tomography.

usually less than 11 g/dL in 10% of patients[59].

STAGING

Staging is the main part for the management and reporting of GBC. Gallbladder carcinoma is staged primarily at the time of surgery. Pathologic staging is decided at the time of surgery when the resection has been performed. Resection area (R) should be reported as it acts as the most important prognostic factor for GBC[60]. American Joint Committee on Cancer has given a TNM Staging System which usually determined by the depth of invasion, expansion of GC into adjacent structures, lymph node involvement and metastatic spread[61,62] The primary stage of GC which decides the treatment is the "T" stage. Surgery is usually performed for T1/T2 (tumor confined to GB wall) if metastasis is absent. Tumors extending beyond the GB wall are considered T3 and T4. T3 tumors can be resected but with en bloc resection of adjacent organs while T4 tumors are unresectable[17]. But a simple cholecystectomy cannot be used to completely remove a T2 stage tumor as there is no serosa on the GB on the side from where it is attached to liver[60]. A minimum presence of three regional lymph nodes is required for accurate "N" staging. Hilar, celiac, periduodenal, peripancreatic and superior mesenteric nodes as well as nodes along the pancreatic head are included in the regional lymph nodes. Lymph nodes outside the hepato-duodenal ligament are regarded as metastatic disease[17]. Metastasis to liver and peritoneum is common and occasionally to the lungs and pleura. Direct tumor invasion to the liver should not be regarded as distant metastasis[60].

IMAGING MODALITIES

Abdominal ultrasound

The most prominent imaging modality to assess symptoms of biliary tract disease along with suspected GC is Ultrasound (US). It can differentiate between carcinoma and chronic cholecystitis with a sensitivity of 44% in early stages of disease[63]. Sensitivity differs for different types as reported by a retrospective study using US in early GC[64]. Ultrasound can easily detect invasion of liver parenchyma and loss of normal tissue interface. The sensitivity and accuracy of US in advanced GC are 85% and 80% respectively[65]. Bach *et al*[66] in their study reported the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in non-resectable carcinoma. The gallbladder wall is not visualized or poorly visualized as a thin echogenic line on ultrasound. An echogenic double-rim effect is produced when the gallbladder wall

becomes thickened from wall edema in inflammatory diseases[67]. Increase in gallbladder wall thickness can be seen in association with many conditions including chronic cholecystitis and neoplasia[68]. Sonography is often the first requested imaging technique in suspicion of gallbladder diseases due to cost effectiveness and easy availability[69-71]. CT and MRI are preferred over US for detection of early lesions and accurate staging and characterization[69,71,72]. US should be used along with other modalities as a complementary tool[73]. Hederström *et al*[63] in their study concluded that US had poor sensitivity to differentiate GBC from chronic cholecystitis. There is significant chance of missing a malignancy. A cholecystectomy in such cases is useful. Another study reported that 6 were correctly diagnosed by surgery or autopsy out of 11 ultrasonographically proved GC. It concluded that sonography can suggest the diagnosis of gallbladder carcinoma, but inflammatory changes in the gallbladder may simulate or mask the signs of malignancy[74]. A study by Wibbenmeyer *et al*[75] suggested that several sonographic findings were more common in patients with GBC in comparison to patients with benign gallbladder pathologies. Evaluation of these signs may be helpful in such conditions[75]. Doppler US has been found to be useful in detecting invasion of GC into the liver, the portal vein, and the bile ducts, but it has limitation in detection of lymph node and peritoneal metastases[66]. Contrast enhanced US may be helpful in improving the diagnosis of GC. The usefulness of contrast enhanced US for differential diagnosis of polypoid GB lesions has been described by Hattori *et al*[76]. Classification of contrast enhanced patterns were described as linear, scattered, and diffuse or branched. It had a sensitivity of 100%, specificity of 76.9% and accuracy of 84.5% in case of diffuse or branched type pattern[76]. US guided fine needle aspiration cytology (FNAC) has been routinely for tissue diagnosis. It has an accuracy of 95% without major complications[77]. FNAC has been reported to have a sensitivity of 88%, specificity of 100%, PPV of 100% and NPV of 52%[78].

Endoscopic ultrasonography

Endoscopic ultrasonography (EUS) has been widely used for peri-operative staging of GC. It looks as a hypoechoic mass with or without GB wall calcifications[79]. The overall accuracy rate of 91.9% has been reported in differentiating neoplastic from non-neoplastic masses[80]. Mitake *et al*[81] also demonstrated the effectiveness of EUS in determination of the extent of tumor invasion and GC diagnosis. They reported an overall accuracy rate for tumor invasion depth to be 76.5% and differentiation between early and advanced stage tumors was possible in 79.5%. Furthermore, the usefulness of EUS has increased by the development of contrast agents. The depth of GC invasion was accurately assessed in 11 of 14 cases (78.6%) by conventional EUS, and in 13 of 14 cases (92.9%) by contrast enhanced EUS in a comparative study. If we compare the conventional ultrasonography to endoscopic ultrasonography, the endoscopic ultrasonography has been observed to be more precise for staging[82,83] A combination of diagnostic methods and biopsies may bring down the incidence of explorative laparotomies performed for GC[71]. Other aids like percutaneous liver biopsies, cholecystocentesis and culture of bile have been suggested in diagnosis of equivocal cases[84]. Use of EUS alone or in combination with EUS-guided FNA of gallbladder can improve the diagnosis of GC[85,86]. EUS-guided FNA is a safe and reliable modality for carcinoma gallbladder[79] .

CT

CT is a common imaging modality for the identification of primary tumor and tumor staging. Hepatic parenchyma is the most commonly invaded site, followed by the bile duct and neighboring organs. CT scan can also accurately determine peritoneal and lymphatic metastasis[87]. Pericholedochal nodes and cystic nodes are commonly involved[88]. Kim *et al*[89] using preoperative CT reported a precision of 71%. Helical CT was suggested to evaluate the spread and depth invasion of GC to assess resectability[90]. There are many advantages of helical CT over conventional CT[52]. In a retrospective study diagnostic precision for T staging was reported to be 83%-86% in comparison to conventional CT[52]. In another study overall accuracy of CT for staging GC was 71% and 79% for T1 and T2 tumors, 46% for T3 tumors, and 73% for T4 tumors. There was statistically significant difference between thickened wall and intraluminal mass type of tumors ($P < 0.05$). The accuracy was (89%) for the intraluminal mass type, (83%) for massive type, whiles it was 54% in the thickened wall type[89]. Another prospective Japanese study using spiral CT reported affectability, specificity, PPV, NPP and the general exactness to be 88%, 87%, 88%, 87%, and 87%, respectively[91]. Although CT is not routinely used to explore patients with gallbladder disease symptoms, it is an important examination for suspected cases of gallbladder carcinoma. The most widely recognized CT finding in gallbladder carcinoma is a mass that fills the greater part of an irregular and distorted gallbladder[92] (Figure 9). Gallbladder carcinoma appears as a symmetric or asymmetric gallbladder wall thickening that may be hard to distinguish from the scarred gallbladder seen in chronic cholecystitis. Non-specific gallbladder wall thickening can be seen in acute and chronic cholecystitis, xanthogranulomatous cholecystitis, adenomyomatosis, diffuse hepatic or systemic disorders like hepatitis, portal hypertension, and congestive heart failure[46,93]. Yun *et al*[87] used dual phase CT to assess thickness as well as enhancement pattern of gallbladder wall seen in gallbladder melanoma as well as chronic cholecystitis in arterial and venous phase. They reported a difference in enhancement patterns of malignancy as compared to chronic cholecystitis using

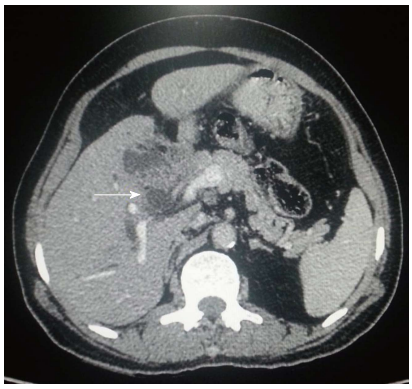

Figure 9 Axial contrast-enhanced computed tomography abdominal section shows heterogenous mass involving gall bladder fossa and leading to obstruction of biliary system due to intraductal spread (arrow).

dual phase CT. Kim *et al*[89] assessed the enhancement pattern of abnormal GB wall thickening using MDCT to differentiate between carcinoma and inflammatory diseases. They concluded that there is a distinct pattern of enhancement of inner wall compared to non-enhancing surface covering. Different signs of gallbladder carcinoma can be found due to biliary obstruction and liver involvement[53]. CT was found to be 85% precise in evaluation loco-regional spread of gallbladder malignancy[94]. It can be useful in guiding aspiration/biopsy from gallbladder in few cases[95]. It is prudent to correlate CT scan findings with clinical and laboratory findings in elderly individuals, particularly women presenting with acute cholecystitis and abnormal liver function. Imaging findings suspicious of carcinoma are diffuse irregular gallbladder wall thickening, intraluminal mass along with enlarged local lymph nodes[96]. Lee *et al*[97] compared the efficacy of ultrasound (US) and CT in cases of intraluminal and infiltrating gallbladder carcinoma with and without gallstones. They found CT to be superior than US. Some authors suggest that combination of CT with ultrasound increases the diagnostic accuracy of gallbladder carcinoma associated with cholecystitis[98].

Magnetic resonance imaging

Initially due to disadvantage of poor spatial and contrast resolution, magnetic resonance imaging (MRI) was not widely used to evaluate GB disease[18]. Nonetheless, with recent advances and introduction of dynamic techniques after the administration of paramagnetic contrast, MR cholangiopancreatography (MRCP) and MR angiography (MRA), have been utilized for tumor staging[70]. Primary GC appears hypodense on T1 weighted images and hyperdense on T2 images with sensitivity of 67%-100% and specificity of 89%-100%[95]. The detection rate of lymph node metastasis was only (57%). The sensitivity and specificity for vascular invasion can be increased by combining MR cholangiography with three-dimensional MR angiography[99,100].

Positron emission tomography

18F-2fluoro-2-deoxy- D-glucose (FDG) uptake by tumor cells gives positron emission tomography (PET) imaging the combined benefit of utilizing metabolic activity and imaging features together. The main drawback of FDG-PET is that it is not yet generally accessible for routine clinical use and the low pervasiveness of GC. Till date, there is little data on the feasible contribution of these techniques in the useful imaging diagnosis of GC. In a recent prospective cohort study in patients presenting with radiologically suspicious gallbladder lesions[101] a staging diagnostic pre-surgical FDG-PET study was performed. Total diagnostic precision was 83.33% for the finding of the primary lesion, 88.89% for the assessment of involvement of lymph node and 85.1% for the assessment of metastatic spread. Karim *et al*[102] examined the efficiency of different imaging techniques employed in GBC diagnosis.

Preoperative evaluation and staging

MDCT is widely available nowadays and has a reported precision of up to 84% in determining the T stage of primary gallbladder carcinoma[103] and 85% in foreseeing resectability due to its capacity to depict hepatic and vascular invasion, lymph nodal and distant metastases[104]. MDCT is commonly performed as unenhanced and contrast-enhanced dual phase, from which multiplanar and 3D volume-rendered reconstruction images are generated to provide complete anatomic information. Additional coronal oblique images may be acquired for surgical management[104]. Kim *et al*[89] claimed that the all-in-one standard protocol of using MR cholangio-pancreatography and contrast enhanced MR angiography may yield a sensitivity of up to 100% with regard to bile duct and vascular invasion. However it is only 67% with regard to hepatic involvement and 56% with regard to lymph node metastases. PET/CT may have a promising role in the diagnosis associated with unsuspected metastases, which might change staging and treatment[105,106]. To date, potential research which specifically assess CT, MRI, and PET/CT in their capabilities to detect and stage gallbladder carcinoma are yet to be executed. The particular spread of gallbladder carcinoma to the liver parenchyma in addition to surrounding internal organs is possibly due to lack of muscularis mucosa in addition to submucosa inside the gallbladder wall structure and primary venous drainage with the liver parenchyma on the hepatic abnormal veins. As per the sixth release of American Joint Committee on Cancer staging manual for gallbladder carcinoma[107] primary gallbladder carcinoma can be delegated T1, kept to the lamina propria or the muscle layer of the gallbladder (T1a and T1b, separately); T2, stretching out to the serosa; T3, puncturing the serosa or directly invading the liver or one other contiguous structure (stomach, duodenum,

colon, pancreas, omentum, extrahepatic bile channels); or T4, invading the primary portal vein, the hepatic artery, or numerous extrahepatic organs. Lymphatic spread is present in more than half of patients at initial finding and first reaches cystic, pericholedochal, hilar, periduodenal, peripancreatic, and predominant mesenteric nodes, which are viewed as regional or N1 nodes. Portacaval, inter-aortocaval, and more distant nodes are viewed as distant or M1 disease. Gallbladder carcinoma can spread through intraductal route along the cystic duct, hematogenous route and neural pathways, and intraperitoneal "drop" metastases[70]. T1 or T2 involvement without nodal metastasis are termed as stage ⅠA or ⅠB respectively. T3 disease without nodal spread are stage ⅡA. T1, T2, or T3 disease with N1 lymph node involvement is characterized as stage ⅡB. A T4 disease without distant metastasis is considered as stage Ⅲ. Any patient with distant disease comes in to the category of stage Ⅳ.

Cytology

A carcinoma at an early stage can be ignored, and the diagnosis can only be made after microscopic examination of paraffin-embedded tissue. Imprint cytology of the gallbladder mucosa is a simple, quick, and excellent method for the detection of GBC[108]. Ultrasound-guided fine-needle aspiration cytology is likewise a safe diagnostic method for GBC[109]. Endoscopic retrograde cholangio-pancreaticography of biliary tree and GBC can also be considered for assessment of clinically suspicious carcinoma[110].

Tumor markers

Nowadays, tumor markers have a significant role in the detection and assessment of GBC. Investigation of CA242, CA15-3, CA19-9, and CA125 are genuinely proficient markers for segregating patients of carcinoma of the gallbladder from cholelithiasis. CA242 and CA125 when utilized together accomplished best sensitivity and specificity. Serum markers appear to be less effective when utilized independently, however it can be a useful complementary tool in combination[111]. No biochemical markers are useful in early detection of GC. Cholestasis and hyperbilirubinemia indicate late stage disease. Carcinoembryonic antigen (CEA) more than 4 ng/mL has a sensitivity of 50% and specificity of 93%[112]. Presence of CA19-9 suggest poor prognosis. A value more than 20 IU/mL has a specificity of 79%. CA19-9 is frequently increased in the presence of biliary obstruction therefore it is less specific in patients of jaundice[112]. Incresed levels of serum alpha fetoprotein (AFP) have been seen in few patients but have little significance[113].

Electrophoretic pattern of proteins

Electrophoretic examination of serum protein has demonstrated protein bands in patients of carcinoma of the gallbladder in comparison to electrophoretic pattern in cholelithiasis[114].

Gallbladder membrane lipids

Fourier enhance infrared (FTIR) spectroscopy is usually very sensitive for the molecular composition of tissues, and has the potential to distinguish premalignant tissues. Lipids have been elevated inside the plasma tissue layer of GBC. This proportion associated with high intensity might be a marker to help in identification of cancer through FTIR[115].

TREATMENT OPTIONS

The only likely curative treatments intended for gallbladder carcinoma is usually surgical resection. Unfortunately, most patients with GBC have unresectable disease. Only 10%-30% of patients can be considered for surgery on presentation[29]. The surgical alternatives for the treatment of GBC have evolved over the years. The methods range from a simple cholecystectomy to a radical or extended cholecystectomy, which incorporates the gallbladder in addition to 2 cm of liver tissue from the gallbladder bed. The radical cholecystectomy has been further modified to incorporate more significant liver resections, like segmentectomies (4b/5), right hepatectomies and trisectionectomy. Extended procedures additionally incorporate regional lymphadenectomy of the porta hepatis and periduodenal and pancreatic stations. Few surgeons incorporate a resection of the bile duct to clear the lymphatics in the porta hepatis. A few specialists now incorporate periaortic lymph node dissection for staging purposes and if the tumor is distal or includes the head of the pancreas, a pancreatico-duodenectomy is added to accomplish R0 resection status[116,117]. Incidental GBC is found during cholecystectomy in 1%-2% of the cases. GBC should be suspected in the event of a tough gallbladder dissection or if there is presence of regional lymphadenopathy. A simple cholecystectomy is definitely an adequate treatment for Tis and T1 stage. Five-year survival for patients with T1 tumors is more than 85% with simple cholecystectomy[117,118]. For the T1 stage patients, the estimation of radical resection depends upon whether it is a T1a tumor or T1b tumor. In a study, the authors compared treatment of simple cholecystectomy and radical resection of T1a patients; however they didn't find any variation in survival or recurrence in the two groups[119]. Additionally, no positive lymph nodes were found in the sampled 147 lymph nodes in 12 patients who underwent radical resection. Simple cholecystectomy is needed for T1a GBC. For T1b (muscle invasion), there is proof that a more aggressive surgical methodology is needed. T1b tumors had lymph node metastases in 15% of cases, though only 2.5% of T1a tumors are accounted to have lymph node association[117]. Extrahepatic biliary resection is advocated for the management of T3 and T4 tumors[120]. The authors propose that the limit for extrahepatic

biliary resection ought to be decreased in patients with penetration of GBC through the subserosa[120]. Adjuvant combination chemotherapy and molecular targeted therapy are emerging as powerful therapeutics choices in those with advanced GBC. These days, adjuvant combination chemotherapy and molecular targeted therapy are regularly utilized as viable treatment for advanced GBC[121,122]. Adjuvant radiation treatment is utilized in locally advanced GBC or gallbladder disease with regional disease and has better survival rate[123,124]. It has been found that chemotherapy did not give effective treatment to unresectable GBC. Different regimens are already studied which include mixtures of 5-FU, leucovorin, mitomycin, adriamycin, in addition to nitrosoureas. A study observed a 64% reaction rate to gemcitabine with or without cisplatin to patients with stage 4 GBCs during phase Ⅱ trial. Average time to development was 28 wk and the average general survival was 42 wk[125]. In other phase Ⅱ trial utilizing a combination of gemcitabine and carboplatin in 20 patients with unresectable GBC, the reaction rate was 37%, average time to development was 34 wk, and the 1-year survival rate was 43%. The study concluded that chemotherapy with mixture of gemcitabine and carboplatin is viable in the treatment of advanced gallbladder carcinoma[126]. Palliative treatment may be done if GBC is found to be unresectable at the time of surgical investigation. The particular rate of biliary obstruction within affected individuals by GBC is higher than 60%[117]. A study concluded that combination of gemcitabin and oxaliplatin works well in inoperable GBC with overall response rate of more than 20%. It depicted that it may even incite complete pathological reaction. One year survival was found in more than 20% patients[127]. Other phase Ⅲ study predicts that survival rate has been found higher in those patients who receive combination of gemcitabine and cisplatin in comparison to gemcitabine alone. Different studies are in process of clinical trials for molecular targeted agents which restrain angiogenesis and EGFR pathways[128]. New effective treatment and drugs are an urgent need for GBC. Recently a new study has been done in which the authors look at the effects of triptolide on GBC cells to identify its anticancer effects. They concluded in their study that triptolide induce apoptosis in gallbladder cells and thus can be used as a potential drug for treatment of GBC[129].

CONCLUSION

The clinical and radiologic diagnosis of gallbladder carcinoma at an early stage is challenging. It is crucial for radiologists to examine the gallbladder in its entirety, especially in patients who are at a greater risk of developing GBC, for important morphologic abnormalities that may suggest carcinoma. Identification of characteristic imaging appearances of primary GBC and comprehension of its pathways of spread and staging criteria will help in formulating appropriate treatment regimens.

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REVIEW

Fluoroscopy guided percutaneous renal access in prone position

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Abstract

Percutaneous nephrolithotomy is a very commonly done procedure for management of renal calculus disease. Establishing a good access is the first and probably the most crucial step of this procedure. A proper access is the gateway to success. However, this crucial step has the steepest learning curve for, in a fluoroscopy guided access, it involves visualizing a three dimensional anatomy on a two dimensional fluoroscopy screen. This review describes the anatomical basis of the renal access. It provides a literature review of all aspects of percutaneous renal access along with the advances that have taken place in this field over the years. The article describes a technique to determine the site of skin puncture, the angle and depth of puncture using a simple mathematical principle. It also reviews the common problems faced during the process of puncture and dilatation and describes the ways to overcome them. The aim of this article is to provide the reader a step by step guide for percutaneous renal access.

Key words: Fluoroscopy; Percutaneous renal access; Percutaneous nephrolithotomy; Learning curve; Kidney

Core tip: This article is a review of the various fluoroscopic guided renal access techniques. It provides an in depth description of the technique with the aim that the urologist can have a step by step guide of the procedure. It gives an anatomical basis of percutaneous renal access and gives description of determining the skin site, angle and depth of puncture. It also describes the difficulties faced and incorporates suggestions to prevent and overcome them.

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INTRODUCTION

Rupel and Brown first reported Percutaneous nephrolithotomy in 1941[1]. Goodwin *et al*[2] described percutaneous trocar nephrostomy in a hydronephrotic kidney in 1955. The technique gained popularity especially after the description by Fernstrom and Johansson in 1976[3-5]. The improvement in endourological equipment and the advancement in techniques resulted in percutaneous nephrolithotomy (PCNL) getting accepted as the gold standard for the treatment of patients with renal stones larger than 20 mm in diameter[6]. Its popularity and acceptance amongst urologists and patients is largely due to the fact that it is minimally invasive and is associated with low morbidity[7]. Initially the procedure was done only in prone position using fluoroscopy guidance. However, in the last couple of decades use of ultrasonography alone or along with fluoroscopy has been used for percutaneous renal access[8]. Various modifications in the position of patient have also been described to overcome some limitations and drawbacks of the percutaneous renal access in prone position[9]. Despite these changes fluoroscopy guided access in prone position is still the most commonly used technique for PCNL[10]. The prone position is associated with a significantly shorter nephrostomy tract length and more potential access sites, which may improve ease and safety of percutaneous renal access[11]. In European countries, urologist establishes their own percutaneous renal access, but in the United States, access is often performed by interventional radiologists. Studies have shown lower stone free rate and a higher complication rate in radiologist performed renal access[12-14]. Despite these facts and the documented safety and efficacy of urologist acquired percutaneous renal access, as few as 11% of urologists who perform PCNL achieve access[15]. This low success rate is attributed to probably a lack of skill[16]. This is probably due to the difficulty in visualizing and mentally imbibing the three dimensional anatomy of the pelvicalyceal system on the two dimensional fluoroscopy screen[17]. The purpose of this review is to describe the various aspects of the technique of fluoroscopy guided percutaneous renal access in prone position. It is the attempt of the authors to provide a step by step guide of all aspects of the technique. Finally, the authors describe their technique in detail and the rationale behind it.

INSERTION OF URETERIC CATHETER

At the beginning of the procedure, a 5 Fr or 6 Fr ureteric catheter is inserted in the collecting system either using a rigid cystoscope (with the patient in lithotomy position) or a flexible cystoscope (with the patient prone). This is used to instill contrast to opacify the system. Also it can be used to flush saline so as to distend the system, flush small gravels during the process of stone fragmentation and at times to pass a glide wire for insertion of a double J (DJ) stent, at the end of the procedure. A Foley catheter is also passed by the side of the ureteric catheter. Both the catheters are secured with each other to prevent inadvertent slipping out of the ureteric catheter.

PRONE POSITIONING

This is an important maneuver which, if not done properly, can result in potentially serious injury to the patient. The ideal way would be to have the patient supine, have a separate trolley by the side of the operating table, shift the patient in supine position to the side trolley, remove the monitoring devices and then make the patient prone on the operating table and immediately connect the devices which have been disengaged or removed. These maneuvers should be done with adequate staff and with proper co-ordination between the anesthesiologist managing the airway, endotracheal tube and neck and the staff managing the chest and torso. Although cervical spine injury during prone positioning under anesthesia is rare, it has been reported with both over flexion and over extension during prolonged procedures[18]. Patients with cervical spine pathology, Down's syndrome or rheumatoid arthritis or patients with myelopathic syndromes are at the greatest risk. Post operative visual loss is an uncommon (0.2% of spinal surgeries in one review) but grave complication of prone surgery[19]. The ability to maintain good ventilation along with the hemodynamic stability throughout the procedure is the challenge which the anesthesiologists face. For a healthy, adequately anesthetized patient, these may be clinically insignificant; however, for those with associated co morbidities, it can be very precarious. Hence, irrespective of whether the procedure is done under general or regional anesthesia, constant vigilant monitoring during the procedure is a must. Anesthesia for PCNL cannot and should not be taken lightly.

POSITION OF PATIENT

Care should be taken to ensure that the pressure points are properly padded and limbs are positioned in a way that undue stretch, especially of the joints is avoided. This would prevent inadvertent injury and stretch of the nerves. The chest and abdomen is supported in a way to ensure free movements for the pulmonary capacity is greater in the prone compared to the supine position[18]. After positioning the flank is properly prepared and the unsterile areas covered with drapes (Figure 1).

ARRANGEMENT OF TROLLEYS

This is as shown in the figure. This helps to have a clear view of the fluoroscopy and endo camera monitors (Figure 2).

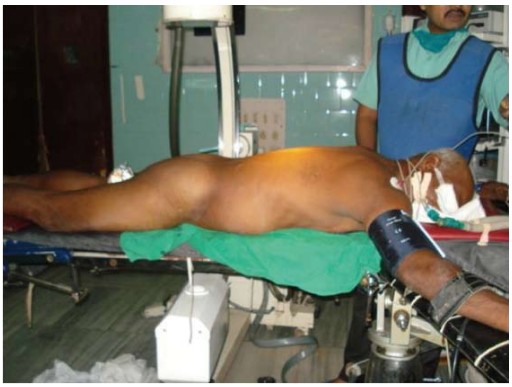

Figure 1 Position of patient.

INSTILLATION OF CONTRAST

Contrast is instilled *via* the ureteric catheter to opacify the pelvicalyceal system and identify the calyx which should be punctured. The contrast should be diluted in ratio of 1:3. The ureteric catheter, if placed in the upper pole, should be pulled down a bit so that it is in the pelvis. This helps in proper filling of all the calyces. The contrast should be instilled slowly to prevent extravasation. There should be continuous fluoroscopy monitoring so that which calyces are filled earlier and which later can be seen and this helps to identify the posterior calyx.

WHICH POLE TO PUNCTURE?

The creation of a proper percutaneous renal access is the gateway to success or disaster in PCNL[10,20,21]. A basic understanding of anatomy is needed to plan this. The kidneys lie on the posterior abdominal wall against the psoas muscle with their longitudinal axis parallel to the oblique course of the psoas at an angle of 13° to 30° to the midline. Also, as the psoas major muscle is cone shaped, the kidneys, in their longitudinal axis have a dorsal tilt with the superior poles being more medial and more posterior than the inferior poles. As the hilar region is rotated anteriorly on the psoas muscle, the kidneys are rotated about 30° posteriorly and hence the lateral aspect of the kidney is posterior to the medial aspect. The kidneys are also angled 30°-50° behind the frontal (coronal) plane with the lower pole anterior to the upper pole[22]. In prone position, the pelvis tends to fall anteriorly on the psoas muscle; hence the lower pole, pelvis and the proximal end of the ureter are placed more anteriorly than the upper pole[23,24]. The calyceal drainage of poles of the kidneys is also very important. Sampaio found that, in the cases he studied, the superior pole was drained by only one midline calyceal infundibulum in 98.6% of cases; the inferior pole was drained by paired calyces arranged in two rows in 58% and by a single mid line calyceal infundibulum in 42% of cases and the mid pole was drained by paired calyces arranged in two rows (anterior and posterior) in 96% of cases[22]. This

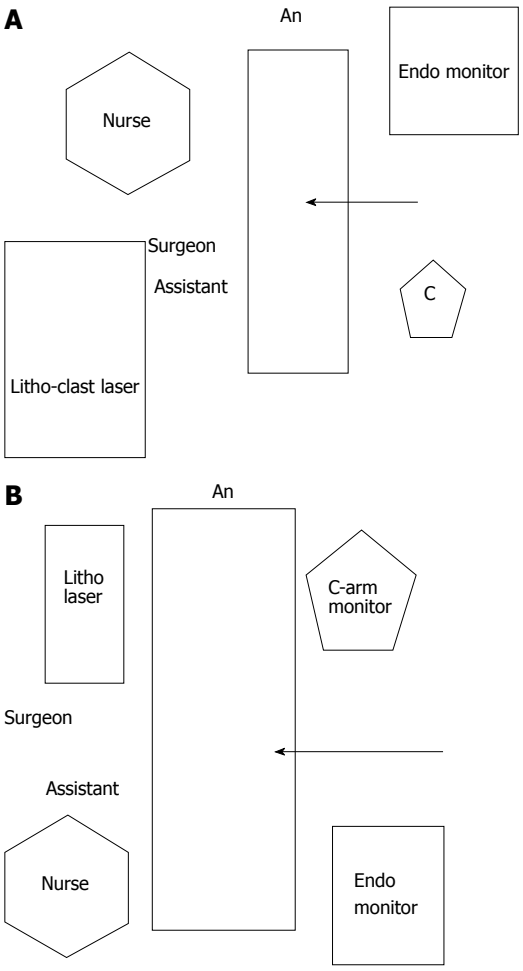

Figure 2 Arrangement of trolleys. A: Arrangement for lower pole puncture; B: Arrangement for upper pole puncture.

has important implications for percutaneous renal access as it will be easier to access endoscopically a polar region drained by a single infundibulum, which usually has suitable diameter, rather than a polar region drained by paired calyces. He also found that for best access to the pelvic-ureteric junction (PUJ) one should choose a pole whose calyx forms an angle of 90° or more with the PUJ[22].

The planning for puncture begins preoperatively by proper assessment of the imaging studies. Traditionally intravenous urography was used for functional and anatomical assessment of the collecting system. Nowadays, CT urography with coronal reconstruction is getting popular[25]. The advantages of the CT scan over intravenous urography is the ability to assess the spatial relationship of the kidney relative to the stone, depict the calyceal anatomy in 3D format to choose the access site, assess risk of pleural or bowel injury and even predict success of a sub costal fluoroscopic access for upper pole puncture. The preoperative detection of heptaosplenomegaly or the presence of retro renal colon allows serious complications related to tract placement to be avoided[26-29]. These advantages are offset by the slightly higher cost and lack of widespread availability of multiplanar CT scans in

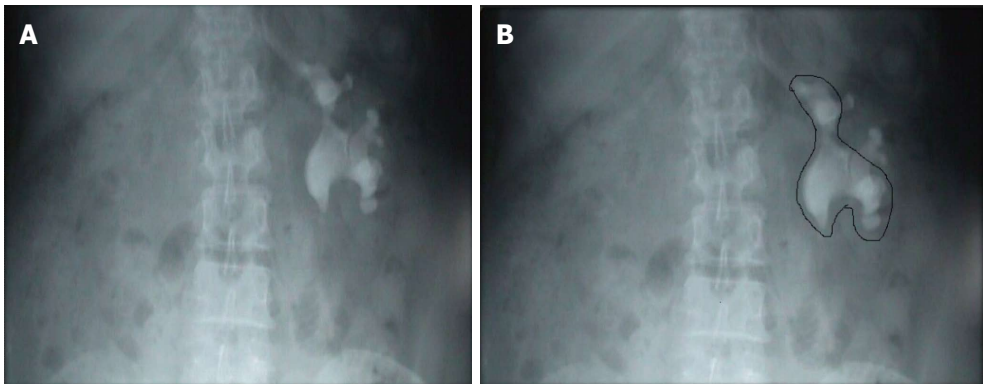

Figure 3 Outline-o-gram. A: KUB showing a Left staghorn calculus; B: Outline-o-gram suggests that most of the calculus can be cleared by the lower puncture and a separate puncture may be needed for the residual fragment.

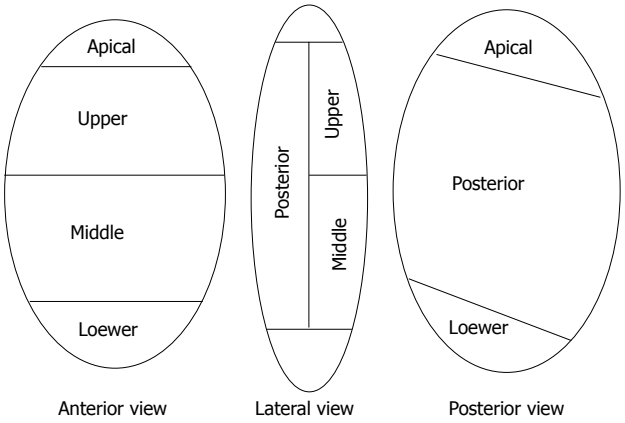

Figure 4 Arterial blood supply of kidney.

developing countries.

Whichever imaging modality is used the urologist has to select a pole for puncture which provides the straightest path along the stone axis and would provide maximum or complete stone clearance. A useful adjunct to make this decision would be to make an "outline-o-gram". As shown in Figure 3A there is a complete stag horn calculus. The "outline-o-gram" as shown in Figure 3B indicates that most of the calculus can be cleared by a lower pole puncture. The mid pole calculus would need a separate puncture or use of a flexible nephroscope. Thus an "outline-o-gram" can serve as a guide to determine which pole to puncture and also to decide whether multiple tracts will be needed.

WHICH CALYX TO PUNCTURE?

The literature is clear about the fact that it should always be the posterior calyx which should be punctured for a safe and complication free access[20,21].

WHY TO PUNCTURE THE POSTERIOR CALYX?

A good understanding of the renal anatomy provides answer to this question. The kidney gets its blood supply from the renal artery which divides into the anterior and posterior branch. These further divide into segmental arteries which supply specific areas of the kidney as shown in the figure (Figure 4). As these arteries are end arteries, there is a zone of relative avascularity between these two divisions, called as the Brodel's line of bloodless incision. The potential for bleeding complications is the least in this area. Due to the renal rotation, the posterior calices are usually oriented with their long axis pointing towards the Brodel's line. Hence puncture of a posterior calyx will traverse this relatively avascular zone[30]. Also, as the patient is prone, it will provide the direct path to the renal pelvis. If an anterior calyx is punctured, there is increased risk of bleeding as it does not traverse through the Brodel's line. More parenchyma is traversed to reach the calyx, resulting in more renal damage. Also, as there will be an acute angle between the line of puncture and the infundibulum, entry in the renal pelvis will be difficult, associated with more torque and thus increased bleeding and damage to the renal parenchyma[20,21].

CALYCEAL ORIENTATION-WHICH CALYX IS THE POSTERIOR CALYX?

The renal papillae drain into the minor calyces which may be simple or compound. There are three drainage zones, upper, middle and the lower pole. Compound calyces are the rule in upper pole, are common in the lower pole and are rare in the middle pole.

Investigators have attempted to differentiate calyces as anterior or posterior solely on the basis of their medial or lateral orientation as seen on IVU. The available anatomical references on this aspect are contradictory, confusing and incomplete. In 1901, Brodel studied corrosion casts of 70 cadaveric kidneys. He depicted the anterior calyces as medial and posterior as lateral[31]. Hodson, in 1972, described exactly the opposite, *i.e.,* the anterior calyces located laterally and posterior calyces located medially[32]. Then, in 1984,

Kaye and Reinke[33] measured calyceal angles from the axial CT images. They concluded that the Brodel pattern is seen in 69% of right kidneys while 70% of left kidneys have a Hodson pattern[33]. Sampaio *et al*[34,35] studied 140 endocasts and found that the anterior calyces are lateral in 28%, posterior calyces are lateral in 19%, and in 53% endocasts the anterior and posterior calyces had varied positions, superimposed or alternately distributed (in one region the most lateral were the anterior calyces and in another the posterior calyces)[34,35]. He found that the calyceal orientation was region dependent. The typical anterior and posterior pattern of the calyces is seen only in the middle pole[22]. The lower pole has this arrangement in only 58% cases while the upper pole almost uniformly has a compound calyceal system[22]. This implies that in the lower and upper pole the calyces are dominantly oriented in the direction of their respective poles. This has been further studied by 3D CT renderings which have also looked at the primary plane of the calyceal group. Miller *et al*[17] found that in the upper pole the primary plane of the calyces in the upper pole was Medial/Lateral and generally neutral relative to the anteroposterior axis of the kidney. As the upper pole is more posterior in the prone position, access *via* any calyx would provide a working tract that parallels the longitudinal axis of the kidney. This would mean access *via* an angle which would allow rigid instruments to reach most of the calyces in the kidney[17]. However, preferably the lateral most calyx should be punctured in the upper pole as puncturing a medial calyx is associated with significant risk of causing injury to the posterior segmental artery[36]. Eisner *et al*[37] studied the lower pole anatomy by CT scans in 101 units. They found that if there were two calices in the lower pole, the medial calyx was anterior in 95% of units while the lateral calyx was posterior in 93% of units. If there were 3 calices in the lower pole, than the medial most calyx was anterior in 93 of units. In such renal units the lateral to most medial, *i.e.,* the second calyx was posterior in 70% of units while the lateral most calyx was anterior in 71% of units. In 31% of cases, no calyx was truly posterior. In these kidneys, though both the calices were anterior, one of the calices was less anterior than the other. Their study showed that regardless of the number of lower pole calices, the most medial calyx on two dimensional imaging is anteriorly facing 94% of times. They recommended that the calyx, just lateral to the medial calyx, the second calyx, is statistically the most likely to be posterior facing and the most posterior positioned calyx[37].

HOW TO IDENTIFY THE POSTERIOR CALYX ON FLUOROSCOPY?

On account of the unreliability of the antero-posterior radiography to determine the optimal posterior calyx for entry additional maneuvers are needed[35]. With the patient in prone position, diluted contrast when instilled will fill the dependent anterior calices first. Thus the posterior calices will be filled later and would appear less dense[21]. Injection of 5-10 mL of air *via* the ureteric catheter also helps to identify the posterior calices as air will preferentially enter these calices when the patient is prone[21,38]. Despite these maneuvers if there is dilemma in indentifying the posterior calyx, movement of the C arm can help to identify the posterior calyx. In the prone position, the posterior calyces move in the opposite direction to the image intensifier on the C arm. If the C arm is rotated towards the surgeon then the posterior calices move away and shorten. *Vice versa*, if the C arm is rotated away from the surgeon then the posterior calices appear elongated. Thus by moving the C arm way from the surgeon one can identify the laterally placed calices as posterior and by moving the C arm towards the surgeon the posterior calices appear more medially placed and appear end on[9].

NEEDLE USED FOR PUNCTURE

A diamond tip needle and not a bevel tip needle should be used for puncture. A diamond tip needle has symmetrical tip which exerts equal force in all directions on the tissue. Hence the tissue is cut in the moving direction of the needle tip. A bevel-tip needle exerts forces asymmetrically so cutting of the tissue occurs at an offset angle depending on the bevel angle, needle flexibility and tissue properties[39]. The size of the needle used for puncture is a matter of debate. The options are a 21 gauge needle (which allows a 0.018 inch guide wire) or an 18 gauge needle (which allows a 0.035 inch guide wire). The 18 gauge needle is stiffer but more traumatic. The 21 gauge needle is less traumatic but less stiff and hence cannot maintain the trajectory adequately. Also, the 0.018 inch guide wire that passes through the 21 gauge needle must be exchanged for a standard 0.035 inch guide wire for subsequent tract dilatation. This requires an extra step, which adds to the complexity of the procedure and increases the risk of loss of access. Weighing the pros and cons of both it would be rational to use the 21 gauge needle when the surgeon is less experienced or if minimizing trauma is the need of the moment. The 18 gauge needle should be used by an experienced surgeon who is confident of attaining access with minimum attempts[40].

WHAT SHOULD BE THE TRAJECTORY OF THE NEEDLE?

Renal pelvis should not be punctured directly as there is very high risk of injuring a retro pelvic vessel (artery and/or vein). Studies by Sampaio have proved beyond doubt that puncture through the infundibulum of a calyx is associated with a significant risk of significant

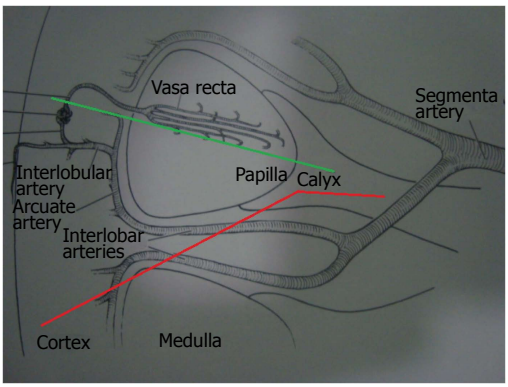

Figure 5 Trajectory of needle during puncture.

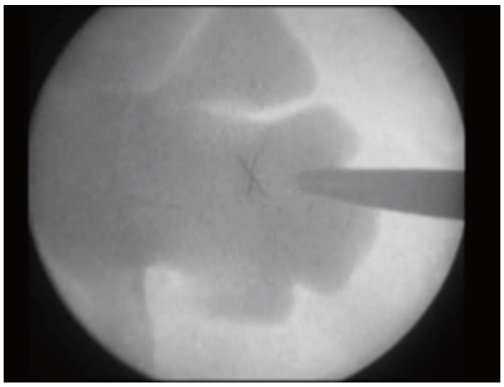

Figure 6 Bull's eye appearance of the needle.

bleeding from interlobar vessels. There is an added risk of through and through puncture of the collecting system. The risk of injury to a major arterial vessel is maximum in the upper pole where puncture of the upper pole infundibulum puncture may cause damage to posterior segmental artery, which is related to the posterior surface of upper pole infundibulum in 57% of cases. Damage to this artery may lead to loss of upto 50% of the renal parenchyma as well as serious hemorrhage[30,35].

The trajectory of the needle during puncture should be such that it aims at the fornix and not at the infundibula (Figure 5). In other words we should aim for the center of the calyx posterolaterally *via* the renal parenchyma. When puncture is made through a fornix, no arterial injury occurs and venous injury occurs in less than 8% cases[30].

CRITERIA FOR A GOOD PUNCTURE

Percutaneous renal access through a calyx must meet five conditions that guarantee safe access and avoids complications[41]: (1) Access should be performed from a posterolateral aspect; (2) Access should be through the renal parenchyma; (3) Access should be towards the center of a calyx posterolaterally; (4) Access should be towards the center of the renal pelvis and as a result of these four conditions; and (5) The trajectory does not damage any major blood vessels.

TYPES OF FLUOROSCOPY GUIDED PUNCTURE

There are two types of fluoroscopy guided puncture techniques-the Bull's eye and the Triangulation Technique. Besides these two there are a number of variations described[42].

BULL'S EYE TECHNIQUE

It is also called the Eye of the Needle technique[20]. The target calyx is identified with the C arm at 0° in the axial plane. Then the C arm is rotated 30° towards the surgeon and the calyx to be punctured would appear end on the fluoroscopy screen. A tilt of 5°-10° in the caudal direction for the lower pole or in the cranial direction for the upper pole may be necessitated to have a circular end on appearance of the target posterior calyx[21]. The position on the skin overlying the selected calyx is then marked and the puncture initiated. The needle is advanced at the end of full-expiration. It is seen as a Bull's eye (as a dot) on the fluoroscopy screen. If a longitudinal segment of needle is seen then it indicates that the trajectory is not correct and adjustment needs to be made accordingly. The C arm may be rotated by few degrees away from the surgeon to get a proper perspective of the depth of the puncture. The needle will now be seen in profile. It is then advanced to puncture the calyx. Free efflux of urine confirms the position in the collecting system[20,21,41,42]. To minimize radiation to the hands, the needle could be held with hemostat, sponge forceps or a purpose-built radiolucent needle holder (Figure 6).

Modifications of the bull's eye technique

Bilen *et al*[43], described the use of an in-line laser pointer to guide renal access in which the laser was attached within the field of the receiving head of the C-arm fluoroscopy unit. Ko *et al*[44] described a further modification using a C arm mounted laser positioning device where the laser beam is focused on the hub of the needle continuously so that the correct alignment is maintained during the puncture without the use of fluoroscopy. This approach may reduce fluoroscopic exposure early in the learning curve. As experience increased the muscle memory leads to maintenance of the correct needle alignment[42].

TRIANGULATION TECHNIQUE

Triangulation technique is the technique of using two known points of reference to locate a third unknown point. It is guided by biplanar fluoroscopy. The medial and lateral plane is assessed with the C arm at 0°. The depth is assessed by rotating the C arm in the

cranial or caudal direction by 30°. The target calyx is identified with the C arm at 0°. Then the line of puncture is aligned with the infundibulum. With the C arm at 0° the needle is introduced through the skin incision. The left and right, *i.e.,* the mediolateral adjustments are made and the needle is aligned with calyx. Then the C arm is rotated 30°, towards the head end for lower pole punctures and towards the foot end for upper pole punctures. The needle is then oriented in the up and down, *i.e.*, the cephalo-caudal position so that the orientation is again towards the desired calyx. When making the adjustments in one plane it is necessary to maintain the orientation of the needle in the other plane. The needle is then advanced with the C arm tilted 30° to give an idea regarding the depth and the respiration suspended at end expiration. After advancing the needle for several centimeters shift the C arm to 0° to see that the trajectory of the needle is still properly aligned to the target calyx in the mediolateral plane. If necessary the needle trajectory can be readjusted to maintain proper targeting. It is imperative that to minimize trauma to the renal parenchyma, the adjustment of the needle plane should be done when the needle is outside the renal capsule and not when the needle is in the parenchyma. A slight jiggle of the needle causing indentation of the desired calyx is a further sign that the trajectory of the needle is correct. If the needle position in the medial-lateral and cephalo-caudal planes is maintained, the needle should enter the targeted calyx[20,24,39-41]. It is preferable to use the 18-gauge rather than a 21-gauge needle with the triangulation technique, as its stiffness provides better stability to help maintain angle of entry[39].

Comparison of Bull's eye and Triangulation technique

In the Triangulation technique, the puncture is along the stone axis, *i.e,* in alignment with the infundibulum. This decreases the need for excessive torque on the renal parenchyma by the rigid instruments, which may cause renal trauma and bleeding[20]. Tepeler *et al*[10] did a comparison of the Bulls' eye and the Triangulation techniques and found no difference between the two as regards operation time, fluoroscopy screening time, duration of hospitalization and blood transfusion rate. They found a slightly greater drop in hematocrit and complication rate in the group undergoing access by the Bull's eye technique as compared to the Triangulation technique. However, the difference was not statistically different.

The advantage of the triangulation technique over the eye-of-the-needle" technique is that the needle cannot be passed too deeply because the depth of advancement is monitored continuously[39]. Also, the triangulation technique alone fulfills the five criteria of a successful puncture[41]. The disadvantage of the triangulation technique is that maintaining both the medial-lateral and cephalo-caudal planes are difficult because both are not being monitored at the same time as in the "eye-of-the-needle" technique. Complex visual spatial skills are required in performing this task when using a C-arm fluoroscopy unit, especially by the novice surgeon[44]. It is at this juncture that multiple attempts are needed by the urologists and excessive use of fluoroscopy occurs especially by a beginner. This is also the aspect which has the steepest learning curve for an urologist getting trained in percutaneous nephrolithotomy[45]. Usually, during the learning curve the problem comes in the assessment of depth with the C arm in the oblique position. Whether the needle is superficial or deep to the calyx has to be ascertained by the surgeon and adjustments made accordingly[46]. The easiest way to determine this would be to place another needle on the skin surface over the target calyx. If the calyx is between the two needles than the puncture needle is deep and should be adjusted superficially. If the target calyx is below the two needles, then the puncture needle is superficial and should be adjusted towards the depth.

Modifications of triangulation technique

Several new approaches and refinements have been described to improve access and reduce the learning curve of the surgeon. Mues *et al*[47] described a geometric model to create a plane of coincidence between the C arm and the needle, each at the same angle of 20°-30° from the targeted calyx, but in opposite directions. For lower pole access, the C arm is rotated cranially 30° from the vertical plane and a needle is advanced from a position distal to the calyx, rotated caudally 30° from the vertical plane. For mid pole and upper pole calyceal access the C arm is rotated 20° away from the surgeon and the needle is advanced from a position lateral to the calyx at an angle of 20° towards the surgeon from the vertical plane. During the procedure the C arm remains fixed and the needle is advanced till the point of coincidence between the calyx and the needle tip is reached. This technique avoids the need for C arm manipulation and thus potentially reduces time need to achieve the puncture[41]. This technique, however, requires a plumb, protractor and ruler to calculate, and confirm the necessary measurements. Also, it presumes that the angle of convergence would be 30° at the lower pole and 20° at the other poles. Considering the wide variations in the structure of the kidney that occurs with the varying degrees of hydronephrosis that occurs, this may not necessarily be always true. Liatsikos *et al*[48] described a technique to take advantage of the ability of triangulation technique to target a calyx from a preselected puncture site. After clearing the calculus from the initial puncture site, the sheath is withdrawn and additional calices which need to be approached for complete stone clearance are punctured from the initial puncture site. A single nephrostomy tube can be left despite multiple entries through the single incision[42,48]. The tracts are from a single site but in

different directions. This does not reduce the chance of complications. Moreover, attempt of maneuvering the rigid nephroscope may increase the torque on the renal parenchyma with resultant increase in bleeding. Mozer *et al*[49] have described a computer generated system which can be used to project the ultrasound nephrostomy tract onto fluoroscopic images virtually. The surgeon, thus, has the benefit of having preview to a three dimensional anatomy while doing the procedure instead of the usual two dimensional fluoroscopy picture. Though exciting, it requires a special system which is not routinely available. Newer C arms utilizing softwares to provide a three dimensional picture have also been used to obtain renal access in animal models[50]. Robot assistance for fluoroscopic percutaneous renal access has also been studied and is under evaluation[51,52]. These futuristic techniques have not gained widespread acceptance A study comparing robot assisted renal access with standard manual access showed that though the mean number of access attempts was comparable, the robot took lesser time to achieve puncture (10.4 min *vs* 15.1 min). However, conversion to manual access was needed in 3 cases where the robot was unsuccessful[53]. A stereotactic localization system with specially designed instruments have been described by Li *et al*[54]. It uses the Pythagoras principle of right angle triangle to calculate the depth of puncture. Then using specifically designed and patented instruments the puncture is made with precalculated depth and angle of puncture. The same instrument is then used for dilatation of the tract. The authors found that their technique is associated with higher efficiency, better stone clearance and lower morbidity, which they attribute to the greater accuracy of the puncture. This technique was not found to be useful when the puncture angle was less than 30°, because the buttocks of the operator would be in the way. Also the authors generally selected the puncture point with the same distance vertically and horizontally, which means at 45° from the skin to the stone. This again makes the principle of puncturing quite rigid as the variations in the pelvicalyceal anatomy may preclude adherence to such rigid principles. Recently, Hatipoglu *et al*[55] described a monoplanar access technique. The chosen calyx is marked with a clamp. For the lower pole puncture the needle is placed 1 cm below and medial to the 12th rib. The needle is placed with a 30° angle to the sagittal plane and is directed toward the desired calyx. If puncture fails, the needle is retracted approximately 1 cm intracorporeally, and its angle of entry adjusted on the same vertical plane and reinserted. For the mid and upper pole puncture the needle is held perpendicular to the spinal column and at a nearly 30° to the horizontal plane to access targeted middle and upper calyces to reach the pelvis and lower poles. The authors proposed that as the C arm is fixed in an anteroposterior position and thus rotation is avoided, the operative time needed for puncture is less[55]. However, there were no upper pole punctures in this study. In the initial learning curve a surgeon would find this technique difficult to master. Also, using a pre fixed puncture point on the skin for all lower pole punctures and directing the needle at a fixed angle of 30° to the sagittal plane may not always be a correct approach especially considering the variation in the position and direction of the lower pole calyces.

Hybrid technique

Rationale thinking suggests that the three most important things needed to achieve a successful percutaneous renal puncture are the site of skin entry, the angle of entry and the depth at which the puncture is achieved. Determining the correct point of skin puncture is important in the triangulation technique because a skin puncture that is too medial or lateral to the desired optimum point of entry would result in a tract of variable length and angle of entry in the calyx. This would interfere with proper access and would cause excessive torque on the parenchyma during maneuvering of the rigid nephroscope in the pelvicalyceal system. The literature does provide guidelines when it comes to determining the site of skin puncture. To avoid injury to the colon, the puncture should be medial to the posterior axillary line but not too medial as it would traverse the paraspinal muscle causing increased postoperative pain and would probably be directly on the renal pelvis without traversing the renal parenchyma. The puncture that is too close to the rib may injure the intercostal nerve and vessels and hence is to be avoided. For lower-pole access, the skin puncture should be 1 cm inferior and 1 cm medial to the tipoff the 12th rib[20,55-57].

We have described the technique of determining the site of skin puncture, which amalgamates the advantages of both the bull's eye and triangulation technique and hence is called as the hybrid technique[41,57]. With the C arm at 0°, the site of skin corresponding to the target calyx is marked as point A. The C arm is then rotated 30° towards the surgeon. The point on the skin corresponding to the target calyx and forming a bull's eye with the needle is marked as point B. In The Bull's eye technique we take a puncture at the point B. However in the Triangulation technique, the puncture is along the stone axis in alignment with the infundibulum. If we take the target calyx as the center of a sphere, then we have an imaginary circle on the skin where the point A is the center of the circle. The distance from point A to B will be the radius of the circle. The radius remains the same irrespective of the direction in which it is measured from the center of the circle. Thus, when we take a line along the stone axis where we intend to take a puncture-the site of skin puncture is marked using this principle. This means that the point B1 is marked on the skin such that the distance from point A to B1 is equal to the distance between A to B, *i.e.*, the radius of a circle with the target calyx being its center. This is how we determine site of skin puncture in triangulation technique[57]

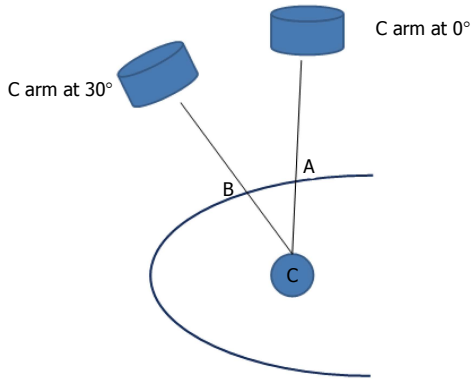

Figure 7 Hybrid technique. Point C is the calyx to be punctured. Point A corresponds to the Point C with the C arm at 0°. Point B corresponds to the point C with the Carm rotated towards the surgeon by 30°. The needle held at Point A or B is seen as a Bull's eye effect on the Carm monitor. The distance between points A and B is measured.

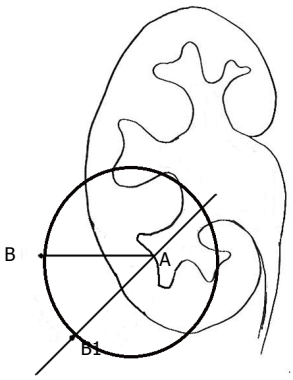

Figure 8 Hybrid technique. "A" is the point on the skin which corresponds to the targeted calyx with the C arm at 0 degree and is the center of an imaginary circle. The distance between "A" to "B1" is equal to the distance between the "A" to "B", *i.e.*, the radius of the circle.

(Figures 7 and 8).

Once the site of puncture is determined the next critical step is to access the centre of a posterior calyx with the needle directed at an appropriate angle. This step of hitting the calyx at the depth often requires maneuvering the C arm in different directions, either towards the surgeon (in bull's eye technique) or in an oblique cephalo-caudal direction (for the triangulation technique) and requires understanding a three dimensional anatomy on a two dimensional fluoroscopy monitor. This step requires maintenance of the needle in one plane while making the adjustments in the other plane and not surprisingly, multiple attempts are needed and excessive use of fluoroscopy occurs, at this step, especially by a beginner[20,45]. Maintenance of needle orientation in one plane while making the adjustment in the other plane is critical for a proper puncture. This is also the aspect which has the steepest learning curve for an urologist getting trained in percutaneous nephrolithotomy[45].

We describe our technique of using a simple mathematical principle to determine the angle and depth of puncture in fluoroscopy guided percutaneous renal access in prone position. We have used it in > 150 cases for lower, mid and upper pole punctures with > 95% success in first attempt and no pleural, visceral or hemorrhagic complications. This has recently been accepted for publication.

In the Bull's eye technique, the angle at which the needle is seen as a dot is the angle at which the puncture is made. Our Hybrid technique utilizes this principle. With the needle at point B and the C arm rotated 30° towards the surgeon and the needle forming the Bull's eye; the angle that the needle makes with the skin surface is measured using a protractor (Figure 9). One needs to take care that the protractor is held parallel to the operating table. Using the principle of sphere and circle as described earlier; if we are hitting the calyx by using the triangulation technique from the point B1-the angle of puncture

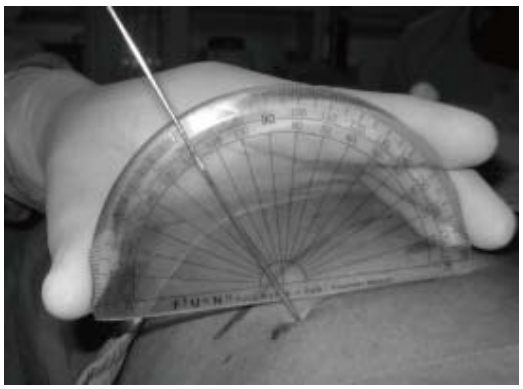

Figure 9 Hybrid technique. The angle which the needle makes with the skin surface is measured using a protractor.

would be the same with probably variations of 1-2 degrees due to the not so perfectly flat contours of the body surface. The third component of the hybrid technique is to determine the depth of puncture. What we have till now is an imaginary triangle (Figure 9) where we know: (1) One side - the distance between point A to B which is marked on the skin; (2) One angle - which is 90° with the C arm at 0°; and (3) Another angle- which is measured using the protractor at the point B.

With this information; by using the Universal triangle solver application from Google play store we can determine the depth. In this application, if we put the two angles and one side then, by the law of sines, it calculates the other two sides and the angle. For example, if the distance AB is 4 cm and the angle calculated by the protractor is 65° and with the other angle always being 90°- by universal triangle solver- the depth will be 9.5 cm.

The same principle can be applied in the triangulation technique. The C arm is brought to 0°. The line of puncture is determined in alignment with infundibulum from point A. On this line the point B1 is marked. From the Point B1 access can be obtained

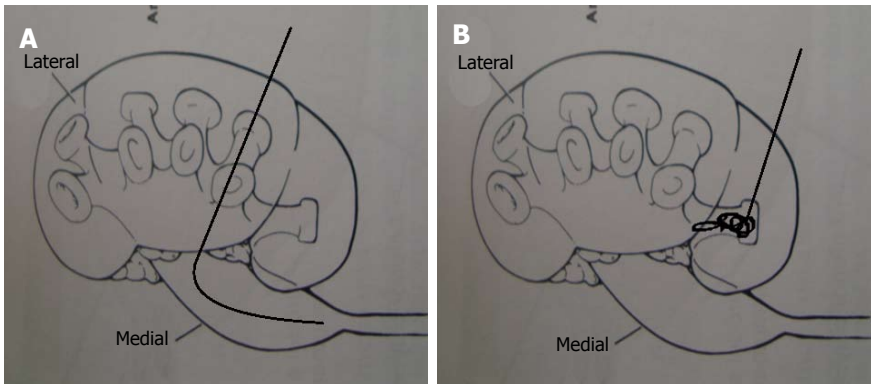

Figure 10 Calyceal puncture. A: If the posterior calyx is punctured than the glide wire passes easily in the pelvis; B: if the anterior calyx is punctured than the glide wire gets coiled in the calyx before it makes its way to the pelvis.

using the triangulation technique (Figure 8). The angle of puncture is as determined by the protractor earlier using the bull's eye principle. The depth of puncture is the same as calculated earlier. As the angle of entry is known and the depth pre calculated, the needle is advanced with the C arm 0° position only (without the need to take it in oblique position) and the puncture is made.

In the technique described by us we have assumed the target calyx as the centre of a sphere. If we have to hit the centre of a sphere from the surface, the distance traversed from any point on the surface to the centre would be the same. Hence once we have marked the point B on the skin surface using the bull's eye technique and then mark point B1 for the skin entry using the triangulation technique; then the distance from C to B or from C to B1 will be the same, *i.e.*, the radius of the sphere. Also the angle of entry from B or from B1 towards point C would be nearly the same, with only minor difference, because of the not so perfectly flat contours of the body.

But, this minimal difference would not cause any major hindrance in achieving access by the technique described because the angle of puncture and thus the trajectory of the needle would not have much variation. This was seen by us in our study. The difference between the calculated and the actual depth ranged from 0-3 mm. Also, as the angle of entry is known the fluoroscopy screening time and the time needed to achieve puncture decreases as multiple movements of the C arm are not required.

The technique described by us is applicable for both the bull's eye and the triangulation method. It describes the three most important things needed to achieve a successful percutaneous renal puncture: the site of skin entry, the angle of entry and the depth at which the puncture is achieved. It relies on simple tools. There could be some errors which could creep in especially if the protractor is not held parallel to the operating table, but this could be overcome easily with minimal experience (and the assistant telling that the protractor is parallel to the table or not). But so far this technique has not been compared with other techniques. The applicability and validation of this technique in the hands of others is yet to be ascertained. This would need a controlled prospective study involving many surgeons of equal experience and comparison with the traditional technique. It would then ascertain whether this technique is associated with a lesser fluoroscopy time, more accuracy and lesser learning curve as proposed by us. The grade of hydronephrosis can affect the puncture with the access being relatively easier for higher grades of hydronephrosis. However, if the angle, *i.e.*, the trajectory of puncture is correct, as described by this technique, the puncture would be easier and precise even in lesser grades of hydronephrosis.

CONFIRMATION OF PUNCTURE OF POSTERIOR CALYX

If air has been instilled during opacification of the pelvi calyceal system, air will be aspirated followed by a free flow saline especially if it is instilled through the ureteric catheter. After this when the glide wire is passed, while maintaining the angle of the needle, in enters the pelvis easily. No manipulation is needed. On the contrary if the anterior calyx has been punctured than the glide wire will be coiled in the calyx, will not enter the pelvis easily or will do so only after much manipulation (Figure 10).

PASSAGE OF GLIDE/GUIDE WIRE

If a 21G needle has been used for puncture than a 0.018 inch guide wire is passed initially which has to be exchanged later for a 0.035 inch guide wire. If a 18 G needle has been used than 0.035 inch glide wire can be passed. Initially a J tip Teflon coated guide wire was used. Nowadays the use of an angled tip hydrophilic glide wire is increasing. The maneuverability, resistance to kinking and ease with which it can be negotiated in the ureter across an impacted calculus or be coiled in a distant calyx are the distant benefits of this wire.

But the slippery nature of the hydrophilic wire makes it prone to displacement. Hence, it should be replaced with a stiffer wire such as a 0.035 inch Zebra or an Amplatz super stiff guide wire[21,41]. Another caution which needs to be exercised in passing the hydrophilic glide wire is to keep it wet. The dried tip can be stiff and cause inadvertent perforation of the collecting system.

INCISING SKIN AND FASCIA

The skin should be incised adequately so that the dilators and desired size of Amplatz sheath can be introduced easily. The correct way of incising the fascia would be to use the knife along the needle under fluoroscopic guidance as a lumbotome. The fascia should be incised in two planes at right angles to each other. This is especially important in patients who have scarring as a result of previous surgery. One may use an 18 G coaxial fascial incising needle (Cook) taking care to avoid lacerating the nearby sub costal or intercostal neurovascular bundle on the inferior rib margin[21].

GUIDE WIRE AND SAFETY WIRE

Traditional teaching gave much emphasis on the placement of a safety guide wire to access the tract in the event of the inadvertent slipping out of the working guide wire[20]. Many surgeons nowadays do not find it necessary to place a second safety wire especially if the guide wire is passed all the way in the bladder and more so it is a super stiff guide wire[21]. However during the learning curve it is prudent to have a safety wire. This can be introduced alongside the initial wire using a dual lumen catheter or 8/10Fr coaxial dilator of the dilatation canula (Karl Storz)[20,21,41]. Dilatation should be done over the stiff wire and not over the slippery hydrophilic wire.

TRACK DILATATION

The dilatation of the tract for creation of the nephrostomy access is an integral step of PCNL. Tract dilatation is performed to increase the size of the percutaneous wire access so that working instruments can be inserted in the pelvi-calyceal system (PCS). The size of the tract should be increased to 24 or 30 Fr size in most cases with the use of specialized dilators. The function of the dilator is to enlarge the tract in a noninvasive manner and to make renal access easier. The dilated tract is then maintained by placement of an Amplatz sheath.

Tract dilatation can be acute or chronic[58]. Chronic dilatation is done by placement of a percutaneous nephrostomy tube, which is gradually dilated over few days by sequentially replacing it by larger tubes. Acute dilatation is done just before the therapeutic procedure. The tract is dilated either by sequential (Amplatz dilators) or telescopic coaxial dilators (Alken dilators). These are rigid dilators. Balloon dilators are also in vogue and have results similar to rigid dilators. The chronic dilatation approach was once the conventional method by which renal access surgery such as PCNL were done; however, in recent years one stage acute dilatation method has become preferred due to its low risks of patient morbidity and decreased time, which allows less room for complications.

Alkens dilators

These are rigid metal dilators that are introduced over a central guide rod. Progressively enlarging coaxial stainless steel dilators help to dilate the tract from the 8 Fr guide rod up to 30 Fr. The guide rod has a round bulbous end prevents the sequential dilators from over-shooting. The advantages of the Alkens dilator system are that it is reusable, hence inexpensive and importantly is able to dilate even when there is dense perinephric scarring[59]. The disadvantage is that the same characteristics that make the Alkens dilator so effective are also the reasons why the rigid metal dilators can do considerable damage.

Amplatz dilators

These are semi rigid plastic dilators that are passed over an 8-Fr PTFE guiding catheter that fits over a 0.035-inch guide wire. They can also be passed over a guide rod. The dilators are passed one after the other, not coaxially like the rigid metal dilators but progressively, by advancing one dilator, removing it, advancing the next larger dilator, and so on until the final tract diameter is achieved. Finally, the working sheath is passed over the final dilator and then the dilator and 8-Fr catheter are removed, leaving the working wire and sheath in place. The dilators are made in increments of 2 Fr, but if the tissue being dilated is soft, then not every dilator needs to be used.

The advantages of Amplatz dilators are that trauma experienced by the collecting system is theoretically less probable than the trauma experienced by the collecting system using rigid metal dilators, but the disadvantage is that bleeding can happen each time a dilator is withdrawn. As these are disposable dilators, they are more expensive than the Alkens dilators.

There have been many comparative studies[59-63] between the two dilator systems but experienced urologists have found no difference between the two systems in terms of safety. Alkens dilators may be preferred in patients who have tight fitting staghorn calculi, as Amplatz dilators need some space in the calyx for dilatation. In calyces that have no space, the dilatation may remain short due to tapered end of the dilator.

Balloon dilators

Here a pressure balloon is used for rapid tract

making[64]. The Amplatz sheath is back loaded on the balloon and is placed once the balloon is adequately distended. The balloon dilator is expensive and may be difficult to use in patients with densely scarred tissue. The dilators may have an advantage when operating on a hyper mobile kidney. As it is a single step dilatation that causes tamponade, the bleeding is expected to be less, but not all studies have documented less bleeding and transfusion as compared to the Alkens and Amplatz dilators[59,65-67].

In an effort to make tract making rapid, easy, and blood less, multiple single step techniques have been described. The simplest is using the largest Amplatz dilators without the initial smaller dilators. In difficult situations where scar tissue is present around the kidney, collings knife or plasma vaporization has been used for tract making[68]. Two new dilatation systems described have been a radially expanding single step dilator system[69] and the 5pang system[70]. Both the systems of the advantage of not removing the needle and hence the dilatation is over s rigid system resulting in less chances of kinking of guide wire. Also the dilatation would be faster. However, so far it has been a single center experience and multi center experience with these two systems has not been described.

The important principles of tract dilatation are: (1) A proper planning of the procedure and correct choice of the calyx of entry is vital for the success of PCNL. This needs a study the radiologic images prior to the procedure; (2) The tract should be dilated only till the minor calyx. If over-dilatation happens, it can traumatize the infundibulum, renal pelvis or uretero-pelvic junction. Trauma to the anterior wall of the PCS can cause significant bleeding that may be difficult to control. It is always better to under dilate than to over dilate and cause trauma; (3) The success of tract making is dependent on maintaining the angle, depth, and the direction of the dilatation; (4) Every step of dilatation should be monitored on fluoroscopy; (5) The collecting system should be kept distended during dilatation by instilling in the system either contrast or saline. This is instilled by the OR assistant through the ureteric catheter; and (6) Adequate lumbotomy is important for safe dilatation.

AMPLATZ SHEATH

The use of an Amplatz Sheath during percutaneous renal procedures has become standard. No matter the type of dilator used, rigid or balloon, or the technique of track dilation, one-step or multi-stepped, an Amplatz sheath is always used. The Amplatz sheath serves many purposes: (1) Amplatz sheath maintains the tract during procedure; (2) It causes tamponade of the tract and reduces bleeding. The beveled end of the Amplatz sheath can be used to tamponade a part of renal parenchyma that is actively bleeding[71]; (3) It protects the renal parenchyma from injury by the instruments used in renal procedures; (4) The use of Amplatz sheath maintains a low-pressure system and reduces fluid intravasation. Maintaining a low-pressure system would be important in patients with infected calculi as the risk of sepsis would reduce; and (5) Amplatz sheath helps in removal of calculi and prevents parenchymal injury by broken ragged stone edges.

WHEN TO DO MULTIPLE PUNCTURES?

The amount of bleeding, parenchymal damage, morbidity as well as the risk to the patient increases with increase in the number of punctures[72]. It is important to plan the first puncture in a way that multiple punctures are avoided. Use of flexible nephroscopy and flexible ureteroscopy would also reduce the need for multiple punctures[73].

Multiple accesses may be needed when treating large and complex stones and staghorn calculi. In this situation the first tract is made in a way that most stone bulk can be removed through it. The accessory tracts can be mini-PCNL tracts for peripheral small calculi. In this situation, upper calyx has an advantage as it affords a direct access to the upper calyx, renal pelvis, all components of the lower calyx and the upper ureter[74]. In selected situations where the calculi are smaller than the neck of the calyx, percutaneous calyceal lavage can be done to flush the calculi in the renal pelvis so that they can be picked up through the primary tract. If necessary, multiple tracts can be safely made in experienced hands with the intent of complete stone clearance[75].

In a complex situation, the plan of management would be as follows: Make the primary tract in a way to clear maximum stone bulk. If access to a flexible nephroscopy with holmium laser is available, use this to prevent additional tracts. If these facilities are not available, percutaneous calyceal lavage, mini-tracts or accessory tracts can be made.

HOW TO MAKE A SAFE SECOND TRACT?

The second or multiple tracts tend to bleed more than the primary tract because when the second tract is made, it is not possible to opacify the system. The puncture is more often directed to the stone and not to the calyx. Also the bleeding and fluid extravasation through the first tract can alter the anatomy. To prevent this, if a second tract is anticipated, it is better to place the guide wires in the calyces where the second tract is expected before the first tract is dilated. The advantage of a pre-placed guide wire is that the proper placement of the tract is possible but the disadvantage is that in some patients this wire may not need to be dilated. The advantages of a proper placed tract far outweigh the risk of a tract made

aiming for the stones.

HOW TO MINIMIZE RADIATION?

The risk of radiation is quite high in patients with stone disease during their evaluation and treatment. Recently, two centers have studied the radiation dose in a patient with a primary acute stone event over a 1 year period. They found average radiation to which these patients were exposed was 29.7 mSv, and 20% of the patients were exposed to > 50 mSv[76]. This dose exceeds the International Commission on Radiological Protection recommendation on limits for occupational exposure to radiation, which is 20 mSv averaged over a 5-year period with not nore than > 50mSv in any single year. In comparison, a typical CT of the abdomen and pelvis without contrast exposes patients to a median of 15 mSv[77]. Fluoroscopy during percutaneous nephrolithotomy is associated with radiation exposure not only to the patient but also to the surgeon and the operation theatre staff[78]. High Body Mass Index, high stone burden, and increasing number of access tracts are associated with an increased radiation exposure. Branched stones and the presence of hydronephrosis are associated with decreased radiation exposure[79,80]. Proper planning of the procedure by an experienced surgeon is very important for reduction of radiation exposure[81]. It is the duty of the surgeon to reduce this health hazard for all concerned. Following steps can be taken to minimize radiation during PNL: (1) The surgeon and the staff must always wear radiation protection gowns, thyroid guards and radiation protection gloves[82]; (2) It is important to limit the time of exposure to minimum necessary. Using short bursts of fluoroscopy and using the "last image hold" feature of the fluoroscopy unit reduces radiation exposure[83]; (3) The image intensifier should be placed as close to the patient as possible, fluoroscopy beam should come from under the table, be focused on the area of interest and a pulsed fluoroscopy mode should be used[25]. The use of air instead of iodinated contrast may further reduce the radiation exposure[84]; (4) Keeping the fluoroscopy unit foot pedal with the surgeon and thinking during and after screening are other small precautions which can decrease the radiation; (5) For lower pole punctures using triangulation technique tilt the C arm cephalad and *vice versa* for upper pole puncture; and (6) Hold the needle in a way that the hands get minimal radiation exposure. Use of an instrument to achieve this would reduce radiation exposure. Needles and dilators that have distance marked on them can help in reducing radiation as the fluoroscopy can be used once the needle-tip is near the kidney[40,85].

PREVENTING VISCERAL INJURY DURING PCNL

Any abdominal organ close to the kidney can be injured during percutaneous renal surgery including the colon, duodenum, jejunum, spleen, liver, and biliary system. Such injury is always an accident and an effort is needed in preventing them. If it happens, early identification and treatment is very vital.

Colonic injury

Colonic injury happens in about 1% percutaneous renal procedures in prone position. It is thought to be due to retro-renal position of the colon. It is more common on the left side when a lower calyx access is attempted[86]. Thin patients, elderly age group, dilated colon, prior colon surgery or disease, and the presence of a horseshoe kidney are additional risk factors[40,87]. It can also happen in patients who undergo significant weight loss in a short time like patients after bariatric surgery, ileal diseases and resections. A recent hypothesis proposed retro-renal colon to be an acquired anomaly[88]. Five patients developed colonic injury in the 2nd stage PCNL. All these patients had a long-standing large hydronephrosis that was initially drained by either a nephrostomy or a DJ stent. They proposed that the colonic mesocolon lengthens over the gradually dilating obstructed kidney. Once the kidney is de-obstructed, the kidney reduces in size but the long mesocolon persists. The colon with the long mesocolon drops posterior to kidney forming a retro renal colon.

Prevention of colonic injury is very difficult. In patients who are predisposed to colonic injury, a pre-operative CT scan in prone position could help identify the position of colon in relation to the proposed tract. Awareness of the colonic gas bubble on fluoroscopy at the time of making access and monitoring any changes in the bubble could help prevent this injury. It would be possible to identify the overlying colon if a sonography guided puncture is attempted.

Liver and splenic injury

Injury to normal sized liver and spleen are very rare during PCNL and are likely to occur if the puncture is above the 10th rib[89,90]. In patients with significant splenomegaly and hepatomegaly pre-operative CT scan could be used to decide a safe access. In rare situations, CT guided access could be made. Pre-operative awareness and planning is the only way to prevent these injuries.

Pleural injury

Pleural injury is a definite risk associated with supra-costal access. All supra costal tracts traverse the diaphragm and hence there is risk of damage to the pleura and lung. The surgeon should be aware of this risk while undertaking Supracostal puncture[91]. The risk increases as the tract moves higher on the intercostal space. The risk that is about 4% in supra 12th rib access increases to nearly 20% in supra 11 rib access[92] .

To understand pleural injury during PCNL, it is

important to understand the pleural anatomy. The parietal pleura crosses the 12th rib such that the medial half is covered by the pleura while the lateral half of the rib is not covered by the pleura. In the mid scapular line, while the parietal pleura is at the level of the 12th rib and the visceral pleura is at the level of the 10th rib,. The parietal and visceral pleura ascend cranially and laterally on the ribs, and further rise in deep expiration[91,93].

To prevent pleural violation[91]: (1) Make the Tract lateral to the mid scapular line; (2) As far as feasible, stay below the 10th rib; (3) Tract making should be performed in deep expiration; and (4) Tract should be kept to the minimum necessary size.

Based on the above mentioned anatomical caveats it would be rational to suggest that tracts below the 11th rib made lateral to the mid scapular line would miss not only the visceral pleura but mal also miss the parietal pleura. Tracts made through the parietal pleura may not be of clinical significance. Use of an Amplatz sheath would further mitigate major complications by preventing leakage of the irrigation fluid in the pleural space.

An anesthetist who understands the procedure and is involved during the procedure is important, as he would maintain the patient in deep expiration when the tract is being made. If the individual case demands higher tract, there is no harm in making it. Use of thoracosopy control would make this safer[94]. The pleural fluid collection, if occurs, can be easily managed by placing a chest drain at end of the procedure. It is vital to check the costo-phrenic angle at the end of the supra-costal access. A clear costo phrenic angle on fluoroscopy at the end of the procedure is a proof that pleura have not been violated[95].

PROBLEMS DURING ACCESS

Failure to opacify the system

Cause: This uncommon occurrence can occur either due to the ureteric catheter slipping out or a tightly impacted calculus preventing passage of contrast across it. Prevention: The ureteric catheter needs to be fixed to the per urethral catheter inserted initially so that it does not slip out while making the patient prone. Using a hydrophilic glide wire also helps in negotiating the wire and then the ureteric catheter across the calculus.

Remedy: For a tightly impacted calculus, where the contrast does not go across it. Keeping the patient in "head-low" position or reducing the concentration of the contrast (increase the dilution) may help some contrast go beyond the blocking calculus. If this does not work, then either an ultrasound guided puncture can be attempted or one can use a Chiba needle to opacify the system. Chiba needle is a much finer needle as compared to the initial puncture needle hence is likely to be less traumatic[96]. The needle is introduced around 2 cm lateral to the vertebral transverse process at L1-2 level. At this site it is likely to hit the renal pelvis. The CT scan images could help in identification of exact site of renal pelvis. Once the PCS is entered with a Chiba needle (confirmed by aspiration of urine from PCS), opacify the system and make the standard tract through the chosen calyx.

Extravasation of contrast

Cause: Extravasation of the contrast is an unfortunate problem. It is important to avoid this situation, as extravasation would happen before the main procedure begins, and would complicate the further access making. The extravasated contrast would make the tract making difficult and also hamper the radiologic confirmation of stone clearance post procedure. The most common cause is when an enthusiastic assistant instills a large volume of contrast under high pressure. Rarely, the contrast may extravasate from an improperly placed ureteric catheter. This would be more common in patients with large impacted ureteric calculi with infection. It may also happen intra-op when the first attempt at needle insertion is not satisfactory and the contrast leaks from the needle puncture site that is made in the collecting system.

Prevention: To prevent extravasation of contrast, inject diluted contrast slowly while keeping the ureteric catheter in the pelvis so that sudden distension of the system with consequent extravasation does not occur. It is extremely important to instruct the assistant to instill a small amount of contrast gradually at a very low pressure. The volume of the normal collecting system is 5-8 mL; hence gradual instillation of small volume is vital.

Remedy: The problem can be salvaged in multiple ways: (1) Give diuretic and wait for the contrast to get absorbed. The concentration of the extravasated contrast would significantly reduce if you wait for about 15 minutes after a frusemide injection; (2) Use concentrated contrast that would help in identification of the PCS through the dilute extravasated contrast. The tract needs to be made fast before the concentrated contrast extravasates and compounds the problem; (3) Use of air-pyelogram to identify the PCS. The similar problem of air extravasation can happen through the needle hole in the cortex; (4) Ultrasound guided percutaneous access is a good option. However, even this technique would be difficult after contrast extravasates. Do not attempt air pyelogram, if you want to do an ultrasonography; (5) Very rarely, it may be desirable to stage the procedure and re-attempt access after 48 h; (6) Grasso *et al*[97] initially described ureteroscopically assisted percutaneous renal access as a salvage procedure in difficult cases. This can be utilized in cases where significant extravasation has occurred. The major hindrance is the availability of a flexible scope, which is not the case in many

developing countries; and (7) Giannakopoulos *et al*[98] have described the use of an angiographic catheter to salvage such situation. A 0.038-inch guide wire is passed through open-end ureteral catheter which is then removed and an angled- tip angiographic catheter is passed. The radiopaque tip of the angiographic catheter is easily seen on the fluoroscopy despite significant extravasation of contrast. A guide wire is then passed through the angiographic catheter. It is manipulated and brought in a calyx which is to be punctured. The angiographic catheter is then brought till the calyx and the puncture is made aiming at the tip of the catheter. The intravenous urogram film or an initial normal fluoroscopic image before extravasation, which is captured on the second monitor of the fluoroscopy unit is very helpful in manipulating the guide wire and catheter in the proper calyx. The correct position of the catheter in a posterior calyx can also be confirmed by rotating the C-arm[98].

Inability to puncture

Cause: Inability to puncture is often a technical problem .This happens either due to the inexperience of the operator or due to technical difficulty commonly while attempting puncture of a non-dilated system. This could be related to incorrect choice of the calyx for puncture.

Prevention: In the initial learning phase, presence of a more experienced colleague goes a long way in minimizing the learning curve and overcoming difficulties during the procedure. It is important to keep the PCS adequately filled for ease of puncture. Ask the assistant to continuously flush fluid in the ureteric catheter so that the system remains distended. If despite multiple attempts it is still difficult, reassess the pre-operative radiological studies and re-plan the puncture. Add a drop of methylene blue or betadine solution to the contrast; aspiration of the colored fluid (blue or brown) from kidney would give confidence of correct puncture[99].

Remedy: In a non-dilated system, fluoroscopy guided puncture is usually feasible using the techniques described above. If the surgeon is worried about trauma to the kidney, then it is prudent to use a 21 G needle for initial puncture and pass a 0.018 inch guide wire, which can be exchanged for a 0.035 inch stiffer guide wire[40]. If these attempts fail, take the help of a senior colleague from the department. An interventional radiologist may help with difficult punctures. Re-planning the procedure under CT scan guidance or ultrasonography guidance may rarely be needed[100] Puncture would also be difficult in a patient who has a very thin renal cortex. The renal cortex tends to move away from the needle or to get tented by the needle rather than getting punctured. Forcefully pushing the needle in, once near the cortex, can help the needle enter the PCS. Forceful insertion would be safe as in this patient with thin renal cortex the PCS is likely to be hugely dilated. Proper care of the wire once inserted is very important in these patients. It is also important to keep a safety wire if possible. If there were a tract loss, it would be very difficult to get inside these deflated hydronephrotic sacs. Puncture will also be awkward in very thin patients. As there is no perirenal fat pad, the kidney tends to get pushed by the needle. A bolster kept below the kidney can hold the kidney in place so that puncture can be made.

Blood at tip of needle and not urine

Cause: It is not uncommon to have made a puncture and after removing the trocar and aspiration have blood and not urine. This happens if the needle is in a blood vessel or needle is in renal parenchyma instead of in the pelvicalyceal system. It can also occur if multiple attempts have been made to achieve access.

Prevention: Avoid trauma by making multiple attempts of puncture using a 18 G needle, instead use a 21 G needle. Follow a proper puncture technique so that we aim at the calyx through the fornix.

Remedy: If after injecting saline through the ureteric catheter, the efflux clears then it suggests that the needle tip is in the collecting system and glide wire can be passed in the collecting system. If the efflux or the aspirated fluid is frank blood then the needle's position should be readjusted. This entails with drawing the needle and adjusting the medio-lateral and anteroposterior position. It is usually the depth of the needle which needs to be adjusted. Either the needle is superficial or deep to the desired calyx. This adjustment is best made after withdrawing the needle outside the parenchyma. Manipulations within the parenchyma cause trauma and should be avoided. The 3 finger technique described by Shergill *et al*[46] is an attempt to help the junior trainee to overcome this difficulty. The tip of the needle should be towards the desired calyx with the C arm in the anteroposterior position or tilted towards or away from the surgeon or tilted in cephalo caudal direction. The surgeon should remember the medio-lateral adjustments should be made with the C arm at 0^0 and the depth adjustment should be made after tilting the C arm towards or away from the surgeon, as in Bull's eye technique or tilting it in the cephalo-caudal direction, as in the triangulation technique. An easy way to determine the depth would be to place another needle on the skin surface over the target calyx. If the calyx is between the two needles than the puncture needle is deep and should be adjusted superficially. If the target calyx is below the two needles, then the puncture needle is superficial and should be adjusted towards the depth. Use of the Hybrid Technique described above minimizes these problems.

Inability to park the guide wire

Cause: This occurs either if the glide wire is outside

the collecting system or if the calyx is completely occupied by a calculus. Rarely, inadvertent puncture of a renal cyst and aspiration of clear fluid can cause a mistaken assumption of a good puncture. But the glide wire does not enter the collecting system in such cases. If an anterior calyx s punctured then also the glide wire will not enter the pelvis easily (Figure 10B).

Prevention: Free flow of urine from the needle usually is a sure shot sign of a correct puncture. A hydrophilic glide wire usually passes easily in the pelvis even across an impacted calculus. As regards inadvertent cyst puncture, the ultrasound findings and the intravenous urogram or the CT scan picture should alert the surgeon regarding such a possibility.

Remedy: Re puncture or re-insert the glide wire if one suspects that the glide wire is outside the system. Injecting diluted contrast or diluted methylene blue can also confirm that the needle is properly positioned in the desired calyx. There may be a calculus blocking the passage of the wire down the ureter, in this situation, the second best place to park the wire would be a distant calyx. If the wire does not coil in distant calyx then keep as much length as possible of the wire in the punctured calyx[99].

Kinking of guide wire

Cause: This usually occurs due to forceful dilatation in the wrong direction and/or against resistance of the initial dilator. Once the guide rod is in place this problem cannot occur.

Prevention: The wire usually kinks at the level of the thoracolumbar fascia. Hence the fascia needs to be incised well before starting the dilatation. Use of super stiff wire is recommended due to its properties to resist kinking more than the PTFE guide wire[101]. The dilatation should be in the correct direction and with adequate force. A simple rule would be to achieve the 2/3 of the progress of the dilator by rotational screwing movements and 1/3 by force. If there is doubt regarding the correct direction then moving the glide wire gives a good indication. If the wire moves freely then it indicates that the direction and trajectory is correct. *Vice versa*, if the glide wire does not move freely then the direction and trajectory needs to be adjusted. This simple friction test can be of immense help in the initial learning of percutaneous renal access. Use of the 5 part PANG needle system largely avoids this problem[70].

Remedy: If a kink has occurred then the initial dilator should be advanced close to the kink and it should be pulled inside the dilator. The correct direction should then be ascertained and further dilation should be done. At times a re-puncture is needed. If a safety wire has been inserted then it can be used for dilatation. Recently Lezrek *et al*[102] have described a use of bi prong forceps to overcome renal mobility and prevent guide wire kinking during tract dilatation.

Under dilatation

Cause: Under dilatation is a condition when the wire was initially well placed but during dilatation the dilators and the Amplatz sheath remained short of the PCS. This usually occurs early during the learning curve. Use of Amplatz dilators is also associated with this as the terminal taper end of the dilator enters the calyx but the amplatz sheath introduced over it does not enter the calyx and remains outside the collecting system. This may cause brisk bleeding as there is a portion of parenchyma that has been partially dilated, which does not have the tamponade effects of the Amplatz sheath. This situation needs rapid management.

Prevention: Flushing saline from the ureteric catheter during the process of dilatation helps in confirming that the dilator/ dilators are within the collecting system by seeing the efflux of saline form the dilators. Small frequent bursts of screening on the C arm confirm the correct position of the dilators.

Remedy: The treatment would depend on the position of the wire. If the wire is still inside the PCS, thread the guide rod on the wire so that the bulbous end of the rod is fluoroscopically positioned in the PCS. Using the flexible guide rod may be easy in this situation. Once the guide rod is placed, use Amplatz dilators to dilate the remaining non dilated tract and the reposition the Amplatz sheath in the collecting system. If the wire has also slipped out and the nephroscope is outside the parenchyma in the perirenal fat, it is important to find the hole in the renal capsule through which partial dilatation had been done. This can be identified as a site of bleeding in the parenchyma. To identify this site one may need to reduce the irrigation pressure of the nephroscope so that venous bleeding is visible. Once the site is identified, place the guide rod through the capsular hole and confirm its position on the fluoroscopy. A guide wire may be placed through the rod to make the access secure. Once this is done, the remaining dilatation could continue with Amplatz dilators. If no bleeding site is identified, for identifying the capsular hole some colored fluid will be needed. Methylene blue or betadine solution can be used. The methylene blue solution should be very dilute. Add just 1-2 drops of methylene blue in 10 cc normal saline. For betadine solution, undiluted betadine or betadine with one-in-one dilution could be used. Flush either solution through the ureteric catheter. Watch for egress of colored solution. Once identified, place a guide rod through that site and continue with the remaining dilatation. If despite colored solution, puncture site cannot be identified, attempts re-puncture and repeat

tract making. This may be difficult as the contrast may extravasate or the calyx may not fill due to leakage of contrast. Choosing an access through another calyx or sonography guided puncture may help. In a rare situation, it may be needed to stage the procedure. The puncture site seals in 48-72 h and a repeat procedure can be done after that time.

Overdilatation

Cause: Over dilatation is a state when the dilators have traversed the opposite wall of the PCS and the Amplatz sheath is now placed anterior to the kidney. Forceful dilatation is the usual reason for this problem.

Prevention: Hold the guide rod firmly and dilate 2/3 by rotation and 1/3 by force. Attempt should be to dilate till the calyx and not till the calculus. It is better to under dilate than to over dilate.

Remedy: The problem with this complication is that when the Amplatz sheath is withdrawn back to get it in the PCS, the dilated anterior wall will not have any tamponade effect and is likely to bleed briskly. Also, the irrigation fluid would leak through the hole in anterior wall. Further Nephroscopy would become difficult as the PCS may collapse due to fluid leakage. The fluid that extravasates would cause significant fluid overload. Even the stone or stone fragments can migrate outside the PCS through the hole in the anterior wall. The Amplatz sheath needs to be withdrawn gradually till the sheath is back in PCS. The further plan after this would depend on the size of perforation and the amount of bleeding. If over dilatation has resulted in a small pelvic perforation than one can get back properly in the system and quickly finish the procedure without causing much extravasation. However if there is a large perforation or significant bleeding than it is prudent to insert a nephrostomy tube, abandon the procedure and live to fight another day. Always a keep a large bore nephrostomy tube during such situations. The second procedure can be staged after 3-4 d, as the perforation usually heals within this period[103].

Loss of tract

Cause: Loss of tract means initially that the tract was well made but during Nephroscopy or lithotripsy, the Amplatz sheath has slipped out of the PCS. This happens when the glide wire slips out before the dilatation is completed. At times during the fragmentation of the calculus, the guide wire comes out and if the amplatz is not held properly then it too can come out of the collecting system.

Prevention: To prevent loss of tract, the guide wire should be adequately parked in the collecting system or passed till the bladder. Use of a safety wire is another maneuver which can prevent a complete loss of tract. Also using a super stiff guide wire instead of a slippery hydrophilic glide wire prevents complete loss of tract.

Remedy: The treatment depends on the position of the guide wire. If either the guide wire or the safety wire is well placed, it is possible to follow the wire endoscopically till the nephroscope is positioned in the PCS; the Amplatz sheath can be threaded over the nephroscope once the scope is well placed. If there is no wire, the steps are same as for under-dilatation. Look for the bleeding site or look for egress of the colored fluid. One important concern of the lost tract is that calculi or calculi fragments may extrude and can get misplaced in the perirenal fat. These calculi can be a source of persistent infection. If possible, an attempt should be made to remove all calculi. During endoscopic exploration of the perirenal space, reduce the irrigation pressure so that patient does not land in fluid overload. If despite care, the extruded calculi are misplaced in the perirenal fat, it is important to document that the radio-opaque shadows seen on post-op imaging are outside PCS. They would cause apprehension to the patient hence proper explanation to the patient is vital.

CONCLUSION

"Good results of surgery are results of good surgery". This adage is most appropriate for a percutaneous renal access, for a correct access decides to a large extent the success of PCNL. More innovations would come in the management of renal calculus and technology would make percutaneous access easier in future; but adherence to the understanding of renal anatomy would still be the base on which a percutaneous renal access would be based.

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Robotics and surgery: A sustainable relationship?

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Abstract

Robotic surgery is increasingly being employed to overcome the disadvantages associated with use of conventional techniques such as laparoscopy. However, despite significant promise, there are some clear disadvantages and robust evidence base supporting the use of robotic assistance remains lacking. In this paper, the advantages and drivers for robotics will be discussed, its drawbacks and its future role in surgery.

Key words: Robotics; Surgery; Simulation; Patient safety

Core tip: Robotic technology is increasingly being employed in surgery to overcome the disadvantages associated with use of conventional techniques such as laparoscopy. However, despite significant promise, robust evidence base supporting the use of robotic assistance remains lacking. Prospective, multicentre randomised controlled trials to evaluate efficacy, long-term outcomes, safety and cost are the next steps before widespread uptake of this technology to treat patients. Moreover, with the unprecedented need for patient safety, it is imperative that adequate training and assessment strategies are in place to bridge the gap between conventional techniques and robotic surgery without harm to patients.

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INTRODUCTION

Robotic surgery is increasingly being implemented to overcome drawbacks associated with the use of conventional techniques such as laparoscopy, especially in complex procedures. However, despite holding significant promise, robotic surgery is associated with some clear disadvantages and robust evidence base supporting robotic assistance remains lacking[1].

The introduction of minimally invasive techniques to general surgery has been described as "the most dramatic change in surgery since the introduction of anaesthesia"[2]. This has led to many procedures being performed exclusively *via* the laparoscopic approach, such as a cholecystectomy. Reasons include reduced blood loss and post-operative pain, reduced risk of infection, reduced length of hospital stay and faster return to daily activities[3]. However, these superior

results are only when the initial learning curve has been taken into account.

Laparoscopic surgery is associated with several challenges. Disadvantages and complications have been well documented[4]. Long, rigid instruments amplify tremor, reduce range of motion and degrees of freedom. This is exacerbated by the fulcrum effect whereby instrument tips move in a direction opposite to those of surgeon's hands[5]. Loss of 3-dimentional (3D) vision and having to view a 2-dimentional image, not directly under the control of surgeon, enhances these difficulties by leading to loss of traditional eye-hand target axis[6]. The laparoscopic technique is associated with poor ergonomics and health problems in surgeons such as nerve injuries[7]. Robotic systems, such as the da Vinci, have thus emerged to overcome few of these limitations.

The 3D, high-definition imaging of robotic technology facilitates stereotactic vision of the operation field and makes depth perception possible[8]. The camera is surgeon-controlled and the area of interest can be magnified up to 10 times. The surgeon's hand movements can be scaled (5:1, 3:1, or 1:1) so that large hand movements are translated into smaller movements inside the patient[9]. Combined with tremor abolition, this facilitates precise surgical manoeuvres. Endowrist instrumentation provides 7 degrees of freedom and improves range of motion, enhancing dexterity, comparable to that attained in open procedures[10]. The surgeon's comfort is increased by the ergonomic sitting position, reducing fatigue (both physical and cognitive) due to exhausting positions or movements often observed in conventional laparoscopy[11]. The intuitive movements in robotic surgery can potentially shorten the learning curve compared to conventional laparoscopy[12]. Thus, less experienced laparoscopic surgeons may acquire skills to conduct robotic surgeries in a relatively shorter time period compared to attaining corresponding proficiency in conventional laparoscopy. Significant progress in robotic applications has been in procedures that cannot be performed by a laparoscopic approach, *i.e.,* cardiac and endovascular surgery.

CARDIAC SURGERY

A Total Endoscopic Coronary Artery Bypass (TECAB) can now be performed using the robotic slave system, da Vinci, from the Left Internal Mammary Artery to the Left Anterior Descending artery without the need for a strenotomy[13]. Successful results have been reported by several groups as a result of reduced post-operative pain, better cosmesis and faster healing due to lack of a strenotomy incision[14]. Procedures requiring extreme precision or fine visualisation, such as coronary anastomosis are facilitated by the high magnification and tremor-free, precise microinstrumentation[15]. Greater patient satisfaction is also reported[16]. Off-pump procedures (*i.e.,* on a beating heart) avoid complications of cardiopulmonary bypass and are associated with a lower incidence of atrial fibrillation, stroke and death in the elderly[17]. Robotic surgery is also useful for mitral valve reconstruction. 3D visualisation allows good view of the ventricle needed for suturing in chordal reconstruction[18]. Greater range of motion facilitates the complex cutting and needle loading angles in the confined space of the left atrium[18].

However, there are disadvantages. Robot-assisted TECAB is a technically demanding and time-consuming procedure. It is associated with a significant learning curve[19]. Nevertheless, it represents a feasible alternative to conventional coronary artery bypass[20].

ENDOVASCULAR SURGERY

Another emerging domain of robotic surgery is that of endovascular robotics. The conventional endovascular catheters present several limitations within the vascular tree. These include their small range of shapes and sizes, difficulty in maneuvering the tip with the lack of stability[21]. Hence, interventionalists have to frequently change catheters and this presents a major risk of vessel trauma or distal embolization as a result of alteration of guidewire position[21]. This is especially critical in the aortic arch, where stroke, as a result of cerebral embolisation, may occur[21].

Riga *et al*[22] demonstrated that Endovascular Aneurysm Repair using a robotically steerable catheter system is feasible and may improve catheter maneuverability, stability and precision. Pre-shaped conventional catheters can rotate around one axis only, presenting a major drawback when fine and controlled movements are required in multiple planes[22]. Conversely, a steerable multidirectional catheter may overcome this hurdle and may be especially useful with regards to anatomically difficult cannulation in fenestrated stent-grafting[23]. This system also minimises operator radiation exposure, as the workstation is located outside the endovascular suite and away from the radiation source. Robotic endovascular catheters may lead to improved accuracy, reduce time and minimise radiation exposure in complex vascular procedures in particular[23]. Moreover, robotic endovascular catheters have been demonstrated to lead to a statistically significant faster skill acquisition in novice surgeons[24]. Hence, there is a potential to shorten the learning curve so that trainees can attempt more complex endovascular procedures earlier and with a greater degree of safety[24]. Yet, transferability of these findings to the operating room (OR) is debatable.

DRAWBACKS AND THE FUTURE

Despite the numerous advantages, robotics in surgery has drawbacks that hinder the widespread

Table 1 Drawbacks associated with robotic surgery

Drawback	Discussion
Cost	The da Vinci system costs approximately $1.5 million with maintenance fees of about $150000 per year[43,44]. Likewise, robotic endovascular catheter systems are expensive, have high maintenance costs, with the additional cost of disposable catheters. However, there is no conclusive data regarding the cost-effectiveness of these robotic systems. Moreover, an economic model, with quality of life adjustment, has not been performed for any of the robotic systems[44]
Evidence	Currently, the evidence for robotic surgery's efficacy and safety is largely from retrospective studies often with small sample sizes or from an institution's initial cases/experiences, where the surgeon may be at the start of his/her learning curve[44]. Hence, conclusions about safety and efficacy must be interpreted with caution
Preparation, floor space and emergencies	The Theatre team must also be trained with the device set-up including troubleshooting problems that may arise during operations. Hence, the robotic surgery venture is likely a time, cost and resource-intensive process[45]. Moreover, considerable floor space is needed, with bulky instruments; this may be problematic and considerable cost may be incurred for renovations before robotic surgery can be employed. Furthermore, in an emergency, there may be a delay in converting to an open procedure since the bulky instruments cannot be as easily removed as in conventional laparoscopy[44]
Unproven efficacy	Current evidence base for efficacy of robotic surgery is mainly from small, retrospective studies. Prospective, multicentre randomized clinical trials to evaluate safety, efficacy, long term outcomes and cost analysis are required to prove that robotic assistance is indeed superior to conventional techniques before its widespread use

Table 2 Definitions of validity and reliability

Type	Definition
Face Validity	Extent to which the simulator resembles real life scenarios
Content Validity	Extent to which the domain that is being measured is being measured by the simulator/assessment tool
Construct Validity	Extent to which a simulator measures the trait it purports to measure
Concurrent Validity	Extent to which the results of the assessment tool correlate with the gold standard for that domain
Predictive Validity	Ability of the simulator to predict future performance
Test-Retest Reliability	Measure of a test to generate similar results when applied at two different points
Inter-Rater Reliability	Measure of agreement between two or more observers when rating an individual's performance

implementation of its usage (Table 1). In particular, the evidence base supporting robotic assistance remains lacking[1]. This extends beyond the examples provided above. A robotic prostatectomy is now the standard of care in many centres; despite only one RCT and substantial publication and selection bias, the results have showed no significant improvement in patient morbidity compared with conventional laparoscopy[25]. Likewise, a Cochrane review showed no differences in safety and efficacy for benign gynaecological robotic surgery compared to conventional laparoscopy[26].

Results from high quality, prospective, multi-centre randomized clinical trials (RCTs) are urgently required to evaluate the true efficacy of robotic surgery. Enhanced patient care may justify any higher costs. For surgeons uncomfortable with advanced conventional techniques, robotic surgery may reduce the time for them to reach procedure proficiency. For experienced surgeons, robotic surgery may enhance precision and decrease physical and mental workload.

With an unprecedented need for patient safety[27], it is imperative that adequate training and assessment strategies are in place to bridge the gap between conventional techniques and robotic surgery without harm to patients. This is especially important now with reduced working hours and training opportunities following calmanisation and introduction of the European Working Time Directive[28]. Possible avenues include: (1) Virtual Reality (VR) simulation; (2) Use of dual consoles; and (3) Training courses.

VR SIMULATION

VR simulation has been well established for conventional laparoscopy and has shown to improve skill transfer to the operating room[29,30]. However, its effectiveness in robotic surgery is less clear[31]. Before a simulator is used, it must fulfil a criterion with regards to validity and reliability (Table 2)[31]. Indeed, a study by Hung *et al*[32,33] showed that the da Vinci Skills Simulator demonstrated content, face and construct validity. The performance of the expert group was superior to intermediate/novice group when evaluating parameters such as overall score, motion economy and time to completion[32]. Specific proficiency-based curricula need to be developed in order to provide structured training with in built measures of assessment. However, while VR simulation for Robotic Surgical Training is a promising tool, data on skills transfer to the operating room is still lacking and further work is required before we can draw any firm conclusions about its efficacy in training. Another promising strategy is use of a dual console.

DUAL CONSOLE

The dual console allows collaboration between the trainee and an experienced mentor[31]. There are two

collaborative modes: (1) "Swap mode" enables the experienced surgeon and the trainee to operate in parallel and switch control of the robotic arms; this facilitates parts of the operation requiring multiple hands, for example vessel isolation[31,33]; and (2) "Nudge mode" enables trainee and mentor to share the two robotic arms which is useful during key parts of the operation whereby the mentor can guide the hands of the trainee[31,33,34]. Marengo *et al*[35] suggested that use of dual consoles might shorten the learning curve and increase trainees' confidence in performing procedures. However, the data for the efficacy of dual consoles is scarce and prospective, RCTs are required to evaluate their true efficacy in surgical training[31].

TRAINING COURSES

Training courses, using animal, inanimate or cadaveric models have shown promise[31]. Assessment parameters include time to setup and operate, complications, errors and quality as determined by the Objective Structured Assessment of Technical Skills score[34,36]. Dulan *et al*[37] have developed a proficiency-based robotic training program that demonstrates construct and content validity as well as feasibility. Further validation of such curricula should be encouraged since we know that for conventional laparoscopy, achieving proficiency ascertains whether a surgeon has the aptitude to perform a procedure; this is not related to the length of training[38]. Aggarwal *et al*[39] demonstrated that a proficiency-based curriculum for laparoscopic cholecystectomy could shorten the learning curve resulting in faster skill acquisition. Moreover, such curricula for robotic surgery may provide the opportunity to exercise deliberate practice that has been regarded as a key practice to enhance and acquire "expert performance"[40,41]. And crucially, proficiency-based curricula may allow standardisation in training and assessment[39].

Finally, future development and innovation in more advanced technology for procedures that are challenging to perform with conventional as well as current robotic technology is warranted with the ultimate aim of improving patient outcome. The new imaging-sensing-navigated, kinematically enhanced robot, a flexible-access robot with integrated multimodal and multi-scale sensing, can enable the surgeon to guide tools into regions of the body that are difficult to access with the current technology[42]. It has already shown promising results *in vivo*, with clinical translation planned in the next couple of years[42].

CONCLUSION

Like when laparoscopic surgery was introduced, establishing the role of robotic surgery will take time and to ascertain which patients are most likely to benefit from it. Prospective, multicentre randomised controlled trials to evaluate efficacy, long-term outcomes, safety and cost are the next steps before widespread uptake of this technology to treat patients.

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Endoscopic treatment of orbital tumors

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Abstract

Different orbital and transcranial approaches are performed in order to manage orbital tumors, depending on the location and size of the lesion within the orbit. These approaches provide a satisfactory view of the superior and lateral aspects of the orbit and the optic canal but involve risks associated with their invasiveness because they require significant displacement of orbital structures. In addition, external approaches to intraconal lesions may also require deinsertion of extraocular muscles, with subsequent impact on extraocular mobility. Recently, minimally invasive techniques have been proposed as valid alternative to external approaches for selected orbital lesions. Among them, transnasal endoscopic approaches, "pure" or combined with external approaches, have been reported, especially for intraconal lesions located inferiorly and medially to the optic nerve. The avoidance of muscle detachment and the shortness of the surgical intraorbital trajectory makes endoscopic approach less invasive, thus minimizing tissue damage. Endoscopic surgery decreases the recovery time and improves the cosmetic outcome not requiring skin incisions. The purpose of this study is to review and discuss the current surgical techniques for orbital tumors removal, focusing on endoscopic approaches to the orbit and outlining the key anatomic principles to follow for safe tumor resection.

Key words: Orbit; Orbital tumor; Endoscopy; Surgery; Approach

Core tip: Recently, minimally invasive techniques have been proposed as valid alternative to external orbital and transcranial approaches for selected orbital lesions. Among them, transnasal endoscopic approaches, "pure" or combined with external approaches, have been reported, especially for intraconal lesions located inferiorly and medially to the optic nerve. Herein we review and discuss the current surgical techniques for orbital tumors removal, focusing on endoscopic approaches to the orbit and outlining the key anatomic principles to follow for safe tumor resection.

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INTRODUCTION

Orbital tumors encompass a broad spectrum of benign and malignant lesions intrinsic to the orbit, like cavernous hemangiomas, schwannomas, hemangiopericytomas, and tumors starting from the skin, sinuses, nose, cranial bones and cerebral parenchima with secondary orbital invasion. Cavernous hemangiomas are the most frequent intraorbital primary tumors in adults, representing 4% of all orbital tumors and 9%-13% of all intracranial cavernous hemangiomas[1].

Exact location within the orbital cavity and size of tumors are crucial elements involved in the surgical planning.

External surgical approaches to the orbit have already extensively been described. Lateral orbitotomy or the transconjunctival approach are usually performed for the removal of small tumors located on the temporal compartment or on the orbital base; supraorbital approach allows the resection of lesions located dorsolaterally; transcranial approaches, like pterional approach, are indicated for large tumors even located medially to the optic nerve.

Recently, minimally invasive techniques have been proposed as valid alternative to external approaches for selected orbital lesions. Norris and Cleasby firstly described the use of the endoscope in orbital surgery in 1981[2]. Endoscopic management of orbital lesions was initially reported in 1985 by Norris *et al*[3]. Thereafter different endoscopic approaches have been described for orbital tumors removal with the aim of reducing the morbidity rate related to a more significant tissue manipulation while preserving cosmesis. Transnasal endoscopic approaches are also well established for different non-tumoral conditions like Graves' ophthalmopathy[4], medial wall fracture[5] and traumatic optic neuropathy unresponsive to steroids[6].

Several authors have reported on different transnasal endoscopic approaches for orbital tumors removal, especially for intraconal lesions located inferiorly and medially to the optic nerve. Most surgeons performed "pure" endonasal approaches; some others described combined "open" orbital and endoscopic surgery[7]. Mir-Salim *et al*[8] removed an intraconal cavernous hemangioma through an endonasal transethmoidal route with the aid of microscope. The expanded endonasal approach allows the removal of all types of skull base tumors, including posterior and medial orbital lesions[9].

Very recently, direct transorbital endoscopic approaches have been described for posterior lateral orbital tumors removal[10].

The aim of the present study is to review and discuss the current surgical techniques for orbital tumors removal, focusing on endoscopic approaches to the orbit.

OPERATIVE TECHNIQUES

External approaches

Lateral orbitotomy, providing a wide exposure of the lateral orbital compartment, is universally indicated for extra- and intraconal lesions situated therein, such as pleomorphic adenomas and cavernous hemangiomas[11-15].

The transconjunctival approach is restricted to smaller basal and medial intra- and extraconal tumors, such as cavernous hemangiomas, schwannomas, hemangiopericytomas, and isolated neurofibromas[15,16]. This approach implies incision of the conjunctiva inferiorly along the corneal edge and caudal opening of the flap[17,18].

The supraorbital approach *via* eyebrow incision is indicated for lesions located superiorly to the optic nerve[19]. This approach is more suited to large extraconal lesions in which added exposure is needed[15].

The pterional approach offers excellent exposure of the posterior orbit and wide visualization of the superior orbital fissure and the anterior temporal fossa, also allowing access to the upper part of the medial orbit[19,20]. The contralateral pterional was considered suitable for lesions located medially and inferiorly to the optic nerve in the posterior intraconal space[20].

Endonasal microsurgical approach

Mir-Salim *et al*[8] described an endonasal transethmoidal approach performed with the aid of microscope in order to remove a cavernous hemangioma. They performed ethmoidectomy and then resected the lamina papyracea between the sphenoid sinus wall, skull base and ethmoid. After mobilization of the medial rectus muscle, the cavernoma was removed under microscopic control.

Endonasal endoscopic approaches

Transnasal endoscopic approach is indicated for intraconal lesions located inferiorly and medially to the optic nerve, especially cavernous hemangiomas, which can be easily manipulated with low risk of rupture thus resulting ideal for the transnasal management[21].

Usually sphenoethmoidectomy is performed by means of a 0° optic with a 18 cm rigid endoscope followed by a maxillary antrostomy to gain access to the floor of the orbit. Then with a 45° optic the bony medial part of lamina papyracea and the floor of the orbit are identified and removed. After careful dissection from the overlying bone, the periorbita is sharply opened with sickle knife and endoscopic microscissors. Then the tumor become visible and is removed after dissection from the periorbital fat.

For intraconal lesions located inferiorly and medially, the dissection corridor is between the medial and inferior rectus muscles. They are identified and

isolated with vessel loop as they insert on the globe and then retracted. Once the intraconal corridor is developed, the tumor is identified and removed with limited bipolar cautery and extensive sharp dissection.

Combined approaches

Campbell *et al*[7] described a combined transcaruncular and a transnasal endoscopic cryo-assisted approach to remove a cavernous hemangioma. The Cryo-probe allowed freezing both at the tumor capsule surface and within the stroma, thus facilitating the endoscopic removal of the fluid-filled lesion.

Tsirbas *et al*[22] performed, through an inferior transconjunctival orbitotomy, an orbital floor dissection subperiosteal to the posterior orbit to identify the anterior limit of the cavernous hemangioma. Then, by means of a transantral endoscopic approach, they removed the posterior orbital floor and gained the posterior orbital periosteum overlying the lesion, which was incised.

Transorbital endoscopic approach

A transconjunctival transorbital endoscopic approach was recently described by Rivkin *et al*[10] for the resection of a pleomorphic adenoma located in the posterior lateral orbit. The authors performed a lateral conjunctival incision, posterior to the lateral rectus muscle. No bone removal, craniotomy, or skin incision were required. Then, under the vision of a 0° endoscope, they made an incision in the periosteum and the tumor was delivered into the periosteal pocket.

The endoscope is a useful adjunct also for the treatment of selected orbital roof lesions, such as cholesterol granulomas, orbital dermoids and Langerhans cell histiocytosis involving the anterior portion of the orbital roof. In these cases bone removal is often needed for adequate visualization behind the superior orbital rim[23].

Table 1 summarizes the hallmarks of aforementioned surgical approaches.

DISCUSSION

Posterior orbit harbors pivotal neurovascular structures like the optic nerve, the ophthalmic artery and vein, and the ocular muscles and their nerves all crowded in a very narrow cone-shaped surgical field[22]. Thus the surgical approach planning is extremely challenging.

The location of the lesion inside the orbit is the most relevant parameter to consider in choosing the surgical approach. The approach is also decided on the basis of the extension and the type of the tumor[20]. The experience of the surgeon especially in endoscopic sinus surgery has also a role in the surgical planning process.

Traditional external orbital and cranial approaches involve risks associated with their invasiveness because they implies manipulation of delicate orbital structures like extraocular muscles, which in some case need to be deinserted, with subsequent impact on extraocular mobility[9].

Moreover lateral orbitotomy and supraorbital approach carry the disadvantage of postoperative scar. At the other hand the transconjunctival approach, which implies incision of the conjunctiva inferiorly along the corneal edge, without bone removal or skin incision, carry the disadvantage of a limited view; thus it is not suitable for large lesions.

The pterional approach offers an optimal view of the posterior orbit and of the upper part of the medial orbit thus providing good control of the optic canal, the superior orbital fissure and the anterior temporal fossa[24]. Extensive tissue manipulation connected with this approach can lead to the risk of injuring the frontal lobe along with intraorbital bleeding, cerebral edema and seizure.

The endonasal microscopic approach to the retrobulbar region described by Mir-Salim *et al*[8] provides a limited view compared with the narrowness and deepness of the surgical field.

Different transnasal endoscopic approaches have been reported on for the treatment of orbital tumors, especially for intraconal lesions located inferiorly and medially to the optic nerve and the extraconal lesions adjacent to the paranasal sinuses.

A safe resection of orbital tumors through an endonasal endoscopic approach requires the respect of some key anatomic principles[9]. First, it is critical to avoid crossing the optic nerve. Thus, tumors that are localized to the superior/lateral orbit are contraindicated for an endonasal approach. Second, entering through the lamina papyracea below the level of the ethmoidal foramina allows sparing of the ethmoidal arteries thus reducing the risk of retrobulbar hemorrhage and vision disturbances. Finally, the dissection should occur between muscle groups rather than through individual muscles for preservation of function.

The avoidance of muscle detachment and the shortness of the surgical intraorbital trajectory makes endoscopic approach less invasive, thus minimizing tissue damage[25,26]. Endoscopic approach carries also the advantage to decrease the recovery time and to improve the cosmetic outcome not requiring skin incisions[26].

Orbital surgery carries the risk of damaging the intraorbital structures because of a local increase of intraorbital pressure. In transnasal procedures, the removal of the lamina papyracea allows partial displacement of orbital content, otherwise collapsed in a not distensible space[25].

The endoscopic surgery also carries some risks and distinct disadvantages. The first one is the lack of three-dimensional vision. However, moving the endoscope actively, thereby providing some sense of depth, can simulate a three-dimensional perception.

Table 1 Hallmarks of the surgical approaches to the orbit

Approach		Ref.	Location	Size	Contraindication	Advantages	Disadvantages
Lateral orbitotomy		Arai *et al*[14] Carta *et al*[11]	Lateral, dorsal and basal to the ON	All	Medial location	Good view	Cosmetic scar
Transconjunctival		Cheng *et al*[16]	Basal and medial intra-extraconal tumors	Small	Medium size and large tumors	Minimally invasive	Limited view
Supraorbital		Maus *et al*[19]	Superior, lateral and medial	All	Basal location	Good view	Cosmetic scar
Pterional		Schick *et al*[24]	Superior and medial	All	Basal location	Good view	Invasive
Contralateral pterional		Hassler *et al*[20]	Superior and medial	All	Basal location	Good view	Invasive
Endonasal microsurgical		Mir-Salim *et al*[8]	Intraconal lesions	All	Lateral location	Three-dimensional view	Long approach distance and limited view
Endonasal endoscopic		Castelnuovo *et al*[25]	Inferior and medial to the ON, paranasal sinuses	Medium	Lateral location	Minimally invasive, better cosmetic outcome, short recovery time	Two visual dimensions, Small operative field
Combined	Transcaruncular and transnasal endoscopic cryo-assisted	Campbell *et al*[7]	Orbital apex	All	Solid consistency	To ablate vascular tumors	Cosmetic scar
	Inferior transconjunct, orbitotomy and transantral endoscopic	Tsirbas *et al*[22]	Posterior orbit, orbital apex	All	Medial location	Improved visualization and limited manipulation within the orbit	Cosmetic scar
Transorbital endoscopic		Rivkin *et al*[10]	Posterior lateral	All	Medial location	Decreased surgical morbidity, improved cosmesis	Two-dimensional view, learning curve

ON: Optic nerve.

Furthermore, the endoscopic sinus surgery allows a limited degree of space for instruments. Considering that the hemostasis could be difficult and risky in a small operative field, the endoscopic transnasal approach should be restricted mostly to benign tumors or inflammatory processes and not be used for highly vascularized tumors[27].

Muscatello *et al*[21] noted that the consistency of cavernous hemangioma is ideal for the transnasal approach, because these lesion maintain their shape and can be easily manipulated without excessive risk of rupture. Moreover, unlike cerebral ones, which are not encapsulated, orbital cavernous hemangiomas are well encapsulated, probably by a specific reaction of the orbital fatty tissue[28] and the extracapsular dissection can be easily performed[23]. Furthermore, they consist of endothelium-lined dilated spaces with a low blood flow; this condition allows the surgeon to manipulate and to remove the lesion piecemeal when this procedure is necessary.

While endoscopic endonasal surgery is limited to intraconal lesions located inferiorly and medially to the optic nerve and to extraconal ones adjacent to the paranasal sinuses, tumors located in the lateral portion of the orbit can alternatively be managed using a transconjunctival transorbital endoscopic approach, as recently described by Rivkin *et al*[10]. Comparing with external approaches, this technique proved to share the same advantages of the endonasal corridor such as decreased morbidity and post-operative pain, reduced hospitalization and improved cosmesis. Similarly, the technique has the limit of a two-dimensional view and the learning curve that may be required for surgeons less familiar with this surgery. Larger tumors or masses with bone involvement may still require standard open approaches.

CONCLUSION

Endoscopic endonasal approach allows a useful and safe route to reach and manage orbital lesions located medially to the optic nerve. More traditional surgical approaches are still widely preferred but imply major surgical morbidity and invasiveness, so they can be avoided whenever an endoscopic endonasal approach should be performed. A multidisciplinary team with expertise in endoscopic techniques is mandatory.

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MINIREVIEWS

Clinical and diagnostic aspects of gluten related disorders

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Author contributions: Masi C analyzed the literature about non-celiac-gluten sensitivity and gluten ataxia revising the other sections; Guidetti E gathered the data about celiac disease revising the other sections; Negrini G analyzed trends in gluten related disorders in general with particular detail to non-celiac gluten sensitivity and revised celiac disease section; Paterini P analyzed current laboratory techniques used for the differential diagnosis of gluten-related diseases revising all of the sections; Tovoli F wrote the draft as a whole armonizing the various sections and updating bibliography; Bolondi L revised the final draft; all of the authors have seen and approve the final version of this paper.

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Abstract

Gluten is one of the most abundant and widely distributed components of food in many areas. It can be included in wheat, barley, rye, and grains such as oats, barley, spelt, kamut, and triticale. Gluten-containing grains are widely consumed; in particular, wheat is one of the world's primary sources of food, providing up to 50% of the caloric intake in both industrialized and developing countries. Until two decades ago, celiac disease (CD) and other gluten-related disorders were believed to be exceedingly rare outside of Europe and were relatively ignored by health professionals and the global media. In recent years, however, the discovery of important diagnostic and pathogenic milestones led CD from obscurity to global prominence. In addition, interestingly, people feeding themselves with gluten-free products greatly outnumber patients affected by CD, fuelling a global consumption of gluten-free foods with approximately $2.5 billion in United States sales each year. The acknowledgment of other medical conditions related to gluten that has arisen as health problems, providing a wide spectrum of gluten-related disorders. In February 2011, a new nomenclature for gluten-related disorders was created at a consensus conference in London. In this review, we analyse innovations in the field of research that emerged after the creation of the new classification, with particular attention to the new European Society for Paediatric Gastroenterology, Hepatology and Nutrition guidelines for CD and the most recent research about non-celiac gluten sensitivity.

Key words: Celiac disease; Wheat allergy; Gluten sensitivity; Non-celiac gluten sensitivity; Gluten-free diet; Gluten; Anti-gliadin antibodies

Core tip: In recent years, there has been a widespread diffusion of gluten-associated symptoms. Current reactions to gluten include, but are not restricted to, celiac disease. This review analyses this interesting epidemiological worldwide phenomenon by discussing the spectrum of gluten-related disorders and focusing on their clinical features and diagnostic criteria. In particular, this paper will cover the most important news from European Society for Paediatric Gastroenterology,

Hepatology and Nutrition guidelines for celiac disease and the state of the art of non-celiac gluten sensitivity.

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INTRODUCTION

In recent years, the prevalence of a wide spectrum of gluten-related disorders (GRDs) has increased. This can be attributed to changes in global dietary habits; many countries are experiencing a progressive westernization of diet as well as worldwide diffusion of the Mediterranean diet, which is based on a large number of foods that incorporate gluten (including wheat)[1]. In particular, consumption of wheat is progressively replacing consumption of rice in many countries in North Africa, the Middle East, and Asia[2]. Corn is still the most consumed cereal in the United States; however, a trend toward an increase in consumption of wheat is clearly evident, with an average of $132.50 spent on wheat products per person[3].

In addition, current wheat varieties have an higher content in gluten compared to the past due to changes directed by both technology and nutritional reasons.

Types of wheat cultivated for thousands of years, such as Triticum monococcum and Triticum dicoccum, contained smaller quantities of the highly toxic peptide 33-mer gliadin[4].

The toxic effects of gluten are mediated in humans primarily by immunologic reactions; however, the absence of proper adaptation of gastrointestinal reactions can also play a role[2]. The mechanization of agriculture and the increasing use of industrial pesticides have encouraged the development of new types of wheat with a higher content of toxic peptides of gluten, constituting a further element in the increasing prevalence of GRDs[1]. Furthermore, bread and bakery products currently contain a higher portion of gluten than in the past because of the reduced time of dough fermentation[5].

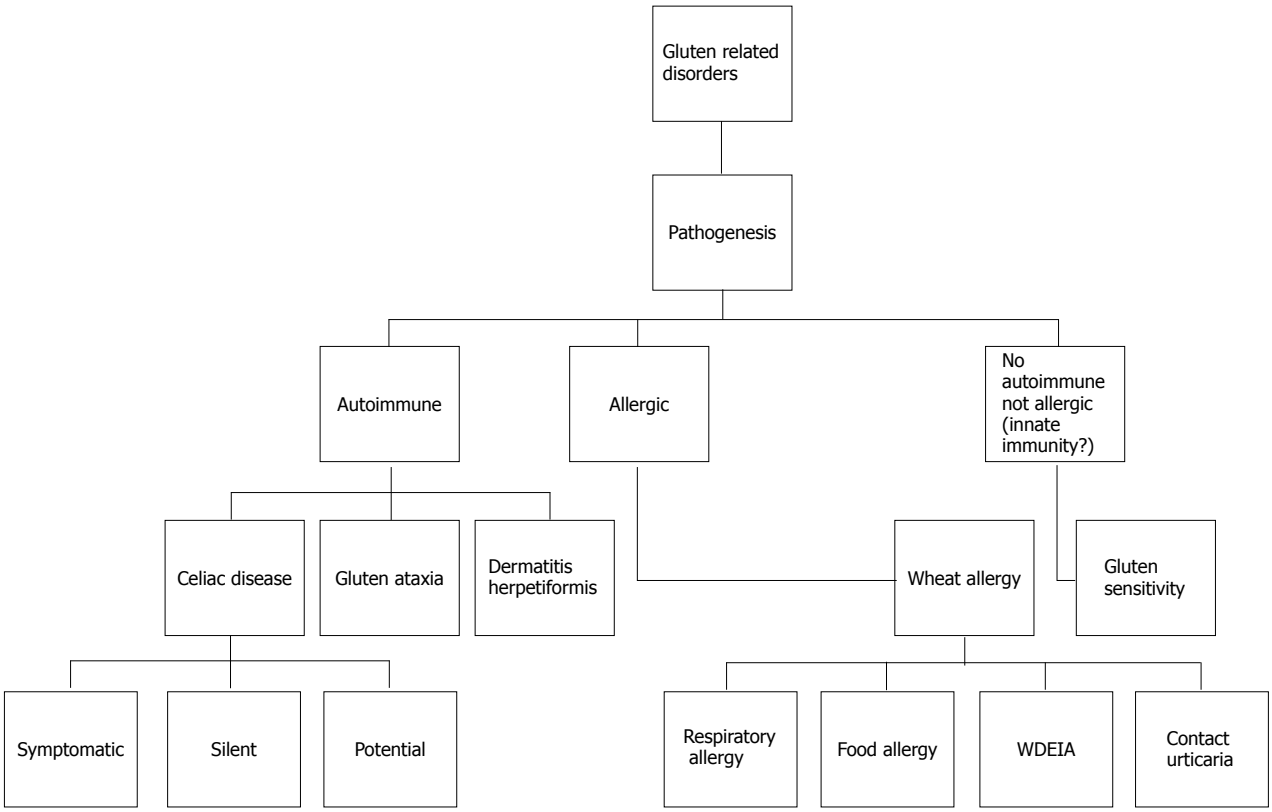
Diagnostic tools for GRDs have progressively improved over time[6,7]. In the 1980s, classification of GRDs was very simple, because celiac disease (CD) and dermatitis herpetiformis (DH) were the only known diseases with a well-documented role of gluten in their pathogenesis. More recently, gluten and other proteins have been recognized as a possible cause of wheat allergy (WA). In addition, more patients with intestinal and extraintestinal symptoms related to ingestion of gluten but without evidence of CD or WA have been identified as potentially affected by non-celiac gluten sensitivity (NCGS), a disorder aknowledged by the scientific community only in recent times[8]. Increasing complexity in the nomenclature and clinical presentation of GRDs has led to the development of a consensus document by a panel of 15 experts on new classification of five heterogeneous GRDs: CD, NCGS, WA, DH, and gluten ataxia (GA)[9] (Figure 1). Each GRD exhibits a unique pathophysiological response to ingestion of gluten, although there can show considerable overlap in the clinical presentation.

The increased prevalence and complexity of GRDs has inevitably sparked growing interest in gluten-free diets (GFDs) in both scientific and non-scientific communities. Although a GFD represents the recommended treatment of GRDs and is believed by many people to be an overall healthier regimen, this is not always the casie. People following a GFD may not meet their nutritional requirements because gluten-free foods may not have the same dietary supplementation as gluten-containing foods[10]. However, a GFD can contribute do meet daily nutritional requirements provided that patients will use a healthful balance of protein, vegetables, fruit, and ancient grains[8]. Even if gluten may not be essential for an healthy diet, an unrequired GFD can be expensive. Furthermore, recognizing gluten-free products can be difficult and time consuming[11,12].

In 2010, the gluten-free food market was worth an estimated $2.6 billion in the United States, showing a steady increase since 2008. This upward trend is expected to continue in the next years[9,13]. The growing gluten-free market may now be between 15% and 20% of the United States population[14]. The increased awareness and knowledge of CD explains only a small fraction of the development of the GFD market, which is probably sustained also by people with different GRDs, such as NCGS and WA. The remaining section of the market includes people who embark on a GFD as occasional users who do not have a medical necessity but believe that popular cereals are unhealthy because of their composition[15]. Consequently, accurate diagnostic criteria for a GFD are needed to distinguish people with a medical condition from those who simply prefer to avoid gluten, leading to different nutrition and follow-up strategies.

CD

CD is an immune-mediated reaction to gluten; it is characterized by an inappropriate T cell- mediated immune response that causes inflammatory injury to the small intestine in genetically predisposed subjects carrying the HLA-DQ2 and/or -DQ8 haplotypes[9]. CD represents a unique model of autoimmune disease because relevant information is known, including the genetic basis (HLA haplotypes) and the triggering environmental factor (gluten)[9]. The disease epidemiology is also well known, with the worldwide prevalence estimated to be 0.6%



Proposed new nomenclature and classification of gluten-related disorders.
Sapone *et al*. *BMC Medicine* 2012; 10: 13 DOI: 10.1186/1741-7015-10-12

Figure 1 New nomenclature and proposed classification of gluten related disorders according to the the II Consensus Conference on gluten related disorders held in London in February 2011.

to 1% of the general population[16-19]. For each person diagnosed with CD, there are at least another five or six people who have not yet been identified, most of whom are adults without gastrointestinal symptoms (representing the so-called celiac iceberg)[20].

CD is a diagnostic challenge for the clinician because it may develop at any age, even in elderly people, and because of its polymorphic clinical presentation. The clinical spectrum of CD includes symptomatic cases with either intestinal or extraintestinal features as well as silent forms revealed only by serological screening. Intestinal manifestations of CD include diarrhoea, weight loss, abdominal distention, and constipation. Extraintestinal symptoms reflect the systemic nature of the disease and include chronic fatigue, anaemia, reduced bone mineral density, aphthous stomatitis, high aminotransferase levels, joint/muscle pain, and spontaneous abortions, epilepsy, peripheral neuropathy[21].

In 2012, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Working Group decided to revise its classification of CD. In particular, the distinction between classic and atypical CD based on symptoms was removed, because atypical signs and symptoms (*e.g.*, anaemia, reduced bone density, neuropathy) can be substantially more common than classic symptoms (*e.g.*, abdominal pain, chronic diarrhoea)[22].

In such a complex clinical picture, case-finding strategies have to be carefully planned. The most relevant scientific organizations in this field, including ESPGHAN, North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition, and American College of Gastroenterology, have identified groups of patients at particularly high risk that should be investigated for CD. Their recommendations are reported in Table 1[22-24]. Diagnostic algorithms for CD consist of initial screening serological tests followed by a confirmatory small intestinal biopsy.

Measurement of serum immunoglobulin (Ig) A anti-tissue transglutaminase antibodies (tTG) has the best diagnostic sensitivity for CD[21,25,26]. Measurement of IgA anti-endomysial antibodies (EMA) is nearly 100% specific for CD, but it is also expensive and operator dependent and therefore is better used as a second-line test[22]. Antibodies to deamidated gliadin peptides (DGP) of the IgG class have been shown to be particularly useful in patients with IgA deficiency and children younger than three years of age[26-28]. Even with the most recent advancements in CD serology, it has been reported that up to 2% of patients with CD do not have any circulating markers of gluten sensitivity, defining a condition of seronegative CD[6].

Since the advent of the Crosby-Kugler capsule, which enabled tissue sampling and histological examination of

Table 1 Populations at risk for celiac disease, in which investigations for celiac disease are indicated, according to the most recent guidelines proposed by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition, North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and American College of Gastroenterology

ESPGHAN	NASPGHAN	ACG
Test children/adolescent with:	Test children/adolescent with:	Test patients with:
Chronic or intermittent diarrhoea, growth failure, weight loss	diarrhea and failure to thrive	chronic diarrhea with weight loss
Chronic abdominal pain, cramping or distension, nausea or vomiting, chronic constipation	abdominal pain, anorexia, constipation, vomiting	post-prandial abdominal pain, bloating
Short stature, delayed puberty	short stature, delayed puberty	other symptoms/signs suspect for CD
dermatitis herpetiformis–type rash	dermatitis herpetiformis	
unexplained abnormal liver biochemistry		unexplained abnormal liver biochemistry
Iron-deficiency anaemia	Iron-deficient anaemia resistant to oral iron	other laboratory signs suspect for CD
repetitive fractures/osteopenia/osteoporosis	osteoporosis	
chronic fatigue, ameorrhoea, recurrent aphthous stomatitis	Dental enamel hypoplasia of permanent teeth	
First- degree family members	First-degree family members	First- degree family members
Type 1-diabetes mellitus	Type 1-diabetes mellitus	Type 1-diabetes mellitus
Other associated conditions[1]	Other associated conditions[1]	

[1]Down syndrome, autoimmune thyroid disease, Turner syndrome, Williams syndrome, IgA deficiency. ESPGHAN guidelines also consider autoimmune liver diseases. CD: Celiac disease; ESPGHAN: European Society for Pediatric Gastroenterology, Hepatology, and Nutrition; NASPGHAN: North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition; ACG: American College of Gastroenterology.

intestinal tissue, small intestinal biopsy has been required to confirm the diagnosis in patients with suspected CD and is considered the gold standard for CD[20]. Interestingly, the most recent guidelines from ESPGHAN propose that it may be possible to avoid intestinal biopsy in children who meet the following criteria: (1) symptoms consistent with CD; (2) serum IgA anti-tTG levels > 10 times the upper limit of normal (confirmed with positive anti-EMA in a different blood sample); and (3) positive HLA-DQ2[22]. It remains to be determined whether this new clinical/laboratory standard functions as well as the gold standard of serology plus biopsy, whether different cutoff values for serology kits should be used, and whether including HLA typing is necessary[29]. Nevertheless, with this notable exception, biopsy is still a required and essential element for the diagnosis of CD according to all of the guidelines[22-24].

Histology alone, however, is not specific for the diagnosis of CD, particularly if villous atrophy is absent. For instance, an increase in the number of lymphocytes in the intestinal epithelium can be found in a number of different conditions, including small bowel bacterial overgrowth, drug-associated enteropathy, infectious enteritis (*e.g.*, giardiasis and Whipple disease), Crohn's disease, autoimmune enteropathy, and enteropathy associated with acquired immunodeficiency syndrome[24]. Genetic studies have identified HLA-DQ2 and -DQ8 as the major determinants of susceptibility to CD. Because these haplotypes are common in the general population, determination of HLA is better suited for ruling out the presence of CD in suspicious cases[22].

In light of the aforementioned complexities, the final diagnosis of CD must be based on a comprehensive evaluation of clinical, serological, histological, and, when indicated, genetic elements. This consideration is known as the "4 out of 5 rule".

As such, four of these five criteria should be satisfied for diagnosing CD: (1) presence of symptoms associated with CD; (2) presence of CD-associated autoantibodies (*i.e.*, tTG, IgA EMA); (3) presence of HLA-DQ2 or -DQ8 alleles; (4) duodenal biopsy demonstrating blunting or absence of villi (Marsh Ⅲ) with > 25 lymphocytes/100 enterocytes (with cluster of differentiation 3+ staining); and (5) melioration of symptoms after a GFD[30].

As we improve our understanding of the pathogenesis of CD, the interplay of genetic, epigenetic, and environmental factors may need to be considered as part of the diagnostic process[29]. In this regard, in recent years, more studies have investigated possible novel biomarkers of CD. Intriguing studies have identified $CD4^+$ gluten-DQ2 tetramers in the peripheral blood of patients with CD after a short gluten challenge[31]. These cells were not present in controls or in patients with CD while on a GFD. More recently, Galatola *et al*[32] reported that a small gene expression panel from peripheral blood monocytes could discriminate between patients with active CD and healthy controls. The intestinal microbiome is another novel field of interest in CD, potentially leading to further understanding of its pathogenic mechanism and to the discovery of new markers of disease. An overall lack of Bifidobacteria and high abundance of Firmicutes were found in children with genetic susceptibility for CD who had early exposure to gluten in a study by Sellitto *et al*[33], which examined a small cohort of at-risk infants and controls up to 24 mo of age. The investigators suggest that there may be predictive ability in measuring such biomarkers. Viitasalo *et al*[34] found significant evidence of high levels of antibodies to ASCA (anti-Saccharomyces cerevisiae antibodies), OmpW (Bacteroides caccae TonB-linked outer membrane protein), and I2 (Pseudomonas fluorescens-associated sequence) in patients with early-stage lesions. Because antibody titres decreased after introduction of a GFD, the investigators proposed that Bacteroides and Pseudomonas species may play a part in the pathogenesis of CD. These results should encourage studies of novel biomarkers as we advance toward the possibility of a biopsy-free diagnosis of CD

because they may add further security to the diagnosis of CD.

DH

DH is a skin disease characterized by a blistering rash and pathognomonic cutaneous IgA deposits[35]. Duhring's original description also included patients with different conditions, such as erythema multiforme and pemphigus.

Differently from other GRDs, the prevalence of DH is higher in men than in women (1.5 to 1.9:1).

DH shares an high prevalence of HLA alleles DQ2 (90%) and DQ8 (5%) with CD[36]. Even if a skin rash or other dermatologic manifestations can be common in untreated CD[37], DH presents with unique characteristics. The usual clinical presentation of DH consists of diffuse, symmetrical, grouped polymorphic lesions consisting of erythema, urticarial plaques, papules, herpetiform vesiculae, and blisters followed by erosions, excoriations, and hyperpigmentation[38,39]. DH most frequently involves the extensor surfaces of the elbows (90%), knees (30%), shoulders, sacral region, buttocks, and face. Itching of variable intensity, scratching, and burning sensation immediately preceding the development of lesions are common.

Gastrointestinal symptoms in patients with DH are uncommon (affecting approximately 10% of patients) and usually mild[38,39]. However, 65% to 75% of patients show a celiac-type intestinal atrophy[9]. Even in patients with apparently normal biopsy specimens, an increased number of intraepithelial lymphocytes can be found consistently with a gluten sensitization[9]. Although often asymptomatic in adults, small bowel involvement in patients with DH can be associated with abdominal pain, diarrhoea, iron deficiency, and reduced growth rates in children[38,39].

Celiac-type serological markers (anti-tTG, anti-EMA, anti-DGP antibodies) can be typically found in DH patients. In a recent study, Borroni *et al*[40] identified IgA anti-epidermal transglutaminase autoantibodies as a promising marker for the serological diagnosis of DH[40].

The revelation of IgA by immunofluorescence staining on biopsy specimens of uninvolved skin analysed is another key diagnostic element[41]. Differently from other autoimmune disorders such as pemphigus, which can display homogeneous linear IgA deposits, IgA are detected as granular or fibrillar deposits in the dermal papillae in DH. Sometimes IgA can be found in linear granular deposits along the basement membrane as well[41].

Final diagnosis of Duhring disease can be made according to the histopathologic findings on skin specimen and presence of celiac related antibodies[41]. Once diagnosis have been formulated duodenal biopsies can be avoided, as it is commonly recognized that DH represents the skin counterpart of CD[41].

GA

GA is perhaps the most dramatic representation of neurological involvement in the setting of an immune response to gluten-containing foods. It has been defined as an otherwise unexplained sporadic ataxia with presence of serological antibodies consistent with a condition of sensitization to gluten[42]. Its predominant clinical manifestations include dysarthria, dysphonia, pyramidal signs, nystagmus and other ocular signs of cerebellar dysfunction (in up to 80% of cases), progressive ataxia of gait associated with myoclonus, palatal tremor, or opsoclonus[43]. Only very few subjects with GA experience any gastrointestinal symptoms; however, almost one-third of these patients have histological evidence for small bowel villous atrophy on biopsy specimens[9]. The precise pathogenic mechanism of GA is not clear, but a number of factors probably play different roles. Vitamin deficiency (in particular, vitamin E and vitamin B1) secondary to malabsorption in the small intestine may play a role; however, there is also evidence for a toxic and immune-mediated response to gluten[44]. Different studies suggest that some subjects with GA have both anti-gliadin antibodies and antibodies reacting with Purkinje cells. The latter marker is not present in patients with other causes of ataxia, resulting a pathognomonic feature of GA[44].

It is believed that the Purkinje cells of the cerebellum share epitopes with gliadin proteins and, as further evidence for the role of the immune system, intravenous immune globulin therapy has been reported to improve ataxia[44]. Recently, tTG6 has been identified in specimens of nervous tissue from patients with GA[45]. The molecular structure of tTG6 is similar to the tTG2 molecule involved in the development of CD and to the tTG3 molecule linked to DH[45]. Interestingly, anti-tTG6 deposits of the IgA class have also been found around brain vessels of patients with GA[45] and circulating anti-tTG6 antibodies appear to be a specific and sensitive marker for GA[46]. In light of these considerations, any subject with an history of progressive cerebellar ataxia of unknown origin should be regarded as possibly affected by GA, and tested for anti-gliadin antibodies (AGA), classical anti-tTG and possibly anti-tTG6 IgG and IgA antibodies[9]. Patients displaying positivity for any of these antibodies should be followed up for one year on a GFD. Stabilization or improvement of the ataxia associated with the negativity of antibodies should also be evaluated after the first year on a GFD[9].

WA

WA can be defined as an adverse reaction of the immune system to the the proteins contained in wheat.

In the vast field of WA a further classification can be made, distinguishing: (1) a classical form of food allergy with involvement of gastrointestinal tract,

skin and possibly respiratory tract; (2) a typical form of inhalant allergy (baker's asthma and rhinitis); (3) wheat- dependent, exercise-induced anaphylaxis (WDEIA); and (4) more rarely, a form of contact urticaria. The role of IgE-mediated reactions in the pathogenesis of baker's asthma and rhinitis has been demonstrated as early as the beginning of the 20th century[47].

Symptoms consisting with baker's asthma have been referred by 4.2% of bakery workers after a single year, increasing after 8.6% after the second working year[48]. Symptoms include rhinitis, skin itching/rash, ocular symptoms (including tearing, itching, and conjunctival injection), respiratory symptoms (including coughing, wheezing, shortness of breath, and sputum production), and "grain fever"[49-51].

Alfa-amylase inhibitors are the considered the most prominent allergen ivolved in the generation of symptoms, but different wheat proteins can play a role[52].

Alimentary WA, in its turn, is a less common disease, however in its most severe forms it can lead to serious reactions, including anaphylaxis and death[53].

In contrast to CD, symptoms of WA are typical for an IgE-mediated allergy, including itching and swelling in the mouth, nose, eyes, and throat; skin rash or swelling; wheezing in the respiratory tract; gastrointestinal symptoms such as cramps, bloating, and diarrhoea; and life-threatening anaphylaxis[54]. Differently from CD, WA does not cause permanent gastrointestinal damage[54].

WDEIA is a separate form of WA that is induced by physical exercise after ingestion of wheat. A specific type of grain protein, ω5-gliadin, acts as a trigger factor. Exercise within three hours of wheat consumption can induce an adverse reaction in susceptible people. In some cases, this can also occur when wheat is consumed directly after exercise[9,53]. Patients with WDEIA can refer different symptoms, ranging from urticaria to anaphylaxis and other severe reactions[55]. Although the mechanism of physical exercise-induced anaphylaxis is indistinguishable, an immediate-type hypersensitivity to water/salt-insoluble fraction of gluten has been considered to underlie this disease[53].

Skin prick tests and IgE assays are considered first-level studies for diagnosis of WA[9]. However, interpretation of these tests can be difficult because different confounding factors should be considered. First, a number of commercial kits for skin prick tests lack in sensitivity because they do not include allergens deriving from the insoluble gliadin fraction. Second, cross reactions with grass pollens may be present (especially in adults), resulting in lower specificity[9]. Testing prick by prick with raw material potentially overcomes these problems; nevertheless a definite diagnosis still requires an oral food challenge as a final test in a number of cases[9].

NCGS

NCGS is the "youngest" member of the GRD family and is characterized by intestinal and extraintestinal symptoms that occur after the ingestion of gluten-containing food in subjects in whom CD and WA have been ruled out. Rapid disappearance of symptoms with a GFD and recurrence a few hours/days after gluten reintroduction are also characteristic of this condition[56].

The existence of NCGS was firstly postulated in 1980 by Cooper *et al*[57], who described an evident amelioration of symptoms (bloating, diarrhoea and abdominal pain) in 6 out of 8 women after gluten withdrawal, in absence of the diagnostic criteria for CD.

After 20 years without further mention of this condition, Kaukinen *et al*[58] reported in 2000 that the majority of patients complaining gluten-related symptoms were non actually classificable as as being affected by CD or WA. Since they had a clear benefit from gluten withdrawal, their condition was called NCGS.

In the past 10 years, NCGS has received growing attention as patients have reported more severe nonspecific symptoms with intake of gluten by accident than seen with classic CD[59]. The recent Consensus Conference on GRD[9] is a clear sign of the scientific interest surrounding this clinical entity, even if little is still known about NCGS, especially when compared with current advancements in CD. For instance, reliable studies regarding the actual prevalence of this condition are still lacking. In current studies, prevalence of NCGS ranges from 0.63% in a primary care program[60] to 6% in a tertiary care centre[9].

The pathogenesis of NCGS is another hot topic. Since it was first described, a link between gluten and symptoms was suggested in subjects with NCGS[57]. Indeed, gluten itself has opioid-like activity because gluten proteins can alter the intestinal transit time in healthy volunteers and its action is reverted by naloxone[61]. Furthermore, an experimental model with transgenic mice gliadin-sensitized demonstrated increased secretion of acetylcholine from the myenteric plexus, with consequential enhancement of muscle contractility and increase in epithelial secretion. Gluten withdrawal was able to revert these abnormalities[62]. However, recent studies suggested that gluten may not be the only triggers of NCGS, with different wheat proteins likely playing relevant roles in this condition. For example, some grains and cereals (such as wheat, rye, and barley) are known to be particularly rich in fermentable oligosaccharides, disaccharides, and monosaccharides and polyols (FODMAPs). In turn, FODMAPS are known to provoke gastrointestinal symptoms in patients with irritable bowel syndrome through mechanisms involving gut microbiota, gas production, and fermentation[63]. Similarly to irritable bowel syndrome, FODMAPs can possibly play a role

in generating both intestinal and extraintestinal manifestations in subjects with NCGS. Recent studies have shown that a diet low in FODMAPs results in improved symptoms in patients with self-reported gluten intolerance, supporting the hypothesis of a major role of FODMAPs compared to gluten[64,65]. Furthermore, wheat amylase and trypsin inhibitors, a complex of proteins in innate immunity, could contribute to the origination of symptoms in NCGS[7].

Because it is unclear which component of wheat-based products is responsible for an individual's symptoms, it may be premature to assign all of the blame to gluten. From a clinical point of view, patients with NCGS may display great variability in gastrointestinal (bloating, abdominal pain, diarrhoea, nausea, aerophagia, aphthous stomatitis, constipation) and extraintestinal symptoms (lack of well-being, tiredness, headache, anxiety, foggy mind, numbness, joint or muscle pain, skin rash, anaemia, dermatitis)[66].

Interestingly, mood disorders in NCGS may recognize similar pathophysiological mechanisms to other neurological manifestations observed in gluten-related disorders such as GA, as reiterated toxic insults might an impaired immunological tolerance (*i.e.*, the so-called "toxicant induced loss of tolerance")[67].

Some papers also suggested a relationship between NCGS and neuropsychiatric diseases, with a particular regard to autism and schizophrenia, however the responsibility of gluten in conditions affecting the nervous system remains hot topic requiring additional studies[68].

Other important clinical aspects of NCGS are its frequent occurrence in first-degree relatives of patients with CD and a straightforward prevalence in female subjects (6:1)[66]. Heterogeneity in clinical presentation with identification of various subgroups of patients has been noticed by some investigators[65,69], possibly reflecting different pathogenic roles for the various proteins and carbohydrates contained in wheat and other gluten-rich cereals. Consequently, it has been speculated that NCGS only provides the best current description of a heterogeneous group of conditions with the common feature of improvement in symptoms on withdrawal of gluten[70]. At this time, it appears that NCGS is not associated with malabsorption or nutritional deficiencies or with any increased risk of autoimmune disorders or intestinal malignancy[24]. Therefore, differently from subjects affected by CD, patients with NCGS should not fear contaminations due to inadvertently introduced traces of gluten.

As noted in the preceding text, before diagnosing NCGS other conditions such as WA or CD should be excluded with appropriate tests during a gluten-containing diet.

WA should be excluded by testing for serum IgE antibodies to gluten and wheat fractions and by skin prick tests, while CD must be ruled out by the negativity of celiac-specific antibodies, *i.e.*, IgA tTG, IgA EMA, and IgG DGP. A duodenal biopsy is also highly recommended because of the possibility of seronegative CD, occuring in 1%-2% of all patients with CD[6].

HLA-DQ2 and -DQ8 aplotypes are present in 50% of subjects with NCGS, representing a low prevalence compared with CD (95%), only slightly higher than in the general population (30%)[9]. Genetic studies investigating non-HLA regions are still lacking and, in general, the immunogenetics of NCGS are still non-existent[71].

Once identified negative criteria for the diagnosis of NCGS, the double-blind, placebo-controlled challenge (DBPCC) was proposed as a first positive diagnostic criterion. DBPCC trials have been highly as a confirmatory test for NCGS because of a possible placebo effect generated by gluten withdrawal[9]. However, a DBPCC is time-consuming and requires a close follow-up of patients and thus is currently used only in research[70].

The identification of other positive criteria and diagnostic markers is of great interest in NCGS. Recently, an elegant retrospective study by Kabbani *et al*[72] analysed 238 patients with symptoms responsive to GFD without prior diagnosis or exclusion of CD, demonstrating that patients with CD and patients with NCGS may have different clinical presentations. In particular, patients with NCGS were less likely to have malabsorptive symptoms, nutrient deficiency, and a personal history of autoimmune diseases, which is consistent with previous reports[24]. Consequently, the investigators concluded that patients who improve with a GFD but have negative findings on serology, lack of malabsorptive symptoms, and absence of risk factors for CD are likely to have NCGS and may not need to routinely undergo diagnostic endoscopy[72]. Nonetheless, these interesting findings need to be validated by future prospective studies.

In regard to serological markers, it was recently shown that 56% of patients with NCGS have circulating IgG AGA antibodies[73], which is consistent with the results from other investigators[69]. Interestingly, after starting a GFD, almost all of the patients with NCGS had normalization of AGA IgG levels, whereas these antibodies were still present in 40% of patients with CD after gluten withdrawal[74]. Strict compliance with and a good response to a GFD, with significant improvement in symptoms, were significantly related to the disappearance of AGA IgG in patients with NCGS[74]. Still, AGA IgG cannot be considered a reliable biomarker of NCGS because it can be detected in multiple different disorders, including autoimmune diseases, as well as in healthy subjects. As the cost of DNA sequencing is spectacularly reducing, it could be interesting for the clinicians to characterize the different subgroups of patients with this condition[71]. Consequently, further research on biomarkers of NCGS

is strongly encouraged.

CONCLUSION

Based on the data presented in this report, it is clear that clinical manifestations of GRDs cover a wide variety of medical specialties, ranging from gastroenterology to allergology and from neurology to dermatology. Within the large family of GRDs, two opposite trends seem evident. First, we have a great deal of information about the clinical presentation, pathogenesis, and diagnostic markers of some diseases (with CD as a prototypic example). Second, there are some more recently accepted conditions, such as NCGS, with many clinical and diagnostic aspects still to be investigated. Consequently, different priorities are required for different situations. In regard to CD, identification of new early microbial or non-microbial markers represents a promising field of interest, eventually leading to an early and non-invasive diagnosis in the future. In the case of NCGS, first we have to understand whether it constitutes a singular entity or only provides the best description of a heterogeneous group of conditions attributable to different wheat-related food constituents. Only studies with more homogeneous groups of patients will be able to provide relevant information on this apparently frequent but still elusive condition.

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MINIREVIEWS

Asthma and metabolic syndrome: Current knowledge and future perspectives

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Abstract

Asthma and obesity are epidemiologically linked; however, similar relationships are also observed with other markers of the metabolic syndrome, such as insulin resistance and dyslipidemia, which cannot be accounted for by increased body mass alone. Obesity appears to be a predisposing factor for the asthma onset, both in adults and in children. In addition, obesity could make asthma more difficult to control and to treat. Although obesity may predispose to increased Th2 inflammation or tendency to atopy, other mechanisms need to be considered, such as those mediated by hyperglycaemia, hyperinsulinemia and dyslipidemia in the context of metabolic syndrome. The mechanisms underlying the association between asthma and metabolic syndrome are yet to be determined. In the past, these two conditions were believed to occur in the same individual without any pathogenetic link. However, the improvement in asthma symptoms following weight reduction indicates a causal relationship. The interplay between these two diseases is probably due to a bidirectional interaction. The purpose of this review is to describe the current knowledge about the possible link between metabolic syndrome and asthma, and explore potential application for future studies and strategic approaches.

Key words: Asthma; Metabolic syndrome; Obesity; Hyperinsulinemia; Dyslipidemia

Core tip: Asthma is a complex syndrome that encompasses multiple phenotypes. The relationship with obesity has been addressed in the past; however, the underlying mechanism of such a relationship seems to be more complex, and not explained by the body weight alone. The metabolic syndrome carries a condition of systemic inflammation that could potentially explain the influence on asthma onset and severity. This is a rather unexplored area that could potentially open new scenario in the diagnostic algorithm and in the strategic approach, with a more comprehensive assessment of the disease.

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INTRODUCTION

Asthma is among the most common chronic diseases worldwide. The disease is poorly controlled despite available therapies in a large proportion of patients[1], with long-term impairment and disability[2-4]. Among factors impairing the control of symptoms and the lack of response to treatment, obesity is to be taken into account, as stated by recent guidelines[5].

It is well recognized that obesity and asthma are epidemiologically linked[6-9]. This relationship is also observed between asthma and other markers of the metabolic syndrome, such as insulin resistance and hypertension that cannot be accounted for by increased body mass alone[9-12]. The World Health Organization has reported that obesity has dramatically increased during the last few decades. In 2009-2010, more than one-third of United States adults (35.7%) were obese[13]. In this scenario, an estimated 300000 deaths per year are directly attributable to obesity, mainly due to heart diseases, diabetes, cancer, obstructive sleep apnea syndrome (OSAS), arthritis, and psychological disturbances, leading to the concept that obesity represents a risk factor for several pathologies in different clinical conditions[14]. In this regard, overweight and obesity have been demonstrated to be associated in a dose-dependent fashion with the risk of having asthma[15], and obesity appears to be a predisposing factor for the asthma onset, both in adults and in children, as assessed by several cross-sectional studies[16]. In addition, obesity could make asthma more difficult to control and to treat; interestingly, weight-loss interventions in overweight severe asthmatic patients have shown substantial improvements in the clinical status, lung function, symptoms, and overall asthma control[8,17,18]. However, the mechanism linking obesity and asthma is still a controversial issue.

The obese-asthma phenotype is characterized by a paucity of airway inflammation. Although obesity may predispose to increased Th2 inflammation or tendency to atopy, other mechanisms that are independent of inflammatory infliltrates need to be considered, such as hyperglycaemia, hyperinsulinemia and dyslipidemia in the context of metabolic syndrome. Metabolic syndrome is defined as a syndrome that involves three of the following characteristics: dyslipidemia (high levels of apoB lipoproteins and triglycerides, and/or low high density lipoprotein cholesterol), an impaired fasting glucose metabolism, hypertension or central obesity[19-21]. Metabolic syndrome is directly involved in the increased prevalence of coronary heart disease, atherosclerotic diseases, and diabetes mellitus type 2[20-22]. Other metabolic abnormalities have been reported in patients with metabolic syndrome (chronic proinflammatory and prothrombotic states, liver disease and sleep apnea)[20-22]. In the literature, some authors consider that the aforementioned criterion is a combination of risk factors rather than a specific syndrome[23]. On the other hand, epidemiological data reveals that there is a high prevalence of metabolic syndrome in both childhood and young adulthood, and pattern seems to be related to several inflammatory diseases including asthma[22].

EPIDEMIOLOGICAL LINK BETWEEN ASTHMA AND METABOLIC SYNDROME

In obese individuals, the risk for asthma in overweight and obese individuals is increased and does not differ with gender[24,25]. In a recent report, Dandona *et al*[26] showed that in obese asthma patients, with or without type 2 diabetes, there is an increased expression of pro-inflammatory mediators. Following gastric bypass surgery and weight loss, the expression of the aforementioned mediators and plasma metabolites fall significantly suggesting that the pro-inflammatory effect of obesity can be downregulated upon adipose tissue reduction. Assad *et al*[27] recently showed that BMI predicts asthma in women more than metabolic sybndrome[28], however, Agrawal *et al*[29] suggested that calculation of parameters was conducted on entirely different scales, thereby limiting comparison of strength. In another study, Brumpton *et al*[11] evaluated the associations of metabolic syndrome with the cumulative incidence of asthma in adults in 23245 individuals after an 11 years follow up (Nord-Trøndelag Health Study 1999-2008), showing that metabolic syndrome predisposes to. In a large mendelian randomization study, Granell *et al*[30] recently found that higher BMI increases the risk of asthma in non-atopic (1.90, 95%CI: 1.19-3.03) and atopic children (1.37, 95%CI: 0.89-2.11).

PATHOPHYSIOLOGICAL MECHANISMS

Obesity-associated asthma is characterized by the presence of neutrophilic airway inflammation, increased morbidity, and resistance to corticosteroids. The mechanisms underlying the relationship between metabolic syndrome and asthma are yet to be fully understood[31]. In the past, these two conditions were believed to occur in the same individual without any pathogenetic link. However, the improvement ~~of~~ in asthma symptoms following weight reduction implies a causal relationship between obesity and asthma[32,33]. The interplay between these two diseases could be based on a bidirectional interaction. For example, obese asthmatics are at higher risk of metabolic syndrome as opposed to obese individuals who do not suffer from asthma, suggesting that asthma *per se* can increase the risk of developing metabolic syndrome[34]. Similarly, metabolic syndrome has been demonstrated to increase the severity of asthma[35,36]. Recently, changes in the expression of pro-inflammatory mediators such as leptin, IL-6, TNF-α, C-reactive protein and adiponectin have been demonstrated in obese asthmatics[37], implying their potential role in the pathogenesis of

obesity-associated asthma. However, due to the paucity of available literature in this area, it appears difficult to draw definite conclusions until additional experimental and epidemiological data are collected.

A cross-sectional study published by Bruno *et al*[38] recently analyzed the influence of BMI on asthma control in subjects with severe forms of the disease, demonstrating that the optimal state of asthma control is lower in obese than in normal weight and in overweight severe asthmatics and the number of asthma exacerbation episodes are significantly higher in obese than in normal or overweight severe asthmatics. These results may be explained with the inflammatory cascade that the adipose tissue generates. Indeed, the obese state is characterized by the so-called low-grade systemic inflammation[38]. Subcutaneous fat is the major source of fatty acids for the liver, and of free fatty acids in the circulating plasma[39,40]. Subcutaneous fat is related to insulin resistance and to visceral adipose tissue[39,40]. Abdominal subcutaneous fat from obese subjects has been reported to be an inflamed adipose state characterized by tissue macrophage accumulation. This pathologic tissue has been associated with impaired local vasodilatation, peripheral hyperinsulinemia, and insulin resistance[39-41]. Macrophage presence in the tissue is associated with an increase of plasma high-sensitivity C-reactive protein (hsCRP) levels and local amounts of TNF-α[39,40]. The precise mechanism of this event remains to be elucidated; however, adipokines have been proposed as important endocrine mediators since they are related to adipose tissue function and modulation. The following proteins are listed as adipokines, which are envisaged as markers of fat body mass and distribution, as well as tissue function: (1) leptin; (2) adiponectin; (3) ghrelin; (4) vaspin; (5) retinol binding protein 4; (6) apelin; (7) progranulin and MCP-1; (8) omentin; (9) resistin and chemerin; and (10) fetuin[42,43]. Adipose derived hormones may represent molecular links between asthma and inflammation. For example, adiponectin is known to exert anti-inflammatory effects, by inhibiting the eosinophil functions. Indeed, pre-treatment with adiponectin has been demonstrated to diminish the eotaxin-mediated chemotactic responses, by binding the adiponectin receptors AdipoR1 and AdipoR2 that are expressed in human eosinophils[44,45]. In addition, adiponectin has been shown to act as a protector to human bronchial epithelial cell that are involved in the pathogenesis of asthma[46].

High serum levels of resistin have been recently documented in asthmatic children[47]. More important, an *in vitro* study showed that the resistin production strongly increases in obese patients with severe persistent asthma[48], providing support to the notion that resistin can be depicted as a pro-inflammatory cytokine mainly in severely obese asthmatics. Conversely, high leptin levels are associated with a more severe disease and this even in non-obese asthmatics[49,50]. Leptin can upregulate systemic inflammation and may lead to an impairment in lung function[51]. Increased expression and secretion of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-12 were detected when exposed to leptin[52]. Also, the systemic inflammation may contribute to drive insulin resistance, endothelial dysfunction and high blood pressure conditions. The results of a survey confirmed that leptin levels were highly associated with asthma especially in premenopausal women independent of BMI[51]. Guler *et al*[53] also suggested that serum leptin concentrations were a predictive factor for asthma in boys, even after adjusting for obesity. Previously, leptin-mediated increased bronchial hyperactivity in obese mice models had been documented[32].

The changes in the adipose tissue in metabolic syndrome favour the production of mediators that modulate the transcription factors. When they are activated by their ligands, they are able to control genes that are involved in intermediate metabolism[54]. In this regard, peroxisome proliferator-activated receptors (PPAR)-gamma agonists may attenuate the upper airway allergic inflammation by induction of T_{reg} cells and inhibiting the proliferation of effector T cells[55].

Diet-induced dyslipidemia may affect the trafficking of immune cells to the lung in diseases such as asthma[56]. In pulmonary physiology, circulating low density and high density lipoproteins (LDL and HDL) are both taken up by specific receptors, and consequently block local cholesterol biosynthesis[56]. Alveolar cholesterol homeostasis has been demonstrated to affect surfactant synthesis in normal lung physiology[56]. Conversely, HDL promotes surfactant production, and lung fibroblast growth. Adipose tissue reduction by diet or surgery, modulation of cholesterol, or glucose metabolism, has an important effect in asthmatic patients. The apolipoprotein E (ApoE)-low density lipoproteic receptor pathway appears to be involved in the pathogenesis of a murine model of allergic asthma[1]. However, the mechanism by which this protein modulates asthma pathogenesis has never been fully elucidated. ApoE has been hypothesized to ~~as~~ negatively modulate the degree of airway hyperresponsiveness[57]. Perhaps, this mechanism can also apply to humans. Low levels of serum HDL were found to be associated with an increased risk for asthma in adolescence[58], and a recent analysis on 85555 adults demonstrated that high triglycerides and low HDL were associated with wheezing, supporting their role as markers of inflammation[59]. Recently, the association between LDL and asthma was investigated by Scichilone *et al*[60], who found that in mild asthmatics, the least pro-inflammatory LDL (LDL-1 and LDL-2) are lower than in healthy subjects, whereas the most pro-inflammatory (LDL-3 and LDL-4) are higher. In addition, the serum concentrations of LDL-3 (most pro-inflammatory) were

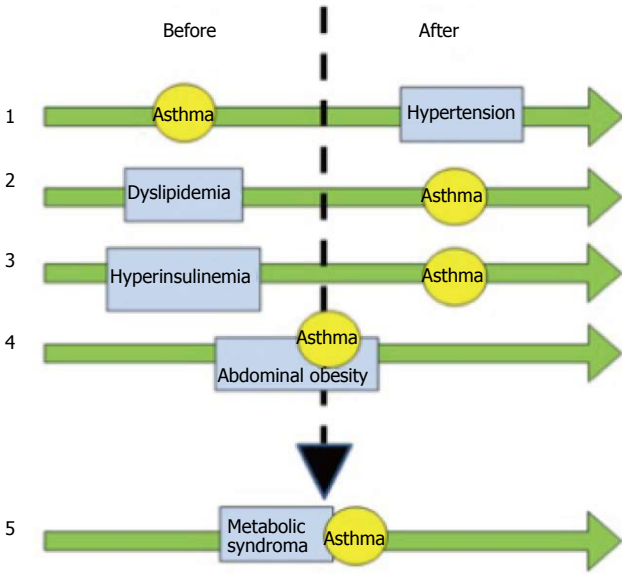

Figure 1 Relationships between asthma and features of metabolic syndrome. The different conditions are divided into "before" and "after" to explain which occurred earlier, implying a causal association. The green arrows describe the temporal evolution and the time when the diseases occurred. Arrow 1 sets asthma as a risk factor for systemic hypertension due to the chronic use of corticosteroids. Arrows 2 and 3 depict the role of dyslipidemia and hyperinsulinemia as risk factors for asthma, due to the abnormalities of the lipoprotein pattern and the influence on the M2 receptors, respectively. Arrow 4 shows the bidirectional association between asthma and obesity. Arrow 5 summarizes the influence of the above-described associations, showing the tight relationship between metabolic syndrome and asthma. Also see text for the explanation.

negatively associated with lung function, suggesting their contribution to the occurrence of the inflammatory changes of the airways[60]. Insulin excess can also directly alter lung cellular physiology and this would represent a fundamental common molecular link between asthma and metabolic syndrome[61]. There is substantial data that mechanistically links insulin and insulin like growth factor-1 to lung development and function. It is conceivable, although not proven, that hyperinsulinemia may lead to development of lung disease, particularly asthma[62]. Experimental studies that directly address this possibility are strongly advocated.

Recent observations seem to focus on the mitochondrial dysfunction as main mediator of the pathogenetic link between metabolic syndrome and asthma. Defective mitochondrial biogenesis in the adipose tissue is well documented in metabolic syndrome[63-66]. However, the involvement of mitochondria alterations among the risk factors of metabolic syndrome and asthma is unknown[67-72].

Oxidative stress on both pulmonary and extra-pulmonary inflammation in obesity may play a major role[73,74]. Oxidative stress is characterized by increased reactive oxygen species (ROS), which induce functional changes of the airways. On this basis, increased oxidative stress may be recognized as a potential mechanism by which obesity results in increased asthma severity. In this regard, the renin angiotensin aldosterone system, a potent inducer of oxidative stress, is often activated in patients with metabolic syndrome, and results in increased levels of angiotensin Ⅱ. Angiotensin Ⅱ seems to be able to determine bronchial hyperresponsiveness[75] and airway remodelling[76]; however, the mechanisms by which this occurs are not yet fully understood.

CURRENT AND FUTURE DEVELOPMENTS

Figure 1 describes the temporal and causal relationships between asthma and features of metabolic syndrome. The role of lipoproteins in the pathogenesis of asthma pathogenesis supported the use of statins in asthmatic patients[77-83]; however, there are still some controversies[84-86]. Even though the aim of statin use in asthmatic patients is related to cholesterol metabolism, most of the reports have highlighted the anti inflammatory properties[77-79,86,87]. An *in vitro* study showed that lovastatin attenuates the differentiation and proliferation of asthmatic bronchial fibroblasts[85], airway smooth muscle cells[86]. Both simvastatin and atorvastatin treatment reduce inflammatory cells in sputum[86]. The mevalonate-dependent and-independent pathways have been identified as potential opportunities for novel treatments with statins in asthma develop new treatments for asthma[78]. Even though statin therapy could be beneficial for a subgroup of asthmatic patients that are either overweight or obese, similar important advantages can be obtained by diet and exercise[20,26]. Biphosphonates could have a beneficial effect in asthmatic patients; alendronate has been shown to have a protective effect by decreasing eosinophil airway inflammation by chemokine secretion, eotaxin, and down-regulating cytokine secretion induced by Th2 and Th17 cells[88]. Retinoic acid[89], retinoids[90] and fenretidine[91] appear to have a beneficial effect on the inflammatory asthmatic response by decreasing the inflammatory milieu. As a consequence, signal transduction pathways inhibition by these compounds could decrease the occurrence of bronchial constriction. Further studies are required to ascertain the possible beneficial effect of new therapeutic elements to control hypertension and endocrine disorders in asthmatic patients with metabolic syndrome. In patients with severe uncontrolled or non responding asthma[92,93], biological therapies seem to be relevant. Interestingly, chemokines and chemokine receptors, CCR3 and CCR4, have been involved in adipose tissue mass increase and insulin resistance[94,95]. Thus, therapy involving chemokines or chemokine receptor inhibition could potentially provide beneficial effects on asthmatic patients with metabolic syndrome[96]. Finally, a therapeutic option in asthmatic patients with diabetes could be represented by thiazolidinediones, oral diabetes medications that selectively activate

PPAR receptor gamma, which have potent anti-inflammatory properties thus reducing the number of exacerbations[97,98].

CONCLUSION

The scope of the current review is to explore the possible link between metabolic syndrome and asthma. Early endocrine disturbances seem to predispose to severe or difficult to treat asthma. Hypertensive and diabetic patients should be screened for respiratory function in the effort to identify cases of airway hyperreactivity or subclinical asthma. Specifically designed studies are needed to address the influence of metabolic syndrome on asthma occurrence and severity, and to unveil the potential underlying common mechanisms. Future studies will hopefully provide convincing evidence on useful therapeutic schemes that today are still unrevealed.

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MINIREVIEWS

Voltage gated calcium channel antibody-related neurological diseases

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Abstract

Voltage gated calcium channel (VGCC) antibodies are generally associated with Lambert-Eaton myasthenic syndrome. However the presence of this antibody has been associated with paraneoplastic as well as non-paraneoplastic cerebellar degeneration. Most patients with VGCC-antibody-positivity have small cell lung cancer (SCLC). Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disease of the presynaptic part of the neuromuscular junction. Its classical clinical triad is proximal muscle weakness, areflexia and autonomic dysfunction. Fifty to sixty percent of LEMS patients have a neoplasia, usually SCLC. The co-occurrence of SCLC and LEMS causes more severe and progressive disease and shorter survival than non-paraneoplastic LEMS. Treatment includes 3,4 diaminopyridine for symptomatic purposes and immunotherapy with prednisolone, azathioprine or intravenous immunoglobulin in patients unresponsive to 3,4 diaminopyridine. Paraneoplastic cerebellar degeneration (PCD) is a syndrome characterized with severe, subacute pancerebellar dysfunction. Serum is positive for VGCC antibody in 41%-44% of patients, usually with the co-occurrence of SCLC. Clinical and electrophysiological features of LEMS are also present in 20%-40% of these patients. Unfortunately, PCD symptoms do not improve with immunotherapy. The role of VGCC antibody in the immunopathogenesis of LEMS is well known whereas its role in PCD is still unclear. All patients presenting with LEMS or PCD must be investigated for SCLC.

Key words: Voltage gated calcium channel antibody; Lambert-Eaton myasthenic syndrome; Paraneoplastic cerebellar degeneration; Onconeural antibodies; Small cell lung cancer

Core tip: Voltage gated calcium channel (VGCC) antibodies are generally associated with Lambert-Eaton myasthenic syndrome, but also with paraneoplastic or non-paraneoplastic cerebellar degeneration. The autoimmune nature of non-tumour Lambert-Eaton myasthenic syndrome is reflected in its association with various HLA subtypes and other autoimmune diseases such as vitiligo, myasthenia gravis and diabetes mellitus. The most common tumour associated with VGCC-antibody-positivity is small cell lung cancer. Knowledge on the relation between cerebellar degeneration and VGCC is limited, and treatment

response is poor in this group of patients.

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INTRODUCTION

Voltage gated calcium channels are immunologic targets for several disease. The calcium channels as a target of the pathogenic antibodies in Lambert–Eaton myasthenic syndrome (LEMS) was first suggested by Fukunaga *et al*[1] in 1983. Subsequent studies showed antibodies against P/Q type calcium channel as the most prominent in these patients[2].

Although voltage gated calcium channel (VGCC) antibodies are generally associated with LEMS, usually seen as a paraneoplastic syndrome with small cell lung cancer (SCLC), rarely non-paraneoplastic cerebellar degeneration may also occur in the presence of this antibody[3,4]. VGCC antibody positivity is observed in 85%-90% of LEMS patients whereas the ratio approaches 100% in LEMS patients with SCLC[5]. Approximately 40% of patients with subacute onset cerebellar degeneration, usually with SCLC, have VGCC antibody positivity[3,6]. Moreover these antibodies can also be detected in SCLC patients without neurological involvement[5].

VGCC

The VGCC is crucial in the depolarization of the cell membrane and cellular influx of calcium in response to action potential. It functions as a secondary messenger in electrical signalization and initiates several cellular mechanisms[7]. They are found in several cells, such as smooth and skeletal muscle fibers, endocrine cells, neurons[7]. The channel also locates on the presynaptic membrane of the axon terminal. VGCC opens by action potential and leads to the entry of calcium ions into the axon terminals. Calcium influx results in movement of acetylcholine vesicles towards the presynaptic membrane and acetylcholine is released into the synaptic cleft. In striated muscles, the VGCC on the membrane of transverse tubules directly activates ryanodine-sensitive calcium channels in the sarcoplasmic reticulum and initiates rapid contraction[7,8].

VGCC is divided into five types: L, P/Q, N, R, T depending on tissues and pharmacological properties[7]. The channel contains 4 or 5 subunits (α1, α2/δ, β and γ).The ion transition pore responsible for the biochemical and electrophysiological properties is the α1 subunit. This subunit contains six helical transmembrane segments (S1-S6) and 4 domains (Ⅰ-Ⅳ)[9] (Figure 1). Ten different α1 subunits have been defined and Cav2.1 α1 subunit is found in P/Q type VGCC[7]. Voltage sensors are located in the S4 segment. The S5 and S6 segments are sensitive to calcium[9]. Antibodies against the S5-6 segments of α1 subunit are detected in 50% of LEMS patients[5]. Other antibodies detected in LEMS patients are against domain Ⅳ and β subunit[5,10]. However, the pathogenic role of β subunit antibodies is still controversial due to its intracellular location.

Antibodies to P/Q type channels are responsible for clinical symptoms of LEMS[5]. Thirty to forty percent of the patients with antibodies to P/Q type channels also have antibodies to N-type channels whereas in 25% patients also have antibodies to L-type channels[5]. Antibodies to N and P/Q type channels are detected in 40% of patients with cerebellar ataxia associated with SCLC[9]. Sry-like high-mobility group box (SOX-1), zic-4, anti-Hu are other antibodies detected in the sera of patients with PCD and SCLC and approximately70% of the patients have one of these antibodies[6].

LEMS

LEMS is the autoimmune disease of the presynaptic nerve terminals. It is a rare disease with a prevalence of 2.3 per million and an incidence of 0.5 per million[11]. It is associated with SCLC in 50%-60% of patients. As the clinical and laboratory features differ in patients with and without SCLC, the disease is divided into two groups as LEMS with SCLC (SCLC-LEMS) and non-tumour (NT-LEMS).The age of onset is 50 years or above and there is a male predominance in patients with SCLC-LEMS. On the other hand NT-LEMS can be seen in all age groups with a peak at the age of 35 and 60 and a female predominance[12].

LEMS hardly occurs in childhood; only 5% of LEMS patients are less than 18-year-old[13]. Our youngest LEMS patient was a eight year-old female.

Pathogenesis

LEMS is a disorder due to antibodies against P/Q type VGCC. VGCC antibodies interact with extracellular S5 and S6 segments of domain Ⅱ, Ⅲ and Ⅳ of α1 subunit and reduce the number of ion channels by cross binding[5,14-16]. The antibodies can also bind to other VGCC types without causing any dysfunction. Although VGCC antibodies usually generate an immune reaction, the response to epitopes varies in LEMS patients[17]. Antigenic modulation followed by clustering and reduction of VGCC leads to reduction in quantal release of acetylcholine in synapses and results in muscle weakness[18]. The down-regulation of the receptors of parasympathetic and sympathetic neurons that cause autonomic dysfunction is also associated with these antibodies.

VGCC antibodies can be detected by radioimmunoassay

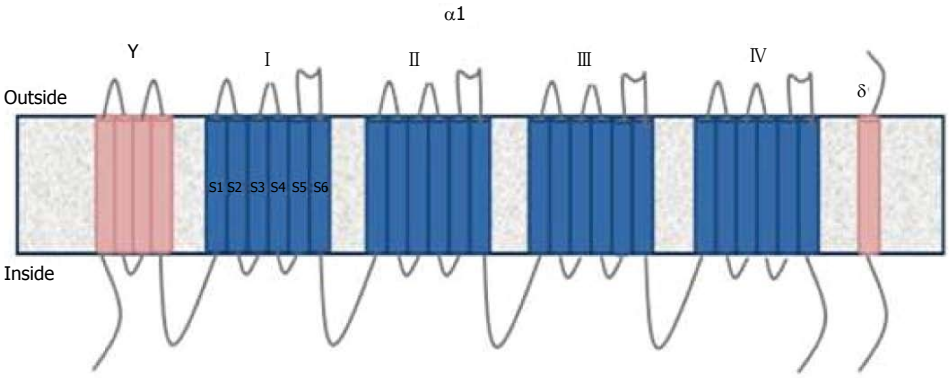

Figure 1 The structure of Voltage gated calcium channels.

in 85%-90% of LEMS patients and 100% of patients with SCLC-LEMS[17,19,20]. P/Q type VGCC are expressed on SCLC cells and this expression results in production of antibodies and cross-reaction with presynaptic VGCC[21]. As the immune reaction is initiated early in tumour development, the diagnosis of LEMS frequently precedes the diagnosis of the SCLC[16]. Besides, VGCC antibodies may be detected in 3%-5% of patients with SCLC without muscle weakness or autonomic dysfunction[16].

Approximately 10%-15% of LEMS patients do not have serum antibodies. However, their serum reproduces LEMS-like symptoms when it transferred to mice. This finding suggests the presence of antibodies at very low concentrations or antibodies against different epitopes of the VGCC that are undetected by routine tests[5]. Antibodies against synaptotagmin, a synaptic vesicle protein, have been found in some seronegative and seropositive LEMS patients[14,22]. Although there is no evidence on their pathogenic role, the blocking of synaptotagmin, which is a Ca^{2+} sensor in the membrane of the pre-synaptic axon terminal may explain the muscle weakness in LEMS[23]. Another such antibody, whose pathogenic role is unclear, is against "sry-like high-mobility group box" (SOX-1) proteins. This antibody is positive in 67% of SCLC-LEMS patients, 22%-32% of patients with SCLC without LEMS and only in 5% of NT-LEMS patients[19].

The role of T lymphocytes in LEMS pathogenesis is unclear. Thymus and other lymphoid organs involved in myasthenia gravis (MG) do not present abnormalities in LEMS and the presynaptic terminals do not reveal T lymphocyte collection[16]. However regulatory T lymphocytes are down-regulated in SCLC-LEMS patients, but not in SCLC patients without LEMS, which suggests the dysfunction of regulatory T lymphocyte in SCLC-LEMS pathogenesis[24].

Unlike SCLC-LEMS patients, the trigger of the immune response is not defined in NT-LEMS.Various HLA associations, also reported with other autoimmune diseases, have been documented: Approximately two-third of patients have HLA-B8 (HLA class Ⅰ) and HLA-DR3, -DQ2 (HLA class Ⅱ) alleles[25,26]. Supporting this phenomenon, monozygous twins have been reported where one has LEMS and VGCC antibodies, and the other, myasthenia gravis and acetylcholine receptor antibodies[27]. On the other hand, HLA association was not found in SCLC-LEMS patients: because tumoral cells do not have strong HLA class Ⅰ antigen expression, these molecules are possibly not involved in the immunopathogenesis[25]. These observations resemble the difference between MG with and without thymoma and reveal the existence of different autoimmune mechanisms in paraneoplastic disorders[16].

Clinical features

The classical triad of LEMS is proximal weakness, reduced tendon reflexes and autonomic dysfunction[16].

Proximal weakness, more prominent in lower extremities, is the first symptom in 80% of patients[16,28]. In the course of the disease, 80% of patients suffer from proximal weakness in both upper and lower extremities[28,29]. Facial, bulbar and distal weakness are also frequent[16]. The weakness is more severe and rapidly progressive in SCLC-LEMS patients[16,28].

Autonomic dysfunction, observed in 80%-96% of patients, is the most common symptom although it is less disturbing than muscle weakness[12,28]. Erectile dysfunction and constipation are more frequent symptoms than urinary retention, dry eye and reduced sweating[5]. The rate and the nature of autonomic symptoms do not differ between SCLC-LEMS and NT-LEMS[16].

On neurological examination deep tendon reflexes are generally reduced or absent. However maximal isometric contraction of the muscle for 10 to 15 s may lead to the appearance of previously depressed or absent deep tendon reflexes and temporarily improve muscle strength, which is called "Post-exercise facilitation". This phenomenon, a characteristic feature of LEMS, is not sensitive[5]. This phenomenon may also mask the reduction of deep tendon reflexes in 40% of patients so the neurological examination must be repeated after a resting period to verify the diagnosis[5].

Associated diseases

The most common co-existence of LEMS is with

SCLC, reported in 50%-60% of LEMS patients[12]. Besides, 0.5%-3% of SCLC patients have LEMS[25]. This co-existence results in a more severe and rapidly progressive neurological disease with shortened survival compared to NT-LEMS which has a near normal survival[16]. Moreover SCLC-LEMS patients tend to be younger than other SCLC patients[19].

Older age, tobacco use, increased ESR support the probability of underlying SCLC. In a Dutch-English cohort study a scoring system called DELTA-P was implemented to predict SCLC in LEMS[12]. According to DELTA-P, dysarthria, dysphagia, chewing or neck weakness(D), erectile dysfunction (E), loss of weight (L), tobacco use (T), age of onset more than 50 (A) and Karnofsky performance less than 60 (P) have a predictive value: patients with a DELTA-P score three or more have higher than 94% risk of having SCLC.

The distinction of SCLC-LEMS from NT-LEMS is important, as treatment options and outcome are different. For this reason, new markers to diagnose SCLC-LEMS are still under investigation. Although SOX-1antibodies are detected in half of the SCLC-LEMS patients, the absence of a commercial kit, and the presence of these antibodies in NT-LEMS limit their use for differential diagnosis[30]. Another study revealed that VGCC antibody against domain IV positivity is more common in NT-LEMS patients than in SCLC-LEMS patients, but this is not used for clinical purposes yet[17].

The co-existence of LEMS with other malignancies such as non-small cell lung cancer, prostate carcinoma, orthymoma has been reported rarely[13] and a random association could not be excluded[5].

In children, the disease is generally non-paraneoplastic; lymphoproliferative malignancy and neuroblastoma are rare associations[13,31]. In accordance with the literature, our pediatric patient did not have any neoplasm.

Other autoimmune diseases such as thyroid disorders, alopecia, diabetes mellitus, MG can occur in NT-LEMS patients probably due to the presence of various HLA subtypes contributing to autoimmunity[32]. In our clinical experience, NT-LEMS patients may have associated vitiligo and myasthenia.

Diagnosis

The time lag between the onset of the symptoms and diagnosis is approximately 4 mo in SCLC-LEMS and 12 mo in NT-LEMS[12,29].

In patients with suspected LEMS, electrophysiological studies with repetitive nerve stimulation are among the most important diagnostic tests. The compound muscle action potential (CMAP) is low after the first stimulation and decreases further after repetitive stimulation at 2-5 Hz[33]. At least 10% reduction in CMAP after low frequency stimulation is considered abnormal and observed in 94%-98% of patients[34,35]. However, this finding may also be present in MG patients. To distinguish these two diseases, nerve stimulation at high frequency (50 Hz) or, as a less painful method for the patient, post-exercise measurement is employed, which increases CMAP by more than 100% in LEMS patients[34,35]. Optimum results will be obtained if treatment is interrupted 12 h before the study and the muscle temperature is above32 ℃.

Although single fiber EMG is a sensitive test, it is used in combination with other tests as it cannot distinguish between MG and LEMS[33]. LEMS patients have increased jitter like MG patients. In case of severe neuromuscular junction dysfunction, the conduction defect in muscle fiber causes a decrease in amplitude and duration of motor unit potential as in myopathies[36].

VGCC antibodies are detected by RIA in 85%-90% of LEMS and in almost 100% of SCLC-LEMS patients[17,19,20]. Although the presence of VGCC antibodies supports the diagnosis of LEMS, the absence of antibodies in a patient with typical clinical features does not exclude the diagnosis.

In 50% of patients SCLC-LEMS, LEMS symptoms precede the diagnosis of SCLC. In addition to scoring systems such as DELTA-P, all patients with the diagnosis of LEMS must undergo computerized tomography of the thorax and positron emission tomography (PET). If the results are negative, the screening must be repeated every 3-6 mo until the second year of the disease[37].

The differential diagnosis of LEMS from seronegative and atypical myasthenia gravis can be challenging. Some clinical findings may be helpful: the progression of weakness is in the craniocaudal direction in MG and the reverse in LEMS; ptosis and facial weakness are less common and severe in LEMS. Electrophysiological studies described above and serological findings assist the clinician in the differential diagnosis.

LEMS with a subacute course can be misdiagnosed as Guillain Barré syndrome (GBS); the presence of sensorial symptoms, neuropathic pain, and elevated CSF protein favor the diagnosis of GBS. Amyotrophic lateral sclerosis may constitute another differential diagnosis, and can be distinguished by the asymmetrical weakness starting in the upper extremities and the presence of upper motor neuron signs.

Treatment

The first choice for symptomatic treatment is 3,4 diaminopyridine[38]. This molecule blocks presynaptic voltage-gated potassium channels and provides a prolonged action potential which increases the quantal release of synaptic acetylcholine[39]. All randomized controlled studies of 3,4 diaminopyridine showed improvement in muscle strength and CMAP amplitudes. The drug is well tolerated although adverse effects like perioral and digital paresthesias and gastrointestinal symptoms are not uncommon. Seizures, which is the most frequent severe side effect, have been reported at high doses exceeding 100 mg/d[40,41]. In our experience, the drug is well tolerated and improves muscle

strength at the dose of 40-60 mg/d.

Other treatments, which can increase the concentration of acetylcholine in synaptic cleft, are pyridostigmine and acetylcholine esterase inhibitors but they are not as effective as they are in MG patients[42].

In case of limited response to 3,4 diaminopyridine, immunosuppressive treatments must be considered. The combination of prednisolone and azathioprine is well studied and documented in LEMS patients[38,42]. Although there is not sufficient data, mycophenolate mofetil, cyclosporine and rituximab are also drugs employed in LEMS treatment[16,43]. Intravenous immunoglobulin (IVIg), another treatment option in paraneoplastic syndromes and MG, can also be used in LEMS. European Federation of Neurological Societies (EFNS) guidelines recommend IVIg in both SCLC-LEMS and NT-LEMS[44]. IVIg is also recommended in pregnant patients, as transplacental transmission of IgG antibodies may cause neonatal LEMS[45]. IVIg is generally preferred as its side effects are rare and it is easily used for the maintenance treatment, which is usually needed in LEMS patients. Plasma exchange whose effect is comparable to IVIg may carry technical difficulties and slightly higher rate of complications[46].

In patients with SCLC, the treatment of the tumour is crucial. The survival of SCLC-LEMS patients is better than in patients with SCLC alone, but there is no relation with VGCC or SOX-1 antibody positivity and survival[17,19]. The better prognosis in these patients may be correlated with the diagnosis time that is earlier in LEMS patients[12,29]. Moreover HLA-B8 positivity is related to prolonged survival in SCLC-LEMS patients[47].

Maintenance of optimal body weight, rehabilitation, frequent examinations for complications such as respiratory infections, and avoidance of drugs impairing neuromuscular transmission are other important aspects of the treatment.

CEREBELLAR DEGENERATION ASSOCIATED WITH VGCC ANTIBODY

Paraneoplastic cerebellar degeneration (PCD) is a syndrome characterized by subacute cerebellar dysfunction[48]. Clinical and pathological features of the syndrome were described by Brain and Wilkinson[49] in 1965 by the evaluation of 13 patients and 6 autopsy cases. Diffuse loss of Purkinje cells is the pathologic hallmark of the disease and usually accompanied by thinning of granular and molecular layers, degeneration of long tracts of spinal cord, dentate and olivary nuclei[4].

Most common neoplasms associated with cerebellar degeneration are lung, breast, ovarian cancers and Hodgkin lymphoma[48]. Onconeural antibodies such as anti-Hu, anti Yo, anti-Ri, anti-CV2, anti-Tr, anti-Ma, anti-Ta, anti zic 4, and anti-mGluR1 as well as VGCC antibody can be detected in PCD[50]. Clinical presentation, neuropathological findings and treatment responses of patients vary according to the type of the onconeural antibody, suggesting distinct immune mechanisms related to different antibodies[4].

PATHOGENESIS

Antibodies against VGCC of the P/Q type or N type are found in 41%-44% of PCD patients, generally associated with SCLC[3,6,9]. The P/Q type VGCC is highly expressed in cerebellar Purkinje cells and in the molecular layer of the cerebellum[9,51]. About 20%-40% of these patients also have clinical or electrophysiological diagnosis of LEMS[3,6]. Neuropathological findings of PCD with LEMS (PCD-LEMS) were reported in 1973 by Satoyoshi *et al*[51] for the first time. In a postmortem study of three PCD-LEMS patients with VGCC antibodies, 70%-80% of reduction in P/Q type VGCC of the molecular layer; loss of Purkinje cells and gliosis in the cerebellar cortex were observed[9,51]. The role of VGCC antibodies in the pathogenesis of PCD is still unclear. In a recent experimental study, antibodies of the IgG type purified from the serum of two VGCC antibody-positive patients with SCLC, one with PCD-LEMS and another patient with isolated LEMS were given to mice intrathecally, the antibodies associated with PCD-LEMS but not from isolated LEMS patients caused cerebellar ataxia in mice[52]. This finding suggests the presence of different epitopes of P/Q type VGCC antibodies which inhibit VGCC's function in cerebellum, or of other additional, yet undiscovered pathogenic antibodies[52].

CLINICAL, LABORATORY AND RADIOLOGICAL FEATURES

Subacute and rapidly progressive gait unsteadiness is the presenting symptom of cerebellar degeneration[50]. Gait and limb ataxia, diplopia, dysarthria are the other prominent symptoms[50]. Sometimes these complaints may preceded by dizziness, nausea and viral infection-like symptoms[50]. Occasionally other signs and symptoms such as dysphagia, nystagmus and sensory deficits can be also seen during the course. Patients who had concomitant LEMS may also show proximal weakness and autonomic symptoms in addition to cerebellar symptoms[4].

The cerebrospinal fluid (CSF) may show mild lymphocytic pleocytosis with elevated protein and oligoclonal bands[48]. VGCC antibodies may also be detected in CSF and there is some evidence of intrathecalsynthesis of the antibodies, and detected in about 25% of the patients[3]. This low percentage may be explained by the absence of CSF analysis in some cases and further studies are needed to increase the rate of antibody presence in CSF.

Initial brain magnetic resonance images or tomography are normal in most patients[4] although in early stages of the disease fluorodeoxyglucose-PET scans may show cerebellar hypermetabolism[50,53], whereas cerebellar

atrophy and cerebellar hypometabolism are seen in the advanced stage of the disease[50].

TREATMENT

Treatment of the underlying malignancy has the priority like other paraneoplastic syndromes[50]. Corticosteroids, plasma exchange, IVIG, tacrolimus and cyclophosphamide are the immunotherapeutic options to be used concurrently with tumour therapy, but most of the cases did not show sufficient improvement despite treatment[50]. Unlike in LEMS, immunotherapy does not result in symptomatic improvement in PCD: this suggests PCD may be associated with irreversible damage of Purkinje cells[3].

NON-PARANEOPLASTIC CEREBELLAR DEGENERATION WITH VGCC ANTIBODY

VGCC antibodies were also found in a few patients with non-paraneoplastic cerebellar degeneration. In a study of the antibody profile of 67 cases with sporadic, late-onset cerebellar ataxia of unknown etiology, VGCC antibodies were found in 12%[54]. Two cases with NT-LEMS who developed cerebellar ataxia during the course of their disease had VGCC antibodies in serum and CSF. Cerebellar symptoms of these patients showed no improvement with different immunotherapies, as in PCD[55]. However a few cases of non-paraneoplastic cerebellar degeneration showed favorable outcome under rituximab and IVIg treatment[56,57].

CONCLUSION

Diseases related to VGCC antibodies are usually associated with SCLC. Therefore, SCLC should be investigated in patients with LEMS and/or cerebellar degeneration. The role of VGCC antibodies in the immunopathogenesis of LEMS is clear, however their role in cerebellar degeneration is not known. Determination of the effect of VGCC antibodies on the pathogenesis of cerebellar degeneration may contribute to the design of more efficient treatment strategies. Therefore, experimental models and pathologic studies that investigate the effect of immune mechanisms at molecular level in the tissue are needed.

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MINIREVIEWS

Evaluation of functional, autonomic and inflammatory outcomes in children with asthma

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Abstract

Asthma is common in childhood. This respiratory disease is characterized by persistent inflammation of the airways even when the child is not in the throes of an attack. Chronic inflammation is caused by an imbalance between pro-inflammatory and anti-inflammatory mechanisms as well as autonomic dysfunction, which plays an important role in the pathogenesis and control of this condition. The impact of these physiopathological aspects leads to inactivity and a sedentary lifestyle, which exerts an influence on functional capacity and control of the disease. The main objective of non-pharmacological therapy is the clinical control of asthma and the minimization of airway obstruction and hyperinflation during an attack. These factors can be controlled with noninvasive ventilation. The aim or the present review was to describe important neural, inflammatory and functional mechanisms that affect children with asthma.

Key words: Asthma; Child; Continuous positive airway pressure; Noninvasive ventilation; Autonomic nervous system; Functional capacity; Inflammatory mechanisms; Evaluation

Core tip: The recurring nature of asthma is related to the clinical control of the disease. Neural and inflammatory mechanisms interfere with this clinical control and affect functional capacity. While the magnitude of an asthma attack cannot be controlled, its clinical impact can be minimized with the use of noninvasive ventilation. Moreover, functional capacity and inflammation can be improved with physical exercise.

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INTRODUCTION

Asthma is common in childhood. This respiratory disease is characterized by persistent inflammation of the airways, which is clinically manifested in the form of recurring cough, shortness of breath, wheezing and chest retraction. These episodes are associated with the obstruction of airflow, which is partially reversible and occurs mainly in the morning and at night[1,2].

Controlling the disease is the main objective of asthma management. Such control refers to the most recent clinical manifestations (previous four weeks) with regard to symptoms, limitations to physical activity, the need for a b_2 agonist and the intensity of airflow limitation as well as the reduction of future risks. Asthma is therefore classified as controlled, partially controlled or uncontrolled. Addressing future risks regards reducing the instability of asthma and exacerbations, the loss of lung function and the adverse effects of treatment[2,3].

The incidence of asthma has doubled in the last 20 years due to the actual increase in the number of cases as well as better recognition of the disease on the part of the medical community. The difficulty in comparing epidemiological data from different countries or even different regions within the same country motivated the International Study of Asthma and Allergies in Childhood, which employed a simple, validated, self-administered questionnaire with a small number of items. The authors of the study evaluated 304796 children aged six to seven years from 42 countries and 463801 adolescents aged 13 to 14 years at 155 centers in 56 countries and distinguished three categories of countries based on the prevalence rates of asthma: weak (less than 5%), medium (5% to 6%) and strong (greater than 10%). Brazil was classified in eight place, with a prevalence rate of 20%[4,5].

Despite the difficulties in diagnosing asthma in children, there is evidence that half of all cases are diagnosed by three years of age and 80% are diagnosed by six years of age. Moreover, one third of the initial symptoms begin before the child has completed one year of life. Although 50% of children with asthma in Latin America exhibit daily symptoms and arousals from sleep, only 10% regularly use medication to control asthma and only treat attacks with an inhaler, while only 13% employ preventive measures and the control of exacerbations[6].

The aim or the present review was to describe important neural, inflammatory and functional mechanisms that affect children with asthma.

PHYSIOPATHOLOGY AND INFLAMMATION IN ASTHMA

Asthma is an inflammatory disease involving the participation of mastocytes, eosinophils, T cells and dendritic cells. Among the different phenotypes, atopic asthma is the most common and is characterized by an increase in eosinophils and total immunoglobulin E (IgE), with the considerable participation of mastocytes and their products in events during the acute phase. These cells also participate in the chronic inflammatory process and bronchial hyperresponsiveness, along with macrophages, eosinophils and T lymphocytes[2]. Macrophages can either increase or diminish the inflammatory process, depending on the stimulus. Alveolar macrophages normally suppress lymphocyte function, but this function may be altered in individuals with asthma following exposure to a triggering factor[7] (Figure 1).

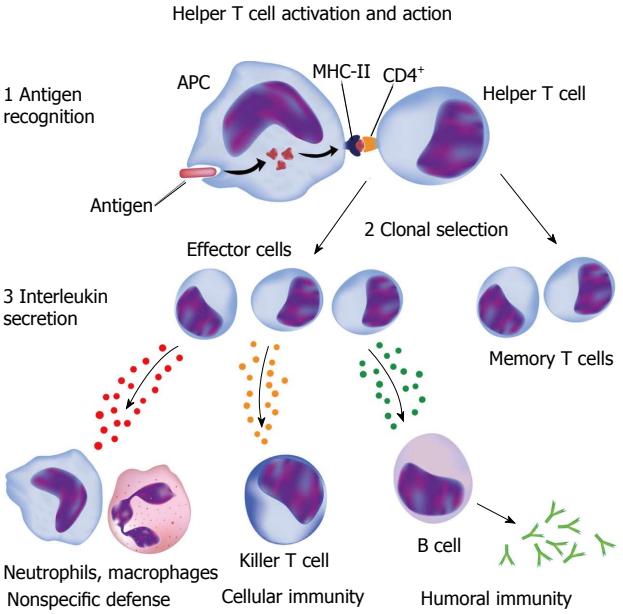

Figure 1 Cellular and humoral response to an antigen. © Can Stock Photo Inc./[Alila].

Eosinophil infiltrate is a physiopathological characteristic of the airways in individuals with asthma and contributes to the differentiation of this disease from other inflammatory conditions. Eosinophils are seen as beneficial in asthma due to their function in inactivating histamine and leukotrienes. However, eosinophils are also involved in injurious processes of the airways tissues, contributing to the development of bronchial hyperresponsiveness.

Chronic inflammation results in the obstruction of distal airways due to the accumulation of secretion and cell debris, the contraction of bronchial smooth muscles, thickening of the epithelial basement membrane and bronchial wall edema[8]. The most striking characteristic of asthma is persistent inflammation of the airways even when the child is not in the throes of an attack. The degree of inflammation is associated with bronchial hyperresponsiveness and symptoms. Chronic inflammation is caused by an imbalance between pro-inflammatory and anti-inflammatory mechanisms[9]. The persistence of this inflammatory condition over the years combined with frequent acute attacks can exert a

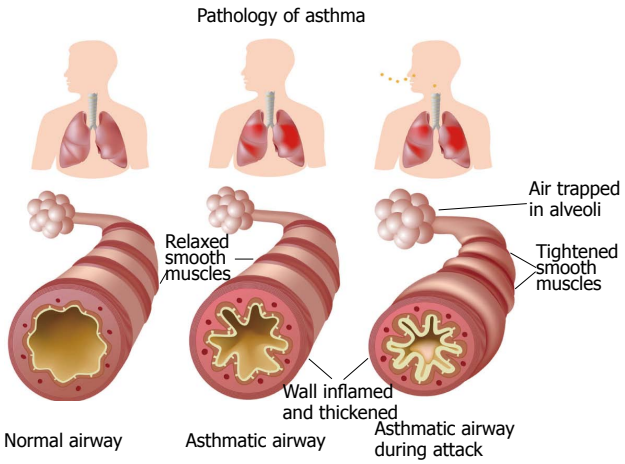

Figure 2 Bronchi in normal conditions, asthma and during asthma attack. © Can Stock Photo Inc./[Alila].

negative influence on lung function (Figure 2).

Inflammation has been the focus of treatment for asthma in the last 20 years. Prior to the 1980s, asthma was seen as recurrent wheezing that responded to b_2 agonists. The inflammatory reaction begins with contact between an allergen (pollutant or virus) and dendritic cells, which activate mastocytes that, in turn, release IgE specific to given allergens. This initial process leads to the release of different mediators. The consequence of this initial phase (four to six hours) of the inflammatory cascade is clinically manifested as bronchospasms[10]. In the late phase of the inflammatory reaction, granulocytes are released by bone marrow and migrate to the target organ (lungs), causing tissue injury and the release of toxins[11,12]. Inflammatory cytokines stimulate the formation of inducible nitric oxide synthase (iNOS). At high concentrations, NO is the major contributor to the inflammatory process in asthma[13] (Figure 3).

Cell apoptosis is maintained activated at all moments of the inflammatory cascade, possibly reducing the reserve of cells that could cease this process. Asthma is characterized by a reduction in apoptotic potential and consequent perpetuation of the inflammatory process. Moreover, the molecules involved in the tissue repair process seem to be relatively ineffective. These events results in chronic inflammation and the structural remodeling of the airways. All these factors seem to be the basis for bronchial hyperreactiveness and other symptoms[10-12].

Differences between childhood and adult asthma

Childhood is the period of greatest incidence of asthma, however the identification of these children still problematic. The clinical syndrome that is recognized as asthma fails to develops in all children and infants with wheezing (in less than half as already stated earlier).

Another difficulty in the identification of asthma in childhood is related to pulmonary function test as

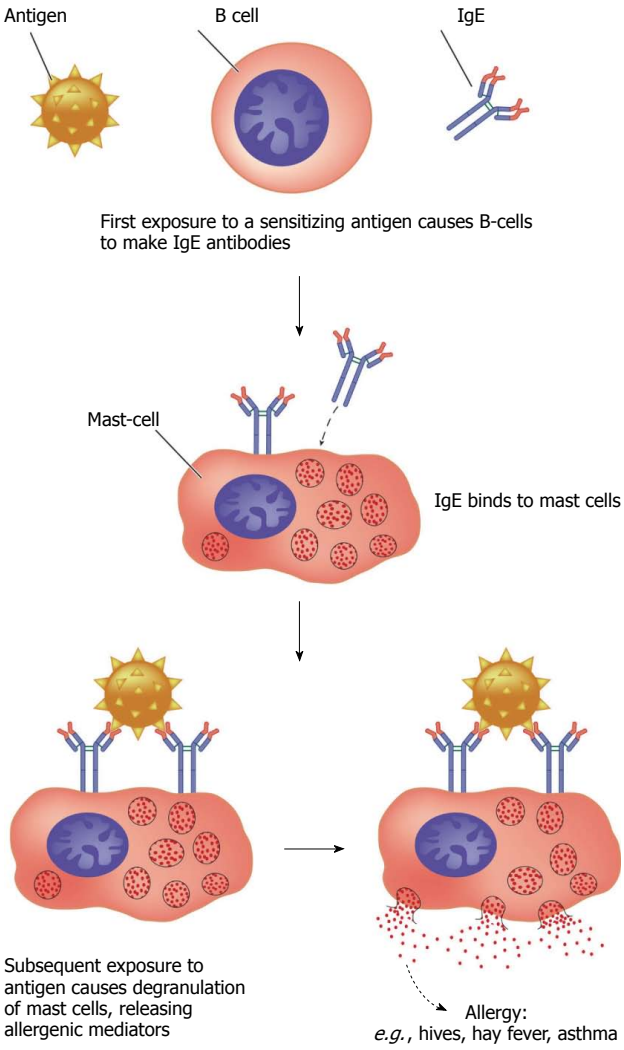

Figure 3 Activation of mast cells and release of IgE. © Can Stock Photo Inc./[Blambs].

well as the documentation of the obstruction and its reversibility objectively and quantitatively in small children. The challenge is to detect by means of non-invasive biomarkers in infants and pre school children who can become asthmatic by facilitating in this way the clinical control and reducing the morbidity of the disease in adulthood.

What is known so far that insults occurred postnatally as aspiration, viral infections or allergic sensitization may alter the airway neural control for long periods[13].

Respiratory infection and asthma in childhood

Various pathogens may cause wheezing in childhood, in particular, respiratory syncytial virus (RSV). Bronchiolitis from RSV increases the risk for recurrent wheezing (more than 3 episodes of wheezing per year) and not persistent (up to 3 episodes of wheezing per year); However, the risk gradually decreases with age and becomes not significant to 13 years of age[14]. These data indicate that RSV infections may contribute substantially to the risk of recurrent wheezing and possibly asthma in childhood and also suggest that

other co-factors (for example: genetic, environmental and development) contribute to the onset or severity of asthma over time.

Respiratory infections and asthma are closely related and children are very susceptible to these infections, but less than half of them develop recurrent wheezing.

Some types of respiratory infection in infancy can stimulate Th1 cells and as regards the pathophysiology of childhood asthma in the balance and the way in which this balance Th1/Th2 is reached in this age group still generate doubts. What is known is that the non atopic child have a reduction of Th2 in the first year of life, already in atopic child Th2 response is associated with the production of IFN-γ in the neonatal period[14].

Atopic asthma is characterized by this imbalance Th1/Th2. Th2 cells when stimulated produces interleukin 4 (IL4) and IL13 that induce the production of IgE by B cells. The IL9 is also produced by Th2 stimulates the proliferation of mast cells which in turn starts the production of histamine, leukotrienes and prostaglandins that lead to an additional activation of eosinophils, basophils and Th2[15].

The endothelial lesion caused by viral infection leads to increased airway permeability facilitating the exposure of allergens to cells in the nerve endings promoting a neurogenic inflammation. Severe respiratory infections increase the recruitment of eosinophils to the airways bringing the risk of persistent asthma in childhood[13].

Fraction of exhaled nitric oxide

Gustafsson *et al*[16] (1991) were the first researchers to isolate the nitric oxide (NO) molecule. According to the authors, NO is formed by the action of NO synthase on the semi-essential amino acid L-arginine. Two basic isoforms are produced: constitutive and inducible[17]. NO synthase performs a number of biological functions, such as neurotransmission, vasodilatation and bronchodilatation as well as playing a role in the immune system. The type Ⅰ constitutive isoform (epithelial) promotes vasodilatation and the type Ⅱ constitutive isoform (neuronal) is responsible for the transmission of stimuli from the central nervous system and autonomic nervous system through the non-adrenergic, non-cholinergic pathway[17,18]. The inducible isoform (iNOS) is stimulated with the perpetuation of the inflammatory process and contributes to this process due to its pro-inflammatory activity. When activated, this isoform promotes an increase in the production of secretion in the airways and a large number of inflammatory cells, favoring necrosis of the ciliated tissue[7,19]. The constitutive isoforms are produced in small amounts that are not detectable in exhaled breath and perform their biological and physiological roles, whereas iNOS is produced in large amounts, has cytotoxic effects and is detectable in exhaled breath (FeNO)[20,21].

FUNCTIONAL CAPACITY

Fear of shortness of breath often makes children with asthma avoid physical exercise, leading to a sedentary lifestyle, psychological and postural problems as well as a poor quality of life. Studies have demonstrated that children with moderate to severe uncontrolled asthma exhibit chronic physical deconditioning and reduced cardiopulmonary capacity[22,23]. Villa *et al*[24] (2011) evaluated children with persistent moderate to severe asthma and found that those with severe asthma exhibited diminished lower limb endurance. Thus, controlled physical activities are needed to prevent the impairment of cardiopulmonary capacity and physical fitness.

Repeated physical activities with varying intensity that last only a few seconds and are intercalated with short rest periods are more appropriate for children. Besides preferring spontaneous activities with a recreational components and considerable variety, children explore the anabolic effects of physical exercise more[25]. Thus, physically-based play activities are indicated for children with asthma. While 40% to 90% of children with asthma exhibited exercise-induced bronchospasms, the regular practice of physical activity is able to improve this symptom, often with no direct impact on lung function[2].

Advances in technology have facilitated the performance of movements and physical activity[26-28]. Interactive video games in the last ten years have contributed to energy expenditure among otherwise sedentary children, with the possible applications regarding lung rehabilitation among children with asthma. However, previous studies have only employed this resource for the training of children without lung diseases[29]. Thus, there is a need for scientific evidence of the benefits of interactive video games for the improvement of physical fitness in this population.

Inflammation and physical exercise

A persistent inflammatory state is a common trait of chronic diseases. Inflammation is indicated by the high concentration of inflammatory markers, such as IL6, tumor necrosis factor alpha (TNFα) and C-reactive protein in the blood plasma[30]. Physical exercise has an anti-inflammatory effect and regular practice seem to offer protection against the development and aggravation of chronic diseases. While children with asthma tend to avoid physical exertion, the practice of physical exercise can be beneficial to this population. However, there are no specific guidelines with regard to the type, intensity, duration and frequency of training[31].

Three mechanisms seem to explain the anti-inflammatory effect of physical exercise. The first is the reduction in visceral fat, as excess fat contributes to the production of pro-inflammatory adipokines,

such as TNF and leptin, as well as the reduction in adiponectin. The second mechanism is the increase in the production and release of anti-inflammatory cytokines, such as IL6, stemming from muscle contractions caused by myosin, which induces different metabolic effects, such as lypolysis and the oxidation of fat, as well as contributing to the homeostasis of glucose during physical exercise. The third mechanism is the reduction in the expression of receptors of monocytes and macrophages, which have a lower inflammatory response to endotoxins in physically active individuals[30-33].

The effect of myokines (IL6 released by the contraction of skeletal muscle) regulates the release of TNFa, resulting in a protective effect[34] (Tilg *et al*[34] 1997). Moreover, IL6 stimulates the release of the anti-inflammatory interleukins 10 and 1ra. IL10 inhibits the production of interleukins 1a and 1b as well as the induction of iNOS[35]. This mechanism may explain the likely effect of physical exercise on changes in the concentration of FeNO.

AUTONOMIC NERVOUS SYSTEM AND CONNECTIONS WITH RESPIRATORY AND CARDIOVASCULAR SYSTEMS

The respiratory and cardiovascular systems are intrinsically linked and the autonomic nervous system (ANS) is one of the pathways that connect these systems[36] (Figure 4).

Moreover, the ANS exerts an important influence on the pathogenesis and control of asthma[37]. Afferent pulmonary nerve pathways regulate cholinergic tone, which is increased when the respiratory rate is increased. This mechanism occurs in response to physiological and physiopathological stimuli. A physiological response occurs when the peripheral respiratory muscles and pulmonary stretch receptors send afferent stimuli to the central nervous system during physical exertion, which results in the reduction in cholinergic tone as well as bronchial dilatation to compensate for the increase in ventilation demand. This mechanism is altered in individuals with asthma[37].

Understanding the relationships between the ANS and respiratory system may help clarify the causes of cardiovascular disorders with a pulmonary origin. From the neuroanatomic standpoint, afferent and efferent neural pathways of the respiratory and cardiovascular systems converge in common regions. Afferent pathways converge in the solitary tract nucleus and efferent pathways converge in the nucleus ambiguous, which is responsible for the generation of the respiratory rate and heart rate[36].

In individuals with asthma, the inflammatory process is increased during periods of exacerbation. The increase in vagal tone during stable periods of the disease may be explained by an attempt to maintain inflammatory control. The ANS exerts an influence on the relaxation and constriction of the smooth muscles of the bronchioles. Relaxation occurs through either the activation of beta receptors of the sympathetic pathway or the activation of the non-adrenergic, non-cholinergic and intestinal peptide pathways. Constriction is mediated by either sympathetic receptors or the vagal cholinergic pathway[37].

Heart rate variability has been used as a measure of vagal autonomic activity and neuroimmunomodulation. A number of studies have addressed the influence of respiration on heart rate variability. Chronic respiratory diseases, such as asthma, demonstrate a link between the inflammatory process and the immune reaction as well as progression with cardiovascular consequences that can contribute to the increase in illness and mortality rates[36].

Asthma and autonomic modulation

The activation of the autonomic mechanism in the respiratory system is due to the reflex response of receptors located in the airways, regulating bronchial contractility[38-41]. Autonomic dysfunction is associated with asthma[42,43], with an increase in bronchial sensitivity to cholinergic constrictors and possibly a reduction in sensitivity to adrenergic b_2 bronchodilators. Besides its essential role in the cardiovascular system, the ANS plays an important role in the regulation of the contraction of bronchial smooth muscles[37].

Asthma severity is directed related to autonomic dysfunction even under conditions in which the patient is not a period of exacerbation. The functions of the ANS in children with asthma also differ in comparison to adults and healthy children[43,44]. This severity is correlated with the magnitude of vagal activation in the stable phase[43]. During periods of exacerbation, children with persistent moderate asthma experience greater action of the sympathetic nervous system, unlike what occurs in stable periods, which conflicts with the hypothesis of hyperactivity of vagal tone in the acute phase[45].

Neural mechanisms are also responsible for the regulation of inflammation. The activation of the vagus nerve is responsible for the inhibition of the activation of macrophages and the synthesis of TNFα as well as the activation of the reticuloendothelial system for the release of acetylcholine[46].

Autonomic modulation and degree of effort in asthma

As mentioned above, asthma progresses with autonomic abnormality during physical effort. The ANS responds to stimuli send by the muscles, lungs and diaphragm for the withdrawal of vagal tone, resulting in an increase in the diameter of the airways. This is a mechanical response to a neural stimulus that allows enhancing the airflow, with a consequent improvement in gas exchange in response to the increase in metabolic demand[37].

Different degrees of physical stress can trigger changes in autonomic modulation in children with

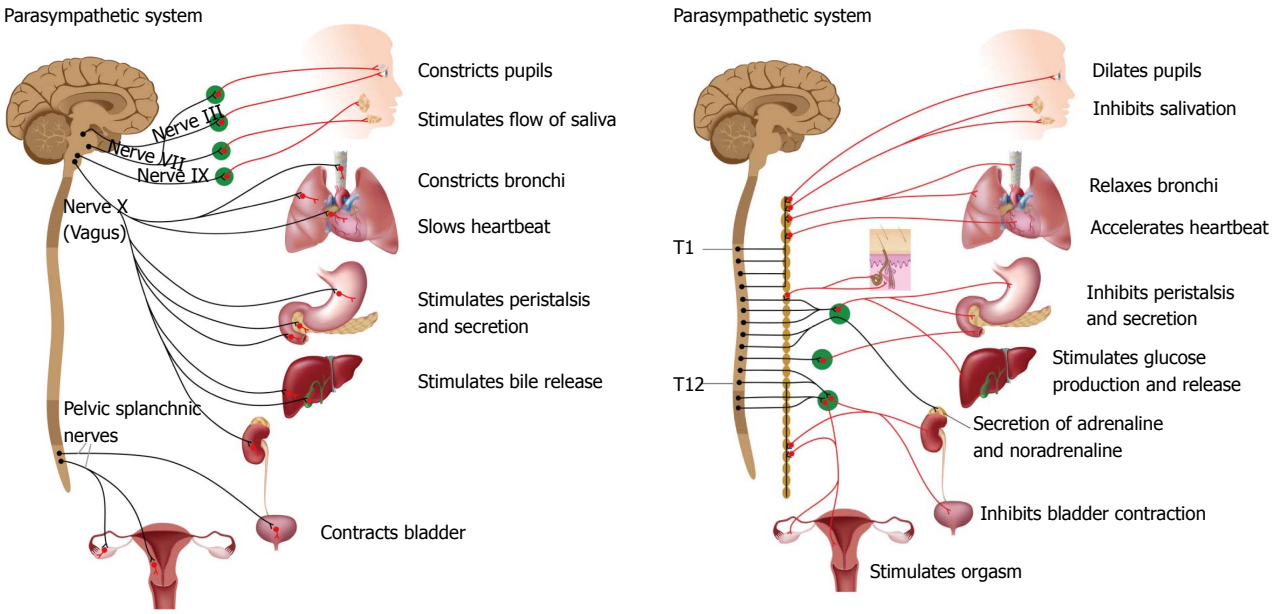

Figure 4 Autonomic nervous system subdivision. © Can Stock Photo Inc./[Alila].

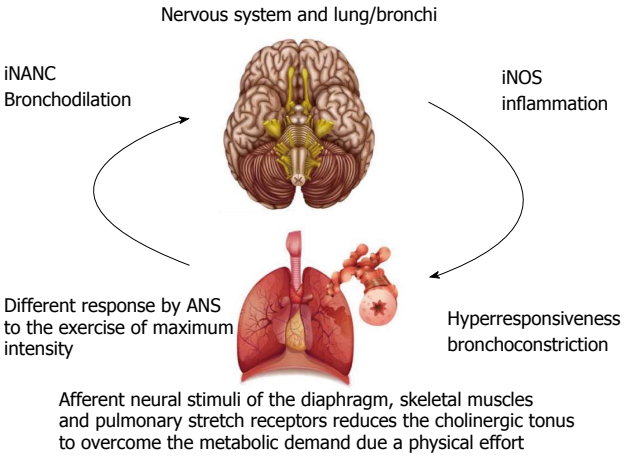

Figure 5 Lung and nervous system interaction. © Can Stock Photo Inc. / [Alexilus and Bluering].

asthma. During physical exertion, the heart rate and cardiac output are increased mainly due to vagal withdrawal stemming from a central command. In the transition from an intense activity involving an increase in heart rate greater than 100 beats per minute, the increase in sympathetic activity is necessary to induce tachycardia as well as increase both heart contractibility and peripheral vascular resistance[47]. During maximum physical effort, this mechanism of vagal withdrawal and sympathetic activation seems to be altered and relatively ineffective in children with asthma. Vagal withdrawal also seems to occur during sub-maximum effort[48] (Figure 5).

Management of shortness of breath and respiratory failure using noninvasive ventilation

During respiratory distress, children with asthma experience bronchial obstruction and hyperinflation, which predisposes such individuals to respiratory failure. Thus, the aims of the management of severe acute asthma include the correction of hypoxemia, alleviation of the airflow obstruction and the reduction of the inflammatory process through medications. The therapeutic goal is to reduce respiratory work and optimize gas exchange. Continuous positive airway pressure (CPAP), which is a form of noninvasive ventilation (NIV), is used to reduce the work of respiratory muscles imposed by hyperinflation of the lungs and produces a change in autonomic modulation stemming from the increase in intra-thoracic pressure[49,50] (Figure 6).

There is scientific evidence of the efficacy of NIV for an asthma attack. Gupta *et al*[51] (2010) report improved respiratory work, fast recovery of lung function as well as reductions in inhaler dose, stay in an intensive care unit and the duration of hospitalization among adults with acute asthma treated with bi-level NIV. CPAP caused changes in both alveolar and intra-thoracic pressures and the activation of pulmonary stretch receptors affects autonomic modulation[37,50]. In a study involving an experimental model, Xue *et al*[52] (2011) found that the increase in intra-thoracic pressure leads to a reduction in bronchial hyperresponsiveness, which persists for up to 24 h following the removal of CPAP. Busk *et al*[53] (2013) found a reduction in bronchial hyperresponsiveness in adults with asthma after seven days of treatment and consider CPAP to be an important non-pharmacological tool for the treatment of this condition.

Parasympathetic nerve stimulation results in both the contraction and relaxation of the bronchial musculature. The non-adrenergic, non-cholinergic

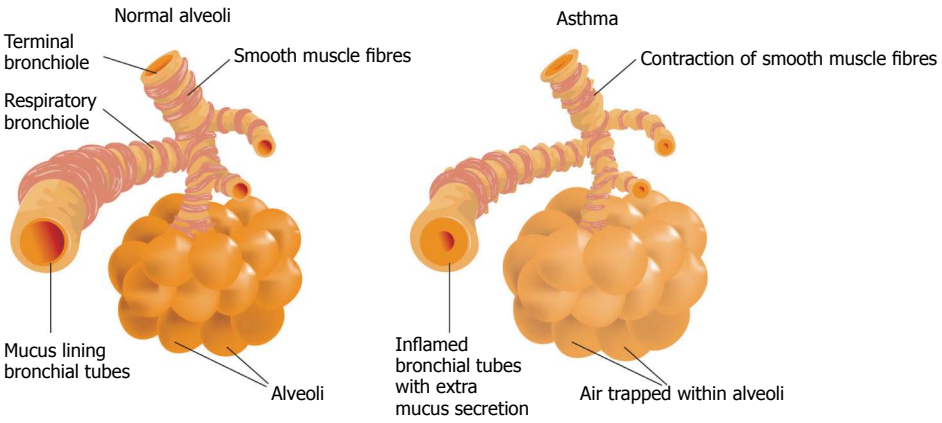

Figure 6 Hyperinflation. © Can Stock Photo Inc. / [Blambs].

pathway (bronchodilatation) is stimulated by pulmonary stretch receptors. Contraction is mediated by acetylcholine and relaxation is mediated by transmitters of the non-adrenergic, non-cholinergic pathway. Cholinergic tone is extremely sensitive to the ventilitory mechanism and is reduced when the respiratory rate is reduced[37]. Thus, CPAP may achieve its positive effects by reducing the respiratory rate and stimulating the non-adrenergic, non-cholinergic pathway through pulmonary stretch receptors. The acute effects of CPAP on autonomic modulation have been demonstrated in other pathological conditions, but there is a lack of scientific evidence regarding the benefits of this method in patients (especially children) with asthma[50].

In a study conducted by our research group[54] involving the evaluation of clinical variables and the ANS in children in the throes of an asthma attack, the activation of vagal tone was found with the administration of CPAP. Parasympathetic activation stimulates both contraction and relaxation of the smooth muscles of the airways. Cholinergic tone is reduced with positive end-expiratory pressure and the reduction in the respiratory rate[37,55].

The findings suggest that the non-cholinergic parasympathetic pathway is activated by the administration of CPAP due to the significant increase in peak flow, which continues after the removal of NIV, suggesting a bronchodilatation response as well as the mechanical stimulation of the opening of the airways. Pulmonary stretch and the activation of the non-cholinergic pathway is believed to assist in the inhibition of the cholinergic bronchoconstriction pathway[37].

CONCLUSION

The recurrent nature of asthma is related to the clinical control of the disease. Neural and inflammatory mechanisms interfere with this control and affect functional capacity. While the magnitude of an asthma attack cannot be controlled, its clinical impact can be minimized with the use of noninvasive ventilation. Moreover, functional capacity and inflammation can be improved with physical exercise.

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ORIGINAL ARTICLE

Case Control Study

Endoscopic ear surgery: A case series and first United Kingdom experience

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Abstract

AIM: To present the United Kingdom's first case series of 70 otological cases of endoscopic and non-endoscopic ear surgeries.

METHODS: Prospective case series incorporating a range of endoscopic procedures performed using a 4 mm, 18 cm rigid endoscope, performed by a single surgeon at a single centre. Primary outcome measures included mean average pre and post-operative air-bone gap hearing thresholds and duration of surgery.

RESULTS: Thirty-eight patients underwent endoscopic assisted ear surgery and 32 underwent non-endoscopic assisted ear surgery. In both surgical groups, there was a significant difference between pre and post-operative mean air-bone gaps (P = 0.02). Mean operating time was comparable between both groups. Eight patients developed post-operative complications.

CONCLUSION: Endoscopic ear surgery can be performed safely in a range of otological procedures. This has the potential to become a well-established surgical option for middle ear surgery in the near future. Advantages and limitations are discussed.

Key words: Endoscopic; Mastoid; Surgery; Imaging; Otology; Cholesteatoma

Core tip: The role of endoscopic ear surgery is yet to be properly established but as more otologists adopt this technique, its role will become much more clearly defined and may lead to widespread use based upon positive outcomes for surgery. As with every new surgical technique, a learning curve must first be overcome before reliable conclusions can be drawn about its use. Our series has shown the benefits of using this technique in limited cholesteatoma disease and in providing a good view during revision mastoid surgery with simple pathology.

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INTRODUCTION

The advantages of using endoscopes in surgery are well described and relate mainly to their portability and ability to provide clear, high quality images[1]. Endoscopes can also be used in theatre and the outpatient setting. In particular, the benefits for middle ear surgery include the ability to visualise poorly seen structures, such as the hypotympanum and sinus tympani, which are often an obstacle during the open-technique approach[2]. In addition, their use *via* the permeatal approach in bypassing a narrow isthmus can provide direct access and a wide view into the middle ear for surgery[3-5]. Benefits of using an endoscope can therefore decrease operating time due to the reduction in time need to gain access into the middle ear cleft[6] and the subsequent closure at the end of the procedure. The disadvantages of endoscopes used in ear surgery include operator dependence (especially in relation to the one-handed technique), restricted views from narrower endoscopes (*e.g.,* 2.7 mm as compared to 4 mm), the ability to manage complications such as bleeding within a narrower operating field, loss of depth perception, limited magnification, and the need for further training in their use[4,5]. Furthermore, when used solely in a permeatal approach, the surgeon must use a one-handed technique for instrumentation and there may be difficulty passing other instruments alongside, even in wide ear canals. Certainly there is no scope for using the operating drill in its present form.

Endoscopic ear surgery can be applied to a variety of operations including; grommet insertion, myringoplasty[2], attic retractions[6], cholesteatoma surgery[7-15], stapedectomy[1,15], benign neoplasms of the middle ear[16] and neuro-otological procedures[4,17,18]. Based on the literature their use has been most commonly described for middle ear disease (cholesteatoma). It has been suggested that preservation of middle ear mucosa by limited surgery using the endoscope can improve the re-aeration of the mastoid cavity leading to better outcomes in surgery[2]. There are also roles in "second look" middle ear surgery using 30 degree endoscopes to check for disease clearance[14,19].

Many of the surgeries described above are derived from international case series from France, Germany, Italy, India, UAE, China, Egypt, Iran and the United States[1,5,12-14,17-24]. We present the first United Kingdom case series that uses a permeatal exclusively endoscopic approach[20].

MATERIALS AND METHODS

We describe a case series of 70 patients who underwent either endoscope-assisted or non-endoscope-assisted ear surgery by a single senior surgeon in a district general hospital. Data collection was carried out prospectively for endoscopic cases and retrospectively for non-endoscopic cases where all cases were performed within a 2 year period (2012-2014). A 4 mm diameter, 18 cm long rigid endoscope was used in all cases. Primary outcomes include mean average pre and post-operative air-bone gap hearing thresholds or duration of surgery, depending on the type of surgery. Pre and post-operative audiometric data using both air and bone conduction (at 500 Hz, 1 KHz, 2 KHz and 4 KHz frequencies) was recorded. Complications were noted. Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc, La Jolla, CA, United States).

Statistical analysis

The statistical methods of this study were reviewed by Virk J, Cambridge University graduate. The dataset was principally descriptive with simple paired t-testing only.

RESULTS

Seventy patients underwent surgery between the ages of 7-85. Of these, 38 underwent endoscope-assisted ear surgery (Group A) and 32 underwent non-endoscope-assisted ear surgery (Group B). All cases were performed under general anaesthesia. Imaging was reviewed prior to surgery. An endoscope was used exclusively for all patients who underwent endoscope-assisted ear surgery, except in parts of an operation which required the use of a microscope (*e.g.*, mastoid portion of modified radical mastoidectomy or canal wall up mastoidectomy). Procedures in Group B patients were preferentially performed with the microscope such as revision stapedectomies under local anaesthetic or those with extensive disease and the endoscope was not used during the procedure. No cases were converted from endoscopic to open operations. Both groups were matched as closely as possible for type of surgery and demographics.

In Group A, 20 patients had had previous surgery to the operated ear (*i.e.*, ipsilateral ear) compared to 7 patients in Group B. A summary of different operations within Group A and B are shown in Table 1. Tables 2-6 summarise data for each operative group.

Overall, air-bone gap closure was achieved within 10 dB in 9 patients (5 Group A *vs* 4 Group B), within 10-30 dB in 18 patients (8 Group A *vs* 10 Group B), over 30 dB in 9 patients (2 Group A *vs* 7 Group B), over-closure in 5 patients (4 Group A *vs* 1 Group B) and no change in 25 patients (18 Group A *vs* 7 Group B). In both groups, there was a significant difference between pre and post-operative mean air-bone gaps ($P < 0.05$) (paired *t* test, $P = 0.036$ group A and $P = 0.002$ for group B) for patients who underwent stapedectomy, where air-bone gap was a primary

Table 1 Summary of procedures

Procedure (including revision surgery)	Endoscopic assisted	Non-endoscopic assisted
	Group A	Group B
Ventilation Tube insertion	1	2
Myringoplasty, tympanoplasty, ossiculoplasty, Tympanotomy	10	10
CSOM and cholesteotoma surgery	15	10
Stapedectomy	11	9
Petrosectomy	1	1
Total	38	32

Table 2 Ventilation tube insertion

No.	Age	Side	Duration (min)	Previous ipsilateral surgery	Pre-op mean air-bone gap[1]	Post-op mean air-bone gap[1]	Closure	Follow up (mo)	Complications
Endoscopic assisted, Group A									
1	15	R	25	No	22.5	0	Within 10-30 dB	4	None
Non-endoscopic assisted, Group B									
1	14	R + L	20	No	25	10	Within 10-30 dB	9	None
2	53	R + L	15	No	20	20	No change	24	Recurrent otitis media with effusion

[1]Mean gap calculated over 4 frequencies (500 Hz, 1 KHz, 2 KHz, 4 KHz).

Table 3 Myringoplasty, Tympanoplasty, Ossiculoplaty and Tympanotomy

No.	Age	Details	Side	Duration (min)	Previous ipsilateral surgery	Pre-op mean air-bone gap[1]	Post-op mean air-bone gap[1]	Closure	Graft material	Follow up (mo)	Complications
Endoscopic assisted, Group A											
1	63	Myringoplasty	R	66	Yes	Dead ear	Dead ear	No change	Conchal cartilage	1	Tragal abscess and otitis externa
2	37	Myringoplasty	L	60	No	15.5	12.5	No change	Temporalis fascia	4	None
3	55	Myringoplasty	L	45	Yes	5	5	No change	Composite tragal graft	4	None
4	16	Revision myringoplasty	R	45	Yes	0	0	No change	Tragal cartilage	5	None
5	22	Tympanoplasty	R	88	No	0	0	No change	Composite tragal graft	12	None
6	45	Tympanoplasty	L	101	Yes	20	15	Within 10-30 dB	Tragal cartilage	4	None
7	32	Tympanoplasty	L	98	No	7.5	6.25	Within 10 dB	Tragal cartilage	2	None
8	46	Tympanoplasty	L	111	Yes	18.75	23.3	No change	Tragal cartilage	2	None
9	35	Tympanoplasty	R	121	No	30	11.25	Within 10 dB	Not stated	3	None
10	34	Ossiculoplasty	R	123	Yes	42.5	12.5	Within 10-30 dB	Not stated	10	None
Non-endoscopic assisted, Group B											
1	12	Myringoplasty	R	55	No	12.5	11.25	Within 10-30 dB	5	5	None
2	9	Myringoplasty	L	130	No	27.5	Not available	Not available	Temporalis fascia	Lost to follow up	
3	30	Revision myringoplasty	L	97	No	16.25	15	Within 10-30 dB	Temporalis fascia	12	None
4	66	Tympanoplasty	L	127	Yes	Not available	Not available	Not available	Not stated	5	Will need ossiculoplasty
5	59	Tympanoplasty	L	114	No	15	40	> 30 dB	Not stated	4	Scarring, false fundus recurrence
6	33	Tympanoplasty	L	174	No	Dead	Dead	No change	Composite tragal graft	5	None
7	10	Tympanoplasty	L	88	No	23.75	21.25	Within 10-30 dB	Temporalis fascia	3	None
8	21	Tympanoplasty	R	92	No	16.25	6.25	Overclosure	Temporalis fascia	10	None
9	63	Tympanoplasty	R	100	No	Dead	Dead	No change	Not stated	3	None
10	50	Revision tympanoplasty	R	101	Yes	0	20	> 30 dB	Tragal cartilage	2	None

[1]Mean gap calculated over 4 frequencies (500 Hz, 1 KHz, 2 KHz, 4 KHz).

outcome.

Mean operating times were as follows; ventilation tube insertion 25 min *vs* 17.5 min in (Group A, *n* = 1 *vs* Group B, *n* = 2), myringoplasty, tympanoplasty,

Table 4 CSOM and cholesteatoma surgery

No.	Age	Details	Side	Duration (min)	Previous ipsilateral surgery	Pre-op mean air-bone gap[1]	Post-op mean air-bone gap[1]	Closure	Graft material	Follow up (mo)	Complications
Endoscopic assisted, Group A											
1	42	Mastoidectomy	L	211	No	7.5	7.5	No change	Temporalis fascia	6	None
2	40	Revision mastoidectomy	R	155	Yes	40	40	No change	Not stated	3	None
3	7	Tympanoplasty and mastoid exploration	L	169	Yes	13.75	5	Overclosure	Conchal cartilage	3	None
4	35	Tympanotomy	L	48	No	25	25	No change	Not stated	2	None
5	18	CWU mastoidectomy	L	195	No	7.5	17.5	Within 10 dB	Conchal cartilage	7	None
6	52	CWU mastoidectomy	L	287	Yes	20	18.75	> 30 dB	Tragal cartilage	6	None
7	13	Revision CWU mastoidectomy	L	188	Yes	11	25	> 30 dB	Tragal cartilage	2	None
8	47	MR mastoidectomy	R	287	No	27.5	23.75	Within 10-30 dB	Not stated	2	None
9	40	MR mastoidectomy	L	223	Yes	Data unavailable	16.25	Within 10-30 dB	Tragal cartilage	2	Post op. pain
10	28	Revision MR mastoidectomy	L	228	Yes	21.25	21.25	No change	Not stated	4	None
11	41	Revision MR mastoidectomy	L	140	Yes	31.25	31.25	No change	Not stated	4	None
12	35	Revision MR mastoidectomy	R	95	Yes	42.5	42.5	No change	Not stated	6	None
13	85	Revision MR mastoidectomy	L	110	Yes	20	20	No change	Not stated	11	None
14	68	Revision MR mastoidectomy	L	155	Yes	45	50	No change	Temporalis fascia	3	None
15	43	Revision MR mastoidectomy		78	Yes	Dead ear	Dead ear	No change	Not stated	4	Transient delayed facial palsy
Non-endoscopic assisted, Group B											
1	8	CWU mastoidectomy	L	220	No	17.5	12.5	Within 10-30 dB	Temporalis fascia	12	None
2	52	CWU mastoidectomy	L	286	No	27.5	25	> 30 dB	Not stated	7	None
3	13	Revision CWU mastoidectomy	L	189	Yes	7.5	30	Within 10-30 dB	Tragal cartilage	5	None
4	70	MR mastoidectomy	L	131	No	13.75	20	> 30 dB	Composite tragal graft	3	None
5	42	MR mastoidectomy	R	255	No	28.75	35	> 30 dB	Temporalis fascia	3	None
6	34	MR mastoidectomy	R	312	No	33.75	37.5	> 30 dB	Composite tragal graft	2	None
7	73	Revision MR mastoidectomy	L	150	Yes	Dead ear	Dead ear	No change	Tragal cartilage	9	None
8	77	Revision MR mastoidectomy	L	179	Yes	2.5	21.25	Within 10-30 dB	Tragal Cartilage	8	None
9	56	Revision MR mastoidectomy	L	251	No	20	10	Within 10-30 dB	Temporalis ascia	4	None
10	78	Revision MR mastoidectomy	R	199	No	Dead ear	Dead ear	No change	Not stated	6	None

[1]Mean gap calculated over 4 frequencies (500 Hz, 1 KHz, 2 KHz, 4 KHz). CWU: Canal wall up mastoidectomy; MR: Modified radical mastoidectomy.

tympanotomy and ossiculoplasty 85.8 min *vs* and 107.8 min (Group A, *n* = 10 *vs* Group B, *n* = 10), *CSOM* and cholesteotoma surgery 171 min *vs* 217.2 min (Group A, *n* = 15 *vs* Group B, *n* = 10), stapedectomy 136.5 min *vs* 175.2 min (Group A, *n* = 11 *vs* Group B, *n* = 9) and petrosectomy 387 min *vs* 253 min (Group A, *n* = 1 *vs* Group B, *n* = 1).

Graft material was used in a total of 30 patients (15 *vs* 15 patients from Group A and B respectively). Choice of graft material varied from tragal cartilage (7 *vs* 5), conchal cartilage (3 *vs* 0), composite tragal graft (2 *vs* 4), temporalis fascia (3 *vs* 6) and fascia lata and fat (1 *vs* 0) from patients in Group A and B respectively.

Table 5 Stapedectomy

No.	Age	Details	Side	Duration (min)	Previous ipsilateral surgery	Pre-op mean air-bone gap[1]	Post-op mean air-bone gap[1]	Closure	Prosthesis	Follow up (mo)	Complications
Endoscopic Assisted, Group A											
1	30	Stapedectomy	L	149	No	28.75	10	Overclosure	SMart piston	9	None
2	57	Stapedectomy	L	119	No	11.25	15	Within 10-30 dB	SMart piston	3	None
3	43	Stapedectomy	R	137	No	35	6.25	Within 10-30 dB	SMart piston	6	None
4	44	Stapedectomy	R	145	No	32.5	10	Within 10-30 dB	SMart piston	4	None
5	32	Stapedectomy	R	150	No	25	26.25	No change	Plastipore PORP	5	None
6	39	Stapedectomy	R	115	No	40	13.75	Within 10 dB	PORP	3	None
7	45	Stapedectomy	R	151	No	38.75	40	Overclosure	Porphexpiston	2	Infection in mastoid cavity
8	33	Stapedectomy	R	125	No	13.75	6.25	Overclosure	SMart piston	5	None
9	37	Revision stapedctomy	L	139	Yes	25	17.5	Within 10 dB	SMart piston	8	None
10	32	Revision stapedectomy	L	142	Yes	60	60	No change	SMart piston	2	Labyrinthitis
11	47	Revision revision stapedectomy	R	129	Yes	16.25	20	Within 10-30 dB	SMart piston	7	None
Non-endoscopic Assisted, Group B											
1	48	Stapedectomy	R	254	No	45	7.5	< 10 dB	fluoroplastic piston	11	None
2	44	Stapedectomy	R	230	No	21.25	Not available	n/a	Fluoroplastic piston	Lost to follow up	
3	45	Stapedectomy	L	118	No	26.25	8.75	< 10 dB	Smart piston	5	None
4	41	Stapedectomy	R	265	No	37.5	13.75	Within 10-30 dB	Smart piston	3	None
5	41	Stapedectomy	L	253	No	33.75	16.25	Within 10-30 dB	Smart piston	13	None
6	42	Stapedectomy	L	98	No	32.5	20	Overclosure	Fluoroplastic piston	22	None
7	40	Stapedectomy	L	169	No	40	5	< 10 dB	Fluoroplastic piston	14	None
8	56	Revision stapedectomy	L	111	Yes	20	10	< 10 dB	Fluoroplastic piston	5	None
9	38	Revision stapedectomy	R	79	Yes	26.25	21.25	> 30 dB	Fluoroplastic piston	8	Planned for revision revision surgery

[1]Mean gap calculated over 4 frequencies (500 Hz, 1 KHz, 2 KHz, 4 KHz). PORP: Partial ossicular replacement prosthesis; n/a: Not Applicable.

Eight patients developed post-operative complications that later resolved including otalgia, recurrent otitis media with effusion, transient delayed facial palsy, labyrinthitis, tragal abscess and tympanic membrane perforation and infection of the mastoid cavity (see Tables 2-6). Three patients were planned for further surgery at follow up. Mean post-operative follow up was 8.8 mo; 2 patients were lost to follow up.

DISCUSSION

Ventilation tube insertion

Only one patient in our case series had ventilation tube insertion lasting 25 min. Though numbers are extremely low, and therefore difficult to analyse, this operation took 7.5 min longer than the mean duration of non-endoscope-assisted surgery. In contrast, a recent study examining 260 endoscopic grommet insertions demonstrated operating times between 5 and 10 min in all cases[2]. Another study has shown that there is no significant difference in duration compared to using a microscope, though it does advocate the use of an endoscope when ventilation tube placement is technically difficult[21].

Myringoplasty, Tympanoplasty and Tympanotomy

This series demonstrates that the endoscope can effectively access the middle ear for these procedures. No further incisions were required and an exclusively permeatal approach was used in all endoscopic procedures. Surgical outcomes were good in all cases (Table 2) with shorter mean operating times as compared to Group B (non-endoscope = assisted surgery), 85.8 min *vs* 107.8 min for Group A *vs* B respectively. This is a fairly accurate representation of true operating time, since the same numbers of operations were performed in each group. There is also evidence that excellent hearing thresholds can be achieved endoscopically, as reported by Balasubramanian and Venkatesan, who achieved pure tone average hearing thresholds of 20 dB in 50 myringoplasties performed endoscopically, further confirming the efficacy of this technique in selected cases[2].

Table 6 Petrosectomy

No.	Age	Side	Duration (min)	Previous ipsilateral surgery	Pre-op mean air-bone gap[1]	Post-op mean air-bone gap[1]	Closure	Graft material	Follow up (mo)	Complications
Endoscopic assisted, Group A										
1	63	R	387	No	Dead ear	Dead ear	No change	Fat, Fascia lata	3	Intraoperative CSF leak; TM perforation
Non-endoscopic assisted, Group B										
1	79	R	253	No	Dead ear	90	No change	Not stated	6	referral for cochlear implant

[1]Mean gap calculated over 4 frequencies (500 Hz, 1 KHz, 2 KHz, 4 KHz). CSF: Cerebrospinal fluid; TM: Tympanic membrane.

CSOM and cholesteatoma surgery

Mean operating time was shorter in Group A compared to Group B (171 min *vs* 217.2 min respectively). Since total number of operations here were not equal (n = 15 *vs* n = 10), it is unreliable to claim the difference between these figures is of clinical significance. Variation in anatomy and pre-operative disease state (*e.g.*, actively discharging ear), will also have implications on duration of operation due to technical difficulty.

Cholesteatoma can vary in anatomical spread and severity of disease. In widespread, severe cases, canal wall up mastoidectomy or modified radical mastoidectomy can be performed. Our case series shows a variation in the number of these procedures between both groups. Performing mastoidectomy exclusively with an endoscope is impossible, and therefore drawing comparisons between these groups is difficult, as the endoscope will not have been used during a proportion of surgery in Group A. However, only the endoscope was used for the entire operation where there were cases of limited cholesteatoma, or recurrent disease in revision surgery. Recent literature supports this, demonstrating that an exclusively endoscopic approach can be very useful as a "second look" surgery in in order to identify residual cholesteotoma[11].

The most widely documented use of endoscopic ear surgery has been for cholesteatoma disease. Some studies have examined the use of the endoscope as an adjunct for surgery. Residual cholesteatoma rates in closed cavity surgery have been documented around 9%, which is comparable to use with a microscope alone[14]. One study examined its use peri-operatively after surgery using the microscope. Residual disease was identified in 65/80 cases, and was documented to commonly occur on the stapes footplate, the stapes crura, and the sinus tympani[13]. The use of the endoscope has also been shown to decrease the rate of "open tympanoplasty" during this surgery. Results for localised attic disease have achieved air bone gap closure within 20 dB in around 90% of patients between 3 and 6 years follow up. Figures of 80% disease-free follow up have also been documented on 27 cases with limited attic retractions[6]. Our series demonstrates relatively efficient use of the endoscope during revision surgery, which highlights the importance of a good visibility during technically challenging operations within the middle ear. However longer follow up is required to confirm its efficacy in these revision cases.

Stapedectomy

Our case series of 11 stapedectomies performed using a 4 mm endoscope at 0 and 30 degrees demonstrated the preservation of the chorda tympani all cases, as well as achieving significant improvement in pre and post-operative air-bone closure (P < 0.05) where thresholds were within < 30 dB for all cases. By comparison, the 9 operations performed without the endoscope, also show significant improvement in pre and post-operative air bone gap (P < 0.05), but with a longer mean duration of surgery (136.4 min *vs* 175.2 min for Group A and B respectively). First described by Poe in 2000, endoscopic stapedectomy has gone on to show promise in other countries across the world, achieving significant improvement in air bone gap by comparing pre and post-operative hearing thresholds[1,15]. Our series is in keeping with this.

Petrosectomy

There are some reports of successful use of the endoscope during cholesteatoma surgery within the petrous apex, as was used for one case in our series[22]. Due to the discovery of a CSF leak intra-opertively, the duration of surgery is much higher compared to the non-endoscopic assisted surgery. It is difficult to compare these two surgical approaches for this operation from this single case series.

Clinical applicability

Endoscopic surgery has also been used in a variety of neuro-otological procedures, including acoustic neuroma surgery. Some centres have also begun using it as the first surgical option or as an adjunct to conventional posterior tympanotomy approach in cochlear implantation[23-25]. Its benefit as an adjunct to conventional surgical techniques where wider exposure is required due to a limited direct vision has been well recognised[4,17,18]. Cadaveric studies using the endoscope alone have also documented superior views of the internal acoustic meatus over conventional

techniques, although clinical applicability for this may well take several years to develop[23,25].

The endoscopic technique in ear surgery undoubtedly gives better quality images and access to blind sacs around the middle ear space that would otherwise not have been visualised adequately using a microscope, irrespective of surgical approach. It is minimally invasive thus providing better cosmesis in patients who do not wish to have a scar. Its use in the outpatient setting has gained popularity by consultant otolaryngologists and junior trainees due to its accessibility, portability and superiority over hand drawn diagrams of the tympanic membrane, which often can be unreliable and inaccurate. In addition, our series demonstrates a role in revision mastoid surgery in particular, where, for example, the cavity can be revised by curettage of a high "facial ridge" entirely endoscopically and permeatally.

The most commonly used rigid endoscopes are 18 cm long and 4 mm (as used for all operations in this case series). Some surgeons find this endoscope difficult to manoeuvre due to its length and larger diameter, and advocate using a paediatric nasal endoscope which is 2.7 mm diameter and 11 cm long[1]. However these endoscopes generate poor views and 3 mm endoscopes are available and better suited for ear surgery. Ideally, an endoscope with a small diameter, and shorter length, possibly with a modification to allow the surgeon to keep two hands free but that retains light intensity within a wider field, would be ideal for operating on the middle ear.

In addition, it is worth nothing that there is a learning curve when using any new technique. This may be improved for otolaryngologists where we regularly use the endoscope during endoscopic sinus surgery for example.

Limitations

The numbers for each operation in our prospective case series is low, leaving the study underpowered. However, this case series serves as a pilot study to open the debate of endoscopic ear surgery in the United Kingdom. To enhance our results, more cases would need to be examined in a similar prospective fashion. Only then could reliable conclusions be drawn from comparing endoscopic and open techniques.

Another limitation is the small number in each group, addressed above in regard of the power of the study, alongside the groups being somewhat heterogeneous particularly in the largest group of mastoid and tympanoplasty surgery. However we need to group the surgeries into a grading from simple to complex and these groupings certainly serve to follow this. The groupings, like the above point, serve to illustrate the possibilities of the endoscope rather than to compare the surgeries themselves. Likewise, including in our series, grommet insertion and petrosectomy demonstrates the utility of the endoscope, despite the few numbers. This will be of value and interest to the readership to investigate further despite the small numbers.

The role of endoscopic ear surgery is yet to be properly established but as more otologists adopt this technique, its role will become much more clearly defined and may lead to widespread use based upon positive outcomes for surgery. As with every new surgical technique, a learning curve must first be overcome before reliable conclusions can be drawn about its use. Our series has shown the benefits of using this technique in limited cholesteatoma disease and in providing a good view during revision mastoid surgery with simple pathology.

COMMENTS

Background

Endoscope assisted ear surgery is increasingly common. However its role has not been properly elucidated. The authors investigate potential roles across a range of otological procedures.

Research frontiers

Minimal access surgery from robotic to endoscopic approaches are being increasingly analysed.

Innovations and breakthroughs

This study highlights the role of endoscope surgery in revision mastoid surgery alongside the more well-established role in stapedectomy. The endoscope allows excellent visualisation of the middle ear cleft and any cholesteatoma.

Applications

The endoscope can assist in mastoid surgery, particularly in revision cases. It also has a role in stapedectomy and other middle ear surgery.

Peer-review

Authors described their experience about endoscopic ear surgery. As mentioned by authors, this surgical procedure has already been described in case series numerically significant.

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CASE REPORT

May-Thurner syndrome: High output cardiac failure as a result of iatrogenic iliac fistula

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Informed consent: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.
Conflict-of-interest: There are no conflicts of interest for any of the authors.

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Abstract

May-Thurner syndrome (MTS) also termed iliocaval compression or Cockett-Thomas syndrome is a common, although rarely diagnosed, condition in which the patient has an anatomical variant wherein the right common iliac artery overlies and compresses the left common iliac vein against the fifth lumbar spine resulting in increased risk of iliofemoral deep venous thrombosis. This variant has been shown to be present in over 23% of the population but most go undetected. We present a patient with MTS who developed high output cardiac failure due to an iatrogenic iliac fistula. The patient underwent an extensive workup for a left to right shunt including MRI and arterial duplex in the vascular lab. He was ultimately found to have a 2.1 cm left common iliac artery aneurysm and history of common iliac stent. We took the patient to the operating room for aortogram with placement of an endovascular plug of the left internal iliac artery and aorto-bi-iliac stent graft placement with CO_2 and IV contrast. Subsequently the patient underwent successful stent placement in the area that was compressed followed by 6 mo of anticoagulation with warfarin. The flow from the fistula decreased significantly.

Key words: May-Thurner syndrome; Cardiac failure; Echocardiogram; Cockett-Thomas syndrome; Iliocaval compression

Core tip: To our knowledge, we describe the first case of high output cardiac failure due to iatrogenic iliac fistula and its management in the setting of May-Thurner syndrome (MTS). In our case, an iatrogenic iliac fistula resulted because of prior stent placement in left iliac vein to prevent deep venous thrombosis (DVT) secondary to MTS. We favored aorto-bi-iliac stent graft placement to prevent the fistula from leaking. In our case, the prior vascular stent placement was a clue to search for the fistula. It is important to note that stent placement to prevent DVT in MTS may result in iatrogenic fistula formation.

Singh S, Singh S, Jyothimallika J, Lynch TJ. May-Thurner

syndrome: High output cardiac failure as a result of iatrogenic iliac fistula. *World J Clin Cases* 2015; 3(3): 318-321 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i3/318.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i3.318

INTRODUCTION

May Thurner syndrome (MTS) or iliocaval compression or Cockett-Thomas syndrome is a fairly common well recognized and rarely diagnosed condition. McMurrich first described this anatomical variant in 1908 and believed the variant was the result of "congenital adhesions" in the common iliac veins. May and Thurner published this syndrome in 1957 and it was widely recognized as MTS in United States. In Europe, however, Cockett, a British vascular surgeon and Thomas published the condition in 1965 and it was termed Cockett-Thomas Syndrome. May and Thurner postulated that the chronic pulsations of the overlying right iliac artery led to development of a "spur" in the vein wall and that this spur would result in partial venous obstruction.

CASE REPORT

A 61 years old male was admitted to our cardiology service for shortness of breath associated with hemodynamic instability (systolic blood pressure, 95mm Hg and diastolic blood pressure, 59 mmHg). His past medical history is significant for hypertension, congestive heart failure, coronary artery disease (post coronary artery bypass graft - 1999), MTS [treated with left iliac vein stent in absence of PE and deep venous thrombosis (DVT) in 2001], chronic pulmonary embolism, protein C deficiency, restrictive lung disease and mild obstructive lung disease. Additionally, the patient smokes half a pack daily for 30 years.

His immediate physical examination revealed hypotension (105/46 mmHg), jugular venous distension of 6 cm, 2/6 pansystolic murmur at the apex, and bilateral pedal edema was noted. Abdomen was soft and non-tender but was distended with normoactive bowel sounds and and liver edge 1 cm below the costal margin. A chest x ray showed mild cardiomegaly with prominence of the central pulmonary arteries (Figure 1). Initial BNP and fibrinogen levels were both elevated (166.7 hh pg/mL and 553 mg/dL). Other blood tests supported renal insufficiency (creatinine 1.68 mg/dl). Echocardiogram revealed diffuse hypokinesia with a low ejection fraction (35%), cardiac output (11.5 L/min), cardiac index (5.54 L/min per square), end diastolic volume (183 mL), end Systolic volume (101 mL), concentric left ventricular hypertrophy, abnormal diastolic relaxation and mild to moderate mitral regurgitation.

Vascular surgery was consulted for evaluation of a possible pelvic shunt/fistula. After extensive workup for a left to right shunt including MRI and arterial duplex, the patient was found to have a 2.1 cm left common iliac artery aneurysm (21.4 mm) (Figure 2). An Inferior Vena Cava duplex and computed tomography (CT) of the pelvis found evidence of atriovenous fistula at the left iliac vein (discovered 11 years after the initial stent was placed in 2001) (Figure 3).

The patient was taken for an aortogram and heparinized with 5000 units of IV heparin. The right common femoral artery was then accessed and a 5-French sheath was advanced over the wire. An Omniflush catheter was then positioned at the level of L1. The patient had evidence of a high volume fistula from within the left common and hypogastric artery on CO_2 aortogram. Given this finding, an additional magnified view was obtained with the Omniflush catheter positioned at the aortic bifurcation. The Glidewire and Omniflush catheter were then used to select the left hypogastric artery. A 6-French Balkan sheath was brought up and over Magic Torque wire and into the left hypogastric artery. The Amplatz 16 mm plug was then advanced into the distal hypogastric artery. This appeared to be distal to any evidence of the fistula. The plug was then deployed in proper position. Next, a marker pigtail catheter was advanced from the right groin. The left groin cutdown was then performed, using a transverse incision. A 5-French sheath was advanced over the wire. A J-wire was advanced into the descending thoracic aorta. This was exchanged for a Lunderquist wire over the stiff wire. A CO_2 aortogram was again performed. This demonstrated the level of the renal arteries. The main body device, Cook Zenith TFB-28-74 was brought over the Lunderquist wire in the left groin. This was deployed down to the level of the contralateral gate. The pigtail catheter was then pulled down and the top cap was deployed securing the position of the graft. Next, a Kumpe catheter was used to select the contralateral limb from the right femoral sheath. The pigtail catheter was spun confirming proper position. An Amplatz wire was advanced from the right groin. A retrograde injection of contrast was performed from the right groin. Next, the contralateral limb, which was a Cook ZSLE-20-39-ZT, was then deployed with care taken to preserve flow to the right hypogastric artery. Next, the remainder of the main body device was deployed from the left groin. The top cap was then retrieved, and the sheath was removed. Due to the incompetence of a valve, the sheath was then replaced with a Gore DrySeal 18-French Sheath. Next, a retrograde injection of contrast was performed from the left groin. The ipsilateral limb, which was a Cook ZSLE-13-90-ZT was brought onto the field and prepped. This was deployed with care taken to ensure 5 cm of overlap into the left external iliac artery. A 32-French Coda balloon was then used to balloon the proximal and distal sites of fixation, as well as all zones of overlap. Wires were exchanged for a soft wire. This demonstrated evidence of a persistent flow within

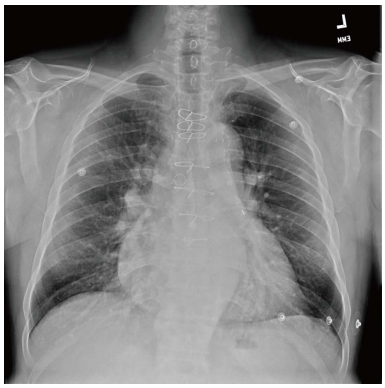

Figure 1 Chest X-ray showing mild cardiomegaly with prominence of central pulmonary arteries.

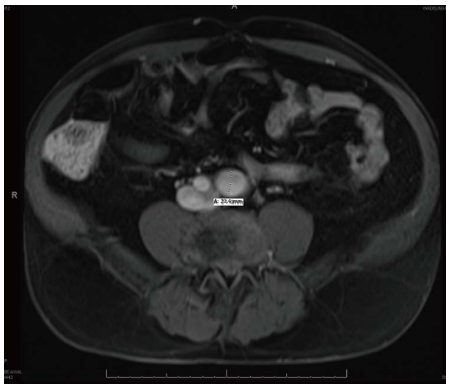

Figure 2 Magnetic resonance imaging of pelvis mark "A" shows 21.4 mm diameter of left common iliac artery.

the fistula that appeared to be less than previous. A completion aortogram was performed. By the end of the procedure cardiac output and cardiac index returned to normal and patient remained stable. Flow through the fistula stopped.

DISCUSSION

MTS is a fairly common, well recognized and rarely diagnosed condition[1]. McMurrich believed the variant was the result of "congenital adhesions" in the common iliac veins[2]. May and Thurner published this syndrome in 1957 and it was widely recognized as MTS in United States whereas Cockett and Thomas published it in 1965 and called the same condition Cockett-Thomas Syndrome in Europe[3]. May and Thurner postulated that the chronic pulsations of the overlying right iliac artery led to development of a "spur" in the vein wall and that this spur would result in partial venous obstruction[4]. Of 430 cadavers, 22% were diagnosed with spurs on left side which is eight times more common than on the right. This came a century after Virchow (1851) first described that thrombosis on the left side was five times more common than on the right side[5]. More recently Kibbe *et al*[6] demonstrated *via* CT the incidence of MTS in asymptomatic patients that correlated with autopsy results reported in the ealy half of twentieth century.

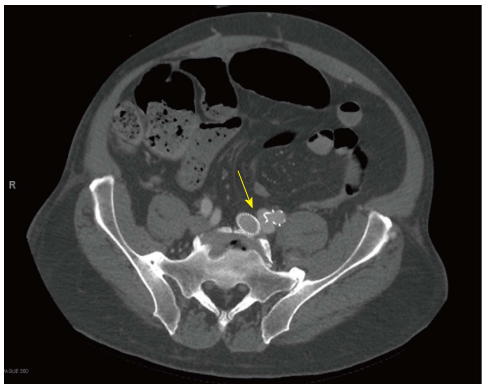

Figure 3 Computed tomography pelvis showing atriovenous fistula of left iliac vessels (yellow arrow) and stent in abdominal aorta.

The goal of treatment of MTS is to reduce symptoms and to reduce the risk of complications. The majority of treatments are geared towards treating DVT. The first known report of treatment of MTS solely by endovascular means was by Berger *et al*[7] in 1995, who successfully placed a venous stent to relieve iliac compression. The initial step in the treatment of DVT in the setting of MTS is thrombectomy with stent placement[8]. Vena Cava filters may be a treatment option for select patients who cannot take anticoagulant medications. Vena Cava filters may not always be used in the treatment of MTS but are used to prevent complications of DVT.

To our knowledge, ours is the first case to report high output cardiac failure due to iatrogenic iliac fistula and its management in MTS. In this case, an iatrogenic iliac fistula resulted because of prior stent placement in left iliac vein to prevent DVT secondary to MTS. We favored aorto-bi-iliac stent graft placement to prevent the fistula from leaking. We managed to successfully reduce cardiac output (11.5 L/min to 8.3 L/min) and cardiac index (5.54 L/min per m sq to 4.0 L/min per squre).

It is important for the practicing physician to note that the identification of high output cardiac failure should lead to a search for the source. In our case, the prior vascular stent placement was a clue to search for the fistula. It is also important to note that stent placement to prevent DVT in MTS can result in iatrogenic fistula.

ACKNOWLEDGMENTS

We thank Prashant Bhensdadia (MD) (Division of Cardiology, Wake Forest Health, 27157), for bringing to notice this interesting case.

COMMENTS

Case characteristics

The patient presented with shortness of breath and bilateral pedal edema.

Clinical diagnosis

The patient was found to high output cardiac failure due to iliac fistula resulting from prior management of May-Thurner syndrome.

Differential diagnosis

Based on patient history, physical exam, and imaging the authors were able to narrow down on the diagnosis by ruling out severe anemia, paget's disease of bone, hyperthyroidism and beriberi.

Imaging diagnosis

The images were obtained by angiography and chest X ray.

Treatment

The patient underwent angiography and stent placement.

Related reports

Berger A, Jaffe JW, York TN. Iliac compression syndrome treated with stent placement. *J Vasc Surg* 1995; 21: 510-514.

Experiences and lessons

It is also important to note that stent placement to prevent deep venous thrombosis in May-Thurner syndrome can result in iatrogenic fistula.

Peer-review

This is an interesting case. English well written, understandable and easily readable.

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CASE REPORT

Intraparotid facial nerve schwannoma: A case report

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Author contributions: Jaiswal A, Mridha AR, Nath D, Bhalla AS and Thakkar A contributed to the writing of the case report in accordance with the standard proposed by the International Committee of Medical Journal Editors.
Ethics approval: This study was case report and so no approval was taken from our institute All India Institute of Medical Sciences, New Delhi.
Informed consent: Consent was taken from the patient at the time of carrying out all their investigations, not again at the time of writing case report.

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Abstract

Facial nerve schwannoma occurring within the parotid gland is a rare tumour. We report a case of schwannoma within the parotid gland in a young female patient, who underwent ultrasound and magnetic resonance imaging (MRI) and subsequent surgical excision of the lesion. The lesion showed hyperintensity on T2-weighted and diffusion-weighted MRI. There was no adjacent lymphadenopathy. Although hyperintensity on diffusion-weighted MRI could suggest malignant tumours, the characteristic "string sign" provided the clue for the diagnosis of schwannoma.

Key words: Parotid; Facial nerve; Schwannoma; String sign; Imaging

Core tip: There is a difference in an approach to surgery for benign and malignant parotid masses. For benign lesions, superficial parotidectomy is done; whereas in a case of malignant tumour total parotidectomy is performed with or without excision of the facial nerve. Clinically, it is very difficult to differentiate them because even malignant tumours have slow growth. Hence, here comes the role of imaging which could suggest the nature of the mass and narrow the differentials.

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INTRODUCTION

Schwannomas of the Facial nerve (FN) are rare benign encapsulated neurogenic lesions. These can arise anywhere along its course[1,2]. Majority of these schwannomas are seen in the intratemporal course of the nerve whereas only 9% are seen in the extratemporal course[3]. In a case series of parotid tumours, schwannomas were found to be very rare accounting for only 2 out of 142 lesions[4]. As presentation is often nonspecific, preoperative

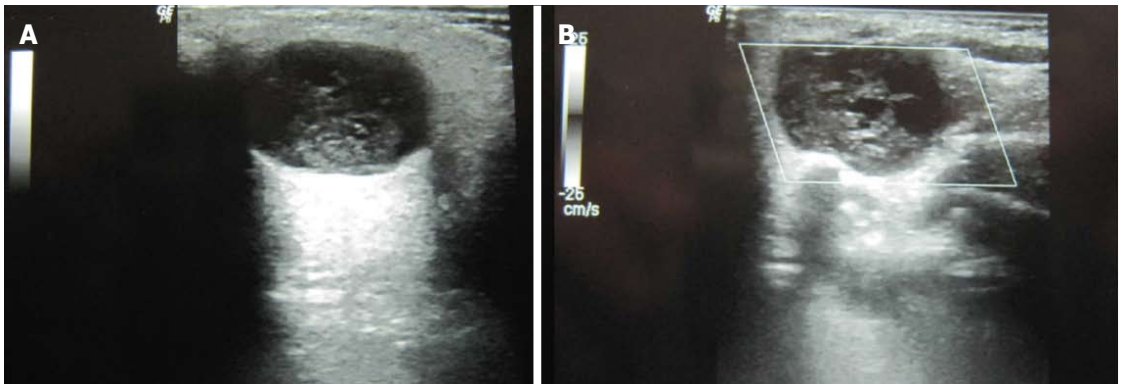

Figure 1 Ultrasound images. A: Left parotid gland shows presence of well defined hypoechoic mass lesion in the superficial lobe with posterior acoustic enhancement; On Color Doppler (B), no internal vascularity could be demonstrated.

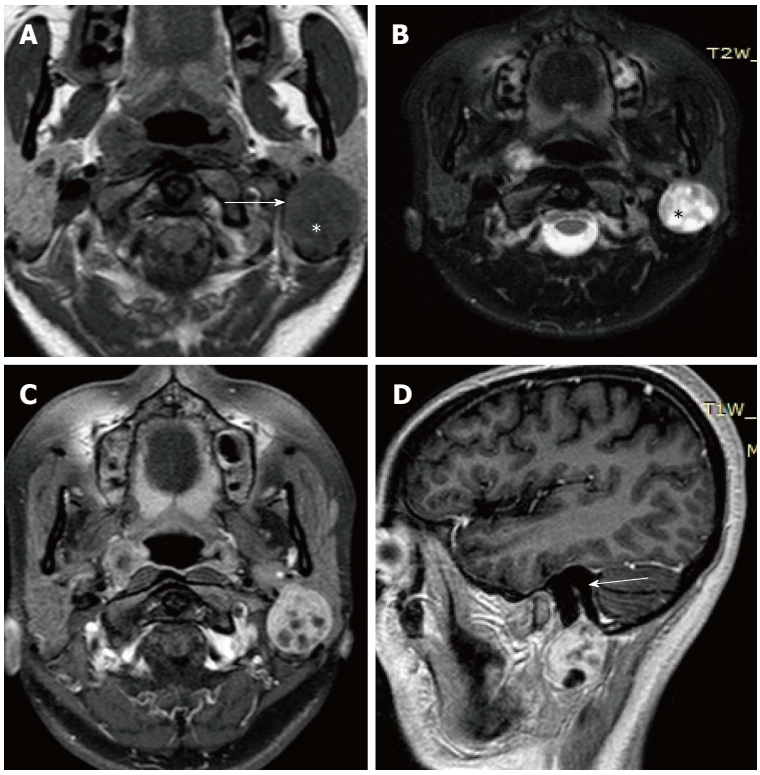

Figure 2 Magnetic resonance imaging images at the level of parotid glands-axial T1W (A), axial T2W (B), Post Gad T1W images in axial (C) and sagittal (D)planes. Arrow in (A) shows presence of well defined intermediate signal intensity mass lesion in the superficial lobe of left parotid with hypointense areas within it (*). These regions (*) are hyperintense on T2W sequence (B) suggestive of myxomatous tissue.On Post contrast images (C and D), the mass enhances homogeneously with few non enhancing areas within. Arrow in D shows the characteristic "string sign" extending along with facial nerve in the stylomastoid foramen.

diagnosis of these tumours is difficult[4].

Clinically these patients do not have any facial nerve dysfunction whereas postoperatively features of facial nerve paresis are common. Hence, it becomes extremely necessary for the surgeon to warn the patient regarding this complication beforehand.

CASE REPORT

A 27-year-old healthy female presented in the surgical clinic with a slow growing painless swelling in the left retromandibular region for the last one year. There was no history of fever or any other constitutional symptoms. Physical examination revealed a soft, non-tender lump measuring approximately 3 cm × 2 cm. Laboratory tests such as complete haemogram, ESR, CRP were found to be within normal limits. Ultrasound examination showed the presence of a well defined, hypoechoic mass in the superficial lobe of left parotid which measured approximately 1.8 cm × 2.3 cm (Figure 1A). The mass showed anechoic areas within it with posterior acoustic enhancement suggestive of cystic component. No calcification or adjacent lymphadenopathy was seen. Color Doppler examination (Figure 1B) did not show any internal vascularity. The differential diagnoses were benign pathologies such as pleomorphic adenoma or less likely an intraparotid lymph node. For further characterisation of the lesion, MR examination of the parotid was performed. MR imaging (Figure 2) revealed a well-circumscribed mass lesion in the left parotid gland. The mass was located just below the stylomastoid foramen with a beak like protrusion into it representing the classic "string sign". T1-weighted

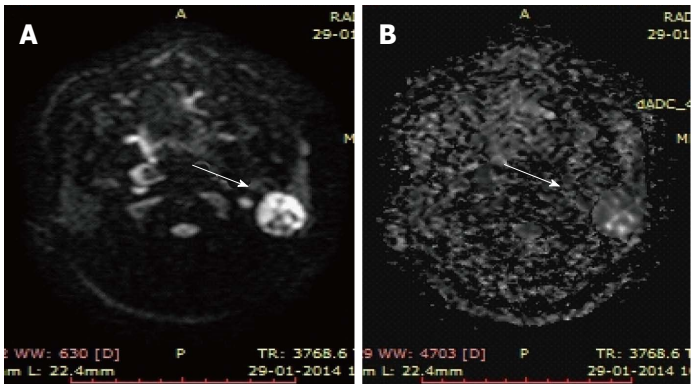

Figure 3 Axial diffusion weighted magnetic resonance imaging. (A) At b = 1000 s/mm^2 hyperintensity is noted throughout the mass (arrow) except the cystic areas. These regions were dark (arrow) on ADC map (B) consistent with restricted diffusion.

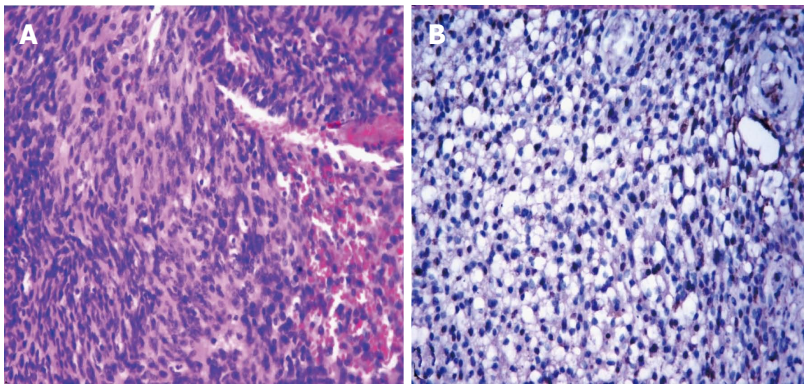

Figure 4 Haematoxylin and eosin stained section shows spindle cells arranged in fascicles (A, × 200). The tumour cells are immunoreactive with S-100 protein (B, × 200).

image (Figure 2A) showed the tumour to be of intermediate signal intensity compared to adjacent muscle, and T2-weighted image (Figure 2B) showed high signal intensity with interspersed areas of lower signal intensity. DWI (Figure 3) showed hyperintensity at b = 1000 s/mm^2 suggestive of restricted diffusion in the solid part of the lesion with facilitated diffusion in the cystic part. On surgical exploration, the mass was found to be in close relationship with the main trunk of facial nerve just below stylomastoid foramen. Postoperatively, the patient developed mild facial paresis. The tumour was histopathologically confirmed to be schwannoma. The spindle cells were immunopositive with S-100 (Figure 4).

DISCUSSION

Schwannomas are benign nerve sheath tumours, composed entirely of differentiated neoplastic Schwann cells. Intraparotid FN schwannoma was first reported by Ibarz in 1927. Since then, fewer than 100 cases of FN schwannomas have been reported. In a study by Fortan *et al*[3], majority of the lesions were found within the intratemporal course, whereas about 9% of the tumours were found in the parotid gland[3]. The frequency of intraparotid schwannomas range from 0.2% to 1.5%[5]. Because of its low prevalence and very few typical clinical and radiological signs associated with it, preoperative diagnosis of intraparotid FN schwannoma is generally difficult.

In a case series of FN schwannomas, the most common clinical manifestation in intratemporal involvement of the nerve was facial nerve dysfunction, whereas in extratemporal course, it was a parotid mass without facial paresis[6].

In patients with a parotid mass, associated facial nerve palsy generally indicates malignancy. But it can also be seen in benign parotid masses such as pleomorphic adenoma and Warthin's tumour. However, none has been reported in intraparotid schwannoma[7].

Similarly in our case, the patient presented with a parotid mass without facial nerve dysfunction, it thus became very difficult to clinically diagnose the schwannoma without the aid of imaging modalities. Ultrasound evaluation in our case showed a well-defined mass with cystic areas within it. Ultrasound when coupled with newer techniques like elastography can help in differentiating benign from malignant parotid masses[8].

MRI images showed that the mass was situated just below the stylomastoid foramen with beaking into the foramen producing the characteristic "string sign". The string sign is due to the vertical orientation of soft tissue on either ends of the mass. The string represents the normal entering or exiting nerve that is in continuity with the nerve sheath tumour.

MRI features described in four cases of facial nerve schwannomas showed heterogeneous lesions that were isointense to brain on both T1- and T2-weighted images[9]. In the present case, the tumour was well defined, isointense and heterogeneously hyperintense to muscle on T1 and T2 weighted images respectively.

Schwannomas may exhibit "target" sign which is characterized by hyperintensity in the periphery

while hypointensity in the centre on T2-weighted images. "Target sign" of neurofibroma is almost pathognomonic[10]. This feature is suggestive of neurogenic neoplasm[11]. In schwannomas, the target sign is due to compactly packed cellular Antoni A regions which is located centrally and loose myxomatous Antoni B regions in the peripheral part[11]. In our case, classical target sign was not observed.

Diffusion weighted imaging features of parotid schwannoma have not been previously described. Restricted diffusion in our case reflects high cellularity of the tumour, supporting the observation that restricted diffusion can be seen in both malignant and benign lesions[12].

Pleomorphic adenomas are the most common tumours of the parotid gland, and a close differential of intraparotid schwannoma due to it being well circumscribed, heterogeneous and hyperintense on T2W sequences[13]. But the presence of "string sign" reasonably excluded the possibility of pleomorphic adenoma in our case.

Adenoid cystic carcinoma, another close differential, is a malignant tumour that has the potential to spread along the nerve sheath[14]. Malignant tumours are hypointense on T2-weighted images and show ill-defined margins on post contrast images[15]. However, T2 hyperintensity and smooth enlargement of the facial nerve canal excludes this diagnosis[14].

In cases of painless swellings of the parotid gland without any neurological involvement, possibility of intraparotid schwannoma should be considered under differentials and the imaging modalities especially MRI revealing characteristic "string sign" further confirms the diagnosis.

COMMENTS

Case characteristics

The patient presented with a slow growing painless swelling in the retromandibular region on left side for the last 1 year.

Clinical diagnosis

The patient's symptoms were nonspecific and presence of painless progressive swelling over a period of 1 year pointed to its benign nature.

Differential diagnosis

Pleomorphic adenoma was ruled out as there was "string sign" showing extension along the facial nerve into the stylomastoid foramen. Adenoid cystic carcinoma was ruled out as the mass showed T2 hyperintensity and well defined margins. Malignant tumours are T2 hypointense with ill defined margins. Even extension into stylomastoid foramen was accompanied by smooth enlargement of the foramen without any irregular erosion.

Laboratory diagnosis

Blood tests were non contributory.

Pathological diagnosis

The excised tumour measured 2.5 cm × 2 cm. Cut surface was fleshy with focal haemorrhage. Microscopic examination showed cellular spindle cells arranged in fascicles. Tumour cells exhibited oval to elongated hyperchromatic nuclei, inconspicuous nucleolus, and fibrillary eosinophilic cytoplasm. Few thick walled blood vessels were seen. No mitosis or necrosis was seen. The tumour cells were immunopositive with S-100; while negative for smooth muscle actin and estrogen receptor. MIB-1 labelling index was < 2%. A diagnosis of schwannoma was given.

Treatment

Under general anaesthesia, excision of the tumour mass was done and sent for histopathological examination.

Related reports

Chung SY *et al* article Facial nerve schwannomas: Computed tomography and magnetic resonance findings published in 1998 in *Yonsei Med J* provide a brief but cumulative overview on the case topic.

Term explanation

Facial nerve schwannoma is a rare neurogenic tumour that arises from the schwann cells of the neurons.

Experiences and lessons

One lesson that the authors learnt from this case was to consider facial nerve schwannoma in the differential diagnosis of parotid mass when a patient presents with painless progressive swelling and imaging shows characteristic "string sign". Restricted diffusion reflects its high cellularity, supporting the observation that restricted diffusion can be seen in both malignant and benign lesions.

Peer-review

Good paper.

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CASE REPORT

Rare case of upper gastrointestinal bleeding in achalasia

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Author contributions: Xie XJ and Zhan SH supervised the patient's diagnosis and treatment; Zhang WW wrote the manuscript; Geng CX revised the manuscript.

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Conflict-of-interest: None.

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Abstract

Achalasia is a prototypic esophageal motility disorder with complications including aspiration-pneumonia, esophagitis, esophageal-tracheal fistula, spontaneous rupture of the esophagus, and squamous cell carcinoma. However, achalasia is rarely associated with esophageal stones and ulcer formation that lead to upper gastrointestinal bleeding. Here, we report the case of a 61-year-old woman who was admitted to our department after vomiting blood for six hours. Physical examination revealed that the patient had severe anemia and mild palpitation in the upper abdomen. CT revealed lower esophageal dilatation and esophageal wall thickening, and an emergency upper endoscopy showed that the esophagus was substantially expanded by a dark round stone, with multiple ulcers on the esophageal wall and a slit in the cardiac mucosa with a large clot attached. The patient's history included ingestion of 1 kg hawthorn three days prior. The acute upper gastrointestinal bleeding was caused by Mallory-Weiss syndrome associated with achalasia and an esophageal stone. For patients with achalasia, preventing excessive ingestion of tannins is crucial to avoid complications such as bleeding and rupture.

Key words: Achalasia; Esophageal stone; Mallory-Weiss syndrome; Upper gastrointestinal bleeding

Core tip: Achalasia is the prototypic esophageal motility disorder that leaves patients at risk for various complications. This is a rare report of long-term achalasia associated with esophageal stone and ulcer formation leading to upper gastrointestinal bleeding caused by Mallory-Weiss syndrome. This paper highlights the importance of avoiding excess tannin ingestion for patients with achalasia to prevent the development of complications such as bleeding and rupture.

Zhang WW, Xie XJ, Geng CX, Zhan SH. Rare case of upper gastrointestinal bleeding in achalasia. *World J Clin Cases* 2015; 3(3): 327-329 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i3/327.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i3.327

INTRODUCTION

Achalasia is the prototypic esophageal motility disorder

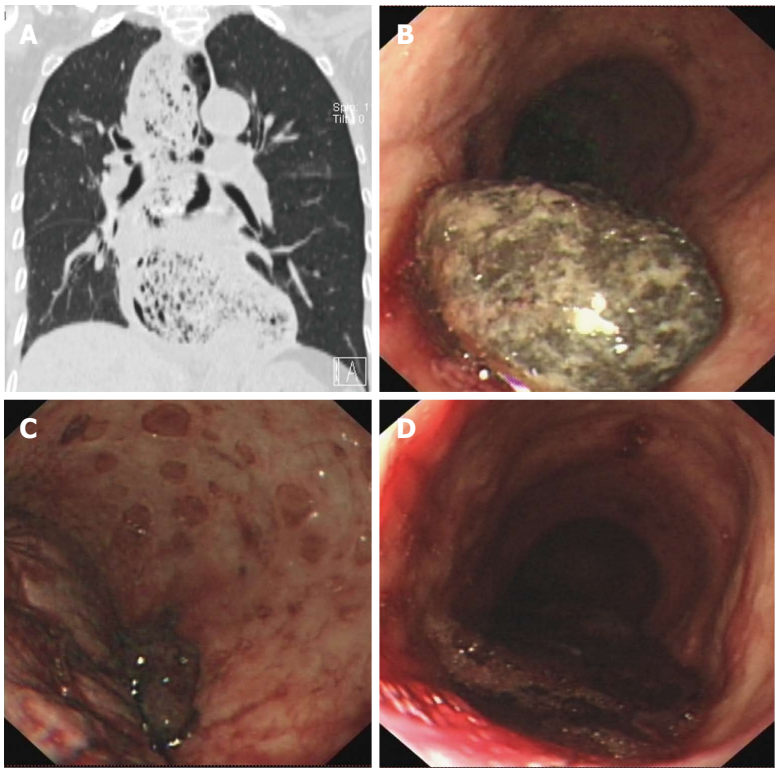

Figure 1 CT and endoscopy imaging. A: CT showed lower esophageal dilatation and esophageal wall thickening; Upper endoscopy revealed; B: A large, dark round stone; C: Multiple ulcers on the esophageal wall; D: A slit in the cardiac mucosa with a large clot attached.

characterized by a hypertensive lower esophageal sphincter with incomplete relaxation upon swallowing, accompanied by aperistalsis of the esophageal body[1]. Patients with achalasia are at risk for developing complications such as aspiration-pneumonia, esophagitis, esophageal ulcers and bleeding, esophageal-tracheal fistula, spontaneous rupture of the esophagus, and squamous cell carcinoma[2]. The prevalence is 10 per 100000 in the United States, involving an equal distribution of men and women of all ages and from all ethnicities[3]. We report a rare case of upper gastrointestinal bleeding in a patient with achalasia that was associated with an esophageal stone, ulcer formation and Mallory-Weiss syndrome.

CASE REPORT

A 61-year-old woman who complained of vomiting blood for six hours was admitted to our department. She experienced dizziness, palpitations, sweating, and fatigue, but did not present with fever, rash or jaundice. Her medical history revealed achalasia that had been present for 30 years, for which she had declined any treatment. When questioned, she reported ingesting 1 kg hawthorn within the past 3 d.

A physical examination indicated that the patient had severe anemia due to pale conjunctiva and nail beds, and mild palpitation in the upper abdomen. Routine blood tests showed: red blood cell count, 1.34×10^{12}/L; hemoglobin, 41 g/L; white blood cell count, 11.25×10^{9}/L; platelet count, 150×10^{9}/L; blood urea nitrogen, 15.99 mmol/L; and creatinine, 98 μmol/L. Lower esophageal dilatation and esophageal wall thickening were revealed upon CT examination (Figure 1A). An emergency upper endoscopy was performed revealing substantial expansion of the esophagus by a dark round stone (Figure 1B), multiple ulcers on the esophageal wall (Figure 1C), and a slit in the cardiac mucosa with a large clot attached (Figure 1D). Endoscopic sprinkling hemostasis and injection of 2.5% sodium bicarbonate were applied to cease the bleeding and dissolve the stone.

DISCUSSION

In achalasia, injury to the lower esophageal sphincter neurons and the loss of the main functional inhibitory neurotransmitter result in a hypertensive sphincter that loses its ability to relax, leading to stenosis of cardia and lower esophagus expansion. Occasionally, patients will present with persistent food retention, and esophageal stone formation can occur in those who have ingested foods rich in tannins, such as hawthorn and persimmon. The pressure of stones can cause multiple ulcers and even upper gastrointestinal bleeding. The discomfort when swallowing and frequent nausea and vomiting can then lead to the development of Mallory-Weiss syndrome.

Currently, there are no curative treatments for achalasia cardia, rather palliative measures are provided, such as oral nitrates or calcium channel blockers, endoscopic pneumatic dilation, injection of sclerosant substances, or surgery[4-6]. However, complications such as bleeding and rupture can be prevented by avoiding excessive ingestion of tannins.

COMMENTS

Case characteristics

A 61-year-old female patient complained of vomiting blood for six hours.

Clinical diagnosis

Acute upper gastrointestinal bleeding from Mallory-Weiss syndrome associated with achalasia.

Laboratory diagnosis

Red blood cell count, 1.34 × 10^{12}/L; hemoglobin, 41 g/L; white blood cell count, 11.25 × 10^{9}/L; platelet count, 150 × 10^{9}/L; blood urea nitrogen, 15.99 mmol/L; creatinine 98 μmol/L.

Imaging diagnosis

CT showed lower esophageal dilatation and esophageal wall thickening. Upper endoscopy revealed a large round stone causing substantial expansion of the esophagus and multiple ulcers on the esophageal wall. A slit in the cardiac mucosa was observed with a large clot attached.

Treatment

Endoscopic sprinkling hemostasis and injection of 2.5% sodium bicarbonate were applied to cease the bleeding and dissolve the stone.

Related reports

Although there are some reports of achalasia combined with esophageal intramural hematoma or esophageal varices, reports of achalasia combined with esophageal stones, ulcer formation and Mallory-Weiss syndrome are rare.

Term explanation

Achalasia is characterized by esophageal aperistalsis and impaired relaxation of the lower esophageal sphincter.

Experiences and lessons

This report not only presents a rare case of upper gastrointestinal bleeding associated with achalasia, but also aims to inform patients with achalasia to avoid excessive ingestion of tannin-rich foods to prevent related complications.

Peer-review

This is a very interesting manuscript reporting a rare case of upper gastrointestinal bleeding as a result of long-term achalasia associated with esophageal stones.

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Bleeding and clotting in hereditary hemorrhagic telangiectasia

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Abstract

Hereditary hemorrhagic telangiectasia (HHT) is a relatively common inherited vascular disorder that was first described in 1864, and is notable for epistaxis, telangiectasia, and arterial venous malformations. While genetic tests are available, the diagnosis remains clinical, and is based on the Curacao criteria. Patients with HHT are at increased risk for both bleeding and clotting events. Because of these competing complications, hematologists are often faced with difficult clinical decisions. While the majority of management decisions revolve around bleeding complications, it is not infrequent for these patients to require anticoagulation for thrombosis. Any anticoagulation recommendations must take into account the bleeding risks associated with HHT. Recent reviews have found that HHT patients can be safely anticoagulated, with the most frequent complication being worsened epistaxis. Large clinical trials have shown that factor Ⅱa and Ⅹa inhibitors have less intracranial bleeding than warfarin, and basic coagulation research has provided a possible mechanism. This article describes the anticoagulation dilemma posed when a 62-year-old female patient with a history of bleeding events associated with HHT was diagnosed with a pulmonary embolism. The subsequent discussion focuses on the approach to anticoagulation in the HHT patient, and addresses the role of the new oral anticoagulants.

Key words: Anticoagulation; Hereditary hemorrhagic telangiectasia; Hemorrhage; Thrombosis; Rivaroxaban; Apixaban; Dabigatran; Warfarin

Core tip: This article reviews an inherited disorder, hereditary hemorrhagic telangiectasia, in the context of a complicated clinical case. It highlights the problem of balancing the risks of bleeding and thrombosis, and raises the question of whether the new oral anticoagulants might provide safer therapy in such patients who need antithrombotic therapy.

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CASE PRESENTATION

A 62-year-old female presented to our hematology clinic with previously diagnosed hereditary hemorrhagic telangiectasia (HHT). The patient had lifelong complications associated with this illness, including recurrent epistaxis and pulmonary arteriovenous malformations (AVMs). The patient had received multiple embolization procedures in the past to prevent complications from her pulmonary AVMs. The epistaxis was debilitating, with episodes occurring up to 5 times per day. Further medical history included the presence of a heterozygous prothrombin gene mutation ($PT^{A20120G}$), discovered from a thrombophilia evaluation for recurrent superficial phlebitis 5 years prior. The patient's more recent medical history included the development of a brain abscess 6 mo prior to presentation, which was treated with surgical evacuation. During her post-operative course, she developed a pulmonary embolism (PE) secondary to a deep venous thrombosis (DVT) in her left lower extremity. Initially, the patient was treated with the insertion of an inferior vena cava (IVC) filter, which was placed because of HHT-related bleeding concerns. The IVC filter ultimately developed extensive thrombosis, and the patient was initiated on warfarin therapy 3 mo prior to presentation. Since warfarin was initiated, she has had an increase in the severity of her epistaxis, but not the frequency. On physical exam, the patient had classic telangiectasia on the tongue and oral mucosa (Figure 1). The clinical question posed to our hematology clinic was how to proceed with anticoagulation for DVT/PE in the setting of a genetic thrombophilia and active bleeding in a patient with HHT.

BACKGROUND

HHT, alternatively known as Osler-Weber-Rendu syndrome, is a relatively common autosomal dominant disorder, with an overall frequency of 1 per 5000 to 10000 individuals[1]. HTT was first described in 1864, but this account did not note a pattern of inheritance[2]. Subsequently, multiple case reports specifically included a familial component in their descriptions[3-5]. In 1909, Frederic Hanes officially used the phrase "hereditary hemorrhagic telangiectasia" for the first time[6].

As the name suggests, the disease is notable for autosomal dominant inheritance (hereditary), bleeding events (hemorrhagic), and visibly dilated blood vessels (telangiectasia). Additionally, most patients are affected by larger AVMs, commonly found in the pulmonary, hepatic, and cerebral vasculature[7]. Specific

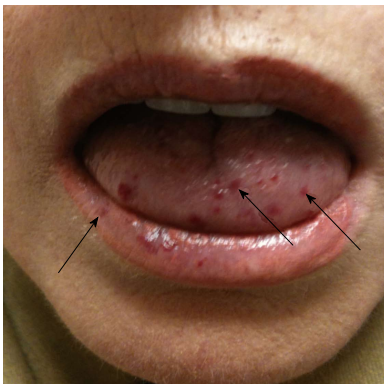

Figure 1 Arrows indicate telangiectatic lesions on tongue and lip of patient.

complications relate to the location of telangiectasia and AVMs, including, but not limited to, epistaxis, gastrointestinal bleeding, visible skin/mucosal manifestations, pulmonary arterial hypertension, pulmonary hemorrhage, and cerebral abscesses. Paradoxically, HHT patients are also burdened by a prothrombotic state due to elevated plasma levels of factor Ⅷ[8]. The balance between hemorrhage and thrombosis is particularly difficult to manage in these patients, and was exemplified in our patient described above.

The underlying pathology in patients with HHT is a primary defect in the vascular wall. The pathophysiology leading to this defect is complex. Patients inherit a mutation in an autosomal dominant fashion; the penetrance of which is highly variable. The three major genes that have been identified are: *ENG* encoding endoglin (HHT type 1), *ACVRL1* encoding activin receptor-like kinase (ALK-1) (HHT type 2), and *SMAD4* encoding Smad4 (HHT in association with juvenile polyposis, JPHT)[9-11]. Over 80% of patients with HHT will have mutations in either the *ENG* or *ACVRL1* gene, with the *ENG* gene accounting for the majority[12]. There is no common mutation in either the *ENG* or *ACVRL1* genes, with over 470 mutations having been described in the *ENG* gene and 375 in the *ACVRL1* gene[13]. Additionally, researchers have been studying two other gene mutations that can cause HHT: *HHT3* and *HHT4*.

Animal models have shed light on how these mutations lead to vascular wall abnormalities. The mutated *HHT* genes described above encode proteins that alter signaling by the transforming growth factor-β superfamily[7]. It is suggested that endoglin, ALK-1, and Smad4 are all part of a common signaling pathway that is altered in HHT. Additionally, studies have shown that vascular endothelial growth factor is increased in HHT patients[14]. In the setting of HHT and an angiogenic stimulus, there is increased proliferation of endothelial cells, excessive vessel branching, and decreased recruitment of mural cells[7]. Ultimately, this process leads to the formation of telangiectases,

Table 1 The Curacao criteria for the diagnosis of hereditary hemorrhagic telangiectasia

Criteria	Description	Percent manifestation
1 Epistaxis	Spontaneous, recurrent	90
2 Telangiectases	Multiple, at characteristic sites:	80
	Lips	
	Oral cavity	
	Finger tips	
	Nose	
3 Visceral lesions	Gastrointestinal telangiectasia	15-30
	Pulmonary AVMs	50
	Hepatic AVMs	30-70
	Cerebral AVMs	10-20
	Spinal AVMs	< 1
4 Family history	Affected first degree relative	
Diagnosis of HHT:		
Definite: 3-4 criteria	Possible: 2 criteria	Unlikely: 0-1 criterion

HHT: Hereditary hemorrhagic telangiectasia; AVMs: Arteriovenous malformations.

which are focal dilatations of postcapillary venules. Once fully developed, these malformed vessels are dilated, convoluted, extend through the dermis, and have excessive layers of smooth muscle without elastic fibers[15,16]. These vessels lack capillaries and connect directly to dilated arterioles. AVMs are similar to telangiectases but have a direct connection between veins and arteries, and are thus much larger. These abnormal HHT blood vessels are prone to bleeding because of their inherently abnormal wall structure, as well as the presence of high perfusion pressures[7].

CLINICAL MANIFESTATIONS

Clinical diagnosis

The diagnosis of HHT remains clinical, although genetic testing has been increasingly utilized. The classic triad of epistaxis, telangiectases, and family history lacks sensitivity and specificity, thus diagnostic criteria were formally created, which are generally referred to as the Curacao criteria (Table 1)[17]. These criteria were recently validated in 263 patients who were screened for HHT and had first degree relatives available for genetic testing[18]. This analysis found that the positive predictive value for a definite clinical diagnosis was 100%, and a negative predictive value for an unlikely clinical diagnosis was 97.7%. Fifty-two study participants had a possible clinical diagnosis, of which 17 (32.7%) had an HHT-causing mutation. Therefore, the utility of genetic testing is most apparent in those with a possible clinical diagnosis. This lends itself to the application of a diagnostic algorithm that can be used to combine the clinical criteria with genetic testing (Figure 2).

Bleeding in HHT

Patients with HHT are at increased risk for both bleeding and thrombosis. Bleeding complications can arise from any location where telangiectases and AVMs are found. The most prevalent form of bleeding in HHT patients is epistaxis, which can be severe and recurrent. Mucosal telangiectases are very common, but their presence is mostly a cosmetic concern. Telangiectases in the gastrointestinal tract are an important source of chronic bleeding, and contribute to the common diagnosis of iron-deficiency anemia found in HHT patients. Perhaps the most important lesions in terms of both morbidity and mortality are pulmonary AVMs. These are present in roughly 50% of HHT patients, although the majority are asymptomatic[7]. The clinical implications of pulmonary AVMs can be divided into two categories: (1) Right-to-left shunting; and (2) Hemorrhage. Right-to-left shunting can result in severe complications, such as brain abscesses and ischemic strokes, as well as less dramatic events such as migraines and dyspnea[19]. Hemorrhagic complications from pulmonary AVMs can include hemoptysis as well as hemothorax. The consequences of pulmonary AVMs can be so severe that current recommendations include screening asymptomatic HHT patients with transthoracic contrast echocardiography (TTCE), or chest computed tomography if TTCE is not available[20]. Cerebral vascular malformations are less common than pulmonary AVMs, but can have devastating effects. Perhaps the most feared complication of HHT is intracranial hemorrhage (ICH), which is most commonly seen with cerebral arteriovenous fistulae (AVF). As vascular lesions in the brain decrease in size, ICH becomes less common. Therefore, AVFs have the greatest risk of ICH, AVMs have intermediate risk, and telangiectasia have the lowest risk. Current guidelines recommend screening for cerebral AVMs with magnetic resonance imaging[20]. One of the more prevalent, but usually asymptomatic, aspects of HHT is hepatic vascular malformations[21]. These can occur as hepatic AVMs (hepatic artery to hepatic vein), hepato-portal VMs (hepatic artery to portal vein), and porto-venous VMs (portal vein to hepatic vein). Lastly, patients rarely may have spinal AVMs that can lead to hemorrhage and subsequent paraplegia.

Thrombosis in HHT

Despite the presence of an overwhelming bleeding propensity, HHT patients also suffer from thrombotic complications. First, they may develop paradoxical thromboembolic stroke from pulmonary AVMs as described above. Second, it seems these patients may also possess an inherent prothrombotic state relating to disturbances in the regulation of coagulation at the endothelial surface. A recent study compared the presence of plasma proteins in HHT-affected adults without a history of thrombosis with non-HHT controls[8]. The researchers found statistically significant elevations in von Willebrand Factor and Factor Ⅷ (FⅧ) in HHT-affected adults. The researchers then evaluated the presence of elevated FⅧ levels in the general HHT population and found that 87 of

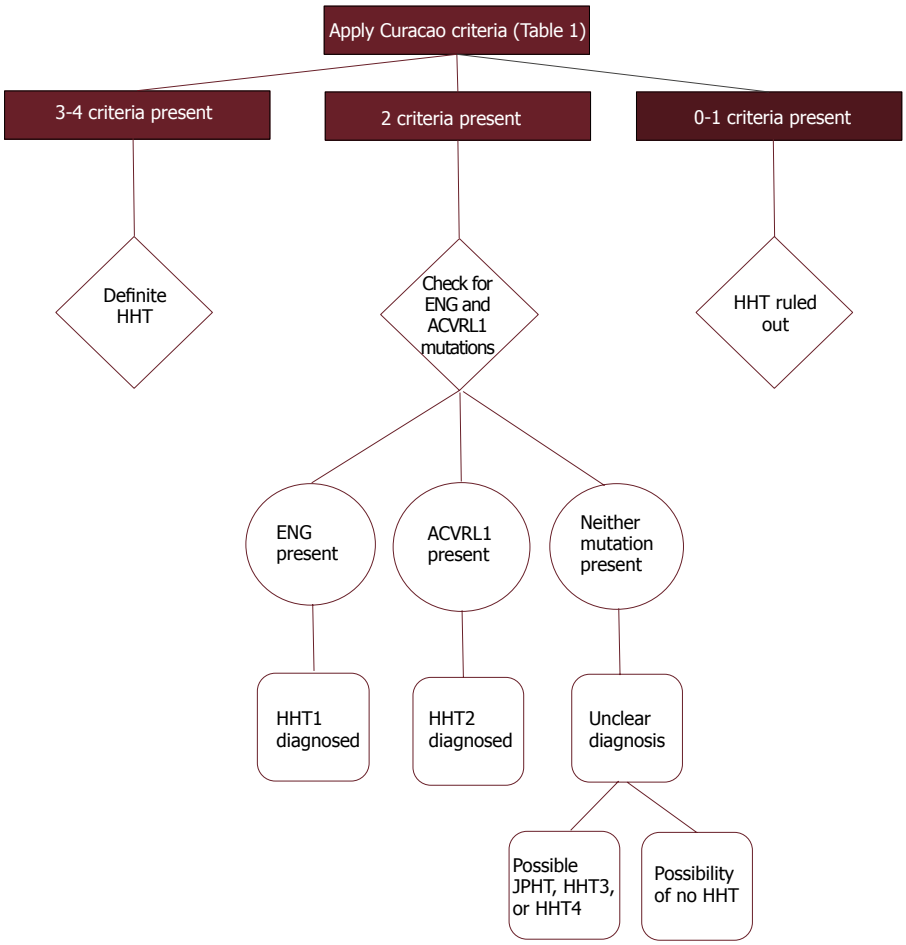

Figure 2 Diagnostic algorithm incorporating the Curacao criteria and genetic testing. HHT: Hereditary hemorrhagic telangiectasia.

125 (70%) individuals had measurements exceeding the upper limit of normal. An inverse correlation was also described between FⅧ level and activated partial thromboplastin time. Furthermore, when compared to HHT individuals with no history of thrombus, HHT individuals who ultimately developed a thrombotic event were more likely to have an elevated FⅧ level when it was initially measured at least 10 mo prior. To summarize the findings of this study, individuals with HHT have higher levels of FⅧ than non-HHT controls, and the degree of FⅧ elevation correlates with future thrombotic risk.

MANAGING THROMBOSIS IN HHT

Traditionally, physicians have been reluctant to treat HHT patients with either antiplatelet or anticoagulant therapy even if otherwise indicated. A recent survey found that 153 of 379 (40.4%) patients with HHT who received antiplatelet or anticoagulant therapy reported no change in epistaxis[22]. Furthermore, 86.9% of patients reported no hemorrhagic events other than epistaxis associated with antiplatelet and anticoagulant use. This survey supports the reasoning that HHT patients who have a strong indication for antiplatelet or anticoagulant use, should not have these agents withheld. Another study examining the use of antithrombotic agents in HHT patients arrived at a similar conclusion[23]. As in the Devlin study, this study found worsening of epistaxis to be the most common complication. There were no new or progressive cases of pulmonary or cerebral hemorrhage, likely because all patients in this study were pre-screened. These data again support the notion that anticoagulation should not be withheld from HHT patients with strong indications for its use. Although bleeding is the major complication associated with all anticoagulant medications, the rate of bleeding varies between agents, and may be of clinical importance in HHT patients.

Choice of anticoagulant agent

For the prior half century, warfarin has been the gold standard of oral anticoagulation. Within the past decade, new oral anticoagulants have been developed and evaluated in clinical trials. Specifically, these agents include the direct thrombin (factor Ⅱa) inhibitor, dabigatran, and the factor Ⅹa inhibitors, rivaroxaban and apixaban (Figure 3). Indications vary by agent, but all have been studied for stroke prevention in non-valvular atrial fibrillation and prevention and treatment of venous thromboembolism (VTE). Interestingly, dabigatran, as well as the factor Xa inhibitors, have

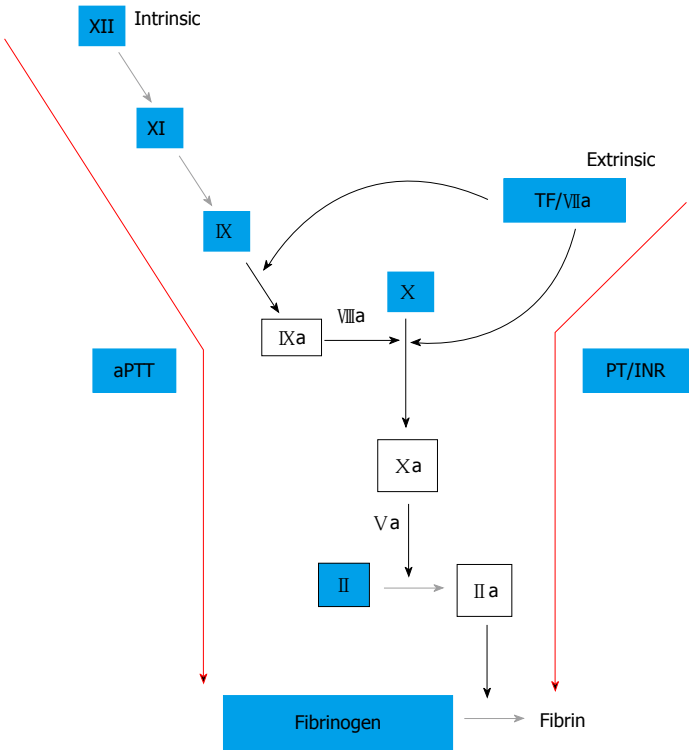

Figure 3 Coagulation cascade. Factors Xa and IIa (thrombin) are the principal targets for the new oral anticoagulants, while factors II, VII, IX, X, and protein C and S are decreased by the vitamin K antagonist, warfarin. Warfarin's effect on the factor VIIa/TF complex is a possible cause of the increased intracranial bleeding seen in warfarin compared to the newer targeted agents.

been associated with reduced intracranial hemorrhage compared to warfarin in patients with non-valvular atrial fibrillation[24-26]. In studies examining the treatment of acute VTE, dabigatran and rivaroxaban had similar bleeding rates compared to warfarin, while apixaban had decreased bleeding relative to warfarin[27-30].

The mechanism by which these new agents lead to similar or improved efficacy in relation to warfarin, while achieving less intracranial hemorrhage, has been evaluated. A recent study compared peak thrombin generation in the presence of both warfarin and dabigatran[31]. This study found that, while the mean lag times were equally prolonged in each group, the peak thrombin level was significantly decreased in the warfarin group. Furthermore, the authors found that in the presence of tissue factor (TF), the peak thrombin level increases. Lastly, it was shown that warfarin has a greater inhibitory effect on peak thrombin level in the presence of TF than dabigatran. When the mechanism of action of warfarin and dabigatran are compared, it is notable that one major difference is the suppressive effect warfarin has on formation of the TF-factor Ⅶa complex which initiates coagulation (Figure 3). Dabigatran, as well as the factor Ⅹa inhibitors, lack this inhibitory effect since these agents blockade the coagulation cascade further downstream. The brain, in particular, has a rich endowment of tissue factor, thus the effect of warfarin is enhanced in the brain relative to dabigatran, which may explain the increased intracranial hemorrhage seen in patient's taking warfarin *vs* the newer target-specific oral anticoagulants. Additionally, there is evidence that some of this effect may be due to reduced drug entry through the blood-brain barrier relative to warfarin[32].

Multiple studies have examined the efficacy of antithrombotic agents in the prevention of recurrent VTE after an initial course of anticoagulation. Both low-dose warfarin and low-dose aspirin have been shown to effectively reduce the risk of recurrent VTE, when compared to placebo, without increasing the risk of bleeding complications[33,34]. As for the new oral anticoagulants, dabigatran and rivaroxaban have been shown to effectively decrease the risk of recurrent VTE, but both increased the risk of clinically relevant bleeding when compared to placebo[35,36]. Notably, dabigatran had a lower risk of major or clinically relevant bleeding when compared to regular-dose warfarin (INR 2-3). Apixaban has been shown to have similar bleeding risk to aspirin when evaluated for stroke prevention in nonvalvular atrial fibrillation[37]. More recently, apixaban was compared at two doses (2.5 mg and 5 mg, twice daily) *vs* placebo in the extended treatment of VTE[38]. In this study, each dose was effective in reducing the risk for recurrent VTE relative to placebo. There was no increased risk of major or clinically relevant bleeding in either dose of apixaban *vs* placebo, or between the two doses.

Due to the inherent bleeding risk in HHT patients, any approach to anticoagulation that may decrease the risk of bleeding would be prudent. Through the use of either a factor Ⅱa or Ⅹa inhibitor, as opposed to warfarin for the acute treatment of VTE, the TF-Ⅶa interaction can be preserved, which may lead to decreased bleeding events, particularly in the brain. Based on the studies reviewed above, it would be reasonable to use either low-dose aspirin, low-dose warfarin, or apixaban to prevent recurrent VTE in a patient at risk for bleeding. Further research is

necessary to fully describe these important differences between anticoagulant agents.

CONCLUSION

The patient presented with a difficult clinical scenario: new venous thromboembolism in the setting of a prothrombin gene mutation and bleeding complications secondary to HHT. The physicians who initially cared for our patient understood this dilemma and opted to place an IVC filter. Unfortunately, this filter thrombosed and the patient was initiated on warfarin. While on warfarin, the patient, like most patients studied, complained of worsening epistaxis, but no major bleeding events.

Our initial approach was to ensure she had been adequately screened for AVMs (pulmonary and cerebral) that could cause severe harm to our patient prior to the initiation of anticoagulation. The next management decision was to address the anticoagulation needs of our patient. Despite her bleeding risk, she had a strong indication for anticoagulation given progressive DVT and PE despite the presence of an IVC filter, as well as a prothrombotic state related to heterozygosity for the prothrombin gene mutation, immobilization, and recent surgery. Our primary options included: continuing warfarin (normal or low-dose); changing to low-dose aspirin; changing to a new oral anticoagulant, such as dabigatran, rivaroxaban, or apixaban; or discontinuing all anticoagulation. At the time of her appointment she had received 3 mo of anticoagulation with warfarin. Despite the role her recent surgery played in provoking the VTE, the presence of a thrombosed IVC filter and heterozygosity for prothrombin gene mutation indicated that she should receive long-term treatment. After 6 mo of anticoagulation, her IVC filter was retrieved as her thrombus burden had declined significantly in her IVC and lower extremities. We hoped retrieval would decrease her risk for recurrent VTE and allow us to eventually discontinue warfarin. Because her epistaxis was more symptomatic on therapeutic anticoagulation, we reduced her INR target range to 1.5-2. After 3 mo of warfarin therapy at a lower target INR, we discontinued anticoagulation completely. Unfortunately, 6 mo after her filter retrieval she developed recurrent right leg pain and a new iliofemoral DVT was identified. Subsequently, a new Celect IVC filter was placed. While recovering from this procedure, the patient developed abdominal pain and was ultimately found to have extensive thrombosis of the portal, superior and inferior mesenteric veins. Therapeutic anticoagulation with warfarin was resumed, and, after six months, her epistaxis became more severe, so her INR range was again reduced to 1.5-2. She has now been on low-dose warfarin for eighteen months and remains free of recurrent VTE or severe epistaxis. If a change to her anticoagulant regimen is warranted in the future, other options include low-dose aspirin and apixaban to prevent recurrent VTE, while minimizing the risk of bleeding.

HHT patients present hematologists with difficult clinical decisions due to the inherent bleeding and thrombotic complications associated with the disease. The majority of management decisions revolve around bleeding complications. When a thrombotic complication arises, anticoagulation recommendations must take into account the bleeding risks associated with HHT. Recent reviews have found that HHT patients can be safely anticoagulated, with the most frequent complication being worsened epistaxis. Patients should be aggressively screened for pulmonary and cerebral AVMs prior to initiating any anticoagulant. Large clinical trials have shown that factor Ⅱa and Ⅹa inhibitors have less intracranial bleeding than warfarin, and basic coagulation research has provided a possible mechanism. In light of this, there is an important role for the use of factor Ⅱa and Ⅹa inhibitors in HHT patients requiring acute anticoagulation. For long-term anticoagulation to prevent VTE recurrence, the agents associated with the lowest risk of bleeding relative to placebo are low-dose warfarin, low-dose aspirin, and apixaban. These agents will have an important role in the long-term prevention of VTE recurrence in patients with HHT.

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MINIREVIEWS

Review of health risks of low testosterone and testosterone administration

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Abstract

Hypogonadism is prevalent in older men and testosterone replacement therapy (TRT) for older hypogonadal men is a promising therapy. However, a number of important clinical concerns over TRT safety remain unsolved due to a lack of large-scale randomized clinical trials directly comparing the health risks of untreated hypogonadism *vs* long-term use of TRT. Meta-analyses of clinical trials of TRT as of 2010 have identified three major adverse events resulting from TRT: polycythemia, an increase in prostate-related events, and a slight reduction in serum high-density lipoprotein cholesterol. There are other purported health risks but their incidence can be neither confirmed nor denied based on the small number of subjects that have been studied to date. Furthermore, subsequent literature is equivocal with regard to the safety and utility of TRT and this topic has been subject to contentious debate. Since January 2014, the United States Food and Drug Administration has released two official announcements regarding the safety of TRT and clinical monitoring the risks in TRT users. Additionally, the health risks related to the clinical presentation of low or declining testosterone levels not been resolved in the current literature. Because TRT is prescribed in the context of putative risks resulting from reduced testosterone levels, we reviewed the epidemiology and reported risks of low testosterone levels. We also highlight the current information about TRT utilization, the risks most often claimed to be associated with TRT, and current or emerging alternatives to TRT.

Key words: Hypogonadism; Epidemiology; Aging; Low testosterone; Testosterone replacement therapy

Core tip: The topic of testosterone replacement therapy which has seen two official announcements for the United States Food and Drug Administration in 2014, is the subject of several large studies both prospective and retrospective, and there is unsettled debate about the safety and efficacy of this treatment. Readers should become familiar with this topic and be aware that further

publications and announcements are likely in the near future.

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LOW TESTOSTERONE EPIDEMIOLOGY AND RISKS

Production of testosterone (T) and serum T concentrations decline as men age. Hypogonadism may be defined either as serum concentration of T (either total T, bioavailable T or free T) or as low T plus symptoms of hypogonadism. The Baltimore Longitudinal Study on Aging reported the incidence of total serum T < 325 ng/dL to be 20% for men in their 60s, 30% for men in their 70s and 50% for men over 80[1]. In an authoritative review by Kaufman and Vermeulen[2] in 2005, similar rates and trends in the reduction of total serum T level were reported. The Massachusetts Aging Male Study reported that 12.3% of men aged 40 to 70 had a total serum T of < 200 ng/dL with 3 or more symptoms of hypogonadism[3]. The Boston Area Community Health Study reported that 5.6% of men aged 30 to 70 were hypogonadal, as defined by total serum T < 300 ng/dL; or, free serum T < 5 ng/dL plus 3 or more symptoms of hypogonadism[4].

Hypogonadism causes a wide range of signs and symptoms including loss of libido, erectile dysfunction, diminished cognitive function, depression, lethargy, osteoporosis, loss of muscle mass and strength[5]. In a literature review on the burden of hypogonadism in adult men, Maggi *et al*[6] demonstrated strong evidence associating hypogonadism with sexual dysfunction and cognitive impairment; and, less compelling evidence associating hypogonadism with depressive symptoms, fractures, and mortality. Several recent studies also reported the health risks associated with untreated hypogonadism, including increased all-cause mortality[7,8], coronary artery disease[9], and stroke[10].

Unfortunately, studying the health risks associated with untreated hypogonadism is often limited by the lack of universally accepted diagnostic criteria, and by study design variations[6].

Hypogonadism does not appear to be identical across racial and ethnic boundaries. In a health screening project among 819 men in Taiwan, the prevalence of hypogonadism (total serum T < 300 ng/dL) ranged from 16.5% for men in their 40s, 23.0% for men in their 50s, 28.9% for men in their 60s, and 37.2% for men older than 70 years of age[11]. The prevalence of hypogonadism among men in Taiwan is higher than the prevalence reported in the Massachusetts Male Aging Study[3], for a similar age group. There are no definitive biological explanations for differences in the epidemiology of hypogonadism. Candidate reasons are related to various lifestyle factors as well as genetic reasons, including the CAG repeat sequence, within the androgen receptor (AR). Rajender *et al*[12] reviewed over 30 studies on the AR trinucleotide repeat and infertility. Overall, for European populations, no significant distinctions were drawn, based on the CAG repeat. However, in Asian populations, four studies indicated a longer repeat was associated with infertility, two indicated no difference was present, and one study reported a shorter length (samples were all oligozoospermic for this subgroup). While there is a suggestion that CAG repeat length may determine androgen responsiveness, this issue is not clearly settled.

Nevertheless, a strong inference remains that race and ethnicity play a role in both the genotype and phenotype as they relate to the epidemiology of hypogonadism.

In summary, the reported prevalence of low T in older men range from 5.6% to 50%, depending upon study design, level of T blood concentration used, and study subjects' age. Combining serum T measurement with signs and symptoms most commonly seen with androgen deficiency are recommended in order to confirm the diagnosis of hypogonadism.

Hypogonadism comes with economic burden as well. An analysis of 8538 men, between the ages of 34 and 65, found direct and indirect cost differences associated with hypogonadism[13]. This study examined an administrative database spanning four years and three months. Men with at least two diagnoses for hypogonadism, or, at least one prescription for testosterone therapy with at least one diagnosis for hypogonadism were considered hypogonadal in the analysis. They were matched against those not satisfying either of the two criteria. Those in the hypogonadal group (n = 4269) had direct health care costs, that exceeded the eugonadal group (n = 4269) by an average of $7100 over the course of the observation window. Due to the expense of treatment for HIV/AIDS, those affected individuals were excluded in another analysis to avoid skewing the difference. The difference in direct costs was then $5579, meaning that the hypogonadal group incurred an additional cost of just over $109 per person per month. Indirect costs went up a little more than $30 per person per month. Examples of mean disease- specific costs for hypogonadal *vs* eugonadal were as follows (these numbers do not exclude those with HIV/AIDS): Cardiovascular and metabolic health: $1453 *vs* $757; Pain: $980 *vs* $365; Mental health: $558 *vs* $176. The hypogonadal group had a higher Charlson's Co-morbidity Index at baseline, with a mean of 0.95, as compared to the eugonadal group at 0.28. In risk-adjusted analyses of costs, the difference in health care costs (both direct and indirect) was $4869, or just over $94 per person per month on average.

This investigation plainly demonstrated higher economic burden and presence of co-morbidities for

hypogonadism. Additionally, it highlighted a potentially serious threat to the interpretation of all observational studies in the testosterone replacement therapy (TRT) field-compliance. Over 31% of individuals in the hypogonadal did not receive TRT during the observation period. Similarly, within the hypogonadal group receiving therapy the proportion of days covered under TRT therapy was only 38% based on medical claims data. These study findings demonstrate that the economic burden for the hypogonadal group is higher than the eugonadal group even when 2/3 of the hypogonadal group did receive the TRT. However, because at least 31% of the hypogonadal group did not receive testosterone replacement, and those that did receive testosterone replacement were covered less than 40% of the time, it's not possible to infer whether or not TRT was mitigating the additional burden. The socioeconomic burden of hypogonadism should be addressed in detail in future observational and clinical trials, to provide robust metrics on the risks, benefits, and costs of TRT.

TRT

An increased awareness of the health risks associated with untreated hypogonadism has caused a substantial increase in TRT utilization in men[14]. The efficacy of TRT has been demonstrated in several randomized clinical trials[15-17] and has shown minor to moderate improvements in lean mass and muscle strength[18,19], increased bone mineral density (BMD)[18,20], modest enhancement in sexual function[21-23], reduced adiposity[18] and lessening of depressive symptoms[24]. In 2011, the estimated sales for TRT were 1.6 billion dollars in the United States[25]. However, significant questions remain regarding the safety of TRT because no large-scale randomized clinical trials have directly compared the health risks of untreated hypogonadism *vs* long-term TRT use[25].

Enthusiasm for TRT utilization has been tempered by concerns regarding the health risks of this therapy. Meta-analyses of clinical TRT trials as of 2010 have identified three major adverse events resulting from TRT: (1) polycythemia; (2) an increase in prostate-related events; and (3) and a slight reduction in serum high-density lipoprotein (HDL) cholesterol[26-28]. Clinical concern over the health risks of TRT was heightened in mid-2013 when a meta-analysis reported increased cardiovascular (CV) risk in men receiving TRT[29]. Similarly, a recent retrospective study reported increased risk of stroke, myocardial infarction, and all-cause mortality in hypogonadal men receiving TRT after angiography[30].

CARDIOVASCULAR AND CEREBROVASCULAR RISKS OF TRT

Two widely established health risks associated with TRT are polycythemia (> 3.5-fold increase in risk)[26,27] and reduced HDL cholesterol[27]. These risk factors represent increased risk for CV and cerebrovascular events. Serious concern regarding the safety of TRT was raised in 2010 when the data and safety monitoring board (DSMB) of a double-blind randomized clinical trial recommended discontinuation of the trial because elderly hypogonadal men (with a high prevalence of chronic disease) experienced an increased incidence of CV events after receiving TRT[31]. This randomized clinical trial was discontinued because 23 participants in the TRT group (approximately 22% of all TRT participants) *vs* 5 participants in the placebo group (approximately 5% of all placebo participants) experienced adverse CV-related events within the first 6 mo. Adverse events ranged from chest pain (n = 1) to myocardial infarction (MI) (n = 3 with one death suspected from MI), with peripheral edema being the most commonly reported side-effect (n = 5).

A large meta-analysis evaluating CV risks associated with TRT (including 27 RCTs and 2994 older men) also reported that TRT increased risk for CV-related events by 1.54 times (odds ratio = 1.54) in comparison to placebo treatment[29]. In a similar but more recent and larger meta-analysis of 75 RCTs on CV risks and TRT, no significant association between CV events (both single and composite events) and TRT was established[32]. However a retrospective study reported that men receiving TRT after angiography (n = 1223) experienced a 29% greater hazard ratio-adjusted rate of MI, stroke, and all-cause mortality (95%CI: 1.04-1.58) at 3 years post angiography *vs* men with untreated hypogonadism (n = 7486)[30]. Finkle *et al*[33] evaluated 55000 patients and reported a more than 2-fold greater risk of MI in men who had received a TRT prescription. These results differ from several smaller analyses reporting heightened CV risk and all-cause mortality in men with untreated hypogonadism[26,28]. This is especially important given that the TRT literature has thus far been equivocal and/or underpowered, despite large, coordinated efforts such as the forthcoming series of trials from Snyder *et al*[34].

As a response to the above study reports, the United States Food and Drug Administration (FDA)[35] the Endocrine Society[36] and the United States Veteran's Administration[37] have called for monitoring and reassessing the health risks associated with TRT, respectively. Since these advisory announcements, a number of literature reports have critiqued the TRT literature, particularly the studies by Vigen and Finkle, for issues associated with the study design, statistical methods, and interpretation of findings[38-40].

Notwithstanding several letters to the editor, and several recent TRT articles showing no increased cardiovascular risk, the United States FDA Joint Advisory Panel voted to change the labeling on testosterone replacement medication until larger studies demonstrate a clinical benefit and account for patient safety[41].

PROSTATE RELATED RISKS OF TRT

Another well-established health risk of TRT is increased incidence of prostate/lower urinary tract-related events (*i.e.*, combined incidence of prostate-biopsy, prostate enlargement, elevated PSA, and prostate cancer)[26]. Several coauthors of this commentary recently conducted a double-blind randomized clinical trial (NCT00475501) and observed that TRT produced a 40% prostate enlargement in older hypogonadal male Veterans over 12 mo[42]. These increased prostate-related risks have raised concern that TRT may increase prostate cancer risk or hasten the development of undiagnosed prostate cancer.

However, no published analysis has reported measurable increases in prostate cancer risk or Gleason score in men undergoing TRT, or in hypogonadal men with a history of prostate cancer undergoing TRT[26,27,43]. Despite this, Calof *et al*[26] estimated that an evaluation of 85862 participants is necessary to detect a hypothetical 20% increase in prostate cancer resulting from TRT. The largest meta-analysis evaluating prostate cancer risk associated with TRT included only 1700 men (*i.e.*, < 2% of the necessary population size)[43].

OTHER PUTATIVE HEALTH RISKS ASSOCIATED WITH TRT

A number of putative health risks have been reported with TRT, including fluid retention[31], gynecomastia[44], liver disorders, and worsening of sleep apnea[45]. These adverse outcomes are worrisome because they represent risks for several serious life-threatening adverse events and for other potentially serious clinical conditions. However, current meta-analyses have not established a definite relationship between TRT and these potential health risks, likely because they lack the statistical power. Additionally, the mechanisms through which T incites the above mentioned health risks are not completely understood, but may result in part from tissue-specific 5α-reduction of T to dihydrotestosterone (DHT)[46] or from the aromatization of T to estradiol. This is especially true in the prostate which highly expresses the type Ⅱ 5α-reductase enzyme. Inhibition of this enzyme via finasteride (a type Ⅱ 5α-reductase inhibitor) or dutasteride (a dual type Ⅰ and Ⅱ 5α-reductase inhibitor) reduces circulating DHT 50%-75% and > 90%, respectively[47], and reduces prostate mass[48] and prostate cancer risk[49]. Our team[42] and others[15] have demonstrated that finasteride also prevents prostate enlargement resulting from high-dose TRT without inhibiting the beneficial musculoskeletal or lipolytic effects of T, indicating the clinical viability of this combination pharmacologic therapy. It is unknown whether other potentially life-threatening health risks and other adverse events discussed above are mediated by the 5α-reduction or aromatization of T.

ALTERNATIVES TO TRT

Given that there is currently no global consensus on the medical approach to testosterone deficiency, it is not surprising that alternative approaches to rectifying low T-levels are great in number, yet also lacking widespread agreement[50]. Several decades of research have been completed evaluating the field of selective estrogen receptor modulators and selective androgen receptor modulators (SARMS). Clomiphene Citrate (CC) is an estrogen receptor modulator that is used in the treatment of male hypogonadism in an off-label capacity. Normally estradiol partially regulates testosterone levels, at the hypothalamus, blunting LH and FSH release from the pituitary. As a selective estrogen receptor modulator, CC interrupts this pathway, and consequently there is a greater stimulation for the production of testosterone in Leydig cells[51].

A cohort of 1150 hypogonadal men were evaluated, and matched to produce a final sample of 93 in three groups: CC, Testosterone Injections (TI), or Testosterone Gel (TG)[52]. Each group consisted of 31 individuals. The research team evaluated changes in serum testosterone and patient satisfaction. All treatment modes were effective at raising T-levels. Changes in T-levels (ng/dL) from pre- treatment to post-treatment were as follows: CC = 247-504, TI = 224-1104, TG = 230-412. Patient satisfaction was equal among groups, though the responses in T-levels were not equivalent. The noted difference was in libido, where injection produced the greatest index of libido on the qADAM questionnaire (4 v. 3 for each comparison of injection v. CC, injection v. TG). CC appears to be a suitable alternative to testosterone supplementation. However, larger randomized clinical trials are needed to determine its proper use, potential safety, and whether this agent effectively mitigates the known side-effects of hypogonadism. Similarly, as reported by Taylor *et al*[51], 104 men received either CC or T-Gel (CC = 65, T-Gel = 39). The CC group had higher post-treatment T-levels, 573 ng/dL v. 553 ng/dL. The monthly cost of T-Gel medication is over three times that of CC (Testim 1%, 5 gm daily = $270/mo, Androgel 1%, 5 gm daily = $265/mo, CC 50 mg every two days = $83/mo). Thus, in terms of cost-effectiveness, CC would appear to be advantageous. However, more research is needed to determine its proper use.

SARMS

The combined research and clinical goals of SARMS are the reductions in catabolic actions initiated by hypogonadism and/or aging in order to preserve skeletal muscle and bone allowing for the individual to maintain functional activities of daily living, reduce fall and fracture risk, and consequent disability. SARMS are of particular interest because of recent guidance documentation[41] on the restriction of exogenous testosterone administration warranted by observational studies indicating an

increased risk of cardiovascular events[30,53]. In light of the recently cited effects on the cardiovascular disease system, SARMS are the most likely candidates to improve skeletal muscle mass in hypogonadal individuals. SARMS are engineered to bind to the androgen receptor without inducing other known side effects (*e.g.*, prostate related events and polycythemia) of TRT. Several SARMS, including JNJ-28330835[54], BMS-564929[55], MK-0773[56], and others have shown a positive levator ani/bulbocavernosus muscle complex/prostate ratio in pre-clinical rodent models, demonstrating an improved anabolic/androgenic ratio is associated with these drugs[57]. The anabolic to androgenic ratio with limited side effects is the therapeutic target of SARMS research.

CONCLUSION

In summary, circulating testosterone concentrations decline throughout the aging process in males[2]. The prevalence of low circulating testosterone (*i.e.*, hypogonadism) is approximately 20% in men between 60-70 years of age and increases to roughly 50% of men over 80 years of age[2]. The use of TRT has increased substantially among men in recent years[14] because of an increased awareness of the risks associated with male hypogonadism (*e.g.*, muscle and bone loss, and increased frailty)[58,59]. However, TRT safety remains of primary concern, as do the potential health risks of untreated hypogonadism. To date, the largest prospective clinical trials that have been conducted on TRT involved only several hundred individuals; as such, they were dramatically underpowered to assess many of the more rare, yet severe health risks that are putatively associated with TRT. Unfortunately, even the largest meta-analyses on adverse events associated with TRT lack sufficient power to detect these and other potentially life-threatening health risks. Additionally, these clinical trials and meta-analyses have only assessed health risks during relatively short-term TRT or for only a very brief follow-up period after the cessation of TRT, which is concerning because once TRT is initiated it is typically continued throughout the lifespan. TRT studies should address additional external factors that may contribute to the reported risks and benefits currently associated with TRT including: diet, exercise, neutraceutical supplementation, sleep, and obesity.

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MINIREVIEWS

Atherosclerosis and the role of immune cells

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Abstract

Atherosclerosis is a chronic inflammatory disease arising from lipids, specifically low-density lipoproteins, and leukocytes. Following the activation of endothelium with the expression of adhesion molecules and monocytes, inflammatory cytokines from macrophages, and plasmacytoid dendritic cells, high levels of interferon (IFN)-α and β are generated upon the activation of toll-like receptor-9, and T-cells, especially the ones with Th1 profile, produce pro-inflammatory mediators such as IFN-γ and upregulate macrophages to adhere to the endothelium and migrate into the intima. This review presents an exhaustive account for the role of immune cells in the atherosclerosis.

Key words: Atherosclerosis; Inflammatory cytokines; Pro-inflammatory mediators; Immune cells; Adhesion molecules

Core tip: Activated endothelium to adhere to the endothelium and move into the intima with the expression of adhesion molecules appears to be an early event in atherosclerosis, which allows mononuclear leukocytes such as monocytes and T-cells. This inflammatory mechanism must be explained before determining a new therapy.

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INTRODUCTION

Atherosclerosis is one of the leading causes of morbidity and mortality arising from coronary artery disease, stroke, and peripheral vascular disease. The pathophysiology of atherosclerosis is best characterized with hyperlipidemia and inflammation[1,2].

For a long time after recognition, atherosclerosis was associated with passive lipid accumulation in the vessel wall. Nowadays we know that atherosclerosis is a chronic inflammatory disorder caused by lipids, particularly low-density lipoproteins (LDLs), and leukocytes[3]. Atherosclerosis is likely to be initiated by the activation of endothelium with the expression of adhesion molecules, and this in turn enables the adhesion of mononuclear leukocytes, such as monocytes and T-cells, to the endothelium and also their transmigration into the intima. At this point, the lesions may be present with rare cells such as dendritic cells (DCs), few neutrophils and

B-cells, and also with smooth muscle cells (SMC), which transform phenotype into synthetic SMC and move into the intima from the media[4].

Polymorphonuclears (PMNs) are recruited and adhered to the endothelium upon the subsequent expression of adhesion molecules, such as E-selectin, P-selectin and intercellular adhesion molecule 1 (ICAM-1)[5]. The endothelial cell expression of selectins and vascular cell adhesion molecule 1 (VCAM-1) is further increased by pro-inflammatory cytokines and mmLDL, and this facilitates the infiltration of the monocytes into the intima[6]. As a result of intimal lipid accumulation, disturbed blood flow, low shear stress, and other stimuli, the transition of monocytes, which are major precursors of macrophages, through the endothelium is allowed by endothelial cells[3]. Endothelial cells and SMCs are triggered by oxidized LDL (OxLDL), and this leads to the secretion of monocytic maturation factors such as monocyte-colony stimulating factor (M-CSF). Monocytes are transformed to macrophages and phagocytose modified lipoproteins, mainly due to the scavenger receptors AI and CD36[7], and then become foam cells[8]. Macrophages may be activated by PMNs following the secretion of tumor necrosis factor (TNF)-α, interleukin (IL)-8, and interferon (IFN)-γ. In addition, the release of myeloperoxidase from granules can stimulate the formation of reactive oxygen species (ROS), as well as the secretion of other pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, IL-8 and granulocyte macrophage colony stimulating factor (GM-CSF) from macrophages. In response, ROS transform the extravasated LDL into OxLDL, consequently forming the foam cell development[9].

Monocyte recruitment and the size of atherosclerotic lesion are bound to decrease if a failure is experienced in adhesion molecules, such as P-selectin, ICAM-1 and VCAM-1, or their interactions with their respective ligands are constrained[10,11].

MONOCYTES AND MACROPHAGES

After the migration from the circulation into the intima of the arterial wall, monocytes are converted to macrophages and DCs. These cells then transform into foam cells by taking up modified lipoproteins[12]. There are three major monocyte subsets in humans[13,14]: the classical $CD14^{++}CD16^{-}$ subset is similar to the mouse Ly6C high inflammatory subset and also presents a high expression of CCR2, and the non-classical $CD14^{+}CD16^{++}$ monocytes are considered to match the $Ly6C^{-}$ cells in mice, which express high levels of CX3CR1 and CCR5 but low levels of CCR2[15]. The third subset, however, is known as the intermediate $CD14^{++}CD16^{+}CCR2^{+}$ subset[16]. Of these, the classical subset includes nearly 90% of the monocytes circulating in humans[17]. The patients with coronary artery disease present with increased amount of pro-inflammatory $CD14^{+}CD16^{+}$ monocytes and serum TNF-α levels[18], and this monocyte subset is in negative correlation with fibrous cap thickness[19].

After chemokinesis, monocytes adhere to and spin on endothelial cells by interacting with E- and P-selectins[20,21]. Lipoprotein-binding proteoglycans are secreted by monocytes in the intima, leading to enhanced accumulation of modified LDL, which carries on inflammation[22,23].

Tissue damage and repair are closely linked to monocytes, and a discrepancy to occur in these processes may have critical results for plaque formation and stability. Importantly, monocytes consist of dissimilar subsets along with different cell surface markers and functional features, and this diversity of components may be associated with the angiogenic processes in atherosclerosis[24].

The formation of atherosclerotic lesions is heavily dependent on the transformation of monocytes into macrophages; for instance, M-CSF-knockout mice show resistance to the development of atherosclerosis[25].

Every phase of the course of disease includes abundant amounts of monocyte-derived macrophages[12], and these cells an important role in lipid accumulation and advancement of atherosclerosis[24]. Also, their crucial role in atherogenesis has been proven by the reduction of lesion formation in monocyte-deficient apolipoprotein E (ApoE) knockout mice and LDL receptor knockout mice[26,27].

The polarization of macrophages towards a specific phenotype has been reported to be positively affected by lipids, growth factors, and cytokines; the M1 macrophages that are classified by means of classical methods may result in plaque vulnerability, whereas the M2 macrophages which are activated by alternative methods may increase plaque stability[28]. The phenotypes of M1/M2 macrophages can be exchanged depending on the conditions of their microenvironment[29].

Many macrophages and dendritic-like cells are known to have membrane-bound lipid droplets in the cytoplasm even at very early phases of atherogenesis. As they comprise lipid deposits, these cells are called "foam cells" and their course of development is initiated when apolipoprotein B-containing lipoproteins (apoB-LPs) are absorbed and processed by phagocytes[21]. While producing matrix metalloproteinases with regards to plaque rupture, macrophages can be primed by oxLDL to develop a foam cell macrophage which bears the characteristics of M1 and M2 activation[28]. Inflammatory cytokines and chemokines that promote inflammation and contribute to the regulation of monocyte/T cell infiltration are generated by macrophages/foam cells[30-33]. With the macrophages in the atherosclerotic plaque, it is possible to generate a wide range of proinflammatory cytokines such as IL-1, IL-6, IL-12, IL-15, IL-18, TNF family members, and MIF, as well as anti-inflammatory cytokines like IL-10 and transforming growth factor beta family members[34,35]. Additionally, IFNγ may trigger the macrophages to produce ROS and neopterin. It has been reported that neopterin levels increased in acute coronary syndrome and neopterin may be useful for the assessment of inflammation related to atherosclerosis[36].

Being the most abundant cell type in atherosclerotic plaques, macrophages have a strong effect on plaque

development and progression due to its overwhelming influence on intra-plaque cholesterol homeostasis, inflammation, necrotic core initiation, and extracellular matrix degradation[37].

Toll-like receptors (TLRs) represent the most comprehensively studied and described type of pattern recognition receptors. TLRs are characterized as type 1 transmembrane proteins involving an ectodomain with leucine-rich patterns that are needed to recognize pathogen associated molecular patterns, a transmembrane region, which determines the locations of the cells, and an intracellular toll interleukin 1 receptor region required for downstream signaling. Up to now, a minimum of 13 TLRs have been described, and each of them present with a degree of specificity for a number of endogenous and exogenous ligands[38]. Expression of TLRs is performed by a number of various cells, such as leukocytes, DCs, and T and B lymphocytes[39]. Atheroma development can be directly influenced by TLRs since the lipid uptake is promoted when the stimulation of macrophages is conducted with TLR2, TLR4 and TLR9 ligands[40,41]. According to recent studies on ApoE$^{-/-}$ mice, even small amounts of TLR4 and TLR2 have positive effects on the deposition of early-stage intimal foam cells in some regions in the aorta which are sensitive to lesion development[42]. The macropinocytosis of lipids in differentiated macrophages can be stimulated by TLR4[43]. Increased expression of scavenger receptors induced by TLR3, TLR4 and TLR9 can be used as a mediator for increased lipid absorption[39,44]. These receptors and their ligands may also interrupt the cholesterol efflux mechanisms, which may have a contributory role in the development of foam cells[28].

THE DENDRITIC CELLS

Dendritic cells, which are antigen-presenting cells (APCs), exhibit a variety of antigens to T cells in addition to initiating and sustaining immune responses as well as inhibiting the activation of T cells. The capacity of DCs in the activation or inhibition of T cells relies on its cytokine production profile and expression of cell surface co-stimulatory molecules. DCs are transformed by activated innate immune receptors, such as the TLR, into APCs that activate T effector cells, whereas, immunological tolerance is produced by antigen presentation which develops when TLR activation is not present. Therefore, DCs play a critical role as a connector between innate and adaptive immune responses[45].

DC has a heterogeneous population with four major categories: conventional DCs (cDCs), plasmacytoid DCs (pDCs), monocyte-derived DCs, and Langerhans cells[46]. Monocytes or DC precursors, which are present in the bone marrow, constitute the two sources of DCs.

Monocytes are completely transformed into monocyte-derived DCs in inflammation and as a reaction to growth factors like GM-CSF or TLR4 ligands. The capacity of presenting antigens along with the ability to cross-present antigens belongs to the DCs that originate from monocytes[37]. DCs are capable of generating a wide range of anti-inflammatory and proinflammatory cytokines. As an example, some proinflammatory cytokines, such as TNF, IL-6, and IL-12, which have been proven to contribute to the atherosclerosis can be generated by TLR binding[47-49]. However, TLR binding may also generate IL-10, which is known as an atheroprotective cytokine[50].

The DCs in mice are best known for their expression of CD11c and they present with healthy mouse aortas, predominantly in the adventitia[51]. In mice, the amount of mRNA expression of CD11c is higher in the sites of the aortic arch susceptible to atherosclerosis, compared to the sites that are resistant to atherosclerosis. Contrary to healthy vessels, most of the DCs in atherosclerotic aortas are localized in the intima[52].

The deposition of CD11c^{+} DCs at the vascular regions prone to atherosclerosis is associated with the increase in the expression of VCAM-1[53]. Mature DCs are more abundant in advanced lesions. High level of expression of human leukocyte antigen (HLA)-DR and interactions with T cells are mostly observed in the sites of the plaque that are predisposed to rupture[54]. The deposition process of the dendritic cells in the intima may be interrupted if the fractalkine receptor CX3CR1 in the aorta is impaired, and this may be an indication that these cells may be transformed from Ly-6Clo monocytes which are known to induce high levels of CX3CR1[55]. OxLDL, in line with the elevation in the production of T cells, functions as an antigen upregulator for the DC expression of HLA-DR and its co-stimulatory molecules[56]. DCs carry out the expression of scavenger receptors (LOX-1, CD36 and CD205) which facilitate their uptake of oxLDL activating the NFκB pathway, and evolution to DCs with a pro-inflammatory cytokine profile[57]. Once DCs are activated by oxLDL in the plaque, they move to secondary lymphoid organs and initiate the clonal proliferation of the T cells that are specific to oxLDL[28].

Following TLR9 activation, it is a common event for pDCs to produce high amounts of IFNα and β, and TLR9 has been reported to contribute to atherosclerosis by promoting macrophage recruitment[58]. The recruitment of monocytes, memory T cells, and DCs to the region of inflammation is reportedly influenced by the CCL2 secreted by DCs[59].

DCs, as prominent mediators of immune responses, may also act as the regulators of innate or adaptive immunity against the potential antigens that are engaged in atherosclerosis[60]. In brief, the roles of dendritic cells in atherosclerosis can be summarized as the induction of chemokines and cytokines, presentation of antigens, and lipid absorption that might trigger inflammation or promote tolerance[37].

T CELLS

The role of adaptive immunity in atherosclerosis was

verified by the presence of antibodies and oxLDL-specific T cells along with the accumulation of oligoclonal T cells in lesions[6,61]. T cells are targeted to the vessel wall in line with macrophages, but to a lesser extent. Activation of T cells in the arterial wall is a reaction to antigens, and after this activation, the T cells initiate the production of pro-inflammatory mediators, by which the inflammatory response is intensified and thus the disease development is worsened[62]. Moreover, most of the pathogenic T cells in atherosclerosis have the characteristics of Th1 since they generate pro-inflammatory cytokines such as IFN-γ and perform the activation of macrophages[63,64]. The reactions mediated by Th1 have harmful effects on the development of atherosclerosis. Vascular smooth muscle cells are recruited by IFN-γ to inhibit the synthesis of collagen, and this leads to harmful effects for the protective thick fibrous cap of the plaque. Also, the activation of monocytes/macrophages and dendritic cells by IFN-γ results in the continuation of the pathogenic Th1 response[30].

Previous studies report that the removal of IFNγ or its receptors leads to a reduction in atherosclerosis, whereas the injection of recombinant IFNγ results in a growth in the size of the lesions[65-67]. The detection of Th2 cells in the atherosclerotic lesions is a rare occurrence. The cytokines produced by Th2 cells include IL-4, IL-5, IL-9, and IL-13. Th2 cells also have contributory effects on the production of antibodies by B cells. As the production of IFN-γ is decreased by these cells, the responses caused by Th2 were thought to be the antagonists of proatherogenic Th1 effects, hence rendering atheroprotection. Nevertheless, how atherosclerotic progress is affected by Th2 pathway has yet to be proven and the role Th2 pathway relies not only on the phase and location of the lesion but also on the method of experimentation to be used[62]. According to some studies on animals, both Th1 and Th2 responses are involved in the progression of atherosclerosis, and lesion formation is started primarily by Th1 activation through a switch towards a proatherogenic response by Th2 in the chronic stage of plaque formation[68]. The expansion and cytokine induction of highly activated effector T cells can also be inhibited by another T cell called TCRγδ$^+$ CD4$^-$ CD8$^-$, and this cell may need to be further analyzed since it is likely to have antiatherogenic characteristics[69,70]. The regulatory T cells (Tregs) have critical roles in the inhibition and suppression of inflammation and also in the regulation of adaptive immune responses. Moreover, these cells can induce tolerance by inhibiting the effector CD4$^+$ and CD8$^+$ T cells[71,72].

IL-10 has been reported to inhibit atherosclerosis, and thus the athero-protective effects of regulatory T cells may be improved when they generate IL-10[73,74]. Studies also report that IL-10 has a protective function in the development and stability of atherosclerotic lesions[72,73].

Th17 lymphocytes represent another T helper subset associated with inflammation, and this subset does not share the same lineage with Th1 and Th2[75]. IL-17 has been demonstrated to have protective and pathogenic effects in a number of autoimmune diseases[76,77].

The main cytokines expressed by Th17 cells include IL-17A and IL-17F along with IL-21 and IL-22. The role of Th17 is still debatable despite the detection of Th17 cells in the atherosclerotic lesions in mice and humans, because both atherogenic and atheroprotective effects have been attributed to these cells[78-80]. IL-17 is also considered to enhance plaque stability since elevated IL-17 induction in human lesions results in a decrease in the number of macrophages, an increase in SMC deposit, and a phenotype with a more fibrotic profile[81].

Proatherogenic profile of IL-17 has been shown previously by many studies[82-85]. In these studies, the evidence for the proatherogenic effect of IL-17 is attributed to the fact that both IL-17 and IFN-γ are expressed by the CD4$^+$ T cells that are separated from atherosclerotic coronary vessels[86].

CD8+ T cells are detected in both murine and human plaques[87,88]. The number of CD8$^+$ T cells is low in the early stages of lesions; however, these cells seem to be the dominant T cell type in the advanced stages of human lesions[88]. CD8$^+$ T cells may have a proatherogenic function since the lesion size was increased and also the recruitment of these cells to the lesion site was promoted when the responses of these cells were stimulated with a CD137 agonist[89].

B CELLS

The responses produced by the Th2 cell have important roles in the activation of B cells, the differentiation of plasma cells, and the production of antibodies that are unique to antigens. B cells are evident in atherosclerotic lesions, but their population is smaller than that of T cells[90]. However, the role of B cells in atherosclerosis remains controversial as two recent studies have reported that the atherosclerotic progression in mice is inhibited when B cells are blocked by the use of an antibody against CD20[91,92]. The evidence that some types of IgM and IgG have atheroprotective effects may suggest that B cells have the ability to protect against atherosclerosis. Moreover, plaques have been detected with both IgM and IgG at all phases of lesion progression[93]. Anti-oxLDL IgM antibodies have been proven to provide protection against atherosclerosis, probably because they achieve oxLDL binding and thus suppress oxLDL absorption by using macrophages and avoid the development of foam cells[94,95]. On the other hand, to what extent the oxLDL-specific IgG is effective remains a controversial issue because both beneficial and inverse effects have been reported in epidemiological studies[96]. OxLDL-specific antibody IgG titers are associated with atherosclerosis[94,97,98], whereas oxLDL-specific IgM titers are related to atheroprotection[99,100]. Nonetheless, the B cell subsets and their roles in atherosclerosis need to be further

analyzed[37].

CONCLUSION

Atherosclerosis is a multiphase process which is characterized with the activation of endothelial cells with the expression of adhesion molecules and monocytes/macrophages, and the transmigration of DCs, T cells and some B-cells into the intima, and also the transfer of modulated types of LDL to matrix components. Monocytes/macrophages are highly abundant and differentiate into foam cells which are rich in modulated LDL.

According to clinical and experimental data, the atherogenic process involves the cells of both the innate and the adaptive immune system, and these cells generate diverse cytokines that may have both pro and anti-inflammatory functions[101-103]. To immunomodulate the atherosclerosis is the primary aim of some clinical studies. Among these, the experimental studies with anti-LDL antibodies and vaccination studies with LDLs are under way[1,104]. Oral administration of oxidized LDLs is reported to be effective on the inhibition of atherosclerosis and production of Tregs in peripheral lymphoid tissues[105]. The functions of immune and inflammatory modulators in the formation and development of atherosclerosis have been better analyzed in recent years and thus provided a deeper insight into these mechanisms. Accordingly, more and more advanced techniques in the diagnosis and prognosis of atherosclerosis, along with new treatment procedures for inflammatory and immune factors, have been developed[106]. However, there is still much to learn about immune cells and their mechanisms affecting atherosclerosis. We believe that further studies investigating immune cells and their mechanisms will help to shed light on atherosclerosis.

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ORIGINAL ARTICLE

Retrospective Study

Endoscopic papillary large balloon dilation for bile duct stones in elderly patients

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Ethics approval: This study was performed after approval of the ethical committee of our institution.
Informed consent: All treatment procedures were performed after obtaining a written informed consent from the patients.
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Abstract

AIM: To investigate whether endoscopic papillary large balloon dilation (EPLBD) can be safety and effectively performed in patients aged ⩾ 80 years.

METHODS: Lithotomy by EPLBD was conducted in 106 patients with bile duct stones ⩾ 13 mm in size or with three or more bile duct stones ⩾ 10 mm. The patients were divided into group A (< 80 years) and group B (⩾ 80 years). Procedure success rate, number of endoscopic retrograde cholangiopancreatographies (ERCP), and incidence of complications were examined in both groups.

RESULTS: Group B tended to include significantly more patients with peripapillary diverticulum, hypertension, hyperlipemia, cerebrovascular disease/dementia, respiratory disease/cardiac disease, and patients administered an anticoagulant or antiplatelet agent (P < 0.05). The success rate of the initial lithotomy was 88.7 (94/106)%. The final lithotomy rate was 100 (106/106)%. Complications due to treatment procedure occurred in 4.72 (5/106)% of the patients. There was no significant difference in procedure success rate, number of ERCP, or incidence of complications between group A and group B.

CONCLUSION: EPLBD can be safely performed in elderly patients, the same as in younger patients.

Key words: Elderly patients; Endoscopic papillary large balloon dilation; Endoscopic sphincterotomy; Large bile duct stones; Choledocholithiasis

Core tip: Endoscopic treatment by papillary large balloon

dilation for large stones can be safely performed in elderly patients, the same as in younger patients.

Sakai Y, Tsuyuguchi T, Sugiyama H, Sasaki R, Sakamoto D, Nakamura M, Watanabe Y, Nishikawa T, Yasui S, Mikata R, Yokosuka O. Endoscopic papillary large balloon dilation for bile duct stones in elderly patients. *World J Clin Cases* 2015; 3(4): 353-359 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i4/353.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i4.353

INTRODUCTION

The number of elderly patients with common bile duct stones has been increasing associated with the global prolongation of life expectancy[1]. Endoscopic retrograde cholangiopancreatography (ERCP) has been recognized as a treatment with less risk and lower mortality than surgery[2]. Endoscopic treatment for choledocholithiasis should be attempted as the first choice of treatment because of its high success rate in addition to its low invasiveness and low incidence of complications[3]. Although a report indicated that ERCP related procedures may be hazardous for patients with common bile duct stones as well as in elderly patients aged ≥ 80 years[4], it is often reported that this procedure can be useful and safely performed even in elderly patients[5-20]. The usefulness of endoscopic treatment using endoscopic papillary large balloon dilation (EPLBD) which is a new papillary treatment using a balloon of large diameter for large bile duct stones or multiple bile duct stones has been recently reported. Most of the reports described the procedure as safe and useful[20-34]. However, there have been some cases of death[35]. Since elderly patients often have an underlying disease, they may follow a fatal course, thus they require special attention[4]. There are few reports on the usefulness of this procedure in elderly patients[20], and it has not been examined sufficiently. This report examined usefulness of EPLBD in patients aged ≥ 80 years.

MATERIALS AND METHODS

The study involved 106 patients (A) with bile duct stones ≥ 13 mm in their short diameter, or (B) multiple (≥ 3) bile duct stones with the smallest more than 10 mm in the shortest diameter, but without confluence stones. These patients were selected from among those with bile duct stones visiting our hospital or our affiliated hospitals from November 2009 to June 2014. Inclusion criteria were patients who could undergo endoscopic sphincterotomy (EST), and who gave their informed consent to the procedure. Exclusion criteria were coagulopathy (international normalized ratio > 1.5), marked thrombocytopenia (platelet count < 50000/mL), patients who could not discontinue the administration of an anticoagulant or antiplatelet agent, patients for whom endoscopic biliary drainage was difficult (Billroth-Ⅱ or Roux-en-Y), patients with the papilla within the diverticulum, patients with stenosis of the intrapancreatic bile duct, stone diameter > 30 mm (shortest diameter), and patients who do not give their informed consent.

We selected 79 patients with new bile duct stones and 27 with recurrent stones. All cases of recurrent stones were those after EST. We performed EST in 65 patients and it had been already performed in 41 patients. Fourteen patients whose stones were not recurrent and EST had been already performed were difficult cases referred to our hospital for a lithotomy. The average diameter of the stones was 14.29 (10-28) mm, the number of stones was 5.73 (1-30) and the diameter of the bile duct was 16.97 (10-28) mm. As for the gall bladder, 77 patients were calculous, one was acalculous and 28 had undergone cholecystectomy. Parapapillary diverticulum was noted in 50 patients. The patients were divided into group A (< 80 years) and group B (≥ 80 years). The clinical background of patients in these 2 groups is shown in Table 1. Patients in group B tended to have significantly more frequently peripapillary diverticulum, hypertension, hyperlipemia, cerebrovascular disease/dementia, respiratory/cardiac disease, or were taking an anticoagulant or antiplatelet agent. There was no significant difference in other factors between group A and group B. One session of treatment lasted up to 60 min after inserting the endoscope. The condition of the patients was observed, and if the patient showed much discomfort, the procedure was completed after inserting the drainage, even while in the process of treatment. Before ERCP, all patients were given a standard premedication consisting of intravenous administration of midazolam (3 to 10 mg), and the dose depended on age and tolerance. Scopolamine butylbromide or glucagon was used for duodenal relaxation. During ERCP, arterial oxygen saturation was continuously monitored using a pulse oximeter. Patients were kept fasting after the procedure for at least 24 h with drip infusion of 2000 mL and remained hospitalized for at least 72 h. For cannulation, catheters PR-104Q, R110Q-1 and PR233Q were used. A 0.025-inch or 0.035-inch guidewire (Jagwire: Microvasive, Boston Scientific Corp., Natick, MA; Revo Wave: PIOLAX, or VisiGlide: Olympus Corp.) was used. The endoscopes used were JF240, JF260V, TJF260V (Olympus Corp.), backward side-viewing endoscope, for patients with no history of gastric resection and patients of Billroth-I. After cholangiography, a guidewire was placed in the bile duct to conduct EST. Clever-Cut3V (Olympus Corp.) was used as the knife for EST. EST was conducted using a single electrosurgical current generator (PSD-20, Olympus Corp.) at a power of 25 watts. For those in which an incision had been already

Table 1 Patient background

	< 80 yr old (Group A)	⩾ 80 yr old (Group B)	*P*-value
Number of patients	59	47	
Male	36	20	NS
Female	23	27	NS
Choledocholith			
Number of stones	5.24 ± 5.52 (1-30)	6.44 ± 6.98 (1-25)	NS
Stone diameter (mm)	14.03 ± 3.00 (10-28)	14.52 ± 4.48 (10-25)	NS
Common bile duct diameter (mm)	16.62 ± 3.52 (10-28)	17.44 ± 3.93 (10-25)	NS
Gallbladder			
Calculous	43	34	NS
Acalculous	0	1	NS
Cholecystectomy	15	13	NS
Primary case	43	36	NS
Recurrence	17	10	NS
Endoscopic sphincterotomy	36	29	NS
Post endoscopic sphincterotomy	23	18	NS
Diverticulum	15	35	< 0.05
Hypertension	23	37	< 0.05
Hyperlipidemia	9	21	< 0.05
Diabetes mellitus	10	8	NS
Chronic respiratory disease	0	5	< 0.05
Cardiac disease	8	18	< 0.05
Chronic liver disease	1	0	NS
Chronic kidney disease	2	3	< 0.05
Anticoagulant/antiplatelet	9	23	< 0.05

NS: Not significant.

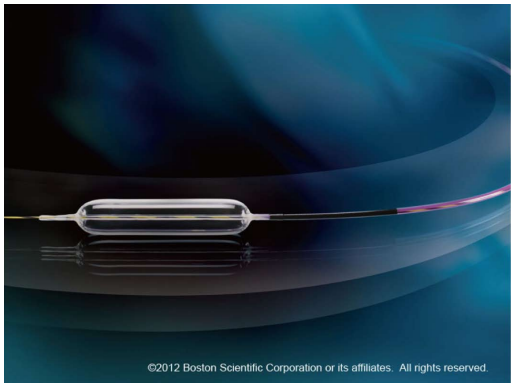

Figure 1 Endoscopic papillary large balloon dilation. CRE 12-20 mm (Wire-guided type 5.5 cm, Boston Scientific Corp., Natick, MA).

made, a guidewire was placed in the bile duct after cholangiography to perform EPLBD. In performing EPLBD, CRE 12-20 mm (Fixed wire type 8 cm or Wire-guided type 5.5 cm: Boston Scientific Corp.) was used depending on the diameter of the bile duct (Figure 1). For balloon dilation, a contrast agent mixed with saline solution at a volume ratio of 1:1 was used to slowly inflate it. Inflation was performed until the notch on the balloon disappeared (Figure 2). However, regardless of the disappearance of the notch, balloon dilation was completed when the papilla was dilated enough for stone removal. The balloon was dilated in a position where it was possible to confirm the tip of the balloon in the papillary side on the endoscopic image, and the position was maintained. After the notch on the balloon disappeared, the balloon was promptly deflated. When we considered it necessary to perform lithotripsy of the stone, we did it without hesitation.

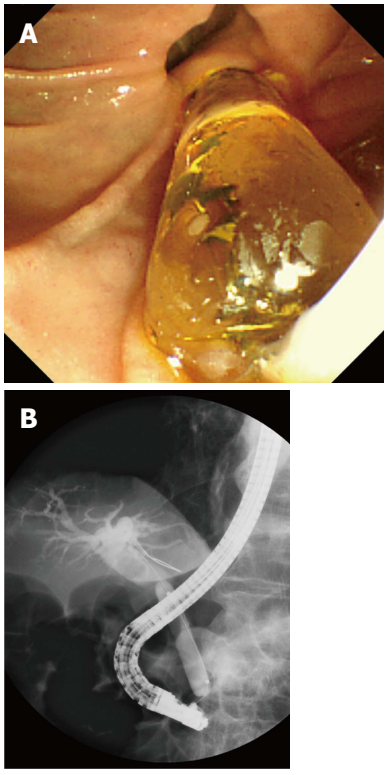

Figure 2 The papilla. A: The papilla was gradually dilated using a large balloon. Dilation was continued until the notch on the balloon disappeared (endoscopic image); B: The papilla was gradually dilated until the notch on the balloon disappeared (fluoroscopic image).

The basket catheter used for lithotripsy was LBGT-7245S (ZEON Medical) or BML-V237QR-30 (Olympus Corp.). Peroral cholangioscopy (POCSL) was performed by mother-baby system using CHF-B260 (Olympus Corp.) as the cholangioscope and Autholith (Northgate) as the electrohydraulic lithotripsy probe. The basket catheter used for collecting stones was FG-22Q or FG-V416Q (Olympus Corp.), LBGT-7245S (ZEON Medical) or BML-V237QR-30 (Olympus Corp.) The balloon catheter used was EXP718200 (ZEON Medical) or FS-QEB-XL-B (COOK). No drainage tube was inserted when lithotomy was successful, while it was inserted when we thought a stone still remained.

Iatrogenic morbidity was assessed according to the criteria of Cotton *et al*[36]. The observation period was 30 d after the procedure and any coincidental event noted during the period was considered as an early coincidental event. All the treatment procedures were performed after obtaining the informed consent from the patients in writing. This study was conducted after the study protocol was approved by the ethics committee of Chiba University.

Statistical analysis

Pearson χ^2 test with Yates correction and Fisher's exact test, when appropriate, were used for statistical analysis of categorical variables. Data were analyzed using SPSS software version 11 (SPSS, Chicago, IL). Differences with a P value of < 0.05 were considered statistically significant.

RESULTS

The success rate of lithotomy in the initial treatment was 88.7 (94/106)%. The final lithotomy rate was 100 (106/106)%. The time necessary to perform lithotomy was 39.4 (10-128) min and the mean treatment frequency was 1.21 (1-4) times. Lithotripsy was needed in 11.3 (12/106)% of the patients. Among the patients requiring lithotripsy, 6 had a highly tortuous bile duct and 6 had significantly large bile duct stones. For lithotripsy, endoscopic mechanical lithotripsy was performed in 10 patients and POCSL in 2. Complications due to the treatment procedure were observed in 4.7 (5/106)% of the patients, including bleeding in 1.9 (2/106)%, perforation in 0.9 (1/106)%, pneumonia in 0.9 (1/106)%, and acute cholangitis in 0.9 (1/106)%. Patients were classified into group A or group B for the analysis of data (Tables 2 and 3). The lithotomy success rate was 88.1 (52/59)% in group A, and 89.4 (42/47)% in group B and that for final lithotomy was 100%, both in group A (59/59)% and in group B (47/47). The lithotripsy rate was 10.2 (6/59)% in group A, and 12.8 (6/47)% in group B. Operation time was 37.59 ± 26.94 (12-125) min in group A, and 42.02 ± 27.12 (10-128) min in group B. The number of ERCP was 1.24 ± 0.683 (1-4) in group A, and 1.17 ± 0.529 (1-4) in group B. The incidence of complications was 6.8 (4/59)% in group A and 2.1 (1/47)% in group B, and there was no significant difference between group A and group B, regarding other parameters.

DISCUSSION

This study showed that elderly patients aged ≥ 80 years often have underlying diseases, however, results of treatment for large bile duct stones or multiple bile duct stones using EPLBD lithotomy were equivalent to those aged < 80 years in terms of success rate, lithotripsy rate, procedure time, number of ERCP, and complications caused by the procedure. EPLBD is the endoscopic treatment for bile duct stones reported by Ersoz *et al*[21] in 2003. Recently, the reports on the results of treatment for choledocholithiasis using EPLBD have been increasing[20-35]. Ordinary endoscopic papillary balloon dilation (EPBD) employs a balloon 4-10 mm in diameter for papillary dilation, whereas EPLBD is performed using a balloon 12-20 mm in diameter. Before this procedure was reported, lithotomy of large bile duct stones or multiple bile duct stones was difficult without lithotripsy of the stone. This procedure has the advantage that compared with EPBD or EST a larger papillary aperture can be obtained. It is reported in randomized controlled trials and meta-analyses that the larger papillary aperture enables easy insertion of the device as well as lithotomy of stones the same size as that of the dilated balloon without lithotripsy of the stones in many patients[34,37,38], which may be advantageous because the duration of the procedure would be shortened[26]. In this study many patients were cured after one session of treatment. There are many reports describing that in the nature of things, elderly patients have many underlying diseases, whereas the comparison of patients aged 80 years or greater with those aged less than 80 years showed the similar tendency[11,19]. Shorter treatment time is naturally an advantage even in young patients, and this study confirmed that a shorter treatment time was desirable for elderly patients because many of them have underlying diseases such as respiratory disease. Shorter treatment time is beneficial for elderly patients. There was no difference in the success rate of the procedure itself, however, many patients aged ⩾ 80 years have peripapillary diverticulum according to a past report[19], and this study also showed elderly patients tended to develop it, thus when performing the procedure, it may be necessary to pay attention to perforation. The presence of peripapillary diverticulum may cause deviation of the course of the bile duct leading to difficulty in cannulation[39]. However, this study revealed that there was no difference in the success rate of the procedure between the two groups. The reason may be due to recent advancement of the endoscope and its related treatment instruments. In this study, EPLBD was performed after EST. There are past reports describing

Table 2 Lithotomy by endoscopic papillary large balloon dilation

ERCP procedures	< 80 yr old (Group A; *n* = 59)	⩾ 80 yr old (Group B; *n* = 47)	*P*-value
Lithotomy success rate			
Initial	52 (88.1%)	42 (89.4%)	NS
Final	59 (100%)	47 (100%)	NS
Lithotripsy	6 (10.2%)	6 (12.8%)	NS
Procedure time: min	37.59 ± 26.94 (12-125)	42.02 ± 27.12 (10-128)	NS
Number of ERCP	1.24 ± 0.683 (1-4)	1.17 ± 0.529 (1-4)	NS

ERCP: Endoscopic retrograde cholangiopancreatography; NS: Not significant.

Table 3 Complications after endoscopic papillary large balloon dilation

Related complications	< 80 yr old (Group A; *n* = 59)	⩾ 80 yr old (Group B; *n* = 47)	*P*-value
Pancreatitis	0	0	
Perforation	1 (mild)	0	NS
Bleeding	2 (mild)	0	NS
Cholangitis	0	1 (mild)	NS
Cholecystitis	0	0	
Others	1	0	NS
Total	4	1	NS

NS: Not significant.

that it is possible to safely perform EPLBD without performing EST[35]. According to this study many elderly patients not only have peripapillary diverticulum but also are taking an anticoagulant or antiplatelet agent, thus if it were possible to safely perform EPLBD without performing EST, the procedure time would be shortened even further and the risk of perforation or bleeding would also be reduced.

Although a shorter procedure time and success of the procedure are very important for elderly patients, a low rate of complications derived from the procedure is also required. Past reports showed that complications caused by this procedure occurred at a low rate[20-35]. In the present study, complications occurred at a low rate and there was no difference between the two groups, suggesting it is possible to safely perform EPLBD even in elderly patients. The most problematic complications among ERCP related procedures is pancreatitis. Although it is reported that pancreatitis is less likely to occur in elderly patients because of their reduced pancreatic function[40], this study revealed there were no such results at all, suggesting that safety of the procedure is not only ensured for elderly patients but also that of the procedure itself is ensured. Reports describing usefulness and safety of EPLBD in elderly patients are currently limited to retrospective studies, thus a prospective study is necessary to confirm our findings.

EPLBD can be safely performed in elderly patients the same as in younger patients.

COMMENTS

Background

The usefulness of endoscopic treatment using endoscopic papillary large balloon dilation (EPLBD), which is a new papillary treatment using a large diameter balloon for large bile duct stones or multiple bile duct stones has been recently reported. Most of the reports have described this procedure is safe and useful. However, there are some cases of death. Since elderly patients are often complicated with underlying diseases, they may follow a fatal course after EPLBD, thus they require special attention. There are only a few reports on the usefulness of this procedure in elderly patients, and sufficient examination has not been conducted. This report examined the usefulness of EPLBD in patients aged ⩾ 80 years.

Research frontiers

The results of EPLBD in patients with bile duct stones ⩾ 13 mm in their short diameter or patients with three or more bile duct stones ⩾ 10 mm in their short diameter were examined.

Innovations and breakthroughs

EPLBD for bile duct stones was reported by Ersoz *et al* in 2003. Recently, its indication has widened. The reports describing the usefulness and safety of EPLBD in elderly patients are currently limited to retrospective studies. A prospective study is necessary to confirm our findings.

Applications

In patients with large common bile duct stones, endoscopic sphincterotomy (EST) + EPLBD is a good alternative to conventional EST. Before this procedure was reported, lithotomy of large stones or multiple stones was difficult without lithotripsy of the stones. Endoscopic treatment by papillary large balloon dilation can be safely performed in elderly patients the same as in younger patients.

Terminology

Treatment by EPLBD, which is lithotomy without lithotripsy for large stones by dilating the papilla using a large balloon, after performing EST has been reported. Endoscopic treatment by papillary large balloon dilation can be safely performed in elderly patients the same as in younger patients.

Peer-review

This is a retrospective study evaluating whether EPLBD can be safely and effectively performed in elderly patients. This study may be of interest to the readers.

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ORIGINAL ARTICLE

Prospective Study

Efficacy of different doses of sugammadex after continuous infusion of rocuronium

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Abstract

AIM: To evaluate the effects of two different doses of sugammadex after maintenance anesthesia with sevofluorane and remifentanil and deep rocuronium-induced neuromuscular blockade (NMB).

METHODS: Patients between 20 and 65 years of age, with American Society of Anesthesiologists physical status classification Ⅰ-Ⅱ, undergoing gynecological surgery were included in a prospective, comparative and randomized study. NMB was induced with an injection of 0.6 mg/kg of rocuronium followed by continuous infusion of 0.3-0.6 mg/kg per hour to maintain a deep block. Anesthesia was maintained with sevofluorane and remifentanil. Finally, when surgery was finished, a bolus of 2 mg/kg (group A) or 4 mg/kg (group B) of sugammadex was applied when the NMB first response in the train-of-four was reached. The primary clinical endpoint was time to recovery to a train-of-four ratio of 0.9. Other variables recorded were the time until recovery of train-of-four ratio of 0.7, 0.8, hemodynamic variables (arterial blood pressure and heart rate at baseline, starting sugammadex, and minutes 2, 5 and 10) and adverse events were presented after one hour in the post-anesthesia care unit.

RESULTS: Thirty-two patients were included in the study: 16 patients in group A and 16 patients in group B. Only 14 patients each group were recorded because arterial pressure values were lost in two patients from each group in minute 10. The two groups were comparable. Median recovery time from starting of sugammadex administration to a train-of-four ratio of 0.9 in group A and B was 129 and 110 s, respectively.

The estimated difference in recovery time between groups was 24 s (95%CI: 0 to 45 s, Hodges-Lehmann estimator), entirely within the predefined equivalence interval. Times to recovery to train-of-four ratios of 0.8 (group A: 101 s; group B: 82.5 s) and 0.7 (group A: 90 s; group B: 65 s) from start of sugammadex administration were not equivalent between groups. There was not a significant variation in the arterial pressure and heart rate values between the two groups and none of the patients showed any clinical evidence of residual or recurrent NMB.

CONCLUSION: A dose of 2 mg/kg of sugammadex after continuous rocuronium infusion is enough to reverse the NMB when first response in the Train-Of-Four is reached.

Key words: Rocuronium; Sugammadex; Neuromuscular block antagonism; Monitoring neuromuscular function; Neuromuscular block rocuronium

Core tip: The release of sugammadex in recent times has been a global shift in the strategy of the reversal of neuromuscular blockade (NMB) induced by aminosteroid neuromuscular blocking. The use of this drug has been increasing slowly, and consequently, we receive more and more questions in regards to its efficacy and safety. In this study we compared the dose of 2 mg/kg to 4 mg/kg sugammadex to reverse the NMB when first response in the train-of-four is reached after continuous infusion of rocuronium. Both doses have been shown to be effective for recovery from NMB.

Soto Mesa D, Fayad Fayad M, Pérez Arviza L, Del Valle Ruiz V, Cosío Carreño F, Arguelles Tamargo L, Amorín Díaz M, Fernández-Pello Montes S. Efficacy of different doses of sugammadex after continuous infusion of rocuronium. *World J Clin Cases* 2015; 3(4): 360-367 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i4/360.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i4.360

INTRODUCTION

Neuromuscular blockade (NMB) is an important technique in modern anesthesia because it improves surgical conditions by suppressing voluntary movements or muscular reflexes. The use of neuromuscular blockers is highly beneficial in determined types of surgery, such as laparoscopy, as it improves the surgical access and the visual field[1]. However, the extended use of NMB is associated with increased postoperative morbimortality due to the risk of residual neuromuscular paralysis or recurarization and the development of subsequent complications[2,3]. Such complications can be reduced by objective monitoring of muscle relaxation and NMB reversal after the surgical procedure.

The release of sugammadex [Bridion®, merck sharp and dohme, Oss, The Netherlands] triggers a change in the way of reversing aminosteroid neuromuscular blocking drugs. Sugammadex is a gamma-cyclodextrin with a lipophilic cavity that traps aminosteroid neuromuscular blocker molecules to form an inactive complex, thus preventing their union with nicotinic receptors and reversing their effects[4,5]. Clinical data suggests that sugammadex has a favorable efficacy and safety profile[4,6,7], allowing a safer and faster recovery-even from deep NMB[8]-than the commonly used combination of acetylcholinesterase inhibitors and anticholinergic agents.

Halogenated anesthetics, such as sevoflurane, increase the effect and duration of rocuronium[9], and this effect is clinically most significant when using a continuous infusion of rocuronium[10]. However, such do not appear to alter the efficacy or safety of sugammadex[11-13]. We hypothesize that a dose of sugammadex could result in a suitable recovery time although it depends on the individual redistribution and elimination of rocuronium as well[14]. The provider has not defined what the ideal dose of sugammadex for reversal the NMB when first response in the train-of-four (TOF) is reached. So, we have designed a study based upon on this hypothesis: after a surgical procedure, a dose of 2 mg/kg sugammadex is comparable to a dose of 4 mg/kg for reversal the NMB induced by a continuous infusion of rocuronium administered when first response in the TOF (T1) is reached.

MATERIALS AND METHODS

Patients and methods

A prospective, randomized and comparative study was designed to include patients undergoing a gynecological surgery, and took place over one year. The study was approved by the Regional Research Ethics Board of Principality of Asturias (Ref 118/2013; approved in August, 2013) and, after being given a verbal explanation, all patients gave their written informed consent. Applicable regulations and good clinical practice guidelines concerning NMB were followed in all cases[15].

The study included patients between 20 and 65 years of age, with American Society of Anesthesiologists (ASA) physical status classification Ⅰ-Ⅱ, who were scheduled for elective gynecological laparoscopy procedures under general anesthesia with sevoflurane requiring NMB with a minimum duration of 1 h, and carried out by the same surgical team.

The sample size was calculated on the basis of data for previous recovery time from NMB to first response in the TOF after sevoflurane anesthesia followed by 4 mg/kg sugammadex[14]. A 50% increase in recovery time was considered to be clinically relevant. To obtain statistically significant results with a probability of

type Ⅰ error (α = 0.05), probability of type Ⅱ error (β = 0.10), and a statistical power of 90%, a total of 22 patients were required. Therefore, 32 patients were recruited to compensate for any possible losses.

Patients were randomized to receive a dose of 2 mg/kg (group A) or 4 mg/kg (group B) after surgical procedure by the responsible anesthesiologist as previously had been determined. A manual randomization method was performed.

Exclusion criteria were as follows: previous known neuromuscular disease, obesity [defined as a body mass index (BMI) $\geqslant$ 30 kg/m^2], allergy to any drug used in the general anesthesia, history of malignant hyperthermia, liver or kidney insufficiency, predicted difficult airways or a previous history of difficult intubation, use of drugs that affect the neuromuscular system (for example: magnesium, anticonvulsants, aminoglycosides), pregnancy or lactation, or any other medical condition which could affect level of consciousness.

Anesthesia and neuromuscular monitoring

All patients received intramuscular 2 mg midazolam as premedication. Standard monitoring was performed once the patients were in the operating room (pulseoximetry, capnography, electrocardiography and noninvasive arterial pressure). Patients were preoxygenated with FIO_2 of 1.0 for 3 min before induction of anesthesia with intravenous propofol (1.5-2.5 mg/kg) and fentanyl (1-2 mcg/kg).

Neuromuscular function was monitored through kinemyography (KMG) in form of the Mechanosensor-Neuromuscular Module Transmission (M-NMT®) (GE Healthcare, Helsinki, Finland) integrated in the Datex-Ohmeda anesthesia machine. The right arm was placed at an angle of 90° to the longitudinal axis of the body and the electrodes were placed on cleaned skin 3-6 cm apart over the ulnar nerve at the wrist. M-NMT was placed on the adductor pollicis muscle. Physical means were used to maintain the peripheral temperature above 35 ℃.

Once the induction of anesthesia was finished and before the administration of rocuronium, the M-NMT monitor was calibrated using 200 μs pulses at a rate of 2 Hz, starting at 5 mA with increments of 5 mA. The maximal current was increased by 15%, yielding the supramaximal stimulation. The 0.6 mg/kg of rocuronium bolus was then injected provided that a first 2 Hz TOF stimulation for 1.5 s yielded four equal responses within 15% of the calibration. When there was no measurable response to TOF stimulation, the patients were intubated and mechanical ventilation was initiated. This initial dose was followed by a continuous infusion of 0.3-0.6 mg/kg per hour of rocuronium which was adjusted to maintain a deep block with a TOF response of zero and PTCs less than 10 for the duration of the procedure. TOF stimulations were repeated every 15 s throughout the study. A PTC mode was initially applied 5 min after obtaining complete NMB and repeated every 6 min. Anesthesia was maintained with sevoflurane 1%-3% end-tidal. In both groups analgesia was provided by remifentanil with a dose of 0.05-0.5 mcg/kg per minute.

Upon completion of the surgery, the administration of sevoflurane, remifentanilo and rocuronium ended. At the reappearance of the T1, every patient received a dose of sugammadex according to the group in which they had been randomized (2 mg/kg in group A, or 4 mg/kg in group B), and they were awoken once complete NMB reversal (TOF ratio $\geqslant$ 0.9) was reached. Neuromuscular monitoring was continued until patients were extubated. Once recovered, they were transferred to the post-anesthesia care unit.

After one hour in the post-anesthesia care unit, a member of the team who was blind to the sugammadex dose that the patient had received, evaluated in each patient the presence of any residual paralysis through neuromuscular monitoring and performed a clinical assessment by signs of muscular weakness and clinical tests (lifting the head for more than 5 s, holding a tongue depressor between the teeth and generalized muscular weakness). The post-anesthesia oxygen saturation, breathing rate and any possible hemodynamic instability as well as the appearance of any adverse effect was also recorded. The same post-surgical analgesia protocol was applied to all patients.

Statistical analysis

Patient baseline quantitative variables in the two groups were compared by two-sided Student *t*-test if they followed a normal distribution. Categorical variables were analyzed by Pearson χ^2 test (or Fisher Exact test if expected count less than 5). Odds ratio (OR) and its CI was calculated if necessary.

The primary efficacy variable was the time (in seconds) between commencing sugammadex administration and reaching recovery of the TOF ratio to 0.9. The time until recovery of TOF ratios to 0.7 and 0.8 were studied too. We used the statistical approaching method described by Rex *et al*[14]. The CI approach was used to demonstrate equivalence in recovery of the TOF ratios between the two treatment groups. Non statistical signification was established if the two-sided 95%CI for the estimated difference of median between group A and group B was within the interval ranging from 0% to 50% of the median of group B. The 95%CI was obtained by using the nonparametric methods of Hodges-Lehmann. Similarly, TOF ratio to 0.7 and 0.8 were studied.

The hemodynamic variables were the evolution of arterial blood pressure (AP) and the heart rate (HR) after sugammadex injection. AP and HR were recorded every 5 min throughout the intervention: previously, during the start of sugammadex, and 2, 5 and 10 min after initiating administration of the drug. Any possible secondary effect associated to its administration was also recorded.

Table 1 Demographic characteristics

Sugammadex (dose)	Group A (n = 16)		Group B (n = 16)		P-value
Age (yr)	43.6	(SD 12.01)	47.1	(SD 14.18)	0.46
Weight (kg)	65.5	(SD 11.22)	60.9	(SD 10.62)	0.25
Height (cm)	163.2	(SD 4.76)	160.1	(SD 5.91)	0.12
BMI (kg/m^2)	24.1	(SD 3.56)	23.2	(SD 3.65)	0.47
Intervention (time-minute)	95.2	(SD 26.91)	94.7	(SD 30.02)	0.96
ASA (1-2)	1.4	(SD 0.51)	1.2	(SD 0.48)	0.28
ASA 1[a]	n = 9	(56.25%)	n = 12	(75.00%)	0.23

Analyzed by student t-test. Both groups are similar. [a]ASA 1 is expressed as percentage of patients with an ASA index of 1 in its group and a Fisher exact test was executed. BMI: Body mass index; ASA: American Society of Anesthesiologists.

Data for AP and HR were analyzed by repeated measure analysis of variance (ANOVA-RM). The within-subjects terms were the AP and HR values for each patient, and the repeated term was the time point (baseline, starting, and minute 2, 5 and 10). Pillai's Trace[16] is calculated for AP and HR and their interactions with sugammadex doses. They were corrected with epsilon multipliers if the assumption of circularity had been violated following Mauchly's test[17]. Lower bound was elected to be the most conservative. Post-hoc analyses were executed. The P-values < 0.05 were considered significant. All tests were 2-sided. Data was analyzed using SPSS 17.0 for Windows (SPSS Inc., Chicago, United States).

RESULTS

A total of 32 patients were included in the study, 16 patients in group A and 16 patients in group B. All descriptive variables are summarized in Table 1. However, AP was not taken in the 10th minute in two patients in each group. Because AP in the 10th minute is a related sample within temporal evolution (the others are AP baseline, pre-sugammadex, minute 2th and minute 5th), only 14 patients from each group were computed (another two were excluded). So, all results were analyzed by per-protocol; however, AP values were lost in two patients in each group for the 10th minute.

The gynecological interventions were fourteen vaginal assisted laparoscopic hysterectomies (43.7%), eleven laparoscopic ovarian cystectomies (34.4%) and seven laparoscopic adnexectomies (21.9%). The two groups were comparable in terms of age, BMI and ASA (Table 1). Surgical time was more than 60 min in all cases.

All patients recovered to a TOF ratio of 0.9 within 3 min (maximum value 175 s). Median recovery time from starting of sugammadex administration to a TOF ratio of 0.9 was 129 s in group A and 110 s in group B. The estimated difference in recovery time between the two groups was 24 s (95%CI: 0 to 45 s, Hodges-Lehmann estimator). This CI was entirely within the predefined equivalence interval (for a median of 110 s in group B = 0 to 52.5 s), so equivalence was assumed. Times to recovery to TOF ratios of 0.8 and 0.7 from start of sugammadex administration were not equivalent between groups. Median time to recovery to a TOF ratio of 0.8 was 101 s in group A and 82.5 s in group B, with an estimated difference of 18 (95%CI: -5 to 39 s, Hodges-Lehmann estimator). 95%CI was out of predefined equivalence interval of 0 to 43.7 s. Median time to recovery to a TOF ratio of 0.7 was 90 s in group A and 65 s in group B, with an estimated difference of 10 (95%CI: -10 to 35, Hodges-Lehmann estimator). So, 95%CI was out of predefined equivalence interval of 0 to 32.5 s. Equivalences were not assumed for TOF ratio 0.8 and TOF ratio 0.7 (Table 2).

There was no significant variation in the AP and HR between the two groups. Although both of them maintained AP and HR within normal ranges the entire time, there was a logical increment of AP and HR as time passed until the effect of anesthetic drugs disappeared. So, post-hoc analyses were statistically significant across the 2nd, 5th and 10th minute within each group (Figure 1).

Based on neuromuscular monitoring and clinical signs, none of the patients showed any clinical evidence of residual or recurrent NMB. Although group B had more adverse events than group A, there was no statistical difference between them (group A: 12.5% *vs* group B: 18.7%, OR = 1.62; 95%CI: 0.23-11.26, P = 0.99). There were no severe adverse effects, even with an increased dosage of sugammadex. As a consequence, in the immediate post-operatory period in group A, there was one case of nausea and another case of pain, while in group B, there was one case of nausea, one case of pain and one patient suffered tremors in lower limbs (Table 3). Habitual symptomatic treatments were adopted and they were effective without any more clinical relevance.

DISCUSSION

Our study suggests that a dose of 2 mg/kg sugammadex is enough for the recovery of NMB induced by a continuous infusion of rocuronium in patients who kept anaesthetized with sevoflurane. This lower dose did not

Table 2 Train-of-four ratio studies

	Group A (n = 14)		Group B (n = 14)					
	Mean	Median	Mean	Median	Assumed calculated interval (increased of 0% to 50% of median in group B)	Estimated difference median by Hodges-Lehmann estimator	95%CI	
TOF ratio 0.9	118.8	129	96.6	105	0 to 52.5	24	0 to 45	Differences not assumed
TOF ratio 0.8	96.7	101	80.1	82.5	0 to 43.7	18	-5 to 39	Assumed
TOF ratio 0.7	78.4	90	66.3	65	0 to 32.5	10	-10 to 35	Assumed

Based on the value of the median of TOF ratio in the Group B, an interval was calculated to establish the acceptable variation of the median values in the Group A (an increase from 0% to 50% respect Group B). The Hodges-Lehmann estimator was calculated for the differences between TOF ratio 0.7, 0.8 and 0.9 medians with their 95%CI. All values are expressed in seconds. If the Hodges-Lehmann 95%CI was contained in the 95%CI based on medians of the Group B, no statistical and clinical differences would be assumed. TOF: Train-of-four.

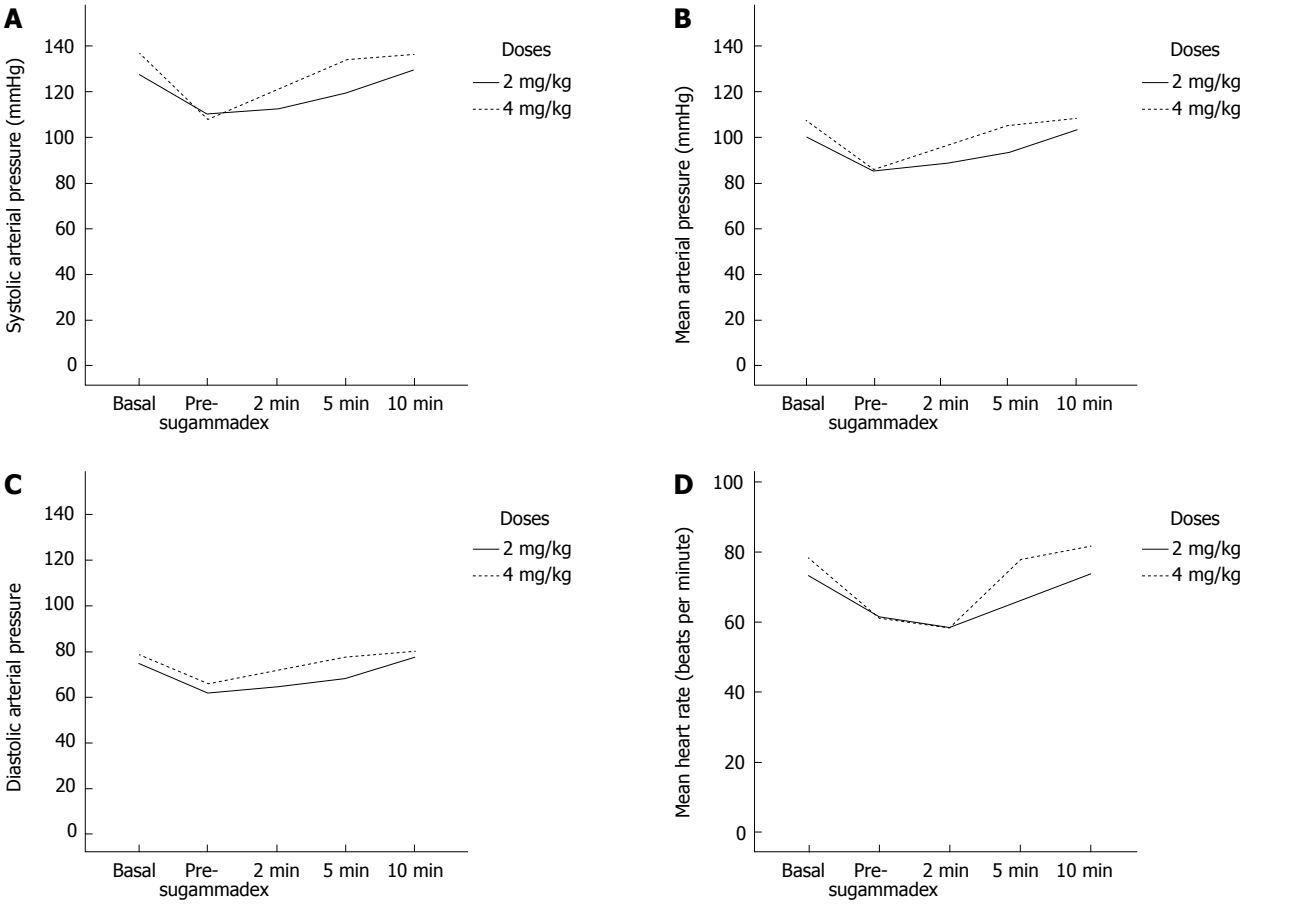

Figure 1 Arterial pressures and heart rate after sugammadex administration. A: Systolic arterial pressure; B: Mean arterial pressure; C: Diastolic arterial pressure; D: Mean heart rate. The increased amount of arterial pressures and heart rate after sugammadex administration was statistically significant as the time passed (post hoc analysis in ANOVA-RM). But the values were in the normal range the entire time. So, it only shows the activity of both administered doses and there was no statistical significance between them.

have any clinically relevant recovery time augmentation or increased risk of residual recurarization. We have not been able to observe other adverse events in our patients.

The main limitation in our study was the lack of rocuronium and sugammadex plasma concentration determinations at different moments of the study. Although previous studies have shown a similar rocuronium pharmacokinetic profile when compared continuous infusion *vs* intravenous bolus dose[18], significant variations in plasma concentrations of rocuronium were also observed in those continuously infused with this drug (highly variable, up to 30% for some patients)[14]. For this reason, neuromuscular transmission monitoring suggested a better option in patients who received continuous infusions of rocuronium as a more realistic approach to the global effect of the drug. This is not routinely used in current

Table 3 Adverse events

Adverse events	Group A	Group B
Arterial hypertension	0%	0%
Arterial hypotension	0%	0%
Bradycardia	0%	0%
Cough	0%	0%
Headache	0%	0%
Nausea	6.20%	6.20%
Pain	6.20%	6.20%
Residual neuromuscular blocking	0%	0%
Vomiting	0%	0%
Others	0%	6.20%

daily monitoring in clinical practice[19,20] and a study published in the United Kingdom in 2007 reported that 62% of anesthetists surveyed had never used monitors to evaluate the effect of NMB[20].

Another point of interest was the use of a dose of 4 mg/kg of sugammadex. It has been demonstrated as preferable in the reversion of deep NMB[8]. The provider recommends a dose of 4 mg/kg if recovery has reached at least 1-2 PTCs, and a dose of 2 mg/kg sugammadex when spontaneous recovery has occurred up to least the reappearance of second response in the TOF[21]. Other authors consider in clinical practice that the appropriate dose of sugammadex for reversing a moderate block (TOF-count 1-3) is 2 mg/kg of sugammadex[22].

A TOF ratio $\geqslant$ 0.9 was used as the main desirable objective variable because a postoperative residual curarization TOF ratio < 0.9 is associated with increased morbidity and extended stay in the post-anesthesia care room[23]. It has been published that with 4 mg/kg of sugammadex, the time to recover a TOF ratio of 0.9 from 1-2 PTCs (induced by a bolus of rocuronium under anesthesia with sevoflurane) was 1.7 min compared to 3.2 min with a dose of 2 mg/kg of sugammadex[12]. However, studies comparing the efficacy of sugammadex in surgical patients when NMB was induced through the infusion of rocuronium are very scarce. Rex *et al*[14] demonstrated that just one dose (4 mg/kg) of sugammadex administered at a NMB to T1, after continuous infusion of rocuronium, was sufficient and safe with both sevoflurane and propofol. This use of continuous infusion of rocuronium has been shown to lengthen the NMB recovery time compared with one single bolus[24], thereby providing a more stable drug concentrations with a constant degree of paralysis. In our series, we find that difference between the means of the TOF 0.9 of both groups is lower than previously described: approximately an increase of only 23% *vs* the estimated published of 88%[12]. This difference can be attributed to different time of reversal of NMB and different procedures.

A limitation of our study is the age of the patients and the kind of surgical intervention (young and gynecological patients). In contrast, these patients were elected because they were attended by the same surgical team; hence similar laparoscopic conditions were expected in all cases. We decided to limit the age to 65 years because, even though reversal from profound block with sugammadex can be performed safely and effectively, there have been reports regarding older patients who recover more slowly than younger ones[25,26]. This slower recovery could be due to age-related decreased cardiac output and muscular blood flow[26].

Another possible bias in our study could be that surgical procedures lasted 60 min. They may be classified as insufficient. Nonetheless, it has been seen that a dose of 2 or 4 mg/kg of sugammadex is sufficient for reversion of NMB, even when deep NMB (1-2 PTCs) is maintained for 2 h or more, with reversal being performed when the second TOF response occurs[8,27].

We also observed the safety of using sugammadex. Adverse events related to the administration of sugammadex have been reported in the literature with an incidence of 14%, the most common being nausea, vomiting, bradycardia, hypertension and hypotension, oliguria, vertigo, headache, cough, dry mouth and intraoperative movements[28]. However, these adverse effects were not related with the use of sugammadex[11,12] or the dose administered. In our series, we found a similar occurrence in the two groups and there was no statistical difference between them. We consider that they were expectable, without direct relationship with the studied drug and not clinically relevant.

It could be supposed that the use of sugammadex would lead to a reduction of adverse events in the immediate postoperative period. They require additional resources and a longer recovery time. So, sugammadex could improve efficiency and reduce the costs related to surgical activities[29,30]. Nevertheless, the reduction of the sugammadex dose to save costs could be a mistake which may lead to other complications, such as the recurrence of NMB after an apparently successful recovery[31]. In this study we do not analyze the economic implications of the lower dose. We think that the group size is too small to establish conclusions, because they were selected and calculated to observe the effect on TOF 0.9 of sugammadex in two different doses. A dose of 2 mg/kg is evidently the half of cost of 4 mg/kg but it is only in respect to a simple drug expenditure and we cannot apply it to the complete surgical procedure and its multiple non-contemplated influent variables.

Only future investigation will make enable us to consider readjusting the currently recommended doses in specific circumstances, without an increase in the risks[32]. So, more studies are necessary in different surgical sceneries to understand all possibilities of sugammadex.

In conclusion, in our study, a dose of 2 mg/kg sugammadex was found to be efficient and safe for reversing the NMB when first response in the TOF is

reached, after a continuous infusion of rocuronium without increasing the risk of residual recurarization. Future studies are required to determine any possible readjustments of doses and the consequent risks that lower doses of sugammadex may cause in the reversal of NMB. In the future, with the absence of plasma level of drugs, neuromuscular monitoring will be essential in the daily anesthetic practice, especially when rocuronium is given as a continuous infusion for the immediacy of its results.

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COMMENTS

Background

The introduction of sugammadex to antagonize non-depolarising neuromuscular blockade (NMB) has led to significant changes in anaesthesia practice. Residual effects of neuromuscular block can have significant clinical consequences and can cause critical respiratory events. The superiority of sugammadex (*vs* neostigmine) for reversing neuromuscular block has now been well established.

Research frontiers

Sugammadex should be dosed according to the prescriber information issued by the manufacturer. The provider recommends a dose of 4 mg/kg if recovery has reached at least 1-2 PTCs, and a dose of 2 mg/kg sugammadex when spontaneous recovery has occurred up to at least the reappearance of second response in the train-of-four (TOF), but they don´t define the ideal dose of sugammadex for reversal the NMB when first response in the TOF is reached.

Innovations and breakthroughs

This study suggests that a dose of 2 mg/kg sugammadex (*vs* 4 mg/kg) is enough for the recovery of NMB induced by a continuous infusion of rocuronium in patients who kept anaesthetized with sevoflurane when first response in the TOF is reached. This lower dose did not have any clinically relevant recovery time augmentation or increased risk of residual recurarization. The authors have not been able to observe more adverse events in the patients.

Applications

A dose of 2 mg/kg sugammadex was found to be efficient and safe for reversing the NMB when first response in the TOF is reached without increasing the risk of residual recurarization. Future studies are required to determine any possible readjustments of doses and the consequent risks that lower doses of sugammadex may cause in the reversal of NMB. In the future, quantitative neuromuscular monitoring is mandatory and increased postoperative vigilance is required in order to identify the problems of incomplete reversal.

Terminology

After injection of a nondepolarizing neuromuscular blocking drug in a dose sufficient for smooth tracheal intubation, TOF recording demonstrates three phases of NMB: intense, moderate or surgical blockade, and recovery. Intense NMB is also called the period of no response because no response to TOF or single-twitch stimulation occurs. Although this phase it is not possible to determinate exactly how long intense NMB will last, correlation does exist between PTC stimulation and the time to reappearance of the first response to TOF stimulation. Moderate blockade begins when the first response to TOF stimulation appears. This phase is characterized by a gradual return of the four responses to TOF stimulation. The return of the fourth response in the TOF heralds the recovery phase. Satisfactory recovery from NMB has not occurred until the TOF ratio is > 0.9.

Peer-review

This is a study comparing the efficacy and safety of two different doses of sugammadex. The paper is well written and designed.

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CASE REPORT

Hair thread tourniquet syndrome in a toe of an 18 mo old girl

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Author contributions: Both authors contributed to this work.
Ethics approval: The study was reviewed and approved by the Spaarne Ziekenhuis Institutional Review Board.
Informed consent: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.
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Abstract

Hair thread tourniquet syndrome is rare and usually affects little children. If the tourniquet is not incised, the affected body part becomes ischemic or even necrotic. An 18 mo old girl was seen in the emergency ward with a painful, red and swollen third toe of the left foot. The toe appeared to be strangulated with a hair, and the diagnosis hair thread tourniquet syndrome was made. After incision of the hair tourniquet the symptoms soon subsided. The diagnosis is easily made if the clinical features are recognized. However, if the tourniquet is not cut through, the affected body part may become ischemic and even necrotic.

Key words: Hair; Thread; Tourniquet; Syndrome; Toe

Core tip: Hair thread tourniquet syndrome is rare and usually affects little children. We present a case of an 18 mo old girl with a strangulated toe. After incision of the hair tourniquet the symptoms soon subsided. The diagnosis is easily made if the clinical features are recognized. However, if the tourniquet is not cut through, the affected body part may become ischemic and even necrotic.

Kuiper JWP, de Korte N. Hair thread tourniquet syndrome in a toe of an 18 mo old girl. *World J Clin Cases* 2015; 3(4): 368-370 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i4/368.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i4.368

INTRODUCTION

Hair thread tourniquet syndrome (HTTS), or hair tourniquet syndrome, is a syndrome in which children experience strangulation of small extremities (fingers, toes or external genitalia) with a hair or thin wire[1,2]. If the strangulation is not recognized, the affected body part becomes ischemic, and can even necrotize in a few hours to weeks[2,3]. In this report, a patient with HTTS of the third toe is presented, for which she was surgically treated.

CASE REPORT

An 18 mo old girl was presented in the emergency ward, after her mother had noticed a constriction

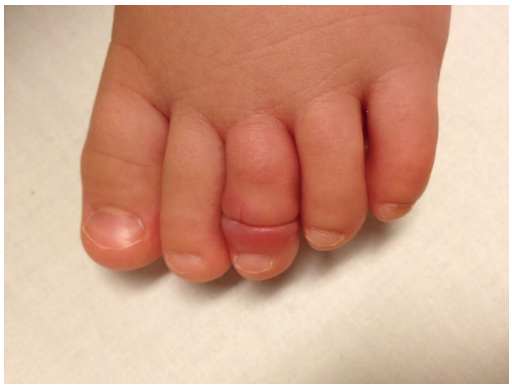

Figure 1 Strangulation of the third toe: Typical clinical picture of hair thread tourniquet syndrome.

around her third toe of the left foot, that morning. She thought this may have had been caused the previous day, when the child's father had removed some hairs from under the toes, after a visit to the swimming pool. The girl was otherwise healthy and did not take any medication. The mother had had a normal partus (C-section), and the girl had afterwards been in the hospital only once, with fever caused by a viral infection.

On physical examination we saw a healthy looking 18 mo old girl, with a strangulation around the third toe of the left foot. No hair or thread could be seen around the toe. Redness and swelling was seen around the strangulation. A normal capillary refill (< 2 s) was seen. Upon palpation, the toe was painful (Figure 1).

An attempt was made to remove (remains of) the strangulating hairs with a stitch cutter, without success. With the idea that possibly all the hairs had already been removed by the father, the patient was sent home, and the mother was advised to return the next day if the strangulation remained.

Indeed, they returned the next day. Physical examination was similar to the previous day, with unchanged strangulation, and still good capillary refill.

It was decided to incise the hair tourniquet in the operating room under general anesthesia. After incision to the bone-medial, between the proximal and distal interphalangeal joint-some hairs were removed.

One day after surgery, the swelling had largely subsided, and the constriction had almost disappeared. After two weeks the girl was seen again, and all symptoms were gone.

DISCUSSION

HTTS is a strangulation of a small limb, usually a finger or toe, or sometimes external genitalia. When the diagnosis is made correctly, treatment is simple and effective. Differential diagnoses may include cellulitis, erysipelas, or other irritation of the skin (for example after being bitten by an insect), or trauma[3].

Approximately a hundred cases of HTTS are previously described, mostly occurring for fingers (24%-47%), toes (25%-43%) or penises (44%)[3]. The typical age of affected children is around 5 years, and fingers are more frequently affected in younger children (up to 1.5 years)[3]. When a toe is affected, this is usually the third or fourth toe[2]. The material causing the strangulation is mostly either nylon or hair (both around 50% of the cases)[2]. However, HTTS in toes is more often caused by hairs[3].

The etiology of HTTS is, as previously mentioned, a hair or thread around a small body part and causing strangulation and sometimes even ischemia or necrosis. The patient can sometimes shed light on the origin of this hair or thread, but in other cases this remains unclear. How a hair can cause such a strangulation is not entirely clear, but one study mentions that that wet hair is longer than dry hair and thus, when drying, a hair tourniquet contracts and thus causes strangulation[3]. With this strangulation, swelling occurs, gradually causing a decrease in arterial blood supply and therefore tissue ischemia and necrosis[2,3].

HTTS diagnosis is easy to make when the clinical picture is recognized. It is however very important that HTTS is not missed, because prolonged HTTS can cause necrosis[3]. Therefore, it is important to always assess capillary refill in such patients[3].

Treatment for HTTS is simple: removal of the strangulating hair or thread. This may be difficult with extensive local swelling, and often surgical incision is required. This incision should be to the bone, to be certain that all the constricting material is dissected[3]. As turned out in this case, after such treatment the symptoms quickly disappear.

COMMENTS

Case characteristics

An 18 mo old girl with a painful swollen third toe was seen at the emergency ward.

Clinical diagnosis

A strangulated third toe was seen, presumably by a hair.

Differential diagnosis

Hair thread tourniquet syndrome is a clinical diagnosis with a very typical representation.

Treatment

Treatment for hair thread tourniquet syndrome consists of incision of the tourniquet.

Related reports

Hair thread tourniquet syndrome usually occurs in little children en generally affects toes, fingers or external genitalia.

Term explanation

Hair thread tourniquet syndrome is abbreviated as hair thread tourniquet syndrome.

Experiences and lessons

Hair thread tourniquet syndrome is a clinical diagnosis and is easily treated when recognized.

Peer-review

This is a nice case and well documented paper about the hair thread tourniquet.

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CASE REPORT

Dysbetalipoproteinemia: Two cases report and a diagnostic algorithm

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Abstract

Dysbetalipoproteinemia is a rare familial dyslipidemia characterized by approximately equally elevated serum cholesterol and triglyceride levels due to accumulated remnant lipoproteins in apolipoprotein E2/E2 homozygotes. It is associated with an increased risk for premature cardiovascular disease. Thus, making a diagnosis of dysbetalipoproteinemia aids in assessing cardiovascular risk correctly and allows for genetic counseling. However, the diagnostic work-up can be challenging. Diagnosis of dysbetalipoproteinemia should be considered in patients mixed dyslipidemia when the apolipoprotein B concentration is relatively low in relation to the total cholesterol concentration or when there is significant disparity between the calculated low density lipoprotein (LDL) and directly measured LDL cholesterol concentrations. Other indices are also informative in the diagnostic process. We present herein two phenotypically different cases (a 44-year-old man with severe hypertriglyceridemia and a 49-year-old woman with mixed dyslipidemia) of genotypically proven familial dysbetalipoproteinemia and a diagnostic algorithm of the disease.

Key words: Dysbetalipoproteinemia; Chylomicronemia; Hyperlipoproteinemia type Ⅲ; Hypertriglyceridemia

Core tip: Dysbetalipoproteinemia is associated with an increased risk for premature cardiovascular disease and its diagnosis may be challenging since its phenotype may significantly vary when specific environmental, hormonal and genetic factors that affect triglyceride (TG) metabolism co-exist. An algorithm with a number of dysbetalipoproteinemia indices may be helpful for the diagnosis of the disease and roughly equally elevated levels of both total cholesterol (TC) and TG and a low apolipoprotein B to TC ratio seem to comprise the two most helpful indices.

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INTRODUCTION

Apolipoprotein E (apoE) in plasma is mainly carried by chylomicrons, very-low-density lipoproteins (VLDL) and high-density lipoproteins (HDL). When associated with these lipoproteins, apoE serves as the ligand for the low density lipoprotein (LDL) receptor and the LDL-receptor related protein on the surface of hepatic cells[1]. In humans, three common apoE isoforms have been described, designated E2, E3, and E4[2,3]. ApoE2 and apoE4 differ from the more frequent apoE3 isoform by a single amino-acid substitution due to a single point mutation, conferring a more acidic or more basic charge to the protein. When compared with the apoE3 isoform, apoE2 has a markedly reduced affinity (< 1%) for the LDL receptor. Only a modest accumulation of cholesterol-enriched lipoprotein remnants of both hepatic and intestinal origin, or β-VLDL is observed in most apoE2/E2 homozygotes, which is not sufficient to cause an elevation of plasma cholesterol and triglyceride (TG) levels above normal. However, in individuals with predisposing genetic, hormonal, or environmental factors, this phenotype is associated with dysbetalipoproteinemia, also known as hyperlipoproteinemia type 3[2,4].

We present herein two phenotypically different cases of genotypically proven familial dysbetalipoproteinemia.

Methods

Blood samples for laboratory tests were obtained after a 12 h overnight fast. All serum laboratory measurements including fasting plasma glucose, creatinine, thyroid hormones, total cholesterol (TC), HDL cholesterol (HDL-C), and TG were determined enzymatically in the laboratory of the University Hospital of Ioannina using an Olympus AU 600 analyzer (Olympus Diagnostica GmbH, Hamburg, Germany). LDL cholesterol (LDL-C) was calculated using the Friedewald equation (provided that TGs were < 350 mg/dL (3.95 mmol/L). Apolipoproteins, serum and urinary proteins were measured with a Behring Nephelometer BN100 with reagents (antibodies and calibrators) from Dade Behring Holding GmbH (Liederbach, Germany). Antinuclear antibodies (MB Laboratories, Sidney, BC, Canada) were assessed by immunofluorescence. A commercial enzyme-linked immunosorbent assay was used to evaluate the levels of anti-extractable nuclear antigen (AESKU Diagnostics, Wendelsheim, Germany), while levels of serum globulins, C3 and C4 fractions of complement and rheumatoid factor were evaluated using nephelometry (Siemens Healthcare Diagnostics, Erlangen, Germany).

VLDL was isolated from plasma by two sequential ultracentrifugations according to the method of Gaw *et al*[5]. In brief, chylomicrons-deficient plasma firstly was prepared by plasma ultracentrifugation in a Beckman SW41 Ti rotor at 20000 rpm for 70 min at 23 ℃. The chylomicrons fraction was carefully removed from the top of the tube and the chylomicrons-deficient fraction was next submitted to ultracentrifugation at density of d = 1.019 g/mL at 45000 rpm for 8 h at 14 ℃. The VLDL fraction floating to the top of the tube was carefully collected.

DNA was extracted from the whole blood specimen according to standard procedures. ApoE genotyping was performed as described by Hixson and Vernier[6]. Polymerase chain reaction (PCR) was used to amplify a 244-bp sequence of the *apoE* gene, including the two polymorphic sites. The PCR product was then digested with the restriction enzyme Hha I and the different genotypes were detected after electrophoresis on 6% NuSieve agarose gel.

CASE REPORT

Case 1

Two years earlier a 44-year-old Caucasian man had received the diagnosis of mixed dyslipidemia based on the following lipid profile; TC: 420 mg/dL, TG: 580 mg/dL, HDL-C: 36 mg/dL. He had no family history of dyslipidemia or established cardiovascular disease, while physical examination was unremarkable. Secondary causes of dyslipidemia were excluded by evaluation of thyroid and renal function, urinary protein excretion, serum protein electrophoresis, erythrocyte sedimentation rate and autoantibodies. A low-fat diet was recommended and patient was given gemfibrozil, 600 mg, twice a day. His lipid profile improved after 2 mo, but he stopped taking the drug and was lost to follow-up. At age 46, the patient had consulted a dermatologist because of the lesions shown at Figure 1 and was referred for serum lipid assessment. He consumed small amount of alcoholic beverages and had stopped smoking 5 years earlier. For the past 6 mo, he experienced symptoms that suggested intermittent claudication. A Doppler ultrasonic study revealed mild stenosis of left femoral artery.

Seen at Figure 1 are the characteristic for dysbetalipoproteinemia tuberous xanthomas over the patient's elbows (they were also present on his knees) and the pathognomonic striated palmar xanthomas, while skin lesions typically associated with chylomicronemia, namely eruptive xanthomas, on his buttocks were also present (Figure 1). Laboratory assessment revealed: fasting plasma glucose: 300 mg/dL, TC: 1055 mg/dL, TG: 2900 mg/dL, HDL-C: 18 mg/dL, VLDL-C: 316 mg/dL, VLDL-TG: 831 mg/dL. The diagnosis of dysbetalipoproteinemia was verified by apoE2/E2 homozygosity genotype.

We suggested the patient to stop fat and alcohol consumption. He received metformin 850 mg twice a day and ciprofibrate 100 mg/d. Four weeks later the patient's skin lesions had regressed significantly and serum laboratory parameters improved (fasting

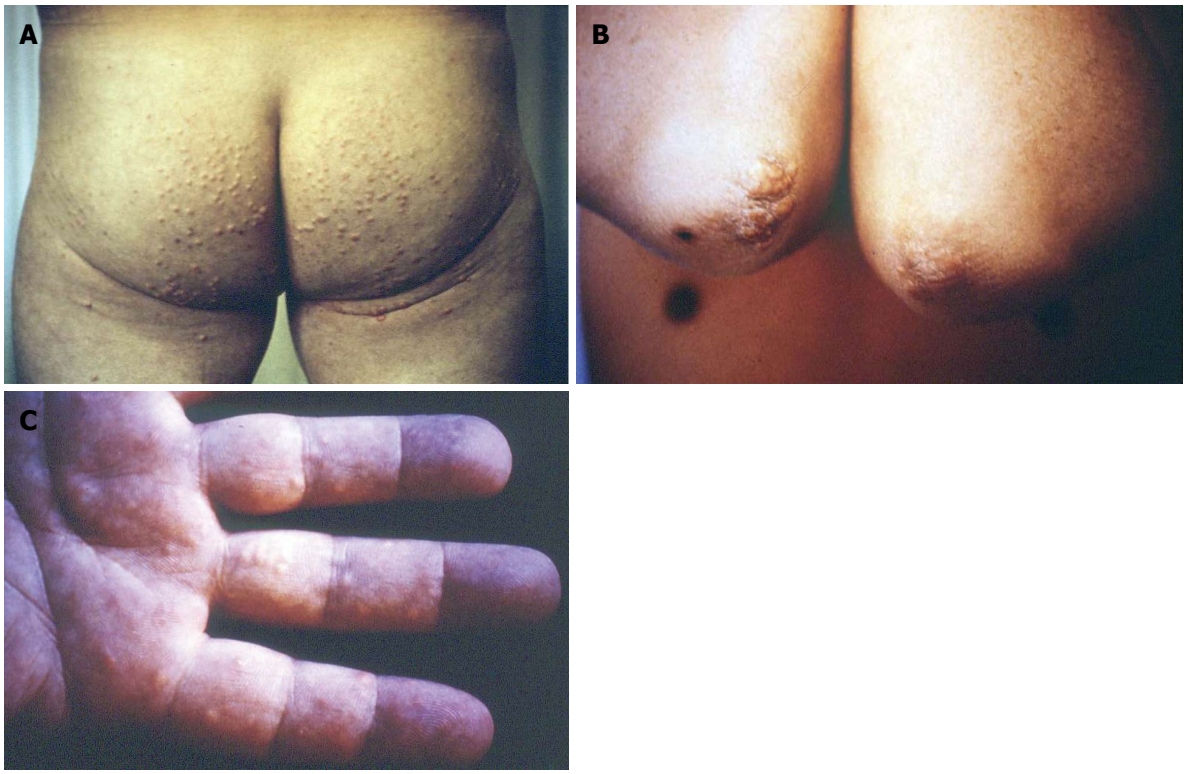

Figure 1 Tuberous xanthomas over the patient's elbows, striated palmar xanthomas and eruptive xanthomas on his buttocks. A: Eruptive xanthomas; B: Tuberous xanthomas; C: Striated palmar xanthomas.

plasma glucose: 150 mg/dL, TC: 412 mg/dL, TG: 754 mg/dL, HDL-C: 27 mg/dL). The dosage of ciprofibrate was increased to 100 mg, twice a day and glimepiride 2 mg/d was added.

Case 2

A 49-year-old Caucasian woman was referred to the Outpatient Lipid and Obesity Clinic of the University Hospital of Ioannina, Greece by her family doctor due to increased TC and TG levels. The patient denied any symptoms indicative of cardiovascular disease but her mother had been diagnosed with peripheral artery disease at the age of 40. In addition the patient had been diagnosed with breast cancer 2 years ago and her body mass index (BMI) was 28 kg/m^2. She was on tamoxifen 20 mg/d and there had been no changes in her medication for the last 11 mo. Clinical examination revealed no pathologic findings, while her electrocardiogram was normal. Treatment naïve lipid profile analysis revealed elevated TC (325 mg/dL), TG (321 mg/dL), LDL-C (214 mg/dL), apoE (147 mg/dL) and lipoprotein a [Lp(a), 47.5 mg/dL] levels, while HDL-C (47 mg/dL), apoA1 (178 mg/dL) and apoB (77 mg/dL) levels were within normal range. Other secondary causes of hyperlipoproteinemia were excluded as in case 1 patient. Dysbetalipoproteinemia was suspected by the equally elevated levels of both TC and TG (TC/TG is approximately 1), before treatment initiation and the low apoB to TC ratio (< 0.33) (Figure 2).We first assessed a number of dysbetalipoproteinemia indices, while the apoE2/E2 homozygosity genotype verified the speculated diagnosis (Table 1, Figure 2).

Patient was advised to switch to a diet low in saturated fats and carbohydrates. She received rosuvastatin 40 mg/d, ezetimibe 10 mg/d and fenofibrate 145 mg/d. Four weeks later patient's lipid profile significantly improved (TC: 238 mg/dL, TG: 160 mg/dL, HDL-C: 50 mg/dL, LDL: 156 mg/dL).

DISCUSSION

Only untreated dysbetalipoproteinemia patients > 30-year-old, like case 1 patient, suffer from diagnostic skin lesions, including tuberous or tuberoeruptive xanthomas on the extensor surfaces of extremities (elbow, knees and buttocks)[7,8]. Striated palmar xanthomas are considered pathognomonic of dysbetalipoproteinemia, but they are not present in all patients (case 2 patient)[8].

Dysbetalipoproteinemia is characterized by increased serum TG and cholesterol rich lipoprotein remnants [mostly intermediate density lipoprotein (IDL) and chylomicron remnants], also known as β-VLDL particles[7,8]. As dysbetalipoproteinemia is associated with increased premature cardiovascular disease demanding aggressive therapeutic management, it is important for the physician to suspect the disease when a mixed dyslipidemia is further characterized by approximately equally elevated levels of both TC and TG (TC is approximately 250-450

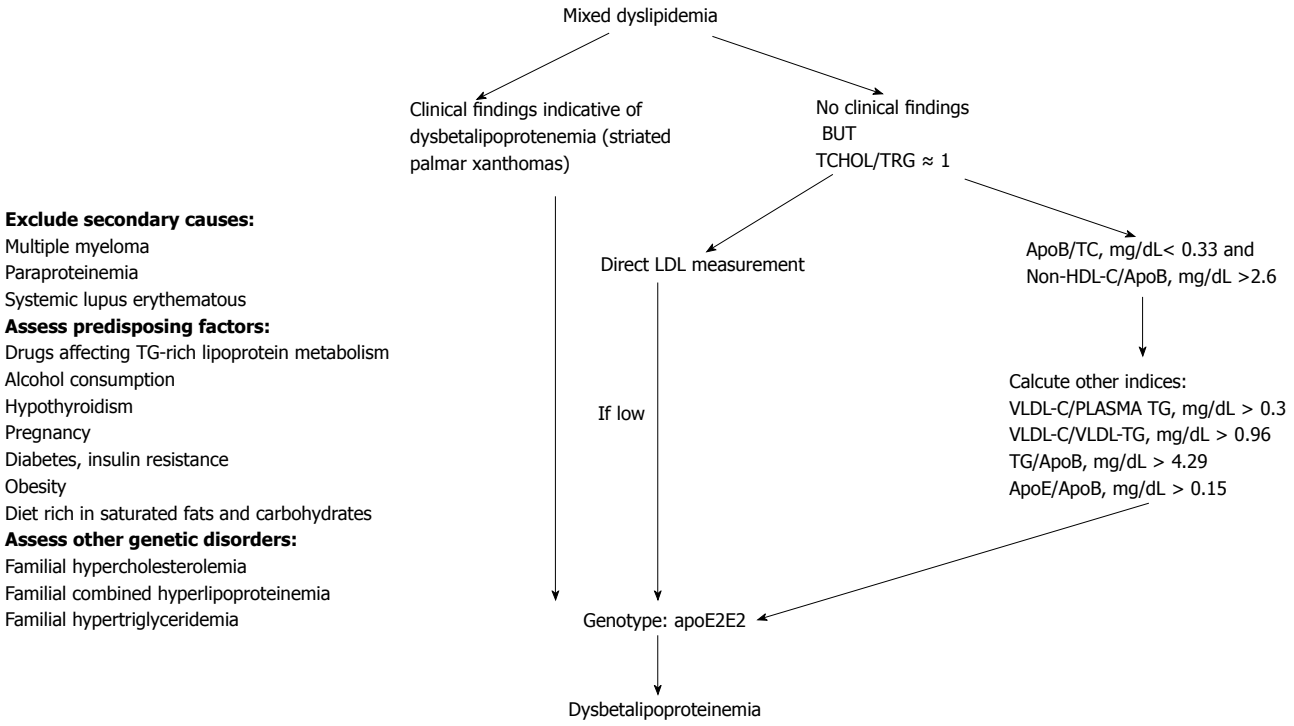

Figure 2 Diagnostic algorithm of dysbetalipoproteinemia. TC: Total cholesterol; TG: Triglycerides; ApoB: Apolipoprotein B; VLDL-C: Very low density lipoprotein cholesterol; VLDL-TG: Very low density lipoprotein triglycerides; ApoE: Apolipoprotein E; Non-HDL-C: Non high density lipoprotein cholesterol.

Table 1 Dysbetalipoproteinemia indices

Index	Patient's 2 values	Indicative for dysbetalipoproteinemia values
TC/TG (mg/dL)	1.01	Approximately 1
ApoB/TC (mg/dL)	0.23	< 0.33
ApoB/non-HDL-C (mg/dL)	0.27	< 0.38
VLDL-C/PLASMA TG (mg/dL)	0.35	> 0.3
VLDL-C/VLDL-TG (mg/dL)	1.06	> 0.96
TG/ApoB (mg/dL)	4.40	> 4.29
ApoE/ApoB (mg/dL)	0.20	> 0.15

TC: Total cholesterol; TG: Triglycerides; ApoB: Apolipoprotein B; VLDL-C: Very low density lipoprotein cholesterol; VLDL-TG: Very low density lipoprotein triglycerides; ApoE: Apolipoprotein E; Non-HDL-C: Non high density lipoprotein cholesterol.

mg/dL, TG is approximately 250-900 mg/dL) and a low apoB to TC ratio (< 0.33) (as it was the case with our second patient)[7,8]. The apoB to TC ratio represents the cholesterol in the circulating lipoproteins, but it does not include the cholesterol that circulates in HDL and other non-apoB-containing lipoproteins[9]. However, the apoB to non-HDL-C ratio was proven to be a less specific dysbetalipoproteinemia index compared with apoB to TC ratio[9]. When available, directly measured LDL-C is lower compared to that of calculated due to impaired conversion of VLDL to LDL[7,8,10]. In addition, an elevated VLDL cholesterol to total TG ratio (> 0.3) is indicative of dysbetalipoproteinemia and was also found in case 2 patient (Table 1)[7,8,10]. However, this ratio should not be used in normolipidemic subjects as they may have elevated ratios. Falsely low ratios, on the other hand, can be found in some dysbetalipoproteinemia patients who additionally have marked chylomicronemia and typical eruptive xanthomas (in the buttocks) as it was with case 1 patient. In this case, patient should be reassessed after several days on a low fat diet. In such cases another point distinguishing dysbetalipoproteinemia from type 5 hyperlipidemia is that the VLDL from a patient with dysbetalipoproteinemia is colored brown, whereas normal VLDL and that from type 2b and type 5 hyperlipidemia is white. Other dysbetalipoproteinemia indices have been also reported including elevated VLDL-C to VLDL-TG (> 0.96) as it represents high levels of cholesterol-enriched VLDL. In addition, elevated apoE to apoB has also been associated with dysbetalipoproteinemia and this index was elevated in our patient (Table 1)[9]. Last, the presence of a broad β band in electrophoresis is diagnostic but it is found in < 50% of cases[11].

Dysbetalipoproteinemia is observed in apoE2 homozygous persons when also a genetic or environmental risk factor for dyslipidemia is also present[7,8]. The disease generally presents after adulthood in men and menopause in women[7,8].

In most cases secondary factors are required for the expression of dysbetalipoproteinemia. These include additional genetic susceptibility variants, or other hormonal or environmental factors, such as obesity, type 2 diabetes, female gender, drugs affecting the metabolism of TG-rich lipoproteins, alcohol consumption or hypothyroidism (Table 2)[8,12]. Of note, case 1 patient was a diabetic man who consumed alcohol, while case 2 patient was an overweight woman who also received tamoxifen, which has been associated with disturbed

Table 2 Factors associated with dysbetalipoproteinemia overt expression

Environmental-hormonal	Genetic	Secondary dysbetalipoproteinemia
Drugs (corticosteroids, tamoxifen, retinoids, antipsychotics)	Familial hypercholesterolemia	Multiple myeloma
Alcohol consumption	Familial combined hypercholesterolemia	Paraproteinemia
Hypothyroidism	Reduced hepatic lipase activity	Systemic lupus erythematous
Pregnancy	Decreased lipoprotein lipase activity	
Diabetes, insulin resistance		
Obesity		
Diet rich in saturated fats and carbohydrates		

TG metabolism. In fact tamoxifen, like estrogens, stimulates the synthesis and secretion from the liver of VLDL, which is the main circulating carrier of TGs, while it decreases VLDL and IDL catabolism as a result of decreasing lipoprotein and hepatic lipase activities. However, the drug can induce only modest elevations in serum TG levels in patients who have a normal lipoprotein metabolism, while it can cause marked hypertriglyceridemia in patients who have a defective TG-rich lipoproteins metabolism[13-15]. Moreover, some patients may possess additional genetic variants or mutations disturbing the metabolic role of apoE[7,8]. On the other hand, less than 5% of dysbetalipoproteinemia patients have dominant mutations in apoE, which *per se* induce mixed dyslipidemia[7,16]. These mutations impair both the chylomicron remnants and IDL particles uptake by liver and the conversion of VLDL and IDL to LDL particles[7]. Of note, patients with dysbetalipoproteinemia show a marked interindividual variation in the serum concentrations of cholesterol and TG, clinically presented as mixed dyslipidemia (case 2 patient) or chylomicromenia (case 1 patient). Furthermore a combination of the apoE2/2 phenotype and additional genetic factors associated with diseases like familial hypercholesterolemia, familial combined hyperlipoproteinemia, or familial hypertriglyceridemia, can determine the expression of dysbetalipoproteinemia[17]. According to the Dutch Lipid Clinic Network criteria proposed by the European Atherosclerosis Society, the diagnosis of familial hypercholesterolemia was not probable in both our patients[18].

Noteworthy, it is important to exclude secondary causes of dysbetalipoproteinemia, including multiple myeloma, paraproteinemia and systemic lupus erythematous can mimic the disease, including the presence of typical xanthomas and the ultracentrifuge findings[19,20]. Thus, a detailed clinical and laboratory assessment is always required.

Dysbetalipoproteinemia patients have increased risk of both coronary artery disease and peripheral vascular disease, even though the LDL-C concentration is low[7,8]. Beta-VLDL is an atherogenic particle that rapidly transforms monocyte-macrophage cells to foam cells; the histologic hallmark of atherosclerosis and xanthomas[21]. Additionally, remnant lipoproteins induce endothelial plasminogen activator inhibitor I expression and activity in cultured aortic endothelial cells contributing to a prothrombotic state[22]. Males with homozygosity for the ApoE2 isoform present with coronary disease at their 4th or 5th decade of their life and there is a predisposition for peripheral vascular disease in these patients[23]. Last, lipoprotein glomerulopathy and pancreatitis in severe hypertriglyceridemic patients with dysbetalipoproteinemia have also been described[24].

Treatment of dysbetalipoproteinemia is the same as for hypertriglyceridemia. Weight loss, diet fat restriction and treatment of secondary factors, such as diabetes and hypothyroidism are important for all dysbetalipoproteinemia patients[7]. In addition, administration of fibrates, statins, omega-3 fatty acids and niacin or their combinations is very effective. However, it has to be underlined that fibrates, with or without statin, seem to comprise the cornerstone of dysbetalipoproteinemia treatment[25].

In conclusion, physician should keep in mind that dysbetalipoproteinemia may present as chylomicronemia when other genetic or environmental causes affecting TG metabolism co-exist. Moreover, dysbetalipoproteinemia has to be suspected in all mixed dyslipidemia cases with equally elevated TC and TG levels (TC/TG = 1) (Figure 2).

COMMENTS

Case characteristics

The two patients presented with dissimilar lipid profile; one presented with extremely high triglycerides (TG) levels and the other presented with equally elevated levels of total cholesterol (TC) and TG.

Clinical diagnosis

The physical signs of the two cases were also dissimilar; one patient presented with tuberous and eruptive xanthomas, while the other patient had no skin lesions.

Differential diagnosis

Type 5 dyslipidemia, chylomicronemia, secondary causes of mixed dyslipidemia and dysbetalipoproteinemia.

Laboratory diagnosis

The first patient had the following lipid profile: TC: 1055 mg/dL, TG: 2900 mg/dL, high density lipoprotein cholesterol: 18 mg/dL, while the second patient had the following lipid profile; TC: 325 mg/dL, TG: 321 mg/dL, low density lipoprotein cholesterol (LDL-C): 214 mg/dL.

Genetic diagnosis

The diagnosis of dysbetalipoproteinemia was verified by Apolipoprotein E2 (apoE2)/E2 homozygosity genotype in both patients.

Treatment

Fibrate with or without statin improved lipid profile in both patients.

Related reports

Dysbetalipoproteinemia is seen in approximately 1 in 10000 people.

Term explanation

Dysbetalipoproteinemia is a rare familial disease characterized by marked elevations of serum cholesterol and triglyceride levels caused by an accumulation of remnant lipoproteins in apolipoprotein E2/E2 homozygotes.

Experiences and lessons

This case report presents the clinical characteristics and lipid profile of dysbetalipoproteinemia and also suggests a diagnostic algorithm. The authors recommend that diagnosis of dysbetalipoproteinemia should be considered in patients mixed dyslipidemia when the apolipoprotein B concentration is relatively low in relation to the total cholesterol concentration or when there is significant disparity between the calculated low LDL-C and directly measured LDL-C concentrations.

Peer-review

This is a nice article that deserves be published.

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CASE REPORT

Saccular trilobed aneurysm of azygos anterior cerebral artery

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Abstract

Multiple saccular or giant aneurysms of azygos anterior cerebral artery (AACA) at the distal segments A2-A5 are very rarely reported. Distal anterior cerebral artery (DACA) aneurysms represent approximately 2%-7% of all cerebral aneurysms. We present the case of an Albanian 62-year-old male, admitted at our service after sudden onset of severe headache and vomiting. Computerized tomography (CT) of the head showed hemorrhage in the front of corpus callosum. CT angiography followed by digitally subtracted angiography (DSA) documented a large necked aneurysm with three lobes at the origin of calloso-marginal artery and a single DACA, also known as AACA. A frontal parasagittal craniotomy was performed. Obliteration of the aneurysm was done only by separate clipping of each three lobes at the respective neck. Postoperative DSA demonstrated complete exclusion of the aneurysm and a regular flow of AACA. The patient recovered uneventfully. Despite it is a rare occurrence, an aneurysm of distal segments of anterior cerebral artery A2-A5, concomitant to AACA should be studied with DSA. In the era of embolization, conserving good microsurgical skills is fundamental for dealing with multilobar cerebral aneurysms, associated with rare anatomical variations.

Key words: Azygos; Cerebral; Aneurysm; Clipping

Core tip: This is a detailed description of radiological and surgical findings in a very rare case of trilobed aneurysm of distal anterior cerebral artery. The characteristics of this aneurysm are exposed and its difficult exclusion by separate three clips is argued with a thorough discussion of the literature.

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INTRODUCTION

The incidence of true azygos distal anterior cerebral artery (DACA) ranges from 0% to 5%[1,2]. The rarest is the form of unpaired DACA. An azygos artery is better studied through carotid angiography with contralateral compression. In the presented case, there was a trilobed aneurysm of A3 segment in a single or azygos variant of DACA.

CASE REPORT

A 62-year-old man showed up at a local hospital complaining of severe headache with sudden onset. Neurological examination revealed nuchal rigidity but no neurological deficits. Unenhanced computed tomography (CT) of the head revealed a hyperdense lesion in front of the knee of corpus callosum interpreted as hemorrhage with edema of the left frontal lobe (Figure 1).

With the event taking place in 2008, a thorough history was requested. Back to 1990 the patient had been admitted at the same rural hospital after an episode of acute headache and loss of consciousness. A CT of the head at that time had shown same hemorrhage as in recent episode, but the patient had been discharged without any in-depth examinations or treatments (Figure 2).

At present episode the patient was promptly transferred at our neurosurgical facility. The CT angiography revealed A3 aneurysm of DACA. Digitally subtracted angiography (DSA) confirmed a trilobed and a complex broad-necked A3 aneurysm at the origin of the callosomarginal (CM) artery (Figure 3), in presence of azygos anterior cerebral artery (AACA).

Cerebral DSA with contra-lateral carotid artery compression confirmed the presence of AACA. Endovascular option of treatment was not considered and microsurgical clipping of the aneurysm with or without a bypass was planned.

Left frontal parasagittal craniotomy anterior to coronal suture was done in order to control at first the distal tract of CMA. Once identified, the CMA led us to the pericallosal artery proximal to the aneurysm. Then the aneurysm with its three lobes was dissected exposing at first the proximal segment of pericallosal artery, although the interhemispheric approach offers small space. After clearing the adhesions, the broad aneurysm neck and its three lobes were exposed, each of which extended in a different direction (Figure 4).

The neck wall of the aneurysm was sclerotic. Single clip closure of the neck was initially tried, but its hard wall shifted the clip away towards the single pericallosal artery reducing its lumen. Therefore separate clipping was deemed the only possible way to exclude the aneurysm (Figure 5). Uneventful postoperative course followed. Carotid angiography documented preserved flow in the parent artery and complete exclusion of the aneurysm (Figure 6).

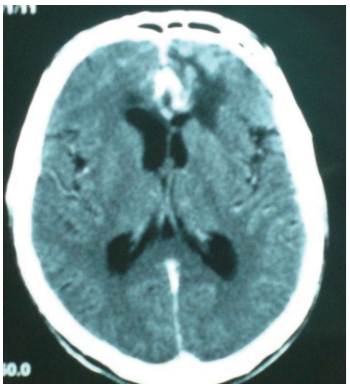

Figure 1 Computerized tomography scan of the year 2008. Second episode of the intracerebral hemorrhage.

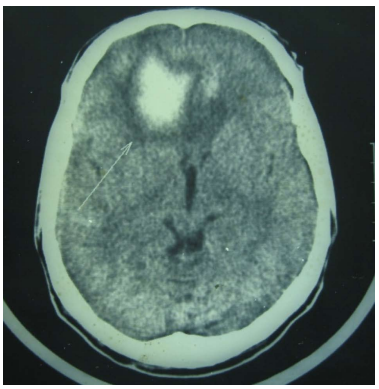

Figure 2 Computerized tomography scan of the year 1990. First episode of intracerebral frontal hemorrhage (white arrow).

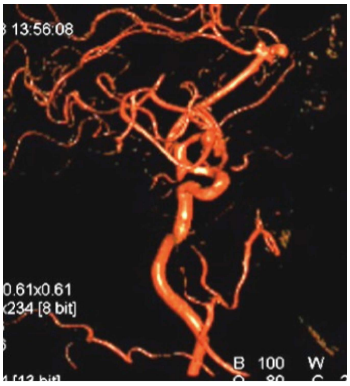

Figure 3 Preoperative digitally subtracted angiography.

DISCUSSION

In 1963 Baptista[2] presented a scale of three groups for the anatomical variations DACA. Years before Wilders coined the term azygos pericallosal artery for the fusion of two A2 arteries. During angiographic and autopsy studies the incidence of this variation is reported from 0.3% to 2%[2-4]. The aneurysms of DACA are seen in 2%-6.7% of the intracranial aneurysms and they are usually saccular, small and single-lobed[2,5-7]. Giant aneurysms of azygos

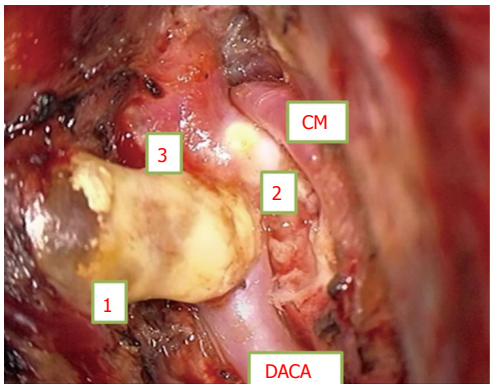

Figure 4 Intraoperative pre-clipping photo. Numbers 1, 2 and 3 refer to respective lobes of the trilobed aneurysm. CM: Calloso-marginal; DACA: Distal anterior cerebral artery/pericallosal artery.

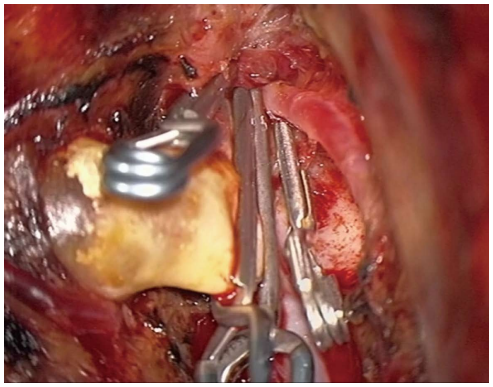

Figure 5 Intraoperative post-clipping photo. Three separate clips exclude the three lobes of the aneurysm.

pericallosal artery and those multi-lobular are extremely rare, and usually situated at the calloso-marginal bifurcation[1,8-12]. Auguste *et al*[13] reported a series of 876 aneurysms surgically treated, with five ACA aneurysms in the presence of an azygos pericallosal artery that represent 0.5% and 1.7% of ACA and ACoA aneurysms of his series. It seems needless to stress the importance of angiography in such cases, not only for a preoperative evidence of the morphology of the aneurysm, but also for the documentation of any variation of AACA, which will influence the surgical technique.

In our reported case preoperative DSA showed a trilobed aneurysm originating from a single neck with different spatial orientation. Single clip closure of the neck was initially tried, but its hard wall shifted the clip away towards the single pericallosal artery reducing its lumen. Therefore we opted for a separate clipping.

The hypothesis of creation of three lobes stands on the blood flow dynamics of the parent artery. The more curved the parent artery, more blood enters the aneurysm.

In case of AACA the blood flow is increased compared with the blood flow of any of the ACAs in a normal anatomical setting. The angled direction of entry and

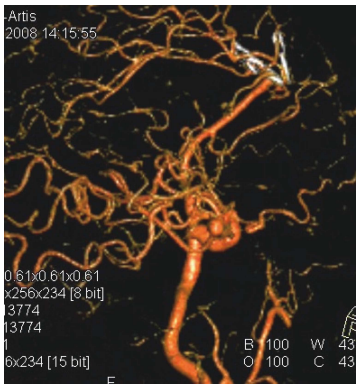

Figure 6 Postoperative digitally subtracted angiography showing preserved blood flow in the pericallosal artery and the exclusion of the aneurysm.

increase of blood flow in the aneurysm space influence the stress over the aneurysm wall. Perioperative morphological study of the single pericallosal artery evidenced the location of the aneurysm at the knee of corpus callosum. At this point the pericallosal artery bends posteriorly. It creates the prerequisite of the scenario mentioned above, that favors aneurysm formation.

In order to explain the origin of such a rare morphology aneurysm, several experimental paradigms are suggested[14]. A larger neck width increases the blood flow rate within the aneurysm. Draining vessels from the aneurysm contribute in the increase of blood flow inside the aneurysm. Both factors increase the pressure on the aneurysm wall and influence multiple lobe formation[14-16].

We suppose that the three lobes in our case were influenced by a change of aneurysm neck and dome, perhaps due to the rupture eighteen years before. We also believe there might be a relationship between the previous supposed rupture of the aneurysm and its rare morphology.

The aneurysm conformation with three lobes, in the background of a very rare anatomical variation of ACA tract, makes our case a clinical rarity. The three lobes of the aneurysm we dealt with are the result of blood flow impact and parent vessel modification after the first hemorrhage, as documented with the CT performed more than a decade prior to the second episode, requiring neurosurgical intervention. We think that vascular neurosurgeons should be prepared to handle such complicated cases.

COMMENTS

Case characteristics

Three lobed azygos anterior cerebral artery (AACA) is an exception. Digitally subtracted angiography (DSA) is the gold standard method of study.

Clinical diagnosis

Repeated episode of subarachnoid hemorrhage was observed in this case.

Differential diagnosis

The differential diagnosis with other type of acute onset of headache was done by computerized tomography scanner.

Laboratory diagnosis

DSA showed AACA aneurysm unsuitable for embolization.

Imaging diagnosis

Surgical clipping was challenged by three lobed shape of the aneurysm, which was excluded only through separate clipping.

Treatment

Excellent surgical skills are necessary for dealing with such rare difficult case.

Peer-review

Interesting case report.

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CASE REPORT

Importance of cardiological evaluation for first seizures

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Author contributions: Choong H and Hanna I collected patients' clinical data; Choong H searched for relevant references from pubmed; Choong H and Beran R wrote the paper.
Ethics approval: The nature of the presentation is not such that ethics approval is required; it is not a clinical trial and is not ethically sensitive.
Informed consent: There is no need to involve informed consent as it is a de-identified report and not involving any patient in any research project or study; it reports clinical experience and all clinicians involved have consented to its release.
Conflict-of-interest: There is no conflict of interest for any of the authors.

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Abstract

This paper reports two cases of long QT syndrome (LQTS) which presented with seizures as their initial feature. Case 1, AB was seen in emergency department with post-partum seizure, discharged and re-presented following cardiac arrest associated with LQTS. Case 2, CD presented initially with tonic-clonic seizure and because of experience with AB, CD was assessed for LQTS which was subsequently confirmed. The legal medicine experience re Dobler v Halverson, which involved a young boy with LQTS, who suffered cardiac arrest without prior diagnosis of LQTS, has reinforced the requirement to seriously consider LQTS as an aetiological factor in first seizure presentations.

Key words: Long QT syndrome; Prolonged QT; Torsades de pointes; Seizure; Epilepsy

Core tip: Long QT syndrome (LQTS), with subsequent cerebral ischemia due to cardiac dysrhythmia, may cause seizures. It is imperative to consider LQTS in patients presenting with first seizure so as to avoid possible brain damage from prolonged cerebral hypoxemia. Failure to recognise LQTS may result in successful suit for negligence if not properly investigated and managed.

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INTRODUCTION

Long QT syndrome (LQTS) represents channelopathies of cardiac potassium/sodium ion channels. Channelopathies may present with seizures and/or risk sudden death because ventricular dysrhythmia known as torsades de pointes (TdP).

CASE REPORT

Case 1 (Year: 2008)

AB was a 26-year-old Asian female, 6 wk post-partum

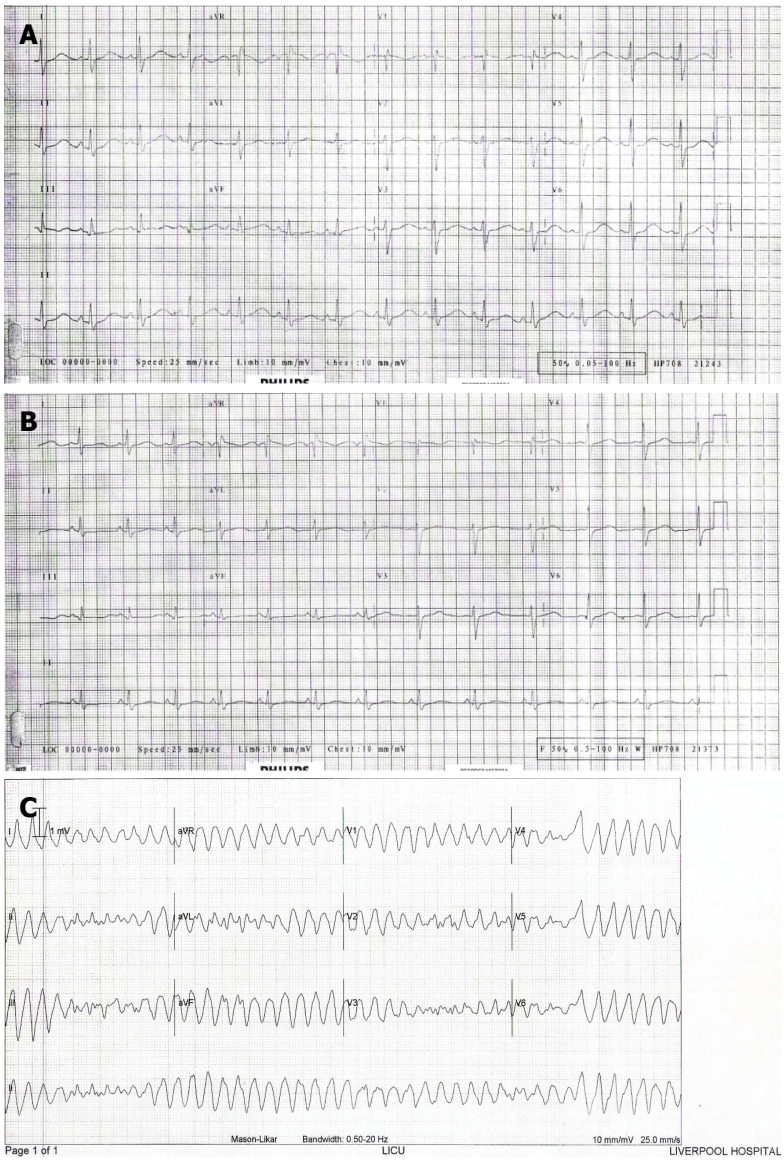

Figure 1 Electrocardiogram of AB. A: Electrocardiogram (ECG) of AB on first presentation showed sinus rhythm, incomplete right bundle branch block (RBBB) and prolonged QTc interval (QTc 526 ms); B: ECG of AB on second presentation showed sinus rhythm, incomplete RBBB and prolonged QTc interval (QTc 505 ms); C: ECG of AB during a syncopal episode on her second admission showed torsades de pointes.

with emergency lower cesarean section at 38 wk into her first pregnancy because of pre-eclampsia and fetal distress. Past medical and family histories were unremarkable and she was not on regular medication.

She had acute "dizziness" when rising from the sitting to standing position, and then collapsed to the floor and was witnessed to be cyanosed. Her husband performed cardiopulmonary resuscitation until the ambulance arrived. Her Glasgow Coma Scale improved with stable vital signs at that time. The paramedics witnessed an episode of generalised tonic-clonic seizure associated with tongue biting that lasted for a few minutes whilst en-route to Liverpool Hospital. In the Emergency Department, she was sedated and intubated because of post-ictal aggression. She was afebrile, blood pressure 127/78 mmHg and pulse rate 70 beats/min. Blood tests were unremarkable with normal computed tomography (CT) pulmonary angiogram, CT brain and CT cerebral venogram. Her electrocardiogram (ECG) showed corrected QT interval (QTc) of 526 ms (Figure 1A), which was above the normal limit for her gender (QTc < 460 ms). She was extubated 24 h later and was back to her normal state. Full neurological examination was unremarkable. No antiepileptic medication was prescribed as this was her first seizure. Electroencephalogram (EEG) and magnetic resonance imaging (MRI) of the brain were organised but she refused to stay in hospital for those investigations and was to be followed by the neurologist.

She was re-admitted the next day after being found unconscious. She was in ventricular fibrillation requiring cardioversion by the paramedics. Her ECG was similar to the previous recording, demonstrating abnormal QTc of 505 ms (Figure 1B) and hence prompting a diagnosis of LQTS. She had further symptomatic polymorphic ventricular tachycardia suggestive of TdP that required cardioversion (Figure 1C). She was subsequently started on beta-blocker therapy and had implantable cardioverter-defibrillator (ICD) for secondary prevention.

Case 2 (Year: 2012)

CD was a 50-year-old Caucasian male with no regular

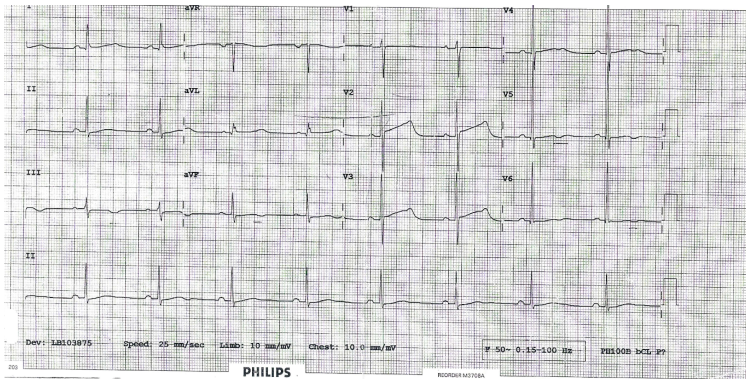

Figure 2 Electrocardiogram of CD showed sinus rhythm and prolonged QTc (QTc 566 ms).

medication. His father died at 57 years old in his sleep.

He presented to Liverpool Hospital with 2 min of witnessed generalised tonic-clonic seizure. This was associated with post-ictal drowsiness but no tongue biting or incontinence. Previously he had three episodes of unconsciousness. The first two were 30 years ago resulting in his being prescribed carbamazepine which he took for a few years but had not taken same for approximately 20 years. He was unable to provide adequate further history nor could he offer more detailed information regarding specific investigations related to those events. Full neurological and cardiological examinations, blood tests, EEG, MRI brain and echo-cardiography were all unremarkable. ECG showed QTc of 566 ms which was above the normal limit for his gender (QTc < 450 ms) (Figure 2) prompting a diagnosis of LQTS. Beta-blocker therapy was commenced. ICD was indicated due to his family history and possible ventricular dysrhythmias that could have accounted for his previous events of loss of consciousness thought possibly to be misdiagnosed as epileptic seizures.

DISCUSSION

Both patients in Cases 1 and 2 presented with generalised convulsive "seizures". AB was initially discharged with conservative management and LQTS was diagnosed on representation. CD was diagnosed because of the experience with AB. This demonstrates that LQTS crosses age, gender and racial boundaries (AB being a young Asian female and CD a middle-aged Caucasian male) demanding a high index of suspicion and consideration of LQTS in all first seizures.

LQTS is a collection of genetically distinct arrhythmogenic disorders resulting in abnormal cardiac potassium and sodium ion channels causing delayed cardiac depolarization[1]. LQTS affects approximately 1 in 2000 people[2,3] and symptomatic cases may present with syncope, seizures or sudden death due to ventricular dysrhythmia known as TdP. These cases are often "erroneously" diagnosed as a primary seizure disorder, having unexplained syncope, or having ill defined "spells"[4,5] which could potentially lead to expensive legal-medicine consequences, such as the Dobler v Halverson case of 2006. This Australian, NSW Court of Appeal, case involved LQTS in a young boy diagnosed by a neurologist as a "faint" without further investigation. Halverson was then managed by his general practitioner (GP), Dr Dobler. Halverson experienced cardiac arrest with severe brain damage and the GP was found negligent for not performing an ECG nor organizing for cardiological assessment.

There is increasing support that seizures, in LQTS, are not solely due to acute cerebral hypoxic-ischemic event secondary to ventricular arrhythmias. It has been proposed that the aetiologies of LQTS and epilepsy may partly overlap *via* a possible link between the cardiac and neural ion channelopathies. It has been demonstrated that patients with LQTS type 2 are more commonly associated with epilepsy, hence supporting the possibility that mutation of *KCNH2* gene responsible for LQTS type 2 may also predispose to seizure activity[5]. Similarly, various case reports and observational studies have suggested that mutation in the *SCN5A* gene, responsible for LQTS type 3, is also associated with epilepsy[6,7]. It follows that initial diagnosis of seizure disorder or epilepsy, with subsequent neurological investigations and antiepileptic treatment, may be inadequate if it does not also include cardiological evaluation. ECG, Holter monitoring and formal cardiological evaluation should become an integral part of a seizure/epilepsy assessment, to identify a subset of patients who also have concomitant LQTS. Failure to do so may predispose the patient to very serious or even fatal consequences and the treating clinician to subsequent personal and legal medicine ramifications.

COMMENTS

Case characteristics

Two cases presenting to hospital after witnessed generalised seizures.

Clinical diagnosis

Both cases had normal physical examination but had prolonged QTc on their electrocardiograms (ECGs), and evidence of torsades de pointes on the ECG for Case 1, prompting the diagnosis of long QT syndrome (LQTS).

Differential diagnosis

Convulsive syncope, secondary to cardiogenic causes; primary or secondary generalised seizure disorder.

Laboratory diagnosis

Both cases had normal routine blood tests, echocardiogram and electro-encephalogram.

Imaging diagnosis

Both cases had normal computed tomography and magnetic resonance

imaging brain.

Treatment

Beta-blocker medication and implantable cardioverter-defibrillator insertion were instigated after the diagnosis of LQTS in both cases.

Related reports

There is a possible link between the cardiac and neural ion channelopathies, hence patients with LQTS may have concurrent primary epilepsy disorder causing seizures rather than solely from the consequences of the ventricular arrhythmias.

Term explanation

TdP refers to Tordes de Pointes which is an uncommon and distinctive form of polymorphic ventricular tachycardia characterized by a gradual change in the amplitude and twisting of the QRS complexes around the isoelectric line; Channelopathies are diseases caused by disturbed function of ion channel subunits or the proteins that regulate them; and *KCNH2* and *SCN5A* are genetic abnormalities found to occur in both inherited epilepsies and LQTS.

Experiences and lessons

Initial diagnosis of seizure disorder or epilepsy, with subsequent neurological investigations and antiepileptic treatment, may be inadequate if it does not also include cardiological evaluation.

Peer-review

The viewpoint of this paper is useful in clinical practice.

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CASE REPORT

Poncet's disease: An unusual presentation of tuberculosis in a diabetic lady

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Sanjay Kumar Mandal, Department of General Medicine, Medical College and Hospital, Kolkata 700073, West Bengal, India
Author contributions: Chakraborty PP and Ray S contributed to conception and design; Ray S and Selvan C contributed to drafting of the article; Selvan C contributed to literature search; Chakraborty PP contributed to analysis and interpretation of data; Bhattacharjee R and Mandal SK contributed to critical revision of the article for important intellectual content; Chakraborty PP, Ray S, Selvan C, Bhattacharjee R and Mandal SK final approval of the article.
Ethics approval: The Institutional Ethical Committee of IPGME and R, Kolkata agreed to give its formal approval to carry out this case study in this institution.
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Abstract

Authors describe a 53-year-old woman who presented to their diabetes clinic with a three week history of multiple painful and swollen joints. She had been diagnosed with type 2 diabetes 5 years back. On examination, both knee joints and left ankle were swollen. A soft tissue swelling appeared over the medial end of the left clavicle few days later. Rheumatoid arthritis, collagen vascular diseases and other common causes of polyarthritis were ruled out by appropriate investigations. Non steroidal anti-inflammatory drugs failed to give satisfactory pain relief and the arthritis persisted. Conventional cultures of synovial fluid samples including cultures for tuberculosis were negative. Computed tomography showed a space occupying lesion involving the left sternoclavicular joint. Fine needle aspiration from the lesion was performed and acid-fast bacilli were demonstrated in the smear using Ziehl-Neelsen stain. The explanation of her arthritis was therefore tuberculous arthritis in left sternoclavicular joint and reactive arthritis in the rest of the joints. A diagnosis of Poncet's disease was considered in her case. We treated her with standard anti-tuberculosis drugs and the arthritis resolved within a few days. She remained symptom-free at her 2 years' follow-up.

Key words: Poncet's disease; Diabetes; Tuberculous arthritis; Reactive arthritis; Acid-fast bacilli

Core tip: Poncet's disease (PD) is a form of reactive arthritis that develops in patients with active tuberculosis (TB). It is a rare, non-destructive parainfective symmetric polyarthritis. In cases of unexplained atypical arthritis associated with non-articular TB, PD should be considered. PD remains a clinical challenge and is essentially a diagnosis of exclusion and requires a high degree of clinical suspicion. Correct identification of this rare complication of TB is required to avoid delayed initiation of appropriate treatment. The dramatic response of arthritis in PD on starting anti-tubercular treatment substantiates the diagnosis. Further studies are required for better

understanding of the pathogenesis underlying PD.

Chakraborty PP, Ray S, Selvan C, Bhattacharjee R, Mandal SK. Poncet's disease: An unusual presentation of tuberculosis in a diabetic lady. *World J Clin Cases* 2015; 3(4): 385-388 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i4/385.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i4.385

INTRODUCTION

The association between of diabetes mellitus (DM) and tuberculosis (TB) is becoming more prominent in developing countries where TB remains endemic and the burden of DM is increasing. Atypical presentations are increasingly being recognized in diabetic patients. The diagnosis of joint TB poses a challenge to the clinicians. Although, septic monoarthritis is a well-known complication of tuberculous infection; active TB may be complicated by a sterile reactive arthritis (ReA), known as Poncet's disease (PD), which is not so common and therefore frequently missed. We report here a diabetic patient who initially presented with oligoarthritis, and later on ended with a diagnosis of PD. A review of the literature on diagnostic and therapeutic aspects involved in PD is also included. This case highlights the need for increased awareness among physicians regarding this rare complication of a common disease to avoid delay in diagnosis and starting the appropriate treatment.

CASE REPORT

A 53-year-old lady presented with a three week history of multiple painful and swollen joints. She had been diagnosed with type 2 diabetes in March 2009. Her diabetes was well controlled and she was on insulin and metformin. Her presentation to the clinic had been prompted by her inability to walk in the previous five days. She had been diagnosed with undifferentiated polyarthritis by a family physician. The joint pains started gradually over three weeks involving left ankle and knee joints. Family history for rheumatic or autoimmune diseases was negative. There was no history of mouth ulcers, eye symptoms, skin rash or genito-urinary symptoms. She denied weight loss, coughing, night sweats but mentioned about low grade fever for few weeks.

On examination, she was in pain and was unable to walk. Her blood pressure was 130/80, pulse rate 90 per minute, respiratory rate 24 per minute and a temperature of 36.80 ℃. The patient's ankle and knee joints were swollen and tender on palpation with limitation of movement. A soft, boggy swelling appeared at the medial end of the left clavicle during her hospital stay clinically resembling a cold abscess. She had no erythema nodosum on examination. No adventitious sound was detected on chest auscultation. There was no lymphadenopathy or hepatosplenomegaly. There was no muscle atrophy. She had no signs of peripheral neuropathy. Fundoscopic examination was unremarkable.

Her initial investigation results showed hemoglobin of 11.9 g/dL, a white cell count of 7.6 × 10^9/L, platelets of 130 × 10^9/L and an erythrocyte sedimentation rate (ESR) of 88 mm/h. Urine dipstick showed no white cells, protein was trace. Laboratory tests results for antinuclear antigen, rheumatoid factor and anti-cyclic citrullinated peptide antibodies were negative. The patient was nonreactive for HIV 1 and 2 by ELISA. The radiographs of the both knees and left ankle were normal. The intradermal skin test for tuberculosis reading was 17 mm, which was considered strongly positive. Synovial fluid was aspirated from the left ankle and both knees and analysis revealed leucocytes between 4.0 and 8.2 × 10^9/L, no crystals and the smears were negative for Gram stain. On bacterial culture of the synovial fluid, no growth was found and synovial fluid cultures for TB were also negative. The plausible explanation of her arthritis would therefore be a form of a reactive arthritis. Since no diagnosis could be made, we went for exploring the neck swelling. Computed tomography scan of the neck was performed which showed a space occupying lesion (SOL) around the medial end of clavicle (Figure 1A). Fine needle aspiration from the lesion showed epithelioid cell collection and multinucleated giant cells on the background of necrotic tissue. AFB stain was positive (Figure 1B).

Treatment with non steroidal anti-inflammatory drugs only gives partial relief. Since there was strong evidences suggesting active joint tuberculosis, she was put on four-drug antituberculosis treatment, including rifampicin, isoniazid, pyrazinamide, and ethambutol. Her joint symptoms showed a remarkable improvement within two weeks of initiation of therapy.

Following treatment with standard anti-tubercular treatment, the arthritis had completely resolved in three weeks period. The consistent association of arthritis with presence of active tuberculosis, the lack of evidence of any other known rheumatic disease and the resolution of symptoms on anti-tuberculosis therapy were all consistent with the diagnosis of PD in our patient. She had remained asymptomatic at 2 years' follow-up.

DISCUSSION

PD or tubercular rheumatism is a form of reactive polyarthritis related with active TB in which no myco-bacterial involvement can be found in the affected bones or joints, and there is absence of other detectable causes of polyarthritis[1-3]. TB reactive arthritis was first described by Poncet[4] in 1897 and named after him as PD. PD is considered a ReA, but the clinical presentation of PD is different from the classical pattern of ReA[5]. Unlike ReA,

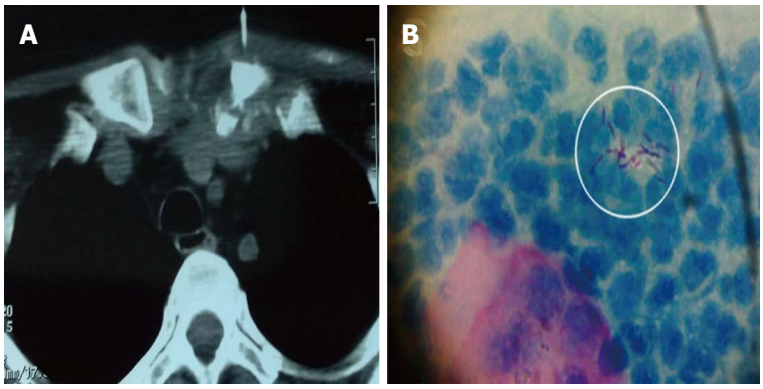

Figure 1 Computed tomography scan of neck showed bone destruction and soft tissue swelling with involvement of articular surfaces of sternoclavicular joint (A); Numerous acid-fast bacilli in the background of necrosis(B) (Ziehl-Neelsen stain, x 1000).

the onset of symptoms in PD prior to the start of arthritis is much longer than only a few weeks, whereas arthritis resolution upon starting of anti-tubercular therapy is generally within a few weeks. Chronic arthritis is not encountered in PD. Although the clinical presentation is somewhat different, the pathogenetic mechanism is considered to be similar. It is thought to occur due to a hypersensitive immune cell mediated response to the tuberculoprotein, resulting in an inflammatory reaction in the joint spaces[2,6]. Due to the rarity of PD despite the frequency of TB, a genetic predisposition has been suggested in the pathogenesis, with links to the HLA DR3 and HLA DR4 haplotypes[7].

Clinically the disease is different to the well recognized TB monoarthritis; a septic mycobacterium infection of a joint leading to its destruction. From the cases previously described, Poncet's arthritis is non-destructive and resolves completely following TB treatment. The arthritis mainly affects the larger joints, with the knee being the most common, followed by the ankle and wrist joints. The axial skeleton tends not to be involved. It is described as a symmetrical polyarthritis but many studies[8] have suggested it to be pauciarticular arthritis, mainly of the larger joints as was the case with our patient. Other common symptoms include lymphadenopathy (mainly cervical and axillary), grumbling fevers (which may be present many weeks prior to developing the arthritis) and skin changes; classically erythema nodosum[9]. In the review of 50 cases report on PD by Kroot *et al*[1] erythema nodosum was present only in 6% of the patients.

The diagnosis is usually one of exclusion and should be considered in all patients with a symmetrical arthritis in TB prevalent regions. Extra-pulmonary TB, particularly lymph node TB, is traditionally thought to be the main culprit[10,11]. The complete resolution of rheumatic symptoms on anti-tuberculosis therapy further confirms the diagnosis. Resolution of the arthritis of PD with anti-tubercular drugs ranged from a week to few months.

To conclude, active tuberculosis needs to be considered in the differential diagnosis of patients presenting with fever and polyarthritis of unclear cause, particularly in regions where the prevalence of tuberculosis is high. The diagnosis of this clinical entity remains a clinical challenge and demands a high index of suspicion. Since not all clinicians are aware of PD, this entity is probably underdiagnosed.

COMMENTS

Case characteristics

A 53-year-old diabetic woman presented with multiple painful and swollen joints.

Clinical diagnosis

The patient's ankle and knee joints were swollen and tender on palpation with limitation of movement. A soft, boggy swelling appeared at the medial end of the left clavicle during her hospital stay clinically resembling a cold abscess.

Differential diagnosis

Septic arthritis, reactive arthritis, rheumatoid arthritis.

Laboratory diagnosis

Her investigation results showed hemoglobin of 11.9 g/dL, a white cell count of 7.6 × 10^9/L, platelets of 130 × 10^9/L and an erythrocyte sedimentation rate of 88 mm/h. Results for antinuclear antigen, rheumatoid factor and anti-cyclic citrullinated peptide antibodies were negative. The intradermal skin test for tuberculosis reading was 17 mm, which is considered a positive test.

Imaging diagnosis

The radiographs of the both knees and left ankle were normal. Computed tomography scan of the neck showed a space occupying lesion (SOL) around the medial end of clavicle.

Pathological diagnosis

Fine needle aspiration from supraclavicular SOL/of the left supraclavicular node showed epithelioid cell collection and multinucleated giant cells on the background of necrotic tissue. AFB stain was positive.

Treatment

The patient was put on four drug anti-tuberculosis (TB) therapy since the evidence was strongly suggestive of active joint tuberculosis.

Related reports

Reviewing the literature, more than 50 case reports were found. In most reports "Poncet's disease" was described as an aseptic polyarthritis, presumably reactive arthritis arthritis developing in the presence of active TB elsewhere.

Term explanation

Poncet's disease is a reactive polyarthritis associated with active TB in which no mycobacterial involvement can be found in the affected bones or joints.

Experiences and lessons

The diagnosis of Poncet's disease remains clinical and is established on excluding other potential causes of arthritis in a patient with active tuberculosis. In cases with unexplained atypical arthritis and non-articular TB, Poncet's disease should be considered.

Peer-review

It is a case report describing Poncet's disease which goes unnoticed and underdiagnosed. The manuscript is scientifically sound and based on the discovery of facts. Data presented are duly supported by appropriate figures.

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CASE REPORT

Unusual histological variant of malignant peripheral nerve sheath tumor with rhabdomyoblastic differentiation

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Author contributions: Shete S, Bolde S, Pandit G and Matkari P diagnosed the case and prepared the first draft of manuscript; Ingle SB prepared the final draft of manuscript and revised the intellectual content and gave final approval of manuscript.
Ethics approval: According to our ethical committee, as it's a single case report on tissue histopathology (not on live patient) no need of approval as there is no question of unethical practice.
Informed consent: As we are presenting only histopathological diagnosis, not doing any experiments on the live object, we have done only diagnosis in pathology lab so no question of disclosing the patients details name /his consent that is also not indicated.
Conflict-of-interest: None is to be declared that is clearly mentioned before submission and also in the copyright form.

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Abstract

Malignant peripheral nerve sheath tumor (MPNST) with rhabdomyoblastic differentiation is called as malignant triton tumor (MTT). It is highly aggressive soft tissue tumor with higher local recurrence rate. MTT has poor prognosis than MPNST. MTT seems to be more aggressive in patients with neurofibromatosis (NF-1). We herein, reporting an interesting case of 55 years male with multiple neurofibromas all over the body since 30 years and multiple café-au-lait spots, diagnosed as NF-1. Since 6 years, he had an enlarged mass in left thigh. Wide excision of mass was done. On histopathological examination revealed the diagnosis of MTT and diagnosis of which was confirmed on immunohistochemistry.

Key words: Malignant triton tumor; Neurofibromatosis-1; Desmin; S-100 protein

Core tip: Meticulous histopathological examination along with immunohistochemistry is the mainstay to arrive at such rare histological diagnosis. The surgical pathologist should keep in mind such rare entity while dealing with such kind of patients.

Shete S, Bolde S, Pandit G, Matkari P, Ingle SB. Unusual histological variant of malignant peripheral nerve sheath tumor with rhabdomyoblastic differentiation. *World J Clin Cases* 2015; 3(4): 389-392 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i4/389.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i4.389

INTRODUCTION

Malignant peripheral nerve sheath tumor (MPNST) is an unusual type of soft tissue sarcomas. It accounts for about 5%-10% of all soft tissue sarcomas [1]. Amongst them malignant triton tumor (MTT) constitutes about 5% of all MPNSTs[2]. MTT is a subtype of MPNST that

has real diagnostic challenge for surgical pathologists with regard to its cell of origin and its relatively aggressive course. It is comprised of malignant Schwann cells coexisting with characteristic malignant rhabdomyoblasts[3]. It occurs in two forms, one is sporadic form and the other is neurofibromatosis type 1 (NF-1) associated form. Majority of the reported cases are of later variety. Immunohistochemically the rhabdomyoblastic elements are typically positive for skeletal muscle markers such as desmin, myoglobin or muscle actin. MTT has a poorer prognosis than MPNST[3]. This poor outcome is mainly attributed to the high frequency of grade Ⅲ histology in this sarcoma[4].

CASE REPORT

A 55-year-old male patient presented with multiple swellings all over the body ranging from 1-3 cm since 30 years (Figure 1). These nodules were histopathologically reported as neurofibromas. A subcutaneous nodule in left thigh was enlarged to attain a present size of 8 cm × 4 cm × 3 cm. He had café-au-lait spots on the trunk ranging from 0.5-1.8 mm. On family history, patient's mother, sister and brother had similar complaints. So he was diagnosed as a case of NF-1. Ultrasonography was suggestive of neoplastic lesion mostly soft tissue sarcoma.

Fine needle aspiration cytology was done and reported as malignant soft tissue tumor with possibilities of malignant fibrous histiocytoma and rhabdomyosarcoma (Figure 2A). Complete resection of the mass was done and the specimen was sent for histopathological evaluation.

On, gross examination showed a mass of 8 cm × 4 cm × 3 cm in size, covered with skin. Cut surface was soft, pale grey in colour with areas of necrosis.

Histopathological examination showed dense areas of malignant spindle shaped cells with oval nuclei with prominent mitoses, marked nuclear pleomorphism along with foci of necrosis. Many nuclei were bizarre and hyperchromatic. Alternating with hypercellular Antoni A areas, there were hypocellular myxoid areascalled as- Antoni B areas. Thick walled congested blood vessels were found. Interspersed within it are seen many Scattered round cells with abundant eosinophilic cytoplasm with atypical nuclei, which were recognized as rhabdomyoblasts (Figure 2B).

The surgical cut margins margins were free of tumor invasion. On Immunohistochemical evaluation, spindle cells showed focal S-100 positivity (Figure 3A) and cells with deeply eosinophilic cytoplasm (rhabdomyoblasts) showed positivity for Desmin (Figure 3B).

After histopathological confirmation patient was under treatment with radiotherapy and doing well without any recurrence on follow up since last 6 mo till date.

DISCUSSION

Peripheral nerve malignant lesions are displaying

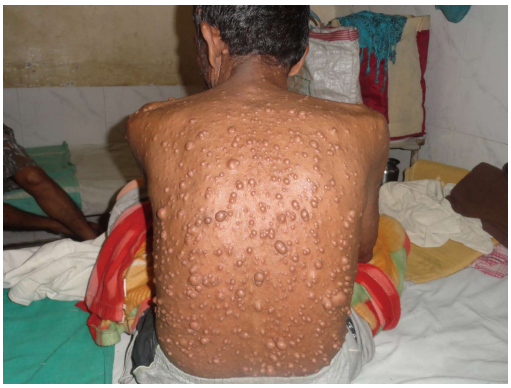

Figure 1 Photograph showing multiple neurofibromas, present all over the body.

differentiation towards Schwann cells, perineural cells, fibroblasts are labelled as MPNST. This term now replacing the earlier terminologies malignant schwannoma, neurofibrosarcoma and neurogenic sarcoma[1]. The diagnostic criteria for NF-1, is presence of two or more of the following signs[1]: (1) Six or more cafe-au-lait macules; (2) Two or more neurofibromas of any type or one plexiform neurofibroma; (3) Freckling in the axillary or inguinal region; (4) Optic Glioma; (5) Two or more Lisch nodules; (6) Osseous Lesion; and (7) First degree relative (parent, sibling, offspring) with NF-1.

MPNST constitutes 5%-10% of all soft tissue sarcomas, about one fourth to one half occur in the setting of neurofibromatosis[1]. Patients with NF-1 have propensity to transform in to sarcoma after a prolonged latent period (10-20 years)[5]. Our patient was diagnosed as having NF-1 and after long latency period of 30 years, he developed MPSNT with rhabdomyoblastic differentiation. MPNST can also arise spontaneously without association of NF-1[4].

MTT is a rare tumor arising from peripheral nerves. It is an autosomal dominant disorder. It has a strong association with neurofibromatosis (type 1). The common sites of occurrence are head, neck, extremities and trunk[4]. The symptoms are mainly attributed to mass effect giving rise to neurological signs and symptoms[4].

In 1973 Woodnelf *et al*[6] proposed the classification of MTT by establishing three criteria for diagnosis: (1) Tumor with peripheral nerve involvement in a patient with NF-1; (2) Majority of the cells in the tumor are Schwann cells; and (3) Presence of Rhabdomyoblasts[6].

Our patient had NF-1 and histology showed all the above mentioned criteria. The pathognomic feature of this tumor is the presence of rhabdomyoblasts. The number of rhabdomyoblasts varies from area to area in the same tumor. They are having abundant eosinophilic cytoplasm. Desmin is demonstrated in the rhabdomyoblasts.

The histogenesis of this unusual tumor is discussed by Masson. He postulated that both cell lines have similar origin, *i.e.*, from less well differentiated neural crest cells[1,7] . The strong relation between neural

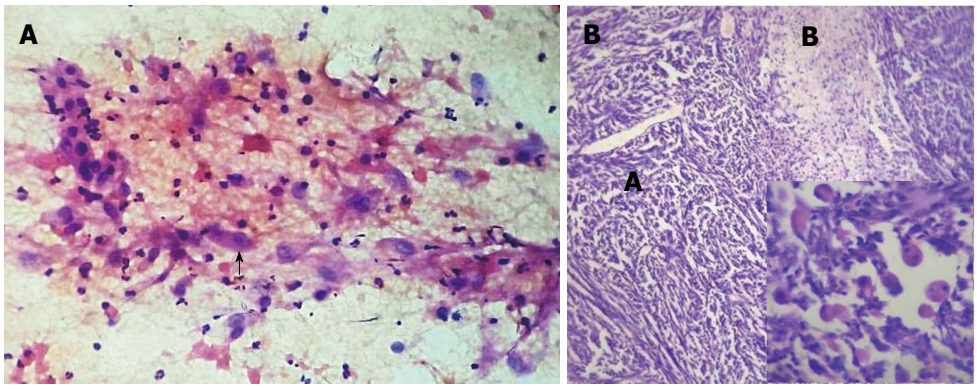

Figure 2 Photomicrograph. A: Photomicrograph of cytological smear showing spindle cells with pleomorphic hyperchromatic nuclei and a plump cell (arrow) with abundant eosinophilic cytoplasm; B: Photomicrograph showing hypercellular areas (Antoni A) of spindle cells having hyperchromatic nuclei and hypocellular areas (Antoni B) (100 ×). Inset shows rhabdomyoblastic differentiation (400 ×).

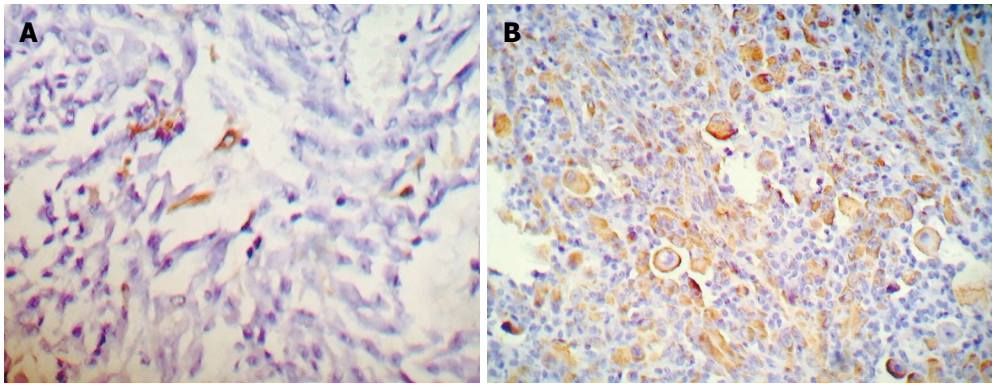

Figure 3 Immunohistochemistry. A: Immunohistochemistry showing focal S100 positivity in spindle cells; B: Immunohistochemistry showing Desmin positivity in rhabdomyoblasts.

tissues and rhabdomyoblastic differentiation has been reported as the development of skeletal muscle differentiation within other neural tumors such as Ocular Medulloblastoma[8], Ganglioneuroblastoma[9,10]. The five years survival rate for MTT is only 11% in contrast to 39% for MPNST[11]. MTT is significantly worse than the usual MPNST. The aggressiveness of MTT is attributed to high grade (grade Ⅲ) nuclear features with high proliferative capacity[4]. Radical excision followed by high dose radiotherapy is the conventional treatment for this unusual tumor[3].

MTT is an uncommon sarcoma which is having high propensity of local recurrence and distant metastases. Histopathologically, the diagnosis of MPNST with mesenchymal differentiation is difficult. So meticulous histopathological examination and immunohistochemical demonstration of neural markers and skeletal muscle markers help to hit the correct diagnosis. Early diagnosis, complete resection of the tumor followed by radiotherapy can help to increase survival of the patient.

COMMENTS

Case characteristics

A 55-year-old male patient presented with multiple swellings all over the body ranging from 1-3 cm since 30 years.

Clinical diagnosis

Multiple neurofibromas.

Differential diagnosis

Neurofibromas, fibrosarcoma, malignant peripheral nerve sheath tumor (MPNST).

Laboratory diagnosis

Fine needle aspiration cytology, histopathology with Immunohistochemistry showing Desmin positivity in rhabdomyoblasts.

Imaging diagnosis

Ultrasonography was suggestive of neoplastic lesion soft tissue sarcoma.

Pathological diagnosis

An unusual histological variant of MPNST with rhabdomyoblastic differentiation.

Experiences and lessons

Careful histological examination along with clinical work up is important to arrive at such unusual diagnosis.

Peer-review

Good overview of the diagnostic challenges in the correct interpretation of this rare tumor.

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EDITORIAL

Epithelial-mesenchymal, mesenchymal-epithelial, and endothelial-mesenchymal transitions in malignant tumors: An update

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Author contributions: Gurzu S designed research and drafted the article; Turdean S analysed and interpreted the epithelial-mesenchymal transition (EMT) literature data in hepatocellular and cholangiocarcinomas; Kovecsi A analysed and interpreted literature data in the field of EMT in gastrointestinal stromal tumors; Contac AO analysed and interpreted data from literature in field of EMT in gastrointestinal carcinomas; Jung I analysed and interpreted data from literature in field of EMT in sarcomas, lung and breast cancer, and approved the final variant of the article.

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Abstract

Epithelial-to-mesenchymal transition (EMT) represents conversion of an epithelial cell in an elongated cell with mesenchymal phenotype, which can occur in physiologic and pathologic processes such as embryogenesis (type 1 EMT), wound healing and/or fibrosis (type 2 EMT) and malignant tumors (type 3 EMT). The proliferation rate, metastasizing and recurrence capacity, as also the individualized response at chemotherapics, in both epithelial and mesenchymal malignant tumors is known to be influenced by reversible switch between EMT and mesenchymal-to-epithelial transition (MET). Although much research work has already been done in these fields, the specific molecular pathways of EMT, relating to the tumor type and tumor localization, are yet to be elucidated. In this paper, based on the literature and personal experience of the authors, an update in the field of EMT *vs* MET in epithelial and mesenchymal tumors is presented. The authors tried to present the latest data about the particularities of these processes, and also of the so-called endothelial-to-mesenchymal transition, based on tumor location. The EMT-angiogenesis link is discussed as a possible valuable parameter for clinical follow-up and targeted therapeutic oncologic management. The paper begins with presentation of the basic aspects of EMT, its classification and assessment possibilities, and concludes with prognostic and therapeutic perspectives. The particularities of EMT and MET in gastric and colorectal carcinomas, pancreatic cancer, hepatocellular and cholangiocarcinomas, and lung, breast and prostate cancers, respectively in sarcomas and gastrointestinal stromal tumors are presented in detail.

Key words: Gastrointestinal stromal tumor; Carcinoma; Gastrointestinal cancer; Hepatic cancer; Sarcoma

Core tip: This review, based on the personal experience of gastrointestinal pathologists, which correlates with literature data, is intended to provide an up-date in the field of epithelial-mesenchymal transition and mesenchymal-epithelial transition in epithelial and mesenchymal-malignant tumors, respectively. The molecular mechanism of these processes and their possible role in tumor progression, metastasis and therapy are presented in detail.

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INTRODUCTION

Because of paucity of data on biological behavior of epithelial and mesenchymal malignant tumors, the process of metastasis is still so poorly understood that it is difficult to block it therapeutically. In recent years, one of the newest described mechanisms that seems to contribute to migratory and invasive properties of tumor cells is the epithelial-mesenchymal transition (EMT)[1-3]. Till now, it is known to be implicated in carcinomas and in some mesenchymal tumors, such as sarcomas and gastrointestinal stromal tumors (GIST)[1-21]. However, the molecular mechanisms underlying EMT and distant metastasis remain somewhat unclear.

In this review we intend to present the main characteristics of EMT in a logical manner, beginning with the basic molecular mechanisms and possibilities of its quantification on histological slides. Then, the particularities of EMT in several carcinomas and sarcomas are explored and a future perspective synthesized.

BASIC INFORMATION ABOUT EMT

Definition of EMT

EMT represents conversion of an epithelial cell in an elongated cell with mesenchymal phenotype, which can occur in physiologic and pathologic processes[22]. Being influenced by several biochemical mechanisms, EMT involves mainly loss of cell polarity, dissolution of cell membrane, disintegration of cytokeratin filaments and desmosomes, and migration of the newly formed cells that have a mesenchymal phenotype[23,24]. Besides increase in migratory capacity, cell resistance to apoptosis also increases with associated cytoskeletal disorders and excessive deposits of extracellular matrix components[23-25].

Classification of EMT

Based on the pathogenetic role, three types of EMT are described. Type 1 EMT, involved in embryogenesis and organ development, mainly designed to generate the primary mesenchyme[23,26]. The first report on type 1 EMT in vertebrata embryogenesis was published in 1995[22].

Type 2 EMT is involved in non-neoplastic processes and genesis, and recruitment of fibroblasts in chronic inflammation-related tissue repair, wound healing, tissue regeneration and fibrosis[23-27].

Type 3 EMT represents cytoskeleton disorders that result from dissolution of cell-cell adhesion, cell scattering and loss of apical-basal cell polarity in malignant tumors[1,2,23,28].

EMT and endothelial-to-mesenchymal transition in non-neoplastic lesions

Type 1 EMT plays important roles in embryo implantation, embryogenesis and organ development[23,26]. At the same time, the reverse mesenchymal-to-epithelial transition (MET) is necessary to generate a secondary epithelium of kidney and other organs[23,24]. The secondary epithelia can remain in this status or can be re-differentiated into several structures such as fibroblasts, connective tissue, astrocytes, adipocytes chondrocytes, osteoblasts, and muscle cells[23,24]. The EMT-to-MET switch is mediated by several genes such as paired box2 (*Pax2*), Wilms tumor1 (*Wt1)*, and *Bmp7*[23]. In the embryonic heart, during formation of the atrioventricular/endocardial cushion, EMT is induced during somitogenesis by the HGF (hepatocyte growth factor) and its c-Met/Crk proteins, secreted by mesenchymal cells[23-25]. EMT is mandatory for the formation of epicardium-derived progenitor cells and their migration in the sub-epicardial space and it is mediated by several factors, such as transforming growth factor-β 1 (TGF-β1) and myocardin[29].

Type 2 EMT was described not only in physiologic repairing processes but also in renal, pulmonary, and hepatic fibrosis and even in pathogenesis of fistulae in Crohn's disease[23-27]. In renal fibrosis, about 35% of fibroblasts originate from tubular epithelial cells, which are detached and mobilized through a damaged basement membrane, and they are responsible for specific lesions such as chronic glomerulonephritis, diabetic nephropathy, lupus nephritis, and Alport syndrome[24]. The other fibroblasts are recruited from bone marrow (15%) and renal stroma (50%)[24]. In cardiac fibrosis, TGF-β1-mediated genesis of fibroblast from damaged vessels-endothelial cells, known as endothelial-to-mesenchymal transition (EndMT), was suggested as the predominant event, as it is involved in cardiac embryogenesis too[25]. In renal fibrosis, it is observed that the EMT-related fibroblasts are SMA-positive myofibroblasts; it is suggested that a combined EMT-EndMT is the key event of any fibrosis[24].

Because TGF-β action and EndMT are experimentally

inhibited and E-cadherin expression is restored in epithelial cells by bone morphogenetic protein 7 (BMP-7), it is supposed that systemic administration of this protein may delay progression of cardiac fibrosis and prevent chronic rejection of transplanted heart[25], besides delaying renal failure[24]. Rapamycin may also ameliorate the amount of fibrotic tissue by blocking the mTOR signaling[30].

EMT in malignant tumors (type 3 EMT)

In malignant tumors, EMT-related invasion and migration of tumor cells in blood flow is associated with poor prognosis, high metastatic rate, and low disease-free survival time[1-21]. In cancer cell lines, EMT is considered when the cells gradually change from epithelial to migratory elongated spindle-like mesenchymal cells with fibroblastic morphology; expression of epithelial markers decreases as tumor cells progressively display the mesenchymal markers[1,2]. Gaining mesenchymal signature facilitates detachment of tumor cells, accompanied by proteolytic digestion of basement membrane, vascular invasion (intravasation), and migration of circulating tumor cells to distant sites[2,3,24].

As not much is known about the cytoskeleton of detached and circulating tumor cells, it is supposed that EMT produces detyrosination of some proteins, such as α-tubulin (storage of Glu-tubulin)[28]. This process, a vimentin-dependent one, induces formation of tubulin microtentacles (microtubules-based membrane protrusions), which are distinct from the well-known actin-based prolongations (lamelipodia, filopodia) and confer on the circulating tumor cells with mesenchymal phenotype the capacity of attachment at the endothelial layers and further extravasation at distant sites[28]. Moreover, Glu-tubulin increases the intravascular life of tumor cells, from 3-5 min to about 16 h, favoring cell extravasation[28,31].

At the same time, in distant metastatic tissue, a reverse MET can be acquired (EMT-MET switching) according to the metastatic microenvironment; regaining of epithelial features allows re-proliferation of tumor cells in these secondary-formed tumor clusters in lung and liver metastases, but not in bone metastatic tissue[2,3,15,18]. It is also worth mentioning that, in primary tumors, in parallel with EMT, the transformed tumor cells acquire a stem cell pattern or activate multidrug-resistant stem cells, and the converted mesenchymal cells that did not migrate in the blood flow are responsible for local recurrence, probably due to a reverse MET in the primary tumor, besides metastatic sites[18,32].

EMT MARKER PROTEINS

Literature describes several markers, which are involved in EMT. During EMT, E-cadherin expression is reduced to near-zero level, E-cadherin-to-N-cadherin (Neural cadherin) switch is installed and other EMT-related markers [vimentin, fibronectin, smooth muscle actin (SMA), desmin, Sox, SNAIL1/SNAIL, SNAIL2/SLUG, Axl, zinc finger E-box-binding homeobox 1 (ZEB1), Notch-1, v-ets erythroblastosis virus E26 oncogene homologue 1 (ETS1), fms-related tyrosine kinase 1 (FLT1), V-set and immunoglobulin domain-containing protein 1 (VSIG), stromelysin-3, Twist, FOXC2, HOXB7, ACTA2, platelet derived growth factor (PDGF), *etc.*] are overexpressed[4,11,32], predominantly at the invasive front[23]. Other epithelial cell-cell adhesion molecules such as claudins (types 3, 4, and 7), α-catenin, γ-catenin, occludin, desmoplakin and plakoglobin are also down-regulated in cells with mesenchymal signature[5,11,32]. Immunohistochemically, E-cadherin and catenins mark the cell membrane; N-cadherin, vimentin, and PDGF present a predominant cytoplasmic expression; Notch-1 and ZEB1 mark the nuclei, whereas SNAIL, SLUG, and Twist mark the cytoplasm, as well as the nuclei of the tumor cells.

It is important to note that some of the transformed cells present only mesenchymal phenotype, whereas other tumor cells display a dual epithelial-mesenchymal expression[32], also known as an amphicrine pattern[33]. As regards the stem cells with malignant potential, it is still unclear if they are identical to the cells with mesenchymal signature, or they represent two distinct cell types; they are marked by both mesenchymal markers and stem cell markers such as CD44 and ALDH (aldehyde dehydrogenase)[34].

SLUG is a zinc-finger transcription inhibitory protein, a member of the SNAIL family (SNAIL 1 and 2) that is primarily described in the neural crest and embryonic mesodermal cells; it also plays roles in type 1 and type 3 EMT[32-36]. SNAIL 1/SNAIL is a transcriptional hypoxia-activated protein that interacts with Wnt- and also with serine/threonine kinase receptor signalling pathways; the tumor cells' invasiveness and resistance to apoptosis are influenced by its overexpression in both tumor cells and stromal fibroblast[32,37]. SNAIL 2/SLUG is involved in cancer progression and suppresses E-cadherin, desmoplakin, and keratin-18 expression, but the exact mechanism is not known; it is supposed to interfere with the Wnt/GSK3β/β-Trcp1 pathway[4].

Vimentin is a mesenchymal marker that can be acquired during EMT but its expression is low and most difficult to be quantified, as compared with other EMT markers that indicate a mesenchymal-like phenotype[1].

The receptor tyrosine kinase Axl is an EMT marker whose mRNA expression is strongly correlated with vimentin. It is involved in EMT of breast, and pancreatic and lung cancers and is expressed more in mesenchymal than in epithelial lines[6-8].

Dismantling of epithelial cell membrane and remodeling of extracellular matrix are also influenced by matrix metalloproteinases (MMP-2, MMP-3, and MMP-9) that act as proteolytic enzymes and may link the EMT with increased invasion and metastasis, and shortened survival time in almost all types of human

cancer[23,24,38].

VSIG, also known as radioiodinated cell surface A33 antigen or glycoprotein A34, is a single-pass 387 amino acid membrane protein involved in cell-cell adhesion[39].

Cathepsin family (*e.g.,* Cathepsin Z located at 20q13.3, Cathepsin X, *etc*.) is also considered to be involved in EMT and to contribute to tumor metastasis through down-regulation of E-cadherin, α-catenin, and β-catenin, and up-regulation of vimentin, fibronectin, MMP-2, MMP-3, and MMP-9[38].

MOLECULAR FACTORS INVOLVED IN EMT

In the last few years, several research works were carried out to understand the mechanism of EMT in both carcinomas and mesenchymal tumors, but data are elusive.

As regards DNA, the next-generation sequencing-based methods proved that the genome-wide DNA methylation reprogramming is not involved in EMT; the progressive EMT and genetic disorders are rather related to altered histone modifications[1,9] and epigenetic mechanisms[11]. Recently, it is suggested that a 76-gene EMT-signature is involved in the metastatic process and that it influences the therapeutical answer of tumor cells[7], but the results are still confusing.

E-cadherin's transcriptional down-regulation is one of the key factors of EMT but other factors, such TGF-β also play important roles in these processes[1,9]. It is worth noting that there are no deletions or mutations of *E-cadherin* gene, but only epigenetic down-regulation or transcriptional silencing which allows its further re-expression in primary or metastatic tumors[18]. E-cadherin is codified by the *CDH1* gene; it interferes with other EMT-related genes, such as vimentin, fibronectin 1 *(FN1), CDH2* (which codifies the protein N-cadherin), *ZEB1* (target of SNAIL), *ZEB2/SIP1*, *K-ras, integrin, Notch,* and *Axl* (a tyrosine kinase inhibitor)[7]. The cancer cell lines with epithelial phenotype are characterized by overexpression of other genes such as *claudins 4* and *7, MUC1, RAB25, SPINT2,* and *ERBB2*[7]. However, some of these molecular markers (SNAIL 1/2, ZEB 1/2, E47) are direct inhibitors of the transcription of *E-cadherin* gene, whereas Twist, Goosecoid, FoxC2, and E2.2 are indirect E-cadherin inhibitors[11,16,17]. At the same time, SNAIL 1/2, ZEB1/2, and Twist are activated by TGF-β, a cytokine, secreted by mesenchymal stromal and inflammatory cells, whereas N-cadherin is activated by Twist[32,40]. If we take into account that TGF-β also induces renal EMT, besides mobility of endothelial cells, followed by EndMT and subsequent renal and cardiac fibrosis[24,25], and regulation of matrix accumulation[32], then we can suppose that stromal fibrosis is generated by an interaction between EMT and EndMT. Moreover, TGF-β is a tumor suppressor in early-stage carcinomas but induces tumor cells' proliferation, migration, and metastases, in advanced stages[32].

Epidermal growth factor (EGF) is a mitogenic factor involved in tumor proliferation and aggressiveness, through its receptor, EGFR. Although *EGFR* and *K-ras* genes' status are used as indicators for targeted therapy with anti-EGFR drugs of several tumors, such as pulmonary and colorectal cancers, the origin of this factor or its prognostic role is not firmly proved. As regards the role of this pathway in EMT, it seems that the Ras-activated/SNAIL/SLUG pathway interacts with FoxC2 and the phosphatidylinositol 3'-kinase (PI3K)/Akt/mTOR axis, at least in the case of colorectal cancer[40].

One of the newest factors found as pivotal regulators of EMT and as negative regulators of E-cadherin are the post-transcriptional gene regulators microRNAs (miRs): miR-21, miR-26b, miR-29c, miR-31, miR-124, miR-212, and the five members of the miR-200 family (miR-200a, 200b, 200c, 141, and 429) with their most prominent gene targets *ZEB1* and *ZEB2* (also known as *SIP1* and *SFHX1B*)[2,10,11,17,18,21,40]. Their overexpression is believed to inhibit EMT in human carcinoma cells and to decrease tumor cell proliferation and migration in the blood flow[2,10,11,17,18,21]. EMT-related interaction of miR-200 family with *p53* gene is also assumed[19].

Their elective affinities for specific types of cancer cell lines are described below.

EMT, ENDMT AND ANGIOGENESIS

The data available on the link between EMT and angiogenesis is so scattered that it does not elucidate if the link is any possible important therapeutic implications. For example, the MMP family members (MMP-2, MMP-3, and MMP-9), which are released by fibroblasts and macrophages, are known to influence both EMT and angiogenesis[23,38], though not proved to mediate the interaction among these two processes. At the same time, EMT, activated *via* SNAIL/Twist, is responsible for the attachment of tumor cells to the activated endothelial cells *via* α-tubulin detyronisation[28]. The transcription factor Twist, a target gene of SNAIL, is reportedly implicated in embryogenesis (EMT type 1), but its hypoxia-activated over-expression is also proved in several human carcinomas[41]. However, its role in carcinogenesis and metastasis is not well defined. In experimental models, Twist inhibition does not decrease tumor cell proliferation rate, but reduces circulating tumor cells significantly[28]. In cancer tissues its expression is increased at the invasion front[41]. Based on these observations, it is supposed that Twist increases direct invasion of tumor cells in the surrounding tissues, and cell penetration inside the endothelial layer, but does not influence tumor growth[28,41]. As a therapeutic target, its inhibition may decrease the rate of metastasis.

Because the endothelium of pre-existing mature vessels, involved in angiogenesis of both epithelial

and mesenchymal tumors, is activated by CD105 (endoglin)[42,43], new studies are necessary to prove if the Twist-attached endothelial cells are also CD105 positive or other mechanism is involved in this attachment.

Most recently, a possible TGF-β1 mediated-EndMT of intratumor endothelial cells is reported[44]. It relates to the cases in which the intratumor endothelial cells lose the cell-cell junction and immunoexpression of CD31 and gain positivity for mesenchymal markers such as α-SMA (smooth-muscle actin)[44]. It is important to note that the endothelial cell-specific miR-302c is proved to inhibit this EndMT, but decreases the tumor cell motility[44]. In previous researches, our team observed inconstant positivity of Kaposi sarcoma cells[42] and gastric tumor cells for the endothelial marker CD105 (Gurzu *et al*[42], personal communication), which could indicate even a possible epithelial-to-endothelial transition or an incidental positivity of epithelial cells with mesenchymal signature.

A relationship between inflammation, angiogenesis and EMT is suggested by COX-2-mediated EMT, which is stimulated by TGF-β through a PGE2-dependent mechanisms[23,35], and also by an MMP-related signaling[23].

Other arguments that favor a link between angiogenesis and EMT-mediated metastasis are that, on the one hand, the endothelial cell-secreted factors, such as EGF, increase cell mobility and inhibit apoptosis, whereas, on the other hand, the EGF-related EMT is more prominent in the perivascular area, but the intraluminal tumor cells exhibit an epithelial phenotype[45]. It is important to prove that the involved vessels present CD105-positive activated endothelium. EGF seems to also induce a stem-like phenotype of EMT-transformed tumor cells (in both intra- and perivascular tumor cells) and SNAIL positivity through PI3k-Akt pathway, whereas down-regulation of EGF in endothelial cells decreases tumor growth rate and the immunoexpression of stem cell markers[45]. The data suggests that antiangiogenic therapy should be based on inhibition of tumor cells from secreting pro-angiogenic substances such as vascular endothelial growth factor-A (VEGF-A), and of the activated endothelial cells to synthesize pro-angiogenic/pro-stem/pro-EMT substances such EGF[45].

EMT IN GASTROINTESTINAL CARCINOMAS

EMT in gastric carcinoma

Fewer than 50% of GC cells express E-cadherin, and SLUG cytoplasmic positivity is reported in about 30% of the cases[36]. Most of the E-cadherin negative cases are hereditary[46] or acquired diffuse/poorly-cohesive gastric carcinomas (GCs)[47]. The 5-year survival rate is strongly related to E-cadherin expression, which is 88.6% in positive cases and 63.5% in cases with loss of E-cadherin expression[36]. As regards SLUG immunoexpression, the 5-year survival rate is about 78% in negative cases and only 54.3% in SLUG positive-GCs[36].

Independent of the histologic type, E-cadherin negative/SLUG positive-GCs are associated with higher risk for lymph node and distant metastases, as compared to the E-cadherin positive/SLUG negative-GCs[36]. The reverse correlation of E-cadherin/SLUG is observed in only 75.5% of GCs, while the other cases present double positivity for E-cadherin and SLUG[36]. In E-cadherin positive-GCs, association of SLUG positivity induces a lower survival rate, as compared to SLUG negative cases (92% *vs* 46.7%)[36].

In Chinese patients, the cell-cell adhesion molecule VSIG is also reduced in GC samples, as compared with paired gastric mucosa, whose decreased levels are proved with PCR, western-blot and IHC examinations[38]. Using IHC methods, total loss of VSIG was found in more than 50% of the cases, especially in GCs with distant metastases, and decreased levels in 46% of them, which are strongly correlated with the overall survival rate and disease-free survival[38].

Cathepsin X expression was detected in *Helicobacter pylori*-infected normal gastric mucosa, which was 3-12 fold up-regulated in 68% of metastatic GCs with EMT, especially in the cases with an intestinal type architecture[38,48].

Twist is especially overexpressed in diffuse type GCs and is correlated with N-cadherin expression; both of them are down-regulated in intestinal type-GC, as compared to paired normal gastric mucosa[49]. N-cadherin is expressed in chief and parietal cells of the normal mucosa. Based on these observations, it was suggested that E-cadherin-to-N-cadherin/Twist switch is specific for carcinogenesis of diffuse type GC, whereas intestinal-type GC is more related to TGF-β-dependent ZEB2 (SIP1) up-regulation/SNAIL weak down-regulation/E-cadherin loss[49]. At the same time, release of TGF-β, which activates the EMT-inductors SMAD 2/3 and PI3K/Akt signaling, is caused by extreme hypoxia[50].

Among the miR-200 family members, miR-141 is reported to have been down-regulated in about 80% of primary GCs, as compared with matched normal gastric mucosa and also in several human gastric cancer cell lines[11,18]. Its overexpression inhibits proliferation of GC cells, but decreased level of miR-141 favors occurrence of distant metastases[18].

As regards the therapeutic approaches, EMT may induce in GCs a Wnt/β-catenin signaling-dependent resistance to adriamycin, which can be reverted by therapy with the proton pump inhibitor pantoprazole[51].

EMT in colorectal carcinoma

In advanced-stage colorectal carcinomas (CRCs), a predominant-epithelial phenotype was reported from the center of primary tumor, and mesenchymal features in the cells from the invasion front[11,20]. As regards SLUG expression, its positivity induced in CRC cells an EMT-related aggressive behavior, but no relation could be proved between E-cadherin and SLUG expression[36,52]. Aggressivity was also induced

by SLUG overexpression in fibroblasts of the tumor stroma[37].

Increased E-cadherin and decreased vimentin-expression were noted in liver metastases, as compared with the primary tumor[11]. This immunophenotype, which indicates an EMT in primary tumor, predominantly in the invasive areas, and a reverse MET in hepatic metastases, seems to be related to altered functions of miRNAs such as miR-21[40], miR-29c[2], miR-31[40], miR-212[10], and some members of miR-200 family[11].

The miR-21 and miR-31 are particularly considered related to the TGF-β-pathway, because of their being targeted by the T lymphoma and metastasis gene 1 (*TLAM1*); their down-regulation increases CRC cells motility and invasion[40].

The miR-29c is down-regulated in primary CRC tumor cells with high invasive potential and re-expressed in hepatic metastases[2]. In CRCs lines, miR-29c decreased the proliferation, migration and metastatic potential of tumor cells, negatively regulated the Wnt/β-catenin signaling pathway and inhibited EMT *via* PI3K/AKT and GSK-3β/β-catenin signaling[2,40]. Immunohistochemical examinations showed increased nuclear β-catenin expression, loss of E-cadherin, up-regulation of T-cell factor/lymphoid enhancing factor transcriptional activity, and increased cell migration and invasiveness in miR-29c knockdown CRC cells, whereas overexpression of miR-29c induced a nuclear-to-cytoplasmic shift of β-catenin[2]. In matched liver metastastases foci, elevated levels of miR-29c induced MET[2]. In CRC, the two target genes of miR-29c and EMT-MET switch are the protein tyrosine phosphatase type IVA 1 and guanine nucleotide binding protein alpha13[2].

Overexpression of miR-212 seems to be responsible for inhibition of CRC cells proliferation; besides, by delaying their migration in blood flow, it becomes protective for both hepatic and lung metastases[10]. On the other hand, low level of miR-212 associates with overexpression of manganese superoxide dismutase, which mediates the EMT of CRC cell lines and induces a more aggressive tumor phenotype and a shorter survival rate of patients diagnosed with CRC[10].

In metastatic CRC of miR-200 family members, the miR-141 and miR-200c, mapped to chromosome 12, were found to be overexpressed in liver metastatic tissue, as compared with primary tumor cells[11]. Owing to gradually decreasing expression of miR-200c in tumor cells, as compared to adjacent normal colonic mucosa, and also in the tumor invasion front, it was found that this molecular factor, which is re-expressed in liver metastases, induces cell proliferation but suppresses cell invasion and migration[11]. On the other hand, high level of miR-200c/141 promoter region in CRC cell lines is associated with epithelial signature, resulting in up-regulation of E-cadherin, down-regulation of vimentin expression, and lower risk for distant metastases[11].

The other two members of miR-200 family, miR-200b and miR-429, which are mapped to chromosome 1, do not influence the EMT in CRC cell lines; miR-200b, but not miR-429, is down-regulated in liver metastatic tissue, as compared with primary tumor cells[11]. However, the most significant member of miR-200 family, involved in EMT and metastatic process of CRC, is miR-200c, which exerts negative regulation of its gene targets *ZEB1, ETS1* and *FLT1*[11]. Although the miR-200 family interacts with *p53* gene in other cancer cell lines[19], no correlation between mi-R200c and p53 was found in CRC[11].

Based on the above-mentioned expression of mi-RNAs, the most recent studies suggest that these molecular factors may serve as potential diagnostic markers and targets for therapeutic strategies in patients with metastatic CRC[2,10,11]. Further *in vivo* studies are necessary to confirm this supposition and to emphasize the relationship between mi-RNAs and other genes such as *p53* and mismatch repair (*MMR*) genes.

As regards the circulated CRC cells, it was proved that, inside the blood vessels, disruption of actin filaments increased cell adhesion, whereas destabilizing cytoskeletal microtubules prevented cell attachment and tumor intravasation at the junctions located between endothelial cells[28,53].

EMT IN PANCREATIC CANCER

In pancreatic cancer cells, EMT is induced by several molecular factors, such as the receptor tyrosine kinase *AXL*[7,8], loss of FOXA1/A2 expression[17], and dysregulation of miR-200 family[17], but this event is not so frequent as the other carcinomas are[17,54]. Moreover, loss of E-cadherin expression, a result of *E-cadherin* gene methylation, is correlated with histological grade of differentiation, which is noticed in about one quarter of well-differentiated ductal adenocarcinomas, half of the poorly-differentiated ones and few or none of the non-cohesive (undifferentiated and signet ring cell carcinomas) cases[17,55]. In chemoresistant well/moderately-differentiated pancreatic adenocarcinomas, E-cadherin loss and a membrane-to-cytoplasm shift of β-catenin expression pattern were noticed in the non-cohesive foci[17,55]. N-cadherin does not mark the normal acinar, ductal, and pancreatic islets; it is expressed in more than 50% of pancreatic carcinomas and is more intense in infiltrating cells from the invasive tumor front, independent of the tumor stage[56]. As regards the other IHC markers that indicate a mesenchymal-like phenotype of the epithelial tumor cells, SLUG was reportedly expressed in half percent of the cases, while SNAIL 1 marked 75% of pancreatic carcinomas. SNAIL 1 presents predominant positivity in the ductal cells of the tumor center and in undifferentiated carcinomas, as compared with the more cohesive carcinomas, whereas SLUG is more intense in the invasive front[56]. Moreover, 50% of the pancreatic carcinoma cells present double positivity for SNAIL and E-cadherin[56]. Twist is negative in 97% of pancreatic carcinomas and its expression is

10-fold higher after exposure of pancreatic cell lines to hypoxia for 48 h[56].

Hypomethylation and overexpression in pancreatic carcinomas cells and elevated serum levels of miR-200a and miR-200b are associated with retention of E-cadherin expression, as also with epigenetic silencing through methylation of *ZEB2 (SIP1)* gene[11,17]. As SIP1 expression is absent in most of the pancreatic cancers, it seems that E-cadherin dysregulation is rather related more to miR-200a and miR-200b than to *SIP1* gene[17]. The serum levels of these miR-factors were noticed to be elevated in patients with chronic pancreatitis, as compared with healthy controls[17]; they can be used as a screening parameter to identify patients with chronic pancreatitis and high risk for ductal adenocarcinoma.

EMT IN HEPATIC CANCER

EMT in hepatocellular carcinoma

The EMT-angiogenesis-stem cell-like phenotype crosstalk is supposed as a key factor of hepatocellular carcinoma (HCC), based on the fact that the number of tumor-associated macrophages, which secrete TGF-β and are involved in angiogenesis, is directly correlated with the number of activated stem cells, as well with EMT-dependent tumor cells invasiveness[57]. Just as other carcinomas do, TGF-β induces EMT through E-cadherin down-regulation and up-regulation of several factors, including twist, N-cadherin, and SNAIL[58].

Cathepsin Z was also reported to be up-regulated and involved in EMT and metastasis of about 43% of HCCs; its increased level was correlated with high serum level of α-fetoprotein and also with poorer 5-year and 3-year survivals[38].

Down-regulation of miR-124 and miR-26b was reported in HCC cells, in cases with a more aggressive behavior, whereas their overexpression suppressed tumor cell proliferation, inhibited EMT and prevented cytoskeletal disorders through blocking formation of stress fibres, filopodia and lamellipodia[21,58]. In HCC, the two target oncogenes, negatively regulated by miR-124 are ROCK2 and EZH2[21].

As regards the therapy, the advanced-stage HCC is currently treated with the multityrosine kinase and angiogenesis inhibitor, sorafenib. Because MET is also involved in metastatic behavior of HCC, the new MET-inhibitor, Tivantinib (ARQ 197), is being tested in the ongoing trials, as a second-line therapy of HCC[59].

EMT in cholangiocarcinoma

Based on the scanty data available, it is believed that the EMT in cholangiocarcinomas is regulated mainly by the transcription factors ZEB2 and S100A4, which are modulated by TNF-α (tumor necrosis factor)[60]. TNF-α also stimulates TGF-β activity[60] which in turn activates other EMT-inducers, such Twist, N-cadherin, and vimentin[61]. Just as other carcinomas do, EMT induces a high rate of metastasis and a short overall survival rate[60], and thus help in blocking TGF-β by BMP-7[61].

EMT IN LUNG CANCER

Lung cancer is one of the tumors that is known for rapid progression accompanied by a large number of genetic and epigenetic changes[1,7]. EMT is identified in about 23% of non-small cell lung cancer (NSCLC) lines and in one third of the patients with metastatic lung cancer. Based on the histology of the cell lines, the squamous pattern associates with a mesenchymal phenotype in about 50% of lines; adenocarcinomas are characterized mostly by epithelial phenotype, and the other patterns (neuroendocrine, large cell carcinoma) by only mesenchymal signatures[7]. Although it was experimentally proved that DNA methylation is not involved in EMT of lung cancer cell lines, after transition, the expression of some DNA-related enzymes, such as methyltransferases 1, 3a, and 3b, and ten eleven translocation (TET1), were significantly altered and some histone methylation was changed[1].

In A549 lung cancer cell lines, during TGF-β-dependent EMT, miRNA relative expression of E-cadherin was progressively lost and the most significant overexpression was attributed to N-cadherin, followed by SNAIL 1 and vimentin[1]. Maximum level of N-cadherin was observed after 96 h of incubation, with significantly increasing levels from 24 to 96 h[1]. SNAIL 1 becomes significantly expressed after 4-12 h of incubation, followed by progressive overexpression in the next 24-96 h; its level is quite low compared to that of N-cadherin[1]. The overexpression of vimentin does not differ significantly at 12, 24, and 96 h. Moreover, its level is maintained at a constant value, which is similar to the levels of SNAIL 1 and N-cadherin, quantified at 10-12 h of incubation[1]. In NSCLCs, SNAIL 1 and vimentin's expressions are reversely correlated with the miR-30a level[62].

In contrast, another experimental study proved that 76 genes were involved in EMT, the main ones being *CDH1* and *vimentin,* followed by several EMT transcription factors such as *FN1, MMP2* (matrix metalloprotease-2), and *ZEB1*; the gene *CDH2* was identified inconstantly and was not included in the EMT molecular signature[7]. In mesenchymal cells from NSCLC lines, *K-ras* mutation, loss of *STK11 (LKB1)*, and *SMARCA4* mutations/deletions are more frequent than in epithelial lines; the last ones rather show CDKN2A and CDKN2B loss[7].

In lung cancer cell lines, EMT also induces down-regulation of some histones methylation, such as DNMT1, DNMT3a, DNMT3b, H3K4me3, H3K9me2, TET2, and TET3, and up-regulation of TET1, and H3K36me3[1].

As regards the prognosis, retention of the epithelial phenotype, proved by E-cadherin positivity in the tumor cells, was associated with longer time to progression

and a longer survival, independent of the mutation status of the *EGFR* (epidermal growth factor receptor) gene[7,12]. *EGFR* wild type/*K-ras*-wild type molecular profile seems to indicate a less aggressive NSCLC[7].

EMT as the therapeutic target in lung cancer

Patients with *EGFR*-mutated NSCLCs showed significant improved outcome after having been introduced to anti-EGFR drugs, such as erlotinib (Tarceva)[12] and gefitinib[13]. However, the *EGFR* wild type and *K-ras* mutated NSCLCs are resistant to EGFR inhibitors, and the data about the effect of these targeted drugs on *EGFR* wild type/*K-ras*-wild type patients is scanty[7].

In NSCLC cell lines, EMT was proved to induce resistance to erlotinib, whereas resistance to Akt/mTOR and PI3K/Akt1 pathway inhibitors was seen in both EGFR wild-type and mutated tumors with mesenchymal signature[7]. However, the mesenchymally-transformed cells are not pan-resistant; they are sensitive to sorafenib and the commonly used chemotherapics such as cisplatin, gemcitabine, vinorelbine, pemetrexed, docetaxel, paclitaxel, and platinum-doublets[7].

On the one hand, retention of the epithelial phenotype (high E-cadherin and low vimentin/fibronectin) is associated with erlotinib sensitivity, even in wild-type *EGFR* and wild-type *K-ras* NSCLCs[7,14]. It was then suggested that E-cadherin's diffuse positivity could be used as an indicator of erlotinib/gefitinib sensitivity, especially in wild-type cases[12]. On the other hand, in culture cell lines with mesenchymal signatures, which are insensitive to anti-EGFR drug gefitinib, restoration of the epithelial phenotype could induce re-sensitivity to this drug[7,13].

The level of the receptor tyrosine kinase Axl increases in mesenchymal NSCLC lines, because the cells, having a dose-dependent synergistic effect with erlotinib, are sensitive to the Axl inhibitor SGI-7079; the maximum synergy level was obtained at high concentrations for both erlotinib and SGI-7079[7].

Based on the foregoing data, a 76-gene EMT signature (including the genes *CDH1, CDH2, vimentin,* and *FN1*) was experimentally designed to predict 8-wk disease control in NSCLC-patients treated with erlotinib, independent of their molecular profile[7]. However, the exact underlying biological mechanism is not fully understood and hence deserves further investigation.

EMT IN BREAST CANCER

EMT in breast[4] is dependent on reducing mi-RNA expression of E-cadherin, which is related to *CDH1* promoter methylation, but not to mutational inactivation[9]. Just as the other carcinomas are, E-cadherin is re-expressed in hepatic metastatic tissues[11], and local recurrence may be dependent on the EMT-MET switch in the primary tumor[32].

Claudins (types 1, 3, 4, and 7) were described to be down-regulated in certain types of breast cancer, such as fibromatosis-like metaplastic carcinoma, a low-grade *EGFR* (exons 18, 19, 20, and 21) wild-type basal-like spindle cell carcinoma with predominant HER-2 and hormone receptors negativity, diffuse positivity for basal keratins and focal vimentin and E-cadherin expression[5]. Fibroblastic-like phenotype of breast carcinoma cells (with negative or low E-cadherin expression), intense angiogenesis (indicated by overexpression of VEGF-A) and chromosome instability can also be induced by twist, *via* Wnt/β-catenin[32,63].

SNAIL 1, known to induce invasiveness in several human cancers, seems to be a hypoxia-protective factor for breast tumor cells, *via* β-catenin activation, which regulates expression of HIF-1 (hypoxia-inducible factor 1)-dependent genes, besides being an inductor of hormone-resistance[32,63]. Based on these facts, it is suggested that SNAIL 1 may be used as an indicator of response of breast cancer to antiangiogenic therapy[64].

Twist expression was proved to be up-regulated in tandem with tubulin detyrosination, in both invasive ductal[28] and lobular-type adenocarcinomas[41], at the invasion front[28,41]. This interaction, which is mainly twist-dependent, seems to promote penetration of tumor cells through the endothelial cell layer, endothelial engagement and, probably, consecutive angiogenesis that favors metastasis[28].

EMT is also induced by the receptor tyrosine kinase *AXL*[6,7], Pyk2 and TGF-β *via* SNAIL 1/2 and ZEB 1/2[32], and it seems to have particular pathways in triple negative breast cancers[32].

As regards miRNA, the miR-200 family-mostly miR-200a/b/c, miR-141, and miR-429-is considered to influence EMT of breast cancer cells with inhibition of the E-cadherin repressors ZEB 1/2[32,65]. An interaction between *p53* gene and miR-200c is also defined in breast cancer cell lines[19]. The miR-21s also influences the EMT phenomenon *via* tumor suppressor gene PTEN and AKT/ERK1/2 axis[32,66]. Besides down-regulation of cyclin-dependent kinase (CDK8), β-catenin targeted-miR-26b is the other reported negative prognostic factor in breast cancer[58,67].

EMT is reported to be involved in breast cancer resistance to tamoxifen; the most down-regulated factor is the methaderin-targeted miR-375[68], whereas miR-519a is up-regulated in tamoxifen-resistant breast cancer cell lines with mesenchymal-like signature[69]. Re-expression of miR-375 and inhibition of miR-519a might serve as potential therapeutic approaches for estrogen receptor-positive breast carcinomas[68,69]. It was also experimentally proved that some contraceptive pills, such as centchroman, may be used to inhibit EMT and to play a dose-dependent antiapoptotic and antiproliferative role in human breast cancer cells, *via* down-regulation of HER2/ERK1/2/MMP-9 signaling[70].

EMT IN PROSTATIC CANCER

Prostatic carcinomas have a predilection for bone metastases, the metastatic cascade being also related

to EMT that is linked with stem cell signature of the prostatic carcinoma cells[15]. Although vimentin and ZEB 1 contribute to EMT, the most involved marker that plays a role in bone metastasis seems to be Notch-1[15].

In liver metastases, prostate carcinoma cells are bound to hepatocytes in an E-cadherin-dependent manner[18]. In primary tumor tissues, EMT is characterized by activation of EGFR and subsequent down-regulation of E-cadherin, whereas, in liver metastases, down-regulation of EGFR signaling induces re-expression of E-cadherin and cell-cell adhesion[18]. In bone metastatic tissue, E-cadherin remains down-regulated and Notch-1 is up-regulated[15].

EMT AND MET IN MESENCHYMAL TUMORS

EMT and MET in sarcomas

Inhibiting MET is one of the therapeutic goals currently being tested in human sarcomas. However, because of the aberrant expression of the EMT markers noticed in many mesenchymal tumors, most of the researchers believe that EMT is involved in grade of recurrence, metastasis rate and overall survival rate[71-75]. To our knowledge, it is more about MET than EMT, but the molecular aspects are neither well explored nor well defined.

In chondrosarcomas, CXCR4 and survivin were tested as candidates for molecule-targeted therapy, and they were found expressed in more than 80% of chondrosarcomas. Besides inducing SNAIL and N-cadherin up-regulation, they are directly correlated with recurrence rate, *via* MEK/ERK and PI3K/AKT signaling[73].

In osteosarcoma cell lines, HIF-1α-mediated hypoxia induces increased level of E-cadherin and decreased vimentin expression. This aberrant pattern is associated with a higher proliferation rate and increased invasivity of the osteosarcoma cells that can be inhibited by resveratrol[74], which has an antiangiogenic effect due to increased nitric oxide production in endothelial cells[76].

In neuroblastomas, several EMT pathways are up-regulated; EMT induces not only aggressiveness but also chemoresistance to doxorubicin[75].

EMT and MET in gastrointestinal stromal tumors

Although they are mesenchymal tumors, one third of GIST cells are proved to express not only the mesenchymal markers such as vimentin and SNAIL, but also as being positive for the cell-cell adhesion molecule E-cadherin and AE1/AE3 keratin[71,72]. Moreover, SNAIL-positive/E-cadherin negative GISTs, independent of vimentin expression, presented a higher risk for distant metastases[71] and keratin/E-cadherin positive-cases were predominantly vimentin/N-cadherin negative[72]. Based on these facts, it is supposed that both EMT and the reverse MET processes are involved in GISTs metastasis.

The post-transcriptional factor miR-137, which is targeted by Twist1, enhances the epithelial cell morphology and it serves as an apoptosis inductor, decreasing GIST cell motility[72]. In conclusion, miR-137 induced-EMT seems to indicate a lower risk for distant metastases and hence may be used as a potential therapeutic tool.

SUMMARY AND FUTURE PERSPECTIVES

Although the literature provides several new insights into EMT of tumor cells, supplementary molecular exploration is necessary for therapeutical reducing the rate of metastasis. The literature review shows that several factors are indeed involved in EMT, most of them having prognostic and/or predictive value. However, the features relating specifically to organ-related carcinomas need further elucidation, because most papers dealing with this subject contain no specific and valuable data.

If EMT is indeed involved in the resistance of tumor cells to specific drugs, such as anti-EGFR substances, tamoxifen, and classic chemotherapics, identification of transition pathways could be of immense help in evolving an appropriate targeted therapy for both carcinomas and malignant mesenchymal tumors, including GISTs.

Additional studies of complex molecular profiling are necessary to elucidate the particularities of EMT in terms of histological type and localization of the tumor and angiogenesis-EMT interaction. Complex studies should also take into account not only cases with E-cadherin loss, but also the particularities of those cases, which show focally decreased expression and aberrant immunohistochemical pattern. We believe that, in the near future, the particularities of EMT will be a very useful parameter for proper clinical follow-up, individualized therapy and a more refined molecular classification of malignant tumors.

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REVIEW

Management of distal humeral coronal shear fractures

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Abstract

Coronal shear fractures of the distal humerus are rare, complex fractures that can be technically challenging to manage. They usually result from a low-energy fall and direct compression of the distal humerus by the radial head in a hyper-extended or semi-flexed elbow or from spontaneous reduction of a posterolateral subluxation or dislocation. Due to the small number of soft tissue attachments at this site, almost all of these fractures are displaced. The incidence of distal humeral coronal shear fractures is higher among women because of the higher rate of osteoporosis in women and the difference in carrying angle between men and women. Distal humeral coronal shear fractures may occur in isolation, may be part of a complex elbow injury, or may be associated with injuries proximal or distal to the elbow. An associated lateral collateral ligament injury is seen in up to 40% and an associated radial head fracture is seen in up to 30% of these fractures. Given the complex nature of distal humeral coronal shear fractures, there is preference for operative management. Operative fixation leads to stable anatomic reduction, restores articular congruity, and allows initiation of early range-of-motion movements in the majority of cases. Several surgical exposure and fixation techniques are available to reconstruct the articular surface following distal humeral coronal shear fractures. The lateral extensile approach and fixation with countersunk headless compression screws placed in an anterior-to-posterior fashion are commonly used. We have found a two-incision approach (direct anterior and lateral) that results in less soft tissue dissection and better outcomes than the lateral extensile approach in our experience. Stiffness, pain, articular incongruity, arthritis, and ulnohumeral instability may result if reduction is non-anatomic or if fixation fails.

Key words: Coronal; Shear; Fractures; Distal; Humerus; Management; Approach; Two-incision

Core tip: Coronal shear fractures of the distal humerus are rare, complex fractures that can be technically challenging to manage. Distal humeral coronal shear fractures may occur in isolation, may be part of a complex elbow injury, or may be associated with injuries proximal or distal to the

elbow. This article aims to summarize the classification, evaluation, management (including surgical approaches, techniques, and post-operative care), and complications of these complex fractures as well as give recommendations on the management.

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INTRODUCTION

Coronal shear fractures of the distal humerus are rare, complex fractures that can be technically challenging to manage[1-3]. They usually result from a low-energy fall and direct compression of the distal humerus by the radial head in a hyper-extended or semi-flexed elbow or from spontaneous reduction of a posterolateral subluxation or dislocation[2,4,5]. Due to the small number of soft tissue attachments at this site, almost all of these fractures are displaced[2]. The incidence of distal humeral coronal shear fractures is higher among women because of the higher rate of osteoporosis in women and the difference in carrying angle between men and women[2,6-11]. Distal humeral coronal shear fractures may occur in isolation, may be part of a complex elbow injury, or may be associated with injuries proximal or distal to the elbow[2,6-10]. An associated lateral collateral ligament injury is seen in up to 40% and an associated radial head fracture is seen in up to 30% of these fractures[2,9,12]. Stable internal fixation restores articular congruity and allows initiation of early range-of-motion movements in the majority of cases[1-3]. Several surgical exposure and fixation techniques are available to reconstruct the articular surface following distal humeral coronal shear fractures. A lateral extensile approach and fixation utilizing countersunk headless compression screws placed in an anterior-to-posterior fashion are commonly used[1-3,7-10]. Stiffness, pain, articular incongruity, arthritis, and ulnohumeral instability may result if reduction is non-anatomic or if fixation fails[3]. We present several methods including a two incision method technique utilizing a direct lateral approach combined with a direct anterior approach. This paper aims to review the literature regarding distal humeral coronal shear fractures and to discuss approach and treatment techniques for these difficult fractures. The search algorithm and search criteria included any original and review articles on the topic of distal humeral coronal shear fractures.

CLASSIFICATION

Bryan and morrey classification

The most common classification of capitellar fractures is the expanded Bryan and Morrey's types Ⅰ-Ⅳ. Type Ⅰ (Hahn-Steinthal) consists of a large fragment of trabecular bone of the articular surface of capitellum and with little to no extension into the lateral trochlea. The type Ⅱ (Kocher-Lorenz) fracture is limited to the cartilaginous articular surface of the capitellum that may include a small fragment of subchondral bone. Type Ⅲ (Broberg-Morrey, Grantham) is a comminuted/compression fracture of the capitellum[13]. Type Ⅳ fracture, later described by McKee, is a shear fracture of the distal end of the humerus that extends in the coronal plane across the capitellum to include most of the lateral trochlear ridge and the lateral half of the trochlea[14].

Dubberley classification

More recent attempts at distal humeral fracture classifications have attempted to characterize capitellar fractures in a manner meant to direct surgical management and potentially predict outcome of injuries. Dubberley *et al*[9] classified capitellar fractures into three types. Type 1 involving primarily the capitellum with or without lateral trochlear ridge, described as a coronal shear fracture equivalent to Hahn-Steinthal. Type 2 is a fracture of capitellum and trochlea in a single piece where the fracture extends in the coronal plane across the capitellum to include most of the lateral trochlear ridge and the lateral half of the trochlea-essentially McKee's-described type Ⅳ Bryan and Morrey fracture. Lastly, type 3 involves fractures of both the capitellum and the trochlea as separate fragments. The fractures are further subdivided into type (A) or (B) depending on absence or presence of posterior condylar comminution, respectively[2,3,9].

Ring classification

Ring *et al*[8] also proposed a five-type system of capitellar fracture classification based on noted injury patterns given that isolated coronal shear fractures were described as rare[2,3,8]. The five types progressively include more distal humeral involvement beginning with Type 1, a coronal shear fracture comprised of single articular fragment that includes the capitellum and the lateral portion of the trochlea. Type 2 includes an associated fracture of the lateral epicondyle. Type 3 involves a further impaction of the metaphyseal bone behind the capitellum in the distal and posterior aspect of the lateral column. Type 4 adds a fracture of the posterior aspect of the trochlea. Finally, type 5 includes a fracture of the medial epicondyle[8].

Evaluation

Evaluation of a patient with a distal humeral coronal shear fracture involves clinical assessment and radiography[1]. A radiographic elbow trauma series consisting of AP, lateral, and radiocapitellar views as well as radiographic images of the wrist and forearm should be obtained requisitely[3]. The double-arc sign on lateral X-ray (Figure 1) of the elbow is pathognomonic of a fracture with substantial

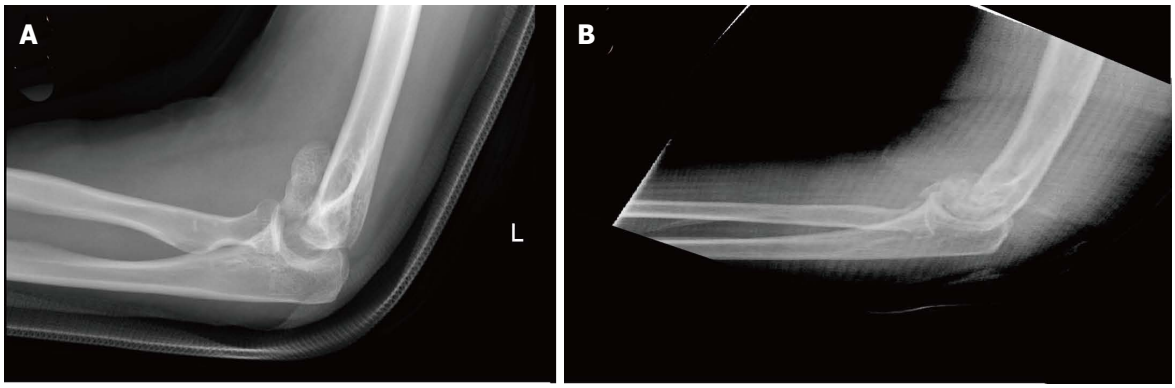

Figure 1 Lateral elbow X-ray of a two patients with distal humeral coronal shear fractures. A: Distal humeral coronal shear fracture-lateral view; B: The double-arc sign is seen, which signifies a McKee's type IV fracture in the Bryan and Morrey classification (involvement of both the capitellum and trochlea).

extension into the trochlea (McKee's type Ⅳ fracture in the Bryan and Morrey classification)[1,2,14]. However, X-rays only have a 66% sensitivity and a negative predictive value of only 63%-67% for detecting fractures beyond the capitellum[1,2,4]. For this reason, a preoperative CT scan of the elbow is recommended to better define the fracture and associated injuries and to guide operative planning[1,2,4,7,14,15]. A thorough neurovascular exam of the upper extremity, assessment of the forearm compartments, and a secondary musculoskeletal survey should always be performed in these patients[3]. Pain and guarding will limit assessment of elbow stability in the office setting or emergency department. As a result, thorough elbow assessment should be conducted at the time of surgical intervention under anesthesia[3]. In general, obtaining imaging of a joint above and below the level of injury will usually reveal bony injuries, but soft tissue injuries require a high index of suspicion and physical examination. This includes palpation of the entire extremity with particular focus on the distal radial ulnar joint and rotator cuff[3,16]. Follow-up examination and MRI scanning may be needed particularly of the shoulder[16].

Non-surgical treatment

Non-surgical management of coronal shear fractures of the distal humerus is reserved only for patients not medically fit for surgery. Otherwise, it is not recommended to treat these fractures non-surgically. Non-operative treatment would involve closed reduction with prolonged immobilization. Such measures often lead to suboptimal results and complications such as chronic pain, mechanical symptoms, and instability[1-3].

Surgical treatment

The goal of surgery is to restore articular congruity and obtain stable fixation, allowing for early range-of-motion to minimize the sequelae associated with non-op treatment: arthritis, pain, stiffness, and instability[2]. Surgical options include open reduction and internal fixation (ORIF), excision, total elbow arthroplasty, and arthroscopic reduction and fixation[2]. These types of surgical treatment and their indications are discussed individually below.

ORIF

ORIF results in good to excellent outcomes [measured by the Mayo Elbow Performance Score (MEPS)] in the majority of patients with coronal shear fractures of the distal humerus[1,2]. Based on many studies, the mean flexion-extension arcs after ORIF of a distal humeral coronal shear fracture range from 96° to 132°[1,3,6-9,17]. Superior results with ORIF are attributed to the fact that it allows anatomic reduction with stable fixation and thus initiation of early range-of-motion exercises[1].

ORIF involves opening the skin *via* a particular surgical approach (discussed in a separate section) to visualize and subsequently fix the fracture. There is currently no consensus on the optimal method of fixation for coronal shear fractures of the distal humerus. Countersunk headless compression screws (along with plate supplementation on the lateral column in cases of comminution extending beyond the articular surface) have been used with success[1,2,7,8,10]. Ruchelsman *et al*[3] recommend placing two screws in a divergent fashion for a Ring's type Ⅰ fracture to ensure rotational control, with sufficient screw spread to avoid iatrogenic fracture of the capitellum. Ring's Type Ⅱ and Ⅲ fractures (posteroinferior/lateral metaphyseal comminution and/or trochlear extension) often require supplemental fixation[3,7-9,18]. This can be accomplished using minifragment Synthes screws (West Chester, PA), threaded K-wires, and bioabsorbable pins for osteochondral capitellum and trochlea fractures < 5 mm, and pelvic reconstruction, precontoured or locking plates to buttress the lateral column in cases of extensive posterolateral comminution[3,8,9,18]. A biomechanical analysis by Elkowitz *et al*[19] has shown that placing headless compression screws in an anterior-to-posterior fashion is superior to placing cancellous screws in a posterior-to-anterior fashion. A follow-up study showed that Acutrak headless compression screws (Acumed, Hillsboro, OR) offered more stability than Herbert screws (Zimmer, Warsaw, IN) when placed

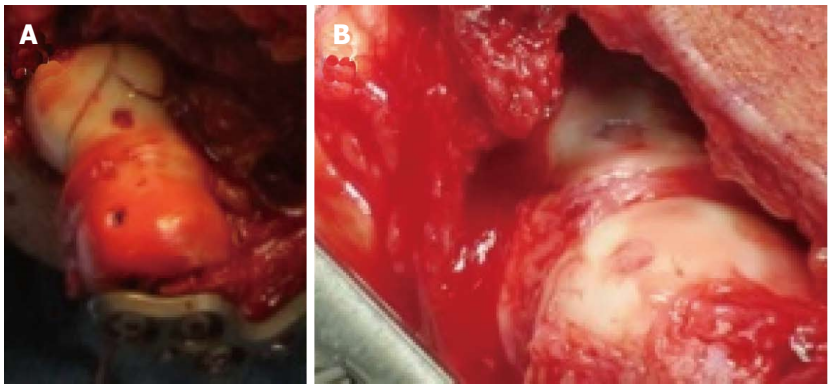

Figure 2 Headless compression screws. A: Holes left by headless screws in the articular surface. This patient later returned to surgery for a contracture release; B: At that time screw holes are shown to be covered with fibrocartilage.

in an anterior to posterior fashion[20]. Placing screws anterior-to-posterior is advantageous because it avoids disruption of the posterior soft tissues and allows better preservation of blood supply[1,21]. Sen *et al*[22] described placing screws in a lateral to anterior fashion to avoid posterior stripping, in addition to an anterior antiglide plate for the capitellum. When posterior comminution is present, supplemental fixation with bone graft might be necessary[2,7-9]. A recent study by Lee *et al*[23] found no significant difference in terms of clinical outcomes and complication rates between orthogonal and parallel plating methods for distal humeral fractures. However, the authors of the study state that orthogonal plating may be preferred in coronal shear fractures (where the posterior-to-anterior fixation can provide additional stability to the intra-articular fractures). Additional studies are needed to explore the association between plating methods and specific fracture patterns.

Our preferred method is to use headless compression screws placed in an anterior-to-posterior fashion with or without a lateral plate for the fixation of coronal shear fractures. There is a Grade C recommendation for this method[1]. Figure 2 shows the appearance of holes created by headless compression screws right after (Figure 2A) and several months after (Figure 2B) insertion.

Excision

Capitellar excision is another option in the treatment of distal humeral coronal shear fractures. This method is associated with complications such as substantial elbow instability, particularly when there is ligamentous injury or the trochlea is involved[1-3]. Grantham *et al*[24] and Mancini *et al*[25] reported poor clinical outcomes and valgus instability plus distal radioulnar joint subluxation, respectively, following capitellar excision. Excision remains an option for small, unfixable fractures, but ORIF should be utilized instead whenever possible[2].

Total elbow arthroplasty

Total elbow arthroplasty is a good option in select elderly patients with fractures deemed unrepairable (Figure 3)[2,26-29]. This is particularly relevant to patients with pre-existing arthritis of the elbow[1,30,31]. McKee *et al*[28] conducted a prospective, randomized, multicenter study in which they compared ORIF with total elbow arthroplasty in forty patients over the age of sixty-five years with displaced, comminuted, intra-articular fractures of the distal humerus. They reported better functional outcomes at two years post-operatively in the group with total elbow arthroplasty based on MEPS and DASH scores. Furthermore, five out of twenty patients (25%) in the ORIF group had to undergo intra-operative conversion to total elbow arthroplasty due to extensive comminution and an inability to achieve stable fixation.

When acute total elbow arthroplasty is anticipated or being considered, it is critical to avoid the olecranon osteotomy operative approach (discussed in a separate section). This approach compromises fixation of the olecranon component and is contraindicated for total elbow arthroplasty[1,28]. Reichel *et al*[29] reported a case in which an ORIF had to be immediately converted to a total elbow arthroplasty following an olecranon osteotomy.

Overall, there is fair evidence that acute total elbow arthroplasty is the preferred treatment for elderly patients (> 65 years of age) with a displaced, comminuted, intra-articular distal humeral coronal shear fracture that is not amenable to stable internal fixation. This gets a grade B recommendation[1].

Arthroscopic reduction and fixation

A case report by Hardy *et al*[32] described arthroscopic-assisted reduction and screw-fixation of a type Ⅰ Hahn-Steinthal capitellum fracture using one viewing portal and two instrumentation portals. According to many authors, arthroscopic reduction and fixation techniques for coronal shear fractures of the distal humerus should be reserved only for simple fractures without comminution (such as type Ⅰ in the Dubberley or Bryan and Morrey classification)[2,32-35].

Postoperative care

After surgical intervention, the patient is put in a long-arm posterior plaster splint or compressive dressing if rigid fixation has been achieved. The patient is usually

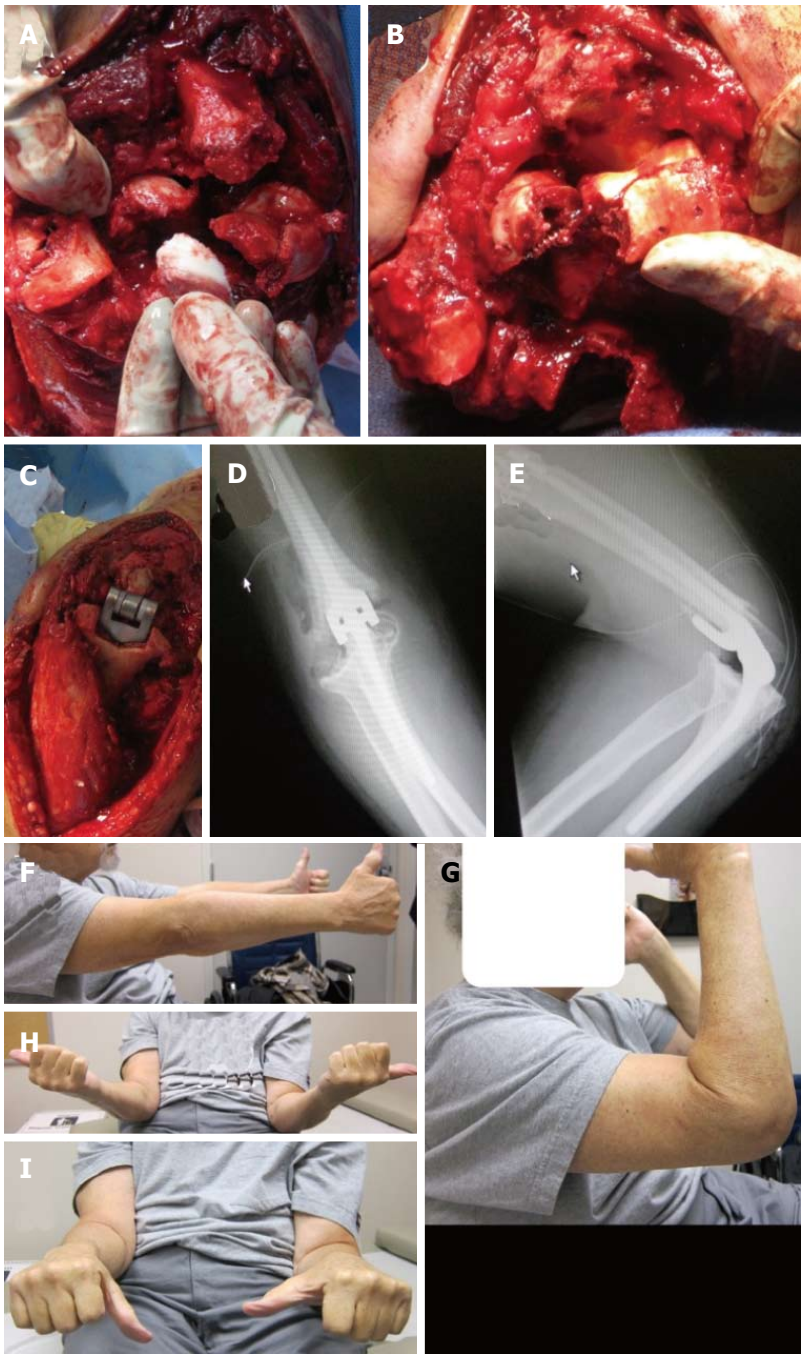

Figure 3 Total elbow arthroplasty. A-E: A non-reconstructible coronal shear fracture in an elderly patient. A total elbow arthroplasty was performed in this patient; F-H: Postoperative range of motion.

seen for his/her first post-operative office visit between seven and ten days post-operatively. The splint and/or compressive dressing are usually removed at this visit. Active and active-assisted range of motion of the elbow and forearm along with formal therapy is also initiated after the first office visit[3].

If the fixation achieved intra-operatively is suboptimal, the patient may be put in a functional brace[3]. If there is concomitant ligamentous or functionally equivalent osseous injuries, then mobilization in pronation is established for lateral-sided injuries and mobilization in supination is established for medial-sided injuries[3,36,37]. When clinical and radiographic evidence of fracture union is evident, strengthening exercises can be initiated[3].

When there is concern about the stability of fixation, delayed or protected mobilization with a hinged elbow brace or cast may be necessary. A hinged brace with gradual reduction of the extension block allows maintenance of radial head congruity with the reduced capitellum. When flexion contracture occurs in the early post-operative period, extension thermoplastic splinting is used[3]. Gelinas *et al*[38] showed that turn-buckle splinting is effective in regaining ulnohumeral motion. If ulnohumeral motion remains poor and there is flexion contracture present, a contracture release can be performed[3].

SURGICAL APPROACHES

Lateral extensile approach

The operative treatment described in majority of publications is performed through the extended lateral Kocher approach. Most authors advocate the extended lateral Kocher approach for fractures without significant posterior comminution or medial column damage[8,10,39].

In the extended lateral approach, the patient is positioned supine and the arm is controlled with a tourniquet. An incision is made from the lateral supracondylar ridge extending over the lateral epicondyle to 2-5 cm distal to the radial head. The common extensor origin is elevated anteriorly. The lateral ulnar collateral ligament (LUCL) should only be elevated if necessary to obtain sufficient exposure of the fracture[8,9,12,14,40-42]. In our practice, we have abandoned elevating the LUCL and place any plates we may use directly over the ligament. If there is an associated fracture of the lateral epicondyle, the fragment should be retracted with the disrupted LUCL[8,43]. In such cases, place a suture through the LUCL; this allows reattachment through holes drilled in the lateral epicondyle after fracture fixation[14]. We commonly suture the LUCL directly over the plate if the ligament is torn or has been elevated.

The incision is then extended between the biceps and triceps proximally and between the anconeus and extensor carpi ulnaris distally. The forearm is then pronated to protect the posterior interosseus nerve (PIN) and the common extensor origin is elevated to create a soft tissue flap. This flap constitutes the vascular supply of the posterior distal humerus and capitellum[12]. The anterior joint capsule can now be elevated and retracted to expose the capitellum and trochlea[14]. The lateral extensile approach is seen in Figure 4 (the 2nd row pictures of both columns). Anterior retractors over the radial neck increase risk of injury to the PIN and should be avoided[12].

Intraoperative difficulty achieving anatomic reduction often signifies posterior comminution of the lateral column not apparent on radiographs. In such cases, and with known posterior comminution, the distal lateral triceps should be reflected from the olecranon to allow the elbow to hinge open, exposing the posterior lateral column[8]. Additionally, some authors elected to use a supplemental posterior midline incision in cases when the medial trochlea could not be visualized well with the extended lateral approach alone[8,42].

Posterior approach and olecranon osteotomy

The posterior approach with an olecranon osteotomy can be used when an articular fracture extends to the medial epicondyle and when there is significant posterior comminution or medial column damage[8,10,17,39]. Additionally, a posterior approach without osteotomy is recommended if future procedures or arthroplasty is anticipated[10]. Dubberley *et al*[9] recommend a posterior approach for all coronal shear fractures with an olecranon osteotomy for some type 2 fractures, and most type 3 fractures.

In the olecranon osteotomy approach, the patient is positioned in the lateral decubitus position with the arm over a bolster and controlled with a tourniquet. A posterior midline incision is made and a single or multiple intermuscular planes are developed to access the capitellum. Lateral planes described in the literature include the Boyd, Kocher, and Kaplan exposures[9,44-46]. Patients with Dubberly type 1 fractures-coronal shear fractures of capitellum and a part of lateral trochlear ridge-can be managed without disruption of the LUCL, a second medial plane, or an olecranon osteotomy[9].

Dubberly type 2 fractures-single fragment fractures of the capitellum that extend into the trochlear groove-often require a second intermuscular exposure through a medial flexor pronator split in order to access the medial trochlea. If reduction remains difficult, it is necessary to disrupt the LUCL allowing the joint to hinge open on the medial collateral ligament. The LUCL should be repaired after fixation using drill holes and a locking suture technique. However, the LUCL should be preserved whenever possible to avoid the risk of instability following repair[9,43].

An alternative to LUCL disruption is an olecranon osteotomy. Dubberly type 3 fractures-comminuted shear fractures of the capitellum and trochlea-often require olecranon osteotomy. After elevation of fasciocutaneous flaps, the ulnar nerve is identified and protected. Then, a chevron osteotomy is created over the olecranon through the bare area. An oscillating saw is used to cut two thirds of thickness of bone and the remaining attachment is carefully separated using an osteotome[8]. We typically place sponge in the ulnohumeral joint and saw directly to the sponge to minimize the kerf of bone removed. This exposes the anterior articular fragments for fixation. The olecranon fragment can be repaired after fracture fixation with two Kirschner wires directed anteriorly to the distal coronoid process and one 18 gauge or two 22 gauge figure of eight tension band wires[8]. Alternatively, the olecranon fragment can be repaired with plate and screw fixation[9].

In studies published to date there are no significant differences in outcomes between the extended lateral approach and the posterior midline approach[3,12,14,17,39]. Given that an olecranon osteotomy creates increased risk of non-union and symptomatic hardware, the procedure is only used for large and or comminuted fractures[9,17].

Anterolateral approach

A few authors advocate for an anterolateral approach because it exposes the capitellum and the trochlea without disruption of the LUCL or an olecranon osteotomy[47,48]. One advocate of this approach recommends an extended lateral approach for isolated fractures of the capitellum and an anterolateral approach for fractures with involvement of the trochlea[47].

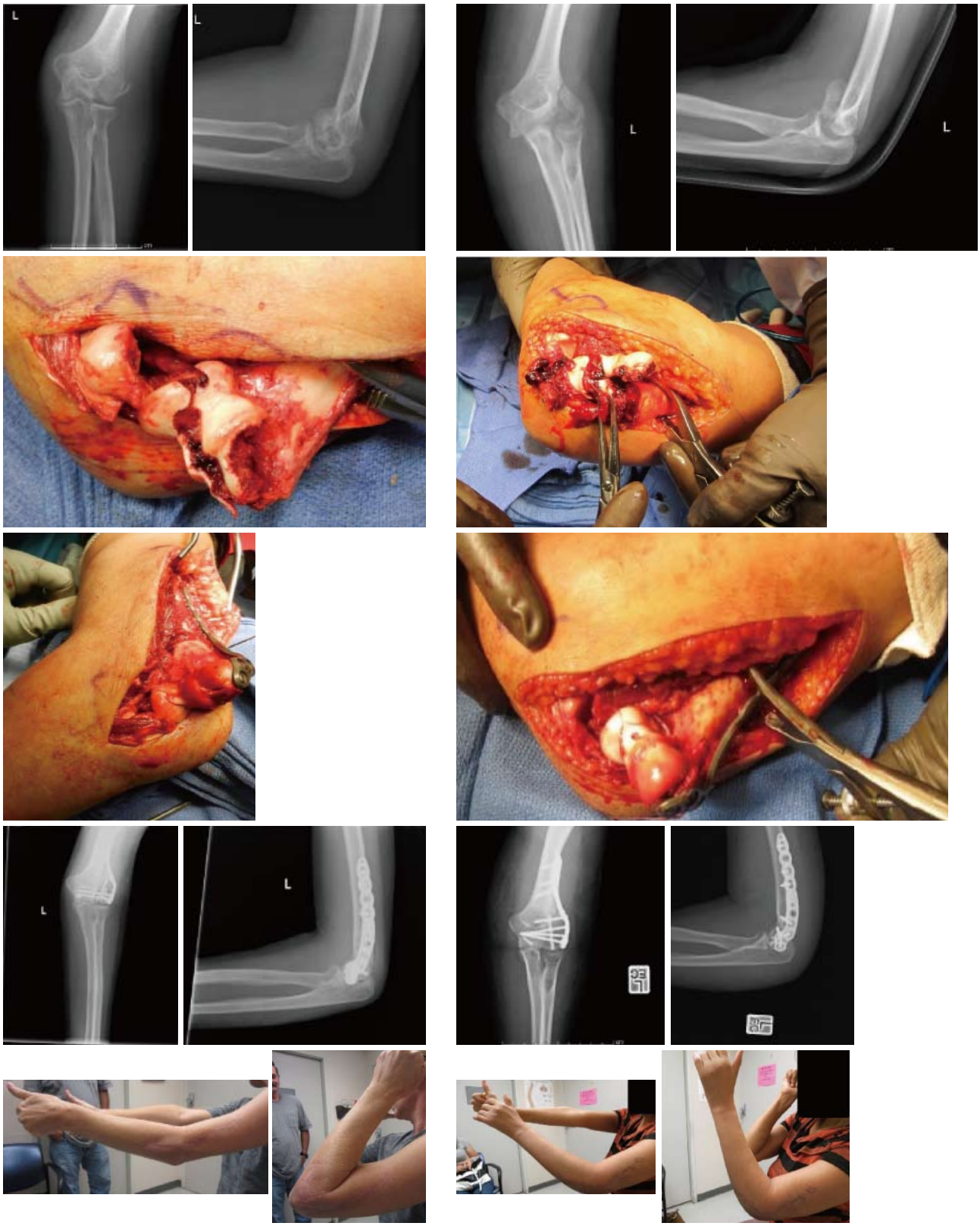

Figure 4 Comparison of outcomes following lateral extensile approach open reduction and internal fixation. Pictures in the left column represent a 52 years old female who fell from a 2 feet wall. Pictures in the right column represent a 45 years old female who fell from a standing height. As can be seen in the first two rows of pictures in each column, both these patients sustained similar Dubberley type 3b fractures. The third and fourth rows in each column shows that good reduction and fixation was achieved intra-operatively in both patients. The last row in each column show maximum range of motion in each patient. The patient in the left column achieved a reasonable outcome, while the patient in the right column had severe stiffness and range of motion deficit which required an operative contracture release. Both patients underwent open reduction and internal fixation of their fractures using the lateral extensile approach for which the lateral collateral ligament was divided. Secondary to poor results, we have abandoned this method and now universally use the technique shown in Figure 4 or utilize olecranon osteotomy.

In this procedure the patient is positioned supine and the arm is controlled with a tourniquet. An incision is made 7 cm proximal to the flexion crease of the elbow between the biceps and brachioradialis and extended along the lateral border of the biceps. At the elbow the incision curves laterally to avoid scarring perpendicular to the flexion crease. The incision continues along the medial border of the brachioradialis 7 cm distal to the flexion crease. The radial nerve is identified and retracted laterally with the brachioradialis. The biceps is reflected medially exposing the anterior joint capsule which is incised vertically. Flexion exposes the capitellum for fracture fixation[47].

Malki *et al*[48] propose a more limited anterolateral exposure with an additional posterolateral stab incision for isolated capitellar fractures. In this approach, an incision is made over the lateral epicondyle and the anterior lateral joint capsule is incised longitudinally. This exposed fracture is manually reduced. Next, a threaded guide wire is inserted radially and directed perpendicular to the fracture. An additional stab incision on the posterior lateral elbow allows for fixation radially and posteriorly. A second wire is advanced parallel to the first wire through this incision until it nearly elevates the articular cartilage. A cannulated drill bit and tap are advanced over the wires and a short cannulated screw is inserted over the wire for definitive fixation[48].

Arthroscopic

There have been four cases reported on arthroscopic repair of coronal shear fractures of the distal humerus: three Dubberly type 1A fractures and one type 3A fracture[32,34,35]. Advocates of arthroscopic fixation report less soft tissue injury and decreased risk of devascularization, infection, and elbow contracture compared to ORIF[32,35]. However, the type 3A fracture that underwent arthroscopic repair developed avascular necrosis at one-year follow up[34]. It has been demonstrated that arthroscopy is a technically difficult but feasible approach for fixation of single fragment coronal shear fractures of the capitellum without comminution or significant involvement of the trochlea[32,34,35]. Given the limited evidence, authors advocate for judicious use of arthroscopic fixation for coronal shear fractures of the distal humerus[34]. It is not possible to perform an adequate comparison of the arthroscopic approach to the extended lateral or the anterolateral approach because there are so few cases in the literature.

In the procedure, the patient is placed in lateral decubitus position, the elbow is flexed to 90 degrees and controlled with a tourniquet. Three incisions are made on the lateral elbow: one for a fluid control system to distend the joint, a second for instrumentation, and a third for the arthroscope. The joint is lavaged and fracture site cleaned with a shaver. The fracture fragment is reduced with a punch and held in place with a K wire prior to fixation with cannulated screws advanced through the articular cartilage[32]. Alternatively, the fracture can be reduced using a K wire and definitively fixed with screws advanced over guide pins perpendicular to the fracture[34,35].

Two incision technique (lateral and anterior approach)

In order to minimize the total soft tissue dissection we have developed a two-incision technique (Figure 5). Of note, we prefer to use a headlight during this procedure. A lateral incision is made over the supracondylar ridge extending distal over the suspected extensor digitorum communis (EDC) tendon. Full thickness fascio-cutaneous flaps are raised. The dissection proceeds in between EDC and extensor carpi radialis brevis (ECRB). The supinator is then elevated off the radial head/neck junction sharply retaining the annular ligament and anterior capsule below undisturbed. A sharp capsulotomy is performed along the lateral distal humerus, stopping at the annular ligament [anterior to the lateral collateral ligament (LCL) insertion], just enough to visualize the fracture. If the capsule is tight and we cannot see the fracture, we divide the annular ligament anterior to the radial portion of the LCL (which lies directly beneath the EDC tendinous origin) to improve our visualization of the fracture[49].

We take great care not to dissect or elevate the LCL. If the fracture is comminuted, there is frequently a shell of bone attached to the LCL. Even in such cases, we do not elevate the LCL but rather apply plate fixation directly over it. Large capitellar fracture fragments are then fixed with headless compression screws. Small, comminuted fragments (usually posterior) are excised if present. We then view the medial extent of the fracture through this lateral incision. At this time, we make an anterior approach as previously described for fixing coronoid fractures[50] but with a more limited dissection to the distal humerus[50,51].

An incision of approximately 3 cm is made beginning at the elbow crease. Dissection proceeds between biceps and the neurovascular bundle. A finger is used to palpate the trochear fracture fragments (typically larger fragments). The brachialis is split at the level of the trochlear fragments through the anterior approach. A longitudinal capsulectomy is then made and the fracture's trochlear fragments are reduced under direct visualization and fixed with headless compression screws. The reduction can be provisionally fixed with several 0.054 K-wires and confirmed through both the lateral and anterior approach prior to placing the headless compression screws. Lateral comminution is addressed by applying a buttress plate directly over the LCL if needed. Fractured fragments are stressed manually and visualized throughout range of motion for stability.

Grades of recommendation for the evaluation, treatment, and post-op care of coronal shear fractures of the distal humerus

The following recommendations for care are adapted from a 2011 review article by Nauth *et al*[1], who

conducted a thorough literature search on the topic of distal humeral fractures: (1) Grade C recommendation for the use of CT scanning in the assessment of coronal shear fractures; (2) Grade C recommendation for ORIF of all displaced coronal shear fractures in patients for whom surgery is suitable; (3) Grade C recommendation for the use of a lateral extensile approach for the fixation of the majority of coronal shear fractures; (4) Grade C recommendation for the use of headless compression screws placed in an anterior-to-posterior fashion for the fixation of coronal shear fractures; (5) Grade B recommendation for acute total elbow arthroplasty in patients > 65 years of age with displaced, comminuted, intra-articular distal humeral fractures not amenable to stable internal fixation; and (6) Grade B recommendation for initiation of early range-of-motion exercises (within 2 wk) following ORIF of distal humeral coronal shear fractures.

Grades of recommendation: (1) A = Good evidence from Level-Ⅰ studies with consistent findings; (2) B = Fair evidence from Level-Ⅱ or Ⅲ studies with consistent findings; (3) C = Poor-quality evidence from Level-Ⅳ or Ⅴ studies with consistent findings; and (4) Ⅰ = Insufficient or conflicting evidence[1,52].

Outcomes

Good to excellent outcomes have been reported for the majority of patients who have undergone ORIF following a distal humeral coronal shear fracture. Outcomes are particularly good when the fracture is isolated to the radiocapitellar compartment[2,3,6-10,12]. One can expect mean pronosupination arcs of 156° to 180°, flexion-extension arcs of 96° to 141°, and flexion contractures of 10° to 28° for these fractures after ORIF[2,6-8,12,14,17,47]. Figure 5 shows one of our patients who achieved excellent outcomes in terms of range-of-motion following ORIF of a distal humeral coronal shear fracture. Of note, we utilized the two-incision technique described above for this patient.

Fractures with significant medial extension or comminution don't do as well, with non-unions occurring in the worse fracture subtype[2,6,9-11,14,47,53]. Dubberley *et al*[9] conducted a cohort study (n = 28) which found significantly inferior functional elbow evaluation scores (based on the American Shoulder and Elbow Surgeons function score and Mayo Elbow Performance Index) with Dubberley type Ⅱ (medial trochlear extension) and Dubberley type Ⅲ (capitellum-trochlea comminution) fractures compared to type Ⅰ fractures[9]. In another study by Ruchelsman *et al*[3,7], patients with McKee type Ⅳ fractures had significantly reduced terminal flexion and net ulnohumeral arc and larger flexion contracture compared to patients with type Ⅰ fractures at two years post-operatively[3,7]. The differences in outcome between the different fracture types may be due to increased severity of injury and/or to the extended surgical approach needed to facilitate exposure in the worse fracture types[3].

Satisfactory clinical and functional outcomes have been reported by some authors following ORIF of McKee's type Ⅳ fractures[3,7,10,11,14,47]. Despite a mean postoperative flexion contracture of 14.5° to 17.5°, a functional arc of ulnohumeral motion is achieved in most of these patients[3,7,14,47].

In a subcohort analysis of 16 patients with distal humeral coronal shear fractures, 5 out of 16 had a concomitant radial head fractures (Mason type Ⅰ and type Ⅱ)[7]. At a mean of 27 mo postoperatively, 2/5 had excellent outcomes, 2/5 had good outcomes, and 1/5 had a fair outcome (based on Mayo Elbow Performance Index). However, compared to the 11 remaining patients with an isolated capitellum and trochlea fracture, patients with concomitant ipsilateral radial head fractures had greater loss of terminal flexion and extension and reduced ulnohumeral arc of motion[3,7]. However, since the sample size in this study was very small, statistical significance cannot be reached and larger cohort studies are needed to compare outcomes of fractures with concomitant radial head fracture with those of isolated capitellum and trochlea fractures following ORIF[3].

A study by *Guitton et al*[6] found good to excellent outcomes in 13 out of 14 (93%) patients at long-term follow-up (median of 17 years) following operative fixation of distal humeral coronal shear fractures. This study shows that outcomes following ORIF are durable[2].

Complications

Complications following ORIF of distal humeral coronal shear fractures in the early post-operative period include stiffness, pain, loss of fixation, instability, infection, neurologic complications (*i.e.*, ulnar neuritis), and hardware complications[3]. Arthritis, malunion, nonunion, avascular necrosis and heterotopic ossification represent complications that can arise later. Overall, complications other than stiffness are rare[2].

Dubberley *et al*[9] found 7 out of 17 patients status post ORIF for Dubberley type Ⅱ or Ⅲ fractures who had elbow contracture with less than functional ulnohumeral motion. Figure 4 compares two patients with similar fractures, one of whom had a good outcome while the other developed severe stiffness and range of motion deficits status post ORIF. It is more important to maintain articular congruity than it is to prevent flexion contracture. The latter, a complication sometimes seen following ORIF of coronal shear fractures of the distal humerus, can be later addressed with contracture release[2]. In a series by Ring *et al*[8], 8 out of 21 patients required a contraction release. There was a mean increase of 42° in ulnohumeral motion following the release. In this same series, two patients who had undergone ORIF through an extended lateral approach developed ulnar neuropathy which required decompression

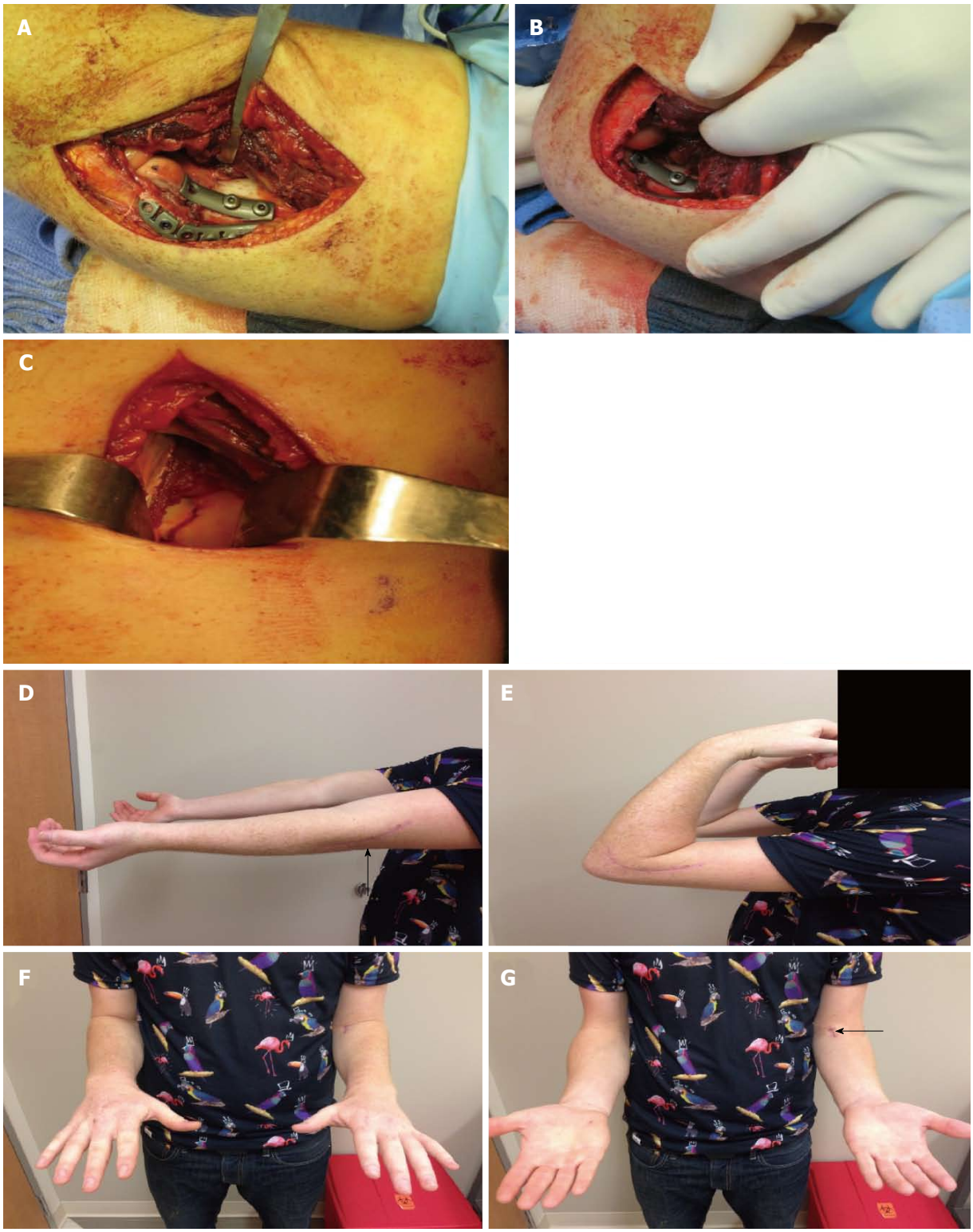

Figure 5 Two-incision approach. A case demonstrating the two incision approach (lateral and medial). A: The lateral collateral ligament is not reflected (the plate is placed directly on top of it). Stable fixation of the capitellar fragment could not be obtained with headless compression screws and a one-third tubular plate was added for stability; B: Flexion demonstrates a mild amount of impingement, which was not symptomatic postoperatively; C: Anterior view demonstrates trochlear fracture line. Even though two incisions are used there is less soft tissue dissection overall than required in other approaches; D-G: Approximate 6 wk follow-up range of motion in this patient.

and transposition. According to Ruchelsman *et al*[3], olecranon osteotomies have been associated with rare hardware complications (such as impingement of hardware in the radiocapitellar joint) which may necessitate screw removal.

Mild to moderate degenerative changes have been

reported in patients having undergone ORIF for distal humeral fractures[3,7,9]. Of 14 fractures treated with ORIF, only seven had Broberg and Morrey radiographic grade Ⅰ or Ⅱ arthritis and two had grade Ⅲ arthritis at a median 17-year follow-up[2,6,8,9].

In a series by Brouwer *et al*[53], 8 out of 18 (44%) patients with Dubberley type ⅢB fractures developed radiographic nonunion while none (0 out of 12) with Dubberley type ⅡA or ⅡB fractures developed nonunion. Of the 8 that developed nonunion, two had infections and were thus considered as failures. The remaining six had good to excellent results in half and fair results in the other half. There was no difference in range of motion compared to the patients who achieved union[2,53]. Dubberley *et al*[9] reported two patients status post ORIF for a type Ⅲ fracture that developed nonunion and had to be converted to total elbow arthroplasty. Total elbow arthroplasty is a salvage option for nonunion/malunion as well as for severe symptomatic post-traumatic arthrosis, articular osteonecrosis, and elbow instability[3].

In a recent study by Lee *et al*[23], only five out of sixty-seven patients with distal humeral fractures developed some degree of heterotopic ossification following ORIF. However, of these patients, only one developed a functional deficit (this patient had suffered from a high trauma injury and had a delayed operation). Overall, clinically significant heterotopic ossification is uncommon and there is insufficient evidence to recommend a prophylactic regimen against this complication[1-3].

CONCLUSION

The aim of this paper was to review the literature on the topic of distal humeral coronal shear fractures and to present several approach and treatment techniques.

Coronal shear fractures of the distal humerus represent significant articular injuries and are usually more complex than suggested by radiographic imaging. CT scans are therefore highly recommended for preoperative assessment of these fractures and treatment planning. The fracture pattern and extent of articular involvement dictate method of surgical exposure and internal fixation technique used for treatment. Open reduction internal fixation through lateral extensile exposures or posterior exposures using variable-pitch, headless compression screws is the treatment of choice for simple fracture types, leading to good to excellent outcomes in the majority of cases. Additional extensile exposures, LUCL disruption, olecranon osteotomy, bone grafting, and supplemental fixation using minifragment screws, column plating, and/or bioabsorbable implants may be required for more complex fracture types. We have found a two-incision approach (lateral and direct anterior) results in less soft tissue dissection and damage than the extensile approaches. Coronal shear fractures with substantial medial extension and posterior comminution generally have worse outcomes. The most common complication following ORIF of these fractures is stiffness (flexion contracture). It should be noted that the literature on distal humeral coronal shear fractures is comprised mainly of level-Ⅲ and Ⅳ studies. Consequently, more prospective, multicenter, large-scale trials are needed to assist surgical decision-making in the future. Furthermore, longer-term data are needed to fully evaluate the incidence and severity of rare complications such as arthritis, osteonecrosis, and heterotopic ossification following these fractures.

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REVIEW

Cardiovascular risk factors for acute stroke: Risk profiles in the different subtypes of ischemic stroke

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Abstract

Timely diagnosis and control of cardiovascular risk factors is a priority objective for adequate primary and secondary prevention of acute stroke. Hypertension, atrial fibrillation and diabetes mellitus are the most common risk factors for acute cerebrovascular events, although novel risk factors, such as sleep-disordered breathing, inflammatory markers or carotid intima-media thickness have been identified. However, the cardiovascular risk factors profile differs according to the different subtypes of ischemic stroke. Atrial fibrillation and ischemic heart disease are more frequent in patients with cardioembolic infarction, hypertension and diabetes in patients with lacunar stroke, and vascular peripheral disease, hypertension, diabetes, previous transient ischemic attack and chronic obstructive pulmonary disease in patients with atherothrombotic infarction. This review aims to present updated data on risk factors for acute ischemic stroke as well as to describe the usefulness of new and emerging vascular risk factors in stroke patients.

Key words: Cardiovascular risk factors; Hypertension; Atrial fibrillation; Diabetes mellitus; Ischemic stroke; Transient ischemic attack; Sleep apnea

Core tip: Prevention of acute stroke by controlling cardiovascular risk factors is a health care priority worldwide for a number of reasons, particularly due to the increasing occurrence of acute cardiovascular events in progressively older segments of the population, the high morbidity and mortality of some stroke subtypes and the economic burden associated to care of acute stroke patients. The frequency of the different cardiovascular risk factors is not equal for all subjects diagnosed of first-ever stroke. For this reason, it is necessary to know the most common profiles of vascular risk factors associated with each individual type of stroke in order to improve primary and secondary stroke prevention strategies. The role of new risk factors, such as sleep-disordered breathing or complex atheromatosis of the aortic arch merits further investigation.

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INTRODUCTION

Cerebrovascular diseases are the first cause of mortality in women and the second cause of death in men in industrialized countries[1-3]. Stroke is the main reason of functional disability. Neurological sequelae are present in 90% of stroke patients, one third of which will not be able to resume daily life activities at the same level than before stroke[1,2]. Cerebrovascular diseases are also an important cause of cognitive impairment and dementia[3]. The high frequency of stroke is illustrated by the cumulative incidence per 100000 persons-year that in Catalonia in 2002 and in the population over 24 years of age was of 218 new cases among males and 127 among females[4]. Therefore, to recognize cardiovascular risk factors and to treat them appropriately is the key to establish primary preventive strategies in non-stroke patients or secondary preventive measures to avoid recurrence in stroke victims.

The etiology of stroke is multifactorial, and therapeutic actions focused on vascular risk factors, particularly in secondary stroke prevention have been shown to reduce the risk of recurrent stroke, as well as the risk of any other coronary or peripheral vascular episode[4,5].

Risk factors for stroke are usually divided into non-modifiable (age, sex, ethnicity, low weight at birth, inherited diseases) and modifiable (hypertension, diabetes mellitus, heart diseases, smoking, dyslipidemia, alcohol abuse, obesity, metabolic syndrome, use of oral contraceptive drugs, hormone treatment in postmenopausal women, clinically silent carotid stenosis, peripheral artery disease, drug abuse, migraine, and other)[3-5].

NON-MODIFIABLE RISK FACTORS

Age, gender, ethnicity/race, low birth weight, family history of stroke and genetics/heredity[6]. In relation to age, in 2006, it was found that 93% of subjects who had suffered a stroke in Spain were older than 64 years of age[7]. Age is a continuous risk factor for the occurrence of stroke and dementia, with a two-fold increase in the incidence and prevalence rates for each successive 5 years after age 65 years. On the other hand, men show a higher incidence of cerebral vascular disease than women. With regard to ethnicity/race[8,9], it has been demonstrated that black patients have a higher incidence of stroke *vs* white patients. Intracranial atherosclerotic disease is more frequent in patients of Asian. Birth weight is inversely associated with coronary heart disease and stroke[9]. The underlying mechanisms of this association are poorly understood but might be related with genetic or nutritional factors[1].

Family history of stroke in a first-degree relative also increases the likelihood of suffering from an acute cerebrovascular event even after adjusting for other vascular risk factors. This increased risk may be due to different mechanisms, including inherited predisposition for stroke risk factors, genetic transmission of susceptibility to stroke, familial-related lifestyle, cultural and environmental factors, and interactions between genes and environmental factors[10].

Different genetic disorders have been associated with stroke. Rare monogenic disorders can cause stroke[11], such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL), cerebral amyloid angiopathy, moyamoya syndrome, Fabry disease, Ehlers-Danlos syndrome type Ⅳ, Marfan syndrome, Sneddon syndrome, mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS) and coagulopathies. However, as with other complex traits, the genetic etiology of common stroke is likely to be polygenic and more related to genetic influences on well-documented risk factors, such as hypertension, dyslipidemia, cardiopathy or diabetes[12,13]. Cerebrovascular events have also been related to polymorphisms of genes that regulates clotting factors, angiotensin-converting enzyme, nitric oxygen synthetase and phosphodiesterase 4D among other[10].

A meta-analysis of genome-wide association studies (the METASTROKE collaboration) identified genetic variants specific to the different stroke subtypes[14]. This study highlights the importance of detailed stroke subtyping in order to maximize success of genetic studies in ischemic stroke and to establish whether different genetic pathophysiological mechanisms seem to be associated with different stroke subtypes.

MODIFIABLE RISK FACTORS

Modifiable risk factors for ischemic stroke are well known. Appropriate treatment of these risk factors has been associated with a reduction of stroke. This allows the implementation of measures for primary or secondary stroke prevention.

Hypertension

Hypertension together with age are leading risk factors for silent or symptomatic cerebrovascular disease[15-17]. High blood pressure multiplies the risk for stroke as much as 4-fold. Both factors are also related to the probability of suffering some degree of cognitive impairment[4]. The risk of cerebral hemorrhage in hypertensive patients is 3.9 times higher than in non-hypertensive individuals. In aneurysmal subarachnoid hemorrhage the relative risk is 2.8 higher[18-22]. The diagnosis and control of hypertension one of the main strategies for primary and secondary prevention of stroke[22-24]. The effect of chronic hypertension on cerebral vessels and tissue (microhemorhages, silent infarctions, white matter lesions and atrophy) also supports a physiopathological mechanism for the association between hypertension and cognitive

Table 1 Distribution by age groups of demographic and cardiovascular risk factors in 2704 consecutive patients with cerebral infarction collected from the "Sagrat Cor Hospital of Barcelona Stroke Registry"[7] *n* (%)

Data	< 65 yr (*n* = 386)	65-74 yr (*n* = 680)	⩾ 75-84 yr (*n* = 1068)	⩾ 85 yr (*n* = 570)
Gender				
Males	270 (69.9)	409 (60.1)	471 (44.1)	186 (32.6)
Females	116 (30.1)	271 (39.9)	597 (55.9)	384 (67.4)
Vascular risk factors				
Hypertension	186 (48.2)	415 (61)	624 (58.4)	276 (48.4)
Atrial fibrillation	38 (9.8)	157 (23.1)	366 (34.3)	246 (43.2)
Diabetes mellitus	90 (23.3)	187 (27.5)	252 (23.6)	103 (18.1)
Dyslipidemia	86 (22.3)	179 (26.3)	161 (15.1)	54 (9.5)
Previous cerebral infarction	42 (10.9)	130 (19.1)	194 (18.2)	102 (17.9)
Ischemic heart disease	37 (9.6)	128 (18.8)	185 (17.3)	85 (14.9)
Smoking (> 20 cigarettes/d)	34 (8.8)	85 (12.5)	132 (12.4)	66 (11.6)
COPD	112 (29)	88 (12.9)	50 (4.7)	10 (1.8)
Peripheral vascular disease	14 (3.6)	54 (7.9)	103 (9.6)	52 (9.1)
Heart valve disease	22 (5.7)	79 (11.6)	86 (8.1)	27 (4.7)
Congestive heart failure	26 (6.7)	46 (6.8)	69 (6.5)	33 (5.8)
Obesity (BMI ⩾ 30 kg/m^2)	7 (1.8)	16 (2.4)	54 (5.1)	71 (12.5)
Oral anticoagulants	20 (5.2)	42 (6.2)	44 (4.1)	12 (2.1)
Alcohol abuse (⩾ 80 g/d)	10 (2.6)	25 (3.7)	41 (3.8)	18 (3.2)
Previous cerebral hemorrhage	38 (9.8)	20 (2.9)	7 (0.7)	1 (0.2)

Data expressed as frequencies and percentages in parenthesis. COPD: Chronic obstructive pulmonary disease; BMI: Body mass index.

impairment[25-29]. As shown in Table 1, according to data of 2704 patients with first-ever ischemic stroke collected from the Sagrat Cor of Barcelona Stroke Registry, hypertension was the main risk factor in the different age groups[7].

Diabetes mellitus

Dyslipidemia, hypertension and obesity are atherogenic risk factors frequently found in type 2 diabetes patients[4,5]. Also, diabetes is an independent risk factor of ischemic stroke of atherothrombotic cause. The influence of diabetes upon increasing the stroke risk is higher in women than in men[30]. Diabetes is the main risk factor following hypertension of cerebral small vessel disease and has been identified as a significant independent variable of symptomatic recurrence in patients with first-ever cerebral infarction of the lacunar type[31,32]. The combination of hypercholesterolemia and hypertension increases the frequency of vascular complications in patients with diabetes.

Heart diseases

Heart diseases are the second cause of acute cerebrovascular events and are diagnosed in one third of patients with stroke[33,34]. Atrial fibrillation (AF) and atrial flutter are the most important and modifiable risk factor, frequently associated with cardioembolic stroke. Cardioembolic infarction is the most severe stroke subtype due to the very low percentage of symptom-free patients at hospital discharge, the non-negligible risk of early recurrent embolic events and the high mortality in the acute stroke phase (27% in the Sagrat Cor of Barcelona Stroke Registry)[33-36]. The prevalence of AF increases with age. It has been shown that 5% of subjects older than 70 years have AF (the mean age of patients with AF is 75 years), and about one fourth of acute strokes in very old patients (> 80 years) are also caused by AF[37,38]. Future embolism is also more frequent in patients with underlying comorbid heart diseases, such as AF and stenosis of the mitral valve. The risk of stroke is 3 to 4 times higher in the absence of organic heart disease or risk factors (lone atrial fibrillation). On the other hand, AF associated to hypertensive heart disease is the most common cardiogenic source of cerebral embolization in industrialized countries[33]. Similar rates of cardioembolism for paroxysmal and chronic AF have been reported, so that preventive therapy should not be different for patients with paroxysmal AF and those with chronic AF[33]. In patients without history of transient ischemic attack (TIA) or stroke, AF carries a risk of stroke of 2%-4% per year. Cardiac emboli arising from cardiac chambers are often large and hence especially likely to cause severe stroke, disability and death[33].

A number of cardiac conditions are potential sources of embolism, such as dilated myocardiopathy, heart valve disease (mechanical prosthetic valve, mitral rheumatic stenosis, infectious endocarditis, marantic endocarditis), left ventricular hypertrophy, atrial myxoma and congenital heart diseases (such as patent foramen ovale, atrial septal aneurysm and ventricular septal defects). Acute coronary syndromes are minor causes of cardioembolism. There is an inverse correlation between ejection fraction of the left ventricle and the incidence of ischemic stroke[33-39].

Cigarette smoking

Cigarette smoking is an independent predictor of cerebrovascular disease in both men and women[40]. Smokers have a relative risk of ischemic stroke of 1.92

times higher as compared to non-smokers. Smoking increases the risk of thrombus formation in narrow arterial vessels and contribute to enhance atherosclerotic plaque burden. Also, smoking increases blood viscosity, fibrinogen and platelet aggregation, and decreases high-density lipoprotein (HDL) cholesterol, which causes direct damage to endothelium and an increase in blood pressure[40-42]. A meta-analysis of 19 prospective studies has shown an association of smoking with dementia and cognitive decline[43]. Finally, there is growing acceptance that passive cigarette smoke increases the risk of stroke[44].

Dyslipidemia

Plasma lipids and lipoproteins [total colesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, HDL cholesterol and lipoprotein (a)] have an influence on the risk of cerebral infarction, but the relationships between dyslipidemia and stroke have not been consistently elucidated[9]. Data from prospective studies in male patients have shown that in the presence of total serum cholesterol values > 240 to 270 mg/dL, there is an increase in the rates of ischemic stroke[45].

In general, the risk of ischemic stroke in both genders is clearly related with dyslipidemia. In men, low HDL levels is a risk factor for cerebral ischemia but data in women are inconclusive. Because high levels of LDL are clearly related with a higher cardiovascular risk, adequate control of LDL cholesterol is recommended (*e.g.,* National Cholesterol Education Program Ⅲ guidelines) in subjects without history of cerebrovascular accident[46].

High triglyceride levels are a component of the metabolic syndrome. In a study of 11117 patients with coronary heart disease, cerebral infarctions were significantly associated with high serum levels of triglycerides and low levels of HDL cholesterol[9].

Alcohol abuse

Chronic heavy alcohol consumption (> 60 g/d) is associated with an increase in the relative risk of stroke [risk ratio (RR) of 1.69 in cerebral ischemia and RR = 2.18 in cerebral haemorrhage][44]. Ethanol is a direct neurotoxin and chronic ethanol abuse causes different neurodegenerative processes, including dementia[47,48]. However, light-to-moderate alcohol consumption (20-30 g/d, equivalent to 1 or 2 drinks per day) is associated with a lower risk of stroke, white matter disease and clinically silent cerebral infarcts[3,44].

Overweight and obesity: Adiposity

Obesity is defined as an increase above 25% of the theoretical body weight according to age and sex. The term "adiposity" refers to the amount of adipose (fat) tissue in the body, and can be considered more precise than "obesity" which is mainly one of the ways to measure adiposity. Adiposity is an energy imbalance between energy intake (calories) and energy expenditure (physical activity and metabolic processes); however, the ideal or normal threshold for adiposity has not been established. Increase in fat tissue is associated with a higher risk of insulin resistance, diabetes, hypertension, dyslipidemia, vascular diseases and other conditions. Persons with a body mass index (BMI) of < 18.5 kg/m^2 are classified as being underweight, between 18.5 and 25.9 kg/m^2 as healthy weight range, between 26 and 29.9 kg/m^2 as overweight and ⩾ 30 kg/m^2 as being obese[49]. Abdominal obesity is commonly measured by either the waist-to-hip ratio or waist circumference and appears to be a more sensitive measure of adiposity and vascular risk. Clinically, abdominal obesity is defined by a waist circumference > 102 cm in men and 88 in women[50]. Weight and abdominal fat reduction is associated with a lowering in blood pressure, and may thereby reduce the risk of stroke. Moreover, there is evidence linking the continuum of adiposity, hyperinsulinemia, and diabetes with dementia[51].

Metabolic syndrome

Metabolic syndrome is defined as te presence of three or more of the following: (1) abdominal obesity as determined by waist circumference > 102 cm and > 88 cm for women; (2) triglycerides ⩾ 150 mg/dL; (3) HDL cholesterol < 40 mg/dL for men and < 50 mg/dL for women; (4) systolic blood pressure ⩾ 130 mm Hg and diastolic blood pressure ⩾ 85 mmHg; and (5) fasting glucose ⩾ 110 mg/dL[52,53]. Hyperinsulinemia/insulin resistance is an important marker of the metabolic syndrome. The metabolic syndrome is a predictor of coronary heart disease, cardiovascular disease (which includes coronary heart disease and stroke) and all-cause mortality. Also, the risk of stroke is higher for patients presenting some of the diseases included in the metabolic syndrome[9].

Asymptomatic carotid stenosis

Approximately between 5% and 10% of men and women over 65 years had > 50% and 1% > 80% asymptomatic carotid stenosis[3], which has been also identified as a risk for stroke[9] and an important clinical feature of underlying ischemic heart disease.

Peripheral vascular disease

Epidemiological studies have shown that patients with intermittent claudication have a high risk of premature death due to ischemic heart disease and stroke. Also, individuals with peripheral vascular disease are at a higher stroke risk[9,54]. History of intermittent claudication, peripheral vascular disease or coronary heart disease in a patient with cerebral infarction indicates the presence of clinically generalized atherosclerosis, according to which the diagnosis of cerebral ischemia of atherothrombotic cause is highly probable.

Postmenopausal hormone therapy

Postmenopausal hormone therapy (estrogen with or without a progestin) should not be used for primary

prevention of ischemic stroke because, paradoxically, the risk of stroke is increased.

The increased risk for vascular outcomes should be taken into account when hormone replacement therapy is used for other indications[9,45].

Oral contraceptive use

The risk of stroke associated with low-dose oral contraceptives (containing low doses of estrogens) in women without additional risk factors (*e.g.,* cigarette smoking or history of thromboembolic episodes) appears low[45]. Women taking oral contraceptives older than 35 years, active smokers, with hypertension, diabetes, migraine headache or history of thromboembolism are at higher risk of stroke[9,45].

Drug abuse

Drug abuse (mainly heroin, cocaine, amphetamins) has been also identified as a risk factor for stroke through different mechanisms, including blood pressure, hematologic, hemostatic and vasculitic-type changes, as well as increased platelet aggregation and blood viscosity[3,9,45].

Migraine

Migraine has been marginally associated with stroke risk in young women but in persons over 60 years an association between stroke and migraine has not been documented[55]. The risk of stroke has been related to underlying pathophysiological mechanisms of migraine (with aura), such as reduced blood flow particularly in the posterior circulation[9]. In young adults, stroke and migraine may be linked by paradoxical embolism through a patent foramen ovale. Patients with migraine also show an increase in platelet–leukocyte aggregation and platelet activation, increasing the risk of emboli formation and suggesting a link at cellular level between stroke and migraine[55,56].

LESS WELL-DOCUMENTED VASCULAR RISK FACTORS

The causal role of these factors in stroke patients remains inconclusive and futher studies are needed to clarify the contribution of each of these factors to the overall stroke risk.

Increase of the apoB/apoA1 ratio

Plasma levels of apolipoprotein B (apoB) is an indicator of very-low density (VLDL) and LDL lipoproteins with proatherogenic properties because each VLDL and LDL particle contains a molecule of apoB. It seems that plasma concentration of apoB is the highest lipid predictor of ischemic heart disease. In patients with TIA, an increase in the apoB/apoA1 ratio would product stroke more strongly (HR = 2.9) than other lipid values, such as apoB (HR = 2.3), total cholesterol (HR = 1.8), LDL cholesterol (HR = 1.5), LDL/HDL ratio (HR = 1.3) and apoA1 (HR = 1.2)[9,45].

Sedentarism

A sedentary lifestyle is associated with an increase in the risk of stroke. Regular physical activity has well-established benefits for reducing the risk of cardiovascular disease, premature death and stroke[9]. The excellent review of Hankey[45] of potential new risk factors for ischemic stroke includes data of a meta-analysis of 23 studies and shows that subjects with high physical activity as compared to those with low physical activity had a lower stroke risk (RR = 0.79). Also, in another study, a reduction in the risk of stroke was reported in relation to moderate physical activity at leisure time (RR = 0.87) and active daily activity $\geqslant$ 30 min daily (cycling/walking to work). The protective effect of physical activity may be partly mediated through its role in reducing blood pressure and controlling other risk factors for cardiovascular disease, diabetes and increased body weight. It has been also related to a reduction of plasma fibrinogen and platelet activity, with increases of both tissue plasminogen activator and HDL cholesterol[3,45].

Insufficient daily fruit and vegetable intake

A meta-analysis of 17 cohort studies showed that risk of stroke decreased by 11% for each additional portion per day of fruit, by 5% for fruit and vegetables, and by 3% for vegetables[3,45]. A higher sodium intake increases the risk of stroke, whereas a higher consumption of potassium was associated with a reduction in the risk of stroke, probably due to the blood pressure lowering effect and mitigation of sodium effects on pressor responsiveness[9,45]. In Asian populations, diets characterized by consumption of cholesterol and saturated fats, as well as low consumption of animal-related proteins have been associated with an increased stroke[9,45].

Psychosocial stress

The risk of stroke is also increased in the presence of stressful live events, including depression[44].

Sleep-related breathing disorders

The presence of sleep apnea is also a risk factor for stroke. Sleep apnea may increase the risk of stroke by leading to or worsening hypertension or ischemic heart disease. Sleep apnea causes reductions in cerebral blood flow, altered cerebral autoregulation, impaired endothelial function, accelerated atherogenesis, hypercoagulability, inflammation and paradoxical embolism in patients with patent foramen ovale. In patients with advanced sleep-disordered breathing, cardiac arrhythmias, atrioventricular block and atrial fibrillation appear when the oxyhemoglobin saturation falls to < 65%. Sleep apnea as a risk factor for

cerebrovascular events is usually underdiagnosed[57-59].

Inflammatory markers

There is increasing evidence of the relevance of inflammation as a physiopathological mechanism of atherothrombotic stroke.

Leukocyte and monocyte counts: In the CAPRIE trial (Clopidogrel *vs* Aspirin in Patients at Risk of Ischemic Events) patients with a history of peripheral arterial disease, stroke, or myocardial infarction or who had a leukocyte count in the highest quartile at baseline showed a higher adjusted risk of recurrent ischemic events compared with those in the lowest quartile (RR = 1.42)[45]. Monocyte count has been shown to be an independent predictor of future atherosclerotic plaque formation in the carotid artery in subjects without preexisting carotid atherosclerosis[3,60].

C-reactive protein: In apparently healthy individuals, high-sensitive C-reactive protein, acute-phase reactant and biological marker of inflammation, has been shown to be an independent risk factor for death of vascular cause, stroke and myocardial infarction[45].

Other biomarkers: An increase in the levels of other inflammatory biomarkers, among which, tumor necrosis factor (TNF) and interleukin 2 (IL-2) are also related to subclinical carotid disease[45]. Inflammatory cells in the atherosclerotic plaque are involved in the release of substances (matrix metallopeptidases) causing rupture of the plaque by instability of the fibrotic layer[45]. An increase of inflammatory mediators have been shown in atherosclerotic plaques in symptomatic as compared to asymptomatic patients[3,45].

Infection

It has been shown that recent infection (within 1 wk) can be a risk factor for stroke and coronary-related complications[3]. This may be partly related to generalized activation of circulating leukocytes, enhancing the tendency for thrombosis at the site of atherosclerotic plaque. Moreover, *Chlamydia pneumonia* DNA or *C. pneumonia* antigen, have been identified in 40% of atherosclerotic plaques[3,45].

Periodontal disease related to seeding of the bloodstream with Gram-negative organisms has been associated with carotid atherosclerosis and the risk of stroke[45]. However, antibiotic treatment has been unsuccessful in the prevention of severe cardiovascular events, at least in patients with established coronary heart disease.

Cytomegalovirus, herpes virus and *Mycoplasma pneumoniae* infection increase the risk of stroke by various mechanisms, particularly by increasing inflammatory mediators, facilitating the coagulation cascade and enhancing expression of adhesion molecules on vascular endothelial cells[3,45].

Helicobacter pylori have also been identified in human atherosclerotic plaque.

These data support the concept of "infectious burden", according to which prolonged exposure to multiple microorganisms during the life period may contribute to the development and activation of the atherosclerotic plaque[9,45].

Fibrinogen

In a meta-analysis of thres prospective studies, patient with fibrinogen concentrations above the median value showed an increased risk of stroke (HR = 1.34) in comparison with patients with serum fibrinogen values below the median, particularly in patients with non-lacunar syndromes (HR = 1.42)[45].

Other biological factors

Hemocysteine: It has been consistently shown a positive and independent association between total plasma hemocysteine and atherosclerotic disease, risk of silent and symptomatic stroke and cognitive decline[45,61]. Also, *MTHFR TT* genotype confers a significantly greater mean hemocysteine levels and greater risk for stroke (OR = 1.26) than carriers of the *MTHFR CC* genotype. Although a standard definition of hyperhomocysteinemia is lacking, it seems that fasting plasma concentrations > 16 μmol/L may be considered indicative of hyperhomocysteinemia[3,55]. Ongoing randomized controlled clinical trials continue to assess the efficacy of homocysteine-lowering treatment for decreasing the risk of ischemic stroke.

Elevated lipoprotein (a): Lipoprotein (a) [Lp(a)] complex has proatherogenic and prothrombotic properties and has been shown to be a risk factor for coronary heart disease[45]. Lp(a) enhances arterial cholesterol deposition, thereby promoting atherogenesis[3,45]. There is an increasing evidence suggesting that high Lp(a) levels may induce cerebral ischemia but findings have not been completely consistent[45].

Lipoprotein-associated phospholipase A_2: High concentrations of Lp-PLA_2 are associated with an increased risk of cardiovascular events, independent of other risk factors, and potentially may increase the stroke risk[45].

Hypercoagulability: Venous thrombosis (but not cerebral infarction) is associated with thrombophilias (hereditary) or acquired hypercoagulable states[45]. Anti-phospholipid antibodies (aPL) is most frequently the cause of arterial thrombosis IgG and IgM anti-cardiolipin antibody and lupus anticoagulant are useful tests to detect aPL. Young women with cerebral infarction have a higher prevalence of aPL.

Therefore, primarily young women who have a history of thrombotic events and meet the laboratory criteria for antiphospholipid syndrome might benefit

from the administration of moderate-intensity warfarin or other antithrombotic therapies as primary or secondary prevention strategies[3,45]. On the other hand, a relationship between stroke and other hereditary hypercoagulable states (protein S or antithrombin Ⅲ, protein C deficiency, *etc.*) has not been reported in most case-control studies[45].

Albuminuria: An increased risk of stroke, death of vascular cause, myocardial infarction and renal dysfunction has been documented in patients with microalbuminuria or proteinuria (30-300 mg/d and > 1 g/d, respectively)[45].

Cystatin C: Cystatin C is a serum marker of renal function that has been shown to be a predictor of stroke, myocardial infarction and vascular death, even stronger in elderly subjects than is creatinine[3,45].

EMERGING VASCULAR RISK FACTORS

Asymptomatic vascular disease

Asymptomatic vascular disease is the first consequence of the impact of uncontrolled progression of cardiovascular risk factors on systemic or cerebral arteria vessels of the organism. Asymptomatic vascular disorders may be viewed as subclinical arterial markers related to cardiovascular risk factors, and are predictors of stroke, myocardial infarction and vascular death in the mid- and long-term. Mild carotid stenosis, carotid intima-media thickness and atheroma of the aortic arch, as well as carotid artery distensibility and endothelial reactivity of the brachial artery have been reported as potential causes of asymptomatic vascular disease[3,44].

Complex atheromatosis of the aortic arch is a risk factor of embolism. There is a significant relationship between atheromatous plaques in the aortic arch and cerebral ischemia, particularly in the presence of protruding plaques ⩾ 4 mm in thick. Morphologically complex plaques (aortic atherosclerotic debris) is considered a high risk embolic source (increased stroke risk by 1.7)[3,44,62-64].

Clinically silent cerebral ischemia

Modern neuroimaging techniques allow the detection of clinically silent cerebral ischemia in the form of white matter hyperintensities, cerebral infarcts, cerebral atrophy or microbleeds, which may be considered stroke risk factors[45]. Most lacunar infarctions are asymptomatic and magnetic resonance imaging (MRI) studies reveal the presence of lacunes in approximately 20%-28% of individuals older than 65 years. Silent lacunar infarction is a risk factor of new infarctions of the lacunar type and cognitive impairment[65-69]. Also, symptomatic progression of lacunar disease has been demonstrated given that at 3 years, between 10% and 50% of patients will show new silent lacunar infarcts in MRI studies. Progression of leukoaraiosis is documented in 40% of patients with lacunar infarct. Therefore, clinically silent small vessel cerebral disease is a stroke risk factor that should be taken into account given the independent role as significant predictor of both symptomatic vascular recurrence and cognitive impairment[70,71].

Other factors

Proteomic risk markers, history of TIA, previous cerebral infarction or primary intracerebral hemorrhage, chronic obstructive pulmonary disease (COPD), chronic liver disease and use of oral anticoagulants are other risk factor for stroke.

RISK FACTORS IN THE DIFFERENT ISCHEMIC STROKE SUBTYPES

Studies of data collected from hospital-based stroke registries have shown that the different etiological stroke subtypes present clearly differentiated cardiovascular risk profiles. In this respect, in the Sagrat-Cor of Barcelona Stroke Registry, clear differences between cerebral infarcts and spontaneous intracerebral hemorrhages were observed[72]. Main risk factors in patients with cerebral infarction are hypertension (54.1%), atrial fibrillation (29.3%) and diabetes (22.6%), whereas in patients with hemorrhagic stroke, the frequency of hypertension was higher (61.3) but the occurrence of atrial fibrillation (15.3%) and diabetes (14.7%) was lower[72] (Table 2). These differences were statistically significant and were consistent with data reported in other studies[73-78]. Also, it should be noted that risk factors in stroke patients present a characteristic profile according to the patient's age, with a high percentage of patients with atrial fibrillation and other heart conditions in the oldest old group (⩾ 85 years) (Table 1).

Moreover, each subtype of ischemic stroke exhibits a distinct vascular risk profile[7] (Table 3). When a logistic regression analysis was performed (Table 4)[7], the risk profile for the subtype of atherothrombotic infarction is characterized by the presence of peripheral vascular disease (OR = 2.28), which is a clear and well-known indicator of generalized atherosclerosis, together with hypertension (OR = 1.84) and diabetes mellitus (OR = 1.66), which are major risk factors traditionally related to cardiovascular and cerebrovascular morbidity of large artery atherosclerosis[36,79]. Other risk factors include history of TIA (OR = 1.50), which should be considered a true neurological emergency due to the early risk of subsequent neurological deterioration (cerebral ischemia)[80], COPD (OR = 1.40), -a clinical entity associated with cigarette smoking and recurrent infection episodes, which may cause an acquired subclinical hypercoagulable status-, previous cerebral infarction (OR = 1.40), the presence of which is associated with an increased risk of recurrent cerebral

Table 2 Comparison of demographic data, risk factors, neuroimaging findings and early outcome between stroke patients with intracerebral hemorrhage and cerebral infarctions ("Sagrat Cor Hospital of Barcelona Stroke Registry" period 1986-2004)[72] *n* (%)

Variable	Intracerebral hemorrhage (*n* = 380)	Cerebral infarction (*n* = 2082)
Age, yr, mean (SD)	72.51 (12.55)	74.97 (12.21)
Age, yr		
< 65	83 (21.8)	329 (15.8)
65-74	112 (29.5)	521 (25.0)
75-84	120 (31.6)	808 (38.8)
⩾ 85	65 (17.1)	424 (20.4)
Gender		
Males	199 (52.4)	987 (47.4)
Females	171 (47.6)	1095 (52.6)
Lacunar syndromes	36 (9.5)	554 (31.4)
Hypertension	233 (61.3)	1126 (54.1)
Diabetes mellitus	56 (14.7)	471 (22.6)
Atrial fibrillation	58 (15.3)	609 (29.3)
COPD	29 (7.6)	166 (8.0)
Magnetic resonance imaging	95 (25)	656 (31.5)
Respiratory events	45 (11.8)	183 (8.8)
Symptom-free at discharge	23 (6.1)	382 (18.3)
In-hospital mortality	107 (28.2)	249 (12.0)
Transfer to a convalescence/rehabilitation unit	64 (16.8)	243 (11.7)
Length of stay, median (IQR)	15 (8-26)	12 (8-20)

Data expressed as frequencies and percentages in parentheses unless otherwise stated. COPD: Chronic obstructive pulmonary disease; IQR: Interquartile range (25^{th}-75^{th} percentile).

Table 3 Cardiovascular risk factors in 2704 consecutive patients with cerebral infarction collected from the "Sagrat Cor Hospital of Barcelona Stroke Registry" according to the different stroke subtypes[7] *n* (%)

Variables	Atherothrombotic (*n* = 770)	Lacunar (*n* = 773)	Cardioembolic (*n* = 763)	Undetermined cause (*n* = 324)	Unusual cause (*n* = 114)
Hypertension	509 (66.1)[b]	525 (71.6)[b]	377 (49.4)[b]	59 (18.2)[b]	31 (27.2)[b]
Atrial fibrillation	120 (15.6)[b]	81 (11.1)[b]	573 (75.1)[b]	25 (7.7)[b]	8 (7)[b]
Diabetes mellitus	242 (31.4)[b]	218 (29.7)[b]	142 (18.6)[d]	24 (7.4)[b]	6 (5.3)[b]
Dyslipidemia	164 (21.3)[b]	166 (22.6)[b]	88 (11.5)[b]	52 (16)[b]	10 (8.8)
Previous cerebral infarction	164 (21.3)[b]	117 (16)	146 (19.1)	31 (9.6)[b]	10 (8.8)[d]
Ischemic heart disease	150 (19.5)[a]	104 (14.2)	163 (21.4)[b]	14 (4.3)[b]	4 (3.5)[b]
History of transient ischemic attack	116 (15.1)[d]	80 (10.9)	73 (9.6)[a]	37 (11.4)	11 (9.6)
Smoking (> 20 cigarettes/d)	87 (11.3)[a]	86 (11.7)[b]	28 (3.7)[b]	41 (12.7)[b]	18 (6.9)
Chronic obstructive pulmonary disease	74 (9.6)	61 (8.3)	62 (8.1)	20 (6.2)	6 (5.3)
Peripheral vascular disease	100 (13)[d]	57 (7.8)	50 (6.6)	3 (0.9)[d]	4 (3.5)[d]
Heart valve disease	11 (1.4)[b]	21 (2.9)[b]	130 (17)[b]	6 (1.9)[d]	6 (5.3)
Congestive heart failure	43 (5.6)	24 (3.3)[d]	72 (9.4)[b]	8 (2.5)[d]	1 (0.9)[a]
Obesity (body mass index ⩾ 30 kg/m^2)	36 (4.7)	47 (6.4)[b]	17 (2.2)[d]	13 (4)	5 (4.4)
Oral anticoagulants	18 (2.3)[a]	7 (1)[b]	63 (8.3)[b]	2 (0.6)[b]	4 (3.5)
Alcohol abuse (> 80 g/d)	26 (3.4)[a]	21 (2.9)	5 (0.7)[a]	10 (3.1)	4 (3.5)
Chronic liver disease	17 (2.2)	15 (2.1)	15 (2)	10 (3.1)	0
Previous intracerebral hemorrhage	9 (1.2)	9 (1.2)	7 (0.9)	6 (1.9)	1 (0.9)

Data expressed as frequencies and percentages in parentheses. [a]$P < 0.05$; [b]$P < 0.001$; [d]$P < 0.01$.

infarcts, and ischemic heart disease (OR = 1.33), which is an epiphenomenon of clinically significant atherosclerosis and a potential cause of recurrent cerebral ischemia.

In lacunar infarction, the vascular profile includes hypertension (OR = 2.64) and diabetes mellitus (OR = 1.55). Both hypertension and diabetes are the main risk factors, a fact that is consistent with previous histopathological studies and data reported in main clinical series of patients published in the literature[25,81-87]. Obesity is also an independent risk factor associated with lacunar infarcts. The frequency of the different risk factors is different in patients with lacunar infarcts from those in patients with lacunar syndromes not due to lacunar infarction[88,89] (Table 5).

In cardioembolic ischemic stroke, cardiac sources of embolism including atrial fibrillation (OR = 20.01), valve heart disease (OR = 5.60) and ischemic heart disease (OR = 2.09) are the most prevalent heart conditions[89,90].

Table 4 Results of multivariate analysis: cardiovascular risk factors independently associated with the different subtypes of ischemic infarction in 2704 consecutive patients with cerebral infarction collected from the "Sagrat Cor Hospital of Barcelona Stroke Registry"[7]

	Odds ratio (95%CI)
Atherothrombotic infarction	
Peripheral vascular disease	2.28 (1.68-3.08)
Hypertension	1.84 (1.53-2.2)
Diabetes mellitus	1.66 (1.36-2.03)
Previous transient ischemic attack	1.50 (1.16-1.95)
Chronic obstructive pulmonary disease	1.41 (1.04-1.93)
Previous cerebral infarction	1.40 (1.12-1.76)
Ischemic heart disease	1.33 (1.06-1.68)
Atrial fibrillation	0.36 (0.28-0.45)
Heart valve disease	0.23 (0.12-0.43)
Lacunar infarction	
Hypertension	2.64 (2.19-3.20)
Diabetes mellitus	1.55 (1.23-1.90)
Obesity ($\geqslant$ 30 kg/m^2)	1.50 (1.01-2.25)
Oral anticoagulation	0.37 (0.16-0.82)
Heart valve disease	0.22 (0.17-0.28)
Cardioembolic	
Atrial fibrillation	20.01 (15.98-25.05)
Heart valve disease	5.60 (3.60-8.71)
Ischemic heart disease	2.09 (1.57-2.78)
Dyslipidemia	0.69 (0.50-0.94)
Diabetes mellitus	0.68 (0.52-0.89)
Hypertension	0.67 (0.54-0.85)
Previous transient ischemic attack	0.66 (0.46-0.95)
Smoking (> 20 cigarettes/d)	0.54 (0.34-0.88)
Obesity ($\geqslant$ 30 kg/m^2)	0.38 (0.20-0.73)
Undetermined cause	
Hypertension	0.12 (0.09-0.17)
Peripheral vascular disease	0.13 (0.04-0.41)
Atrial fibrillation	0.15 (0.10-0.23)
Diabetes mellitus	0.21 (0.13-0.32)
Ischemic heart disease	0.24 (0.13-0.42)
Heart valve disease	0.34 (0.14-0.80)
Previous cerebral infarction	0.61 (0.40-0.93)
Unusual cause	
Atrial fibrillation	0.15 (0.07-0.32)
Diabetes mellitus	0.17 (0.07-0.38)
Ischemic heart disease	0.21 (0.08-0.58)
Hypertension	0.27 (0.18-0.42)

In patients with cerebral infarctions of undetermined cause (or essential) and in patients with infarctions of unusual cause (haematological disorders, infections, vasculitis and other entities), classical cardiovascular risk factors are less frequent[91,92].

Finally, the role of emerging and less-well documented stroke risk factors as described above is still inconclusive but further characterization in well-designed clinical studies will add knowledge to develop more tailored primary and secondary preventive strategies in stroke.

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Table 5 Comparison of cardiovascular risk factors in lacunar infarction and in lacunar syndromes not due to lacunar infarcts[89] *n* (%)

Datos	Lacunar syndromes not due to lacunar infarction	Lacunar infarcts	*P* value
Total patients	146	733	
Male sex	82 (56.2)	423 (57.7)	0.73
Age, yr, mean ± SD	72.9 (12.6)	74.1 (10.2)	0.285
Age $\geqslant$ 85 yr	26 (17.8)	110 (15.0)	0.393
Risk factors			
Hypertension	107 (73.3)	525 (71.6)	0.683
Diabetes mellitus	31 (21.2)	218 (29.7)	0.037
Heart valve disease	10 (6.8)	21 (2.9)	0.017
Ischemic heart disease	23 (15.8)	104 (14.2)	0.623
Atrial fibrillation	44 (30.1)	81 (11.1)	0
Congestive heart failure	4 (2.7)	24 (3.3)	0.737
Previous transient ischemic attack	12 (8.2)	80 (10.9)	0.331
Previous cerebral infarction	16 (11)	117 (16)	0.123
Head traumatism	6 (4.1)	6 (0.8)	0.006
Peripheral vascular disease	17 (11.6)	57 (7.8)	0.124
Obesity (BMI $\geqslant$ 30 kg/m^2)	8 (5.5)	47 (6.4)	0.671
Alcohol abuse (> 80 g/d)	7 (4.8)	21 (2.9)	0.34
Smoking (> 20 cigarettes/d)	19 (13)	86 (11.7)	0.663
Dyslipidemia	29 (19.9)	166 (22.6)	0.46

Data expressed as frequencies and percentages in parentheses.

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REVIEW

Tick-borne encephalitis: A review of epidemiology, clinical characteristics, and management

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Abstract

Tick-borne encephalitis is an infection of central nervous system caused by tick-borne encephalitis virus transmitted to humans predominantly by tick bites. During the last few decades the incidence of the disease has been increasing and poses a growing health problem in almost all endemic European and Asian countries. Most cases occur during the highest period of tick activity, in Central Europe mainly from April to November. Tick-borne encephalitis is more common in adults than in children. Clinical spectrum of the disease ranges from mild meningitis to severe meningoencephalitis with or without paralysis. Rare clinical manifestations are an abortive form of the disease and a chronic progressive form. A post-encephalitic syndrome, causing long-lasting morbidity that often affects the quality of life develops in up to 50% of patients after acute tick-borne encephalitis. Clinical course and outcome vary by subtype of tick-borne encephalitis virus (the disease caused by the European subtype has milder course and better outcome than the disease caused by Siberian and Far-Easter subtypes), age of patients (increasing age is associated with less favorable outcome), and host genetic factors. Since clinical features and laboratory results of blood and cerebrospinal fluid are nonspecific, the diagnosis must be confirmed by microbiologic findings. The routine laboratory confirmation of the tick-borne encephalitis virus infection is based mainly on the detection of specific IgM and IgG antibodies in serum (and cerebrospinal fluid), usually by enzyme-linked immunosorbent assay. There is no specific antiviral treatment for tick-borne encephalitis. Vaccination can effectively prevent the disease and is indicated for persons living in or visiting tick-borne encephalitis endemic areas.

Key words: Tick-borne encephalitis; Diagnosis; Epidemiology; Clinical manifestations; Treatment; Prevention/vaccination

Core tip: Tick-borne encephalitis (TBE) is the most common tick-borne central nervous system infection in Europe and Asia. It is caused by three subtypes of TBE virus: European, Siberian and Far-Eastern. Because of relatively severe clinical course, the absence of etiologic treatment, considerable proportion of patients with incomplete recovery after acute illness, as well as due to increasing incidence it represents a growing public health problem that could be substantially reduced with vaccination.

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INTRODUCTION

Tick-borne encephalitis (TBE) is an important viral infection of the central nervous system in Europe and in several regions in Asia. It is caused by the Far-Eastern, Siberian and European subtype of tick-borne encephalitis virus (TBEV). Although the disease is preventable by vaccination, the incidence has been increasing during the last few decades and consequently represents a growing health problem in almost all endemic European and Asian countries. With the increase of tourism TBE is becoming a problem also outside endemic regions[1].

Most cases occur during the highest period of tick activity, in Central Europe mainly from April to November[2-4]. TBE caused by the European virus subtype usually presents as meningitis or meningoencephalitis and has a biphasic course in approximately two-thirds of patients. Up to 50% of patients have long-term sequelae[4]. An occasional clinical manifestation is an abortive form of the disease[5]. Treatment is based on the symptomatic measures. However, TBE can be successfully prevented by safe and highly effective vaccine[4].

ETIOLOGY

The neurotropic TBEV was first described as the cause of TBE by Zilber more than 75 years ago[6]. It is a spherical lipid-enveloped RNA virus, a member of genus Flavivirus within the Flaviviridae family. The mature virion is composed of 3 structural proteins-capsid (C), membrane (M), and envelope (E). Protein E is a major antigen which induces production of neutralizing antibodies. It can be inactivated by pasteurization[4].

The genetic analysis shows the existence of three TBEV subtypes named as European, Siberian, and Far-Eastern subtype[2]. They are genetically very closely related; variation in amino acids sequences between subtypes is 5%-6%[7]. In spite of the pronounced genetic similarity of the subtypes the illness caused by individual subtype is not completely equivalent to those due to the other subtypes.

EPIDEMIOLOGY

TBE is endemic in Europe, Siberia, far-eastern Russia, northern China and Japan. During the past few decades endemic regions have expanded, and within many endemic areas the number of reported cases increased. The increase in the reported incidence rates is thought to be a result of complex interplay of social and ecological factors as well as due to increased medical awareness and advanced diagnostics[8,9].

The European TBEV subtype is predominantly found in Europe, but has also been identified in west Urals, and in Siberia, whereas the Siberian TBEV subtype is found in Siberia, the Baltics, and northern Finland. The Far-Eastern TBE virus subtype is endemic in far-eastern Asia and Japan, and has been identified also in central and eastern Siberia[1,2,10,11].

In Europe and Asia between 10000 and 15000 TBE cases are reported annually[12]. The number is very likely underestimated because in many countries notification of the disease is not mandatory and only in a subset of the countries TBE case definition is in place. TBE is endemic in 27 European countries, and is a reportable disease in only 16 countries[1,12,13]. European countries with the highest incidence of the disease in the period 2005-2009 were Slovenia (14.1 cases per 100000 inhabitants per year), Estonia (11.1), Lithuania (10.6), and Latvia (8.8)[1]. Pronounced yearly variations of registered TBE cases occurred. According to the latest available epidemiological data for Slovenia the incidence of the disease in 2013 was 15.0 cases per 100000 inhabitants[14].

The primary reservoirs and hosts of TBEV in nature are small rodents; humans do not play any role in the maintenance of TBEV in nature and they are only accidental hosts. TBEV is transmitted to humans mainly by hard tick bites; in Europe the principal vector is *Ixodes ricinus* (*I. ricinus*), in parts of Eastern Europe, Russia and in far-east Asia the vector is *Ixodes persulcatus* (*I. persulcatus*) whereas in Japan Far-Eastern TBEV subtype has been demonstrated in *Ixodes ovatus* ticks. In endemic areas in Central Europe approximately 0.1% to 5.0% of ticks harbor the virus (depending on the time of the year and geographical location); in Siberia infection rates of up to 40% are reported for *I. persulcatus*[4,15]. In Slovenia, the prevalence of ticks infected with TBEV was found to be 0.47%; 0.54% in 2005 and 0.43% in 2006[16]. Approximately 1% of all TBEV infections in humans are probably acquired by consuming infected unpasteurized milk or milk products from infected livestock, particularly goats[2]. This means of transmission has to be considered particularly in cases of local epidemics. Outbreaks due to oral virus transmission are more common in Eastern Europe and the Baltic states than in Central Europe[17,18]. A few cases of laboratory-acquired TBEV infections have been documented in the literature[19].

TBE cases usually happen in the warm months between April and November, which is also the period of the highest tick activity[20]. In Central Europe, where the dominant tick species is *I. ricinus,* a two-peak distribution of TBE cases can be seen (first in June and July, second in September and October), whereas in the Ural region, Siberia and the Far East, where *I. persulcatus* is widespread, cases as a rule occur in May and June[21]. In all age groups men are affected more frequently than women[14,22-24]. On average, 10%-20%

of all reported cases of TBE occur in children[25]. It should be pointed out that due to its unspecific clinical presentation TBE in children is often missed and is diagnosed as aseptic meningitis of unknown etiology[26].

With increasing of tourism, TBE has become a more global problem. Therefore, it should be included in the differential diagnosis of the central nervous system infections not only for those living within an endemic region but-in case of an appropriate epidemiological history-also in patients living outside endemic areas. The risk of travel-associated TBE depends on the season of travel, degree of unprotected outdoor exposure as well as on consuming unpasteurized dairy products. TBE surveillance data available in Austria shows that an overall risk of acquiring TBE for a non-vaccinated tourist, staying in a highly endemic region for 4 wk during the TBEV transmission season, has been estimated at approximately 1 case per 10000 person-months of exposure, which is approximately equivalent to the risk of contracting typhoid fever or malaria while traveling in India[27,28].

PATHOGENESIS AND PATHOLOGY

After an infected tick bite TBEV replication occurs locally. Dendritic skin cells (Langerhans cells) are assumed to be the first cells for viral replication and to transport the virus to local lymph nodes. From this initial site the TBEV than disseminate to extraneural tissues, especially spleen, liver and bone marrow, where further multiplication maintains viremia for several days. During the viremic phase (which clinically corresponds to the initial phase of TBE) the virus probably reaches the brain[29,30]. The exact mechanism by which TBEV breach the blood-brain barrier is not known; four possible routes have been postulated: (1) peripheral nerves; (2) highly susceptible olfactory neurons; (3) transcytosis through vascular endothelial cells of brain capillaries; and (4) diffusion of the virus between capillary endothelial cells. The primary targets of TBEV infection in central nervous system are neurons[31].

According to rather limited information the neuropathological findings are nonspecific. Cerebral and spinal meninges usually show diffuse infiltration with lymphocytes and sometimes neutrophils. The most extensive meningeal inflammation is in the vicinity of the cerebellum. Pathological lesions which consist of lymphocytic perivascular infiltrations, accumulation of glial cells, nerve cells necrosis, and neuronophagia are localized in the grey matter and are most often present in the medulla oblongata, pons, cerebellum, brainstem, basal ganglia, thalamus, and spinal cord. Rarely, oligodendrocytes are infected. In the motor area of the cerebral cortex degeneration and necrosis of the pyramidal cells, lymphocytic accumulation, and glial proliferation are present[29,32].

MANIFESTATIONS OF TBEV INFECTION

The large majority of infections with TBEV are asymptomatic; published data suggest that the ratio of asymptomatic infections is between 70% and 98%[4,33]. However, the proportion of asymptomatic cases is hard to ascertain because patients with mild clinical signs and symptoms may remain undiagnosed.

The incubation period of TBE ranges from 2 to 28 d and is usually 7-14 d. After alimentary TBEV transmission the incubation period is as a rule shorter, usually 3 to 4 d[20]. Investigation of a recent small outbreak of TBE after drinking raw goat milk infected with TBEV in Slovenia revealed infection with the virus in four out of four exposed individuals. Of them, three developed symptomatic infection 2 to 3 d after the milk consumption, while the one who had been vaccinated against TBE remained healthy[18].

In about 75% of patients with TBE due to the European TBEV subtype the disease has a typical biphasic course. The majority of patients with monophasic course of the disease has central nervous system involvement (meningitis, meningoencephalitis), while a small fraction has a febrile illness with headache but no meningitis (*i.e.,* the initial phase of TBE not followed by the second, meningoencephalitic phase of the disease), named abortive form of TBE or "febrile headache"[4,5,20,34].

The initial phase correlate with viremia and usually presents with non-specific symptoms such as moderate fever, headache, body pain (myalgia and arthralgia), fatigue, general malaise, anorexia, nausea, and others[3]. This phase lasts for 2 to 7 d and is followed by amelioration or even an asymptomatic interval that usually lasts for about 1 wk (1-21 d). Than the second phase appears: in approximately 50% of adult patients it presents as meningitis, in about 40% as meningoencephalitis, and in around 10% as meningoencephalomyelitis[4,34].

Meningitis, encephalitis, myelitis

Meningitis and encephalitis are the most frequent clinical forms of TBE. Meningitis typically manifests with high fever, headache, nausea and vomiting; many patients have photophobia, and some vertigo. Meningeal signs are present in most of patients. Encephalitis can be manifested by impaired consciousness ranging from somnolence to stupor and, in rare cases, coma. Other manifestations comprise personality changes, behavioral disorders, concentration and cognitive function disturbances, tongue fasciculations and tremor of extremities; very rarely focal or generalized seizures, delirium and psychosis develop. Flaccid pareses, that are a typical characteristic of meningoencephalomyelitis, usually arise during the febrile phase of the disease, and are occasionally preceded by severe pain in the affected muscle groups. The upper extremities are more often affected than the lower extremities and the proximal segments more frequently than the distal ones. Patients with pareses of respiratory muscles rather commonly require artificial ventilatory support. Involvement of the central portions of the brainstem and medulla oblongata are associated with poor prognosis. Myelitis usually occur with encephalitis, and only very rarely as the only manifestation of TBE[4,20,35,36].

In patients with TBE the involvement of cranial nerves has been reported. Published data suggests that cranial nerve involvement is rare and mainly asymmetrical, that its occurrence varies with the severity of clinical presentation of TBE, and that in most cases it has a favorable outcome[24,37,38]. Cranial neuritis most commonly affects ocular, facial, pharyngeal and vestibular nerves[4,20]. Of 1218 adult patients with TBE, who were hospitalized at the Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia from 2003 to 2009, 11 (0.9%) developed peripheral facial palsy during the course of the disease (nine had unilateral and two had bilateral facial nerve involvement; no one had a central facial palsy) and in three of them TBE was associated with borrelia infection[39]. In regions where TBE and Lyme borreliosis are endemic, concomitant infection with TBEV and *Borrelia burgdorferi* sensu lato should be considered.

Occasionally, patients with TBE have pronounced variability in heart rate or other signs of autonomic nervous system dysfunction[40].

Abortive form of TBE

Data on this manifestation of TBE are limited. It manifests with moderate fever, headache, fatigue, and other symptoms of initial phase of the disease that are not followed by nervous system involvement. The fever typically endures for several days, and the outcome of the disease is excellent[41,42]. In Central Europe the majority of patients with the initial phase of TBE develop the second, central nervous system phase of the disease. In 2002, Lotric-Furlan *et al*[5] published a prospective clinical study on the etiology of febrile illnesses after a tick bite. Among 56 patients with confirmed TBE, only one (2%) had an isolated initial phase, whereas all the others (55, 98%) diagnosed at the time of the initial phase developed the second phase of the disease with pleocytosis.

In Russia this clinical manifestation is named "fever form," and is reported to represent up to 50% of all clinical presentations of TBE[43].

TBE with normal cerebrospinal fluid cell counts

A patient with encephalitis and serologically confirmed TBEV infection but without CSF pleocytosis has been reported[44]. In larger studies of serologically proven TBE, CSF pleocytosis was found in all patients[3,4,24,33]. This finding however, might be due to selection bias because in these studies CSF pleocytosis was one of the inclusion criteria for the diagnosis of TBE.

Chronic progressive form of TBE

There is no agreement on the existence of chronic TBE. Cases of a chronic progressive form of TBE have been identified in Siberia and the Russian Far East. This form of TBE is believed to be caused by the Siberian TBEV subtype. Both mutation in the *TBEV NS1* gene as well as an inappropriate T-cell immune response have been implicated to be associated with chronic progressive disease[3]. According to information from Western Siberia 1.7% of patients with acute TBE develop a chronic progressive form of the disease[45]. Clinical presentations include Kozshevnikov's epilepsy, lateral sclerosis, progressive neuritis, progressive muscle atrophy, and a Parkinson-like disease. Pronounced dissimilarities in the incubation period, in time to the onset of individual neurological signs/symptoms, and in the survival time after the onset of the disease have been reported[2]. Nevertheless, progressive form of TBE is most probably not present or is extremely unusual in disease caused by European TBEV subtype. In the study carried out in Lithuania, where both, European and Siberian TBEV subtypes are present, progressive course was noted in two out of 133 consecutive patients with acute TBE[24].

Post-encephalitic syndrome

TBE may cause long-lasting morbidity which often has an impact on patients' quality of life and, sometimes, necessitates an alteration of lifestyle. Many nonspecific neurological/neuropsychiatric symptoms and residual neurological dysfunctions have been reported in some prospective and several retrospective studies, but findings are hard to compare due to diverse study designs, distinct definitions, and variable follow-up times. Most studies also failed to comprise a control group; as a result findings are difficult to interpret because of unclear distinctions between post-encephalitic syndrome, other sequelae of TBE, and symptoms present in general population. Published data suggest that 40% to 50% of patients after acute TBE develop a post-encephalitic syndrome[46]. The most frequently reported symptoms have been cognitive disorders, neuropsychiatric complaints (such as apathy, irritability, memory and concentration disorders, altered sleep pattern), headache, hearing loss and/or tinnitus, disturbances of vision, balance and coordination disorders, and flaccid paresis or paralysis[24,37,38,47-49].

Lithuanian prospective clinical follow-up study showed that 46% of patients with TBE had sequelae 1 year after the onset of acute illness[24]. In a review by Haglund and Günther four retrospective and four prospective studies on the long-term morbidity from TBE were included. Up to 46% patients suffered permanent sequelae at long-term follow-up[47]. In 2009, Misić Majerus *et al*[48] published a prospective study on TBE post-encephalitic syndrome. Of 124 patients aged 16-76 years, who were followed for at least 3 years, 49 (40%) developed moderate or severe post-encephalitic syndrome lasting for up to 18 mo. Permanent sequelae were seen in 14 (11.3%) patients: spinal nerve paresis in 5, hearing impairment in 6, dysarthria in 2, and severe mental disorder in 1 patient.

CLINICAL COURSE OF ACUTE DISEASE AND LONG TERM OUTCOME

Course of acute TBE and its long-term outcome depend upon the subtype of TBEV infection. The disease caused

by the European TBEV subtype usually has a biphasic course, with a severe neurologic deficit in approximately 10% of patients, and a case-fatality rate of less than 2%[4,20]. The abortive form of TBE is rare-the initial phase most of the time move on to the second phase of the disease[5]. Long-lasting sequelae are identified in up to 50% of adult patients[46]. Infections with Far Eastern TBEV subtype often cause an illness with a gradual onset, more severe course, higher rates of severe neurologic sequelae, and a fatality rate of 20%-40%. Little information with a respect to the clinical course of the disease is available for Siberian TBEV subtype. The case-fatality rate is 2%-3% and some reports from Russia suggest an association with a chronic progressive form of TBE[2,21].

Published data suggest the relationship between age of patients and severity of TBE. The disease caused by European TBEV generally has milder course and better outcome in children than in adults. Nevertheless, TBE cases with severe clinical course, permanent sequelae and even death have been described not only in adults but also in children[23,50]. The predominant form of TBE in children and adolescents is meningitis. A summary of 8 studies on 1169 children with TBE, published from 1963 to 2005, showed that meningitis was present in 802 (69%), meningoencephalitis in 356 (30%), and meningoencephalomyelitis in 11 (1%) patients. Twenty out of 945 patients (2.1%) had long-term neurologic sequelae[25]. Limited information is available on the relationship between age and severity of TBE in adults. Mickiene *et al*[24] reported about this correlation but no precise data were given. Comparison of patients over an under 60 years revealed several differences in the course and outcome of TBE and corroborated previous postulation that TBE is a more severe disease in the elderly[22].

Some clinical studies have shown that TBE with monophasic presentation is associated with a more severe course of the acute disease[3,51-55].

The comparison of clinical course of TBE in unvaccinated and vaccinated patients did not reveal significant differences in disease severity[56].

The outcome of TBE is associated with clinical presentation. The risk of incomplete recovery is higher for patients who have more severe clinical illness during acute phase of TBE[24].

In recent years, genetic factors with potential impact on the course and outcome of TBE have been of scientific interest, resulting in several investigations. For example, the assessment of the role of chemokine receptor CCR5 indicated that *CCR5*Δ23 allele may predispose for TBE[57]. Barkash *et al*[58,59] reported on the association between *CD209* gene promoter region polymorphism and predisposition to severe forms of TBE, and on possible association between 5 OAS single nucleotide polymorphisms and the outcome of TBEV infection in a Russian population. Interesting new findings on the role of host genetic factors in TBEV infections may appear in future.

DIAGNOSIS

A case of TBE is delineated by the presence of: (1) symptoms/signs indicating meningitis or meningoencephalitis; (2) an elevated cerebrospinal fluid cell count (> 5×10^6 cells/L); and (3) microbiologic evidence of TBEV infection (the presence of specific IgM and IgG antibodies)[60].

Blood and cerebrospinal fluid analysis

In patients with TBE blood and cerebrospinal fluid findings are nonspecific. In the first (viremic) phase of TBE leukopenia and/or thrombocytopenia is established in approximately 70% of patients, rarely abnormal liver function test results are seen[61]. In the second phase of the disease mildly elevated leukocyte count may be present in peripheral blood (rarely > 15×10^9/L); erythrocyte sedimentation rate and concentration of C-reactive protein are normal in the majority of patients but may be elevated, particularly in some long-lasting severe cases. Cerebrospinal fluid examination typically reveals elevated leukocyte counts (usually lower than 500 cells/mm^3), a normal glucose concentration, and a normal to slightly elevated protein concentration. Early in the course of the disease neutrophils may predominate, while later cerebrospinal fluid profile is characterized by a predominance of lymphocytes. Elevated lymphocyte counts may last for several weeks after clinical improvement[20,62].

Microbiological investigations

At the time when neurological symptoms/signs occur TBEV has already been cleared from the blood (TBEV is present in blood in the initial but not in the meningoencephalitic phase of the disease) and is only very exceptionally present in cerebrospinal fluid. Consequently, isolation of TBEV from blood and detection of viral RNA by reverse transcriptase PCR in blood and cerebrospinal fluid of patients with TBE have a limited diagnostic yield and are as a rule not used in clinical practice[63]. Reverse transcriptase PCR assays is mainly limited to the initial phase of the disease and could be a useful method in a diagnostic procedure of febrile illness occurring after a tick bite in areas where several tick-borne diseases are present[64].

The routine laboratory confirmation of the TBEV infection is based mainly on the demonstration of specific antibodies in serum (and cerebrospinal fluid), usually by highly sensitive and specific enzyme-linked immunosorbent assay[63,65]. In the majority of patients specific serum IgM and IgG antibodies are present at the beginning of the meningoencephalitic phase of the disease; rarely only IgM antibodies to TBEV are found in the first serum sample. In such cases a second serum sample has to be tested 1-2 wk later, because the demonstration of IgM antibodies alone does not suffice for the diagnosis. TBEV IgM antibodies can be detected in the serum for several months (up to 10 mo or even longer) after acute infection, whereas TBEV

IgG antibodies persist for a whole life, and mediate an immunity that prevents symptomatic reinfection[63,66]. In cerebrospinal fluid specific IgM and IgG antibodies are detectable several days later than in serum, and in almost all cases by day 10[63,67].

However, some limitations are necessary to take into account when using and interpreting serological testing. Specific TBE IgM antibodies may be detectable for several months after acute TBEV infection (as well as in some persons after the first two doses of primary immunization) and may lead to erroneous interpretation in case of another central nervous system disease/infection within this time period[63,66]. Because of the close antigenic relationship between TBEV and other flaviviruses cross-reactive antibodies are induced by infections or vaccinations. This may pose a diagnostic challenge in people vaccinated against yellow fever or Japanese encephalitis and in travelers having acquired dengue, West Nile or other flavivirus infections[68]. Such potential problems in TBE serodiagnosis can be resolved by the quantification of IgM antibodies in a single serum sample taken at the time of hospitalization. High IgM values (> 500 Arbitrary Units) are indicative of a recent infection with TBEV. Lower IgM values, however, may require the analysis of a follow-up sample and/or a specific neutralization assay to exclude the possibilities of long persisting IgM antibodies after infection or those induced by vaccination as well as cross reactive IgM antibodies[69]. Carefulness in the interpretation of TBE serology is also needed in patients with meningoencephalitis or meningitis who had been vaccinated against TBE. In the majority of patients with vaccination breakthroughs serological response is distinct from those found in unvaccinated persons with TBE and consequently vaccination breakthrough cases may be overlooked. These cases are characterized by a delayed development of specific IgM response (during the initial week of the meningoencephalitic phase of TBE specific IgM antibodies are usually not detectable) associated with a rapid increase of specific serum IgG antibodies[70-72]. For a reliable diagnosis of TBE in persons previously vaccinated against TBE, demonstration of intrathecal production of TBEV antibodies is required[34].

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of TBE is extensive and includes a wide variety of central nervous system infections due to other infectious agents as well as noninfectious diseases.

In the initial, viremic phase of TBE, when a patient present with fever, headache, arthralgia, myalgia, and malaise the differential diagnosis may include various viral syndromes; if nausea, vomiting, diarrhea, and anorexia are present, gastroenteritis is a possible explanation. When signs and symptoms of central nervous system involvement develop, TBE needs to be differentiated from encephalitis or aseptic meningitis due to many other viruses. Differential diagnosis comprises also other tick-borne diseases such as Lyme borreliosis, babesiosis, human granulocytic anaplasmosis, tick-transmitted rickettsioses, and tularemia[20].

In many endemic regions TBEV geographically overlaps with bacterial tick-borne pathogens such as *Anaplasma phagocytophilum* and *B. burgdorferi* s.l. Both diseases caused by the two agents, human granulocytic anaplasmosis and Lyme borreliosis, are treatable with antibiotics, and caution must be taken to distinguish them from TBE[20]. Concomitant TBEV and *B. burgdorferi* s.l. infections, as well as concomitant TBEV and *A. phagocytophilum* infections have been described[60,73-76].

The initial, viremic phase of TBE and human granulocytic anaplasmosis have a similar clinical and laboratory presentation. Both diseases are characterized by fever and headache, and both are typically associated with thrombocytopenia and leukopenia; in certain patients also mild abnormalities in liver tests are present. Nevertheless, a clinical report on chills, myalgia and arthralgia, and laboratory findings of elevated concentration of C-reactive protein and lactate dehydrogenase values point to the diagnosis of human granulocytic anaplasmosis and not in favor of the initial phase of TBE[77].

TREATMENT

There is no specific antiviral treatment for TBE. Patients as a rule need hospitalization and supportive care based on the severity of signs/symptoms, and usually encompasses administration of antipyretics, analgesics, antiemetics, maintenance of water and electrolyte balance, and if necessary administration of anticonvulsive agents. In patients with neuromuscular paralysis leading to respiratory failure, intubation and ventilatory support are necessary. In a large prospective German study, 12% of patients were treated in intensive care unit and in 5% assisted ventilation was required[38]. Among Slovenian patients with TBE, hospitalized at the Department of Infectious Diseases, University Medical Centre Ljubljana, in years 2000 to 2004, 6.9% were hospitalized in intensive care unit and 22.5% of them needed mechanical ventilation[22].

Cerebral edema is a potential complication of acute viral encephalitis that aggravates the clinical picture and portends poor neurologic outcome. Patients who have significantly raised intracranial pressure are often treated with intravenous mannitol and/or steroids[78]. Mannitol produces a shift of fluid from the edematous brain back into the intravascular space and subsequently increases circulation volume and improves cerebral perfusion pressure, and lowers intracranial pressure through cerebral autoregulation. It also acts on erythrocyte membrane fluidity and through reducing blood viscosity improves blood flow and oxygen delivery. A rebound phenomenon in intracranial hypertension occurs in about 5% of patients. To avoid complications,

it is frequently recommended that mannitol no longer be administered when the serum osmolality exceeds 320 mOsm/L[78,79]. Despite a rather common clinical practice to treat patients who have significantly raised intracranial pressure with intravenous mannitol, there are no reliable (comparative) studies substantiating the usage of mannitol in patients with TBE.

It has been demonstrated that the use of dexamethasone results in a reduction of brain edema in acute viral encephalitis[80]. In the Baltics and some Eastern European countries patients with TBE are quite common given corticosteroids. The use of steroids is based on the impression that they produce a good and rapid clinical response in patients with TBE, but the validation of the impression in controlled studies has been limited. In a Lithuanian study corticosteroids were used in 81 out of the 133 patients with TBE. Among patients with mild, moderate, and severe disease, 39.7%, 70.7%, and 100% received corticosteroids, respectively. Hospitalization was significantly prolonged among these patients, compared with patients who received only symptomatic treatment. Because all patients with severe disease received corticosteroids it was hard to assess their role in the outcome of TBE[24]. In the group of Polish patients with TBE dexamethasone was used in 54.8% of patients with meningitis, in 69.6% of patients with meningoencephalitis and in 78.3% of patients with meningoencephalomyelitis. In this study the duration of hospitalization was significantly longer only in patients who received dexamethasone longer than 10 d[49]. Perhaps corticosteroid treatment is effective in certain cases, but until the results of the randomized studies are available they cannot be recommend as a standard treatment approach[24,49].

A case report on a patient with severe TBE who substantially improved after application of high dose intravenous immunoglobulins late in the disease course[81], and reports on the successful treatment of encephalitis due to other arboviruses with high doses of intravenous immunoglobulins, prompted Růžek *et al*[82] to propose the implementation of randomized controlled treatment study on the efficacy of high dose intravenous immunoglobulins in patients with severe TBE. Such a proposal should not be interpreted as an indication for treatment of TBE with immunoglobulins.

NONSPECIFIC PREVENTIVE MEASURES

Nonspecific preventive measures comprise pasteurization of milk, reduction of tick population, and personal protective procedures.

Since milk from endemic regions may contain TBEV, pasteurization, and avoiding consumption of unpasteurized milk and dairy products, prevent infection of humans[3,18].

Population of ticks can be reduced by impacts on the environment (*e.g.,* by regular cutting grass around the house, by usage of acaricides, and/or by control of deer population).

Nonspecific personal preventive measures include avoidance of ticks (which means avoidance of contact with vegetation, especially in deciduous and mixed forests with a rich understory and a layer of decaying vegetation on the ground that provides sufficient humidity for the development and survival of ticks), wearing light-colored clothing (light colors enable that ticks are better noticeable) with long sleeves and slacks stuck in socks or footwear (to diminish tick access to the skin), use of repellents, careful examination of the whole body for the presence of ticks, and removal of the attached ticks as soon as possible[83]. However, TBEV is present in salivary glands of the infected tick and may be transmitted from the saliva of an infected tick within a few minutes after attachment[3]. Although the recommended personal measures appear to be self-evident for the prevention of tick-borne diseases including TBE, the effectiveness of certain measures is limited, questionable or has not been properly assessed. Additional problem is that only a small proportion of exposed persons follow the recommended procedures in everyday life[84,85].

VACCINATION

Historically, immunoglobulins containing gamma globulin against TBEV were used as a prophylaxis against TBE within 96 h after a tick bite in the TBE endemic regions (post-exposure prophylaxis). However, due to reports indicating a more severe disease course in children who had received the immunoglobulin[86-88] and because protection was rather unreliable[88], the usage of the immunoglobulins (passive immunization) in European Union has been abandoned[89]. Nevertheless, according to recent studies in Russia, timely single administration of a specific immunoglobulin preparation in a dose of 0.05 mL/kg body weight was protective in approximately 80% of the cases[90]. Further analysis of these findings is needed.

Active immunization is the most effective way to prevent TBE[3,4]. Given that TBE is a zoonosis, that the source of infection is an infected animal, and that TBEV is transmitted by a tick bite and does not spread from human to human, vaccination enables only individual protection. Consequently, high immunization rate of a population in a given environment does not protect persons who are not vaccinated.

Recommendations for TBE vaccination

Because TBE incidence varies within and between different endemic areas recommendations for public vaccination strategies should be based on risk assessments conducted for individual region.

World Health Organization (WHO) recommends vaccination to people of all age groups, including children, in highly endemic areas (≥ 5 cases/100000 per year). In regions where the pre-vaccination incidence of TBE is low to moderate (5 year incidence < 5 cases/100000 per year) immunization should target

persons in the most vulnerable groups. The WHO also recommends TBE vaccination for people travelling from nonendemic areas to rural endemic areas up to altitudes of 1400 m[91].

Central European Vaccination Awareness Group strongly recommends the introduction of universal TBE vaccination for persons > 1 year old for all countries at very high risk of TBE infections. For countries with a very low risk of TBE, recommendations for TBE vaccination should only apply to persons travelling to endemic regions[92].

Vaccines

Two vaccines against TBE, FSME-IMMUN® and Encepur®, are registered in Europe. They contain inactivated European subtype of TBEV-FSME-IMMUN is based on strain Neudorf 1, Encepur on strain K23. Both vaccines prevent not only the disease caused by the European but also those caused by the Siberian and Far-Eastern subtype of TBEV. Procedures used in the preparation of the two vaccines are similar (viruses are grown in chick embryo fibroblast cells, are inactivated by formaldehyde and are purified), and in both vaccines the adjuvant is aluminum hydroxide[89]. Both vaccines are registered for adults and children aged 1 year and older (vaccines for children are called FSME-IMMUN 0.25 mL Junior, and Encepur Kinder, respectively).

In addition to European vaccines, two vaccines based on Far-Eastern subtype of TBEV are registered in Russia (TBE-Moscow and EnceVir); also in these vaccines viruses are grown in chick embryo cells and are inactivated with formalin. Another vaccine, which is produced and used in China, is also based on the Far-Eastern subtype of TBEV[89].

Vaccination schedule

Several vaccination schedules exist; all of them consist of primary (basic) vaccination followed by booster doses. For a complete primary (basic) vaccination three doses, usually with an interval of 1-3 and 5-12 mo between first and second, and second and third dose, respectively, is required. Immunity is maintained with booster doses: the first booster dose is administered 3 years after completion of the primary vaccination, later on one dose is needed every 5 years. Due to the deterioration of the immune response in persons aged > 60 years (FSME IMMUN) or > 50 years (Encepur) boosters are recommended at 3 years intervals in this age group[89].

Vaccination can begin at any time, but immunization with the first two doses is preferably carried out in the winter months to achieve protection before tick activity. Reports on individual cases of severe forms of TBE in subjects who had received only one dose of vaccine against TBE, had historically been an additional reason to start vaccination (receipt the first two doses) in winter. Subsequent information showed that incomplete vaccination does not pose an increased risk for severe disease as compared to unvaccinated persons of the comparable age.

When it is desired to achieve protection in a short time, "fast schedule" can be used in accordance with the manufacturers' instructions[3,4,85]. In contrast to classic approach, in "rapid vaccination" the second dose is usually administered 14 d instead of 1 to 3 mo after the first dose. Protection efficacy is comparable to that seen after classical schedule; however, scientific data on "quick schedule" is less comprehensive than for the conventional approach.

In a person who had not received the recommended doses according to the schedule but with longer intervals, vaccination does not need to be started again from the very beginning but continue with missing doses[93]. Example: if someone had received only two doses of TBE vaccine 5 years ago and afterwards forgot to get the third dose, the vaccination should proceed with the third dose of basic vaccination and then with booster doses according to schedule. Or: a person who had received complete basic vaccination but for 15 years had not obtained any booster dose, does not need to repeat basic vaccination but just get the first booster dose and then continue immunization with booster doses according to the recommended schedule. Longer intervals between doses generally do not reduce antibody concentrations after completion of TBE vaccination, but protection in the period before the delayed dose is less reliable[93].

Persons who had acquired TBE are esteemed to be protected against the disease and do not need vaccination.

Mode of application, dosages

TBE vaccine is administered intramuscularly into the deltoid muscle; in young children it can be given in the anterolateral thigh. The vaccine may be administered simultaneously with other vaccines (live or inactivated) but not on the same place[89].

Doses depend upon the age of the recipient; the age limits for vaccines available in Europe slightly differ. In subjects younger than 16 years, the dose of the FSME-IMMUN vaccine is 0.25 mL, while for persons who have 16 years or are older 0.5 mL is recommended; in subjects younger than 12 years, the dose of the Encepur vaccine is 0.25 mL, while in older the dosage is 0.5 mL.

Efficacy and safety

Both European vaccines are safe and effective; particularly voluminous data exist for FSME-IMMUN vaccine of which more than 100 million doses were used.

Fourteen days after the second dose of basic vaccination protective antibodies develop in about 85% of the subjects, while after three doses more than 98% of persons with normal immunity are protected[89].

In Austria, where the vast majority of the population is vaccinated, the incidence of TBE declined dramatically. The estimated protection after vaccination (field

effectiveness) is more than 98% for persons vaccinated according to the recommended program and more than 90% for those who received basic vaccination, but were later not vaccinated according to the planned schedule[94].

Side effects are mild and relatively rare. They are more frequent after the first dose of vaccine than with later doses. Most commonly local pain and tenderness on pressure at the injection site take place; redness and swelling occur less often. Short-term fever after vaccination is rare in adults but relatively common in young children. Neurological complications are very rare[89].

Storage

The vaccine must be stored in a refrigerator at a temperature between 2 ℃ and 8 ℃. Storage at higher temperatures and freezing are not suitable[89].

Limitations and contraindications

Vaccination is not carried out in subjects with acute febrile illness.

Vaccination is contraindicated in the case of: (1) A severe allergic reaction after previous dose of TBE vaccine; (2) Information on severe allergic reactions to vaccine components (in addition to the active ingredients, TBE vaccine also contains remnants of formaldehyde, neomycin, gentamicin and protamine sulfate); and (3) Anaphylactic hypersensitivity to eggs (TBE viruses are grown in chick embryo fibroblast cells).

Controlled clinical trials to assess the safety of TBE vaccine during pregnancy are not available; thus, pregnant women are vaccinated only after a careful individual assessment of the potential risks and benefits. There is also no sufficient data on the safety of vaccination during lactation. It is not known whether the vaccine components are excreted in human milk or not, however, mother's antibodies produced after vaccination against TBE are probably present in the milk and consequently breast-feeding baby may come in contact with them. Given that TBE vaccines are based on inactivated virus, the damage of fetus or breast-feeding child is highly unlikely.

Although there is no evidence that vaccination may trigger autoimmunity or worsen the course of autoimmune diseases, caution is needed in subjects with an autoimmune disease since information to ensure the safety of vaccination in this group of patients is limited[89].

The manufacturer of the vaccine and some recommendations suggest that in immunocompromised persons the efficacy of vaccination is verified by serological testing approximately four weeks after the second dose, and that-in case of an inadequate antibody response-the second dose is repeated and followed by the third dose in accordance with the normal vaccination schedule. According to some suggestions similar approach may apply also to all subsequent doses. Although the approach appears logical, there is no convincing clinical data to substantiate its use. As a rule the effectiveness of protection after vaccination against TBE is not verified by the detection of antibodies against TBEV in serum.

CONCLUSION

TBE is an important tick-borne central nervous system infection in Europe and Asia. Due to relatively severe clinical course combined with the absence of etiologic treatment, considerable proportion of patients with incomplete recovery after acute illness and increasing incidence, it represents a growing (public) health problem that could be substantially reduced with vaccination.

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Multidetector computed tomography of temporomandibular joint: A road less travelled

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Abstract

This article reviews the imaging anatomy of temporomandibular joint (TMJ), describes the technique of multi-detector computed tomography (MDCT) of the TMJ, and describes in detail various osseous pathologic afflictions affecting the joint. Traumatic injuries affecting the mandibular condyle are most common, followed by joint ankylosis as a sequel to arthritis. The congenital anomalies are less frequent, hemifacial microsomia being the most commonly encountered anomaly involving the TMJ. Neoplastic afflictions of TMJ are distinctly uncommon, osteochondroma being one of the most common lesions. MDCT enables comprehensive evaluation of osseous afflictions of TMJ, and is a valuable tool for surgical planning. Sagittal, coronal and 3D reformatted images well depict osseous TMJ lesions, and their relationship to adjacent structures.

Key words: Temporomandibular joint; Temporomandibular joint trauma; Congenital anomalies of temporomandibular joint; Temporomandibular joint arthritis; Multi-detector computed tomography

Core tip: This pictorial review describes the common as well as uncommon bony afflictions of the temporomandibular joint (TMJ) with classical images. Bony afflictions of the TMJ constitute a significant bulk of lesions around this joint. However, very little literature is available on imaging evaluation of non-articular disc, osseous disorders. Computed tomography (CT) is the workhorse for evaluation of osseous lesions around the joint and this article focuses on the CT evaluation of these lesions, and also on optimal imaging strategy, which is essential for surgical planning.

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INTRODUCTION

Temporomandibular joint (TMJ) is a complex joint essential for the uniquely human -function of speech[1], and the functions of mastication and swallowing. A number of congenital, traumatic, inflammatory, and rarely neoplastic disorders can affect the TMJ causing symptoms like pain, trismus, malocclusion and facial asymmetry. It is crucial to distinguish between different TMJ disorders as the etiology of the disorder determines the clinical course, management and prognosis.

It has been difficult to evaluate the TMJ on conventional radiographs because of overlap by bones of skull base and face. With the advent of multidetector row computed tomography (CT) technology, it has become possible to acquire thin slices, do multiplanar reformatted reconstructions, and hence image the joint in exquisite detail.

ANATOMY

The TMJ is composed of osseous and soft tissue components[2,3]. The osseous component is formed by the temporal bone superiorly that forms the glenoid fossa and the articular tubercle, and by the mandibular condyle inferiorly. The joint capsule and the articular disc are the soft tissue components of the joint. The articular disc has a thin central part and thick anterior and posterior bands[3,4].

MULTI-DETECTOR CT TECHNIQUE

The patient lies supine on the CT table and is instructed not to move or swallow during the scanning process. The scan should start at the level of inferior orbital margin and end at the level of tip of the chin. The scan area must include the external auditory canals. Slice thickness and slice interval for acquisition should be 0.5-1 mm. Sagittal images are reconstructed from the raw data perpendicular to the plane of mandibular condyles as seen on axial plane, and coronal images are reconstructed parallel to the condyles as seen on axial plane (Figure 1), with a slice thickness and inter-slice gap of 2-3 mm[5].

TEMPOROMANDIBULAR DISORDERS

The term "Temporomandibular disorders" encompasses a variety of lesions which involve the TMJ, muscles of mastication, and the adjacent musculoskeletal structures of the head and neck[6]. Most patients with Temporomandibular disorders are children and young adults (less than 20 years of age in our experience). The articular disc, the masticatory muscles and other soft tissues around the TMJ are best evaluated by MRI, and are beyond the scope of discussion of the present article. The other groups of disorders affect the glenoid fossa and the mandibular condyle and include a spectrum of congenital, traumatic, inflammatory and neoplastic diseases, and these are discussed in detail here.

Congenital and developmental disorders

Embryology: The TMJ develops between 7 and 11 wk of gestation. The mandibular condyle and the squamous temporal bone are formed by intramembranous ossification and the articular disc is formed by condensation of mesenchyme in the region of TMJ[7]. Hypoplastic mandibles are seen in Turner syndrome, hemifacial atrophy, and Silver Russell syndrome (a syndrome of intrauterine and post natal growth retardation, facial dysmorphism and clinodactyly) and whereas hyperplastic mandibles are seen in Marfaan's syndrome and Proteus syndrome (a syndrome characterized by highly variable, asymmetric overgrowth of tissues that may include macrodactyly, vertebral anomalies, connective tissue nevi and vascular malformations).

Hemifacial Microsomia (Goldenhaar syndrome-Oculo-Auriculo-Vertebral syndrome): Hemifacial microsomia is a developmental anomaly that affects 1 in every 5600 live births[8]. It is the second most common developmental anomaly of the face and cranium after cleft lip and cleft palate[8]. It arises due to aberrant development of the first branchial membrane and the first and second branchial arches. Abnormal development of the mandible, nose, ear, lip and soft palate are the hallmarks of this condition (Figure 2); associated anomalies include upper limb defects, tetralogy of Fallot, CNS malformations, renal agenesis or malposition, and speech and hearing disorders[8,9]. Asymmetric development of the mandible is the sine qua non for the diagnosis of hemifacial microsomia Association with chromosomal anomalies as deletions of 5p, 6q, duplications of 22q, and trisomies 7, 9, 18, 22, and maternal use of drugs as thalidomide and retinoic acid have been described[8,9].

An accurate pre-operative assessment of the mandible is mandatory for good surgical outcomes and multi-detector computed tomography (MDCT) plays a valuable role here by depicting the anatomy of the mandible and the glenoid fossa in exquisite detail. The OMENS classification system (Orbital asymmetry, mandibular hypoplasia, ear deformity, nerve involvement, soft tissue abnormality)[10,11] is used to describe the mandible in patients with hemifacial microsomia-the degree of dysmorphism increases from grade 1 to grade 5. Grade 1 is a normally shaped but small mandible; Grade 2 is a small mandible with abnormal shape, with Grade 2A assigned to a condyle in normal position and Grade 2B assigned to an infero-medially and anteriorly displaced condyle; Grade 3 is complete aplasia of the ramus and condyle, glenoid fossa and TMJ.

Non syndromic congenital mandibular hypoplasia: Mandibular hypoplasia is a common craniofacial

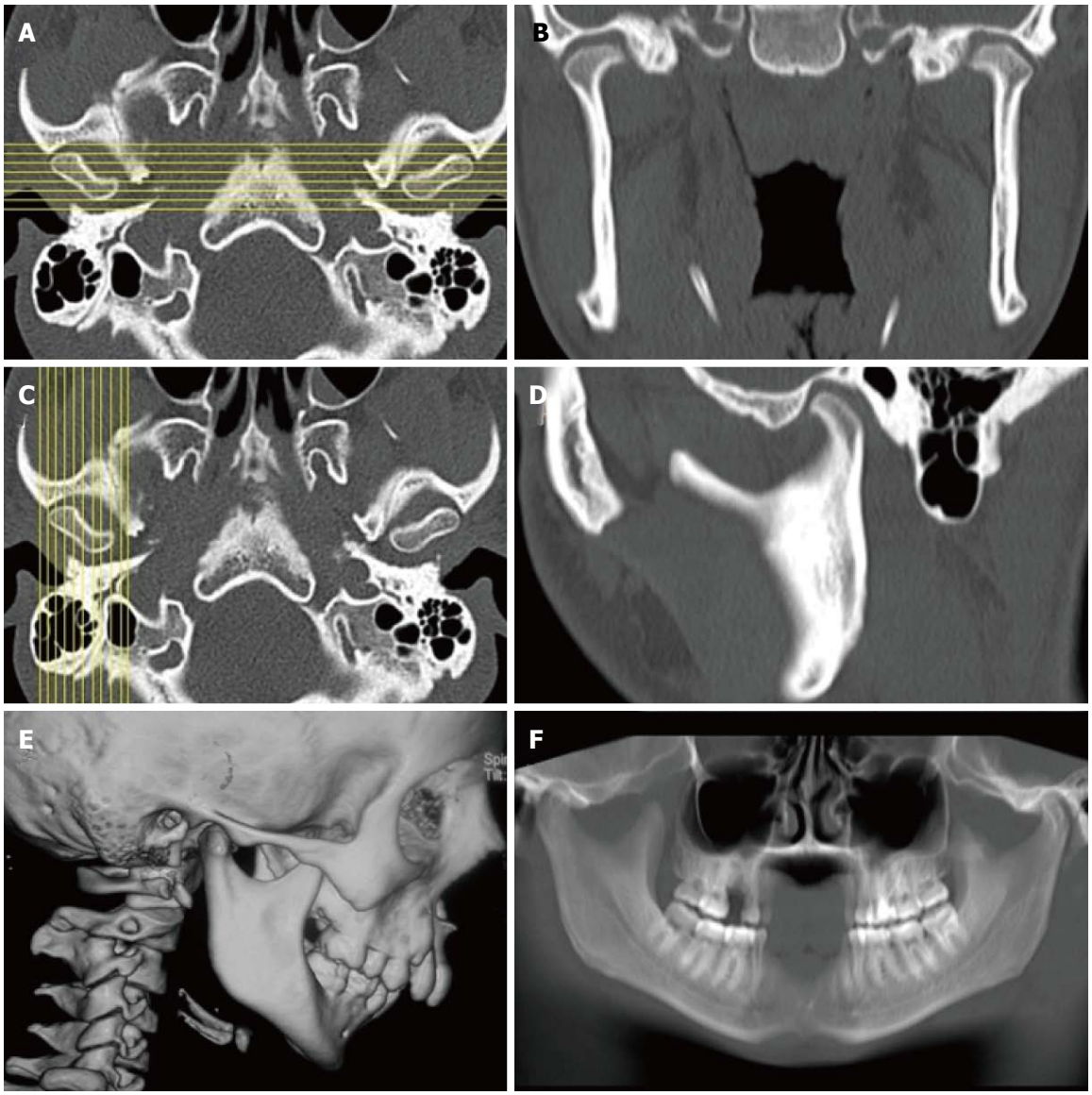

Figure 1 Technique of reconstruction. A, B: Sagittal images are reconstructed perpendicular to the plane of the glenoid fossa; C, D: Coronal images are reconstructed perpendicular to the plane of sagittal images; E, F: The joint anatomy is well depicted in 3D reformatted and reconstructed panoramic image.

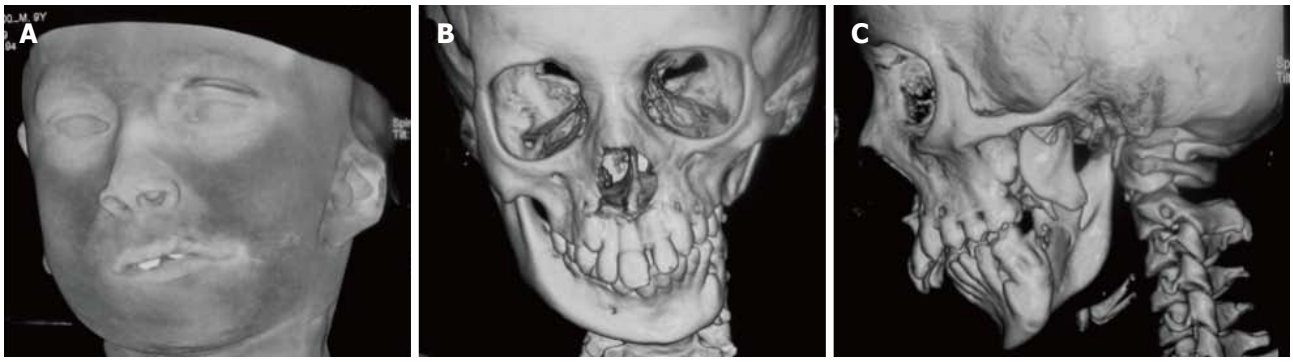

Figure 2 Goldenhaar Syndrome in a young boy. Shaded surface display (A) image shows micrognathia, downward slanting left palpebral fissure and hypoplastic left pinna. 3D reformatted images show the normal right hemimandible (B) and hypoplastic body and ramus of left hemimandible with underdeveloped mandibular condyle and glenoid fossa (C).

anomaly and can be congenital, developmental, or acquired in origin, and may be unilateral or bilateral (Figure 3). Acquired causes of hypoplasia include oncologic defects, radiation damage, trauma, and hemifacial atrophy. Treatment consists of initial surgical moving of mandibular components into predetermined

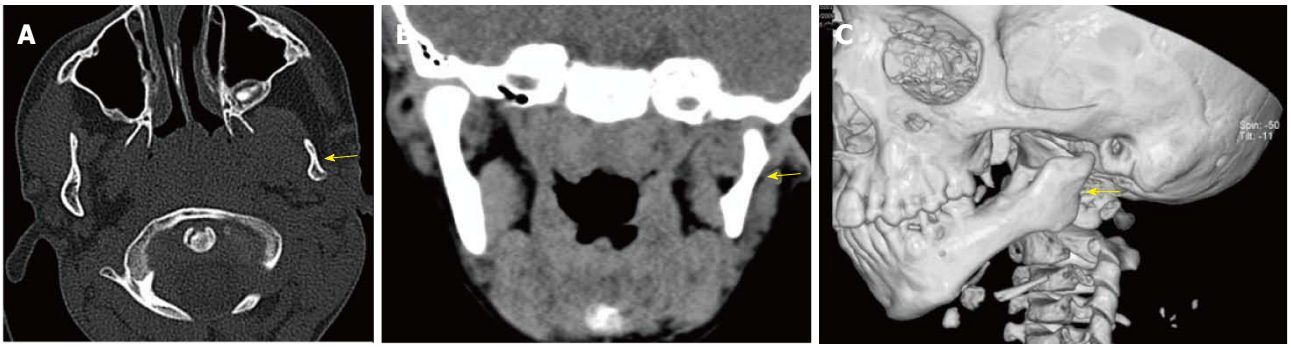

Figure 3 Mandibular hypoplasia in a 7-year-old boy. Axial (A), coronal (B) and 3D reformatted (C) images show show small left hemi-mandible with hypoplastic mandibular condyle and glenoid fossa (arrows).

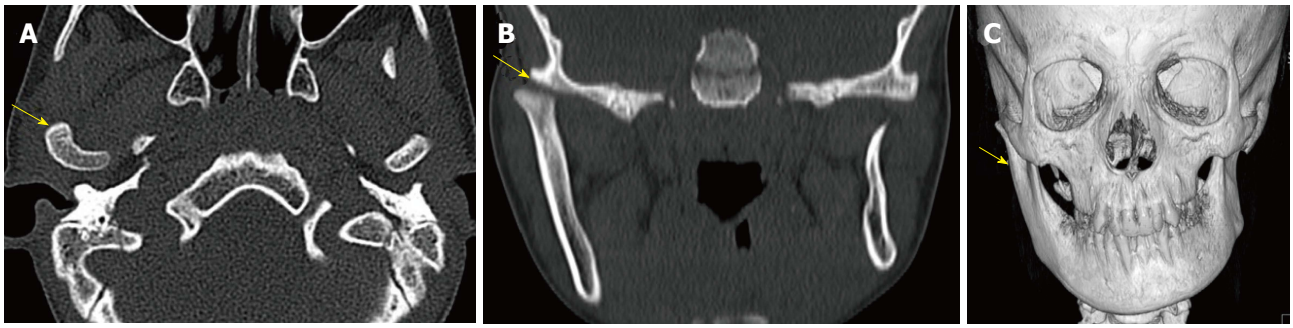

Figure 4 Condylar hyperplasia. Axial (A), coronal (B) and 3D reformatted images (C) show enlarged left mandibular ramus and condyle (arrows).

optimum followed by orthodontic treatment to correct dental malocclusion. MDCT provides the road map for surgical planning.

Condylar hyperplasia: Condylar hyperplasia is a disorder of uncertain etiology seen in patients between the ages of 11 and 30 years[12]. The mandibular condyle continues to grow relentlessly even though the normal growth period has ended (Figure 4). The condition can be treated by condylectomy during the period of active growth; and surgical mandibular repositioning for persisting symptoms after growth has stopped. MDCT defines the size of the condyle, and its relationship to adjacent structures.

Osteochondroma may cause similar symptoms and signs as condylar hyperplasia and must be distinguished. Osteochondromas are seen as localized to a part of the condyle on CT, whereas diffuse enlargement of the condyle is seen in condylar hyperplasia.

Trauma

MDCT with axial and coronal reformatted images is the modality of choice for evaluation of acute trauma to TMJ[3,13]. The temporomandibular joints, the mandible, the maxilla, and the paranasal sinuses can be evaluated in a single scan. The Strasbourg Osteosynthesis Research Group classification defines 3 main types of condylar fractures: diacapitular fracture through the condylar head (DF), fracture of the neck of the condyle, and fracture of the base of the condyle (CBF)[14]. Fractures of the condylar head are further classified as-extracapsular or intracapsular; undisplaced or displaced (Figure 5). On imaging, the radiologist should describe the type of fracture, relationship of the fractured fragment to the mandible as well as glenoid fossa, angulation, presence of vertical compression, and damage to the soft tissues, *i.e.*, the articular disc and the joint capsule.

Condylar fractures lead to anterior and medial displacement of the condylar process due to unbalanced action of the lateral pterygoid muscle which inserts into the condylar process, articular disc and capsule of TM joint. On clinical examination, the mandible is seen deviated towards the side of fracture with anterior open bite and malocclusion. If a condylar fracture is diagnosed, one must carefully look for associated fractures of skull base that can lead to CSF otorrhea and complications as meningitis.

Most of the fractures of condylar head are managed by closed reduction. The indications for open reduction are: displacement of the condyle into the external auditory canal or middle cranial fossa, lateral displacement of the condyle, inability to occlude, open wound with gross contamination or foreign body within the joint[14,15].

Arthritides

The most common arthritides affecting the TMJ are degenerative and traumatic arthritis; rheumatoid, metabolic, infectious arthritis, and spondyloarthropathies

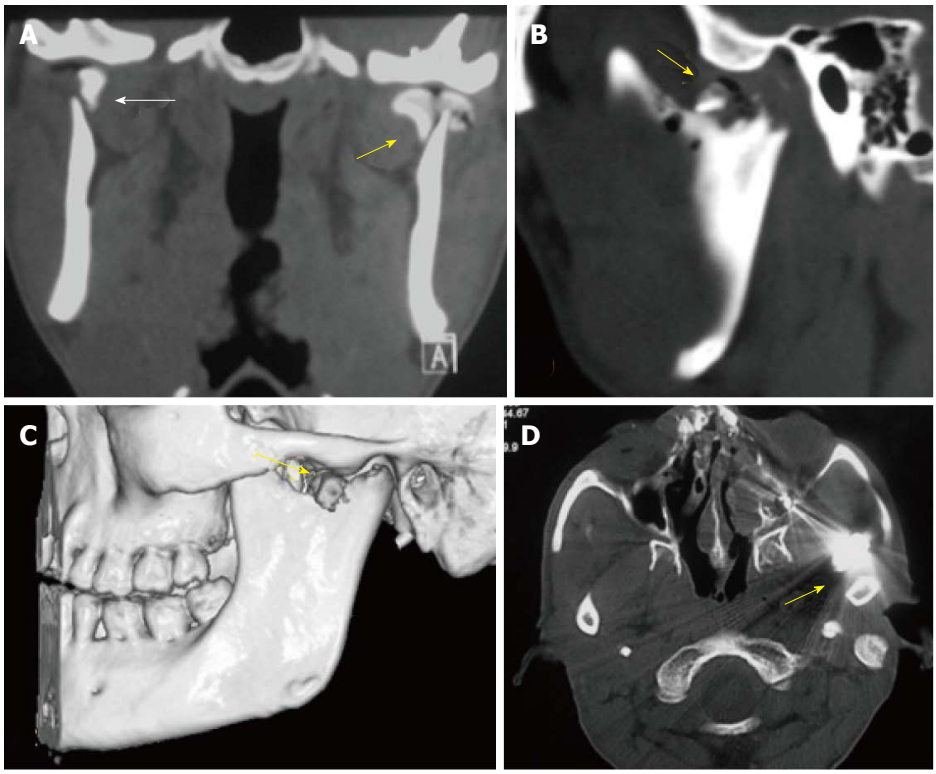

Figure 5 Condylar fractures in different patients. Coronal (A), sagittal (B) and 3D (C) reconstructed CT images depict bilateral displaced, extracapsular (right- bold arrow), and intracapsular (left- arrow) condylar head fractures with involvement of the articular surface on the left side. The intra-articular air on the left side indicates an open wound (arrow). Open joint wound with a foreign body (bullet in another patient- D) or gross contamination are indications for open surgery.

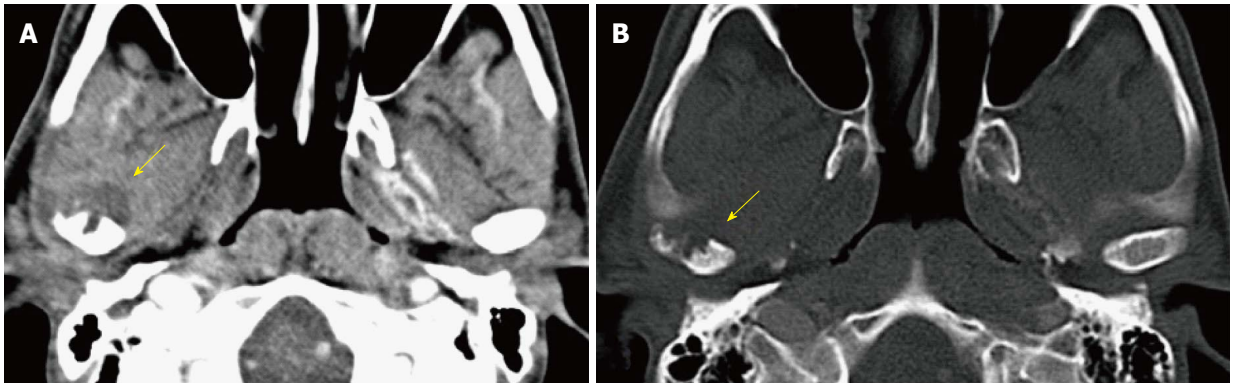

Figure 6 Pyogenic arthritis in a 33-year-old woman with fever and pain around right ear. A: Soft tissue edema with loss of fat planes (arrow) around right temporomandibular joint is seen in axial computed tomography (CT) image (A); B: Bony erosions in the condyle are well seen in axial CT image in bone window setting (arrow).

are less seen frequently[3,16,17]. Degenerative arthritis is secondary to internal derangement of the joint due to articular disc abnormalities. Arthritis after trauma is seen in untreated, ignored fractures of the condyle and these frequently progress to ankylosis.

Infective arthritis is seen secondary to other infections of the head and neck (Figure 6). Patients present with an acutely painful and swollen joint with malaise, and CT reveals erosions of the articular surface of condyle and/or glenoid fossa, and inflammatory changes in surrounding muscles. Rarely, a sequestrum may form within the joint (Figure 7). If untreated, it may progress to fibrous or bony ankylosis.

Rheumatoid involvement of the TMJ is characterized by synovial proliferation and secondary erosive changes of the bone, with destruction of the condyle and articular eminence[17]. Eventually, bony ankylosis ensues with destruction of the intervening soft-tissue structures.

TMJ ankylosis

The term "ankylosis" is defined as joint fixation or fusion leading to chronic, painless restriction of joint movements. It may also cause facial asymmetry and deviation. Trauma and infection are the most common causes of TMJ ankylosis[18]. The initial traumatic insult may not be apparent and intra-articular hematoma leads to scarring and excessive bone formation, eventually causing fusion of the joint. TM joint infection is mostly due to contiguous spread from otitis media and mastoiditis, hematogenous spread is less common. Other rare causes include inflammatory arthritis (juvenile rheumatoid arthritis[19], psoriatic arthritis[20], ankylosing spondylitis[21]), or iatrogenic after surgery.

TMJ ankylosis can be classified into two categories:

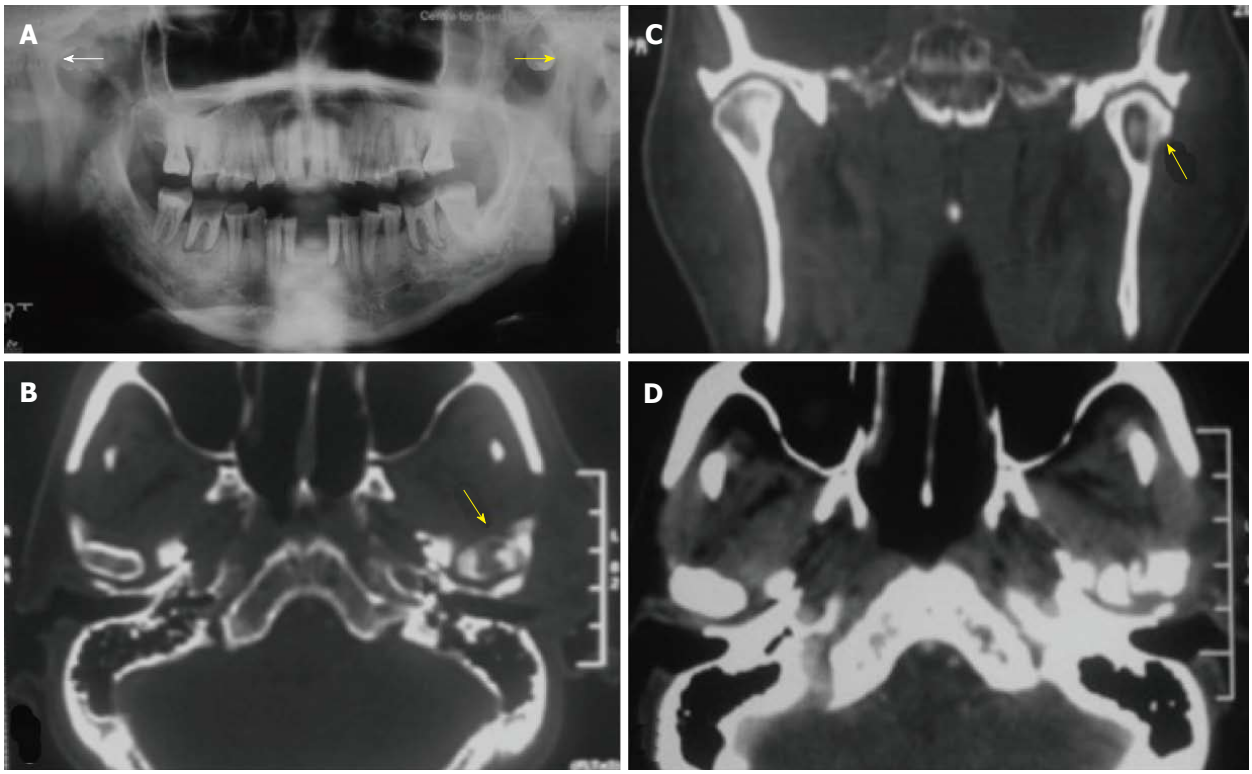

Figure 7 Chronic osteomyelitis of temporomandibular joint in a 55-year-old patient. Sclerosis of left mandibular condyle is seen in panorex radiograph (A); a bony sequestrum is seen within the left mandibular condyle in axial (B) and coronal (C) reformatted computed tomography (CT) images; axial CT section in soft tissue window (D) shows inflammatory changes in tissues around the temporomandibular joint.

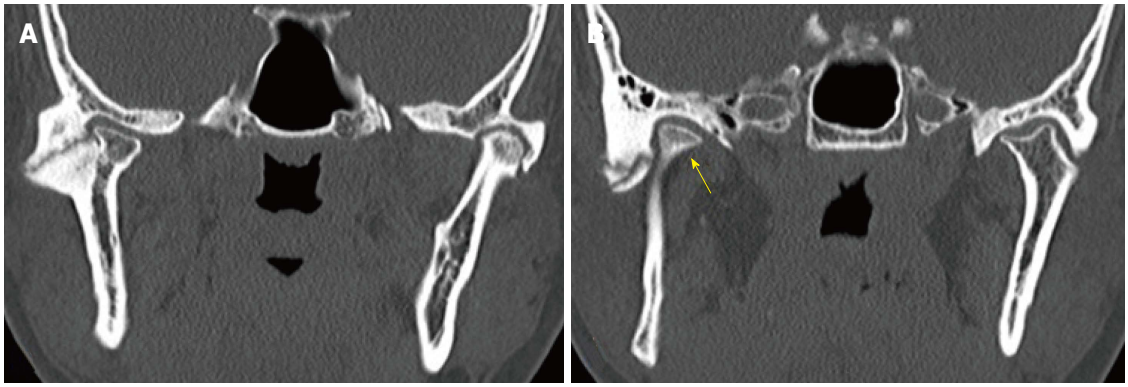

Figure 8 Post traumatic ankylosis in a 28-year-old male patient. Coronal reformatted multi-detector computed tomography images demonstrate ankylosis and pseudo-joint formation in both temporomandibular joints (A); mandibular condyle is displaced anteriorly and medially (B). The referring clinician needs to know the relationship of the condylar mass to adjacent skull base structures.

type Ⅰ, medially angulated condyle with deformed articular fossa and a mild-to-moderate amount of new bone formation; and type Ⅱ, no recognizable condyle or fossa but instead a large mass of new bone[22]. Type I is etiology-specific and seen after antecedent trauma. TMJ ankylosis is surgically treated by excision of the ankylosis, with or without autogenic, allogeneic or alloplastic graft replacement. MDCT provides a detailed three-dimensional image of the condylar mass and its relationship to adjacent structures of the skull base, which is essential for surgical planning (Figure 8).

Tumors and tumor like conditions

Neoplastic lesions rarely involve the TMJ. Osteochondroma, osteoma, eosinophilic granuloma, chondrosarcoma and bone cysts have been described in the literature[3,23]. Synovial chondromatosis, pigmented villonodular synovitis (PVNS), pseudogout, fibrous dysplasia, hyperparathyroidism and giant cell reparative granuloma are some tumor like conditions which may involve the TMJ[23]. Contiguous tumors of external ear and parotid, and metastases from carcinoma breast, kidney, lung, colon and rectum and lymphoma may also

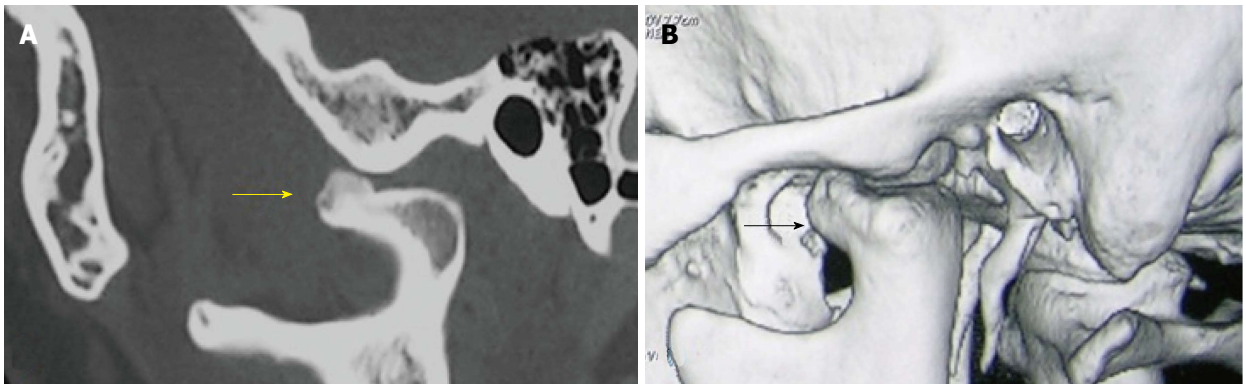

Figure 9 Osteochondroma in a 32-year-old man presenting with trismus. Sagittal (A) and 3D reconstructed (B) images demonstrate a bony outgrowth arising from the mandibular condyle (arrow), just beneath the articular eminence.

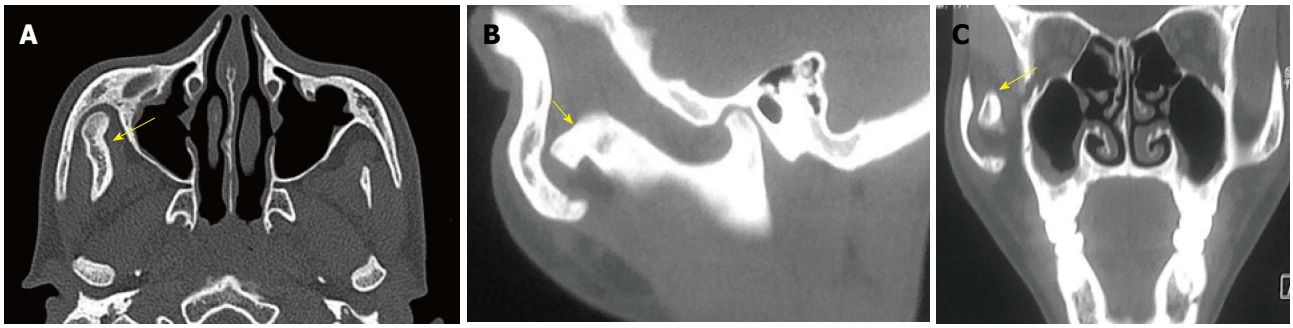

Figure 10 Jacob's disease in a 17-year-old boy presenting with restricted mouth opening. Axial (A), sagittal (B) reformatted computed tomography images depict "pseudo-joint formation" between enlarged coronoid process of mandible and zygomatic arch (arrows).

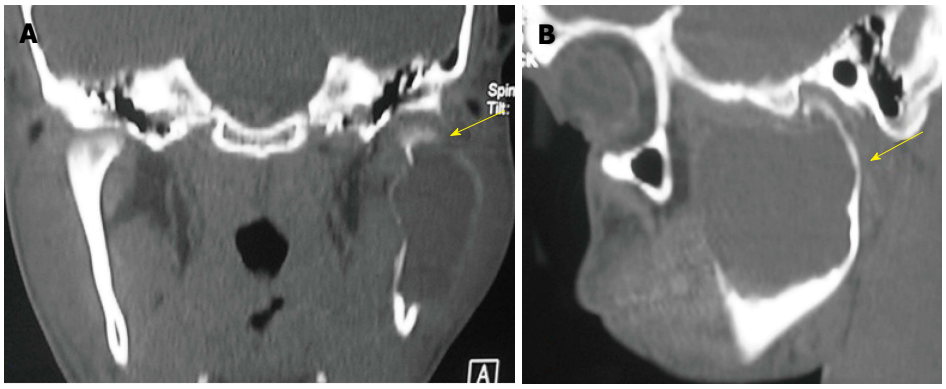

Figure 11 Aneurysmal bone cyst of the mandible in a 17-year-old boy present with left cheek swelling since one year. Coronal (A) and sagittal (B) reformatted computed tomography images depict a large, expansile, lytic lesion involving left mandibular condyle and ramus.

involve TMJ[3].

Of the above tumors, osteochondroma of the mandible deserves a special mention (Figure 9). It is an indolent tumor that is seen at the condyle or the coronoid process tip. It may become large enough to present as a facial mass or it may cause trismus. An MRI should be performed if there is a rapid change in the size of mass or pain develops in a previously asymptomatic mass. A cartilaginous cap that exceeds 2 cm in thickness on MRI is suspicious for a malignant transformation to chondrosarcoma, although such a transformation is quite rare.

Jacob's disease (first described by Jacob in 1899), is a rare entity in which a pseudo-joint develops between a sessile coronoid process osteochondroma or a hyperplastic coronoid process, and the zygomatic process of maxilla causing severe restriction of jaw movement[24]. The length of the coronoid processes can be accurately measured on MDCT and changes in the zygomatic arch are also well depicted (Figure 10).

Aneurysmal bone cyst of the TMJ is another uncommon, tumor-like non-neoplastic affliction of the Temporomandibular joint (Figure 11). It usually affects children and young adults less than 20 years of age.

The patients present with slowly increasing swelling in the cheek and preauricular region. The lesion may be unilocular or multilocular on imaging and has a non-specific imaging appearance. It is characterized by presence of well defined blood spaces lined by endothelium on histology. MDCT evaluates the lesion and defines its extent of (involvement of condyle, mandibular ramus, temporal bone, presence of breach of cortex) and is necessary for deciding surgical management (curettage *vs* block resection).

CONCLUSION

Trauma, internal derangement with possible sequelae (osteoarthritis), and inflammation are the most common of pathologic conditions involving the TMJ. MDCT enables comprehensive evaluation of osseous afflictions of TM joint, and is a valuable tool for surgical planning.

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SYSTEMATIC REVIEWS

Intraoperative laparoscopic complications for urological cancer procedures

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Data sharing: No additional data are available in order of the kind of narrative literature review.

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Abstract

AIM: To structure the rate of intraoperative complications that requires an intraoperative or perioperative resolution.

METHODS: We perform a literature review of Medline database. The research was focused on intraoperative laparoscopic procedures inside the field of urological oncology. General rate of perioperative complications in laparoscopic urologic surgery is described to be around 12.4%. Most of the manuscripts published do not make differences between pure intraoperative, intraoperative with postoperative consequences and postoperative complications.

RESULTS: We expose a narrative statement of complications, possible solutions and possible preventions for most frequent retroperitoneal and pelvic laparoscopic surgery. We expose the results with the following order: retroperitoneal laparoscopic surgery (radical nephrectomy, partial nephrectomy, nephroureterectomy and adrenalectomy) and pelvic laparoscopic surgery (radical prostatectomy and radical cystectomy).

CONCLUSION: Intraoperative complications vary from different series. More scheduled reports should be done in order to better understand the real rates of complications.

Key words: Intraoperative complications; Laparoscopy; Surgical complication; Urology; Cancer

Core tip: We decided to perform this literature review to light and to arrange the intraoperative rates of laparoscopic urological cancer complications, which are such as messy in the different manuscripts published. This idea leaves from an urological team which performs more than 150 laparoscopic procedures per year since 2005.

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INTRODUCTION

The consequences of errors during laparoscopic surgery are unpredictable and can vary from no adverse effects to fatal events. An adverse event is the result of the combination of an active failure and a penetration of all the defences and safeguards which ought to be in place to prevent it, in these terms, safe surgery is dependent on systems designed to prevent all errors. Most of the intraoperative complications are consequence of consecutive and cumulative errors without individual consequence but globally result into a complication. Surgeon, team, technique, technology/device and organization errors are different kind of mistakes that can finally explain an intraoperative complication[1].

Some risk factors have demonstrated statistical significance to develop a surgical complication related to laparoscopic surgery for urological cancer: type of procedure (*i.e.,* partial nephrectomy, radical cystectomy or radical prostatectomy), abnormal renal function and more than 4 h of surgery. Other conditions as body mass index, ASA score, previous abdominal surgery or surgeon experience have not demonstrated significant association. However there is a trend to decrease of complications when surgical experience increases[2].

General rate of perioperative complications in laparoscopic urologic surgery is 12.4%. The rate of complication of Laparoscopic Radical Nephrectomy is 14%, Laparoscopic Partial Nephrectomy is 23%, Laparoscopic Nephroureterectomy is 22.1%, Laparoscopic adrenalectomy 10%, Radical Prostatectomy 6.7% and Radical cystectomy 33.3%-48.3%. General Intraoperative complications were rated in 4.7%. Nevertheless, most of the manuscripts published do not make differences between pure intraoperative, intraoperative with postoperative consequences and postoperative[2,3].

We pretend to review the rate of intraoperative complications that requires an intraoperative or perioperative resolution. In some cases we describe the possible resolution and the possible prevention.

MATERIALS AND METHODS

A literature review of Medline database was completed. There were no limits of date. English published papers were reviewed, for non-English papers only the abstract were visualized. A combination of the following key words was used: laparoscopy, intraoperative complications, pathologic processes, radical nephrectomy, partial nephrectomy, nephroureterectomy, adrenalectomy, radical prostatectomy and radical cystectomy. From the diverse literature manuscripts and abstracts searched, the authors selected 40 manuscripts to review. The research was focused on intraoperatory laparoscopic procedures inside the field of urological oncology. Laparoscopic complications for other non-oncological conditions were excluded (pyeloplasty, simple nephrectomy, living donor nephrectomy, adenomectomy or bladder augmentation), likewise postoperative complications were also excluded. Only pure laparoscopic procedures were included; removing hand assisted, ablative or single port approaches. Most of the manuscripts were high volume retrospective monoinstitution series, there are some review articles, a few meta-analysis of retrospective data and single cases of extremely rare conditions.

RESULTS

We expose the results with the following order: retroperitoneal laparoscopic surgery (radical nephrectomy, partial nephrectomy, nephroureterectomy and adrenalectomy) and pelvic laparoscopic surgery (radical prostatectomy and radical cystectomy).

Retroperitoneal laparoscopic surgery

Radical nephrectomy: The general rate of intraoperatory complications during a laparoscopic radical nephrectomy varies from 1.7% to 16%. The conversion to open surgery due to notorious bleeding or technical difficulty is 2.5%. The intraoperatory complications can be systematized as follows.

The rate of complications linked with trocar placement varies from 0.09% to 0.27%. There are no normalized devices to perform the pneumoperitoneum and most of the surgeons use the Veress needle, the optical port and the Hasson port, with a rate of injuries of 0.18%, 0.27% and 0.09% respectively[4].

The injuries reported are bowel, solid organs, vessels from the abdominal wall and diaphragm. Intraoperatory laparoscopic repair by suturing and hemostatic agents (fibrin glue, bio glue, bio patches) are used in many cases[5].

The Mayo Clinic series described two complications related to specimen handling and retrieval. One specimen was fractured by the Endo-Catch bag when the kidney was not completely entrapped prior to closure. The other specimen was a partial nephrectomy in which part of the tumor was fragmented during dissection. In this fragmented specimen fractures were seen within the tumor but the whole mass was still completely removed and surgical margin status was negative[6].

It is the most frequent intraoperative complication with a rate of 2.37%[3]. Most described lesions are small and large bowel injuries during the aperture of the Toldt line (0.8%) and duodenum during the right hilium dissection, these lesions should be repaired immediately when noticed and in most cases intracorporeal knots are adequate, minor thermal injuries may be managed with observation. Resection and anastomosis or ileostomy/colostomy is in some cases necessary. Prevention of these lesions could be maintaining the electric devices with 1-2 cm far from the bowel during the aperture of the field, followed by

blunt dissection as aspiration devices when the proper plane line is opened. There are also described indirect lesions of the bowel by local ischemia due to accidental enclosure of mesenteric artery; it required end to end vascular anastomosis and bowel resection. It is also reported partial ischemic lesions of splenic flexure of large bowel managed conservatively[6,7].

Laparoscopic operations involving the left kidney and adrenal gland may be complicated by pancreatic injury owing to the proximity of the pancreatic tail to the surgical field[8]. Pancreatic injury is described in 0.69% left laparoscopic urology procedures and 2.1% during left radical nephrectomy. No cases of injury of the right side of pancreas have been described for urological procedures. In most cases the injury was not noticed during the intervention and the solution was conservative with nasogastric tube, parenteral nutrition and somatostatin, according with the general surgeon experience, most of the pancreatic fistulas closes within 2 wk[9]. If detected intraoperatory, endovascular stapler can be used or over sewn, also tissue glue can be used as adjunctive agent[8]. Possible prevention methods are a complete mobilization of spleen, transecting the splenophrenic attachments and allowing the pancreas and spleen to move together; just like being attentive when the features significantly distort the normal anatomy.

The injury of the spleen is reported in 1.4% of left radical nephrectomies. In most cases can be managed conservatively with electric devices and haemostatic agents but if the lesion is large might requires open conversion. The prevention could be a gentle traction to avoid tearing[6].

Liver injury and gallbladder injury are described in 1.1% and 1.4% respectively. There are no reports found about the mechanism of injury (except of the optic port placement[7]), management and prevention[3].

Chylous ascites is a rare complication caused by unrecognized interruption of the cisterna chyli or other major retroperitoneal lymphatic channels and establishment of a lymphoperitoneal fistula. It is described in surgery for renal cancer as the third cause[10]. The overall incidence of chylous ascites was 5.1% (32 of 622 cases), including 4 severe refractory cases (0.6%). The incidence was higher in those who underwent lymphadenectomy (13.9% with lymphadenectomy and 4.0% without lymphadenectomy). Only 1 patient underwent explorative laparotomy due to persistent severe chylous ascites despite 8-wk conservative management. Most cases were successfully managed conservatively by total parenteral nutrition and a low fat diet. To prevent this complication the authors suggested meticulous clipping of all perihilar and retroperitoneal fibrous fatty tissue during major vessel dissection, especially for left nephrectomy or extensive lymphadenectomy[11].

There are three mechanical devices to control the renal hilium: vascular staples, metallic clips and polymer clips. It is described the estimated total device-related complications in 1.1% for staples, 2.0% for metallic clips and 0.2% for polymer clips.

Food and Drug Administration report of 2172 cases does not conclude that one device is safer than another. Analysing the intraoperative complications over the total complications there are 352 failures noted: staples (63%-223 total complications: 1% death, 22% severe bleeding, conversion to open surgery 35%), metallic clips (33%-111 total complications: 1% death, 2% severe bleeding, conversion to open surgery 7%) and polymer clips (5%-18 total complications: 17% death, 22% severe bleeding, conversion to open surgery 44%)[12].

The leading causes of failure reported in stapling devices were staple line malformation (47%) and locking up (29%). In titanium clips, jamming/feeding difficulties (27%) and trouble closing or "scissoring" clips (26%) were the most common. In locking clips, dislodgement (44%) was most frequently reported.

The presence of accessory polar arteries not identified during the hilium dissection is a common cause of bleeding. Grasping the stump and positioning a clip are described as an intracorporeal solution[5].

Sometimes calcified and atherosclerotic arteries are the cause of absence of closure or arterial rupture. Preoperative TC examination is mandatory and these conditions must be taken into account because intraoperative resolution is difficult to perform[7].

Defining intraoperative haemorrhage as bleeding which requires intraoperative blood transfusion or open conversion, it was described as 2.2%-2.8% in the biggest series[2] and it is the second most frequent intraoperative complication for radical nephrectomy. Separately, venous bleeding is 1.8% and arterial bleeding is 1%. It is one of the most described causes of open conversion. Increasing pneumoperitoneum pressure and using Haemostatic agents (bio glue, fibrin glue, fibrin patches) could be used as first option; intracorporeal knotting solution requires high volume of cases and huge experience. No air emboli were noted in the literature review[13].

The pleural injury is described in 0.4%-0.6% of upper abdominal laparoscopy. Eleven cases were noticed during radical nephrectomy (9 radical and 2 cytoreductive). When noticed, the surgeon can directly watch the defect or can indirectly watch the diaphragm billowing, which is named floppy diaphragm. Sometimes the damage of pleura is unnoticed anyway and sometimes is intentional because of tumoral infiltration. The rupture can be treated intracorporeally with running suture, with a technique defined by Cleveland Clinic[14]. There is also the possibility of conservative management with torax tube and postoperative surveillance. When the rupture is unnoticed the management should be conservative with thorax tube, this condition used to be done when organs as spleen or liver are covering the defect[14,15].

Partial nephrectomy: Most of the considerations for radical nephrectomy could be added to partial

nephrectomy in terms of adjacent organs and diaphragm for upper pole masses, instead, the control of the hilum and the parenchymal bleeding have distinctive features for this kind of technique. The rate of intraoperatory complications is 5.5% and the rate of open conversion is 2%-6%[16].

There are many devices designed to control de ischaemia of the kidney (laparoscopic bulldog, Satinsky clamp and the Rumel tourniquet), including the possibility of no hilar control for small exophytic masses not exceeding the diameter of 2 cm[17]. Injury of the renal vessel may compromise the entire procedure as well as the long term results.

Severe intraoperatory bleeding is described to be 3.5%-3.8% in different laparoscopic series[18,19].

In order to prevent urine leakage and vascular fistula/pseudoaneurim, two layers of suture should be performed. First line approximation of the interstitial tissue with running suture with two absorbable clips at the beginning and the end of the suture to earn ischaemia time. Parenchyma repair is performed with a second running suture, secured with Hem-o-locks with the same plan as first described. To ensure from slipping first hem-o-lock is enforced with by a knot and two clips are sited at the end of the suture. It is also described the use of haired sutures for this procedure[16]. Replacing the knotting by Hem-o-lock sutures let speeding up the procedure. Haemostatic agents as fibrin glue, bio glue or patches can add haemostasis and minimize the bleeding.

Radical nephroureterectomy: Most of the considerations for radical nephrectomy could be added to nephroureterectomy with the special complications of nerve injury and vascular injury during the dissection of the distal ureter. There are 3 techniques described (open, endoscopic, pure laparoscopic) for the management of distal ureter and each one have got their one special intraoperative complications. The rate of intraoperatory complications is 5.4% and the rate of open conversion is 2.3%[20,21].

The complications and their prevention should be similar to radical nephrectomy, instead it is described a rate of neural injury of 0.8%, during the middle and distal ureter dissection. Also a perforation of diverticulum at sigmoid sigma was specifically reported[7].

Adrenalectomy: The average of complication during laparoscopic adrenalectomy in high volume laparoscopic series is 3.2%, varying between 2.1% and 7.8% in different laparoscopic series. The open conversion rate varies between 0.3%-9.6%, and most frequently explained by technique problems than bleeding[22-24].

For this procedure are described diaphragmatic lesions in 1.16%. The Cleveland series described 1 non intentional diaphragmatic lesion and 4 intentional lesions during a transthoracic adrenal approach. Laparoscopic repair was attempted and a goretex patch placement was performed for transthoracic adrenalectomy. A special laparoscopic technique is described for this issue[14].

It is the most common intraoperative complication described (1.3%-1.74%). The vessels involved by order are adrenal vein, cava vein and renal vein. In most cases is the cause of open conversion, when possible intracorporeal laparoscopic suturing is attempted[22].

It is the second intraoperative complication 0.04%-6.3%. The authors of the series consulted noticed 4 spleen injuries (controlled with hemostatic agents and two of them required open conversion), 2 hepatic injuries (controlled with hemostatic agents), 1 intestinal injury and 1 pancreatic laceration. Nevertheless, pancreatic injury has been described in 8.6% during left adrenalectomy[8].

Laparoscopic pelvic surgery

Laparoscopic radical prostatectomy (LRP) is probably considered the most complex technique of minimally invasive urologic surgery. Paradoxically intraoperative complications in these procedures are rare, regardless of the technique used, either transperitoneal, extra-peritoneal robot-assisted or single port. Laparoscopic radical cystectomy (LRC) is a more recent technique, with smaller series, and perhaps thereby, relatively contemporary publications report higher rates of complications. Published data are very incomplete, as most of the published series refer only to postoperative complications, without naming those occurring during surgery and resolved without further impact that increased surgical time.

Conversion to open surgery: Conversion from laparoscopic to open surgery should be considered a complication in itself, although it has many causes. The rate of conversion to open surgery for LRP is estimated at 0.2%-1%, with no demonstrated differences between classical laparoscopy and robot-assisted laparoscopy[25]. This meta-analysis shows data published since 1990, and probably in contemporary series the percentage is even lower, among other factors, because the lack of experience in open surgery in younger surgeons. In the first communications on the LRC conversion rates reached 10%[26], while contemporary series show conversion rates of 0%[27]. It is necessary to note that although the more recent series include learning curves have the advantage of the technique is mature, and surgeons already experienced in PRL.

Intestinal injuries: Bowel injuries during pelvic laparoscopy should be divided in intestinal rectal injuries and intestinal non rectal injuries. Non rectal intestinal lesions are rare in the RLP, from 0.02% to 0.14%[25]. In the series of LRC no bowel injuries are reported. It seems unlikely that no intestinal injury happen during cystectomy, so there is probably not a correct record of complications[26,27]. Overall intestinal lesions occur after the creation of pneumoperitoneum with a

Veress needle, in the blind placement of the first port, and can be largely avoided by creating the access by minilaparotomy. They can also occur in the blind part of the field, tearing with the clamps of the assistant. In these two first cases may be missed during surgery, causing significant postoperative morbidity. Less common are those caused by release of adhesions, which also tend to be diagnosed and easily treated with a suture without further consequences.

Rectal lesions occur during dissection of the backplane, usually in the most distal part of the lateral pedicles and the prostatic apex. It is the most frequent intraoperative complication of LRP and occurs in 1.1% of cases, but shows an important heterogeneity among published series, from 0.2% to 8%. This heterogeneity may be explained by the inclusion in publications of learning curves at the beginning of the art.

The series of robotic surgery show significantly lower rates of between 0.2% and 0.4%. These differences should be taken with caution, because in general the series of LRP are mature series, by surgeons with much previous experience, subsequent in time and with a very high number of cases per surgeon[28]. For anatomical reasons in LRC rectal injuries occur almost exclusively in male patients, and the authors have not found reports of rectal injury in women.

Rectal injuries reported in LRC are isolated. Older series report rates of up to 20%, but with very few cases, while contemporary series report a rate of about 0%-1%[27-30]. The data from this series, however, are probably incomplete, since only recorded injuries causing clinical complications postoperatively. Risk factors for rectal injury are: history of radiation therapy, hormone therapy, infection, previous prostate or rectal surgery and advanced tumor stage, all of them controversial. The surgeon's experience is inversely related to the risk of rectal injury, but this risk never disappears[31]. Most rectal lesions are diagnosed by direct vision, in which case it must be repaired immediately. There is consensus that the closure must be performed in two planes with absorbable suture and checked for leaks by blowing air through a rectal probe. For rectal injury during LRC, peritoneal flap placement on the rectal lesion is simple and generally must be performed, but is mandatory in cases where an orthotopic bladder is made. In the LRP interposing a flap of tissue between the bladder and rectal levels or performing a colostomy is not essential in primary watertight closures, although it seems advisable in patients with lesions larger than 2 cm or history of radiotherapy[32]. Injuries not diagnosed during surgery may present as early postoperative abdominal septic conditions, but more often they are diagnosed several days or even weeks after surgery by pneumaturia, fecaluria and urinary tract infections. An important gesture to avoid postoperative morbidity in rectal lesions is anal dilation. The rectum is a high-pressure reservoir. Anal dilatation decreases this pressure for at least the first few days, the critical period of healing[26]. Although its efficacy is not fully tested in the literature, many authors advise, and is a simple and quick gesture. Another controversial item is the utility of the previous bowel preparation, exclusively mechanical, or associating antibiotics. Although its value does not clearly shown, most authors still use[32].

For the treatment of rectal injury during prostatectomy the protocol described by Blumberg offers a simple and practical algorithm[33].

Ureteral injuries: The anatomical relations of the ureter make it susceptible to be injured during prostatectomy or cystectomy. Its frequency is very low in LRP series published between 3% and 0.04%, which is supported by population studies[34] without showing differences between the results of LRP and robot-assisted LRP. There is a clear inverse relationship with the number of cases performed by the surgeon, but often occur after overcoming the learning curve. The most common site of injury is the juxtavesical ureter; which is injured during the section of the posterior bladder neck or during dissection of the seminal vesicles[35]. If they are diagnosed during surgery, most cases require ureteral reimplantation or end to end anastomosis, which only determines an increased surgical time. The problem is that the majority of ureteral injuries go unnoticed, leading to increased postoperative morbidity, increased hospitalization time and often the need for further reoperation. As risk factors for ureteral injury during prostatectomy have been reported: history of infections, transurethral resection of the prostate, the presence of prostatic middle lobe and large prostate sizes. For more proximal lesions described risk factors include abdominal surgery, radiotherapy and extended pelvic lymphadenectomy[36]. Intraoperative ureteral injuries are not described in the literature as a complication of LRC, probably because the surgical technique can be adapted to the length of ureter feasible.

Neurological injuries: Obturator nerve injuries occur in 2% of patients underwent LRP with significant variability among published series, from 0% to 4%. In robotic frequency series appears to be less, 0.4%, but this difference does not seem significant[25].

In the series of cystectomy no injuries obturator nerves are described[37]. The injury usually occurs during the performance of pelvic lymphadenectomy, and heterogeneity of the published data is probably due to variability in the indications and extent of lymphadenectomy. If the section is made with a cold cut with not much tissue destruction can be attempted immediately by epineural nerve repair points[38]. If a thermal injury occurs is probably necessary to discard the injured nerve ends, which prevents direct anastomosis without tension. In that case have been described good results by immediate repair with interposition of a segment of sural nerve[39]. In the event that goes unnoticed is easily diagnosed in the early postoperative by the functional impairment resulting

denervation of the adductor. Treatment is then based on the rehabilitation and physiotherapy, with varying results but almost never complete.

Vascular injuries: Vascular lesions in radical prostatectomy described in the literature are rare, 0.1%-0.7% for laparoscopic surgery, and the series of robot-assisted LRP are even more rare, the 0.03%-1%[25]. In cystectomy communications vascular injuries are also punctual, but in most of the series do not appear as a complication. Among the published data, it is noteworthy Castillo's series, with a percentage of 11% intraoperative vascular lesions, including iliac venous injury, iliac artery injury and one epigastric artery injury. Despite the high number of vascular lesions does not describe other intraoperative complications[40]. Generally the vascular injuries occur in the external iliac vein while performing lymphadenectomy. Unlike what happens in retroperitoneal surgery in most cases it is possible to repair by laparoscopic suturing, mainly without further consequences[26,40]. The keys for the control of a venous injury are: bleeding control with pressure, raising the pressure of CO_2, obtains a good control dissection and proximal and distal to the lesion ends and finally a careful suturing.

DISCUSSION

Most of the papers reviewed described retrospective personal or institutional series. In general, there is no clear separation between intra and postoperative complications, making difficult to centre in some aspects as technique errors in order to avoid them in the future. In this terms, it should be necessary to assess the surgical complications in normalized forms as Clavien for postoperative and Satava for intraoperative. The results on retroperitoneal laparoscopy are based on huge series with many years of experience with the exception of adrenalectomy which is both performed by general surgeons and urologists. In laparoscopic pelvic surgery, the results on prostatectomy are deep and the rate of intraoperative complications is lacking. The laparoscopic cystectomy is a less studied technique with current result still heterogeneous. In spite of the high index of perioperative complications, the rate of intraoperative complication is also lacking.

COMMENTS

Background

The authors decided to perform this literature review to light and to arrange the intraoperative rates of laparoscopic urological cancer complications, which are such as messy in the different manuscripts published. In general, laparoscopic complications are described as postoperative but it is infrequent to find manuscripts regarding specifically intraoperative complications and their possible prevention or solution. The authors think it is important the existence of a document to sum up this issue, mainly for urologist used to perform this kind of procedures by this approach. This idea leaves from an urological team which performs more than 150 laparoscopic procedures per year since 2005.

Research frontiers

Nowadays laparoscopy is one of the most frequent approach for the treatment of urological oncology field. It should be necessary the development of a systematic review to statistically report the complications of the most frequent procedures. It also should be necessary the cooperation between high volume institution to light the annual incidence and prevalence of the intra and postoperative complications, clearly separated in order to futures reviews.

Innovations and breakthroughs

A narrative review of the intraoperative complications for the most frequent urological cancer procedures. From people point of view, there is no other similar manuscript summarizing intraoperative laparoscopic complications in English medical literature.

Applications

To compare the local rate of complications with the general rate reviewed and to inform to young urologist that start in laparoscopy the possible problems that they can find.

Terminology

Laparoscopy: is an operation performed in the abdomen or pelvis through small incisions (usually 0.5-1.5 cm) with the aid of a camera. It can either be used to inspect and diagnose a condition or to perform surgery.

Peer-review

The manuscript is interesting and the authors have performed a good study.

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CASE REPORT

Pedunculated colonic lipoma prolapsing through the anus

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Author contributions: Ghanem OM, Heitmiller RF and DiRocco JD designed the study; Ghanem OM, Slater J and Singh P collected patient's clinical data and analyzed the data; Ghanem OM, Slater J, Singh P, Heitmiller RF and DiRocco JD wrote the manuscript; Heitmiller RF and DiRocco JD critically revised the manuscript; all authors declare that they contributed to this article and that they all approve its final submitted version.
Ethics approval: Medstar Health Institutional Review Board has reviewed and approved this study.
Informed consent: A written informed consent was obtained prior to the reporting of this case.
Conflict-of-interest: Each author certifies that he or she has no commercial associations that might pose a conflict of interest in connection with the submitted article.

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Abstract

Colorectal lipomas are the second most common benign tumors of the colon. These masses are typically incidental findings with over 94% being asymptomatic. Symptoms-classically abdominal pain, bleeding per rectum and alterations in bowel habits-may arise when lipomas become larger than 2 cm in size. Colonic lipomas are most often noted incidentally by colonoscopy. They may also be identified by abdominal imaging such as computed tomography or magnetic resonance imaging. We report a case of a sixty-one years old male who presented to our emergency room with a 6.7 cm × 6.3 cm soft tissue mucosal mass protruding transanally. The patient was stable with a benign abdominal examination. The mass was initially thought to be a rectal prolapse; however, a limited digital rectal exam was able to identify this as distinct from the anal canal. Since the mass was irreducible, it was elected to be resected under anesthesia. At surgery, manipulation of the mass identified that the lesion was pedunculated with a long and thickened stalk. A laparoscopic linear cutting stapler was used to resect the mass at its stalk. Pathology showed a polypoid submucosal lipoma of the colon with overlying ulceration and necrosis. We report this case to highlight this rare but possible presentation of colonic lipomas; an incarcerated, trans-anal mass with features suggesting rectal prolapse. Trans-anal resection is simple and effective treatment.

Key words: Colorectal lipoma; Rectal prolapse; Trans-anal resection

Core tip: Colorectal lipomas are typically asymptomatic. They are incidentally found on colonoscopy or radiologic imaging. This report portrays a rare presentation of colonic lipomas as an incarcerated prolapsed mass through the anus, and highlights trans-anal resection as a simple, safe and effective treatment. Thus, it describes an uncommon pathology with a unique presentation that sets a diagnostic and therapeutic challenge.

Ghanem OM, Slater J, Singh P, Heitmiller RF, DiRocco JD.

Pedunculated colonic lipoma prolapsing through the anus. *World J Clin Cases* 2015; 3(5): 457-461 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i5/457.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i5.457

INTRODUCTION

Lipomas of the gastrointestinal (GI) tract were first described by Bauer in 1757[1]. Although they are rare findings, cases of these benign tumors have been reported throughout the world for many years. Colonic lipomas originate from the connective tissue of the intestinal walls and are most commonly submucosal[2]. Chronic irritation or inflammation as well as the excessive accumulation of adipose tissue secondary to the underdevelopment of arterial, venous and lymphatic circulation, are a few of the speculated factors associated with the formation of these tumors[3]. Lipomas occur with greater frequency within the ascending colon, but can be present in any part of the GI tract from the hypopharynx to the rectum[4]. Generally, colonic lipomas are solitary, well-delineated and sessile masses[5]. They predominately affect females and those within the 6th-7th decade of life[3,6-8]. Although malignancy is rare in these masses, complications including, hemorrhage, infarction, obstruction, and intussusception can occur[2,5,8]. Thus, symptomatic lesions are routinely removed through surgical interventions such as endoscopic excision, segmental resection or hemi-colectomy in an open or laparoscopic fashion[9,10]. Unfortunately, at present there are no clinical trials which validate the most appropriate methods by which colonic lipomas should be diagnosed and treated. Much of patient management is thus dependent on case reports discussed within literature. This paper presents a unique report of a sixty-one years old man with a prolapsed ano-rectal mass that was identified as a submucosal lipoma of the rectum after trans-anal surgical excision. By analyzing this case, we will attempt to review the common practices used in managing patients with colonic lipomas.

CASE REPORT

A sixty-one years old male patient with a diagnosis of schizophrenia and chronic constipation presented to the emergency room with a prolapsed ano-rectal mass. The patient noticed the propulsion of the mass while defecating four hours prior to his presentation to our hospital. Associated anal pain and minimal bright red blood per rectum were reported; however, all remaining review of systems was negative. The patient had no previous history of gastrointestinal symptoms or pathologies-denies change of bowel habits and any history of hemorrhoids or prolapse. He had never undergone a colonoscopy. On physical examination, the firm, well circumscribed, tender, hyperemic mass

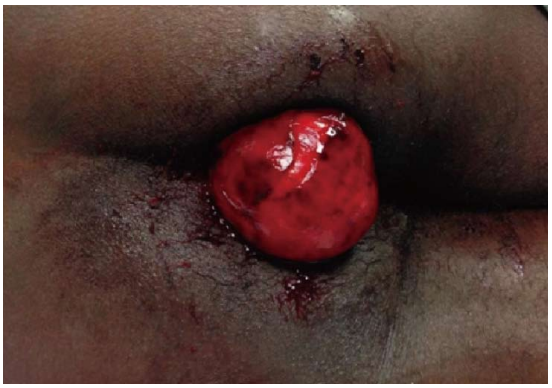

Figure 1 Protruding mass from anal canal in the emergency room (left lateral decubitus position).

was noted to be 6.7 cm × 6.3 cm in size (Figure 1). There appeared to be a layer of superficial necrotic tissue. It did not appear to be originating from the anus or hemorrhoidal tissue as a limited digital rectal exam was able to identify this as distinct from the anal canal. It was irreducible despite attempts by emergency room and surgical staff (manipulation, squeezing and pushing). Abdominal exam was unremarkable and no systemic signs of infection were noted. Preoperative laboratory studies including hematology, chemistry and coagulation profiles were all within normal ranges. The differential diagnosis included other types of prolapsed neoplastic lesions or an atypical presentation of either thrombosed internal hemorrhoids *vs* rectal procidentia.

The patient was admitted to the colorectal surgical service. Given the patient's pain and discomfort and given that the mass was irreducible, the patient was taken to the operation room for exam under anesthesia with planned resection. After sedation, the patient was placed in a high lithotomy position. Digital rectal examination and further manipulation of the mass identified that the lesion was pedunculated with a long (mucosal origin could not be identified) and thickened stalk (2 cm in diameter). As the mass was retracted externally, the stalk could be visualized and was transected by a 60 mm laparoscopic linear cutting stapler (Figure 2). The mass was completely removed and the specimen was sent to pathology. Afterwards, examination of the anus and the distal rectum was performed with an anal retractor. The staple line was not visualized and there existed no active hemorrhage. The bowel was not adequately prepped for endoscopic evaluation so we planned on postoperative colonoscopy after complete bowel preparation.

Post-operatively the patient was stable and denied any pain. He tolerated food, passed gas and also had a bowel movement. The patient was discharged home on post-operative day one with instructions to follow up with a colonoscopy in 2 wk.

Pathology report determined the mass to be a 6.7 cm × 6.3 cm × 4.8 cm polypoid submucosal lipoma of the colon with overlying ulceration and necrosis (Figure

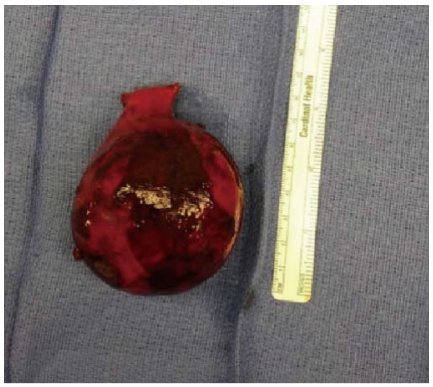

Figure 2 Gross view of the resected specimen.

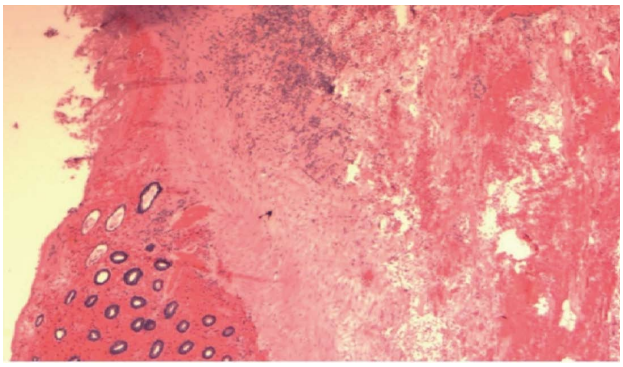

Figure 3 Histopathology (HE, 2 ×) showed colonic lipoma; the colonic mucosa (left side) has atrophy and ischemic necrosis with hemorrhage.

3). No malignancy was identified.

DISCUSSION

Lipomas are the second most common benign tumor of the colon after adenomatous polyps[3,5,9] with an incidence of only 0.035%-4.4%[6,9]. Colonic lipomas most commonly occur between the ages of 50-65[3,6-8] and as Jiang *et al*[6] noted can have up to a 66.7% female predominance. They are most commonly located within the ascending colon (61%)[3,6,7] followed by descending colon (20.1%), transverse colon (15.4%), and least commonly the rectum (3.4%)[3,6,11,12].

Three types of colonic lipomas exist: submucosal, which account for 90% of all intestinal lipomas[2], subserosal and mixed[3]. Grossly, these masses can present as rounded, sessile or pedunculated lesions with smooth mucosal surfaces and a yellow color[3-8]. Histologically, a fibrous capsule is found surrounding the adipose tissue giving the masses a lobulated appearance[3,6]. Ulceration, granulation and fat necrosis has also been noted in the overlying mucosa of many of colonic lipomas[6,8], as identified within the lipoma found in this case report. Over 94% of colonic lipomas are asymptomatic[2,8,13] with many incidentally found during colonoscopic screening, surgery or autopsy[6,9]. Colonic lipomas are more prone to be symptomatic when they are greater than 2 cm in size[4-6,14]. More specifically, 75 percent of patients with "Giant Lipomas"-larger than 4 cm are symptomatic[15]. Symptomatic patients may have abdominal pain (42.4%), bleeding per rectum (54.5%) and alterations in bowel habits (24.2%)[6,7]. Other reported conditions may include constipation, hemorrhage, intussusception, obstruction[2,4,5,8] or anemia[2,4,16,17]. Spontaneous expulsion of these lipomas in the stool has been rarely reported[1,4,9,13,18,19] but is attributed to self-amputation of the lipoma at its stalk[9,20]. Self-amputation typically occurs in giant and pedunclated lipomas and can be caused by intussusception, procedures such as endoscopy or by other idiopathic processes[13,18,19,21].

The rare presentation of colonic lipomas makes the diagnosis a difficult task. They are often misdiagnosed as a rectal prolapse or as a colonic malignancy[3]. One initial radiographic test for colonic lipomas is a barium enema which identifies a well-defined, smooth and radiolucent mass causing an intraluminal filling defect which elongates during peristalsis (the "squeeze sign")[4,5,7,14]. Unfortunately, although barium enema is sensitive for lipomas, it is not specific to these masses and may mistake them for other endoluminal neoplastic masses[6]. Endoscopic ultrasonography is another commonly used test displaying lipomas as hyperechoic colonic lesions. It is helpful in identifying the involvement of the muscularis propria and serosa[3,5,7,9]. Information regarding the depth of the lesion in the wall of the colon is particularly important in deciding whether the lesion is capable of being safely excised endoscopically.

Three classic signs for colonic lipomas have been described during colonoscopy: the "tent sign" (lifting the overlying mucosa of the lipoma with forceps to create a tent-like shape), the "cushion sign", (forceps causing indentation of the lipoma which is resolved with their removal) and finally the "naked fat sign" (extrusion of adipose tissue from the lipoma during biopsy)[3,6,14]. However, Andrei *et al*[3] noted that although colonoscopies are successful in identifying typical lipomas, they are less sensitive for lipomas with atypical qualities such as overlying ulceration or necrosis as seen in the lipoma described in this report. In addition, colonoscopic biopsies often fail to obtain adequate samples of lesions as normal mucosa or even ulcerated and necrotic tissues can cover the adipose tissue necessary for diagnostic testing[6,14]. In situations where obtaining histologic determination of the mass is relevant, needle biopsy under endoscopic ultrasound guidance provides a safe option. The literature indicates that the study of choice for colonic lipomas is computed tomography (CT) scan[3,5,14]. This test visualizes well-defined, ovoid and homogenous intramural lesions with a fat density between -40 and -120 UH[3-5,8]. CT is specifically useful when lipomas are large (> 2 cm)[6,7] and in lipomas with associated complications such as necrosis, infarction or intussusception[2,5,8,22]. Nonetheless, the diagnostic value of CT scan is limited

by the size and partial volume of the lesion. Smaller lesions are more likely to be missed by CT while, increases in partial volume, due to the added volume of fecal matter and soft tissue make some masses appear larger than normal on CT imaging[3,14].

The treatment of colonic lipomas involves observation for asymptomatic cases and surgical intervention for lipomas with symptoms or associated complications[23]. Depending on the lipoma size, location and the presence or absence of complications, surgeons decide on endoscopic *vs* surgical intervention[8]. Endoscopic excision with snare electrocautery is the treatment of choice for lipomas smaller than 2 cm in size[5,6,9,14,23,24]. Higher risk of perforation has been reported with the endoscopic excision of lipomas larger than 2cm in size[6,9]. Newer instrumentation and techniques developed for endoscopic submucosal resection of adenomatous lesions make endoscopic resection more feasible for larger lesions. Segmental colectomy with lipectomy is the gold standard for uncomplicated lipomas larger than 2 cm[3,25]. More radical approaches to resection-hemicolectomy-is usually reserved for lipomas with wide implantable bases, deeper lesions such as those that originate in the subserosal layer, and those with excessive bleeding or associated intussusception[9]. Finally, laparoscopic removal of lipomas is also an available option for treatment[10] and is a superior tool in cases when endoscopic removal is unsafe, ineffective, or cannot obtain negative margins[26]. These surgeries have been found to cause less post-operative pain and quicker recoveries when compared to open colectomies[9,10,13,26-28]. However, Boler *et al*[26] do acknowledge that laparoscopic resection is limited by the inability to definitively locate certain lipomas. Yet, preoperative colonoscopic injection of a colonic mural marking agent such as india ink or intraoperative colonoscopy can aid in the localization of the lipoma. Overall, surgical intervention is the most effective treatment for colonic lipomas and there have been no reported recurrences in the current literature[9].

In our case, the colonic lipoma was protruding through the anus and was still attached by a viable stalk. To minimize patient morbidity and expedite therapeutic resolution of his symptoms, we resected this incarcerated tumor by a transanal approach. As of the time of creation of this manuscript, the patient has declined colonoscopic evaluation.

Colorectal lipomas most commonly present as asymptomatic, incidental findings on colonoscopy and are treated by either local endoscopic or surgical resection. Our patient demonstrates that they can also present acutely as an incarcerated, trans-anal mass with features suggesting rectal prolapse. We present this case to highlight this possible presentation. Trans-anal resection is simple and effective treatment.

COMMENTS

Case characteristics

A 61-year-old male patient presented to the emergency room with a prolapsed ano-rectal mass.

Clinical diagnosis

A firm, well circumscribed, tender, hyperemic ano-rectal mass was noted to be 6.7 cm x 6.3 cm in size.

Differential diagnosis

Prolapsed neoplastic lesion, thrombosed internal hemorrhoid, rectal procidentia.

Laboratory diagnosis

Hematology (CBC), Chemistry (BMP) and Coagulation profile (PT INR. PTT) were all within normal range.

Pathological diagnosis

Pathology report determined the mass to be a 6.7 cm x 6.3 cm x 4.8 cm polypoid submucosal lipoma of the colon with overlying ulceration and necrosis.

Treatment

The prolapsed ano-rectal mass was resected trans-anally by the use of laparoscopic linear cutting stapler.

Related reports

Very few cases of colorectal lipomas were reported with a similar presentation (prolapsed ano-rectal mass) and management (ano-rectal excision).

Term explanation

Lipoma is a benign tumor of the adipose tissue. Rectal procidentia is another term for rectal prolapsed.

Experiences and lessons

The authors report this case to highlight this rare but possible presentation of colonic lipomas; an incarcerated, trans-anal mass with features suggesting rectal prolapse. Trans-anal resection is simple and effective treatment.

Peer-review

The manuscript is well written and the result of the management is acceptable.

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CASE REPORT

Small bowel diverticulitis with severe anemia and abdominal pain

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Abstract

The current case report is related to a male patient with diabetes, obesity [body mass index (BMI) 33], hypertension and recurrence of anemia associated to melena and deep asthenia. M.P., a 60-year-old obese individual, was referred to our department by the primary care unit (PCU) of our hospital for severe anemia (Hemoglobin 6.5 g/dL) associated to episodes of melena and abdominal pain. In the past 5 mo the patient referred to the local hospital 3 times for episodes of melena (hemoglobin levels showed anemia 9.8 g/dL) but the main gastroenterological exams were completely negative (colonoscopy and gastroscopy). The PCU of our Hospital, after stabilization of the main parameters and blood transfusion for the low levels of hemoglobin, referred the patient to gastroenterologists: the patient was subjected to both colonoscopy and gastroscopy that were negative. Due to the condition of acute severe hemorrhage the patient, during the first 3 h from the access to the PCU, was subjected to arteriography that did not reveal any hemorrhagic foci or vascular alterations. The video capsule for the study of the small bowel showed the presence of blood beginning from the third portion of duodenum but deep gastroscopy did not reveal it. The patient was then subjected to double balloon endoscopy that revealed a severe diverticulosis of the small bowel with blood from the diverticula. The entero-tomografia computerizzata confirmed the diagnosis and revealed an extension of the diverticula for almost the entire small bowel (no diverticula in the colon). The patient was subjected to wide spectrum antibiotic therapy with resolution of the symptoms and stabilization of hemoglobin levels. The surgeon suggests no indication to surgery for the wide area involved from the disease and potential high risk of complication due to the high BMI. At home, the patient started a monthly therapy with rifaximin and probiotics associated to mesalazine. At present, after 12 mo from the last episode of hemorrhage, the patient is in good clinical condition, reduced his body weight of about 7 kg and the hemoglobin levels appear in slow progressive increase (last measurement 13.2 g/dL).

Key words: Small bowel; Diverticulitis; Abdominal pain; Anemia; Intestinal bleeding

Core tip: The current case report adds an additional tool for the treatment of small bowel diverticula. Although the best recognized treatment is represented by surgical approach, the current case demonstrates the possibility of effective treatment by pharmacological approach. The pharmacological approach allows to treat patient with high surgical risk and all patient with contraindication to surgery; moreover the small bowel preservation avoid all the symptoms and signs of malabsorbtion, inevitably occurring after surgery.

De Minicis S, Antonini F, Belfiori V, Lo Cascio M, Marraccini B, Piergallini S, Mosca P, Macarri G. Small bowel diverticulitis with severe anemia and abdominal pain. *World J Clin Cases* 2015; 3(5): 462-465 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i5/462.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i5.462

INTRODUCTION

Small bowel diverticulosis is an uncommon pathology and may exist in two different forms: congenital or acquired. The congenital form is represented exclusively by Meckel's diverticulum, which is a true diverticulum located on the antimesenteric border of the small bowel, to a variable distance from ileo-cecal valve between 40 to 80 cm[1]. Conversely, the acquired forms of the small bowel diverticula are also named false diverticula: they are lesions consisting of mucosa, submucosa, and serosa without a tunica muscularis[2].

Multiple diverticulosis of the jejunum represents an uncommon pathology of the small bowel that is often asymptomatic. An incidence of about 0.5%-2.3% found in small bowel contrast studies and of 0.3%-4.5% found in autopsies have been reported in the literature[3,4].

In a few percentage of case this condition may lead to several and unspecific symptoms such as malabsorption, anemia, chronic abdominal pain and discomfort. Major complications may be characterized by diverticulitis, hemorrhage, obstruction and perforation, which are described in very selected number of cases[5-7].

The management of symptomatic jejunal diverticulosis is a challenge for clinicians. We herein report a case of a 60-year-old man presented at the primary care unit (PCU) of our hospital for acute abdominal pain associated to vomiting and deep asthenia in a patient with diabetes, obesity [body mass index (BMI) 33], hypertension.

CASE REPORT

M.P., a 60-year-old obese individual, was referred to our department by the PCU of our hospital for severe anemia (Hemoglobin 6.5 g/dL) associated to episodes of melena and abdominal pain.

In the past 5 mo the patient referred to the local hospital 3 times in 5 mo for melena associated to deep asthenia and one episode of lipotimia (hemoglobin levels showed anemia with values respectively of 9.5, 9.1 and 9.8 g/dL); in the described episodes, both colonoscopy and gastroscopy were negative for the identification of blood sources.

At the present, M.P. occurred to the PCU of our hospital for the forth episode of abundant melena associated to abdominal pain and low-pressure levels (100/50 mmHg).

The main laboratories examinations showed a 6.5 g/dL of hemoglobin levels associated to low levels of iron, with no additional alterations.

The patient was transfused with red packed blood cells and the clinical conditions were stabilized. The patient was referred to the gastroenterologist when the main parameters were: cardiac frequency 90, Saturation 97%, PA 110/70 mmHg.

Both Gastroscopy and colonoscopy were completely negative for the presence of lesion potentially involved in bleeding.

During the first 4 h from the access to the PCU, taking into consideration the gastroscopy and colonoscopy previously executed in other hospital, the patient was subjected to arteriography that did not reveal any alteration or potential sources of bleeding.

After adequate preparation, the patient repeated the colonoscopy that showed regular mucosa and no alteration able to justify massive bleeding. The patient performed video-capsule endoscopy examination that revealed the presence of mucosal areas with blood, extended from the upper part of the jejunum for several centimeters, with no other specifications.

In order to better investigate the small bowel, the patient was subjected to double balloon examination that revealed the presence, just down to the Treitz ligament, of a pattern with numerous and giant diverticula of jejunum.

The entero-tomografia computerizzata (TC) and small bowel contrast study (Figure 1) subsequently confirmed the jejunal diverticula, located in the in the right and upper part of the abdomen, apparently related to most of the jejunum; the same diverticula were particularly tick with edema of the mucosal layer, as typical sign of inflammation.

We first referred the case to the surgical department of our Hospital for evaluation: but no criteria for emergency procedures were present, since no active bleeding was occurring at that time; on the other hand, the high number of comorbidities strongly contraindicated any potential surgical procedure.

Due to this analysis and the hemodynamic condition of stability, we decided to start treatment with rifaximine (1200 mg/die), probiotics and mesalazine (1600 mg/die); with the objective to use at home a monthly-based therapy characterized by 1 wk of rifaximine and probiotics and 2 wk of mesalazine.

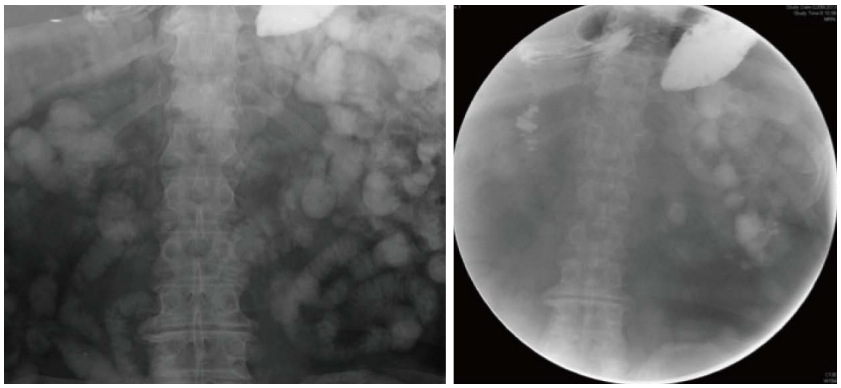

Figure 1 Small bowel contrast study: Imaging of diverticula of the jejunal diverticula, located in the right and upper part of the abdomen.

No additional episodes of bleeding occurred, with a progressive increase of hemoglobin; the patient was discharged after 12 d with no major complications.

At the present, after 12 mo of follow-up, no additional episodes of bleeding occurred, the level of hemoglobin reached 13.2 g/dL and no episodes of abdominal pain were referred. The patient continues treatment with rifaximine, probiotics and mesalazine on a monthly base.

DISCUSSION

Jejunal diverticulosis was described for the first time by Somerlingin 1794 and by Sir Astley Cooper in 1807. These false diverticula are commonly located on the mesenteric border of the jejunum[8]. The main feature that jejuna diverticula shares with colonic diverticula is that mucosal herniation occur through gaps in the muscle layers[9].

Regarding the portion of the bowel interested by the pathology: Jejunal diverticula may be the only site in the gastrointestinal tract or being associated to colonic diverticula (35%), duodenal diverticula (26%) and esophageal diverticula (2%)[7,8]. Small bowel diverticula are more frequent in male and its prevalence increases in elderly[10].

The low prevalence and the poor symptoms described in the clinical practice may lead to misdiagnosis, or relatively delayed-diagnosis[11].

In the specific clinical case reported in this manuscript, the patient was deeply studied and carefully evaluated with second level examinations only at the forth access to the PCU and only in the condition of severe anemia (hemoglobin 6.5 g/dL) and probably active bleeding. Furthermore, the condition of bleeding necessarily bring the attention to the endoscopic evaluation of the intestinal tract, such as gastroscopy and colonoscopy, that may delayed the time of diagnosis without solving the main problem.

In the specific case the TC imaging analysis was performed only in a condition of hemodynamic stabilization of the patient and overall secondarily to the double-balloon endoscopic examination. The evidence-based flow chart presumably leads to a different approach in the general management of the pathology: in hemodynamically stable patient, endoscopic techniques (gastroscopy or colonoscopy) may be used. Secondarily, small bowel contrast studies and computed tomography scans are able to visualize such regions and thus establish the diagnosis[12]. In our case we preferred to directly perform the double balloon instead of waiting for the radiologic imaging techniques.

As reported in literature, hemorrhage from jejunal diverticula generally presents as lower gastrointestinal bleeding that may be acute or chronic with iron deficiency anemia noted[13].

Although the treatment of choice in bleeding is represented by surgical resection of the source of bleeding with primary anastomoses, the case report described had several contraindication for surgical approach: first, the history of cardiopathy and the low rate of pulmonary reservoir; second, the obesity that greatly limits the feasibility of the technical procedure; third, the lack of a specifically localized source of bleeding, that do not allow to perform a selective resection of the bowel, but may lead to the resection of a too large portion of the bowel[14].

Due to the high number of contraindications to the surgical approach, we decided proposing the patient for pharmacologic treatment.

The drugs used mainly consist in the same protocol existing for the colonic diverticula, characterized by rifaximine, probiotics, and mesalazine cyclically, on a monthly-based therapy.

To date, no exhaustive data are provided in literature on the efficacy of the current used pharmacological therapy and no study of comparison between potential pharmacological therapy and surgical treatment are otherwise described[15]. Furthermore, it is important to take into consideration the high surgical risk due to several comorbidities occurring in this specific patient. Surgeon of our department presented to the patient the potential effect of the surgical therapy and the possibility to obtain a complete resolution of the pathology informing the patient on the main risks of the procedure. In the specific case, the patient refused to perform additional examinations and expressed the clear choice of non-surgical treatment.

Both rifaximine and mesalazine represent an

off-label therapy in the specific case of small bowel diverticula and no study, as far as we know, are present in literature discussing this issue[16].

In conclusion, in the general management of small bowel diverticula, acutely showing complication such as hemorrhages, the treatment of choice should be considered the surgical procedure with the resection of the tract of intestine affected by the bleeding[17]. However, it is important to take into consideration two major points occurring in this patient: active bleeding with anemia without the possibility to specifically localize the source of bleeding; second, the presence of major contraindications to surgery[18].

The current case report describe a single patient that referred to the hospital with a severe anemia in the course of acute intestinal bleeding from small bowel diverticula, with no indications to surgery. In the specific case report, the patient referred to the PCU 4 times in the time lap of 8 mo. At the current state, the last acute episode of bleeding was observed 12 mo ago, the hemoglobin levels are progressively increasing (last value was 13.2 g/dL) only with oral administration of iron.

Thus, in the flow chart of therapeutic opportunities for patients with small bowel diverticula, even with previous complication of bleeding, pharmacological therapy option should be considered, especially in the presence of major contraindications for surgical procedures.

COMMENTS

Case characteristics

Patient develops sever anemia and abdominal pain.

Clinical diagnosis

Double-balloon endoscopy revealed the presence of small bowel diverticula.

Differential diagnosis

Signs and symptoms may suggest all the different conditions of upper gastrointestinal (GI) bleeding.

Laboratory diagnosis

Low hemoglobin levels represent the main sign.

Imaging diagnosis

Double balloon endoscopy and radiographic GI imaging revealed the presence of diverticula.

Pathological diagnosis

Melena and anemia represent the main features of the clinical case.

Treatment

Mesalazine and wide spectrum antibiotics.

Related reports

The majority of case report of complicated small bowel diverticula suggests the resolution of the pathological condition by surgical approach.

Experiences and lessons

The pharmacological treatment in complicated small bowel diverticula should be considered as a treatment option in addition to surgical approach, particularly in those patients that present absolute contraindication or high risk to surgery.

Peer-review

It is comprehensive and elaborating.

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CASE REPORT

Extremely unusual case of gastrointestinal trichobezoar

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Conflict-of-interest: None to be declared.

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Abstract

Trichobezoars (hair ball) are usually located in the stomach, but may extend through the pylorus into the duodenum and small bowel (Rapunzel syndrome). Rapunzel syndrome remains uncommon; with fewer than 40 cases reported. To the best of our knowledge, this case may be the first well-documented case with a length of 75 cm. They are almost always associated with trichotillomania and trichophagia or other psychiatric disorders. In the literature several treatment options are proposed, including removal by conventional laparotomy, laparoscopy and endoscopy. Herein, we are reporting an interesting case of an 18-year mentally retarded girl with history of trichotillomania and trichophagia who presented to our emergency department with a history of central abdominal pain associated with vomiting and constipation for five days. An examination showed a trichobezoar requiring emergent surgical intervention, and indicating the need for psychiatric treatment. The trichobezoar was treated successfully by laparoscopy.

Key words: Giant Trichobezoar; Therapy Endoscopy; Trichotillomania; Rapunzel syndrome; Laparoscopy

Core tip: Laparoscopic management is ideal for trichobezoar due to an improved cosmetic appearance, fewer postoperative complications, and reduced hospital stay. It has a better outcome with many benefits over laparotomy and is slowly becoming the treatment of choice. After trichobezoar removal, prognosis is good if psychiatric therapy to control habitual trichophagia is successful.

Jatal SN, Jamadar NP, Jadhav B, Siddiqui S, Ingle SB. Extremely unusual case of gastrointestinal trichobezoar. *World J Clin Cases* 2015; 3(5): 466-469 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i5/466.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i5.466

INTRODUCTION

A trichobezoar is an unusual condition hair bundles in stomach and small intestine, leading to intestinal obstruction usually affecting younger females[1,2].

It is mainly associated with a psychiatric disorder, trichotillomania having tendency of pulling hairs and significant hair loss. The large number of patients with this disorder are having problem of depression, anxiety and poor self-image[3]. The feel pleasant while pulling the hairs. The prevalence of the condition is 0.06% to 4%[4]. Due to smoothness, the ingested hairs resist digestion as well as peristalsis and get accumulated in the gastric mucosal folds. Eating of hairs for a long period leads to trichobezoar. Its main location is in stomach. However, in some cases, there is extension in to small intestine and exceptionally in to large intestine, then the condition is labeled as Rapunzel syndrome. Rapunzel syndrome was first discovered by Vaughan *et al*[5] in 1968. Fragmentation and dislodgement lead to obstruction[6-8]. As they are clinically asymptomatic, many times there is delay in diagnosing the condition. Due to availability of newer techniques of removal, *i.e.,* Laparoscopy and endoscopy, routinely laprotomy is not preferred[7-9]. Herein, we are reporting a similar interesting case of trichobezoar, removed on laproscopy.

CASE REPORT

A mentally retarded an adolescent girl (18 years age) came to Jatal Hospital and Research Centre with history of trichotillomania and trichophagia. She was eating her own hairs since last 10 years. She was presented with central colicky abdominal pain along with episodes of vomiting for 7 d. she was clinically conscious, with signs and symptoms of mild dehydration, anemic and had no signs of jaundice. The systemic parameters are normal.

Patient's upper gastrointestinal diagnostic endoscopy was performed and showed heavy large trichobezoar (hairball) in the stomach (Figure 1). Computed tomography (CT) scan was not done, in view of poor economical status of the patient and also as it was not indicated. Accordingly, patient was planned for emergency laparoscopic management.

A 10 mm umbilical port (telescopic port) was used having 30° telescope. Two 5 mm ports, one at right hypochondriac region and the other at midaxillary line was used. The one used at mid-axillary line was an operating port of 10 mm. Fourth port, *i.e.*, a 5 mm port at epigastric region to retract the left lobe of liver was used (Figure 2).Laparoscopic gastrostomy was performed with the help of harmonic scalpel. There was a giant trichobezoar extending from stomach through pylorus upto the jejunum (Rapunzel Syndrome). Successful complete removal of trichobezoar was done laparoscopically. The length of the trichobezoar was 75 cm. (Figure 3). On follow up patient is doing well since one month and she is under treatment of psychiatrist.

DISCUSSION

Continued hair ingestion in the patients of trichophagia forms a tight, growing hair balls leading to obstruction in the intestine. After accumulation the hair traps the viscous materials, *i.e.,* mucin, blood that forms tight and compact growing hairballs, not easy to remove[10]. In addition, the gastric churning helps to trap new hair into already formed hairballs. Mucus gives the hairball a shiny surface. Due to denaturation of proteins in the acidic environment of stomach; the color of the ball remains black. The foul smelling breath that results from fermentation of fats[11]. Many times, the patient remains asymptomatic for many years, until the point of obstruction[11]. Subsequent gastrointestinal blood loss leads to anemia.

Depending on the composition they are classified as: Trichobezoars. Phytobezoars, Medication bezoars and Lactobezoars. Phytobezoars are more common that composed of indigestible fruits, vegetable fibers, skin, or seeds[12]. Phytobezoars are mainly associated with a history of previous gastric surgery, conditions of reduced gastric acidity, delayed motility or poor gastric mixing. Medication bezoars composed of undigested tablets or semiliquid drugs. Lactobezoars are predominantly seen in lowbirth- weight or premature babies fed with a highly concentrated milk formula during the first weeks of life[12].

Rapunzel syndrome is defined as a bezoar extending down in to small intestine and exceptionally in to large intestine leading to obstruction[11]. Rapunzel syndrome is very unusual with less than 40 cases documented[11]. It is a reflection of psychiatric illness and no associated with gastrointestinal motility and first discovered by Vaughan *et al*[5].

Various modalities are used for diagnosis of this rare condition. A contrast upper gastrointestinal series often diagnostic of trichobezoars. They are also easily diagnosed on abdominal ultrasonography and or computed tomography scan[12,13]. However, upper GI endoscopy is an effective diagnostic tool to confirm the presence of a trichobezoar. Endoscopy also helps the clinician to differentiate between a trichobezoar and another foreign body[14].

Several treatment modalities are available for the treatment of trichobezoar, *i.e.,* open surgery, Laparoscopic or endoscopic removal by mechanical fragmentation or using chemicals[11]. The main drawback of above methods is they are not useful in large trichobezoars[14].

Previously open surgery and or laprotomy was a treatment of choice[14]. However, they have post-operative complications, *i.e.,* perforation, infection of wound, pneumonia etc[14]. Other surgical complications are upper digestive tract bleeding, anemia, bowel intussusception and rarely death.

With the availability of newer less invasive modes, laparoscopic removal is preferred but it seems to be difficult. The advantages are less hospital stay, cosmetically accepted and minimal complications[14]. However, there are chances of spillage of hairs into the peritoneal cavity and requires more time[10,14].

To conclude psychiatric treatment and follow up

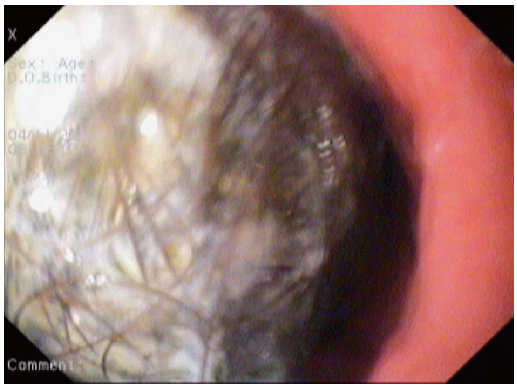

Figure 1 Upper gastrointestinal endoscopy shows a large hairball.

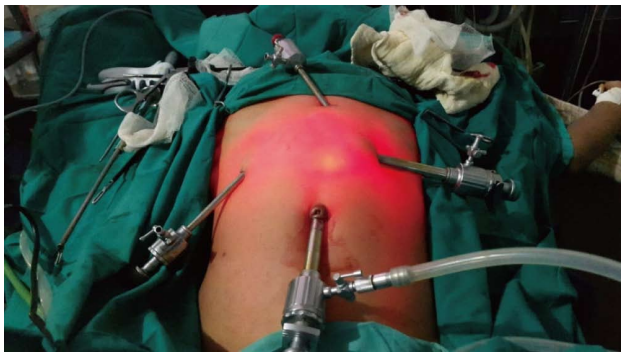

Figure 2 Showing laparoscopic ports.

is very important in the treatment and prevention of recurrence of Rapunzel syndrome. The long term prognosis is excellent[11].

COMMENTS

Case characteristics

Complained of central colicky abdominal pain with episodes of vomiting and constipation.

Clinical diagnosis

Trichobezoars.

Imaging diagnosis

Computed tomography scan was not performed due to the poor economical status of the patient and also as it was not indicated.

Pathological diagnosis

Trichobezoars.

Treatment

Laparoscopic removal followed by psychiatric therapy is ideal and is successful to control habitual trichophagia in this case.

Peer-review

The authors have performed a good study, the manuscript is interesting.

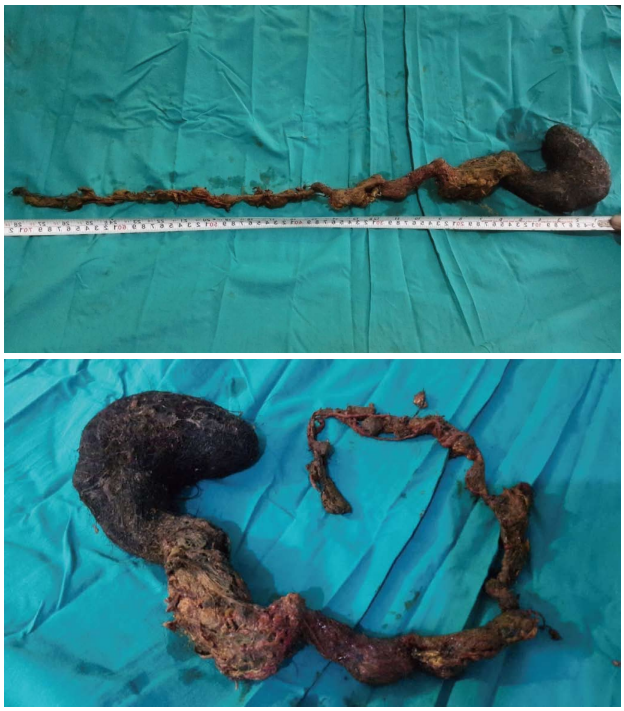

Figure 3 Large trichobezoar measuring 75 cm in length.

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CASE REPORT

Congenital pulmonary airway malformation: A report of two cases

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Abstract

Congenital pulmonary airway malformation (CPAM), previously known as congenital cystic adenomatoid malformation is a congenital disorder of the lung similar to bronchopulmonary sequestration. In CPAM, usually an entire lobe of lung is replaced by a non-working cystic piece of abnormal lung tissue. This abnormal tissue will never function as normal lung tissue. The underlying cause for CPAM is not known. It occurs in approximately 1 in every 30000 pregnancies. The association between CPAM and malignancy has been well documented. There is a small risk (0.7%) of *malignant transformation* within the cyst. So early diagnosis and surgical resection is important to prevent the grave complications. Herein, we are reporting two interesting cases of CPAM and one belonged to Type Ⅱ and other belonged to Type Ⅲ of Stocker's classification.

Key words: Congenital pulmonary airway malformation-Type Ⅱ; Congenital pulmonary airway malformation-Type Ⅲ; Surgical resection

Core tip: Congenital pulmonary airway malformation (CPAM) is a rare disease with various clinical presentations and having the risk of future malignant transformation. Early pulmonary resection for asymptomatic CPAM is required and recommended to make a definitive diagnosis and determine the prognosis of the disease.

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INTRODUCTION

Congenital pulmonary airway malformation (CPAM) is rare condition with a reported incidence of 1:25000 to 1:35000. About 15%-50% of cases of congenital cystic lung disease are reported to be CPAM[1-3].

This condition was first discovered by Stoerk[1]

in 1897. The abnormality is mainly attributed to a maturation defect. It is classified into 5 major types based on clinical and pathological features. It is important to make early pathological diagnosis by surgical excision in congenital cystic lung diseases to determine the prognosis. Most CPAM lesions are manageable with the proper assessment, diagnosis and surgical interventions[4].

CASE REPORT

Case 1

A male baby was born to primigravida mother with a history of 8 mo amenorrhoea. The mother's prenatal parameters are within normal limits. The baby was having high grade fever and respiratory symptoms. High resolution computed tomography of thorax revealed approximately 4 cm × 3.7 cm × 4.8 cm sized round to oval lesion involving right lower lobe. It showed multiple tiny communicating cystic areas in the central portion and solid peripheral portion. Patchy consolidation was noted surrounding this lesion. Possibilities of CPAM (Type Ⅱ) or pulmonary blastoma were suggested.

Clinical diagnosis of low birth weight with early onset sepsis and CPAM Type Ⅲ was kept. Lobectomy was planned and performed. The resected specimen of right lower lobe of lung was sent for histopathological evaluation.

Right lower lung lobectomy specimen was solid, firm in consistency measuring 6 cm × 5 cm × 3 cm in size. The cut surface showed solid gray white area with slit like opening and few small cysts measuring smaller than 0.3 cm in diameter. Foci of large areas of haemorrhages were also noted (Figure 1).

Microscopy revealed small cystic and predominantly solid areas. Cystic areas showed bronchiole like structures lined by simple cuboidal epithelium which are surrounded by alveolar spaces (Consistent with immature lungs). Some alveoli and cystic spaces filled with acute inflammatory exudates with microabscess formation. Intraalveolar haemorrhages were also noted (Figure 2).

Finally, the case was diagnosed as CPAM [congenital cystic adenomatoid malformation (CCAM)] Type Ⅲ with acute pneumonia and focal intraalveolar hemorrhages.The patient is doing well on follow up till date since 4 mo.

Case 2

A 31 years old female $G_3P_2L_2$ with history of nine months of amenorrhoea came to the outpatient department with USG abdomen and pelvis report suggestive of CCAM with Polyhydramnios, displacing the heart towards left. Labour was induced. After birth baby did not cry, for which resuscitation was done but in vain, the baby was cyanosed. Clinically it was diagnosed as; female child with Very Low Birth Weight with CPAM. Baby was shifted to NICU (Neonatal intensive care unit). Baby was initially responding but after few hours, succumbed to death and autopsy was performed.

Autopsy findings revealed Heart and Lung together weighing 40 grams. Left lung measured 5 cm × 2 cm × 1 cm. It was firm in consistency. Right lung measured 7 cm × 3 cm × 3 cm. Pleural surface of right lung and cut surface showed tiny cysts ranging in size from 0.5-2 cm in diameter involving the entire parenchyma of right lung. No other anomalies were noted.

Microscopically sections studied from right middle and lower lobe showed multiple, cystically dilated abnormal bronchiole like structures lined by cuboidal to columnar epithelium resting on thin fibromuscular wall. Sections from left lung and right upper lobe show evidence of hyaline membrane disease characterised by alveoli and airways lined by eosinophilic membrane.

On autopsy findings, the case was finally diagnosed as CPAM Type Ⅱ (Figure 3).

DISCUSSION

CPAM is an unusual condition characterised by immature, malformed lung tissue with cystic appearance. CPAM is seen mainly in newborns, still born infants and is an unusual condition in children beyond infancy. It is a hamartomatous lesion that is usually symptomatic in first few days of life. The patients with CPAM can present as neonates with severe, progressive respiratory distress due to cyst expansions. Hydrops may be present[3].

Exact etiology of CPAM is not known, it is to be considered as hamartomatous malformation and abnormal proliferation of the pulmonary tissue at different sites.

It has been proposed that designation of this lesion "CCAM" be changed to "CPAM" to reflect the fact that the lesions described as cystic are present in only 3 of the 5 types and "Adenomatoid" only in one type (Type Ⅲ). CPAM more accurately encompasses all five types of this classification[1].

CPAM Type 0-Acinar dysplasia/agenesis is rare malformation largely incompatible with life. Lungs are small, firm with diffusely granular surface. Microscopically, it shows bronchus like structures with muscle, glands and numerous cartilage plates and loose, vascular mesenchymal tissue[1].

CPAM Type Ⅰ-It accounts for nearly 65% of cases. It is operable with good prognosis. Grossly, lesion is predominantly cystic type (measuring 3-10 cm in diameter) surrounded by smaller cysts. Microscopically, the large, thin walled cysts are lined by ciliated pseudostratified columnar epithelium with some mucin producing cells. The wall composed of fibromuscular and elastic tissue and occasional cartilage plate[1].

CPAM Type Ⅱ-it accounts for 10%-15% of cases and mainly seen in first year of life. It has poor prognosis

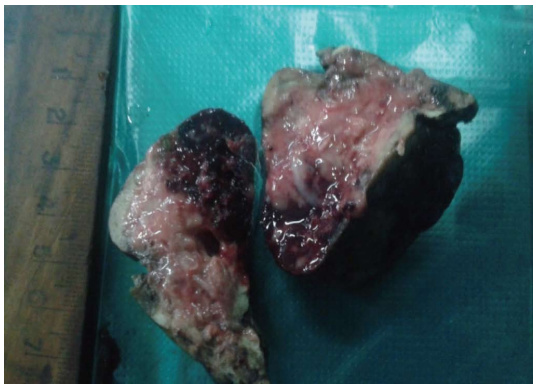

Figure 1 Congenital pulmonary airway malformation Type III-Cut section of lung showing solid areas with few slit like spaces with focal areas of haemorrhages.

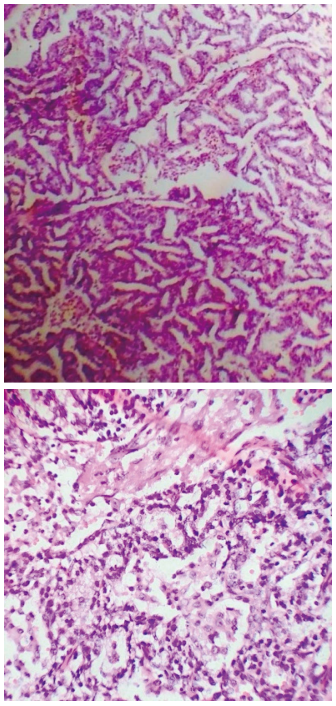

Figure 2 Photomicrograph showing congenital pulmonary airway malformation Type III with pneumonia. Neutrophilic infiltrate in alveoli and cystic spaces. (HE × high power).

because it is frequently associated with other congenital anomalies. Grossly lesion is composed of medium sized cysts measuring 0.5 to 2.0 cm in diameter that are evenly distributed and blend with the adjacent normal parenchyma. Our second case belonged to this type of CPAM. Cysts were arranged in back to back fashion and bronchiole like structures and lined by cuboidal to columnar epithelium with thin underlying fibromuscular layer. Mucous cells and cartilage plates were absent. CPAM Type 2 has been noted in nearly 50% cases of extralobar sequestration[1].

CPAM Type Ⅲ- Chin and Tang (1949) have described this lesion. It is infrequent and accounts only about 5% of cases. It is small cystic or solid type, exclusively seen

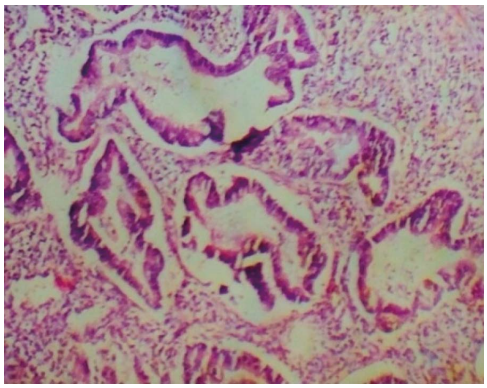

Figure 3 Photomicrograph showing congenital pulmonary airway malformation Type II-Bronchiole like structures are lined by cuboidal to columnar epithelium with back-to-back arrangement (HE × high power).

in first few days to months of life with characteristic male preponderance. It is commonly associated with maternal polyhydramnios, foetal anasarca. So it has high mortality rate. Increased Alfa-fetoprotein level can be noted in second trimester. Grossly cysts are small measuring less than 0.2 cm in diameter, affecting large bulky mass involving an entire lobe or even an entire lung. Microscopically the lesion resembles an immature lung devoid of bronchi. It consists of irregular, stellate shaped bronchiole like structures lined by cuboidal epithelial cells, surrounded by alveolar ductules and saccules that are lined by cuboidal epithelium giving the adenomatoid appearance that is why originally named as CCAM. Mucous cells, cartilage, rhabdomyomatous cells are not seen[1,4].

CPAM Type Ⅳ-It is hamartomatous malformation of the distal acinus and accounts for 10%-15% of cases with an age range of newborn to 4 years. This lesion involves a single lobe. Grossly large, thin walled cysts are lined by flattened epithelium-alveolar lining cells with underlying loose, fibrovascular mesenchymal tissue[1].

Clinical examination and chest X-ray invariably identify CPAM. On antenatal ultrasonograpy CPAM lesions can be identified in a population of infants who are asymptomatic at birth[5,6]. Most of these CPAM (10%-15%) are associated with other congenital anomalies, *e.g.*, CPAM Type Ⅱ is associated with bilateral renal agenesis, extralobar pulmonary sequestration, cardiovascular malformation, *etc*.

The lack of bronchial cartilage distinguishes CPAM from bronchogenic cyst and the distinctive rows of mucous cells from simple foregut cyst[2].

Congenital lobar emphysema can be distinguished from CPAM by the presence of bronchovascular markings extending to the periphery of the involved lobe and by atelectasis of adjacent tissue[7].The distinction from a sequestration of the intralobar variety may be difficult but a systemic blood supply would favour sequestration[8].

The association between CPAM and malignancy has

been well documented. Malformation and proliferation cause hamartomas over the tracheobronchial tree. Type Ⅰ CPAM may involve malignant transformation of mucinous bronchioloalveolar carcinoma[9-11]. Type Ⅱ CPAM may involve malignant transformation-Rhabdomyosarcoma. Type Ⅲ CPAM requires examination of the entire lesion to exclude pulmonary blastoma by confirming whether or not sarcomatous differentiation is present in the solid parts[8,12,13].

Surgical resection in asymptomatic infants is more beneficial with fewer complications compared with intervention following development of symptoms. It is the gold standard for management of CPAM for both pathological diagnosis and treatment[6].

COMMENTS

Case characteristics

Case 1: The baby was having high grade fever and respiratory symptoms; Case 2: It was female child with very low birth weight (LBW) with Congenital pulmonary airway malformation (CPAM).

Clinical diagnosis

Case 1: Clinical diagnosis of LBW with early onset sepsis and CPAM Type Ⅲ was kept; Case 2: Clinically it was diagnosed as; female child with very LBW with CPAM.

Differential diagnosis

Case 1: Pulmonary blastoma; Case 2: Diagnosed on autopsy.

Imaging diagnosis

Case 1: On CT diagnosis of pulmonary blastoma or CPAM were suggested; Case 2: Diagnosed on autopsy.

Pathological diagnosis

Case 1: Finally, the case was diagnosed as CPAM (congenital cystic adenomatoid malformation) Type Ⅲ with acute pneumonia; Case 2: On autopsy findings, the case was finally diagnosed as CPAM Type Ⅱ.

Treatment

Case 1: Lobectomy was planned and performed; Case 2: Died immediately.

Experiences and lessons

Early pulmonary resection for asymptomatic CPAM is required and recommended to make a definitive diagnosis and determine the prognosis of the disease.

Peer-review

This is an interesting case report.

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CASE REPORT

Composite pheochromocytoma masquerading as solid-pseudopapillary neoplasm of pancreas

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Author contributions: Gupta G, Godhi S, Saran RK and Saluja SS equally contributed; Gupta G and Godhi S wrote paper; Srivastava S and Saluja SS contributed in management of the patient; Saluja SS and Mishra PK revised the paper along with managing the case.
Ethics approval: We did not take any ethics committee approval as we are describing a histological surprise and not conducting an experimental study.
Informed consent: A written informed consent was taken from the patient and his relatives.
Conflict-of-interest: All the authors declare that they do not have any conflict of interest in the subject of the case report.

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Abstract

Pheochromocytoma and ganglioneuroma form rare composite tumours of the adrenal medulla comprising less than 3% of all sympathoadrenal tumours. We present a case of intraoperatively detected adrenal medullary tumour of composite pheochromocytoma and ganglioneuroma diagnosed on histopathology, in a normotensive patient. A 50-year-old male with a past history of chronic obstructive pulmonary disease presented with abdominal pain and significant weight loss since one month. Ultrasound and contrast-enhanced computed tomography abdomen revealed a large lobulated lesion in the distal body and tail of pancreas suggestive of solid and papillary neoplasm of body and tail of pancreas. Intra-operatively, a 15 cm × 10 cm solid lesion with cystic areas was seen arising from the left lower pole of the adrenal gland pushing the pancreas which appeared unremarkable. In our case, exploratory laparotomy with tumour excision was done. Extensive sectioning and microscopic examination of this adrenal tumour confirmed a diagnosis of composite Pheochromocytoma with Ganglioneuroma on histopathology. Immunophenotyping with S-100 further supported the diagnosis. The goal of this report is to increase the awareness of this rare disease and to further identify its variable presentation.

Key words: Ganglioneuroma; Pheochromocytoma; Adrenal

Core tip: Adrenal composite pheochromocytomas may present in various ways without hypertension and conclusive symptoms. We present a case of intraoperatively detected adrenal medullary tumour of composite pheochromocytoma and ganglioneuroneuroma diagnosed on histopathology, in a normotensive patient. The goal of this report is to increase the awareness of this rare disease and

to further identify its variable presentation.

Gupta G, Saran RK, Godhi S, Srivastava S, Saluja SS, Mishra PK. Composite pheochromocytoma masquerading as solid-pseudopapillary neoplasm of pancreas. *World J Clin Cases* 2015; 3(5): 474-478 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i5/474.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i5.474

INTRODUCTION

Composite tumours of the adrenal medulla combining features of pheochromocytoma and ganglioneuroma are extremely rare. These tumours typically combine features of pheochromocytoma or paraganglioma with those of ganglioneuroma, ganglioneuroblastoma, neuroblastoma, peripheral nerve sheath tumour or neuroendocrine carcinoma. Most cases of composite pheochromocytomas are functional, with symptoms related to increased levels of catecholamines or corticotrophin-releasing hormone or their metabolites. Associated symptoms usually include headache, palpitation, excessive perspiration and hypertension in majority of the cases. We report a case of composite pheochromocytoma with ganglioneuroma in a normotensive patient who presented with an abdominal lump, diagnosed on histopathology clinically masquerading as solid-pseudopapillary neoplasm in tail of pancreas.

CASE REPORT

A 50-year-old male, known case of chronic obstructive pulmonary disease (COPD) for 12 years, presented with lump in abdomen associated with pain since one month. He also had history of significant weight loss. There was no associated history of hypertension or diabetes mellitus. On chest examination he had decreased breath sounds in left basal region and expiratory wheeze was noted. Per abdomen examination revealed an ill defined, non-tender mass in the left hypochondrium extending to the left lumbar region. Ultrasound done showed a solid cystic mass which appeared to arise from pancreas. Contrast enhanced computed tomography (CT) scan confirmed a lobulated mass lesion measuring 10.8 cm × 8.1 cm × 9.1 cm arising from body and tail of the pancreas suggestive of a solid-pseudopapillary neoplasm of the pancreas (Figure 1A). At endoscopic ultrasound the mass appeared to originate from body of pancreas with displacement of the splenic vessels (Figure 1B).

Pre-operatively, as per the advice of the chest physician, the patient was stabilised with antibiotics and bronchodilators and further taken up for surgery under moderate risk. Intra-operatively, a highly vascular solid lesion with cystic areas pushing the pancreas, splenic artery and splenic vein upwards probably arising from the retroperitoneum was noticed. Since the patient had fluctuation in blood pressure on handling the tumour, a pheochromocytoma was suspected. Patient was stabilized with nitroglycerine and adrenal infusion and the surgery was completed. Right kidney and adrenal were unremarkable. Post-operatively the patient was extubated on day 2. On Post-operative day 3 (POD-3), the patient started developing breathlessness. He was re-intubated on POD-4 as his blood gas levels deteriorated. Arterial blood gas analysis showed features of type I respiratory failure. The patient died on POD-6.

The surgical specimens were formalin fixed and paraffin embedded. The sections were stained with routine hematoxylin and eosin stain. Immunohistochemical staining was performed by the streptavidin-biotin-peroxidase method and diaminobenzidine as chromogen. The antibodies used included S-100, Synaptophysin, Vimentin, Chromogranin and neurofilament protein. Appropriate positive and negative controls were performed.

Grossly, the left adrenal tumour was well-encapsulated and lobulated measuring 13.5 cm × 10 cm × 6 cm, and weighing 310 g. Cut-surface of the tumour showed multiloculated cystic areas along with a few solid and hemorrhagic areas. Histopathological examination showed a well-encapsulated composite tumour comprising of pheochromocytoma with ganglioneuroma (Figure 2A). The pheochromocytoma component (accounting for 10% of the mass) comprised of polygonal intermediate cells with amphophilic cytoplasm arranged in well-defined nests separated by delicate fibrovascular stroma (Zellballen pattern). These tumour cells had moderate to abundant granular eosinophilic to amphophilic cytoplasm, round to oval nuclei and single prominent nucleoli. The ganglioneuromatous component predominated (accounting for 80%-90% of the mass), and showed sheets of mature ganglion cells surrounded by fascicles of Schwann-like cells (Figure 2B). Areas of haemorrhage and necrosis were noted within the tumour. Normal compressed adrenal was found at periphery.

Immunohistochemically, the pheochromocytoma component was strongly positive for synaptophysin (Figure 2C). However, the gangliocytes were strongly positive for neurofilament protein and vimentin (Figure 2D and E). The sustentacular cells of the pheochromocytoma component and the schwannian cells of the ganglioneuroma component showed characteristic staining of S-100 (Figure 2F). MIB-1 index was noted to be less than 1%.

DISCUSSION

Composite tumours of the adrenal medulla are rare accounting for less than 3% of sympathoadrenal tumours, and typically comprise of a pheochromocytoma component along with a non-pheochromocytoma component which can include ganglioneuroma, ganglioneuroblastoma, neurofibromatosis-1 and rarely schwannoma. Less than 50 cases have been reported

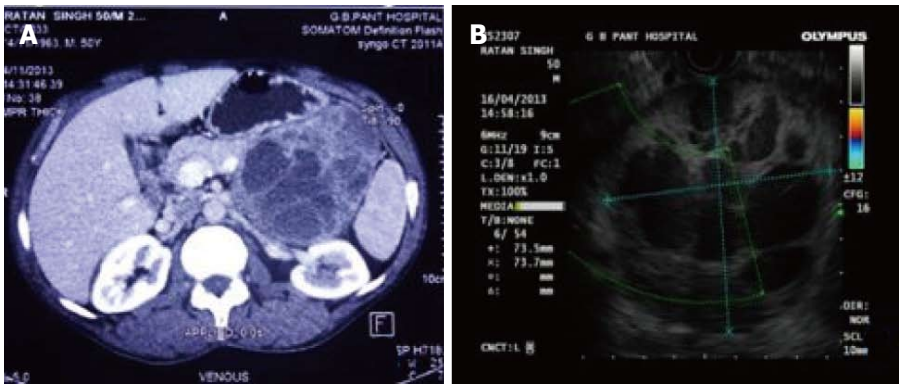

Figure 1 Contrast enhanced computed tomography and endoscopic ultrasound for pancreas. A: Contrast enhanced computed tomography shows 10.8 cm × 8.1 cm × 9.1 cm solid cystic mass arising from body and tail of the pancreas; B: Endoscopic ultrasound showing a well-encapsulated heteroechoic mass inseparable from tail of pancreas.

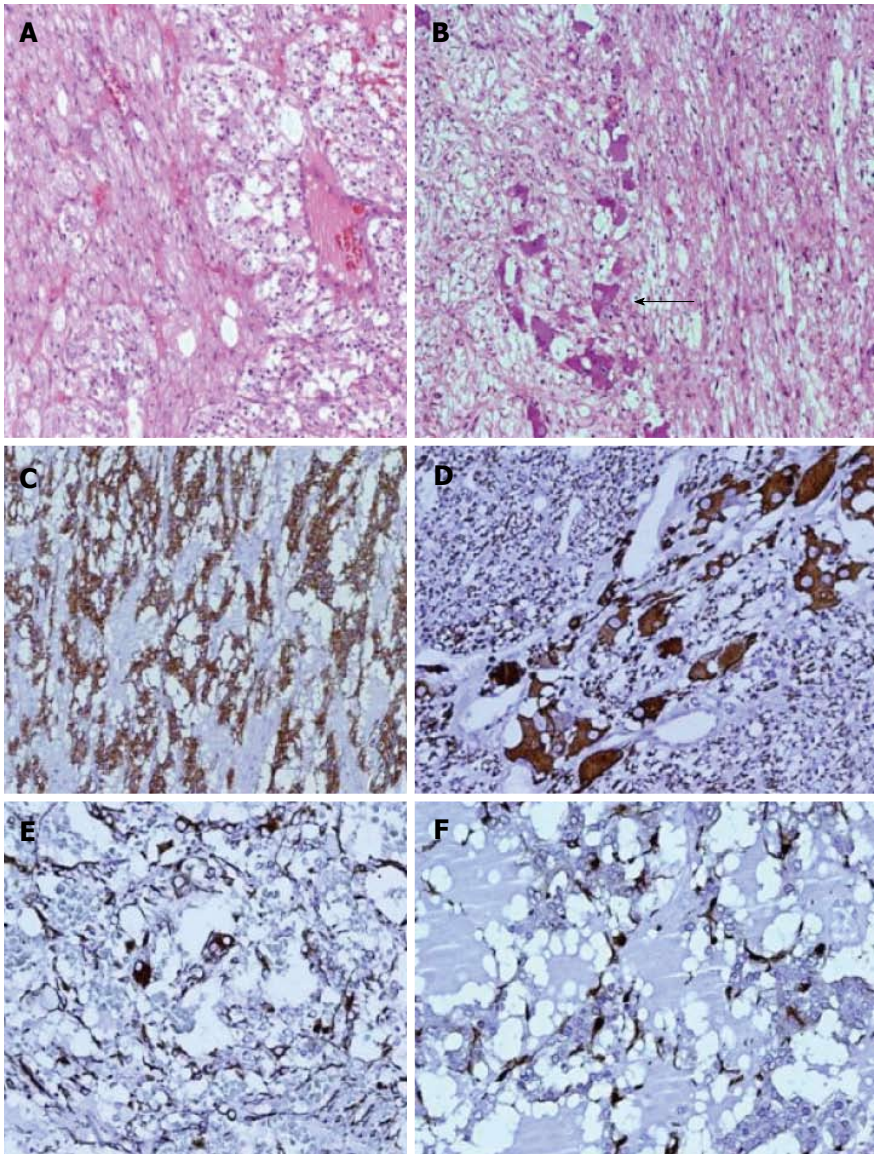

Figure 2 HE stain. A: Scattered ganglion cells with plump cytoplasm surrounded by intersecting bundles of Schwann cells (HE stain × 200); B: Two distinct components of the tumour–pheochromocytoma comprising of atypical polygonal chromaffin cells and ganglioneuroma component (arrow) with sheets of mature ganglion cells surrounded by fascicles of schwann cells (HE stain × 200); C: The chromaffin cells were strongly positive for synaptophysin (× 100); D: The ganglion cells were strongly positive for neurofilament protein (× 100); E: The gangliocytes were strongly positive for vimentin (× 200); F: The sustentacular cells of the pheochromocytoma component showed characteristic staining of S-100 (× 200).

in medical literature. Pheochromocytoma represents a tumour that originates from the adrenal medullary chromaffin cells, while ganglioneuroma is known to originate from autonomic ganglion cells or its precursors. However, both the chromaffin and the ganglion cells arise from a common embryonic progenitor which is the neural crest.

Clinical features

The age range for composite pheochromocytomas is 14-74 years (median 50 years). Patients with composite tumours tend to be older than pure pheochromocytomas. Mostly, 90% of these tumours are localized in the adrenal gland and the remainder in the urinary bladder, organ of Zuckerkandl, or elsewhere

in the retroperitoneum. Shawa *et al*[1] described 9 patients with composite pheochromocytomas and concluded that composite pheochromocytomas; and pheochromocytomas alone are indistinguishable clinically, biochemically and on imaging. Clinically pheochromocytoma can be suspected with its classical symptoms of headache, sweating and palpitations with paroxysmal or sustained hypertension. Kragel *et al*[2] found hypertension in 4 of 13 patients with composite pheochromocytomas. In a review of composite pheochromocytomas by Khan *et al*[3], 76.3% were functional. As 3/4th of these patients have symptoms of pheochromocytoma, preoperative biopsy is not advisable. Tischler *et al*[4] also has similar conclusions stating that the clinical presentation is usually as those of pure pheochromocytomas and so are the profiles of catecholamines and their metabolites. Neural components may cause a watery diarrhea-hypokalemia-achlorhydria syndrome due to increased production of vasoactive intestinal polypeptide. However, in our case, a normotensive male patient presented with an abdominal lump with no symptoms referable to pheochromocytoma.

In some patients the absence of endocrine abnormalities and symptoms of pheochromocytoma cannot be explained. One of the reasons for this is the autoregulation of the pheochromocytoma cells by the ganglion cells in the ganglioneuroma component which was first described by Aiba *et al*[5].

Imaging

Various imaging modalities can be used to differentiate the pancreatic tumours from adrenal masses and help the clinician to make a precise diagnosis and customize treatment accordingly. Abdominal ultrasound carries a diagnostic sensitivity of 87%-90% and 96% for detecting pancreatic and adrenal tumours respectively[6]. The above results differ slightly with those of other imaging modalities like contrast-enhanced CT, having a sensitivity of 89% and 92% respectively. A fine-needle aspiration biopsy guided by endoscopic ultrasound may provide tissue diagnosis in patients who are not surgical candidates, with a sensitivity of 92% and a specificity of 100%[7]. Shawa *et al*[1] in his series of 9 patients described the CT scan features of pheochromocytoma-ganglioneuroma composite tumour (PC-GN). These tumours have 43.5 Hounsfield units (HU) and there was significant enhancement on postcontrast venous-phase imaging. On delayed-phase complete wash out was seen but around 1/3rd of patients showed no washout. Five tumors (83%) were heterogeneous and four tumors (67%) had cystic components. The composite pheochromocytomas cannot be distinguished from pure pheochromocytomas based on CT scan and have similar morphology and pre and post contrast density values[1].

Pathology

Most case reports that describe composite pheochromocytomas have made diagnosis based on post operative histopathological examination and immunohistochemistry. Thus precise preoperative diagnosis is not possible in composite pheochromocytomas. Immunohistochemically, useful battery of markers include chromogranin A, synaptophysin and catecholamine biosynthetic enzymes tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT). In addition staining for S-100 protein, will identify Schwann cells and sustentacular cells. Neurofilament protein will identify axon like processes.

Prognosis and management of composite tumours

In the study by Shawa *et al*[1], the PC-GN composite tumor in one of the patients did not show any growth over 6 years. In addition 6 of their patients had no PC-GN recurrence during a median follow-up of 29 mo according to findings on serial abdominal imaging and/or levels of fractionated plasma-free metanephrine. According to the literature, one in eight patients with PC-GN developed recurrent disease with distant metastasis during a median follow- up of 15 mo[3,8]. Two other reported cases of PC-GN had distant metastasis at the time of diagnosis[1,4]. Including the series by Shawa *et al*[1] the rate of malignancy in PC-GN composites is almost 8%, compared with 13% to 25% in PC[9,10]. Thus, the presence of a GN composite in PC tumors does not seem necessarily to imply a worse prognosis. Malignancy in pheochromocytomas cannot be confidently predicted on the basis of clinical, biochemical, radiological or histopathological features. The only criterion that may help is the presence of distant metastasis. However, malignant tumours generally have an extraadrenal location, greater tumour weight (> 80 g), larger size (> 5 cm), exhibit high mitotic activity along with vascular/capsular invasion[4]. In our case, the tumour was largely benign as there were no histopathological features suggestive of malignancy, and MIB-1 index was < 1%. The only described treatment is surgical excision and no adjuvant chemotherapy or radiotherapy has been advised[11].

Association with genetic disorders

Of the 45 reported cases in the literature, 8 (18%) cases were associated with genetic disorders (6 cases of NF1, 1 case of MEN2A, and 1 case of Von Hipple-Lindau). Kimura *et al*[12] explained the association between NF1 and PC-GN composites by noting that neurofibromin insufficiency may induce abnormal proliferation of Schwann cells and produce neurotrophins that cause pronounced proliferation of the PC and ganglionic cells through autocrine and paracrine loops. This process eventually results in the formation of composite PC-GNs.

COMMENTS

Case characteristics

Non hypertensive/non-diabetic 50-year-old man had abdominal pain × 1 mo with anorexia and weight loss. He had an ill defined lump in left hypochondrium

and lumbar region.

Clinical diagnosis

Body tail pancreatic mass.

Differential diagnosis

Retroperitoneal tumour, renal cell carcinoma.

Laboratory diagnosis

Laboratory tests were inconclusive.

Imaging diagnosis

On ultrasound complex cystic pancreatic body tail mass. Computed tomography scan suggest Solidpseudopappilary mass body tail pancreas supported by endoscopic ultrasound findings.

Pathological diagnosis

Composite pheochromocytoma.

Treatment

He underwent surgical excision of the tumour with intra-operative haemodynamic stabilization with adrenaline and GTN.

Related reports

Composite pheochromocytomas with ganglioneuromas are rare tumours and less than 50 cases have been reported with 3/4th of the cases having symptoms of pheochromocytoma unlike this case who had only pain abdomen as his main complaint

Term explanation

PC-GN: Pheochromocytoma-ganglioneuroma.

Experiences and lessons

Composite tumours are rare in occurrence. Although they present with clinical features similar to pheochrocytoma in ¾ cases but asymptomatic tumours can mimic solid pseudopapillary tumours or other retroperitoneal tumours depending upon their location. Imaging characteristics are similar to pheochrocytoma but can have an overlap with solid pseudopapillary tumour. Treatment is surgical excision and they carry a good prognosis.

Peer-review

The case report is interesting and well written.

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EDITORIAL

Crohn's disease with gastroduodenal involvement: Diagnostic approach

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Abstract

Crohn's disease (CD) is a chronic idiopathic inflammatory disease of gastrointestinal tract characterized by segmental and transmural involvement of gastrointestinal tract. Ileocolonic and colonic/anorectal is a most common and account for 40% of cases and involvement of small intestine is about 30%. Isolated involvement of stomach is an extremely unusual presentation of the disease accounting for less than 0.07% of all gastrointestinal CD. To date there are only a few documented case reports of adults with isolated gastric CD and no reports in the pediatric population. The diagnosis is difficult to establish in such cases with atypical presentation. In the absence of any other source of disease and in the presence of nonspecific upper gastrointestinal endoscopy and histological findings, serological testing can play a vital role in the diagnosis of atypical CD. Recent studies have suggested that perinuclear anti-neutrophil cytoplasmic antibody and anti-Saccharomycescervisia antibody may be used as additional diagnostic tools. The effectiveness of infliximab in isolated gastric CD is limited to only a few case reports of adult patients and the long-term outcome is unknown.

Key words: Gastrointestinal tract; Crohn's disease; Isolated gastric involvement; Perinuclear anti-neutrophil cytoplasmic antibody; Anti-Saccharomycescervisia antibody

Core tip: The stomach is rarely the sole or predominant site of Crohn's disease (CD) accounting for less than 0.07% of all gastrointestinal CD. Serological testing and meticulous histopathological examination by excluding other causes of granulomatous gastritis can play a vital role to arrive at the correct diagnosis.

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INTRODUCTION

Crohn's disease (CD) can affect any region from mouth to the anus. Isolated Gastroduodenal involvement is an extremely unusual event. The CD is diagnosed usually on the basis of clinical, laboratory, upper gastrointestinal (GI) scopy and histopathology. The anti-Saccharomycescervisia antibody (ASCA) is relatively good specific marker with minimal sensitivity. However, it is difficult to diagnose it in patients with isolated involvement of stomach and duodenum. In such circumstances other granulomatous conditions must be excluded with careful evaluation of the patient to hit the accurate pathological cause[1,2].

The famous criteria to diagnose this rare condition are: (1) evidence of noncaseating granulomas on histopathology; and (2) confirmation of changes of Crohn's disease on endoscopy or radiography[3-10].

EPIDEMIOLOGY

Incidence

It occurs in 0.5% to 4% patients of CD[3-6]. Isolated stomach and duodenum involvement accounts for less than 0.07% of all cases of CD[1].

Pattern of involvement

Most patients show involvement of terminal ileum and distal segment of large intestine[4,5,7]. Contiguous involvement of stomach and duodenal involvement is most common (60%)[6,10-12].

PATHOPHYSIOLOGY

For pathogenesis of isolated gastric CD multiple hypothesis were postulated: (1) the hygiene hypothesis relatively less trained and weak immunological system leading to ineffective immune response to newer antigens; (2) the environmental factors *i.e.*, geography, smoking, drugs, diet are also main contributing factors[13,14]; (3) immune mechanism - It is being postulated that the immune reactivity in this disease is due to "loss of immune tolerance" to self antigens of intestinal flora, resulting into an inappropriate granulomatous immune response of Chron's disease[15,16]; and (4) role of chemical mediators - interferon-γ, interleukin (IL)-12, IL-18 and increased expression of T-bet[17-19]. T-cells are not undergoing apoptosis[20-25].

CLINICAL PRESENTATIONS

Age

The disease mainly seen in the age group 30-40 years[6].

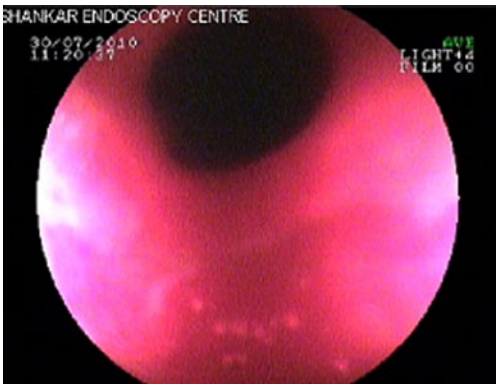

Figure 1 Endoscopic findings include patchy erythematic, gastric outlet narrowing.

Sex predilection

Male to female ratio is 1.2:1[6,12].

Symptoms and signs

Majority of the patients are usually symptomless[9]. Most of the patients are presenting with pain in epigastric region, relieved by antacids and food intake[4,9,11]. In cases with stricture formation persistent pain, nausea and vomiting are common[4]. Many times,it may simulates acid peptic disease clinically[4]. Acute blood loss may rarely occur[4,9,11,26,27].

Uncommon presentations

Uncommon presentations of CD may manifest as a single symptom or sign, such as impairment of linear growth, delayed puberty, perianal disease, mouth ulcers, clubbing, chronic iron deficiency anemia or extra-intestinal manifestations preceding the gastrointestinal symptoms, mainly arthritis or arthralgia and rarely osteoporosis[2]. In such cases, the diagnosis is challenging and can remain elusive for some time.

DIAGNOSTIC EVALUATION

Radiological signs

Aphthous ulcer is the early feature on radiography[28]. The characteristic features are presence of nodularity in the mucosa giving classic appearance of "cobblestone"[4]. Radiography examination using double-contrast medium is useful in cases with stenoses or strictures which are mainly seen in advanced disease[6,12,27,29,30]. A barium enema should be done in suspected cases of gastro colic fistula[4].

Endoscopy

Endoscopy with biopsy is an effective diagnostic modality[6,9,27,30]. Endoscopic findings include patchy erythema, gastric outlet narrowing (Figure 1) mucosa is friable, thickening of mucosa and ulcerations linear as well as aphthous[4,7,9,12]. The ulcers of CD are typically linear or serpiginous in contrast to the peptic

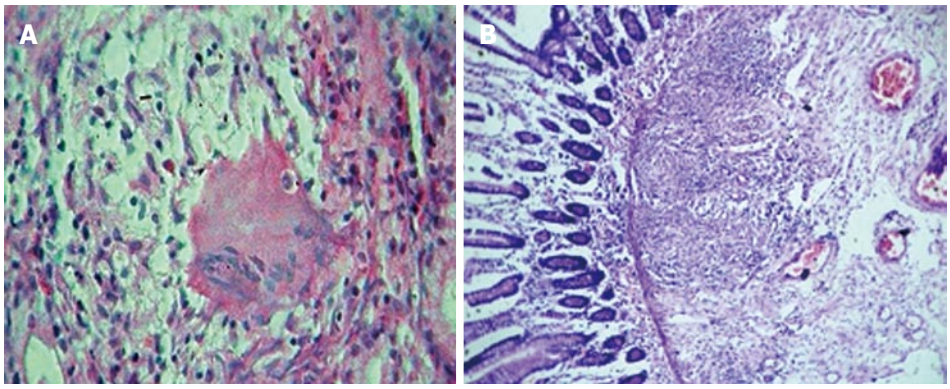

Figure 2 Biopsy showing non-caseating granulomas and oedema in the submucosa (HE × 10). A: Non-caseating granulomas; B: Oedema alongwith granulation tissue.

ulcers[27]. In cases with diffuse stomach involvement a linitis plastica appearance,is seen[31,32]. Sophisticated endoscopic features such as, bamboo-joint-like appearance and notched sign can be seen[33].

Biopsy findings

The biopsy findings are often nonspecific. Exclusion of other causes of granulomatous lesions is important. Granulomas without caseation are noted in 5% to 83% of cases (Figure 2)[9,12]. The differential diagnosis of granulomatous gastritis are *H. pylori* infection, gastric sarcoidosis, tuberculosis, syphilis, *etc*[7,9,32]. So presence of granuloma is not a definitive criterion to arrive at the diagnosis. *H. pylori* negative chronic gastritis is common feature.

Additional histological features are mucosal edema, crypt abscesses, lymphoid aggregates and fibrosis[32-34].

Serological markers

Currently, it has been stated that perinuclear anti-neutrophil cytoplasmic antibody (pANCA) and ASCA can be used as supportive diagnostic tools. Indeed, ASCA is detected in 55%-60% of children and adults with CD and only 5%-10% of controls with other gastrointestinal disorders. This finding pANCA highlights the relatively good specificity but poor sensitivity of ASCA as a marker for CD. pANCA on the other hand is more specific to ulcerative colitis.

Genetic studies

In addition, some *NOD2/ CARD15* gene polymorphisms were found to be associated with CD with gastroduodenal involvement. It is possible that these genes might also help to support the diagnosis in the atypical presentation of CD in the future[2].

DIFFERENTIAL DIAGNOSIS

The differential diagnosis includes corrosive gastritis due to ingestion of lye, gastric scirrhous carcinoma, Ménétrier's disease. Pseudolymphoma, amyloidosis can also mimic CD[29]. Although Ménétrier's disease can involve the entire stomach and produce ulcérations, it does not cause transmural disease[29]. Malignant and infiltrative processes are to be ruled out by the histological findings.

TREATMENT

Medical treatment

Proton pump inhibitors in combination with steroids are the first line of treatment in active CD. Some of the studies proved steroid-induced remission in active disease[10,11,35-39]. But, 6-Mercaptopurine and azathioprine are proved to be helpful to maintain steroid induced remission.

Balloon dialation

Strictures are treated successfully with balloon dilation[4,5,40-43].

Surgical intervention

Some of the patients requires surgical intervention, where patients are not responding to medical treatment[44]. Other situations are massive and persistent upper gastrointestinal hemorrhage, gastric outlet obstruction, and fistula or abscess formation[4,5,7,10,12,45]. The important indication is duodenal obstruction[6]. The surgical modalities of treatment include bypass surgery with gastrojejunostomy[6,7,9]. Gastrojejunostomy with highly selective vagotomy is an ideal line of management[44]. Delayed gastric emptying is a postoperative complication seen in 24% of cases, but this may be seen in stricturoplasty also[6,46,47]. Additional post operative complications are anastomotic leak, enterocutaneous fistula, intraabdominal abscess, and stomal ulceration[48].

CONCLUSION

To conclude, CD with isolated gastric involvement is an extremely unusual event in clinical practice. Endoscopic biopsy along with battery of laboratory tests is an effective tool to hit the correct diagnosis by exclusion of

various causes of granulomatous gastritis. This prevents untoward mortality and/morbidity related to disease and treatment.

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REVIEW

Giant cell arteritis: Current treatment and management

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Abstract

Glucocorticoids remain the cornerstone of medical therapy in giant cell arteritis (GCA) and should be started immediately to prevent severe consequences of the disease, such as blindness. However, glucocorticoid therapy leads to significant toxicity in over 80% of the patients. Various steroid-sparing agents have been tried, but robust scientific evidence of their efficacy and safety is still lacking. Tocilizumab, a monoclonal IL-6 receptor blocker, has shown promising results in a number of case series and is now being tested in a multi-centre randomized controlled trial. Other targeted treatments, such as the use of abatacept, are also now under investigation in GCA. The need for surgical treatment is rare and should ideally be performed in a quiescent phase of the disease. Not all patients follow the same course, but there are no valid biomarkers to assess therapy response. Monitoring of disease progress still relies on assessing clinical features and measuring inflammatory markers (C-reactive protein and erythrocyte sedimentation rate). Imaging techniques (*e.g.*, ultrasound) are clearly important screening tools for aortic aneurysms and assessing patients with large-vessel involvement, but may also have an important role as biomarkers of disease activity over time or in response to therapy. Although GCA is the most common form of primary vasculitis, the optimal strategies for treatment and monitoring remain uncertain.

Key words: Giant cell arteritis; Therapy; Disease management; Glucocorticoids; Immunosuppressive agents

Core tip: Giant cell arteritis (GCA) is the most common form of primary systemic vasculitis. Treatment with high doses of glucocorticoids should be initiated as early as possible to prevent ischaemic manifestations, such as blindness (occurring in up to 20%). However, glucocorticoid therapy leads to significant toxicity in over 80% of the patients. Various steroid-sparing agents have been tried, but robust scientific evidence of their efficacy and safety is still lacking. Not all patients follow the same course, but there are no valid biomarkers

to assess therapy response. The authors review the optimal strategies for treatment and monitoring of patients with GCA.

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INTRODUCTION

Giant cell arteritis (GCA), also called temporal arteritis, is the most common form of primary systemic vasculitis, with an overall incidence of 15-25 per 100000 per year[1]. It affects large and medium-sized blood vessels with a predisposition for the cranial branches derived from the carotid artery; in approximately 50% of cases, the aorta and its major branches may also be involved[2,3]. It typically affects individuals aged above 50 years and is two to four times more common in women than men[4,5]. Polymyalgia rheumatica (PMR) can be present in up to 50% of the cases, beginning before, simultaneously or after the clinical manifestations of GCA, suggesting they are different spectrums of the same disease process[6].

Due to the intense myointimal proliferation and vessel occlusion, which in up to 20% of the cases may lead to blindness (usually permanent), GCA is considered a medical emergency[7,8]. Treatment with high doses of glucocorticoids should be initiated as early as possible to rapidly control inflammatory symptoms and prevent ischaemic manifestations, such as jaw claudication, visual loss and stroke. However, the burden of high-dose glucocorticoids is considerable, especially in the elderly, with over 80% of the patients experiencing significant treatment related side-effects[9]. Proven *et al*[10] have reported a high number of major adverse advents related to long-term glucocorticoid use in GCA: posterior subcapsular cataract (41%), bone fractures (38%), infections (31%), hypertension (22%), diabetes mellitus (9%) and gastrointestinal bleeding (4%).

Moreover, the optimal duration and doses of glucocorticoid treatment varies from patient to patient, as well as the need to add other immunosuppressant agents to control disease activity and reduce glucocorticoid toxicity.

The key issues in managing GCA after its diagnosis are prompt institution of correct therapy; recognition and amelioration of the adverse events related to immunosuppressant medications; and rapid identification of disease activity and flares.

Our purpose is to review the current therapeutic options, guidelines and clinical trials in GCA, as well as to discuss follow-up strategies and potential biomarkers for this condition (Figure 1).

MEDICAL TREATMENT

In GCA medical treatment is required for induction and maintenance of remission.

Induction

Glucocorticoids: Glucocorticoids remain the cornerstone of treatment in GCA since their discovery in the 1950s[11]. They should be prescribed immediately after the diagnosis of GCA is suspected, and in most cases are able to provide complete symptomatic relief within 24-48 h[12].

Despite their importance, there are no clinical trials comparing different glucocorticoid dosing regimens. Most clinicians will base their practice on personal experience and on the European League Against Rheumatism (EULAR) and British Society for Rheumatology (BSR) guidelines[9,13]. These guidelines were based on an extensive literature review of the available evidence, including published data from randomised controlled trials, and full consensus by expert opinion.

The EULAR guidelines recommend 1 mo of high-dose glucocorticoid therapy (prednisolone 1 mg/kg per day, maximum 60 mg/d) for induction of remission and pulsed intravenous methylprednisolone for patients with early onset of visual symptoms (dose not specified). The BSR guidelines advise prednisolone 40 to 60 mg (at least 0.75 mg/kg) daily until the resolution of symptoms and laboratory abnormalities for patients with uncomplicated GCA (without visual loss or jaw claudication); 500 mg to 1 g of intravenous methylprednisolone per day for 3 d for patients with visual loss or a history of amaurosis fugax; and at least 60 mg prednisolone daily for patients with established visual loss. Daily dosing is more effective than alternate day dosing, but single or divided daily doses have shown comparable results[14].

Induction treatment with high-dose pulsed intravenous (IV) methylprednisolone (15 mg/kg for 3 consecutive days followed by oral prednisone dose of 40 mg/d) has been suggested for all patients with GCA to allow faster tapering and a lower cumulative steroid dose in a double-blind, placebo-controlled, randomized trial involving 27 patients[15]. Although a greater number of patients were able to reduce their oral prednisolone to 5 mg/d by week 36 with this regimen, the small sample size was not sufficient to draw conclusions regarding the differences in steroid-related adverse events; therefore, these results should not be generalized. Larger studies are needed to address this issue[16].

The most frequent type of eye involvement in GCA is anterior ischemic optic neuropathy[17], but other visual manifestations can also occur (Table 1). A degree of controversy exists regarding induction treatment by either high-dose pulsed IV methylprednisolone or oral prednisolone in GCA patients with visual symptoms. There are patients who despite being given high doses of IV methylprednisolone still develop visual loss. This might be explained by the latent period of up to 5 d

Table 1 Eye manifestations in giant cell arteritis

Anterior ischemic optic neuropathy
Posterior ischemic optic neuropathy
Arterial occlusion (central retinal artery, branch retinal artery or cilioretinal vessels)
Amaurosis fugax
"Cotton-wool spots" (microinfarcts of the retinal nerve fiber layer)
Diplopia (involvement of muscles, cranial nerves, or brainstem)
Ocular ischaemic syndrome (hypotension, ischaemic iritis)

Adapted from Ness *et al*[17].

between starting treatment and controlling the arteritic process in the wall of the posterior ciliary arteries; as well as by the decreased perfusion pressure in the vascular bed of the optic nerve head that makes it very prone to ischaemia due to any minor fall of the systemic blood pressure[18]. Another proposed explanation is that glucocorticoids may have a pro-coagulant effect by enhancing platelet activation[19], but this needs further confirmation. Although conflicting data exist[18,20-22], most clinicians, especially ophthalmologists, will prescribe IV steroids when presented with a patient with GCA with acute visual impairment.

Other immunosuppressive therapy: The key to successful induction therapy is to initiate glucocorticoids as quickly as possible, given their rapid onset of action. Other immunosuppressive treatments prescribed at presentation of the disease have been tried, particularly with the aim of allowing a faster withdrawal of steroids or help controlling severe manifestations of the disease; however, results have been conflicting and generally disappointing[23-25].

Nevertheless, when a patient has an unacceptable high-risk of glucocorticoid-related side effects, such as concomitant severe osteoporosis and poorly controlled high blood pressure or diabetes mellitus, it might be feasible to add another immunosuppressive (*e.g.*, methotrexate[26]) at the onset of the disease to allow a safer and faster tapering of glucocorticoids.

Maintenance

Glucocorticoids: Glucocorticoid reduction should be considered only in the absence of clinical symptoms, signs and laboratory abnormalities suggestive of active disease. The tapering regimen is variable; it is highly dependent on the clinician's personal experience, disease severity and response to treatment, use of concomitant immunosuppressive agents, the patient's compliance and the occurrence of steroid related toxicity. Nevertheless, the BSR[13] has proposed a standard tapering scheme after 1 mo of treatment: reducing by 10 mg of prednisolone every 2 wk to 20 mg, then another 2.5 mg every 2-4 wk to 10 mg, followed by a decrease of 1 mg every 1-2 mo.

During steroid tapering, flares occur in up to 50% of patients, requiring escalation of glucocorticoids and a more prolonged treatment course. An increase of 5-10 mg/d of prednisolone is usually sufficient to treat a common relapse; however, in the presence of ocular or neurological symptoms an increase to the original induction dose (0.75-1 mg/kg per day) should be considered.

There are no reliable predictors to determine treatment duration. Hernández-Rodríguez *et al*[27] have suggested that the intensity of the systemic inflammatory response at baseline may influence the number of disease relapses and time needed to safely withdrawal steroids. Different studies have shown different treatment durations[28-30], but typically 2 to 3 years are necessary for the patients to be weaned off glucocorticoids without any clinical features of active disease. In some cases, particularly when the disease is recurrent or there is secondary adrenal insufficiency, the treatment duration may exceed 5 years[31,9].

Other immunosuppressive therapy: The search for an effective disease-modifying agent for the treatment of GCA has proven elusive. Few clinical trials have been performed; the number of patients enrolled is limited and the duration of follow up is often short. A number of drugs have been studied, with disappointing results to date.

However, given the significant burden of morbidity associated with long term glucocorticoid treatment, current BSR guidelines for the management of GCA recommend consideration of the early introduction of methotrexate or alternative immunosuppressant therapy following a relapse[13] and EULAR guidelines for the management of large vessel vasculitis recommend that an immunosuppressant agent should be considered for use in large vessel vasculitis as adjunctive therapy[9].

Of the limited available evidence, methotrexate may be of benefit in the management of GCA. Three prospective randomised double blind placebo controlled trials have addressed this issue. In 2001, Spiera *et al*[23] randomised 21 patients with newly diagnosed GCA to high dose corticosteroids plus methotrexate (up to 20 mg once weekly) or placebo. At study completion there were no differences between the methotrexate and placebo groups with regard to cumulative steroid dose, relapse or adverse events[24]. In a similar study, in the same year, Jover *et al*[26] randomised 42 patients to high dose corticosteroid and methotrexate (10 mg per week) or placebo. Significant differences were reported in terms of reduction in relapse (one relapse $P = 0.02$, multiple relapses $P = 0.004$) and lower mean cumulative steroid dose ($P = 0.0009$) for the methotrexate treated group. Adverse events were similar in the two groups. In 2002, Hoffman *et al*[32] randomised 98 patients with newly diagnosed GCA to glucocorticoids plus either methotrexate (up to a maximum of 15 mg once weekly) or placebo. No differences were observed in cumulative steroid exposure or in the number of adverse events between the methotrexate and placebo treated groups. One patient in the methotrexate treated group relapsed *vs*

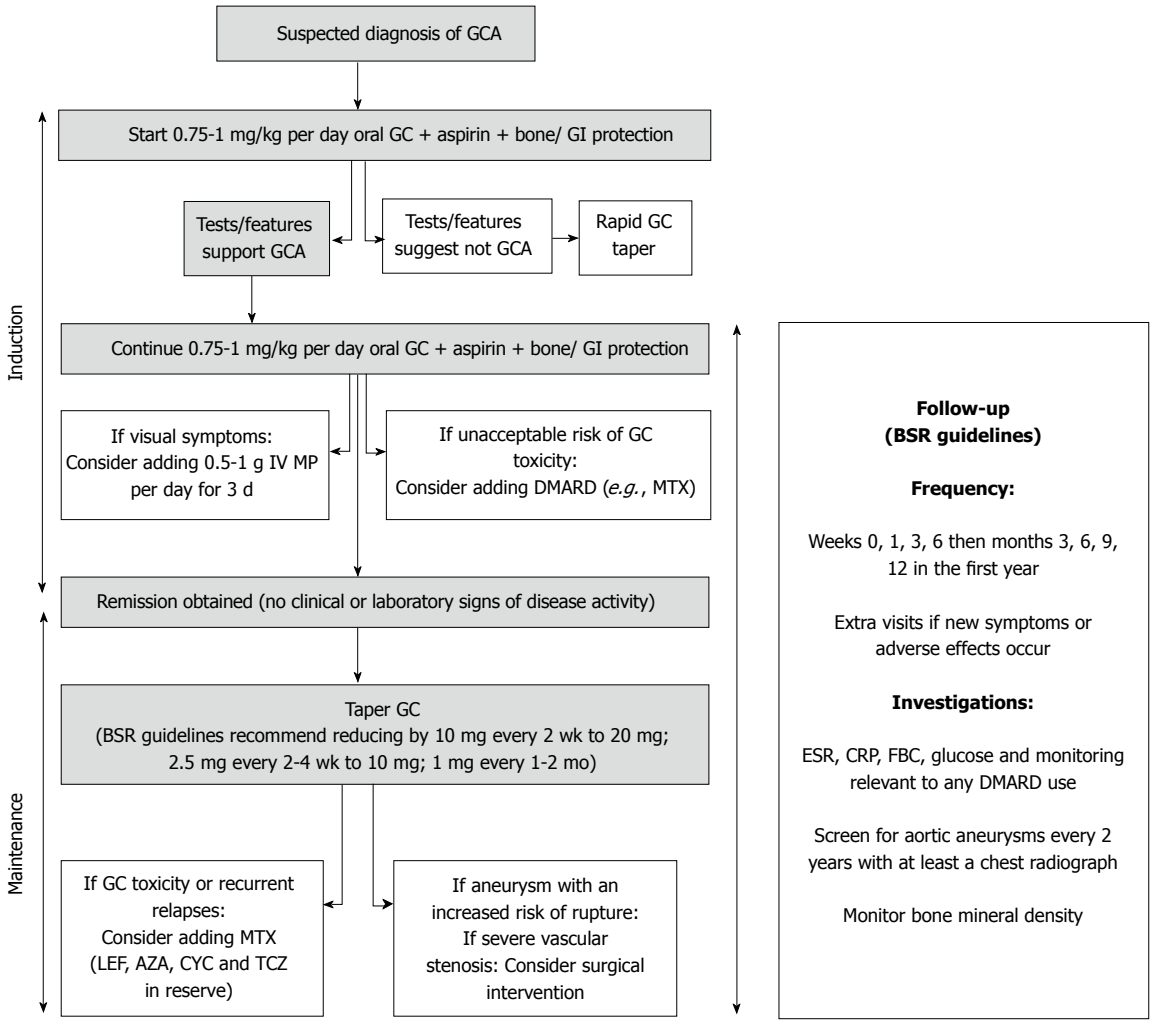

Figure 1 Current schema for giant cell arteritis treatment. AZA: Azathioprine; BSR: The British Society for Rheumatology; CRP: C-reactive protein; CYC: Cyclophosphamide; DMARDs: Disease-modifying antirheumatic drugs; ESR: Erythrocyte sedimentation rate; FBC: Full blood count; GC: Glucocorticoids; GI: Gastrointestinal; GCA: Giant cell arteritis; IV: Intravenous; LEF: Leflunomide; MP: Methylprednisolone; MTX: Methotrexate; TCZ: Tocilizumab.

five patients in the placebo group. A subsequent meta-analysis of individual patient data from these three trials demonstrated a modest reduction in relapse and glucocorticoid exposure in the methotrexate treated groups. However, adverse events remained similar between the groups[33].

Azathioprine is often considered as a potential steroid- sparing agent in GCA. Evidence supporting its use is limited. Only one double blind randomised placebo controlled trial was performed, which included 31 patients with GCA or PMR, or both (not differentiated) randomly assigned in a double blind fashion to either a standard glucocorticoid treatment schedule plus placebo or standard treatment plus azathioprine (150 mg once daily). At week 52 the treatment arm had a statistically significant reduction in steroid requirements ($P < 0.05$). However, the maintenance steroid dose was low in both arms at week 52, and the differences observed (1.9 ± 0.84 mg *vs* 4.2 ± 0.58 mg) while statistically significant are in practical terms of limited clinical significance[34].

Two small cases series have suggested that leflunomide has a steroid sparing effect in patients with PMR and GCA[35,36]. Adizie *et al*[36] demonstrated the efficacy of leflunomide in patients with difficult to treat disease. However, these results require replication in prospective randomised placebo controlled trials.

A number of retrospective studies have looked at cyclophosphamide use in patients with GCA, particularly in those who were steroid resistant, dependent or toxic and who had failed either methotrexate or azathioprine. Overall a significant sustained response was demonstrated (in up to 80%), with lowering of steroid doses. However, substantial treatment related adverse events observed limits it routine use[37-40].

Neither hydroxychloroquine nor cyclosporine have demonstrated any benefit in clinical trials in the management of GCA[41-43].

TNF-α is upregulated in GCA with elevated serum levels in active disease and increased expression of TNF-α in the temporal artery wall of patients with GCA[44]. However TNF-α inhibition in GCA has proven disappointing. Three randomised double blind placebo controlled trials of TNF-α inhibitors (infliximab, adalimumab and etanercept) have failed to show promise

in the treatment of patients with GCA. Neither the infliximab nor the adalimumab studies met their primary endpoints and the infliximab trial was stopped prematurely following the interim analysis[25,45]. Nevertheless, patients on etanercept did have a statistically significant lower cumulative steroid dose after 1 year; however, given that only 17 patients in total were enrolled in this study, firm conclusions cannot be made[46].

More recently, treatment with tocilizumab, a monoclonal IL-6 receptor blocker, has shown potential in a number of case studies and case series in the treatment of patients with PMR and GCA in terms of improvement of clinical symptoms and reduction in the acute phase response[47-50]. GiACTA is a multicentre, randomised, double-blind, placebo controlled trial designed to test the ability of tocilizumab to maintain disease remission in GCA and is currently ongoing[51].

Adjuvant therapies

Antiplatelet agents: The use of antiplatelet agents in GCA is controversial. There are no randomised controlled trials that have evaluated the use of aspirin as an adjuvant treatment in GCA. However, in addition to its antiplatelet effects, aspirin may have a disease modifying effect in GCA. One of the signature cytokines driving vascular inflammation in GCA is Interferon gamma (IFNγ). IFNγ is produced by Th1 cells and its production is relatively steroid resistant with very high doses of glucocorticoid required for effective suppression.

Using a SCID mouse chimera model, Weyand *et al*[52] have demonstrated that IFNγ production by T cells was suppressed by high dose aspirin. Nesher *et al*[53] in a retrospective review of 175 patients with GCA in 2004 found a significantly increased risk of ischaemic events in patients who were not on aspirin prior their diagnosis of GCA (29% *vs* 8%). Similar results were reported by Lee *et al*[54] with 16% of patients who were on aspirin at the time of their diagnosis having an ischaemic event *vs* 48% of those who were not on aspirin. Other studies have not demonstrated any clinical benefit from the use of aspirin[55,56]. Some of the discrepant findings may be explained by a higher burden of ischaemic heart disease in some cohorts, which is of itself associated with a higher risk of developing a subsequent ischaemic event. Nevertheless, the use of low-dose aspirin (75-150 mg/d) is routinely recommended for patients with GCA in the absence of contraindications[13].

Statins: Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the most powerful class of lipid lowering drugs to date, widely used in medical practice. Apart from their lipid lowering effect, additional pleotropic effects have been discovered, which include anti-inflammatory and immunomodulatory properties. Statins are capable of the following actions: suppressing the expression of major histocompatibility complex class II antigen induced by IFNγ in various cells; decreasing T-cell activation and proliferation; down-regulating endothelial adhesion molecules; and reducing circulating inflammatory molecules and cytokines such as IL-6, IL-8, IL-1β, TNF-α, as well as acute phase proteins (CRP). Moreover they restore endothelial cell function and decrease muscle cell proliferation in the vessel wall, which in turn prevents intimal hyperplasia[57,58]. Given the pathophysiology of the disease, statins could influence the inflammatory process in GCA, since some of the inflammatory pathways may be shared with atherosclerosis. Narváez and colleagues conducted a retrospective follow-up study with 121 patients with GCA treated with or without statins, which found no significant benefit from their use[59]. By contrast, in another retrospective study of 594 patients (GCA and controls), patients receiving statins were less likely to develop GCA; however, these drugs did not appear to modify the clinical presentation or disease course in patients who actually developed GCA[60]. To date there are no formal recommendations on the use of statins in patients with GCA.

Bone protection: The treatment of GCA requires both long-term and high dose glucocorticoid therapy. Oral glucocorticoid treatment with the equivalent of > 5 mg prednisone daily can lead to a reduction in bone mineral density and a rapid dose-dependent increase in the risk of fracture[61,62]. Calcium in isolation appears to have little effect in preventing bone loss in patients starting glucocorticoids[63] although when combined with vitamin D, it is an appropriate adjunctive treatment[64]. Bisphosphonates are indicated in accordance with local guidelines. For example, the BSR advise weekly bisphosphonate therapy for all patients with GCA[13], but American College of Rheumatology recommends a stratified approach according to the FRAX score[65].

Gastrointestinal protection: Given the high doses and long term duration of glucocorticoid therapy in patients with GCA, gastrointestinal protection is recommended with proton pump inhibitors, especially if concomitant risk factors are present such as NSAID use, and older age[13,66]. However, in clinical practice it is often advisable to discontinue NSAID use (apart from low dose aspirin) whilst the patient is receiving glucocorticoids.

Future therapies

Advances in immunology have revealed that a number of molecules are important modulators in the pathophysiology of GCA. Patients with GCA, refractory to glucocorticoids, have a higher expression of pro-inflammatory cytokines such as s IL-1β, TNF-α, and IL-6. Mice lacking the interleukin 1 receptor antagonist (*IL-1ra*) gene developed large vessel vasculitis[67], suggesting that IL-1 inhibition could be a therapeutic option in patients with GCA. Ly *et al*[68] have reported

three patients with GCA, refractory to glucocorticoids, who were successfully treated with anakinra, showing improvement in clinical and/or inflammatory markers (two patients also showed radiologic improvement). However, randomized control trials are needed to determine the true efficacy and safety of this drug. Activated T-cells are believed to have a critical role in the development of large-vessel vasculitis. Abatacept, a signal modulator of T-cell activation, is being evaluated in a randomized study of patients with active GCA or Takayasu's Arteritis[69].

SURGICAL TREATMENT

Extracranial involvement in GCA, or large-vessel GCA (LV-GCA), has been described in 30%-80% of cases, varying according to the imaging modality performed[70,71]. However, most patients with LV-GCA improve with medical treatment alone, making the need for surgical interventions uncommon.

The risk of aortic aneurysm is higher in patients with GCA when comparing with the general population (twofold increased risk in the United Kingdom[72]), and the aneurysms are more likely to occur late in the disease course. Given there are no validated guidelines on surgical repair of aneurysms in patients with GCA, most strategies are based on the recommendations of atherosclerosis-related aneurysms. Surgical intervention should be considered in case of symptomatic aneurysm; ascending aorta aneurysm >5 cm in diameter; descending aorta aneurysm > 6 cm; abdominal aorta aneurysm > 5.5 cm; and an aneurysm which has grown > 0.5 cm within a 6 mo period[73].

In addition, revascularization procedures (*e.g.*, angioplasty, stenting or bypass surgery) due to artery stenosis are rarely required. Although narrowing of important arteries, such as the subclavian artery, may compromise distal tissue viability, the development of extensive collateral circulation over time is usually sufficient to maintain adequate tissue viability, even when ischaemic symptoms, such as limb claudication or loss of large vessel pulses, are observed. There have been some case reports of successful revascularization surgery, but with common restenosis[74-76]. When necessary, surgical treatment should be performed in the quiescent phase of the disease and in experienced centres[9].

Aortic structural damage in GCA is associated with a trend towards increased mortality (of any cause)[77]; however, the comparison between surgical outcomes in GCA and other causes of aortic disease has not been evaluated.

MANAGEMENT

Not all patients with GCA respond to therapy in the same way, but there are no valid biomarkers to assess treatment response. Several potential molecular and imaging biomarkers have been investigated.

Molecular markers

Changes in the conventional inflammatory markers (CRP and ESR) do not consistently reflect disease activity[78]; however, they are still the laboratory tests used routinely to monitor the effects of therapy. Serum levels of IL-6 have been found to be more sensitive than ESR for indicating disease activity in untreated and treated patients with GCA[79]. Additionally, circulating pentraxin 3 (PTX3) and vascular endothelial growth factor (VEGF) levels have been recognized to be significantly increased in patients with very recent optic nerve ischaemia[80], with VEGF levels responding well to treatment[81]. Antibodies against ferritin have also been suggested as potential activity markers for GCA, particularly in patients without cranial artery involvement[82]. However, further studies with larger series are warrant to understand the potential role of these serum markers in the assessment of GCA.

Imaging

Imaging techniques, especially for patients with extracranial involvement, have an important role in monitoring patients with GCA.

Ultrasound: Three meta-analyses have reported the high value and validity of ultrasound in diagnosing GCA[83-85], and we have recently completed patient recruitment for a large multicentre study looking at ultrasound as a diagnostic tool for GCA - TABUL study (Temporal Artery Biopsy *vs* ULtrasound in diagnosis of GCA)[86]; however, the role of ultrasound as a measure of disease activity is still unclear. In small case series and case reports, abnormal ultrasound appearances have been reported to resolve within 2 d of starting glucocorticoids[87] or alternatively, to persist for 11 wk despite treatment[88], allowing correlation of imaging changes with clinical response. In the TABUL study, we performed a cross-sectional analysis of 131 patients with GCA and positive ultrasound halo (dark area around arterial wall); the size of the halo was found to be smaller in patients who had received more days of glucocorticoid treatment, as well as correlating with the presence of ischaemic symptoms, supporting the early use of ultrasound as a potential prognostic marker and monitoring tool[89]. In addition, Czihal *et al*[90] documented that the clinical pattern of patients with extracranial GCA and cranial GCA, all identified by ultrasound, substantially differs, with visual impairment inversely correlated to the frequency of extracranial GCA (Figure 2).

Magnetic resonance imaging/magnetic resonance angiography: Magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) can demonstrate the presence of increased wall thickness, oedema; mural contrast enhancement is highly

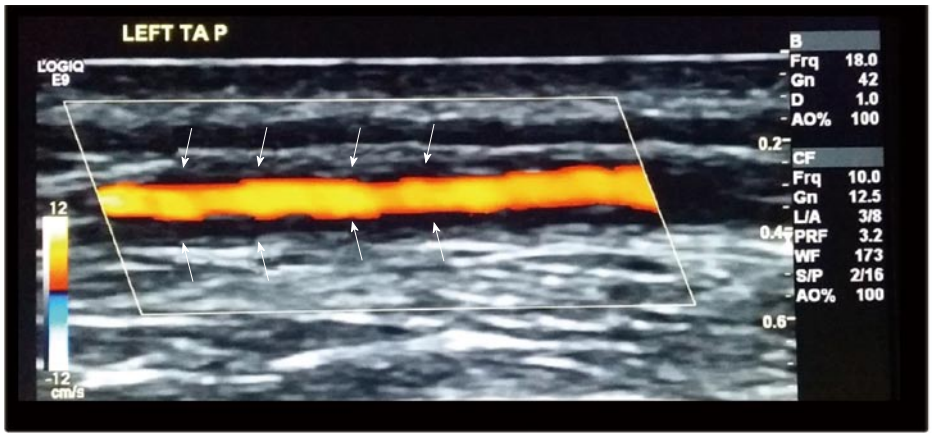

Figure 2 Ultrasound of the left temporal artery showing a dark halo (arrows) around the vessel wall of the parietal branch compatible with vascular inflammation.

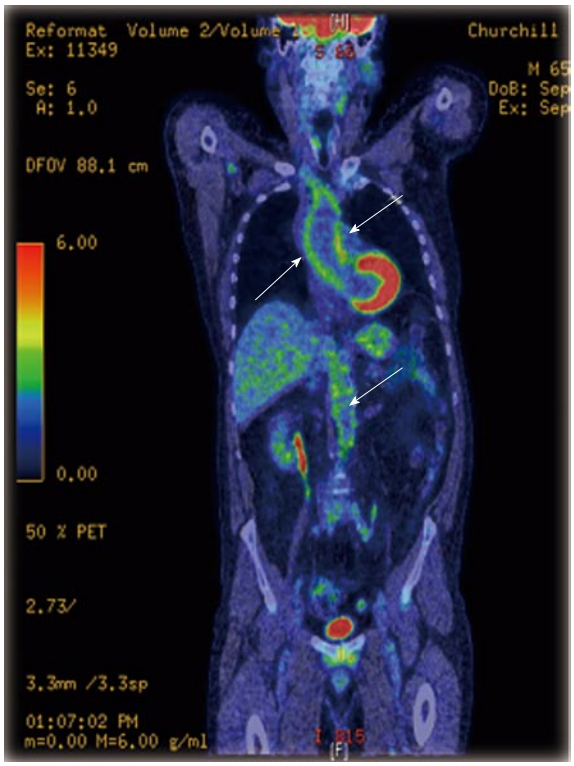

Figure 3 Whole body positron emission tomography-computerised tomography scan of a patient with large vessel vasculitis, showing increased fluorodeoxyglucose uptake in the ascending and abdominal aorta (arrows).

suggestive of vascular inflammation. These imaging modalities can be used in patients with GCA, not only to verify extra-cranial involvement, but also to evaluate temporal arteries. High-resolution MRI of the cranium has been reported to detect biopsy-positive GCA with high sensitivity[91,92], but future research is needed to validate this technique for diagnosis of cranial GCA.

There are still controversies regarding the use of MRI/MRA to monitor patients with extracranial GCA. Although it has great value for assessing aortitis and potential associated aneurysms and stenoses, MRI has failed to correlate well with clinical measures of disease activity[93-95]. In addition, false-positives may occur due to increased mural contrast enhancement as a result of vascular remodelling.

Because MRI/MRA are not invasive and do not involve ionizing radiation, they are widely used for monitoring GCA, despite their limitations.

18F-Fluorodeoxyglucose positron emission tomography: 18F-Fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) is a functional imaging technique which is very sensitive for diagnosing inflammatory changes in vessels with a diameter greater than 4 mm[96] (Figure 3). When compared to MRI, it appears to be more sensitive in detecting early vascular inflammation and correlates better with changes in disease activity over time[93,97,98]. However, like MRI, the relationship between PET findings and the prediction of disease activity or relapse is also inconsistent[93,97], which can be partly explained by the lack of standardized and validated criteria for disease activity in large vessel vasculitis.

The main limitations of this modality are: the lack of a uniform definition of vascular inflammation based on the FDG uptake; inability to provide information regarding wall structure or luminal flow; inability to visualize the temporal arteries; the use of large amounts of ionizing radiation (typically 15-20MSv per scan); and the limited access to this technique by most health institutions.

Computerised tomography/computerised tomography angiography: In GCA the main role of computerised tomography/computerised tomography angiography is to assess large vessel involvement or late complications of the disease, such as vessel stenosis, occlusions or aneurysms. This imaging modality has been proposed to evaluate response to treatment in Takayasu patients[99], but to our knowledge the same has not been considered in GCA.

Chest radiograph: The main use for regular chest

radiographs in patients with GCA is to monitor for potential aortic aneurysms. Although the BSR recommends its performance at least every 2 years[100], we have recently demonstrated that the risk of aneurysm development as a result of GCA is actually quite low[72]; if an aneurysm is suspected, more advanced imaging modalities (described above) should additionally be obtained in order to confirm the diagnosis and evaluate possible treatment measures.

Follow-up

The frequency for patient follow-up should be guided by their clinical manifestations and adverse advents. The BSR recommends follow-up during the first year at weeks 0, 1, 3, 6, then months 3, 6, 9, 12 and if new symptoms or adverse effects occur[13]. At each visit bloods tests for ESR, CRP, full blood count, glucose as well as monitoring relevant to any DMARD use should be performed. In practice, this is often not achievable in secondary care and therefore involvement by the patient's primary care physician is usually required. Screening for aortic aneurysms and monitoring bone density may be indicated in high risk individuals (*e.g.*, older male smokers have the highest risk of aortic aneurysm).

CONCLUSION

Despite the severe consequences of untreated GCA, such as blindness, there is no consensus on the optimal therapeutic strategies for this disease. Early initiation of glucocorticoid treatment is essential; however, the value of additional steroid-sparing synthetic or biologic agents to avoid the common glucocorticoid adverse effects or obtain quicker remission is still uncertain. We do not know how many and which synthetic DMARDs should be used before considering a biologic agent, because there are no valid and specific biomarkers to assess therapy response in GCA. Potential biomarkers which require further validation include circulating levels of IL-6 and VEGF as well as imaging assessments, such as ultrasound. Further investigation is needed to establish the role of these biomarkers, which can assist in the development and testing of innovative targeted therapies whose effects can be more reliably measured.

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MINIREVIEWS

Improving efficiency and saving money in an otolaryngology urgent referral clinic

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Abstract

A closed loop audit of the ear nose and throat (ENT) urgent referral clinic at a London hospital was conducted assessing the number of patients reviewed, referral source, appropriateness of referral, presenting complaint and assigned follow-up appointments. Data was sourced from clinic letters and the patient appointment system over a 3-mo period. The initial cycle analysed 490 patients and the subsequent cycle 396. The initial audit yielded clinically relevant and cost effective recommendations which were implemented, and the audit cycle was subsequently repeated. The re-audit demonstrated decreased clinic numbers from an average 9.8 to 7.2 patients per clinic, in keeping with ENT United Kingdom guidelines. A 21% decrease in patient follow-up and 13% decrease in inappropriate referrals was achieved. Direct bookings into outpatient clinics decreased by 8%, due to correct referral pathway utilisation. Comparisons of all data sets were found to show statistical significance $P < 0.05$. We reported a total financial saving of £32490 in a period of 3 mo (£590 per clinic). We demonstrated that simple guidelines, supervision and consultant-led education which are non-labour intensive can have a significant impact on service provision and cost.

Key words: Otorhinolaryngologic diseases; Quality of health care; Clinical audit; Practice guideline; Total quality management; Ambulatory care facilities

Core tip: The implementation of simple clinical guidelines, a transparent referral pathway to the ear nose and throat urgent referral clinic, and consultant led education to both juniors and referring specialties has demonstrated an improved service provision whilst being more cost effective and efficient.

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INTRODUCTION

The ear nose and throat (ENT) urgent referral clinic is a fundamental and well established service provided by otolaryngology department. This service is heavily depended upon by primary care providers and emergency departments to avoid unnecessary admissions and inpatient reviews[1]. With Accident and Emergency presentations rising to 21.7 million in 2012/13, a fifty percent increase in the last decade, urgent referral clinics such as that provided by the ENT department are increasingly pressurised[2]. Improving the efficiency of these clinics whilst ensuring that standards are maintained is a necessary adaptation. The services provided in ENT urgent referral clinics can vary significantly from one trust to another with no gold standard or national guidelines[3]. However, historically, these clinics are booked beyond their capacity due to high service demand. This can lead to erroneous direct bookings into consultant led two week wait clinics. This has an evident threat to patient safety but also a significant financial implication. The inevitable result of this high demand is non-adherence to clinic appointments and to time, resulting in poor patient satisfaction which is a key indicator of quality of care[4]. The source of this extra demand cannot be attributed to the increasing pressures on the accident and emergency department alone. The ENT urgent referral clinic itself can generate unnecessary patient follow-up which is a well-recognised source of financial pressure as funding is typically only available for a limited number of follow up appointments.

AUDIT CYCLE

A closed loop audit was performed with implementation of new guidelines after the initial cycle, highlighting key areas of improvement. Local guidelines were constructed on the basis of our findings.

Data for each audit cycle was sourced from clinic letters, each over a period of 3 mo, conducted at Northwick Park Hospital. The initial audit analysed 490 patients and the subsequent re-audit 396 patients. The primary outcome measure of this study was the number of patients reviewed in each urgent referral clinic and whether these were in line with the standards set by ENT United Kingdom[5].

Data pertaining to the following was also evaluated: (1) the source of the original referral; (2) the presenting feature; (3) the number of follow up appointments for each patient; and (4) the appropriateness of the referrals and whether patients were diverted towards main outpatient clinics, ultimately causing a loss of funding.

Data was analysed using GraphPad prism v5.0 (San Diego, California, United States) with a P value of < 0.05 considered statistically significant.

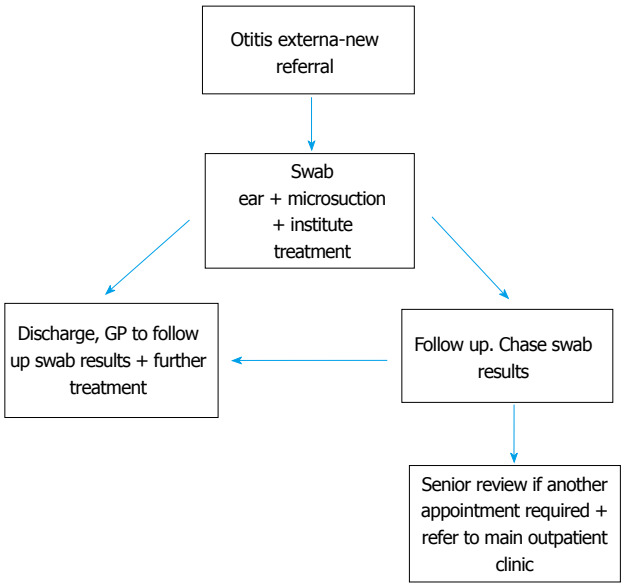

Figure 1 Otitis externa protocol.

SUMMARY OF DATA FROM INITIAL AUDIT CYCLE

All patients reviewed in the urgent referral clinic in a 3-mo period were analysed both in the initial and subsequent re-audit.

The initial audit analysed 490 patients during a 3-mo period from the urgent referral ENT clinic at Northwick Park Hospital. An average of 9.8 patients were reviewed in each clinic. Thirty-two percent (158 patients) were listed for follow up appointments after assessment in the urgent referral clinic. Twelve percent (59 patients) were direct outpatient bookings rather than registration through the "choose and book" service and therefore bypassed the appropriate referral and funding pathway. Re-referrals *via* the GP only consisted of 0.2% (1 patient) which is a source of income generation for the trust. Eighteen percent (89 patients) of referrals were deemed to be inappropriate for urgent referral review by a designated senior clinician and were suitable for either GP review or non-urgent ENT review. The most common presentation to the ENT urgent referral clinic was otitis externa consisting of 26% of patient reviews. The others included 16% nasal trauma, 15% removal of a foreign object and 11% with epistaxis which are consistent with similar audits conducted[3].

Based on the above findings from the initial cycle, the following guidelines were implemented: (1) clinical protocols were produced for ENT juniors regarding accepting, reviewing and organising subsequent follow up for patients; (2) clinical guidelines for the management of common ENT pathologies, *i.e.,* otitis externa were produced (Figure 1); (3) a safety net

Table 1 Summary of data including both initial and subsequent re-audit

Patients per clinic	First cycle 9.8		Re-audit 7.2	
	Percentage	No. of patients	Percentage	No. of patients
Follow ups	32%	158	11%	45
Direct outpatient bookings	12%	59	4%	15
Re-referrals	0.2%	1	4%	15
Inappropriate referrals	18%	89	5%	21
Total patients		490		396

Table 2 Estimated savings and income

Cost of follow up per patient	£190	
Income per clinic patient reviewed	£190 + tariffs for procedures performed (discontinued after 2 follow ups)	
Re-audit	Savings £29830	Income £2660
Overall	£32490 over 3 mo £590 per clinic	

system for earlier senior review of patients that had been followed up more than once and those who were immunocompromised[6]; (4) clear instructions to GPs on discharge paperwork from Accident and Emergency/wards requesting for an appropriate referral to a consultant led ENT clinic; (5) education regarding the follow up of patients more than once and deterrence from booking into main outpatient clinic appointments unless clinically indicated; and (6) education for local GPs and emergency department services regarding the referral pathway (combined contribute 95% of referrals received) by regular otolaryngology consultant led sessions.

SUMMARY OF DATA FROM SUBSEQUENT RE-AUDIT

The implemented guidelines overall had a significant effect on outcomes (Table 1). The number of patients per clinic decreased to 7.2. A significant decrease in the number of patient follow-ups from 32% to 11% (158 to 45 patients) was noted after the implementation of new clinical guidelines relating to common ENT presentations (Figure 2). This also led to a reduction in the number of referrals deemed to be inappropriate from 18% to 5% (89 to 21 patients). The booking of patients directly into main outpatient clinics decreased from 12% to 4% (59 to 15 patients); this was previously a significant source of financial loss. As a result of education regarding the referral system and clearer instructions to GPs, the number of re-referrals increased from 0.2% (1 patient) to 4% (15 patients).

The above results showed statistical significance between the initial audit and the re-audit in all comparisons ($P < 0.05$, Table 1).

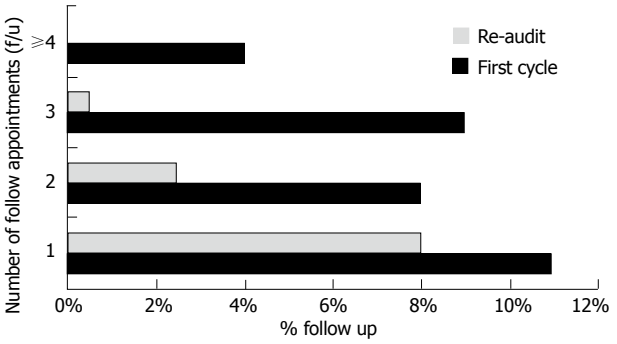

Figure 2 Comparison of number of follow up (f/u) appointments in first and second cycles of audit, $P < 0.0001$.

FINANCIAL IMPLICATION

The above results demonstrate a definite clinical significance, whilst simultaneously equating to a financial saving. The implemented changes described above and subsequent re-audit demonstrated a saving of £29830 over a 3-mo period. Income generated for example by re-referrals equated to £2660. Total savings amounted to £32490 over a 3-mo period, or £590 per clinic (Table 2).

ROLE OF THE URGENT REFERRAL CLINIC

The fundamental role of the urgent referral clinic is variable and each NHS trust provides a different service with no pre-determined gold standard. There are no specific national guidelines which outline the remit of service which should be provided by urgent referral clinic, nor the manner in which referrals should be accepted. Similar audits have been conducted which show an improvement in service provision with a shift from open access to rapid access clinics and the formation of robust criteria to avoid what are deemed to be inappropriate referrals[3,7]. The overall common denominator is that these clinics are heavily depended on by primary and secondary care with a significant referral number. This can lead to abuse of the service and subsequently poor patient satisfaction.

SYNOPSIS OF KEY FINDINGS

Clinical activity decreased from 9.8 to 7.2 patients per clinic between each audit cycle with 21% decrease in patient follow up and 13% decrease in the number of referrals deemed inappropriate for review in the urgent referral clinic. Direct bookings into outpatient clinics decreased by 8% with a greater number of referrals taking the correct pathway. The implemented changes resulted in a total financial saving of £32490 in a period of 3-mo or £590 per clinic.

CLINICAL APPLICABILITY/CONLUSION

Urgent referral clinics are historically serviced by junior members of the ENT team which can lead to a greater duration of patient consultations but also a tendency towards greater clinical caution and therefore follow-up. This study demonstrates that the implementation of simple changes such as an established protocol (Figure 1) for the management of otitis externa (the commonest presentation), guidance and education to general practitioners and emergency department staff regarding the referral pathway, can all translate to an improvement in the service provided, by reducing clinical activity. This has subsequently resulted in a financial saving whilst maintaining a high standard of clinical care.

The results of this audit have a 2 fold consequence. A reduction in the overall number needed to be seen in the urgent referral clinic (primary outcome), with patients seen by the correct services. This volume reduction is in keeping with ENT United Kingdom guidelines and has resulted in clinics running to time, a higher patient satisfaction whilst permitting juniors to seek advice for complex cases[5]. Secondly, we have demonstrated that service improvements are not mutually exclusive to financial saving/income and one may run in parallel with the other through the implementation of simple guidelines, supervision, education and frameworks which are non-labour intensive. More globally we have highlighted the importance of clinical governance in driving forward service provision with an awareness of the finite resources available within our National Health Service.

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MINIREVIEWS

Nasobronchial interaction

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Abstract

Upper and lower airways can be considered as a unified morphofunctional unit. In this paper, nasobronchial interactions are evaluated based on literature.To discuss nasobronchial interactions, literature review from PubMed since 1982 is evaluated. Data base was including the terms "nasobronchial interaction, nasal and bronchial". Asthma and rhinosinusitis may be associated with environmental factors and immunological predisposition. Treatment of rhinosinusitis may decrease asthma exacerbations. It was concluded that "one airway, one disease"-concept may be accepted when considering naso-bronchial interaction. Asthma treatment should also mean treating the nose as good as treating patients with nasal symptoms. To reach the succesful results ıt should be associated with evaluation of lung functions.

Key words: Nasal; Bronchial; Nasobronchial interaction; Reflex; Airway

Core tip: Upper and lower airways may be accepted as unified morphofunctional unit. This concept is defined as nasobronchial interaction.

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INTRODUCTION

Upper and lower airways may be accepted as unified morphofunctional unit. Likewise rhinosinusal disorders and lower airway diseases have interrelation. Inflammatory cytokines may play a role for the interaction between cells[1].

During infections of upper respiratory tract such as rhinosinusiitis, asthma development may occur[2,3]. Atopic dermatitis in children, and also allergies to the

food may be the first sign for developing of allergic rhinitis (AR) and astma[4].

In this paper, nasobronchial interactions were evaluated thoroughly by the means of literature review from PubMed since 1982. Data base was depended on the terms of nasobronchial interaction, nasal and bronchial.

NASAL FUNCTON

Nose works as "air-conditioner". Cold air through induces bronchospasm in asthmatic patients. Nose humidifies the air which inhaled nasally. In allergic rhinitis patients, exposure to allergens increase bronchial hyper-reactivity[5-8].

MECHANISMS FOR NASO-BRONCHIAL INTERACTION

Impaired nasal function, pulmonary aspiration of nasal contents, the nasal-bronchial reflex and increased absorption of inflammatory mediators in the blood stream maybe responsible for lower airway dysfunction in AR[7]. They also cause the interaction between the nose and the lung. After nasal allergen application, no allergen deposition was shown in the lungs by radiolabeled allergen[6]. Cold and dry air exposure may cause immediate bronchoconstriction[8]. Nasal provocation with methacholine also increase lower airway resistance in asthma and AR patients[9].

Mast cells and eosinophils are the major effector cells in AR and asthma[10,11]. Eosinophils migrate to the tissues from the blood and this process is depend on the expression of cytokines and adhesion molecules[12]. Vascular cell adhesion molecule-1 and E-selectin increased after nasal provocation[13]. After bronchial provocation, mast cell degranulation and increase of of basophils occur in the nasal mucosa[14].

Impaired nasal function

In AR, mouth breathing was observed related to the nasal obstruction. Therefore, with the lower nasal filter function, increased allergen exposure develops. This process is resulted in hyperresponsivity of the airway. If additional chronic rhino-sinusitis and/or nasal polyposis were present, surgery help to improve nasal functions. In these patients, asthma control could be done better[15]. When nose blocked with a clip, response to cat-allergen was not detected in patients with cat allergy[16].

Aspiration of nasal contents

Inhale particles may be removed by the help of the mucociliary clearance. With this function and beat of the cilia, particles were carried toward the pharynx. In AR patients, secretions contain inflammatory mediators and cells[7,17]. When allergens inhaled, there was swelling of the nasal mucosa[18].

Nasobronchial reflex

The trigeminal nevre is responsible for afferent sensory innervation of the nose. Efferent parasympathetic fibers are carried in the Vidian nevre. Vagal nevre supports afferent and efferent innervation of the lower airways[19]. Sneezing, coughing or bronchoconstriction occur with the help of reflex mechanisms. The receptors were present in the nose, trachea, larynx and respiratory tract. The are sensitive to the mechanical or chemical factors. Cold dry exposure of nasal mucosa can cause immediate bronchoconstriction in asthmatic patients[6,20,21].

Nasobronchial reflex is another mechanism for interaction between upper and lower airways. Receptors are localize in the nose, sinuses and pharynx. The signals were transferred to the medulla by trigeminal, facial and glossopharyngeal nerves[22]. In the medulla, connections with vagal nevre were performed and bronchoconstriction occur[23].

Triggers of the reflex

In infectious conditions of the nose and lungs, virus and bacteria may trigger the reflex system. Smoking, pollutant agents in the work places and environment may cause chronic inflammation[24]. Beta-blockers or aspirin, cold dry air exposure, and physical exercise may also trigger the AR and asthma[25].

Increased absorption of inflammatory mediators in the blood stream

Increase in the eosinophil count is detected in AR and asthma patients in the blood[26]. Nasal provocation, performed with methacholine, also increases the lower airway resistance. It may be said that systemic mediators may induce lower airway resistance[9]. In AR and asthma, inflammatory cells and progenitors may play a role[27-29].

RHINOSINUSITIS AND ASTHMA

Symptoms of rhinosinusitis

Syptoms of rhinosinusitis are known as nasal congestion, discharge, purulence and postnasal drip; hyposmia, facial pressure, fever, halitosis, dental pain and headache. Chronic rhinosinusitis (CRS) may damage to the mucociliary clearance. CRS is an independent risk factor for asthma[2,3,30,31].

Epidemiology

Rhinosinusitis and asthma were coexistently detected in 34%-50% of patients. In asthmatic patients, concomitant rhinosinusitis were present up to 84%[24].

Clinical appearence

In rhinosinusitis patients, there is the possibility of having asthma. When nasal disease was treated, asthma control maybe easier due to the reducing of

bronchial hyperresponsiveness. Therefore, therapeutic approach to asthma and rhinosinusitis should be planned together[32].

Treatment

Medical treatments or surgery for rhinosinusitis help the reduce of respiratory symptoms and impove the asthma. Cold air inhalation causes the decrease FEV1 in asthmatic patients[5].

AR AND ASTHMA

AR is a risk factor for asthma development[33]. Whereas, in same cases, asthma starts first. Exercise induces bronchoconstriction in most asthmatic patients. It may be related to to alterations in the osmolarity of the liquid covering the epithelial layer. This process may be resulted release of chemical mediators originating in mast cells[34].

Epidemiology

In 19%-38% of AR patients, there are concomitant asthma. Moreover, 30%-80% of asthmatic patients also have AR. Rhinitis symptoms were reported in 98.9% of allergic asthmatics and in 78.4% of non-allergic asthmatics[35]. Bronchial hyperreactivity was observed in most of the AR patients[36]. In AR petients, 40% have involvement of the lower airways. Additionally, allergic asthma patients have concomitant rhinitis symptoms as a 80%[37,38].

Potential mechanisms

The allergic response starts with the uptake of the antigen by antigen presenting cells, in particular dendritic cells[39,40]. Dendritic cells present the antigen to T lymphocytes in the regional lymph nodes[41,42]. B cells recognize antigen with surface immunoglobulin (sIg) receptors. The T cell receptor recognizes antigens and activation of the it stimulates the naive T helper cell, a ThO cell. It differentiates to either a Th1 or a Th2 subset[43,44].

Activation of Thl cell causes the release of IL-2 and lNF-y. Additionally IL-4, IL-5, IL-1O and IL-13 are also produced by Th2 cells. Th2 cytokines are also involved in IgE synthesis (IL-4)[45,46].

Nerve growth factor (NGF) and NGF receptors are expressed in the nasal mucosa[47]. In patients with AR and allergic asthma, serum levels[48-50]; and nasal and bronchial fluid levels of NGF increase[51-53].

Microbial stimuli

Exposure to endotoxins reduces the risk of developing allergic rhinitis and asthma during the first years of life. Chlamydia pneumoniae infection has been suggested as a possible causative factor for asthma[54]. It was also reported as protective against the development of asthma in children[55]. In infancy, bacterial infection is associated with a reduced prevalence of atopic eczema, allergic rhinitis and asthma[56]. Viral respiratory infections stimulate nasal allergic inflammation in atopic patients; and virus-induced airway hyperresponsiveness develops[57].

Treatment

The medical treatment of AR reduces the risk of asthma-related events in asthmatic patients[58]. Nasal corticosteroids stabilize bronchial hyperreactivity in allergic rhinitis patients with seasonal asthma[37,59]. The leukotriene receptor antagonists are less effective than antihistamines and nasal steroids in upper airway disease[60,61].

Allergen immunotherapy reduces asthma symptoms. This therapy also improves nasal disease[62,63]. By immunotherapy, progression of allergic rhinitis to bronchial asthma may be prevented. Specific immuno-therapy with seasonal allergic rhinitis significantly reduced the asthma development risk[64,65].

UPPER AIRWAYS AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

The correlation between chronic obstructive pulmonary disease and upper airway inflammation are reported. The relation between nasal and bronchial inflammation by estimating the IL-8 concentration were reported[66].

CONCLUSION

Upper and lower airways are thought as one functional entity. Local allergen exposure of the respiratory system induces mucosal inflammation. A "unified airway" concept suggests that the upper and lower airways function as a single unit[67]. The nasobronchial interaction may be suggested as present in airway. Trigger agents for naso-bronchial reflex are important to initiate the symptoms.

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MINIREVIEWS

Effect of bariatric surgery on humoral control of metabolic derangements in obese patients with type 2 diabetes mellitus: How it works

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Abstract

Obesity and diabetes is a co-pandemic and a major health concern that is expanding. It has many psychosocial and economic consequences due to morbidity and mortality of this disease combination. The pathophysiology of obesity and related diabetes is complex and multifactorial. One arm of this disease process is the genetic susceptibility. Other arm is dependent on the intricate neuro-humoral factors that converge in the central nerve system. Gut hormones and the adipose tissue derived factors plays an important role in this delicate network. Bariatric surgery provides the only durable option for treatment of obesity and furthermore it provides a remission in the concomitant diseases that accompany obesity. This review provides a brief insight to all these mechanisms and tries to deduce the possible reasons of remission of type 2 diabetes after bariatric surgery.

Key words: Type 2 diabetes; Morbid obesity; Bariatric surgery

Core tip: Metabolic surgery in obese individuals results weight loss and beneficial effects on type 2 diabetes mellitus and metabolic syndrome.

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INTRODUCTION

Obesity is considered as abnormal accumulation of

Table 1 World Health Organization classification of obesity

Classification	WHO class	BMI (kg/m^2)
Underweight		≤ 18.5
Normal		18.5-24.9
Overweight		25.0-29.9
Obesity	I	30.0-34.9
	II	35.0-39.9
Extreme obesity	III	40

Adapted from Aronne[4]. WHO: World Health Organization; BMI: Body mass index.

excess energy as fat tissue that results in major health problems and reduced life span of the individual[1]. Obesity is a major health problem and is now considered a worldwide pandemic. Its incidence increasing in an alarming rate and by 2015 around a million people is expected to be overweight worldwide[2]. Furthermore, it is considered as a government political problem for it results in economic, social losses due to morbidity and mortality during the course of the disease[3]. The risk stratification of obesity is made according to World Health Organization's proposed classification of obesity (Table 1)[4]. Increasing body mass index (kg/m^2) the morbidity and mortality rate of the patient increases. Obesity causes many concomitant systemic diseases. Among the diseases that occur with obesity are diabetes, hypertension, coronary artery diseases, dyslipidaemias, non-alcoholic fatty liver disease and metabolic syndrome are pronounced[5]. There are many problems when dealing with obese patient. For since it is a multifactorial disease the treatment is very hard furthermore there are many social security related problems when dealing with bariatric patient. There are also psychosocial implications against obese people such as job interview bias, unavailability of public bills and inability of social security coverage for obesity related diseases[6].

Obesity is multifactorial disease and many neuro-humoral factors are orchestrated in appetite control and energy haemostasis of the individual. Detailed review of these factors is out of the scope of this text. Here we will examine briefly the central nervous system related and gut humoral factors related control mechanisms of the appetite of the individual and furthermore we will give detailed information regarding type 2 diabetes mellitus (T2DM) and obesity pandemic. Later on, we will try to summarize the effects of bariatric surgery on humoral factors of obesity and diabetes.

Role of central nervous system in control of appetite

Central nervous system as a pivotal point in orchestration of anorectic and orexigenic signals received from the periphery. Mainly the arcuate nucleus of the central nervous system integrates all the input[7]. This area of the brain contains neuropeptide YY rich neurons. The main orexigenic stimulus comes from Ghrelin and the anorectic stimuli come from the Glucagon like peptide-1 (GLP-1), plasma peptide tyrosine tyrosine 3-36 (PYY3-36), cholecystokinin and *etc*. All these stimuli integrate at the arcuate nucleus and this causes the individual to seek food in discrete time points between daily activities[8-10]. This is one of the main points that is deranged in the obese individual and a continuous eating behaviour occurs in the obese patient.

Peripheral axis

Peripheral axis consists of gut related hormones with vagus and the adipose tissue related humoral factor; namely the adipokines. These are all potent short and long terms stimuli for the control of energy haemostasis in the individual and as a result of this there is the food seeking behaviour characteristics of humans (and most vertebrates) ae determined.

Role of gut related hormones in obesity

Several factors secreted from the gastrointestinal tract regulate the caloric intake and the food seeking behaviour of the individual[7,8]. There are factors that increase the adipogenesis and there are counteracting factors that reduce the appetite and reduce adipose tissue formation. Main orexigenic factors secreted from the gastrointestinal system are ghrelin and insulin. The counteracting mechanisms on the other hand are GLP-1, NYY3-36, *etc*[9,10]. Furthermore vagus and the autonomic system are important afferent inputs of the gastrointestinal system to the arcuate nucleus forming the gut-brain feedback mechanisms[11,12].

Ghrelin is a 28-amino acid peptide. It has been shown to be produced from the fundic mucosa. In animal models, it was shown that ghrelin increased feeding and weight gain and had an orexigenic role in energy balance. The clock genes *PER1* and *PER2* ghrelin levels peak before meals and quickly decrease following meals. Arcuate nucleus in the central nervous system exhibits ghrelin receptors[13,14].

Insulin has many roles in energy balance. Serum glucose levels elevated after a meal stimulate insulin release by pancreatic beta cells. Additional secretagogues of insulin are amino acids such as alanine, glycine, and arginine, acetylcholine produced from vagal nerve endings, and incretins such as GLP-1 and glucose-dependent insulinotropic polypeptide[7,8]. Although central effects of insulin is reduced appetite and weight loss in preclinical studies in obese individuals show higher basal insulin levels[8].

GLP-1 has an important role in increasing secretion of insulin from the pancreas[7]. Both *in vivo* and *in vitro* researches have showed that GLP-1 increase insulin secretion in the beta cell. Moreover, glucagon secretion is inhibited by GLP-1 while insulin sensitivity is increased[7,15].

PYY3-36 is an anorectic factor on the arcuate nucleus through the Y2 receptors expressed at the neuronal level. It shows peak levels 1-2 h following the meals and the rise in serum levels are observed within 15 min following eating[8,16]. PYY3-36 anorectic effect is possibly facilitated by the vagus nerve[16].

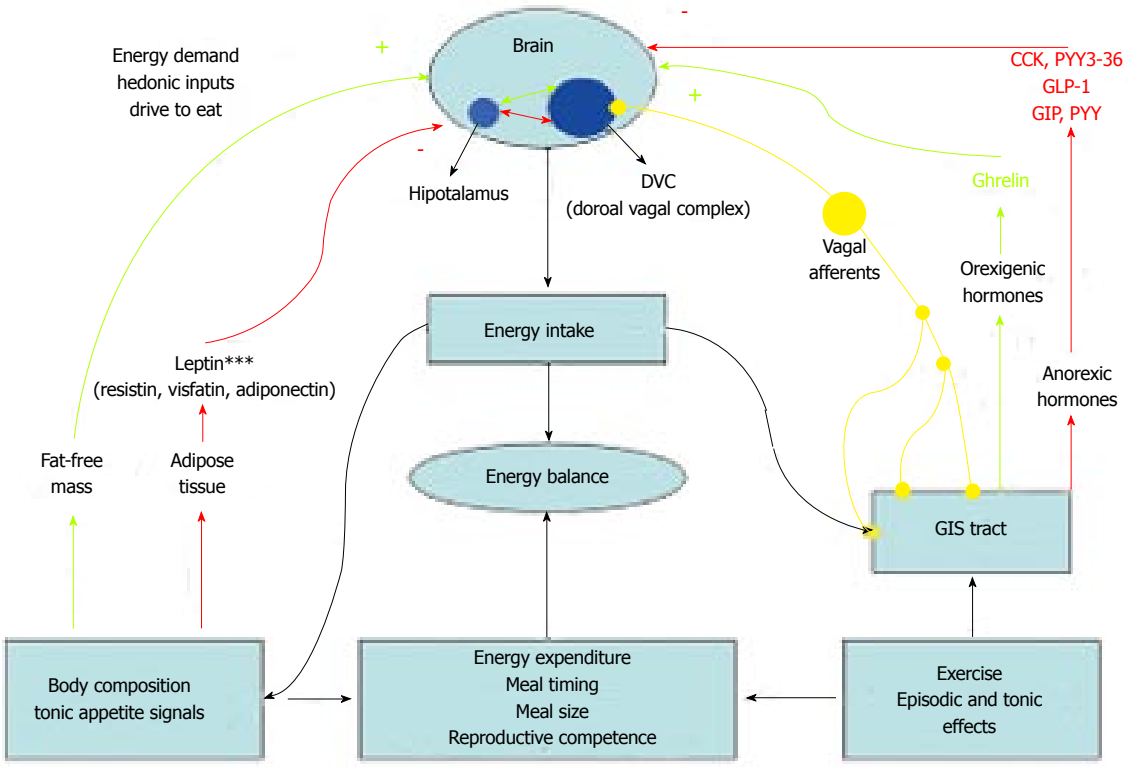

Figure 1 Factors produce a tendency towards insulin resistance and diabetes in the obese patients. DVC: Dorsal vagal complex; CCK: Cholecystokinin; GLP-1: Glucagon-like peptide-1; PYY: Peptide YY; GIP: Gastric inhibitory polypeptide.

Role of adipose tissue in obesity

Leptin and adiponectin are the two main humoral factors secreted from the adipose tissue and that play a role in the energy haemostasis of the individual. They all together form the adipose-brain feedback axis[8].

Leptin is produced in white and brown adipose tissue, placenta, ovaries, skeletal muscle, stomach, breast, bone marrow, pituitary, and liver[8]. Engineer and Garcia have showed that leptin affects hypothalamus, where it inhibits NPY/AgRP receptor neurons while stimulates α-MSH neurons[17]. In contrast to ghrelin, leptin acts on appetite and energy balance[7,18]. Harvey *et al*[8] has reported leptin resistance in obese patients.

Adiponectin is secreted from white adipose tissue[8]. Serum adiponectin levels are negatively correlated to serum insulin and glucose levels, body fat mass, and waist-to-hip ratio. It has been shown that fasting adiponectin levels have been reduced in the obese individuals[19]. Nevertheless, the response to a meal appear to be exaggerated in obese subjects.

Relationship between obesity and diabetes

Obesity is associated to many medical conditions, probably the most disturbing may be T2DM. Both obesity and T2DM are mainly related with insulin resistance[3]. The nonesterified fatty acids (NEFAs) secreted from adipose tissue in obese population may lead to the theory that insulin resistance and β-cell dysfunction are probably related[20,21]. Adipose tissue affects body metabolism by secreting hormones, glycerol, leptin, cytokines, adiponectin, proinflammatory substances, and NEFAs. Increased NEFA secretion is detected both in obesity and T2DM, and it is related with insulin resistance in both situations. In humans, insulin resistance starts to develop shortly after an acute increase of plasma NEFA levels.

Intra-abdominal fat is linked to the genes that secrete specific types of proteins responsible for the production of energy[22,23]. Omental adipocytes secrete larger amount of adiponectin than the subcutaneous-derived adipocytes[24,25]. Furthermore, adiponectin secreted from omental adipocytes is negatively associated with weight gain. The excretion of NEFAs to different tissue may be affected by their source. Abdominal fat is considered more lipolytic than subcutaneous fat, and it does not respond simply to the antilipolytic action of insulin, which makes intra-abdominal fat more significant in causing insulin resistance and diabetes[26]. All these factors produce a tendency towards insulin resistance and diabetes in the obese patients (Figure 1).

Fecal fat, and fecal biliary acids in obesity

The cytotoxicity of fecal bile acids is associated with their concentration in fecal water, and particularly is related to the concentration of secondary bile acids[27,28]. Total bile acid levels in fecal water were decreased meaningfully in the course of orlistat treatment. The decrease in fecal water bile acids during orlistat treatment mainly was caused by a large reduction in deoxycholic acid. Small reductions were observed in fecal water with both the orlistat and placebo groups for all the other bile acids. This is relevant in that the

secondary bile acids, which include deoxycholic acid, are the bile acids most frequently associated in colonic cell hyperproliferation[29,30].

BARIATRIC SURGERY AND OBESITY RELATED METABOLIC CHANGES

Bariatric surgery consists of various interventions which can be divided as restrictive, malabsorptive, or combined restrictive and malabsorptive. The number of bariatric interventions (*i.e.*, metabolic surgery) for the treatment of obesity is in exponential increase. This is partially due to effective and long-lasting weight loss; in addition, a good deal of improvement of co-morbidities after surgery compared with diet and physical activity[7]. In this subsection we will briefly evaluate the changes in the levels of above mentioned factors with respect to various bariatric procedures and at the end try to draw certain conclusion regarding the metabolic effects of bariatric (metabolic) surgery. We will briefly summarize the effect of bariatric surgery on each of the adipose and gut humoral factors.

In the case of ghrelin there are many report regarding Roux-en-Y gastric bypass (RY-GBP) and the serum ghrelin levels detected in various set points starting from ranging between 14 d postoperatively to 2 years[31,32]. Most of the researchers have found decreased ghrelin levels postoperatively and these results have been compared to non-resectional restrictive procedures such as adjustable gastric banding (AGB). Therefore we can say that the final effect of procedures involving gastric transection reduces the serum ghrelin levels[33,34].

The changes in ghrelin after sleeve gastrectomy (SG) were measured in different studies. Shak *et al*[35] stated that fasting ghrelin levels are decreased up to 5 years of follow-up. Moreover, some studies tried to evaluate and compare the effects of RY-GBP or AGB with the SG on fasting ghrelin levels, which showed to be decreased[36]. These studies showed that the ghrelin suppression after both SG and RY-GBP may be part of the mechanism that contributes to diabetes remission[35,36].

Serum insulin levels sow rapid drop with RY-GBP, biliopancreatic diversion and duodenal switch and SG however with AGB although insulin drops the incretin effect is not observed[7,35-38].

A strong GLP-1 response was reported 10 years after RY-GBP, suggesting a long-lasting effect. Furthermore, in T2DM patients, an improved GLP-1 response to meal intake is not enough to preserve normal glucose tolerance in the long term after RY-GBP. Similarly, some studies have shown unaffected fasting GLP-1 and a noteworthy increase in response to a glycemic challenge[39,40]. Studies have shown a significantly increased fasting level of PYY3-36 following RY-GBP. Following the AGB, fasting PYY3-36 or meal-stimulated response is variable and inconclusive[41,42]. Data regarding the effects on leptin are inconclusive and therefore research regarding area is urgently needed. Furthermore, adiponectin studied in RY-GBP in only one study and was found be increased following surgery[43]. This study is not enough to draw conclusions and therefore multi-centric high patient volume studies are needed to evaluate the role of metabolic surgery on adipose-brain axis in obesity.

Clinically all these changes in the humoral effects that the bariatric surgery produces is seen as remission of diabetes in obese individuals postoperatively. Even in patients who continue to have diabetes postoperatively have a better quality of life due to reduced medications and a better glycaemic control. This has been extensively studied and currently here even meta-analysis regarding this subject showing very good results in almost all procedure types[44-47].

CONCLUSION

Metabolic surgery in obese individuals results weight loss and beneficial effects on T2DM and metabolic syndrome.

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MINIREVIEWS

Clinical overview of hypertensive crisis in children

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Abstract

Hypertensive emergencies and hypertensive urgencies in children are uncommonly encountered in the pediatric emergency department and intensive care units, but the diseases are potentially a life-threatening medical emergency. In comparison with adults, hypertension in children is mostly asymptomatic and most have no history of hypertension. Additionally, measuring accurate blood pressure values in younger children is not easy. This article reviews current concepts in pediatric patients with severe hypertension.

Key words: Hypertensive crisis; Hypertensive urgency; Hypertensive emergency; Blood pressure

Core tip: Hypertensive crisis in children is a disease easily mismanaged in the emergency department. The physician should carefully search for evidence of end organ injury to distinguish between hypertensive emergency and hypertensive urgency. Only patients with hypertensive emergency require immediate reduction in markedly elevated blood pressure to prevent and arrest progressive end organ damage. In all other patients, the elevated blood pressure can be lowered slowly using oral agents, *i.e.*, esmolol, nicardipine, labetalol and fenoldopam. All young children should receive complete examinations to look for the underlying cause of secondary hypertension.

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DEFINITIONS OF HYPERTENSION IN CHILDREN: HYPERTENSIVE CRISIS AND HYPERTENSIVE ENCEPHALOPATHY

Hypertension in children older than 12 mo was defined as blood pressure (BP) level > 140/90 mmHg, as in adults, until the updated definition of "The fourth report on the diagnosis, evaluation and treatment of high blood pressure in children and adolescents" in 2004[1]. Hypertension is identified when the systolic BP (SBP) or diastolic BP (DBP) is greater than or equal to the 95th percentile for gender, age and height: stage 1 hypertension is SBP or DBP within the range of the 95th percentile to the 99th percentile plus 5 mmHg; stage 2 hypertension is greater than the 99th percentile plus 5 mmHg. Height and gender revealed no statistical difference from the previous study[2-4].

Hypertensive crisis is defined as a severe elevation in BP, classified as hypertensive emergencies and hypertensive urgency[5]. Hypertensive encephalopathy is a hypertensive emergency and characterized by an abrupt or prolonged elevated BP that overcomes the autoregulatory capacity of the cerebral vasculature. It appears as severe hypertension associated with headache, altered mental status, seizure, visual disturbances or stroke, and the lesion may be revealed as reversible posterior leukoencephalopathy[6-11].

EPIDEMIOLOGY

Although the prevalence of hypertension tends to be increasing today, pediatric hypertension still accounts for about 0.5%-1% in children and its incidence is obviously less in younger children and infants[12,13]. Until now, data of the incidence of hypertensive crisis in children have not been analyzed enough to give a definite result, but in adults, approximately 1% of hypertensive individuals have been reported to have hypertensive crisis[14].

ETIOLOGY

Generally, primary hypertension is identifiable in children and adolescents, whereas secondary hypertension is more common in younger children. In primary hypertension, there is a strong association of high BP with being overweight and BMI should be calculated as part of physical examination, with the marked increase in the prevalence of overweight children.

In newborn infants with hypertension, the most likely definable causes are renal artery thrombosis or stenosis, congenital renal malformation, or coarctation of the aorta[15]. In children between infancy and 6 years of age, coarctation of the aorta, renal parenchymal diseases and renal artery stenosis are the three most common causes of secondary hypertension. In children older than 6 years, renal artery stenosis and renal parenchymal diseases are the leading causes of diastolic BP over 90-100 mmHg. Primary hypertension accounts for 90% of the causes of hypertension in patients aged over 15 years[16-19].

PATHOPHYSIOLOGY

In the long term in children, high BP levels could be associated with the early development of cardiovascular changes[20]. In the acute phase of hypertensive crisis, rapid increases in systemic vascular resistance could be precipitated as a result of increases in the circulating vasoconstrictor substances, including norepinephrine, angiotensin Ⅱ, or anti-natriuretic hormone[21]. Arteriolar fibrinoid necrosis may induce a consequence of the severely elevated BP, precipitating endothelial damage with resultant end organ ischemia. Ischemia could trigger the further release of vasoactive substances, causing further vasoconstriction and myointimal proliferation[21]. This may appear to be an important part[22]. Activation of the renin-angiotensin system could also be highly involved[23-25].

CLINICAL MANIFESTATIONS

Compared to adults, the clinical presentations of hypertensive crisis in children are more likely asymptomatic[26]. Our previous study reported that no specific clinical manifestation correlated with the age factor[27]. Patients with hypertensive crisis in different age groups did not have specific different clinical presentations in this study[27]. In addition, the signs of end organ dysfunction are hypertensive encephalopathy, acute left ventricular failure and acute myocardial ischemia, papilledema, elevated liver function tests, *etc.* For children with complaints of any symptoms such as persistent headache, nausea/vomiting and altered mental status, hypertensive crisis should be ruled out immediately to prevent further damage. According to some clinical analyses, the related risk factors for hypertensive encephalopathy were male gender, stage 2 hypertension and some clinical symptoms. In pediatric patients with hypertensive crisis caused by essential hypertension, symptoms of chest tightness and no family history of hypertension may show a lower risk for hypertensive encephalopathy[27]. Moreover, the recurrence was 29.1%[27].

EVALUATION

Detailed medical history taking and complete physical examinations are both required in children presenting with suspected hypertensive crisis. In laboratory tests, serum electrolytes, complete blood counts, blood urea nitrogen, creatinine and urinalysis should be considered for children with suspected hypertensive crisis. Chest radiograph and electrocardiogram may be also performed in patients with chest pain and tachypnea. A brain computed tomography scan may be needed in

hypertensive children with neurological signs[28].

MANAGEMENT

Pediatric patients with hypertensive crisis require immediate and appropriate reduction in BP levels, whereas patients with hypertensive urgency require a slower rate of reduction in BP levels over 24 to 48 h. However, rapidly decreasing BP levels may result in decreasing the blood flow of organs, causing ischemia and infarction[29-31]. In patients with hypertensive encephalopathy combined with chronic hypertension, it is important to reduce the mean arterial pressure gradually during the first hour[32]. Accordingly, patents with hypertensive emergency should be treated in an intensive care unit. The drugs of choice should depend on the end organs involved and the monitoring environment. Once the reductions in mean arterial pressure to less than 20% or to a DBP of 100 mmHg have been reached, oral maintenance therapy may be given instead of the intravenous agent. Another important part of BP control in hypertensive crisis is volume depletion. Adequate fluid replacement will restore organ perfusion.

The agent of choice should depend on the clinical presentation and the severity of the hypertensive crisis. The preferred agents include esmolol, labetalol, fenoldopam and nicardipine, but phentolamine and trimethaphan camsylate may be less commonly used today. Nevertheless, they appear to be useful in some particular situations, such as catecholamine-induced hypertensive crises, such as pheochromocytoma[33,34]. Sodium nitroprusside could be used in patients with acute pulmonary edema and/or severe left ventricular dysfunction and in patients with aortic dissection. However, this agent has some limitations for application in the pediatric ED because of the requirement of intra-arterial BP monitoring and protection from light[35]. Short-acting, immediate-release oral nifedipine and sublingual nifedipine have been used for effective reduction of severe elevated BP, but some studies cautioned against it as being potentially dangerous in patients with hypertensive crises. Clonidine and angiotensin-converting enzyme inhibitors are long acting and difficult to titrate, but these agents may be still useful in some clinical conditions[36].

CONCLUSION

Hypertensive crisis in children is a rare but important disease easily mismanaged in the ED. Pediatric patients with hypertensive emergencies need immediate reduction of BP levels to arrest further end organ damage. For patients with hypertensive urgency, BP may be lowered slowly.

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CASE REPORT

Paraspinous hemolymphangioma associated with adolescent scoliosis

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Abstract

Lymphangiomas are a group of benign malformations of the lymphatic system, and hemolymphangioma (or hemangiolymphangioma) is a rare congenital malformation of the lymphatic system with vascular involvement. These benign malformations are most commonly diagnosed at an early age, and may be present as a part of an associated syndrome. In this case report, we describe the first case of adolescent scoliosis associated with a large, paraspinous hemolymphangioma. A 15-year-old girl with an incidental finding of a paraspinous hemolymphangioma is presented along with her history, physical exam, radiographic findings, and operative course. The possible pathogenesis, treatment approach, and clinical dilemmas are also discussed. Given the well-known relationship between tumors and scoliosis, a benign paraspinous vascular and lymphatic tumor may be responsible for the presence of scoliosis in a small number of patients.

Key words: Case report; Scoliosis; Etiology; Hemolymphangioma; Tumor

Core tip: This case report describes the first case of adolescent scoliosis associated with a large, paraspinous hemolymphangioma. Given the relationship between tumors and spinal deformity, a benign paraspinous vascular and lymphatic tumor may be responsible for scoliosis in a small number of patients.

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INTRODUCTION

The association between paraspinous tumors and scoliosis is well recognized in clinical orthopaedics. To our knowledge, there are no reports in the current literature of an association between paraspinous hemolymphangioma and scoliosis. A lymphangioma is a congenital or post-traumatic malformation of the lymphatic system that is inherently benign, and typically found by palpation or with symptomatic compression of nearby anatomic structures. This entity has been classified into four types: capillary lymphangioma, cavernous lymphangioma, cystic lymphangioma (hygroma), and hemolymphangioma[1]. However, it may also be classified by cyst size as microcystic, macrocystic, or mixed[2].

Hemolymphangioma is the least common subtype, and has been found in the genitalia[3,4], gastrointestinal tract[5,6], head and neck[7,8], and extremities[9,10]. In the literature, there is also one report of a retroperitoneal hemolymphangioma without associated scoliosis[5], as well as a case series of bone lymphangioma associated with spinal deformity in the international literature[11]. Secondary transformation of lymphagioma to lymphangiosarcoma is rare, and when present, it is most often due to radiation therapy[12]. Treatment for symptomatic tumors includes marginal excision, aspiration and drainage, cryotherapy, injection of sclerosing agents, and radiotherapy[12]. However, recurrence is common, even after meticulous excision[10]. For asymptomatic lesions, a conservative approach may be taken in the form of serial imaging.

In this case report, we describe the first case to our knowledge of adolescent scoliosis associated with a large, paraspinous hemolymphangioma. The diagnosis and operative course of this patient is presented, in addition to a discussion of the pathogenesis and clinical management of this condition. We discuss various clinical dilemmas associated with this case, and we suggest a modification in the work-up of adolescent scoliosis in the setting of a strong family history of lymphatic tumors.

CASE REPORT

A 15-year-old girl presented for consultation regarding her scoliosis. Several years ago, the patient had been prescribed a brace for a 26-degree curve, but she admitted to non-compliance with this regimen. The patient was born at full-term by vaginal delivery. She had delayed puberty, and at the age of 15, she was 145 cm tall and weighed 34 kg. The patient was active in sports and did not complain of back pain. She had no family history of scoliosis or spine surgery. However, her maternal heritage was significant for several relatives with "hemangiomas," including her mother who had a large and irregular lesion of the left upper extremity (Figure 1).

On physical exam, the patient demonstrated a right thoracic rib prominence of 10 degrees on Adams forward bending test. There were no cutaneous lesions present, and the remainder of her physical exam including neurological exam was unremarkable. Routine scoliosis radiographs were obtained (Figures 2 and 3), and demonstrated a right thoracic curve. No additional imaging was indicated at that time[13]. At this point, the patient's presentation and curve pattern seemed consistent with adolescent idiopathic scoliosis (AIS), and given the severity of disease, the patient was indicated for a posterior spinal fusion from T2 to L1.

In the operating room, a standard posterior approach to the spine was performed, and the posterior elements were exposed. A Lenke probe was used to instrument the right pedicle of T11 (Figure 4), at which point, an immediate flow of thin, white fluid was noted through the tract. As a result, cultures were obtained, a drain was placed, and a decision was made to postpone the definitive procedure until the etiology of this fluid was determined.

The intra-operative Gram stain and microscopic analysis demonstrated moderate white blood cells without the presence of bacteria. Similarly, AFB, fungus, aerobic and anaerobic cultures were negative for any organisms. An magnetic resonance imaging (MRI) was also obtained that demonstrated a multilobulated, prominently vascularized lesion in the retroperitoneum from T7 to L5 (Figures 5 and 6). These pathologic and radiographic findings were reviewed with an expert pediatric surgical oncologist at a leading cancer center, and based on the patient's strong family history, intraoperative finding of lymph, and the lesion's characteristics on advanced imaging, a diagnosis of hemolymphangioma was made by the specialist. At that point, there was no indication for acute oncologic management, and there were no contraindications to proceeding with spine surgery. After discussion with the patient and her family, the decision was made to move forward with the definitive procedure. The patient underwent an uncomplicated posterior spinal fusion from T2 to L1 as previously planned (Figure 7). Care was taken to avoid the left pedicles of T7 to T9, as these levels corresponded to the largest portion of the hemolymphangioma seen on MRI. The patient recovered uneventfully, and was discharged without further incident. She was advised to follow-up with a pediatric oncologist for further surveillance and management. At one-year follow-up, the patient reported no symptoms, and her most recent radiographs revealed a stable correction with intact hardware.

DISCUSSION

At the patient's initial presentation, the curve pattern and clinical behavior of her spinal deformity seemed consistent with AIS. However, the immediate proximity of this large malformation to the spine suggests an association with the patient's spinal deformity, and warrants further discussion regarding the etiology and

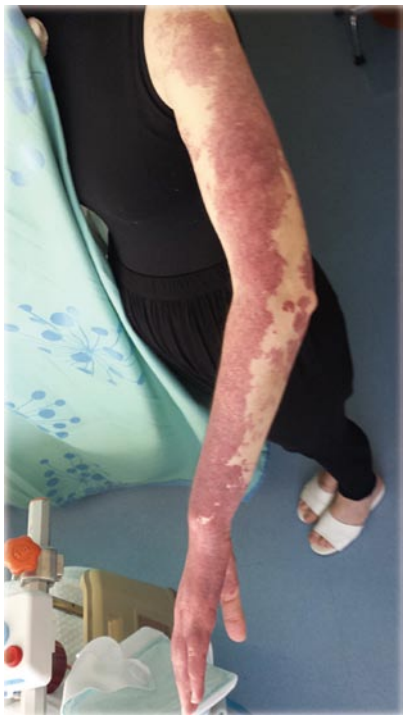

Figure 1 Vascular malformation, left upper extremity of mother.

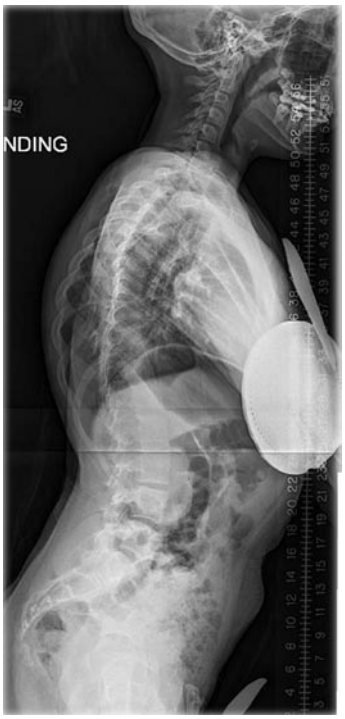

Figure 3 Pre-operative lateral X-ray.

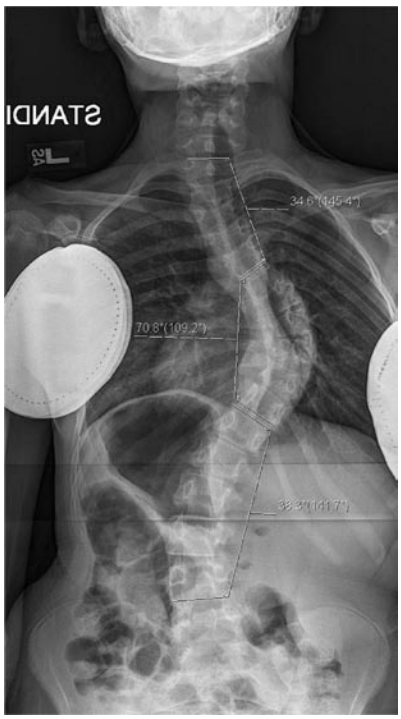

Figure 2 Pre-operative AP X-ray.

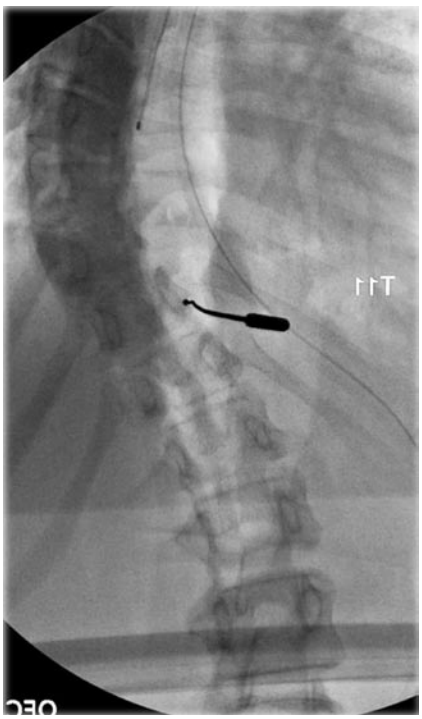

Figure 4 Intraoperative X-ray from initial procedure at right T11 pedicle.

management of this patient's scoliosis.

Several tumors within the spine have been shown to be associated with scoliosis and spinal deformity, most commonly osteoid osteoma and osteoblastoma[14,15], but also astrocytoma, ependymoma, epidermoid cyst[16], and bone lymphangioma[11]. However, there have been no reports of spinal deformity associated with paraspinous hemolymphangioma. In the case of osteoid osteoma and osteoblastoma, the tumor is more commonly located on the concavity of the curve. As a result, the deformity is caused by an asymmetric inflammation of the paraspinal muscles leading to asymmetric spasm[17]. In the case of bone lymphangioma, the spinal deformity is thought to result from a loss of spinal stability[11]. This patient's tumor was also predominantly located on the concavity of

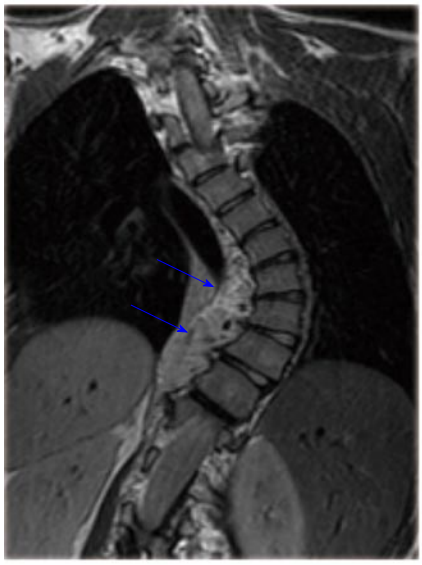

Figure 5 Coronal T2-weighted magnetic resonance imaging.

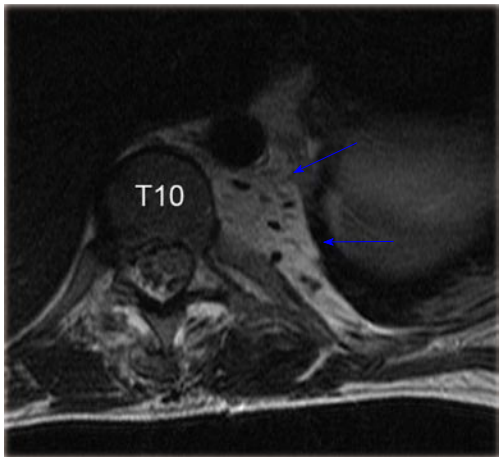

Figure 6 Axial T2-weighted magnetic resonance imaging.

the curve and her spinal stability was compromised suggesting a similar pathogenesis. In addition to an asymmetric inflammatory response and loss of spinal stability, unilateral disruption of growth or alteration of soft tissue dynamics may also have played a role in the pathogenesis of this patient's spinal deformity.

This case also presents several interesting clinical dilemmas. The first dilemma is whether an MRI should have been obtained pre-operatively. Since this patient was healthy and lacked any neurological signs or atypical features, a pre-operative MRI did not appear to be indicated[13]. However, given the findings of this case report, it is reasonable to obtain a pre-operative MRI in patients with a strong family history of lymphatic tumors. Secondly, the unexpected intra-operative finding of a thin, whitish fluid from the pedicle raises an important dilemma regarding surgical decision-making. In this instance, a drain was placed and the etiology was further investigated. The staged procedure allowed the surgeon to review the patient's case with experts

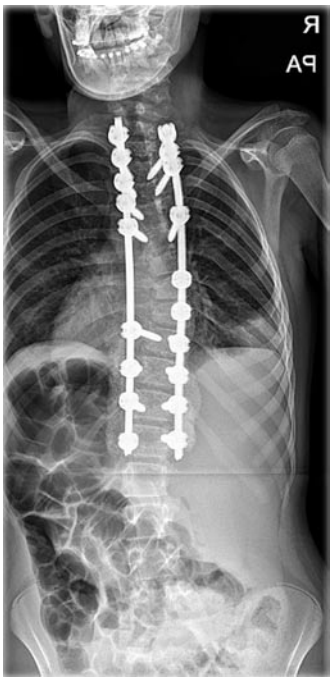

Figure 7 Post-operative AP X-ray.

and colleagues, and proceed when malignancy and infection were ruled out. Once the clinical diagnosis of a benign lesion was made, the surgeon was able to safely proceed with the planned procedure. Ultimately, this patient's treatment was similar to the originally prescribed surgical management, with the exception of fewer pedicle screws being included in her instrumentation. Lastly, it is unclear whether the patient will benefit from treatment of her hemolymphangioma, since the natural history of this condition is benign and there are several risks associated with treatment, including malignant transformation and a high likelihood of recurrence[10,12]. Nevertheless, it is clear that this patient will require close follow-up to monitor for symptoms as well as recurrence of her deformity.

Overall, this case report describes the first case of a paraspinous hemolymphangioma associated with adolescent scoliosis. The natural history of hemolymphangioma is typically benign, however, it may be associated with the development of scoliosis in a small number of patients. The diagnostic work-up and surgical management of scoliosis may need to be modified in patients with a known history or strong family history of lymphatic tumors.

ACKNOWLEDGMENTS

We would like to thank Michael P La Quaglia, MD, for his expertise in the oncologic diagnosis and management of this patient.

COMMENTS

Case characteristics

A 15-year-old girl presented with scoliosis.

Clinical diagnosis

On physical exam, the patient had a right thoracic rib prominence of 10 degrees on Adams forward bending test, she had no cutaneous lesions, and her neurological exam was unremarkable.

Differential diagnosis

The possible diagnoses included adolescent idiopathic scoliosis or pathologic scoliosis.

Imaging diagnosis

The patient's initial radiographs demonstrated a right thoracic curve, and magnetic resonance imaging (MRI) demonstrated a multilobulated, prominently vascularized lesion in the retroperitoneum from T7 to L5.

Pathological diagnosis

The intra-operative Gram stain and microscopic analysis demonstrated moderate white blood cells without the presence of bacteria, and AFB, fungus, aerobic and anaerobic cultures were negative for any organisms.

Treatment

After consultation with an expert pediatric oncologist, the patient underwent a posterior spinal fusion from T2 to L1. However, care was taken to avoid the left pedicles of T7 to T9 as these levels corresponded to the largest portion of the hemolymphangioma seen on MRI.

Related reports

There have been no previous reports of adolescent scoliosis associated with a large, paraspinous hemolymphangioma, however there have been several reports of spinal deformity associated with spinal tumors.

Term explanation

Hemolymphangioma is a rare congenital malformation of the lymphatic system with vascular involvement.

Experiences and lessons

This case report presents the diagnostic work-up and surgical management of adolescent scoliosis associated with a large, paraspinous hemolymphangioma. Several clinical dilemmas are also discussed such as the need for advanced imaging, surgical decision-making, and post-operative management. An MRI may be indicated in patients with a strong family history of lymphatic tumors, and close collaboration with a surgical oncologist and other colleagues is required to ensure an optimal surgical outcome.

Peer-review

The case is rare, and the correction is well done. It is a nice paper.

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CASE REPORT

Advanced Anderson-Fabry disease presenting with left ventricular apical aneurysm and ventricular tachycardia

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Author contributions: Poulin MF, Shah A, Trohman RG and Madias C designed the report and contributed to the writing and editing of the paper; Poulin MF and Shah A collected the clinical data.

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Ethics approval: Rush University Medical Center Institutional Board Review.
Informed consent: The patient gave her verbal informed consent prior to study inclusion.
Conflict-of-interest: All authors have no conflict of to report.

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Abstract

A 54-year-old female with Anderson-Fabry disease (AFD)-R342Q missense mutation on exon 7 in alpha-galactosidase A (*GLA*) gene - presented with sustained ventricular tachycardia. Imaging confirmed the presence of a new left ventricular apical aneurysm (LVAA) and a significantly reduced intra-cavitary gradient compared to two years prior. AFDcv is an X-linked lysosomal storage disorder caused by GLA enzyme deficiency. The phenotypic expression of AFD in the heart is not well described. Cardiac involvement can include left ventricular hypertrophy (LVH), which is typically symmetric, but can also mimic hypertrophic cardiomyopathy (HCM). Left ventricular apical aneurysm is a rare finding in HCM. We suggest a shared mechanism of LVAA formation in AFD and HCM, independent of the underlying cardiomyopathy. Mechanisms of LVAA formation in HCM include genetic predisposition and long-standing left ventricular wall stress from elevated intra-cavitary systolic pressures due to mid-cavitary obstruction. Both mechanisms are supported in this patient (a brother with AFD also developed a small LVAA). Screening for AFD should be considered in cases of unexplained LVH, particularly in patients with the aneurysmal variant of HCM.

Key words: Anderson-Fabry disease; Sustained ventricular tachycardia; Left ventricular apical aneurysm; Hemodynamic compensation; Transthoracic echocardiography; Magnetic resonance imaging; Hypertrophic cardiomyopathy

Core tip: Left ventricular apical aneurysm (LVAA) is a very rare phenomenon in patients with Anderson-Fabry disease (AFD); however, this patient with genetically confirmed AFD presented with a new LVAA. The authors believe that formation of the LVAA is the result of long-

standing elevated intra-cavitary systolic pressures and possibly genetic predisposition, similar to what can be seen in hypertrophic cardiomyopathy.

Poulin MF, Shah A, Trohman RG, Madias C. Advanced Anderson-Fabry disease presenting with left ventricular apical aneurysm and ventricular tachycardia. *World J Clin Cases* 2015; 3(6): 519-524 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i6/519.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i6.519

INTRODUCTION

Anderson-Fabry disease (AFD) is an X-linked lysosomal storage disorder caused by alpha-galactosidase (GLA) enzyme deficiency. The phenotypic expression of AFD in the heart is not well described. Cardiac involvement can include left ventricular hypertrophy (LVH), which is typically symmetric, but can also mimic hypertrophic cardiomyopathy (HCM). We report the case of a middle-aged female previously diagnosed with AFD who developed a left ventricular apical aneurysm (LVAA), which has been rarely reported in AFD. We suggest a shared mechanism of LVAA formation in AFD and HCM, independent of the underlying cardiomyopathy.

CASE REPORT

A 54-year-old female with AFD and well controlled hypertension presented with chest pain, palpitations and dizziness after missing two doses of metoprolol. Her initial electrocardiogram (ECG) displayed sustained monomorphic ventricular tachycardia (VT) with a cycle length of 214 ms. The VT had a right bundle, superior axis morphology suggesting a left ventricular (LV) apical origin (Figure 1)[1]. She initially received a bolus of amiodarone that failed to terminate the VT. Post direct current cardioversion, serial ECGs showed atrial fibrillation with persistent ST segment elevation in the inferior leads (Figure 2). A coronary angiogram demonstrated non-occlusive coronary artery disease. Transthoracic echocardiogram (TTE) revealed that her ejection fraction was newly reduced from 75% to 40% and identified a thin-walled LVAA that was not seen on TTE two years prior (Figure 3A and B). The mid and basal segments of the LV still had marked concentric hypertrophy with systolic mid-cavitary obliteration; however, the intra-cavitary gradient at rest was significantly lower than on the prior study; it had decreased from 71 to 16 mmHg (Figure 4). Contrast-enhanced cardiovascular magnetic resonance (CMR) confirmed these findings and demonstrated late gadolinium enhancement (LGE) of the aneurysmal LV apex extending to the lateral and inferolateral walls, consistent with myocardial scarring (Figure 5). The patient was restarted on metoprolol and had no further episodes of VT. A defibrillator (ICD) was implanted prior to discharge.

DISCUSSION

AFD is a rare, X-linked lysosomal storage disorder caused by GLA enzyme deficiency, which results in an abnormal accumulation of sphingolipid catabolites in multiple organs, including the heart. Although the prevalence of AFD is likely underestimated, mutations are present in 1:6000 to 1:40000 females. Clinical manifestations in heterozygous females vary widely from no apparent clinical disease to full expression of the disease[2]. Cardiac involvement can lead to myocardial hypertrophy, restrictive cardiomyopathy, coronary artery disease, arrhythmias (atrial and ventricular) as well as aortic and mitral valvulopathy[3]. Our patient's disease manifested in childhood with acroneuropathy and hypohydrosis, and subsequently progressed to include cutaneous lesions, lacunar infarcts and LVH. A few years prior to presentation she was diagnosed with hypertension, but there was no manifest renal involvement from AFD. Her blood pressure was well controlled with a combination of an angiotensin converting enzyme inhibitor and a beta blocker. The diagnosis of AFD was established at age fifteen by genetic testing with a R342Q missense mutation on exon 7 in the *GLA* gene. A brother had the same mutation. The R342Q mutation has been shown to lead to a complete loss of the GLA activity, and to be associated with a classical phenotypic presentation of AFD[4,5].

Despite a relatively early diagnosis, our patient had been reluctant to initiate AFD therapy until her symptoms worsened. Enzyme replacement therapy with Agalsidase beta (Fabrazyme®, Genzyme, Cambridge, MA, United States) had been initiated eighteen months prior to presentation. She received monthly infusion of 65 mg for one year, followed by an infusion of 60 mg every 2 wk. Despite being compliant with the treatment, her disease slowly progressed, with worsening of her neuropathy, cutaneous lesions, development of cerebral vasculopathy and formation of the LVAA.

LVH is a relatively common finding in AFD and has been shown to correlate with severity of the disease. LVH in AFD can mimic HCM and has been identified among the causes of late onset HCM in women. In addition, women tend to present at a later age than men, and often have milder symptoms, making the diagnosis of AFD more challenging in female patients[6,7]. The pattern of hypertrophy in AFD is typically symmetric, as opposed to asymmetric with preferential septal involvement in HCM. Contrast-enhanced CMR typically demonstrates evidence of LGE, particularly in the LV subepicardial and mesocardial inferior and inferolateral segments. The LGE is believed to be the result of both intra-myocyte accumulation of sphingolipids and fibrosis[8]. Development of a LVAA is uncommon in HCM (reported in only 2% of patients) but portends a poor prognosis with a high adverse event rate, including sudden death,

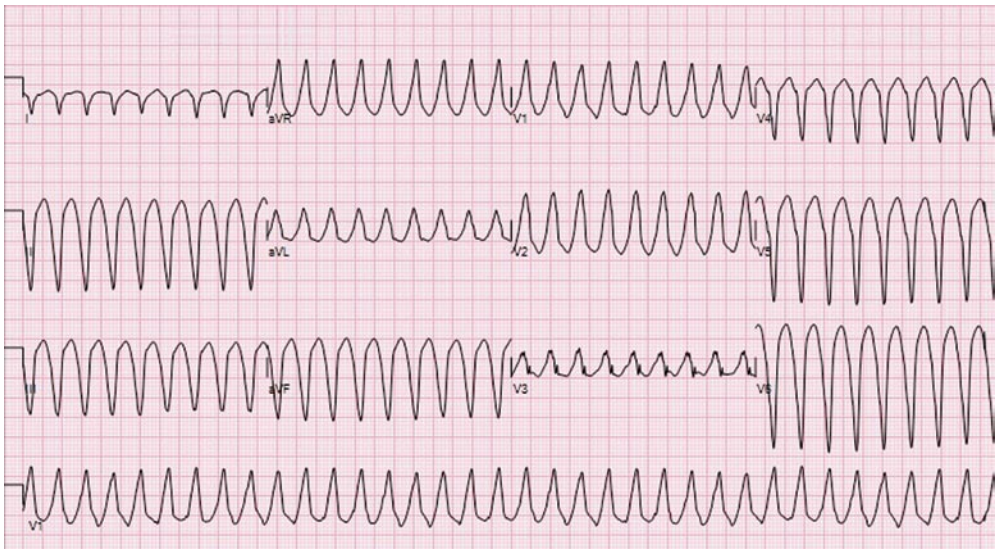

Figure 1 12-lead electrocardiogram (5 mm/mV) during ventricular tachycardia. The QRS shows a right bundle morphology with superior axis, suggesting a left ventricular apical source.

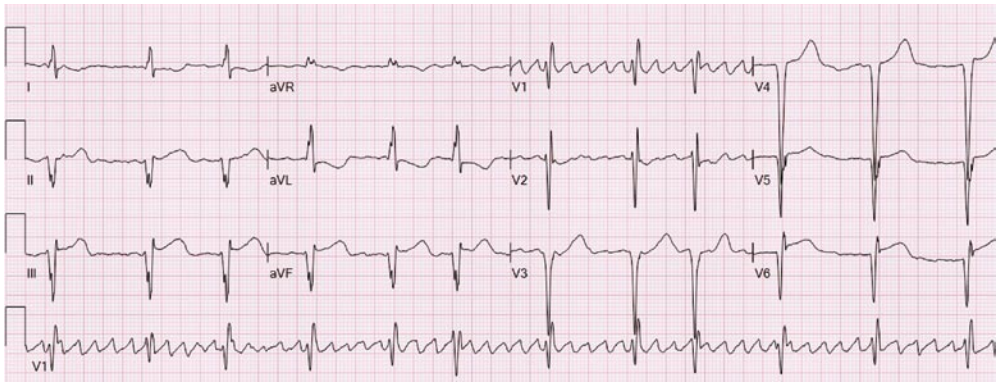

Figure 2 12-lead electrocardiogram (10 mm/mV) following direct current cardioversion. ST segment elevation is seen in the inferolateral leads.

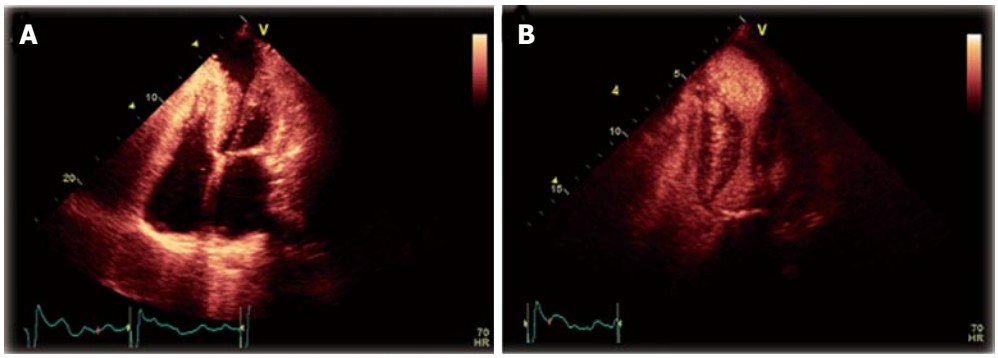

Figure 3 Transthoracic echocardiogram. A: Transthoracic echocardiogram (TTE), apical four-chamber view, demonstrating concentric left ventricular hypertrophy with mid-cavitary obliteration at end-systole, as well as a large left-ventricular apical aneurysm (LVAA); B: TTE apical two-chamber view, with ultrasound contrast demonstrating stasis within the LVAA with a circular flow pattern, as well as decreased perfusion of the apex.

appropriate ICD discharges, thromboembolic stroke, and progressive heart failure[9].

In contrast to AFD, HCM is a relatively common genetic disease and is the most common cause of sudden cardiac death in young people. Its estimated prevalence is 1 in 500. In HCM, the inheritance pattern is autosomal dominant. The mutant proteins that cause HCM are incorporated into intact filaments of the sarcomere and have been described as "poisonous peptides". Local environmental factors such as pressure, protein defect and modifier genes interact to induce the subsequent phenotype[10]. In HCM, development of ST

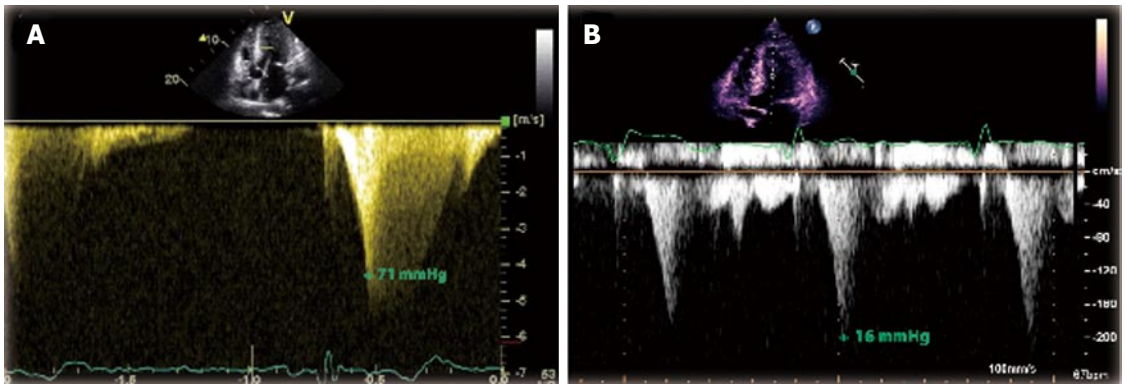

Figure 4 Transthoracic echocardiogram continuous Doppler. A: Transthoracic echocardiogram continuous Doppler flow across the left ventricle. The mid-cavitary gradient at rest from two years prior to the left-ventricular apical aneurysm (LVAA) formation (71 mmHg) is shown; B: Over time, flow across the mid-cavitary obliteration decreased and the peak pressure declined after the formation of the LVAA (16 mmHg).

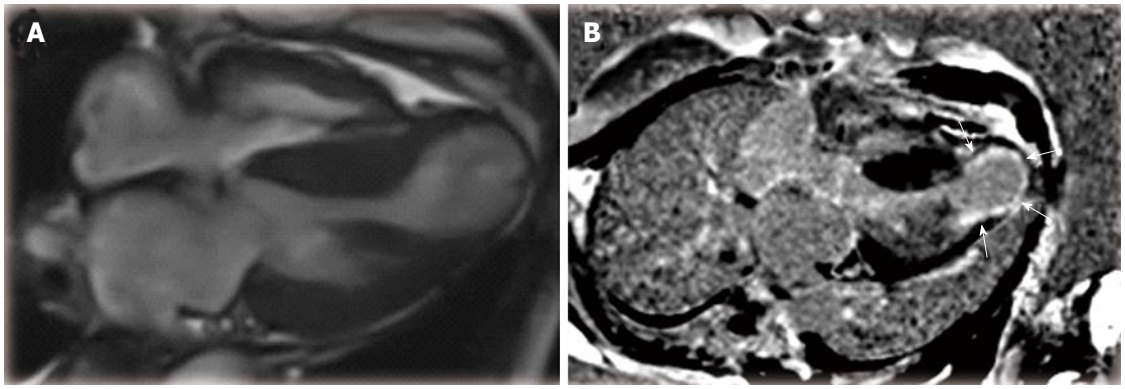

Figure 5 Contrast-enhanced cardiovascular magnetic resonance. A: Long-axis four-chamber cardiovascular magnetic resonance in systole showing concentric left ventricle hypertrophy with mid-cavitary obstruction and large apical aneurysm; B: Late gadolinium enhancement (LGE) image of the left-ventricular apical aneurysm consistent with myocardial scarring (arrows) with extension of LGE into the inferolateral segments (a pattern typically seen in Anderson-Fabry Disease).

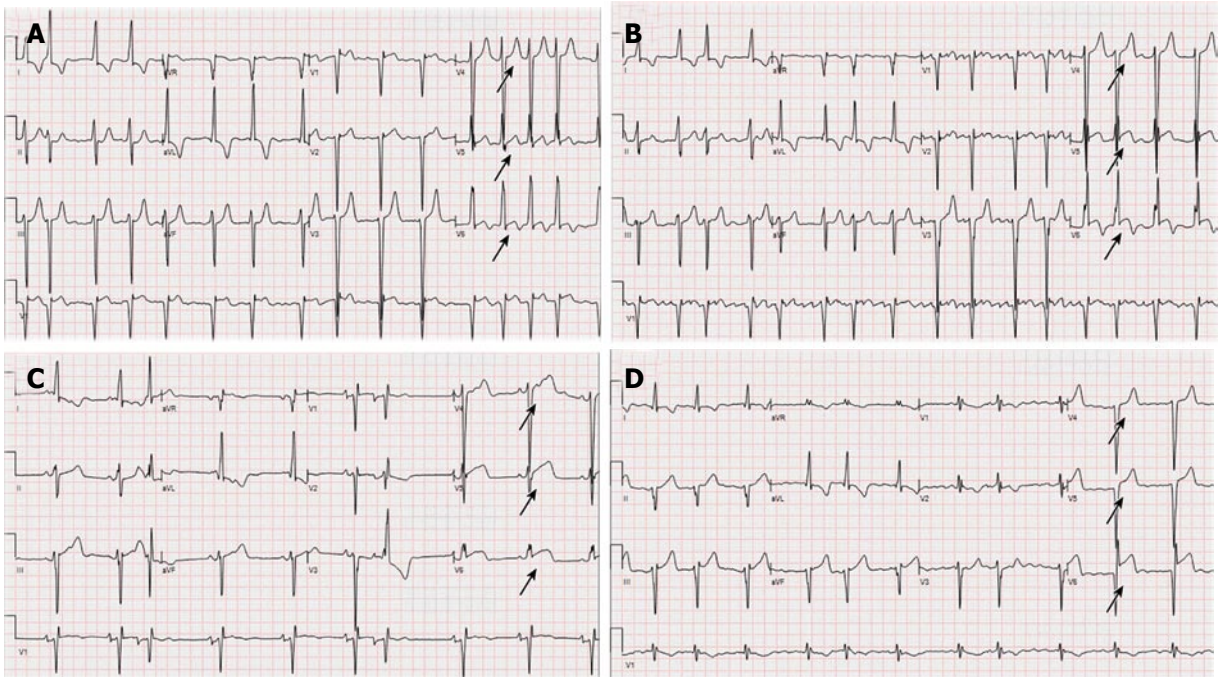

Figure 6 Serial electrocardiograms from this patient (A) 4 years prior, (B) 2 years prior, (C) 1 year prior, and (D) 3 mo prior are presented. The development of ST elevation in leads V4-V6 (arrows) can be seen, and correlates with the formation of the left ventricular apical aneurysm.

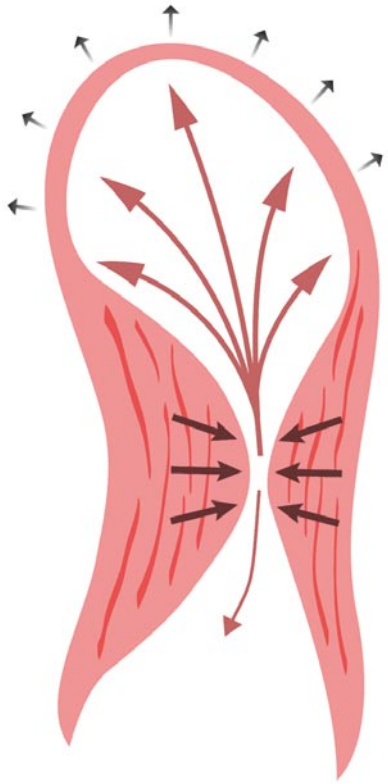

Figure 7 Illustration of blood flow direction during left ventricular systole. While some blood is ejected towards the outflow tract (single arrow), the flow is initially greater towards the apex, which is the area of least resistance (arrows from mid-cavity to the left-ventricular apical aneurysm), resulting in a lower gradient across the mid-cavitary obliteration as the aneurysm size increases.

elevation in leads V4 through V6 has been associated with LVAA formation[11]. Similar findings were seen upon reviewing our patient's serial ECGs (ranging from 4 years to 3 mo) prior to her presentation (Figure 6). To the best of the authors' knowledge, the development of LVAA has only been previously described once in AFD. In a recent report, the case of a middle-aged female diagnosed with HCM and LVAA is described. That patient was later found to have a missence mutation in the *GLA* gene (p.R118C) confirming the diagnosis of AFD[12].

The proposed mechanisms for LVAA formation in HCM are genetic predisposition and long-standing LV wall stress from elevated intra-cavitary systolic pressures as a result of mid-cavitary obstruction[8]. Both mechanisms are potential contributors in our patient, as a brother with AFD also developed a small LVAA prior to his death. We hypothesized that the formation of an apical aneurysm may be considered a hemodynamic adaptation to sustained elevation of systolic intra-cavitary LV pressure. The apex can dilate in response to the chronically elevated wall pressures during systole, with some blood flow directed towards the aneurysm, which offers less resistance than the mid-cavitary obliteration (Figure 7). Over time, this results in decreased flow across the mid-cavitary obstruction and a concordant smaller peak pressure gradient[13]. This phenomenon is exhibited in our patient by the considerable fall in her peak intra-cavitary gradient after the LVAA developed. Of note, our patient had chronic atrial fibrillation and it could be speculated that the LVAA formation was the result of a thromboembolic event. However, this scenario seems unlikely given that she had been appropriately anticoagulated with coumadin for years and had no other evidence of (non-cerebral) systemic embolization.

The phenotypic expression of AFD in the heart is not well described and can mimic even rare forms of HCM. The findings in this case suggest a shared mechanism(s) of aneurysm formation in AFD and HCM, independent of the underlying cardiomyopathy. Screening for AFD should be strongly considered in cases of unexplained LVH, particularly in patients with the aneurysmal variant of HCM. As previously demonstrated in HCM, it is likely that LVAA formation in AFD portends a poor prognosis.

COMMENTS

Case characteristics

A middle-aged female with Anderson-Fabry disease (AFD) presented with chest pain, palpitations and dizziness for a few hours after missing two doses of her beta-blocker.

Clinical diagnosis

She was found to have sustained ventricular tachycardia originating from the left ventricular (LV) apex, and further imaging confirmed the presence of a new LV apical aneurysm (LVAA).

Differential diagnosis

The differential diagnosis included aneurysm from hypertrophic cardiomyopathy (HCM), aneurysm from prior infarct, apical diverticulum and Takotsubo cardiomyopathy.

Laboratory diagnosis

The diagnosis of AFD was established by genetic testing with the presence of R342Q missense mutation on exon 7 in the *GLA* gene.

Imaging diagnosis

A transthoracic echocardiogram revealed that her intra-cavitary gradient had significantly decreased from two years prior and identified a new LVAA, which was confirmed by a cardiovascular magnetic resonance imaging.

Pathological diagnosis

The findings in this case suggest a shared mechanism(s) of aneurysm formation in AFD and HCM: genetic predisposition and long standing LV wall stress from elevated intra-cavitary systolic pressures as a result of mid-cavitary obstruction.

Treatment

She was placed back on her beta blocker and a defibrillator was implanted prior to discharge.

Related reports

Left ventricular hypertrophy in AFD can mimic HCM, however the pattern of hypertrophy is typically symmetric in AFD as opposed to asymmetric with preferential septal involvement in HCM. Development of a LVAA is uncommon in HCM (reported in only 2% of patients) and has only been (recently) reported once in AFD.

Term explanation

Development of a LVAA is a very rare phenomenon in patients with AFD.

Experiences and lessons

The findings in this case suggest a shared mechanism(s) of aneurysm formation in AFD and HCM, independent of the underlying cardiomyopathy. Screening for AFD should be strongly considered in cases of unexplained LVH, particularly in patients with the aneurysmal variant of HCM.

Peer-review

Authors showed a case of AFD complicated by apical aneurysm and ventricular

tachycardia. This report is written and presents well.

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CASE REPORT

Variant of multiple sclerosis with dementia and tumefactive demyelinating brain lesions

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Author contributions: Hamed SA solely contributed to this work.

Ethics approval: The study was reviewed by and received exemption from the Assiut University Hospital Institutional Review Board.
Informed consent: The study participant provided written informed consent for inclusion in this case report.
Conflict-of-interest: The author declared no potential conflicts of interest.

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Abstract

We describe an unusual clinical and diagnostic feature of a patient with multiple sclerosis (MS). A 25-year-old woman was admitted to the Neurology department (December 2009) with one month history of rapid cognitive deterioration. She had poor cognition, dysphasia, reduction in visual acuity and temporal pallor of the optic discs. She had prolonged latencies of P100 component of visual evoked potentials (VEPs). Magnetic resonance imaging (MRI)-brain showed multifocal large (⩾ 3 cm) white-matter hypointense lesions in T1W and hyperintense in T2W and fluid-attenuated inversion recovery images and patchy enhancement. A diagnosis of tumefactive MS was given. She received two consecutive 5-d courses of 1 g daily intravenous methylprednisolone for 2 mo and oral prednisolone in dose of 80 mg twice/daily in between. At the 3rd month, Mini Mental State Examination and VEPs returned to normal but not the MRI. Patient continued oral steroids after hospital discharge (March 2010) for 9 mo with significant MRI improvement after which tapering of steroids started for a year. The patient refused immunomodulation therapy due to her low socioeconomic status. Neither clinical relapse nor new MRI lesions were observed throughout the next 4 years. In spite of the aggressive course of tumefactive MS variant, good prognosis may be seen in some patients.

Key words: Tumefactive multiple sclerosis; Acute dementia

Core tip: Multiple sclerosis (MS) is the most common cause of progressive neurologic handicap in young adults. MS is typically presented by sensory, motor and visual dysfunctions, abnormal visual, auditory brainstem, somatosensory and motor evoked potentials, elevated cerebrospinal fluid proteins and oligoclonal bands, and abnormal neuroimaging of the brain and spinal cord. In the literature, atypical clinical and radiological presentations or variants have been described in adults with MS which may pose diagnostic difficulties. However

and in spite of the aggressive course of its tumefactive variant, good prognosis may be seen in some patients on corticosteroids.

Hamed SA. Variant of multiple sclerosis with dementia and tumefactive demyelinating brain lesions. *World J Clin Cases* 2015; 3(6): 525-532 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i6/525.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i6.525

INTRODUCTION

Multiple sclerosis (MS) is a chronic immunologic disease in which inflammation, demyelination and axonal damage are the main pathologic features. The exact etiology of MS is unknown, however genetic susceptibility may be contributed[1]. MS has a prevalence of more than 30 cases per 100000 people[2,3]. Typically, the majority of patients with MS (approximately 70%) are young adults with an age range between 20-40 years particularly females (75%), while the remaining 10% and 30% of cases occur before the age of 20 years and after the age of 40 years, respectively[3]. MS has often nonspecific initial symptoms; however, typically, MS is presented by sensory, motor and visual dysfunctions. MS is usually diagnosed by demonstrating evidence of clinical and/or radiographic dissemination of the disease in time and space[4,5]. The typical MS plaques appear in magnetic resonance imaging of the brain (MRI) as multiple periventricular homogenous ovoid lesions ranged in size from 3 to 16 mm, often oriented perpendicular to the long axis of the ventricular system and have no mass effect. Other locations include the optic nerves, corpus callosum, centrum semiovale, pons, cerebellar peduncles or hemispheres, brainstem and spinal cord[5]. Nearly 85% of cases with MS develop relapsing-remitting MS (RRMS) course in which relapses usually occur on average once every other year. Nearly 50%-90% of cases with RRMS develop secondary progressive MS within 10-25 years, 10% develop primary progressive MS and 5% develop acute attacks on top of steadily progressive neurologic decline[6]. Pharmacologic treatment of MS include immunosuppressants and immunomodulators[7].

Some cases of MS may pose a diagnostic difficulty due to atypical clinical and neuroimaging manifestations which mimic other fulminant central nervous system (CNS) conditions as inflammatory/infective conditions and intracranial neoplastic and non-neoplastic space occupying lesions (SOLs).

This paper describes an adult woman who presented for the first time with mental confusion and rapid deterioration in different cognitive functions. She also had atypical imaging features.

CASE REPORT

At December 2009, a 25-year right handed woman was admitted to our department with a one month history of mental confusion and rapid cognitive deterioration without previous history of systemic infection or a stress-related factor. Her family reported that the patient was unable to do her daily home duties, neglected care for herself and her children and looked blind and had poverty of speech. She was referred by a neurologist who reported abnormal computed tomography (CT) of the brain with large multifocal hypodense lesions (Figure 1). On neurological evaluation upon admission, she looked apathetic, had mixed dysphasia and had marked diminution of visual acuity (hand movement) with bilateral dilated pupils which were less reactive to light and bilateral temporal pallor of the optic discs. She had normal motor and peripheral sensory examination and bilateral flexor planter responses. She had bilateral prolonged P100 component of the visual evoked potentials (VEPs) indicating demyelinating optic neuropathy (Figure 2). Conventional magnetic resonance imaging of the brain (MRI-brain) showed bilateral multiple subcortical superficial and deep white-matter large (≥ 3 cm) lesions in the frontal, parietal, temporal and occipital lobes which were hypointense in T1-weighted and hyperintense in T2-weighted and fluid-attenuated inversion recovery (FLAIR) images with minimal perifocal edema and mass effect in spite of large sizes and bilaterally of the lesions. They demonstrated incomplete or patchy contrast enhancement with gadolinium (Figure 3). Spinal MRI was normal. The patient had normal routine laboratory blood testing which included complete blood count, blood sugar, serum electrolytes, liver and kidney function tests, erythrocytic sedimentation rate, rheumatoid factor, antinuclear antibodies and anti-double stranded DNA (anti-ds DNA) antibodies. The patient was given a diagnosis of tumefactive MS. After admission, she received 1 g daily intravenous methylprednisolone for 5 d. Oral prednisolone in a dose of 80 mg twice daily was given for 1 mo. As no clinical improvement was observed, another dose of pulse steroid therapy was given which was also followed by

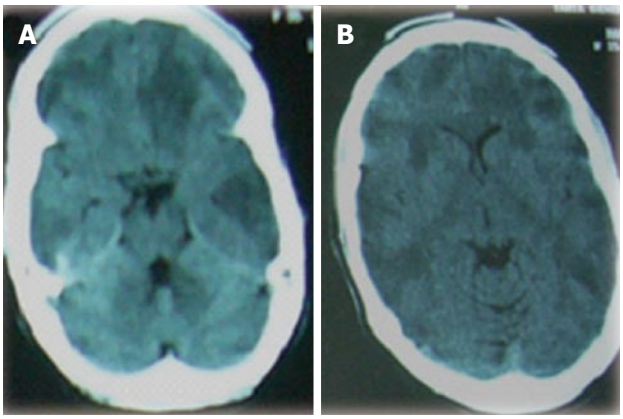

Figure 1 Cranial computed tomography brain showing axial views (A, B) with bilateral multifocal large hypodense lesions in the frontal, parietal, temporal and occipital lobes.

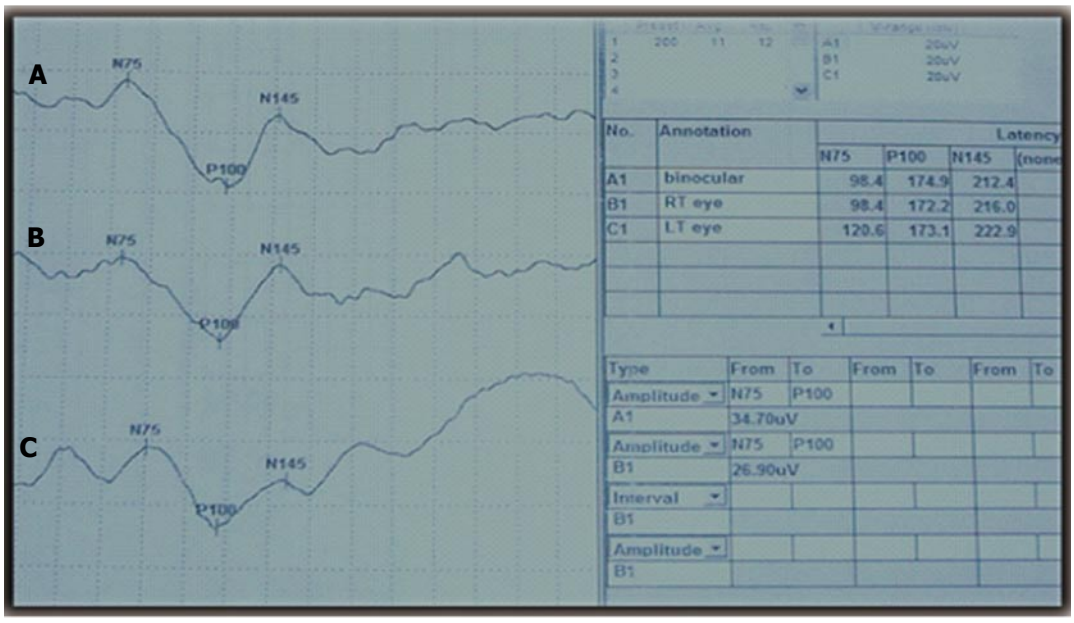

Figure 2 Visual evoked potentials showing prolonged absolute latencies of the P100 component of the right eye (A), left eye (B) and binocular (C).

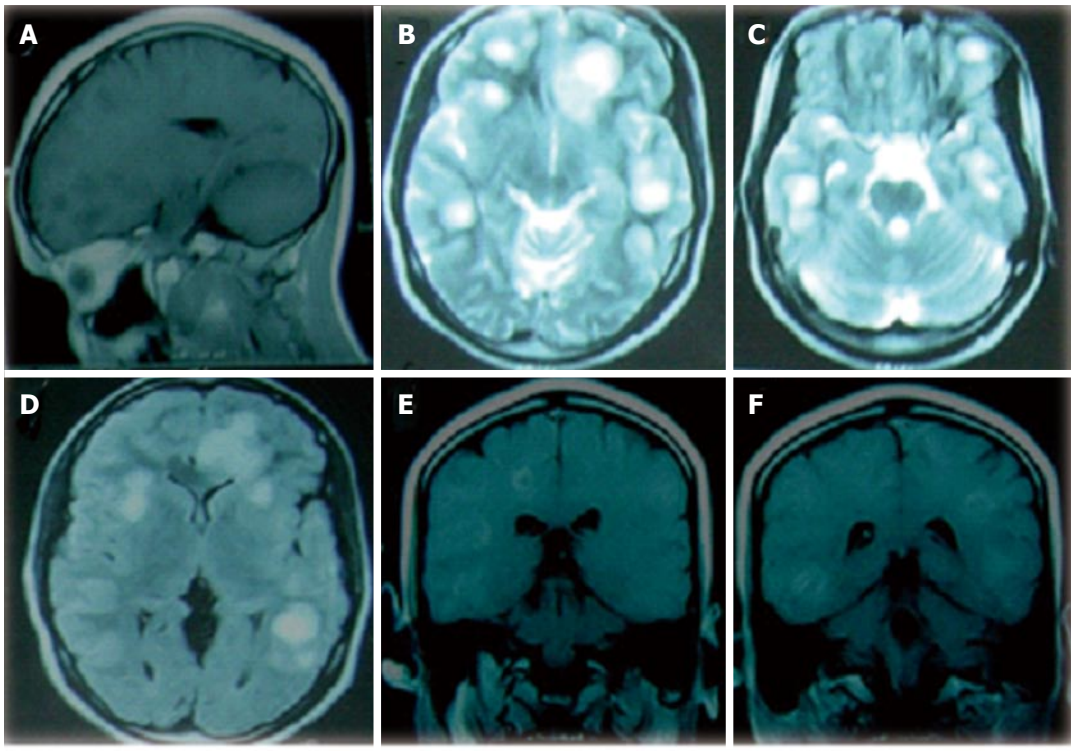

Figure 3 Cranial magnetic resonance imaging brain (on admission) showing (A) sagittal T1-weighted view with multiple hypointense lesions in the frontal and parietal regions, (B, C) axial T2-weighted images showing multifocal large hyperintense lesions in the frontal, parietal, temporal and occipital lobes, (D) axial fluid-attenuated inversion recovery-weighted image showing hyperintense lesions with minimal perifocal hypointense rim (edema), (E, F) coronal T1-weighted contrast enhanced views with patchy enhancement of the hypodense lesions in the temporal, parietal and frontal lobes bilaterally.

80 mg twice daily oral prednisolone. Near the end of the 3rd month of treatment, improvement started with rapid regression of patients symptoms in the form of improvement of cognition and vision, after which the patients was discharged from the hospital (March 2010). Clinical and MRI follow ups were done every 3 mo. We observed lack of significant MRI improvement in spite of the clinical improvement (Mini Mental State Examination = 30/30) after 6 mo from the onset of the condition (Figure 4). The patient was informed that she is in need for a disease modifying therapy to optimize therapy and prevent relapses (as IFNβ-1a/b, glatiramer acetate, mitoxantrone and natalizumab which are available in our country) but she refused due to the

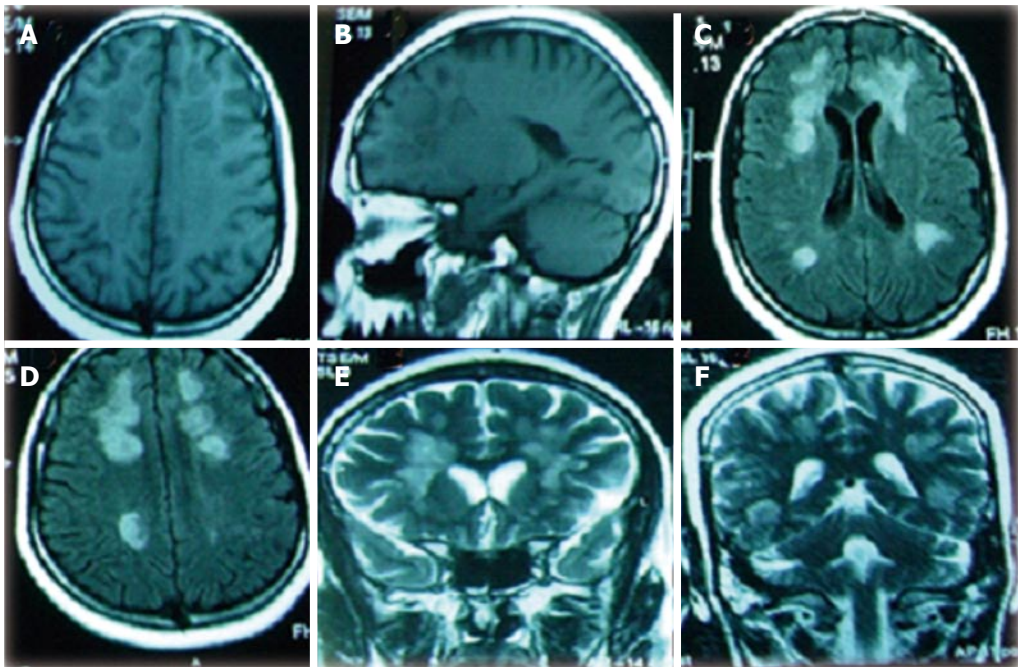

Figure 4 Cranial magnetic resonance imaging brain (after 6 mo of follow up) showing (A, B) axial and sagittal T1-weighted views with multiple hypointense lesions in the frontal, parietal, temporal and occipital regions, (C, D) axial fluid-attenuated inversion recovery-weighted images showing multifocal hyperintense lesions, (E, F) coronal T2-weighted images showing multifocal bilateral hyperintense lesions.

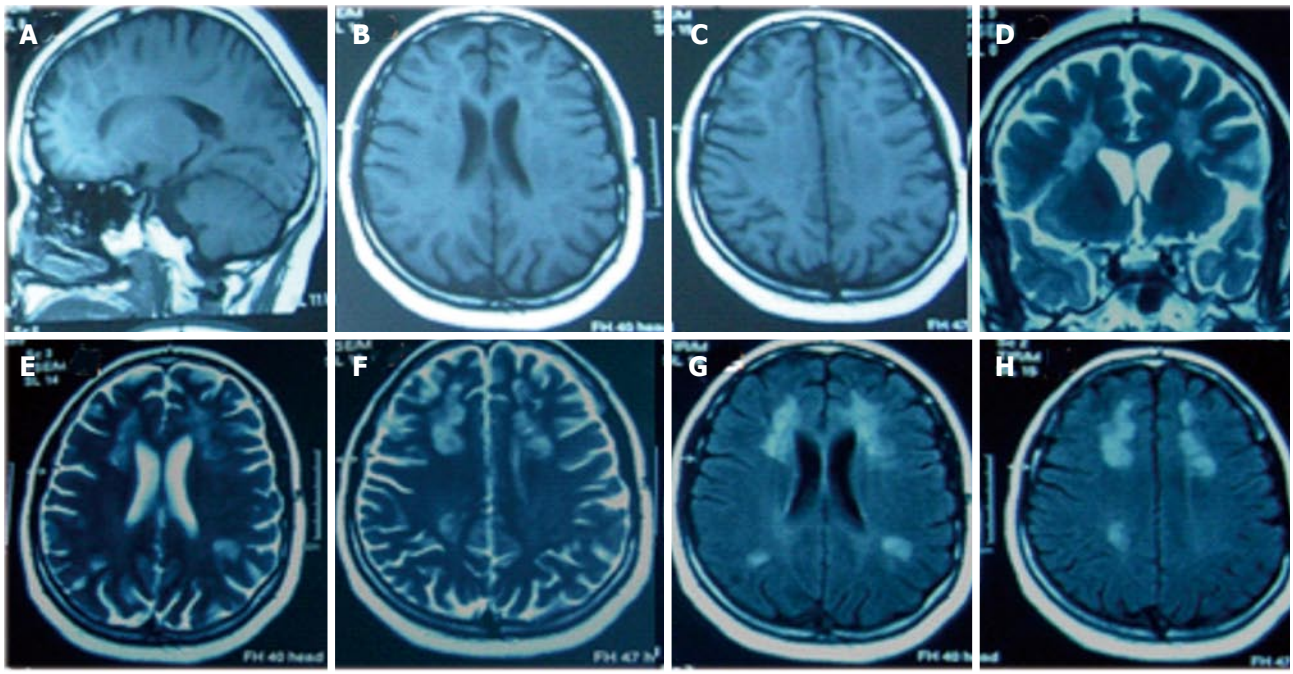

Figure 5 Cranial magnetic resonance imaging brain (after one year of follow up) showing (A, B, C) sagittal and axial T1-weighted views with bilateral multiple hypointense lesions in the frontal, parietal, temporal and occipital regions, (D, E, F) coronal and axial T2-weighted images showing multifocal bilateral hyperintense lesions, (G, H) axial fluid-attenuated inversion recovery-weighted images showing multifocal hyperintense lesions. In all views, lesions had reduced sizes compared to those at 6 mo follow up.

low socioeconomic status and she had no insurance to cover the cost of such expensive course of treatment. Significant reduction of the sizes of the MRI lesions was observed after one year of onset of the condition (Figure 5). Neither clinical relapse nor new MRI lesions were observed throughout the following 4 years of follow up.

The protocol of the study was approved by the local ethical committee of Assiut University Hospital which was in accordance of the principles of Helsinki and informed consent was obtained from the patient to publish her detailed clinical, laboratory and imaging data.

DISCUSSION

For our patient, tumefactive MS (or a tumor like demyelination) was established as diagnosis. Tumefactive MS is one of the atypical clinical and radiological presentations or variants described in adults with MS. It nearly represents 7% of cases with MS[8]. These variants are collectively named central nervous system inflammatory demyelinating diseases (CNS IDD) and include tumefactive MS[8-14], acute disseminated encephalomyelitis (ADEM)[15,16], Marburg's MS[17], Balo Disease (or Balo concentric sclerosis)[18] and Devic's disease or neuromyelitis optica[19]. In general, these variants have monophasic presentations and commonly aggressive or fulminant clinical course and do not show dissemination in time and space which is a typical feature for MS. Most commonly, these atypical aggressive attacks of variants of MS may present as the first demyelinating event in the course of MS. The management of these variants often prompts hospital admission and intensive care monitoring.

The diagnosis of tumefactive MS was done based on the acute onset (developed over days or weeks) of the condition, the predominance of cognitive impairment[8-14], the radiological evidence of multiple bilateral large rounded white matter lesions, absence of cortical involvement, absence of mass effect[9], patchy enhancement with gadolinium[14], regression with corticosteroids and lack of MRI evidence of new lesions on follow ups[8]. The severe cognitive impairment was due to great burden imposed by the large bilateral multifocal brain lesions. This also could explain the presence of aphasia and/or agnosia and/or apraxia (bilateral superficial and deep subcortical lesions). The marked diminution of vision in the patient was due to the presence of demyelinating optic neuropathy as evidenced by the VEPs, however, the possibility of the presence of field defects due to the involvement of the optic radiations in the temporal, parietal and occipital lobes of the cerebral hemispheres could not be excluded as additional cause of diminution of vision although visual field was not tested due to marked cognitive impairment. The enhanced lesions represented the areas of active inflammation, while the unenhanced lesions represented the chronic phase of the inflammatory process[20].

In general, tumefactive MS represents 1-2/1000 of cases of MS[21] with high frequency in adult females[14]. It is defined by the presence of single (frequent) or multiple large sized brain masses ($\geqslant$ 2.0 cm in diameter)[9], associated with perilesional edema and mass effect[14]. The common clinical presentations of tumefactive MS include headache, cognitive abnormalities, mental confusion, impaired consciousness, aphasia, apraxia, cerebellar symptoms, visual field defects and/or seizures. With contrast, tumefactive demyelinating lesions usually appear as open-ring (directed toward the cortical surface or to the basal ganglia) or closed rings[14,20] or have diffuse, homogeneous, punctate, or concentric enhancement[14]. The CSF may have high levels of immunoglobulin G (IgG) index and oligoclonal bands. CSF may also be normal in fulminant conditions and short duration of the disease[22]. Abnormal visual (VEPs) and somatosensory-evoked potentials may present in 33%-60% of cases with tumefactive MS[14]. Acute treatments of tumefactive MS include intravenous methylprednisolone and/or plasma exchange, rituximab and natalizumab followed by immunomodulatory agents[12,23,24]. The prognosis and course of tumefactive MS remain controversial. Some studies reported clinical and radiological improvement of tumefactive demyelinating lesions with no future development of typical recurrent relapsing MS[8]. In contrast, few reported development of clinically definite MD[14,25,26].

The differential diagnosis related to the presented case may include: (1) other variants of MS as ADEM, Marburg's MS and Balo concentric sclerosis; (2) CNS vasculitis; (3) intracranial infection/abscess or granuloma as tuberculoma; and (4) intracranial neoplastic SOLs as multifocal glioma, metastasis and primary CNS lymphoma (PCNSL).

ADEM is an acute multifocal monophasic inflammatory demyelinating disorder of the brain and spinal cord which should not progress beyond 3 mo. Although ADEM often occurs in the pediatric population, however adult cases have been reported. ADEM is often preceded by viral upper respiratory tract infection. ADEM is typically presented by encephalopathy. Other features may include vomiting, fever, headache, motor, sensory, and cerebellar symptoms, optic neuritis, seizures and aphasia[15,16]. Characteristic MRI features of ADEM include multifocal and diffuse hyperintense lesions in the gray and white matter of the brain and spinal cord in T2-weighted and FLAIR images and have no gadolinium enhancement[15,27]. Marburg's MS is a very rare form of atypical MS which rapidly progress and often leads to rapid disability followed by death[17]. It is caused by the severe axonal loss. Balo concentric sclerosis is a rare and rapidly progressive variant of MS. It usually first appears in adulthood. Symptoms may progress rapidly over several weeks or more slowly over 2-3 years. Symptoms may include headache, seizures, gradual paralysis, involuntary muscle spasms , and cognitive loss. Balo concentric disease is so named because of the pattern of concentric layers formed by the damaged myelin tissues. Some patients have been known to spontaneously recover from this disease[18]. CNS vasculitis is inflammatory disease of the blood vessels of the brain or spinal cord. It is often secondary to connective tissue diseases or systemic autoimmune diseases as systemic lupus erythematosis (SLE), polyarteritis nodosa and rheumatoid arthritis and Behçet's syndrome[28,29], but it can occur without associated systemic disease. Symptoms of CNS vasculitis may include headache, delirium, disorientation, forgetfulness

or confusion, recurrent focal or generalized seizures, aphasia, apraxia, motor and sensory abnormalities, gait disorder, optic atrophy and encephalopathy. The MRI brain picture consistent with vasculitis include multiple ischemic brain lesions which appear as periventricular hyperintense dot-like areas in T2-weighted, FLAIR- and diffusion-weighted MRI imaging, multiple subcortical white matter hyperintense lesions in the frontal, temporal, parietal and occipital lobes, and abnormal enhancement of the leptomeninges. Magnetic resonance angiography may show string-of-beads stenosis of the carotid and vertebro-basilar arteries. Treatment of CNS vasculitis include immunotherapy as methylprednisolone, methotrexate, cyclophosphamide and intravenous immunoglobulins (IVIGs)[30]. Patients with intracranial infection/abscess/granulomas commonly have history of risk factors as immunocompromised state, dental abscess, pulmonary abscess, intravenous drug use, etc. Fever and abnormal labs (as high erythrocytic sedimentation rate or C-reactive protein) are clues for presence of infection. Presentation is usually of acute onset and secondary to symptoms of increased intracranial pressure (ICP), and seizures and focal neurological deficits are common. MRI-brain usually show ring enhancement which is often complete with regular margin[31]. Intracranial tuberculomas are relatively common in developing countries. It represents about 10%-30% of all intracranial masses of cases of infective intracranial SOLs. Seizures and focal neurological deficits are common clinical presentations. Systemic tuberculosis may be absent in up to 70% of cases. MRI-brain may show multiple intracranial enhancing SOLs particularly ring enhancement or target sign[32]. Intracranial neoplastic SOLs which mimic tumefactive MS include multifocal glioma, metastasis and PCNSL. Multifocal glioma usually presents insidiously with progressive neurologic deficit. Seizures are the common presentation in nearly one fourth of the patients of supratentorial lesions. Increased ICP is the common presentation of posterior fossa lesions[33]. Nearly fifteen percent of cases with cerebral metastasis have no previously known cancer. The primary cancers commonly involve the lung, breast, kidney, and gastrointestinal tract. Increased ICP, seizures and focal neurological deficits are common presenting manifestations[34]. PCNSL is a rare brain tumor that comprises only 1% of all intracranial neoplasms. Nearly 80% of cases of PCNSL have single supratentorial lesions. In one-third of the cases, PCNSL lesions are located deeply in the white matter while 10% are present in the posterior fossa. PCNSL is characterized by varied nonspecific neurologic deficits. The most common and early presenting manifestations are mental change, somnolence and paresis. Nearly 90% of cases with PCNSL have hyperdense or isodense lesions in CT brains and hypointense or isointense in T1-weighted MRI images. Only 40% have hyperintense lesions in T2-wighted images with mild edema and mass effect. Ring enhancement is more frequent in solitary lesions but meningeal enhancements is rare[35].

The atypical clinical and radiological presentations of MS may sometimes pose a considerable diagnostic difficulty. However and in spite of the aggressive course of its tumefactive variant, good prognosis may be seen in some patients.

ACKNOWLEDGMENTS

I would like to thank the patient and her mother for their approval to publish the results of this case presentation.

COMMENTS

Case characteristics

A 25-year-old woman with history of acute dementia and blindness.

Clinical diagnosis

The patient had marked cognitive impairment, dysphasia, reduction in visual acuity and temporal pallor of the optic discs.

Differential diagnosis

Other variants of multiple sclerosis (MS) as ADEM, Marburg's MS and Balo concentric sclerosis; central nervous system (CNS) vasculitis; intracranial infection/abscess/granuloma and neoplastic lesions as primary CNS lymphoma.

Imaging diagnosis

Magnetic resonance imaging-brain showed multifocal large ($\geqslant$ 3 cm) white-matter hypointense lesions in T1W and hyperintense in T2W and fluid-attenuated inversion recovery images and patchy enhancement.

Pathological diagnosis

A demyelinating disease.

Treatment

Intravenous methylprednisolone in a dose of 1 g/d for 5 d was administered for two consecutive months followed by oral prednisolone in a dose of 80 mg twice daily was given for 1 mo. As no clinical improvement was observed, another dose of pulse steroid therapy was given which was also followed by 80 mg twice daily oral prednisolone for 9 mo followed by tapering of the steroid dose over the next year.

Related reports

Usually acute treatments of tumefactive MS include intravenous methylprednisolone and/or plasma exchange, galatiramer acetate, mitoxantrone and natalizumab followed by immunomodulatory agents as IFNβ-1a/b.

Term explanation

MS is a chronic immunologic disease in which inflammation, demyelination and axonal damage are the main pathologic features.

Experiences and lessons

In this case report, in spite of the fulminant presentation, good prognosis was seen with corticosteroids.

Peer-review

Interesting article on the scientific and practical level, text is well wrote and easily comprehensible with clear figures.

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CASE REPORT

Intestinal type gastric adenocarcinoma with unusual synchronous metastases to the colorectum and bladder

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Author contributions: Seow-En I performed the literature review wrote the draft manuscript; Seow-Choen F performed the surgery and critically revised the manuscript; all authors read and approved the final manuscript.

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Informed consent: The patient provided written consent prior to study enrolment.
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Abstract

A 75-year-old male presented with difficult defecation and increasing urinary frequency over a few months. He had a significant history of previous partial gastrectomy for gastric carcinoma 20 years prior. Computed tomography of the abdomen and pelvis showed extensive lymphadenopathy, a gastric mass and rectal as well as bladder wall thickening with bilateral ureterohydronephrosis. Normal looking serosal surfaces of the bladder and bowel were seen on laparoscopy and a defunctioning ileostomy was created. Gastroscopy revealed a malignant mass while cystoscopy and sigmoidscopy found extensive tumour growth lining the mucosal surfaces. Biopsies from all sites were compatible with intestinal type adenocarcinoma of gastric origin with few signet ring cells. Metabolic response to palliative chemotherapy was good and the patient's symptoms have improved on follow-up four months post ileostomy. We discuss the immunohistochemical profile of the tumour and review the literature.

Key words: Gastric adenocarcinoma; Intestinal-type; Metastasis; Colorectum; Bladder

Core tip: Although exceedingly rare, metastases to the colorectum and bladder can occur with primary gastric adenocarcinoma. Unusual sites of spread are more often associated with diffuse type or signet ring cell gastric carcinoma but can occur with intestinal type as well. Site specific symptoms should alert the clinician to the possible locations of spread so as to allow prompt diagnosis. CK7 and CK20 profiles may help to establish the origin of the metastatic tumour if in doubt.

Seow-En I, Seow-Choen F. Intestinal type gastric adenocarcinoma with unusual synchronous metastases to the colorectum and bladder. *World J Clin Cases* 2015; 3(6): 533-537 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i6/533.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i6.533

INTRODUCTION

Gastric adenocarcinoma commonly spreads to the peritoneum, liver, lungs and intra-abdominal lymph nodes. Multiple as well as unusual sites of metastases have been described more often with diffuse type or signet ring cell gastric cancer. We describe the first case of intestinal type gastric adenocarcinoma with few signet ring cells presenting with synchronous colorectal and vesical metastases. Laparoscopic findings were also unusual as the serosal surfaces looked grossly normal but extensive intraluminal masses were found on endoscopy. Metabolic response to palliative chemotherapy was good and the patient's symptoms have improved on follow-up four months post defunctioning ileostomy.

CASE REPORT

A 75-year-old Indonesian male presented to our clinic with the complaints of difficult defaecation and increasing frequency of micturition for a few months' duration. He had a history of previous partial gastrectomy for gastric cancer in Indonesia more than 20 years prior. Full histology from the initial surgery was unavailable. Abdominal examination was unremarkable apart from mild abdominal distension. On rectal examination, extra-anal nodular masses were felt causing tight stricturing of the anal canal extending proximally. The anal mucosa felt intact.

Serum CEA and CA 19-9 were markedly elevated at 31.6 μg/L (normal 0-5 μg/L) and 30523 U/mL (normal 0-37 U/mL) respectively. AFP level was normal. Contrast enhanced computed tomography of the abdomen and pelvis revealed extensive retroperitoneal lymphadenopathy including para-aortic and aortocaval nodes as well as bilateral iliac lymphadenopathy. In addition a gastric mass was suspected. Rectal wall thickening causing luminal constriction and bladder wall thickening with bilateral ureterohydronephrosis were noted. The liver appeared normal.

During diagnostic laparoscopy, the transverse colon down to the visualised rectum was found to be thickened and rigid. The bladder appeared normal externally (Figure 1) but was thickened with intravesical masses palpable *via* laparoscopic manipulation. The appendices epiploicae of the transverse colon and the median umbilical ligament also appeared thickened (Figure 1) and biopsies were taken from both sites. Moderate greenish ascites was noted. No peritoneal or serosal nodules were seen and the small intestine and stomach were grossly unremarkable on laparoscopy. The previous gastrojejunostomy anastamosis site looked normal. A defunctioning ileostomy was created to alleviate intestinal obstruction.

As no definite intra-abdominal masses were found on laparoscopy, oesophago-gastroduodenoscopy (OGD) and sigmoidoscopy were performed at the same sitting. OGD showed a mass at the lesser curve of the stomach and sigmoidoscopy revealed anal stricturing and extensive tumour lining the rectal mucosa (Figure 1). The scope was unable to pass beyond 8 cm from the anal verge due to the tight intraluminal growths. A cystoscopy was done which showed the entire bladder mucosa to be covered by mucosal tumour growth.

Biopsies from all sites including the gastric, rectal and bladder tumours as well as the appendices epiploicae and median umbilical ligament were compatible with adenocarcinoma of gastric origin (Figure 2). This was of intestinal type based on both the Lauren as well as world health organization (WHO) classification. In view of the length of time between the patients's initial gastric tumour and the current primary lesion occurring in the remnant stomach it was not felt to be a recurrence. Immunohistochemical studies revealed the tumour cells to be positive for CK7, CK19 and CDX2 while negative for CK20 and CK5 (Figure 3). Mucicarmine staining showed intracytoplasmic mucin positivity in only few tumour cells (Figure 4). Uroplakin and HER2/neu were negative. *KRAS* and *BRAF* gene mutations were not detected. No features of a second primary tumour were found.

F-18 fluorodeoxyglucose positron emission tomography coupled with a multi-slice low-dose computed tomography scan (FDG PET/CT) was performed with FDG uptake at the site of gastric tumour and retroperitoneal lymph nodes. Additionally, hypermetabolic para-oesophageal, anterior mediastinal, paratracheal and left supraclavicular lymph nodes were seen. There were also foci of increased FDG uptake in the T2 and T3 vertebrae consistent with bone metastasis. There was no significant FDG uptake seen in the liver.

The patient underwent initial chemotherapy with 5-FU and bevacizumab. Three further cycles of chemotherapy was given using 5-FU, bevacizumab and oxaliplatin with paclitaxel added to the second cycle.

FDG PET/CT prior to the third cycle showed good metabolic response with interval decreased FDG uptake in the stomach. Previously noted FDG avid nodes in the neck, thorax, abdomen and pelvis all showed interval resolution, metabolic resolution or decreased uptake. Interval resolution was also observed in T2 and T3 vertebrae. Serum CA 19-9 also came down to 3900 U/mL.

The patient showed clinical improvement with resolution of urinary symptoms and decrease in abdominal distension with good ileostomy output 4 mo from surgery at the time of writing. He has been on regular follow-up and is planned for further chemotherapy.

DISCUSSION

Gastric adenocarcinoma is the fourth most common cancer worldwide, accounting for 8% of the 12.7 million new cases of cancer diagnosed in 2008[1]. More than 50% of these patients present with unresectable locally

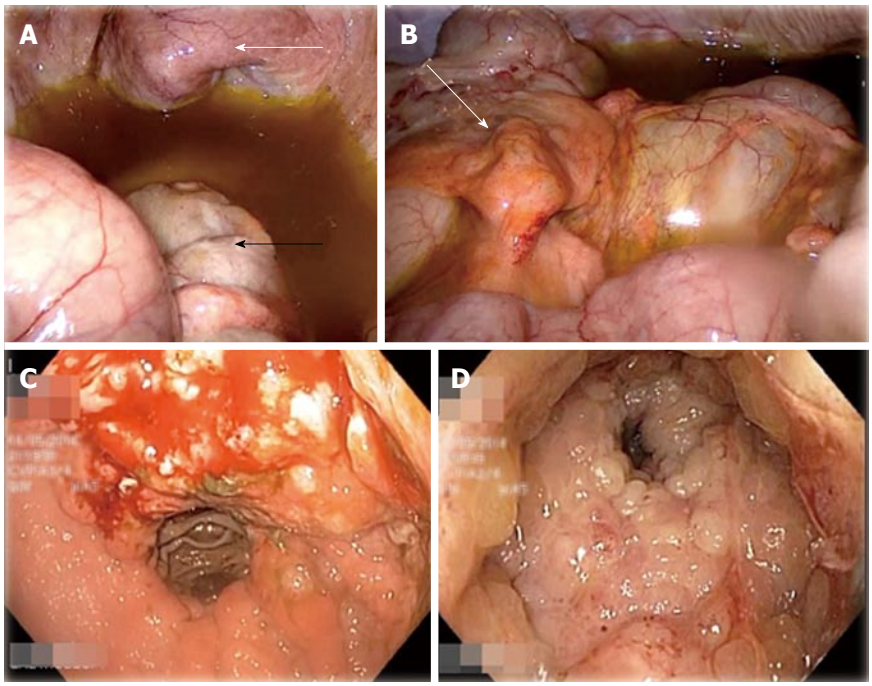

Figure 1 Laparoscopy examination. A: Laparoscopic view of the pelvic organs; Bladder (white arrow) and rectum (black arrow) look grossly normal. Moderate amount of greenish ascites seen; B: Transverse colon serosa normal looking except for thickened appendices epiploicae (white arrow); C: Gastric tumour seen at lesser curve on EGD; D: Extensive circumferential rectal mucosal tumour with luminal narrowing.

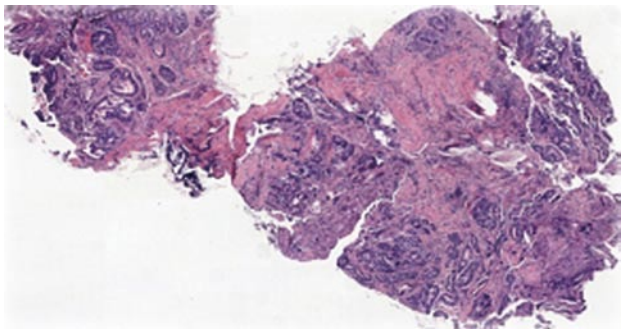

Figure 2 Gastric biopsy showing intestinal type gastric adenocarcinoma.

advanced or metastatic cancer[2], and out of those resected with curative intent, 40%-65% of cancers will recur[3]. Seventy-nine percent of recurrences occur within the first 2 years of resection, 94% within 4 years and rarely after[4].

Patterns of gastric metastasis have been well studied. The most common sites of metastasis include the liver due to portal drainage, the lungs due to lymphatic drainage, the peritoneum, and the intra-abdominal lymph nodes. Reports of spread to colonic or bladder mucosa have been rare in literature. 39 cases of gastric linitis plastica with metastases to the colon were described from 1936 to 1989; the bladder was involved in three cases[5]. To our knowledge, only four cases of gastric cancer with colorectal metastases[6-9] and five cases with vesical metastases[10-13] were described in the English language literature in the past 10 years. No other reports of concomitant colorectal and vesical metastases were found.

Six out of these nine recent cases of gastric adenocarcinoma with colorectal or vesical metastases were poorly differentiated diffuse type based on the Lauren classification with predominant signet ring cells. Two of the remaining cases were well differentiated intestinal type[6,10], while the last was an undifferentiated type with signet ring and intestinal differentiation[8]. Diffuse type adenocarcinoma have been shown to have an increased propensity for disseminated spread to multiple organs, in particular to the peritoneum, while intestinal type carcinoma more frequently spreads to the liver. Frequency of abdominal lymph node involvement was similar for both types[14]. In a 2001 radiological study of 23 patients with intestinal metastases from gastric adenocarcinoma, 70% were linitis plastica and 74% were poorly differentiated or signet ring cell type[15]. It is clear that an association exists between linitis plastica/diffuse type or signet ring type histology and unusual sites of metastases.

This report is the first described with synchronous colorectal and vesical metastases from intestinal type gastric adenocarcinoma. Few signet ring cells were present in the histological specimens and less than the required 50% to be labelled as signet ring carcinoma as per the WHO classification. This case was also unusual as the rectal and bladder metastases were only obvious intraluminally and initially suggestive of *de novo* carcinoma. While the serosal biopsies were positive for cancer, no obvious masses were visible on the serosal aspect during laparoscopy.

Immunohistochemical analysis can be useful in establishing location of the primary tumour if in doubt. In this case the $CK7^{+}/CK20^{-}$ profile of the tumour was

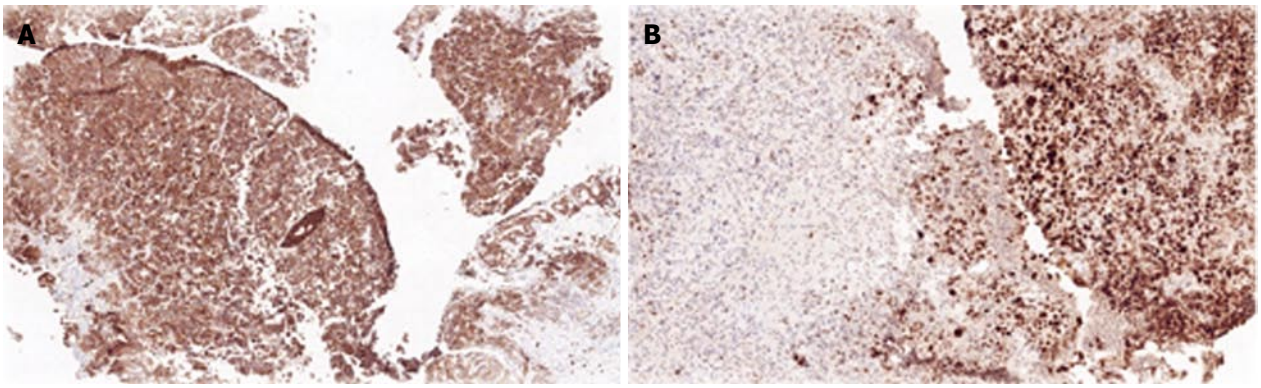

Figure 3 CK7 with strong cytoplasmic positivity (A) and CDX2 with diffuse and strong nuclear positivity (B).

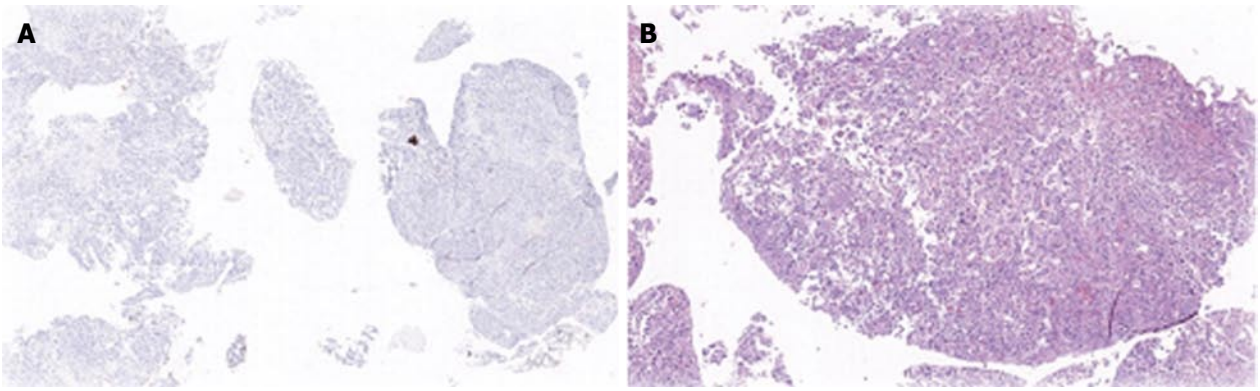

Figure 4 CK20 negative (A) and few signet ring cells seen with mucicarmine staining (B).

indicative of a primary gastric carcinoma as opposed to a colorectal origin, with more than 90% of rectal primaries expressing CK20[16]. CDX2 is a sensitive and specific marker for adenocarcinoma of the gastrointestinal tract, and is associated with both gastric intestinal metaplasia and intestinal type gastric carcinoma. CDX2 positive gastric adenocarcinoma have been demonstrated to have better outcomes and prognosis as compared to CDX2 negative tumours[17]. A $CK7^{+}/CK20^{-}/CDX2^{+}$ profile was compatible with adenocarcinoma of pancreaticobiliary origin as well; this was ruled out by PET/CT.

In conclusion, albeit very rare, colorectal and vesical metastases can occur from a primary gastric adenocarcinoma. Although previously more significantly associated with linitis plastica, diffuse type or signet ring cell carcinoma, multiple sites of metastases to unusual locations can also occur with intestinal differentiation. Further studies can be done to shed light on the mechanism of such spread and may likely be derived only from analyses of isolated case reports. Site specific symptoms should alert the clinician to the possible locations of spread so as to allow prompt diagnosis and appropriate treatment.

COMMENTS

Case characteristics

A 75-year-old male presented with difficult defecation and increasing urinary frequency over a few months.

Clinical diagnosis

Rectal examination revealed nodular masses causing tight stricturing of the anal canal while endoscopy showed a gastric mass and intravesical tumour growth.

Differential diagnosis

Metastatic gastric adenocarcinoma *vs de novo* rectal or bladder carcinoma.

Laboratory diagnosis

Serum CEA and CA 19-9 were markedly elevated at 31.6 μg/L (normal 0-5 μg/L) and 30523 U/mL (normal 0-37 U/mL) respectively.

Imaging diagnosis

Computed tomography showed a gastric mass, rectal and bladder thickening as well as extensive lymphadenopathy.

Pathological diagnosis

Histological analyses of all biopsy specimens were compatible with metastatic gastric adenocarcinoma of intestinal type differentiation based on Lauren classification.

Treatment

A defunctioning ileostomy was performed to relieve impending obstruction and the patient underwent palliative chemotherapy with good response.

Related reports

Incidences in literature of colorectal or bladder metastases from a primary gastric adenocarcinoma are rare. Most reported cases are associated with diffuse type adenocarcinoma or linitis plastica rather than intestinal type.

Term explanation

Cytokeratins are intermediate filament proteins found in normal epithelium, which have preserved expression in neoplastic cells. This allows the detection of specific cytokeratins to be useful tools in determining the origin of metastatic carcinoma.

Experiences and lessons

Multiple sites of metastases to unusual locations can occur with intestinal type gastric adenocarcinoma and site specific symptoms should alert the clinician to

the possible locations of spread.

Peer-review

It is a very unsual case of gastric Ca. with long time distal relapse in colon, bladder and huge abdominal lymph nodes. Specially remarkable is that primary tumor ocurred 20 year ago.

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CASE REPORT

Primary signet ring cell carcinoma of the appendix: A rare case report

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Author contributions: Kulkarni RV and Siddiqui S prepared the manuscript; Ingle SB critically revised the intellectual content and gave final approval of manuscript.

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Abstract

Primary adenocarcinoma of the appendix is a rare malignancythat constitutes < 0.5% of all gastrointestinalneoplasms. Moreover, primary signet ring cell carcinomaof the appendix is an exceedingly rare entity. In the present report, we describe a rare case of primary signet ring cell carcinoma of the appendix with ovarian metastasesand unresectable peritoneal dissemination occurring in a 45-year-old female patient. She was clinically misdiagnosed as torsion of ovarian cyst. She underwent appendicectomy and unilateral salpingo-oophorectomy.Histopathology revealed signet ring cell carcinoma and a right hemicolectomy was done. She then received palliative systemic chemotherapy with 12 cycles of oxaliplatin, 5-fluorouracil, and leucovorin (FOLFOX-4). The patient is doing well till today on follow up without progression of disease 10 mo after beginning chemotherapy.

Key words: Appendix; Primary; Signet ring cell carcinoma; Ovarian metastasis

Core tip: Meticulous histopathological evaluation along with clinical correlation is strongly recommended in such unusual circumstances. The clinician and surgical pathologist should keep in mind this rare entity as a differential diagnosis.

Kulkarni RV, Ingle SB, Siddiqui S. Primary signet ring cell carcinoma of the appendix: A rare case report. *World J Clin Cases* 2015; 3(6): 538-541 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i6/538.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i6.538

INTRODUCTION

Primary adenocarcinoma of the appendix, first invented in 1882, and constitutes 0.12 cases per one million

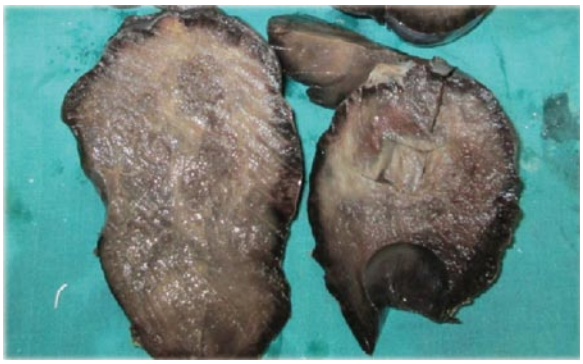

Figure 1 Showing twisted ovarian cyst.

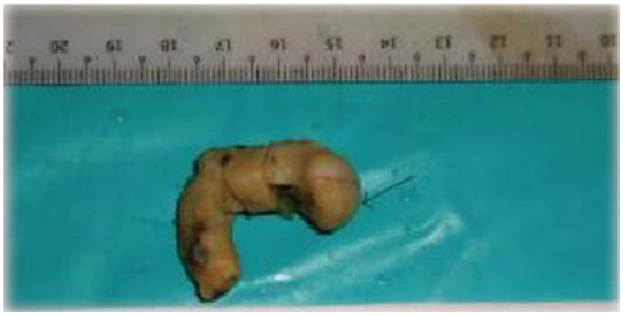

Figure 2 Showing appendix.

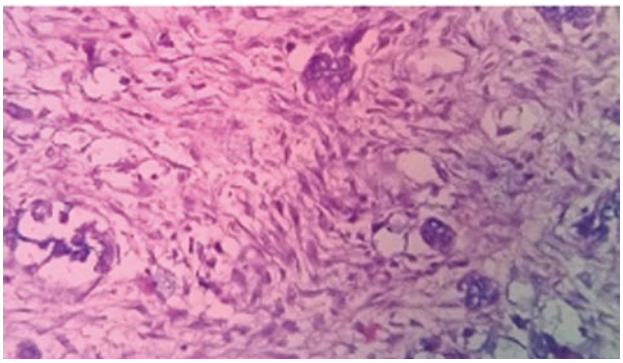

Figure 3 Signet ring cells in left ovary.

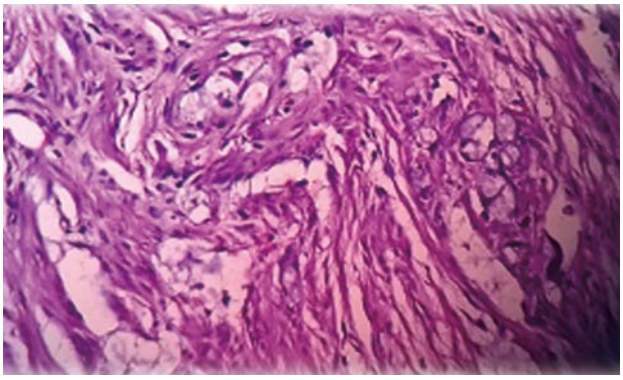

Figure 4 Showing signet ring cells in the appendix.

people per year[1]. Primary signet ring cell carcinoma is an extremely unusual event in surgical practice[2]. Clinically it simulates acute appendicitis and difficult to distinguish from it[3,4]. So, it is a difficult task to diagnose it on clinical grounds. Usually the diagnosis is confirmed on histopathology of a surgically-removed inflamed appendix[5].

CASE REPORT

A 46-year-old female admitted in YCR Hospital Latur with persistent right lower quadrant abdominal pain. Baseline blood tests showed neutrophilic leukocytosis. Per abdominal examination revealed right abdominal distension. The patient was misdiagnosed as twisted ovarian cyst and emergency laparotomy was planned and performed. At laparotomy, the appendix appeared severely inflamed so the patient underwent an appendectomy and unilateral salphingo-oopherectomy. On gross examination, left large encapsulated, dark brown and smooth ovarian mass measuring 16 cm × 13 cm × 9.5 cm was found. The cut surface showed large hematoma with grey white visible areas. Attached fallopian tube measured 5 cm in length (Figure 1).

Appendix measured 6 cm in length. External surface was thickened and congested. Cut section showed obliterated lumen filled with gelatinous material (Figure 2). Microscopic examination of both left ovarian mass revealed signet ring cells showing an intracellular mucin to vacuolated cytoplasm shifting the nuclei towards periphery (Figure 3). Some extracellular mucin was seen infiltrating the stroma.Large areas of hemorrhage and necrosis are noted. In appendix the signet ring cells and extracellular mucin was seen invading the mucosa, submucosa and muscularis propia (Figure 4). Thus the case was finally diagnosed as infiltrating adenocarcinoma of the appendix with "signet ring cells" differentiation, with signs of infiltration of peri-appendicular fat. The tumor cells were immunopositive for CEA, cytokeratin 20 (Figure 5), MUC2, and CDX-2 (Figure 6). The patient underwent colonoscopy for the evaluation of synchronous disease, which was negative. Later computed tomography (CT) scan was done and an 8 cm × 5 cm lesion in right adnexal region was noted so the patient subsequently underwent right hemicolectomy with total abdominal hysterectomy and unilateral salphingo-oopherectomy and no residual carcinoma with negative lymph nodes was found.

DISCUSSION

Appendiceal adenocarcinoma is an unusual malignancy[1]. The reported prevalence rate is 0.3%[6]. Signet ring carcinoma constitutes only 4% of all neoplasms of appendix[2]. Malignant carcinoids are mainly found in younger age group (mean age, 38 years)[1]. The mean age of occurrence of mucinous adenocarcinoma is 60 years, while that of signet ring cell carcinoma is 62 years with male: female ratio 1:1[1].

According to International Classification of Diseases

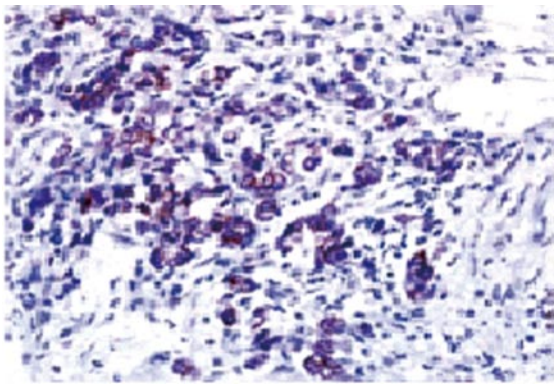

Figure 5 CK20 positive tumor cells.

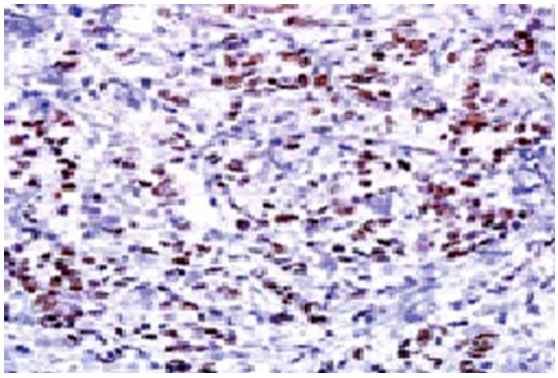

Figure 6 Presentations of CDX positivity by tumor cells.

for Oncology appendiceal tumors are divided in to five classes: Mucinous adenocarcinoma, colonic type adenocarcinoma, goblet cell carcinoma, signet ring cell carcinoma and malignant carcinoid/adenocarcinoid[1,7]. Adenomas are the premalignant lesions[8,9]. The signet ring cell carcinomas are usually frequent in the stomach and intestine. In our case, the tumor cells were immunopositive for cytokeratin 20, CDX-2, MUC-2, and CEA. The CDX-2 marker is the key to confirm the final diagnosis[10].

Most of them are clinically low-grade tumors with indolent behavior. The overall 5-year survival rate is 20.5%. As per previous workers except for signet ring cell carcinoma and malignant characinoid, the histopathological variant does not affect the survival rate[11]. In fact the extent of the disease at the time of diagnosis is an important determinant of prognosis of patient. As per previous studies, the prognosis of patients with diffuse, peritoneal metastases is worse, with a 5-year survival rate of 6.7%-14%[12,13]. However, according to some workers signet ring cell carcinoma and poorly differentiated adenocarcinoma of the appendix had high propensity for development of metastasis with a 5-year survival rate of only 7%. So, signet ring cell carcinoma is considered as a separate tumor type in the appendix in view of its poor prognosis. Right hemicolectomy is the treatment of choice for all microscopic types of appendiceal carcinoma, even in cases with perforation. In case of metastasis treatment modalities are systemic chemotherapy along with intraoperative intraperitoneal chemotherapy, peritonectomy and cytoreductive surgery[1,5].

To conclude, meticulous histopathological examination of appendix is mandatory during exploratory laparotomy for ovarian masses and for diagnostic procedure. Exclusion of signet ring cell carcinoma from other carcinoma subtypes is of particular importance as it has an extremely poor prognosis and is usually diagnosed in advanced stages.

COMMENTS

Case characteristics

A 45-year-old woman presented to the Emergency Department of YCR Hospital Latur with persistent right lower quadrant abdominal pain.

Clinical diagnosis

Clinically diagnosed as twisted ovarian cyst.

Differential diagnosis

Twisted ovarian cyst was the clinical differential diagnosis and signet ring carcinoma of either colon, stomach or appendix were the histological differential diagnosis.

Laboratory diagnosis

Primary signet ring carcinoma of appendix with secondaries in the ovaries.

Imaging diagnosis

Computed tomography (CT) scan was done and an 8 cm × 5 cm lesion in right adnexal region was noted so the patient subsequently underwent right hemicolectomy with total abdominal hysterectomy and unilateral salphingo-oopherectomy.

Pathological diagnosis

Primary signet ring carcinoma of appendix with secondaries in the ovaries.

Treatment

Right hemicolectomy.She then received palliative systemic chemotherapy with 12 cycles of oxaliplatin, 5-fluorouracil, and leucovorin (FOLFOX-4). The patient is doing well till today on follow up without progression of disease 10 mo after beginning chemotherapy.

Experiences and lessons

Meticulous histopathological examination of appendix is mandatory during exploratory laparotomy for ovarian masses and for diagnostic procedure.

Peer-review

The authors have performed a good study, the manuscript is interesting.

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EDITORIAL

Where is hidden the ghost in phantom sensations?

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Abstract

The term phantom sensations (PS) refers to sensations in a missing body part. They are almost universal in amputees and can be both painful and not painful. Although PS have been frequently described in limb amputees, they can also occur in other clinical conditions and several pathophysiological interpretations have been proposed, with a predominance of theories based on a central origin. Actually, different mechanisms are able to create a phantom sensation. After an amputation, PS are frequently generated by the genesis of ectopic action potentials in the interrupted nerve fibers but the PS generator can also be more proximal. Sometimes PS are not created by the stimulation of somatosensory fibers with a missing territory, but they can be the result of central sensitization or neuroplastic changes that allow for the convergence of impulses coming from different body parts (referred sensations), one of which is missing. In conclusion, PS can be generated by both neuropathic and non-neuropathic mechanisms developed in the amputated body part or in other parts of the nervous system. Since these mechanisms are not pathognomonic of amputation there are no hidden ghosts to look for in phantom sensations. The only interpretative rule is just to follow the pathophysiological principles.

Key words: Phantom sensations; Phantom pain; Neuropathic pain; Referred pain; Pain pathophysiology

Core tip: The term phantom sensations (PS) refers to sensations in a missing body part. They are almost universal in amputees and can be both painful and not painful. Several pathophysiological interpretations have been proposed, with a predominance of theories based on a central origin. Actually, PS can be generated by both neuropathic (ectopic) and non-neuropathic (referred) mechanisms developed in the amputated body part or in other parts of the nervous system. Since these mechanisms are not pathognomonic of amputation there are no hidden ghosts to look for in phantom sensations. The only interpretative rule is just to follow the pathophysiological principles.

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The term phantom sensations (PS) refers to sensations in a missing body part, phenomena that obviously appear paradoxical (but also intriguing) for the most part of the people. A literary example is in the very famous book "Moby Dick, or the whale" (1851) by Herman Melville, who describes in a short sentence the PS of the Captain Achab who had a leg amputated by the whale: "here is only one distinct leg to the eye, yet two to the soul".

PS are almost universal in amputees and can be both painful and not painful[1-3]. More precisely, patients can describe their PS in several ways, according to the anatomical and pathophysiological characteristics of the amputation: burning[4], tingling[3] or painful[3] sensations, illusory limb movement[5], visual hallucinations[6], and so forth.

Although PS have been frequently described in limb amputees, they can also occur in other clinical conditions such as after orchiectomy[7], mastectomy[8], tooth' s root canal treatment[9], penis amputation[10], ocular evisceration or enucleation[6].

Several pathophysiological interpretations have been proposed for PS, with a predominance of theories based on a central origin, including psychiatric explanations[11]. Actually, different mechanisms (neuropathic or non-neuropathic) are able to create a phantom sensation in a missing body part.

It is largely accepted that any neuropathic mechanism is characterized by the ectopic generation of action potentials in somatosensory afferent fibers[12]. In amputees, neuropathic pain mechanisms of PS can be localized at the level of amputation or more proximally. Sometimes they are strictly linked to the amputation, sometimes not.

After an amputation, PS are frequently generated by the genesis of ectopic action potentials in the interrupted nerve fibers, as demonstrated by human microneurographic recordings[13].

Nevertheless, several studies suggested that the PS generator can be proximal to the amputation site. On a pathophysiological point of view, this is not at all strange. In physiology, it is well known that the direct (ectopic) stimulation of a sensory nerve fiber induces a sensation localized in the territory of the stimulated fiber, *i.e.*, in the body part where the receptors are located. When Penfield and Rasmussen described for the first time the sensory homunculus, they reported patients' sensations evoked in different body parts during the electrical stimulation of the somatosensory cortex[14]. All that considered, when the territory of the stimulated nerve fibers is missing, the adequate ectopic stimulation of somatosensory nerve fibers always creates a phantom sensation, wherever the stimulation is applied.

Several examples can be given. For instance, in a recent paper, selective peripheral nerve blockades suggested a major role played by dorsal root ganglia in the generation of PS in a group of amputees[15].

Very interesting is also the recently described case of a patient with an old hip disarticulation amputation due to a malignant sarcoma[16]. After 1.5 years from amputation, this patient started to complain a severe phantom limb pain, mainly localized at the right phantom thigh. Computed tomography and magnetic resonance imaging showed the presence of a metastatic spinal mass involving the L3 vertebra with stenosis of the right lateral recess. Importantly, the resection of the vertebral mass completely resolved the phantom limb pain, demonstrating that the pain generator was in the sensory nerve fibers compressed at the lateral recess of the lumbar spine and not at the site of amputation.

Moving proximally in the central nervous system, the electrical stimulation of the thalamus during functional stereotactic mapping constantly evoked various PS, including pain, in a group of amputees[17].

Sometimes PS are not created by the stimulation of somatosensory fibers with a missing territory, but they can be the result of central sensitization or neuroplastic changes that allow for the convergence of impulses coming from different body parts (referred sensations), one of which is missing. All that happens because the conscious representation of the body lies in the activation of one or more parts of the sensory cortex, independently from what really occurs in the periphery. This seems to be clearly confirmed in patients with arm amputation where the stimulation of face and trunk can evoke a phantom sensation. Since face and trunk are close to the hand in the cortical representation of the human body, the interpretation for this referred sensation was again the change of cortical representation after the amputation[18].

On a neurobiological point of view, great importance has been attributed to the rearrangement of the central nervous system in response to the loss of inputs coming from the periphery[19], but it is important to underline that any injury can induce a change in the cortical body representation, independently from PS occurrence[20].

It is also worth highlighting that referred sensations are not neuropathic per se and can also be observed in healthy subjects, although only in special situations[21]. Since the beginnings of the 20th century it was clear that a painful sensation can be complained in a part of the body as a consequence of a disease in another[22]. This is confirmed by several studies on experimental pain demonstrating how the intense stimulation of some tissues is able to evoke a painful sensation not only in the stimulation site, but also at a distance from it[23,24]. Nowadays referred pain is truly considered a rather common complaint in several clinical conditions.

Moreover, the (traumatic) amputation of a body part is not necessary for the development of PS as demonstrated by the evidence that patients with congenital limb absence can experience PS after minor trauma or minor surgery[25,26].

Interestingly, cortical representation changes can also explain other quasi-phantom phenomena. For instance, in patients with complete spinal cord injury the stimulation of body parts above the lesion can evoke a

sensation below the injury level[27].

All that considered, referred sensations can thus represent an additional pathophysiological basis of PS in amputees.

In conclusion, PS can be generated by both neuropathic and non-neuropathic mechanisms developed in the amputated body part or in other parts of the nervous system.

Since these mechanisms are not pathognomonic of amputation there are no hidden ghosts to look for in phantom sensations. The only interpretative rule is just to follow the pathophysiological principles. In this respect, since PS are generally very stressful for patients, according to Sherman[4], physicians have an important role in easing the patients' suffering by educating them about the PS pathophysiology in order to explain that their sensations are not so strange as they appear.

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REVIEW

Treatment strategies for multiple sclerosis: When to start, when to change, when to stop?

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory condition of the central nervous system determined by a presumed autoimmune process mainly directed against myelin components but also involving axons and neurons. Acute demyelination shows as clinical relapses that may fully or partially resolve, while chronic demyelination and neuroaxonal injury lead to persistent and irreversible neurological symptoms, often progressing over time. Currently approved disease-modifying therapies are immunomodulatory or immunosuppressive drugs that significantly although variably reduce the frequency of attacks of the relapsing forms of the disease. However, they have limited efficacy in preventing the transition to the progressive phase of MS and are of no benefit after it has started. It is therefore likely that the potential advantage of a given treatment is condensed in a relatively limited window of opportunity for each patient, depending on individual characteristics and disease stage, most frequently but not necessarily in the early phase of the disease. In addition, a sizable proportion of patients with MS may have a very mild clinical course not requiring a disease-modifying therapy. Finally, individual response to existing therapies for MS varies significantly across subjects and the risk of serious adverse events remains an issue, particularly for the newest agents. The present review is aimed at critically describing current treatment strategies for MS with a particular focus on the decision of starting, switching and stopping commercially available immunomodulatory and immunosuppressive therapies.

Key words: Multiple sclerosis; Disease-modifying therapy; Treatment start; Treatment switch; Treatment stop; Interferon beta; Glatiramer acetate; Azathioprine; Natalizumab; Fingolimod

Core tip: Disease-modifying therapies for multiple sclerosis (MS) modulate or suppress with different mechanisms the autoimmune process that underlies the

disease. Patients with relapsing MS may benefit from treatment but individual response to a given therapy and adverse events occurrence are largely unpredictable and many cases need to change several drugs to stabilize their disease. Nevertheless, a high proportion of patients evolve to a progressive phase, which is not responsive to any existing therapy. As opposed, some cases have a benign course without treatment. A critical review of strategies for starting, switching and stopping disease-modifying therapies for MS is here presented.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic neurological disease of unknown cause sustained by a widespread inflammatory process within the central nervous system (CNS) leading to multifocal demyelination and axonal loss mostly in the white matter but importantly also in the grey matter of both brain and spinal cord[1]. Clinical manifestations are heterogeneous depending on the anatomical location of inflammatory lesions, and are expression of acute demyelination which can fully or partially resolve, of chronic demyelination and neuroaxonal injury, that are generally irreversible, or both. Based on the predominance of episodic acute demyelinating events or of the chronic neurodegenerative process, the clinical course is defined either relapsing-remitting, which represents around 60% of prevalent cases, or progressive (primary if progression starts from onset or secondary if it begins after a preceding relapsing-remitting phase). About 10% of MS cases have a primary progressive (PP) course, while transition to the secondary progressive (SP) phase occurs in around half of RR MS patients, generally decades after clinical onset. An initial acute episode of neurological disturbance that is suggestive of MS but does not fulfill diagnostic criteria is defined clinically isolated syndrome (CIS), which is the typical presentation of relapsing forms of MS, although many patients may remain asymptomatic and free of disease-defining brain/spinal cord MRI activity for several years after a CIS has occurred[2,3].

MS predominantly affects young adults of female sex (female to male ratio 2.5:1 or greater), although the disease may begin in children and subjects over the age of 60. Caucasians are more frequently affected and the prevalence of the condition varies profoundly across different areas of the world, roughly following an increasing gradient from the equatorial zone - where it is below 5 cases per 100000 inhabitants - to the poles, reaching rates over 130 cases/100000 in several regions of Northern America, Europe and Australia[4-6]. Epidemiological studies indicate that genetic susceptibility, infections (particularly Epstein-Barr virus), reduced sun light exposure/blood levels of vitamin D, cigarette smoking, obesity, and increased dietary salt intake are risk factors for developing the disease but have not yet a completely established causative role[7]. Although the etiology of MS remains unknown, there is strong biological evidence of an autoimmune pathogenesis sustained by migration of peripheral T and B cells - reactive against one or more unidentified myelin or neuronal antigens - into the CNS, in which lymphocytes induce and maintain inflammation also through persistent microglia activation among other mechanisms that cause demyelination, axonal loss, and ultimately neuronal death[8].

Currently disease-modifying therapies (DMTs) for MS approved by the European Medicine Agency (EMA) and Food and Drug Administration (FDA) include interferon beta (IFNB) 1-a and 1-b, glatiramer acetate (GA), mitoxantrone, natalizumab, fingolimod, teriflunomide, dimethyl fumarate, and alemtuzumab. In addition, azathioprine and cyclophosphamide are used off-label or approved in some countries for MS treatment as a consolidated indication not initially registered (Table 1). Also methotrexate and rituximab are used as an off-label option in some cases. All mentioned agents act by modulating and/or suppressing the immune system at various levels and with different mechanisms of action, the description of which is beyond the scope of this review[9]. As a general rule, available DMTs have a favorable impact on relapsing-remitting MS, while they have no significant benefit in progressive MS in which neurological disability continues to worsen over time[10]. Even in relapsing-remitting MS, the efficacy, tolerability and safety profile vary greatly across treatments, ranging from combinations of modest effect and excellent safety to options that are highly effective but at increased risk of serious adverse events, which may be fatal in rare cases[11]. These include but are not limited to: cardiomyopathy and acute leukemia after long-term treatment with mitoxantrone; natalizumab-associated progressive multifocal leukoencephalopathy (PML); bradyarrhythmias, macular edema, and varicella-zoster virus infections occurring with fingolimod therapy; autoimmune thyroiditis, thrombocytopenia, and glomerulonephritis induced by alemtuzumab. Ideally, optimal treatment responders should be free from relapses, disability worsening and adverse events, outcomes that are difficult to assess experimentally in the long term given the relatively short duration of clinical trials for a lifelong condition such as MS. As a consequence, surrogate outcomes - mainly represented by brain MRI measures - have been increasingly used in trials for the last 20 years to demonstrate the biological activity of MS therapies[12,13]. However, the precise correlation between short-term effect on MRI measures and long-term clinical changes remains to be fully elucidated[14-16]. In addition, MS may have an extremely

Table 1 Main characteristics of available disease-modifying therapies for multiple sclerosis

Agent	Indication and line of therapy	Dosage, route and frequency	Clinical efficacy in placebo-controlled phase III trials	Tolerability issues	Safety issues
Interferon beta 1b	RR MS; SP MS with relapses; CIS First line	250 mcg *s.c.* every other day	34% reduction of ARR over two years (RR MS) 50% risk reduction of conversion to CD MS at two years (CIS) No statistically significant effect on disability progression	Flu-like syndrome; injection site reactions	Hepatotoxicity; myelotoxicity; autoimmune thyroiditis; microangiopathy; epileptic seizures (rare)
Interferon beta 1a	RR MS; CIS First line	30 mcg *i.m.* once a week	18% reduction of ARR over two years (RR MS) 44% risk reduction of conversion to CD MS at two years (CIS) No statistically significant effect on disability progression	Same as above	Same as above
Interferon beta 1a	RR MS; CIS First line	44 mcg *s.c.* three times a week	32% reduction of ARR over two years (RR MS) 45% risk reduction of conversion to CD MS at two years (CIS) 30% reduction of progression of disability at two years (RR MS)	Same as above	Same as above
Peginterferon beta 1a	RR MS First line	125 mcg *s.c.* every two weeks	36% reduction of ARR over one year	Same as above	Same as above
Glatiramer acetate	RR MS; CIS First line	20 mg *s.c.* every day	29% reduction of ARR over two years (RRMS) 45% risk reduction of conversion to CDMS at three years (CIS) No statistically significant effect on disability progression	Injection site reactions; post-injection reaction (chest pain, flushing and dyspnea)	Cutaneous necrosis; anaphylaxis (rare)
Mitoxantrone	RR MS; SP MS; PR MS Second or third line	12 mg/m^2 *i.v.* every three months or 8 mg/m^2 *i.v.* every month	65% reduction of relapse risk over two years (mostly in RR MS)[98] 66% reduction of risk of disability progression at two years (mostly in RR MS)[98]	Nausea/vomiting; amenorrhea/infertility; alopecia; blue discoloration of sclera and urine	Infusion site tissue necrosis; myelotoxicity; infections; cardiotoxicity; acute leukemia
Natalizumab	RR MS Second line	300 mg *i.v.* every four weeks	68% reduction of ARR over two years 42% reduction of progression of disability at two years	Headache	Infusion associated reactions; anaphylaxis; infections; hepatotoxicity; progressive multifocal leukoencephalopathy
Fingolimod	RR MS Second line (first line in the United States)	0.5 mg per *os* every day	48%-54% reduction of ARR over two years 30% reduction of progression of disability at two years	Fatigue; headache	Bradyarrhythmias after first dose; lymphopenia; viral infections (VZV); macular edema; hepatotoxicity; hypertension
Teriflunomide	RR MS First line	14 mg per *os* every day	31%-36% reduction of ARR over one year or more 26%-32% reduction of progression of disability at one year or more	Nausea; diarrhea; alopecia	Myelotoxicity; hepatotoxicity; infections; peripheral neuropathy; pancreatic fibrosis; teratogenicity (requires accelerated elimination procedure)
Dimethyl fumarate	RR MS First line	240 mg per *os* twice a day	44%-53% reduction of ARR over two years 38% reduction of progression of disability at two years	Flushing; gastrointestinal symptoms; pruritus	Lymphopenia; progressive multifocal leukoencephalopathy
Alemtuzumab	RR MS Second or third line	12 mg/d *i.v.* for five days followed by 12 mg/d *i.v.* for three days one year after the first course	49%-55% reduction of ARR over two years compared to *s.c.* interferon beta 1a 42% reduction of progression of disability at two years compared to *s.c.* interferon beta 1a	Infusion associated reactions; myalgia; arthralgia; irregular menstruation	Infusion associated reactions; cytokine release syndrome; lymphopenia; infections; autoimmune thyroiditis; thrombocytopenic purpura; glomerulonephritis
Azathioprine[1]	MS of all types First or second line	2.5 mg/kg per *os* every day	23% relative risk reduction of the frequency of relapses over two years No statistically significant effect on disability progression at two and three years[98]	Gastrointestinal symptoms; photosensitivity; irregular menstruation/reduced fertility	Myelotoxicity; hepatotoxicity; lymphopenia; infections; acute pancreatitis; increased toxicity in subjects with thiopurine methyltransferase deficiency; malignancies (cumulative dose > 600 g)
Cyclophosphamide[1]	SP MS; PP MS Third line	1 g *i.v.* over three days or 500 mg *i.v.* over five days	No statistically significant effect on disability progression at two and three years[98]	Nausea/vomiting; amenorrhea/infertility; alopecia	Myelotoxicity; hepatotoxicity; infections; hemorrhagic cystitis; bladder cancer

[1]The use of these drugs for the treatment of multiple sclerosis is off-label in most countries. ARR: Annualized relapse rate; CD: Clinically definite; CIS: Clinically isolated syndrome; PP: Primary progressive; PR: Progressive-relapsing; RR: Relapsing-remitting; SP: Secondary progressive.

Table 2 Critical factors affecting the decision of starting disease-modifying therapies for multiple sclerosis

Factors suggesting not to start a DMT	CIS with favourable prognostic factors
	RR MS with no relapses in previous two years, no disability, and no evidence of MRI activity (potential "benign" case)
	Progressive forms of MS with no relapses or evidence of MRI activity
	Pregnancy planning
	High risk of low adherence to treatment
Factors suggesting to start a first line DMT	CIS with unfavourable prognostic factors
	RR MS with at least one relapse in previous two years but less than two relapses in the last year, low residual disability, and/or active MRI
Factors suggesting to start a second line DMT	RR MS with at least 2 disabling relapses in the last year
	Progressive forms of MS with relapses and/or active MRI

DMT: Disease-modifying therapy; CIS: Clinically isolated syndrome; MS: Multiple sclerosis.

variable clinical course both within and between subjects, who may show extremely active and break-through disease despite treatment or, on the contrary, very mild forms or phases not necessarily requiring a potentially harmful and costly pharmacological therapy[17].

Here we will discuss current and potential strategies to start, change and stop disease-modifying MS therapies in the clinical practice.

WHEN TO START TREATMENT FOR MS?

Primum non nocere

To avoid overtreatment, it is important to start on a DMT MS patients who carry the highest probability of optimal therapy response, making decisions based on multiple factors, including evidence of efficacy and safety profile of drugs, disease course and activity, expected adherence and preferences of the individual case (Table 2)[18-20]. Placebo-controlled randomized trials of IFNB and GA in patients with CIS have shown that active treatment significantly delays conversion to definite MS and prevent accumulation of new brain lesions on MRI[21-25]. However, there is little or no significant benefit of early *vs* delayed therapy on worsening of neurological disability in the open-label extension phase of these trials up to 10 years after study initiation[26-28].

Randomized trials of DMTs for relapsing-remitting MS included patients who had experienced at least one or two relapses in the previous one or two years prior to randomization and showed that all therapies significantly reduce relapse rate over 2-3 years of treatment with largely different effect size depending on the specific drug considered (Table 1)[29-45]. Comparisons between old and new drugs or between pivotal and recent trials are limited by the changed profiles of MS subjects enrolled in clinical trials who are now generally in earlier phases of disease and with much lower clinical and MRI activity compared to patients included in studies between 1988 and 2000[46].

When taking the decision of treating a patient with MS for the first time, clinicians choose either an escalation or an induction approach[10]. The first consists of starting with a first-line medication - intended as a moderate-efficacy high-safety drug - and switching to a second-line treatment (more effective but also with more safety risks) in case of unsatisfactory response to the first line: this is reasonable in most patients seen in the clinical practice who present with mildly or moderately active disease. The induction approach is the initial use of a highly effective second-line treatment in order to obtain the rapid remission of a very active disease, which justifies the risk of serious adverse events. This strategy is intended for MS cases with frequent (*i.e.*, two or more per year) and severe relapses who are at increased risk of rapid accumulation of disability.

IFNBs, GA, teriflunomide, and dimethyl fumarate are considered first-line therapies, while natalizumab, alemtuzumab, are mitoxantrone are second-line or third-line drugs. Fingolimod is approved as a second-line treatment in the EU and as first-line in the United States, Canada and other countries[47]. Azathioprine and cyclophosphamide, which are not registered for MS treatment, are used by clinicians as first-line and second-line medications, respectively. Among first-line drugs, differences exist in terms of efficacy and tolerability, although direct comparison data are limited. Existing evidence indicates that high dose IFNB (particularly IFNB 1-a 44 mcg subcutaneously three times a week) is more effective than low dose IFNB, *i.e.,* IFNB 1-a 30 mcg intramuscular once a week[48,49]. However, high dose IFNB and GA have similar efficacy on clinical parameters, while they slightly differ in terms of impact on MRI measures, that is greater for IFNB than GA, and tolerability profile[50-53]. There is less experience worldwide with dimethyl fumarate given its recent introduction to the market. One of the pivotal studies included a group of GA-treated patients as reference arm: MS subjects receiving the experimental drug or GA had similar statistically significant reductions of relapse rate, while differences in disability progression at 2 years were not significant, compared to placebo[42]. Teriflunomide has shown a similar efficacy to high dose IFNB and, as dimethyl fumarate, has the advantage of being an oral medication[54]. Recently, an independent comparative study has shown that azathioprine is not inferior to IFNBs in relapsing-remitting MS in terms of relapse rate and disability progression reduction, confirming the utility of an old and safe drug as a low cost and oral administration treatment option for this

Table 3 Critical factors affecting the decision of changing current disease-modifying therapy for multiple sclerosis

Factors suggesting to switch from a first line DMT to another	Tolerability/safety issues Suboptimal efficacy with disease activity not suitable for escalation to a second line DMT Persistent high-titre neutralizing antibodies in patients treated with interferon beta
Factors suggesting to switch from a first line to a second line DMT	RR MS patients experiencing at least one relapse and with an active MRI during the previous year on treatment RR MS patients transitioning to the secondary progressive phase with evidence of relapses or MRI activity
Factors suggesting to switch from a second line DMT to another or to a third line DMT	RR MS patients continuing to experience relapses Progressive forms of MS with relapses and/or active MRI despite treatment Safety issues (*e.g.*, patients on natalizumab at high risk of developing progressive multifocal leukoencephalopathy)
Factors suggesting to switch from a second line to a first line DMT	Tolerability/safety issues Risk perception of patient

DMT: Disease-modifying therapy; RR: Relapsing-remitting; MS: Multiple sclerosis.

condition[55].

Natalizumab, fingolimod, and mitoxantrone are consolidated second-line DMTs, which can be used as initial treatment in patients with aggressive MS requiring an induction approach. In addition, EMA and FDA recently approved alemtuzumab with the indication for "active" MS. In patients not previously treated with other medications, all the mentioned drugs strongly reduce the frequency of attacks compared to standard first-line therapy (around 50% relapse rate decrease *vs* IFNB) and have a profound effect on MRI activity measures[44,56-58]. However, the benefit on disability progression appears less robust and consistent across studies.

There are no approved DMTs for the PP form of MS[59-61], which carries the worst prognosis. For this reason, some patients - particularly in presence of rapid neurological worsening, superimposed relapses and evidence of inflammatory activity on brain/spine MRI - are treated off-label with immunosuppressants such as cyclophosphamide or mitoxantrone, based on the possible efficacy on disability progression suggested by some randomized trials[36,62].

WHEN TO CHANGE TREATMENT FOR MS?

Evidence-based data and guidelines on criteria and timing for DMT change in MS are limited and choices of clinicians on this matter are often based on observational reports and guided by good clinical practice (Table 3). In fact, MS patients who start a DMT discontinue it in a proportion ranging from 30% to 80% for various possible reasons[63]. One of the biggest challenges is the definition of treatment response/failure. An easy-to-apply and fairly validated tool is the Rio score, which combines clinical and MRI parameters to predict disability progression over five years[64,65]. In any case, MS patients receiving a first-line DMT who continue to have a similar relapse rate compared to the pre-treatment phase, have persistent MRI activity, and/or show irreversible neurological disability worsening, have a sub-optimal response and a therapy switch needs to be considered[66]. Second-line options for these cases are natalizumab, fingolimod and alemtuzumab, considering potential differences across drugs in efficacy and safety profiles[37-39,56,57,67,68].

For patients on first-line DMT with evidence of partial response but not fulfilling requirements for escalation to a second-line treatment (*e.g.*, isolated persistent MRI activity) or with adverse reactions/tolerability issues that affect patient safety or quality of life, a so called "lateral" switch to another first-line DMT is justified, *e.g.*, shifting from low-dose to high-dose IFNB (or the reverse in case of side effects), from GA to IFNB or *vice versa*[69,70]. In the near future switching from IFNB or GA to one of the newest oral agents such as teriflunomide and dimethyl fumarate will likely become very common. An additional option is switching from IFNB or GA to azathioprine.

Some authors suggest that patients treated with IFNB should be monitored for the serological status of neutralizing antibodies (NABs) both in cases in which suboptimal efficacy is suspected and with stable disease: persistent high-titer NABs positivity reflects IFNB biological activity loss, is associated with a higher risk of disease activity, and indicates the need of switching to a non-IFNB therapy[71]. Although NABs assay is not routinely performed in all IFNB-treated patients in all Centers, positivity is currently reported in less than 10% of cases on IFNB 1-a and over 30% of subjects receiving IFNB 1-b[72].

Finally, one has to consider the possibility or necessity of changing a second-line or third-line treatment in a patient with MS. If a patient continues to experience relapses and - more importantly - shows disability progression, a DMT change is needed as well as in case safety concerns arise during treatment. MS patients on fingolimod with break through disease will typically switch to natalizumab if this is safe, or to "rescue-therapy" with cyclophosphamide, which is also a possible option for cases not responsive to natalizumab, although this rarely occurs and should raise the suspicion of NABs presence[73]. Anyway, this scenario will likely change in the next future as the use of alemtuzumab catches on as a third-line or earlier therapeutic strategy. A debated issue in the community of MS neurologists is changing therapy in patients treated with natalizumab and at risk of developing PML,

since treatment discontinuation is associated with a high risk of disease reactivation[74]. However, also switching to another DMT, including fingolimod, does not prevent relapse occurrence and MRI worsening in many cases, particularly if new therapy start is delayed[75-77]. Other strategies, such as continuing natalizumab with a strict surveillance of early PML signs[78], or shifting to a third-line option such as cyclophosphamide or alemtuzumab are being adopted in some Centers, although it is not excluded that PML risk could be carried over by prolonging immunosuppression after natalizumab[79].

WHEN TO STOP TREATMENT FOR MS?

Effective DMTs are essential to guarantee the highest possible well-being to people with MS. For the same reason there are circumstances in which ongoing DMT should or must be stopped to avoid that risks or costs overcome benefit. Given the nature of MS, DMT discontinuation is usually temporary but in some cases it can be permanent[19,80].

First, DMT must be stopped when a serious adverse event potentially correlated to treatment occurs or is suspected, in particular if it is life threatening since MS itself does not lead to a meaningful increase of mortality. Several MS therapies, especially among the newest, expose patients to the risk of infectious, hematologic, cardiac, and neoplastic complications that are potentially lethal and must be monitored carefully[81]. If a DMT is discontinued for this reason, a treatment change has to be considered with caution since other drugs with similar mechanism of action may interfere with recovery of the adverse event or even aggravate it. In some cases a precautionary interruption of treatment, which may be temporary or prolonged, is dictated by factors that are known to increase the risk of certain adverse events. This is the case of PML risk during natalizumab in patients with anti-JCV antibodies positivity, previous immunosuppressive exposure, and treatment duration of 2 years or more[68]. Other examples include: risk of opportunistic infections in patients treated with fingolimod or dimethyl fumarate and persistently low lymphocyte count in the peripheral blood[82,83]; risk of cardiotoxicity and leukemia for patients treated with mitoxantrone[84]; increased risk of cancer with immunosuppressive cytotoxic therapies prolonged for more than 3 years in the case of cyclophosphamide or more than 10 years for azathioprine[85,86]. Beside serious adverse events, DMTs may cause "minor" side effects and tolerability issues that disrupt patient quality of life[87]. Cases not obtaining a satisfactory management of such symptoms or not perceiving treatment benefit that justifies undesired effects generally have low adherence to the prescribed medication. This is known to be a risk factor for poor control of disease activity and progression: if lack of adherence to treatment cannot be improved DMT has to be discontinued[88].

Pregnancy is another event that requires immediate DMT interruption in women with MS who, however, must be carefully informed of the need of adequate contraception prior to and during treatment, of the possibility that some DMTs may reduce fertility, and of the importance of becoming pregnant when the disease is as stable as possible[89]. Treatment cannot be resumed during breast-feeding meaning that nursing mothers should be advised of stopping breast-feeding and (re)starting therapy only in presence of disease activity or in case of aggressive course prior to treatment interruption. Pregnancy planning requires DMT discontinuation with the appropriate timing according to the pharmacokinetic of the specific drug[90]. IFNB and GA may be continued until few weeks in advance or even up to conception; natalizumab, fingolimod and dimethyl fumarate should be stopped at least two months prior to planned conception; cytotoxic agents, such as mitoxantrone and azathioprine, need to be discontinued at least three months in advance. In addition to therapy interruption, patients on teriflunomide are required to undergo an accelerated elimination procedure with colestyramine or activated charcoal at least two months before conception (in case of unexpected pregnancy the procedure must be started immediately)[91]. For patients on alemtuzumab pregnancy program appears more complex as the effects of a single five-days course of the drug may last up to four years; however, based on pharmacokinetic data, maintaining contraception for at least four months after last alemtuzumab administration is currently recommended[92]. Data and guidelines regarding paternity planning for men with MS receiving DMT are lacking. Treatment interruption is generally not recommended for IFNB and GA, since the outcome of pregnancies fathered by patients receiving those drugs does not differ from general population[93]. However, male patients receiving therapies with mutagen potential that could lead to an increased risk of fetal malformations should be encouraged to avoid conception while on treatment.

Although it might be difficult to establish, MS patients who gradually accumulate irreversible disability without experiencing relapses and MRI inflammatory activity - *i.e.,* have transitioned to the SP phase of the disease - do not benefit significantly from any of currently available DMT, which should be therefore discontinued in this group of subjects[94]. On the other hand, for treated patients with prolonged stable disease and no apparent side effects DMT discontinuation is not recommended because the disease could reactivate. However, available data have been obtained from few patients treated for less than three years who had high pre-treatment MS activity and were not selected according to an a priori definition of stable disease[95]. In this context, patients treated with natalizumab represent an exception because it has been consistently reported that treatment interruption even in cases with no sign of MS activity for several years, frequently leads to disease reactivation - with a very severe clinical

picture in some cases - soon after stopping therapy[96].

CONCLUSION

General consensus and detailed guidelines on starting, changing and stopping DMTs for MS are lacking. Recently, an effort to guide the use of DMTs based on evidence from the literature with the aim of improving access to therapies for MS patients, led to a consensus paper by the MS coalition[97].

Based on current evidence and good clinical practice principles, we suggest the following.

When to start treatment for MS?

First-line DMT should be started in patients with a diagnosis of relapsing MS (according to 2010 McDonald's criteria) and at least one documented attack in the previous two years; as for the choice of the specific drug, high dose IFNB 1-a and GA are the preferred options among established injectable therapies, although oral therapies such as azathioprine, teriflunomide and dimethyl fumarate have at least comparable efficacy.

First-line DMT may be initiated in patients with a CIS or MS with a single attack and dissemination in space and time according to 2010 McDonald's criteria in presence of factors known to be associated with poor prognosis, such as male sex, incomplete recovery from attack, prominent neurological efferent systems involvement, and more than nine lesions on brain MRI (good clinical practice point - there is no evidence that subgroups of patients with such features are significantly protected by DMTs against long-term disability progression).

DMT-naïve MS patients experiencing at least two disabling relapses in the last year and with an active MRI scan should be treated with a second-line regimen, such as fingolimod or natalizumab; also alemtuzumab may be considered for patients with aggressive disease from onset.

Available DMTs are of no utility in PP MS, although cases with rapid progression, superimposed relapses and active MRI might benefit from immunosuppressants such as mitoxantrone, cyclophosphamide, or methotrexate.

When to change treatment for MS?

Given the current availability of multiple options, a DMT change needs to be considered in any MS patient with suboptimal response: in case of one or more relapses during the previous year on a first-line DMT, particularly in case of incomplete recovery, switching to a second-line medication is appropriate, while isolated MRI activity and/or increased relapse frequency not qualifying for second-line escalation are conditions for switching to another first-line DMT; patients relapsing while on fingolimod may be switched to natalizumab, or the reverse (although natalizumab is expected to reduce relapse rate more than fingolimod based on indirect comparison); alternatively, these cases may be shifted to a third line of treatment such as alemtuzumab or intravenous cytotoxic immunosuppressants.

Patients on IFNB who develop persistent high-titer NABs need to change treatment even if disease is stable.

Subjects with intolerable side effects from their current medication need to be switched to another DMT within the same line of treatment.

Patients receiving natalizumab for more than two years who are anti-JCV antibody positive and previously received cytotoxic immunosuppressants should be switched to another DMT due to the significantly increased risk of PML; possible options include fingolimod, alemtuzumab, cyclophosphamide, and less convincingly first-line DMTs; to minimize the risk of disease reactivation the wash-out interval should be shortened as much as possible.

When to stop treatment for MS?

DMT must be stopped in case a serious adverse event potentially related to the drug occur or is likely to occur, in patients becoming pregnant, and in subjects who are not adherent to treatment.

DMT should be also discontinued in patients with confirmed disability progression over one year in the absence of relapses and new/enhancing lesions on MRI; these subjects have progressive MS, which does not respond to any DMTs, and priority should be given to symptomatic treatment, physical therapy, and management of disability.

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REVIEW

From variome to phenome: Pathogenesis, diagnosis and management of ectopic mineralization disorders

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Abstract

Ectopic mineralization - inappropriate biomineralization in soft tissues - is a frequent finding in physiological aging processes and several common disorders, which can be associated with significant morbidity and mortality. Further, pathologic mineralization is seen in several rare genetic disorders, which often present life-threatening phenotypes. These disorders are classified based on the mechanisms through which the mineralization occurs: metastatic or dystrophic calcification or ectopic ossification. Underlying mechanisms have been extensively studied, which resulted in several hypotheses regarding the etiology of mineralization in the extracellular matrix of soft tissue. These hypotheses include intracellular and extracellular mechanisms, such as the formation of matrix vesicles, aberrant osteogenic and chondrogenic signaling, apoptosis and oxidative stress. Though coherence between the different findings is not always clear, current insights have led to improvement of the diagnosis and management of ectopic mineralization patients, thus translating pathogenetic knowledge (variome) to the phenotype (phenome). In this review, we will focus on the clinical presentation, pathogenesis and management of primary genetic soft tissue mineralization disorders. As examples of dystrophic calcification disorders Pseudoxanthoma elasticum, Generalized arterial calcification of infancy, Keutel syndrome, Idiopathic basal ganglia calcification and Arterial calcification due to CD73 (NT5E) deficiency will be discussed. Hyperphosphatemic familial tumoral calcinosis will be reviewed as an example of mineralization disorders caused by metastatic calcification.

Key words: Ectopic mineralization; Pseudoxanthoma elasticum; Pseudoxanthoma elasticum-like syndrome; Generalized arterial calcification of infancy; Keutel syndrome; Idiopathic basal ganglia calcification; Arterial calcification due to CD73 deficiency; Hyperphosphatemic familial tumoral calcinosis; Etiology; Phenotype

Core tip: Ectopic mineralization disorders represent a broad range of phenotypically heterogenous diseases, often leading to significant morbidity and mortality. Involving a complex interplay between different pro-osteogenic mediators and inhibitors of calcification, the mechanisms of ectopic mineralization are progressively being unveiled. Though current knowledge is beyond any doubt the tip of the proverbial iceberg, insights already have significant implications in the diagnosis and daily management of these patients. As such, ectopic mineralization diseases are a fine example of translating variome data to the clinic. Here, we will discuss prototype hereditary ectopic calcification diseases with respect to their presentation, diagnosis and management.

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Table 1 Causes of metastatic/dystrophic calcification and ectopic ossification

	Metastatic calcification	Dystrophic calcification	Ectopic ossification
Primary	Primary hyperparathyroidism Pseudo(pseudo)hypoparathyroidism HFTC	PXE PXE-like syndrome GACI Keutel syndrome IBGC ACDC AI	Fibrodysplasia ossificans progressiva
Secondary	Sarcoidosis Vitamin D intoxication Milk-Alkali syndrome Secondary hyperparathyroidism Renal failure Hemodialysis Tumor lysis Therapy with vitamin D and phosphate	Scleroderma Dermatomyositis SLE	Nonhereditary myositis ossificans

ACDC: Arterial calcification due to CD73 deficiency; AI: Amelogenesis imperfecta; GACI: Generalized arterial calcification of infancy; HFTC: Hyperphosphatemic familial tumoral calcinosis; IBGC: Idiopathic basal ganglia calcification; PXE: Pseudoxanthoma elasticum; SLE: Systemic lupus erythematosus.

INTRODUCTION

Physiological biomineralization is a complex multifactorial metabolic process, which in normal conditions is restricted to the extracellular matrix (ECM) of specific body structures, namely the bones, teeth, hypertrophic growth plate cartilage and calcified articular cartilage[1,2]. The intracellular and extracellular mechanisms, underlying physiological biomineralization, rely on a balanced interplay between mineralization inhibitors and propagators (Figure 1)[2,3]. Although in physiological circumstances calcium and inorganic phosphate (Pi) concentrations exceed their solubility in most human tissues, this does not result in mineralization of soft tissues, suggesting that these tissues possess regulatory mechanisms preventing mineral deposition. Mineralizing tissues must be able to modulate these mechanisms to enable calcification[2], but should also contain anti-mineralizing factors to prevent escalation of the calcification process leading to excessive and uncontrolled mineral deposits[1,2]. When these regulatory mechanisms are inadequate, ectopic mineralization, *i.e.,* inappropriate biomineralization in soft tissues, occurs and causes a spectrum of ectopic calcification disorders (Table 1)[2,4].

Uncontrolled mineralization occurs frequently in response to tissue injury or a systemic mineral imbalance. This leads to the development of a calcified lesion, which can occur throughout the body, though tissues as articular cartilage, the cardiovascular (CV) tissues and kidneys seem particularly prone[3,5,6]. Unlike physiological mineralization deposits, which only contain calcium phosphate crystals such as hydroxyapatite, ectopic mineralization depositions may also contain other calcium salts, including calcium oxalates or octacalcium[4].

Regarding the initiation of and pathogenetic mechanisms underlying ectopic mineralization several hypotheses have been proposed (Figure 1): (1) increasing evidence is found that soft tissue calcification can be initiated in matrix vesicles (MVs), extracellular membrane particles (approximately 20-200 nm in diameter), which have a key role in the normal physiological mineralization process[3]. MVs contain calcium-binding non-collagenous matrix proteins, such as secreted phosphoprotein 1 (SPP1; OMIM*166490), which can boost mineralization *in vitro*[7]. MVs initiate mineralization in 2 phases: (1) initial formation of hydroxyapatite in the MV itself: after budding from the plasma membrane, tissue-nonspecific alkaline phosphatase (TNAP; OMIM*171760) activity induces an increase of extracellular Pi concentration, which then enters the vesicles *via* sodium-dependent inorganic phosphate transporters (PiTs). This is followed by calcium influx into the MVs, which is enabled by annexin A5 (ANXA5; OMIM*131230) and phosphatidyl serine (PS), located at the MV inner membrane leaflet[1,3]; and (2) propagation of the calcium salts in the ECM: in the MVs hydroxyapatite crystals continue to grow, eventually rupturing the MV membrane. As a result, the crystals are exposed to the ECM, inducing their further expansion[3,8]; pathological calcification can also be influenced by ectopic osteogenic and chondrogenic signaling, leading to the activation of multiple pro-mineralization proteins[9]. This conversion of tissue-specific cells to bone-like cells has been mainly

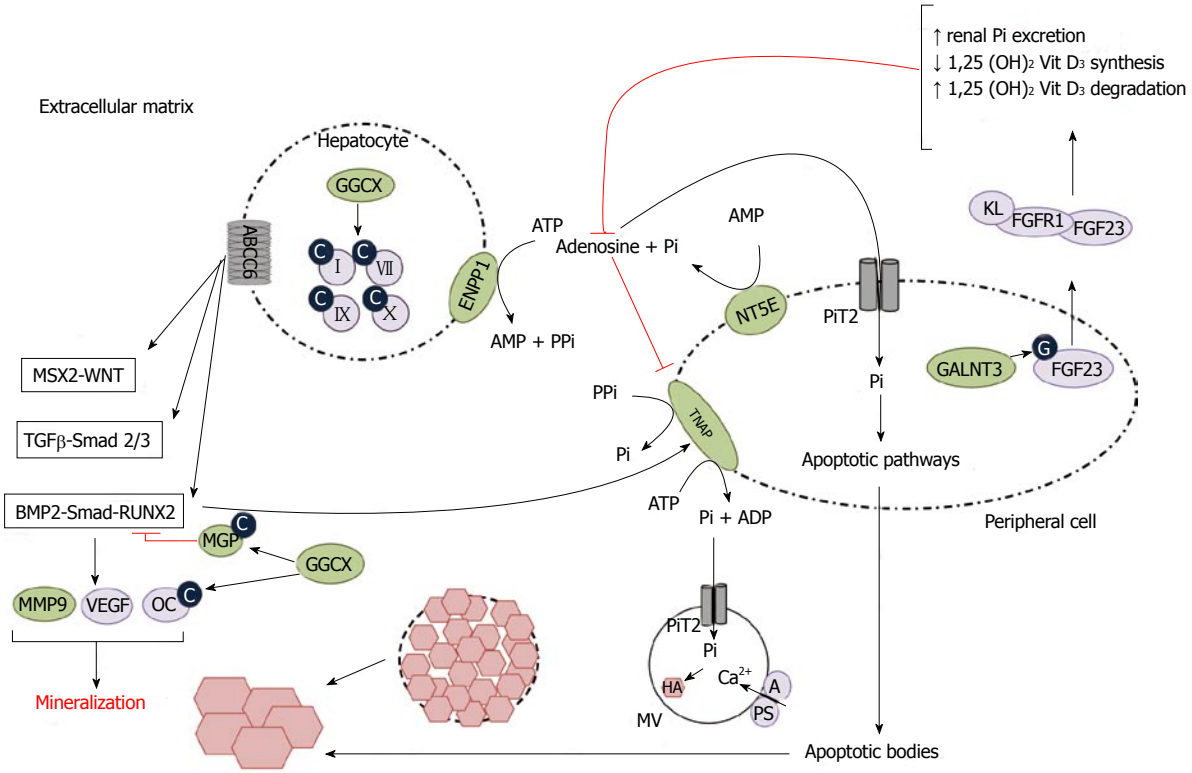

Figure 1 Schematic representation of the pathophysiological mechanisms leading to ectopic mineralization. Hepatocyte: Impairment of ABCC6 function leads to upregulation of pro-osteogenic pathways (MSX2-WNT, TGFβ-Smad 2/3, BMP2-Smad-RUNX2), upregulation of their downstream targets and eventually to ectopic mineralization. GGCX carboxylates and hence activates multiple targets, such as coagulation factors and MGP, the latter being a potent BMP2-inhibitor and hence mineralization inhibitor. When GGCX function is impaired, these targets stay inactive, leading to increased mineralization. ENPP1 converts ATP to AMP and PPi, the latter being a mineralization inhibitor. Impairment of this conversion and hence a decrease in the PPi level leads to increase in ectopic mineralization. Peripheral cell: After glycosylation by GALNT3, FGF23 forms a complex with FGFR1 and KL (coreceptor) which leads to increased renal excretion of Pi, a pro-mineralizing agent and decreased 1,25 dihydroxyvitamin D3, causing a decrease in intestinal Pi absorption. NT5E converts AMP to Pi and adenosine, which inhibits the pro-mineralizing TNAP. Impairment of NT5E function leads to increased TNAP activity and decreased PPi concentration, hence leading to ectopic mineralization. Pi is internalized into the peripheral cell by PiT2 and leaves the cell through apoptotic bodies, which cause ectopic mineralization through apoptotic pathways (not shown). In MVs an influx occurs of Pi *via* PiT2 and of Ca^{2+}, which is facilitated by A and PS. This leads to an accumulation of growing hydroxyapatite crystals, eventually causing the MVs to burst and the crystals to grow in the extracellular matrix. A: Annexin A5; ABCC6: Adenosine triphosphate-binding cassette, subfamily C, member 6; ADP: Adenosine diphosphate; AMP: Adenosine monophosphate; ATP: Adenosine triphosphate; BMP2: Bone morphogenetic protein 2; C: Carboxyl; Ca^{2+}: Calcium 2+; ENPP1: Ectonucleotide pyrophosphatase/phosphodiesterase 1; FGF23: Fibroblast growth factor 23; FGFR1: Fibroblast growth factor receptor 1; G: Glycosyl-; GALNT3: UDP-N-acetyl-alpha-D-galactosamine: Polypeptide N-acetylgalactosaminyltransferase 3; GGCX: Gamma-glutamyl carboxylase; HA: Hydroxyapatite; KL: Klotho; MGP: Matrix gla protein; MMP9: Matrix metalloproteinase; MSX2: Muscle segment homeobox, drosophila, homolog of, 2; MV: Matrix vesicle; NT5E: Ecto-5-prime nucleotidase or CD73; OC: Osteocalcine; Pi: Inorganic phosphate; SLC20A2: Solute carrier family 20 (phosphate transporter), member 2; PPi: Inorganic pyrophosphate; PS: Phosphatidyl serine; RUNX2: Runt-related transcription factor; Smad: Mothers against decapentaplegic, drosophila, homolog of; TGFβ: Transforming growth factor β; TNAP: Tissue-non-specific alkaline phosphatase; VEGF: Vascular endothelial growth factor; WNT: Wingless-type MMTV integration site family; II, VII, IX, X: Vitamin K-dependent coagulation factors; 1,25 $(OH)_2$ Vit D3: 1,25-dihydroxyvitamine D3 (calcitriol).

described in vascular calcification, and is probably due to the common mesenchymal origin of vascular smooth muscle cells (VSMCs) and bone cells[1]; (3) apoptosis or programmed cell death is accompanied by the release of apoptotic bodies, which exteriorize PS to the outer membrane of the apoptotic body and therefore face the ECM. There, PS may bind calcium, resulting in an accumulation of calcium and phosphate, as is also seen in MVs, thus contributing to physiological and pathological mineralization[1,10]. Another potential apoptosis pathway includes elevated phosphate levels to induce VSMC apoptosis, a process that is possibly caused by downregulation of growth arrest-specific 6 (Gas6; OMIM*600441) and B-cell CLL/Lymphoma (BCL2; OMIM + 151430), with subsequent caspase 3 activation[11,12]; and (4) reactive oxygen species (ROS), highly reactive oxygen-containing molecules, are formed as byproducts of normal oxygen metabolism and has important roles in cell signaling and metabolism. Nonetheless, if ROS concentration surpasses a critical threshold, oxidative stress, accompanied by important cell damage, can occur[13]. Potential sources of ROS in soft tissues are nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) oxidase, nitric oxide synthase (NOS), xanthine oxidase, cytochrome P450 and cyclo-oxygenase; in addition, mitochondrial dysfunction may also lead to the formation of ROS. ROS possibly causes soft tissue mineralization through either the IκB-NF-κB pathway (inhibitor of κB - nuclear factor kappa-light-chain-enhancer of activated B cells), upregulation of the pro-osteogenic bone morphogenetic protein 2 (BMP2; OMIM*112261) pathway and/or osteogenic conversion

of soft tissue cells[1].

These pathophysiological mechanisms are however not mutually exclusive and display significant crosstalk[1].

Ectopic soft tissue mineralization is a common finding in aging and several common disorders, including atherosclerosis, hypertension, diabetes, chronic kidney disease and autoimmune diseases, and can be related to significant morbidity and mortality in each of these. It has been shown that vascular calcification correlates with an increased risk of myocardial infarction and that it is an independent risk factor for death in patients with coronary artery calcification[14,15]. However, in these complex, multifactorial disorders, multiple genes are likely to contribute, with each gene having only a small effect[16]. Contrary, in primary genetic mineralization disorders mutations in a single gene or few genes can cause an often extreme and life-threatening phenotype. Though individually rare, as a group they affect a considerable number of patients with important impact on quality of life and high morbidity and mortality rates.

Ectopic mineralization disorders are conventionally classified based on the mechanism through which the mineralization takes place: *i.e.,* metastatic or dystrophic calcification or ectopic ossification (Table 1)[14]: (1) metastatic calcification, due to hyperphosphatemia and/or hypercalcemia; (2) dystrophic calcification, which occurs in diseased (metabolically impaired or dead) tissue under normal calcium and phosphate homeostasis[1]; and (3) ectopic or heterotopic ossification, leading to true bone formation[1,4,17,18].

For many of these disorders, important advances have been made in defining their clinical presentation (phenome), their (molecular) etiology (variome) and the correlation between both. This has led to novel insights and perspectives for the management and treatment of the patients, but also supports the complexity of the pathophysiology of soft tissue mineralization.

This review will focus on the clinical presentation, pathogenesis and management of primary genetic soft tissue mineralization disorders due to dystrophic (Pseudoxanthoma elasticum, Generalized arterial calcification of infancy, Keutel syndrome, Idiopathic basal ganglia calcification, Arterial calcification due to CD73 deficiency) or metastatic calcification (Hyperphosphatemic familial tumoral calcinosis).

PSEUDOXANTHOMA ELASTICUM

Pseudoxanthoma elasticum (PXE; OMIM#264800) is a rare, autosomal recessive connective tissue disorder, resulting from ectopic mineralization and fragmentation of elastic fibers. The prevalence of PXE is estimated between 1/25000 and 1/100000 with a carrier frequency of 1/80, although this may be an underestimation due to the variability of the phenotype, which in some cases may hinder the diagnosis[19-21].

Clinical characteristics

PXE primarily affects 3 organ systems, *i.e.,* the skin, the eyes and the CV system, albeit with important inter- and intrafamilial variability in severity[19,21,22]. Usually the skin symptoms are the first to arise, though they are not present in all patients, presenting as soft yellowish papules in flexural body areas (*i.e.,* neck, axilla, elbow, groin and knees) (Figure 2A-E)[19]. These solitary papular lesions can coalesce into larger plaques. Loss of resilience may give the skin a wrinkled aspect and can cause an esthetic burden[1,19]. Less frequently, mucosal lesions (usually at the inner lower lip) are present (Figure 2F)[19]. The emergence of additional inelastic skin folds[1], especially in neck (and thigh) area(s) can also cause functional problems, *e.g.,* when sleeping or riding a bicycle[1,19].

The most common ocular features in PXE patients are peau d'orange and angioid streaks (AS), which themselves cause no functional impairment (Figure 2G). In later stages choroidal (subretinal) neovascularization (CNV) occurs and these neovessels may rupture, causing retinal hemorrhage (Figure 2I). Symptoms will include metamorphopsia and vision loss, which can be permanent if left untreated. More recently, chorioretinal atrophy, subretinal fluid independent from CNV, pattern dystrophy-like changes, debris accumulation under the retinal pigment epithelium, reticular drusen and a decreased fluorescence on late phase indocyanine green angiography were described[23].

CV symptoms, usually arising when patients are 30-40 years old, include accelerated coronary and peripheral artery disease (hypertension, myocardial infarction, intermittent claudication), diastolic cardiac dysfunction and gastrointestinal hemorrhage[24]. In 15% of PXE patients ischemic stroke may occur, at an average age of 49[1,24]. Heterozygous carriers usually develop neither skin nor eye symptoms but can suffer from accelerated atherosclerosis and (mild) diastolic dysfunction of the heart[24].

To date, no correlation has been established between the PXE phenotype and mutations in the main causal gene adenosine triphosphate (ATP)-binding cassette, subfamily C, member 6 (*ABCC6*; OMIM*603234), which complicates the prediction of the evolution and severity of the symptoms in an individual patient[19,25]. The absence of a reliable genotype-phenotype correlation suggests that other genes, so-called modifier genes, may play an important role in influencing the disease course, apart from other factors such as lifestyle, environmental factors and - although less probable - dietary habits[19]. Thus far, only one promising modifier gene, vascular endothelial growth factor A (*VEGFA*; OMIM + 192240) has been identified for the PXE retinopathy[26].

Pathogenesis

PXE is caused by mutations in the *ABCC6* gene, enco-

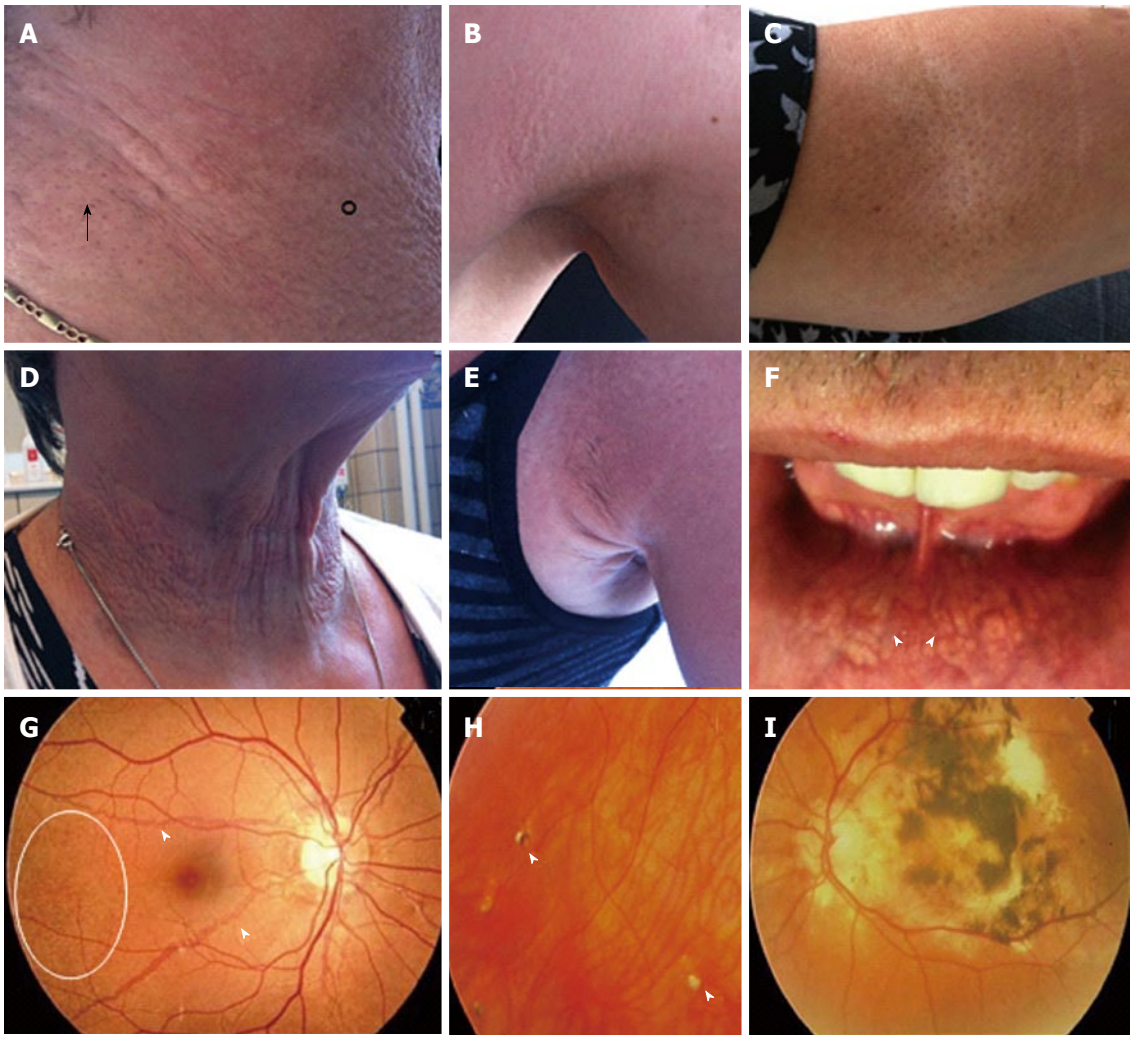

Figure 2 Dermatological (A-F) and ophthalmological (G-I) manifestations of pseudoxanthoma elasticum. A, B: Flexural areas can show papular lesions (°) and coalesced plaques of papules (arrow); C: Cutaneous peau d'orange; D, E: Additional skin folds; F: Yellowish, reticular pattern on the mucosae of the lip (arrowed); G: Ocular fundi show peau d'orange (circle) and angioid streaks (arrowed); H: Comets and comet tails (arrowhead); I: Choroidal and subretinal hemorrhage.

ding an ATP-binding efflux transporter, the substrate and (patho)physiological role of which are yet to be elucidated[27]. The ABCC6 transporter is mainly expressed in the liver and kidneys while only minimally present in the organs affected by PXE[28,29]. This led to the hypothesis that PXE is a metabolic disorder in which a defective transporter causes inefficient transport of one or multiple substrates into the bloodstream[28,29]. As a result, a deficiency of vitamin K (VK) -dependent and -independent mineralization inhibitors occurs, favoring ectopic soft tissue mineralization[23,30,31]. The metabolic hypothesis was reinforced several times, until very recently Ziegler *et al*[32] reported that a conditional, liver-specific *Abcc6*$^{-/-}$ mouse model does not develop ectopic mineralization and concluded that mineralization in PXE occurs through a liver-independent mechanism. This would correspond with a second, so-called cellular, hypothesis which states that the local environment in the affected organ systems is altered; in this respect it was shown that PXE fibroblasts suffer mild chronic oxidative stress because of overexpression of oxidative stress-favoring mediators[31,33].

More recently, 3 pro-osteogenic pathways, *i.e.*, BMP2-Smad (mothers against decapentaplegic, drosophila, homolog of; OMIM*601366)- runt-related transcription factor 2 (RUNX2; OMIM*60021) and transforming growth factor β2 (TGFβ2; OMIM*190220)-Smad2/3 pathways and the MSX2 (muscle segment homeobox, drosophila, homolog of, 2; OMIM*123101)-canonical WNT (wingless-type MMTV integration site family; OMIM*164820) pathway which are associated with vascular mineralization, were found to be upregulated in the skin and eyes of PXE knock-out mice and in PXE patients (Figure 1)[34]. The relevance of BMP2-Smad-RUNX2 signaling was alluded on by previous observations in PXE, including the low levels of carboxylated (active) matrix gla protein (MGP; OMIM*154870)/gamma-carboxyglutamic acid, a potent inhibitor of BMP2, the upregulation of several target genes of RUNX2 such as SPP1, osteocalcin, matrix metalloproteinase (MMP9; OMIM*120361), TNAP and VEGFA, the influence of oxidative stress on BMP2

Table 2 Differential diagnosis of pseudoxanthoma elasticum manifestations[1,19,47,49,53-59]

Disease	Distinct differences with PXE
Beta-thalassemia (PXE phenocopy)	Severe anemia Reduced production of hemoglobin
PXE-like syndrome (*AR*; *GGCX* gene)	More severe cutaneous phenotype not restricted to flexural areas Vitamin K-dependent coagulation factor deficiency
GACI (*AR*; *ENPP1* gene)	Onset in infancy or early childhood Arterial stenosis Early-onset severe myocardial ischemia High mortality rate in early childhood
Fibroelastolytic papulosis, Treatment with D-penicillamine	No ophthalmological or CV phenotype
Buschke-Ollendorf syndrome (*AD*; *LEMD3* gene)	Skeletal manifestations (osteopoikilosis, stiff joints, osteosclerosis) No ophthalmological or CV phenotype No mineralization
Solar elastosis	Dermatological features (lentigines, mottled pigmentation, actinic keratoses, telangiectasias, xerotic texture) No ophthalmological or CV phenotype No mineralization
Late-onset focal dermal elastosis	Onset in 7th to 9th life decade No ophthalmological or CV phenotype
Cutis laxa	No ophthalmological or CV phenotype Histopathology: scarce and mottled elastic fibers, no mineralization
A(R)MD (age-related macular degeneration)	No AS No CV or dermatological phenotype Less unique lesions (outer retinal tabulation or Bruch's membrane undulation)
Presumed ocular histoplasmosis	No AS No CV or dermatological phenotype

AD: Autosomal dominant; AR: Autosomal recessive; A(R)MD: Age-related macular degeneration; AS: Angioid streaks; CV: Cardiovascular; ENPP1: Ectonucleotide pyrophosphatase/phosphodiesterase 1; GACI: Generalized arterial calcification of infancy; GGCX: Gamma-glutamyl carboxylase; LEMD3: Lem domain-containing protein 3; PXE: Pseudoxanthoma elasticum.

expression and the overexpression of RUNX2 in calcified cardiac tissue of the *Abcc6*-related dystrophic cardiac calcification mouse[34-36]. Furthermore, apoptosis was identified as an important process in PXE contributing to mineralization, by activation of BCL2 and multiple caspases[34].

Some insights in the dysfunction of pro- and anti-mineralizing factors in the PXE pathogenesis, have been described in the PXE murine model and/or PXE patients. Several local pro-mineralizing factors seem to be upregulated *in vitro* and/or *in vivo* (TNAP, BMP2) while mineralization inhibitors, such as ecto-5-prime-nucleotidase or CD73 (NT5E; OMIM*129190), SPP1, ankyrin (mouse, homolog of) (ANKH; OMIM*605145) and VK-dependent calcification inhibitors, were found to be less expressed[37-39].

Besides local factors, systemic inhibitors of mineralization such as Fetuin A and more recently inorganic pyrophosphate (PPi), were shown to be less abundant in PXE. PPi is a potent endogenous inhibitor of vascular calcification, both *in vitro* and *in vivo*, which was already shown to be downregulated in PXE fibroblasts, thus promoting pro-calcifying stimuli leading to tissue mineralization[40,41]. Jansen *et al*[42] found low PPi serum levels in both *Abcc6*$^{-/-}$ mice and PXE patients, and concluded that an impaired ABCC6 transporter negatively influences PPi efflux from hepatocytes to the hepatic circulation, though the exact mechanism is poorly understood.

Diagnosis

The diagnosis of PXE is a clinical one to begin with, based on the presence of typical skin and/ or fundus changes. While the skin lesions of PXE can be mimicked macroscopically by other disorders (Table 2), the presence of peau d'orange and/or AS in the ocular fundus can be considered pathognomonic. Diagnostic confirmation can be obtained by skin biopsy, showing shortened, fragmented elastic fibers as well as mineral deposits in the mid-dermis using H&E (hematoxylin and eosin) and von Kossa staining. Molecular analysis of the *ABCC6* gene detects both causal mutations in approximately 95% of patients[1,33,43,44]. Sanger sequencing is still the gold standard, but should be complemented with multiplex ligation-dependent probe amplification, to detect larger deletions and insertions[45]. If no or only one *ABCC6* mutation can be identified, it is worthwhile to screen for gamma-glutamyl carboxylase (*GGCX*; OMIM*137167) mutations as digenic inheritance has been described[46]. Furthermore, the initial presentation of the *GGCX*-related PXE-like disorder with coagulation factor deficiency (see below) can be identical to PXE[47,48]. Sequencing of the ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*; OMIM*173335) gene is useful when no *ABCC6* mutations can be detected in a patient with a histologically confirmed diagnosis of PXE; digenic inheritance of an *ABCC6* and *ENPP1* mutation has so far not been reported[49]. This increasing number of genes which may cause PXE as well as the potential importance of modifier genes, brought about a gradual shift from Sanger sequencing towards the more recently introduced next generation sequencing[26,47,50-52].

Differential diagnosis

The differential diagnosis of PXE manifestations is summarized in Table 2.

Management

To date, PXE management is mainly symptomatic, focusing on prevention and treatment of complications[19]. For ophthalmological complications, preventive measures include wearing glasses and avoiding sports and activities with a (relative) high risk of (head) trauma or increased pressure[19,21,60]. Once fundus changes have appeared, an annual control by an ophthalmologist is important, as well as weekly self-examination using the Amsler Grid. If distortion or metamorphopsia occurs,

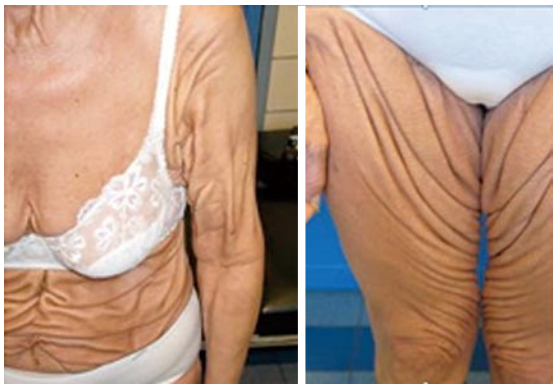

Figure 3 Cutaneous features of a pseudoxanthoma elasticum-like patient with increased amount of generalized thick leathery skin folds.

the patient should contact his/her ophthalmologist immediately[20,23]. Timely treatment with anti-VEGF antibodies, such as bevacizumab or ranibizumab, were shown to be successful in forcing back neovessels and preserving visual acuity[1,20,30]. Prophylactic anti-VEGF therapy has however not been proven to be advantageous[19].

The prevention of CV complications consists of controlling traditional CV risk factors (*e.g.,* smoking, obesity, hypercholesterolemia and diabetes)[1]. Upon diagnosis, a baseline screening should be performed with measurement of blood pressure, assessment of biochemical CV risk factors, echocardiography, determination of the ankle-brachial index and duplex ultrasound of the arteries of the neck and lower extremities. If hypertension is found, further assessment with 24-h blood pressure monitoring and an exercise test should be done. Further CV management is tailored based on the results of this screening, usually comprising an annual checkup by a cardiologist and if necessary initiation of secondary prevention[24]. Since heterozygous carriers suffer CV complications more frequently compared to the general population, they should also undergo a baseline CV screening and regular checkups by a cardiologist[24]. Furthermore, the use of anticoagulants, aspirin and nonsteroidal anti-inflammatory drugs should be avoided as they may elevate the risk of gastrointestinal bleeding[1]. When complications do occur, standard interventional or surgical procedures can usually be applied[24,61].

For the skin problems no prevention is possible and therapeutic options are scarce. When functional problems arise, mainly due to excessive skin folds, plastic surgery can be attempted[61]. Possible post-surgery complications include slower wound healing and apparition of skin lesions in the scars[61-63]. Recently, Salles *et al*[64] described a PXE patient in which skin lesions in the neck were successfully treated with fractional carbon dioxide laser therapy. The post-laser reaction - redness, pain, swelling and crusting - was the same as seen in normal skin. After a follow-up of 2 years, the treatment showed an overall satisfactory esthetic result, showing improvement of the skin texture, irregularity, volume and distensibility. Moreover, lipofilling to reduce esthetically disturbing skin folds, especially in the neck region, is being evaluated in an experimental setting[65].

PXE-LIKE SYNDROME WITH MULTIPLE COAGULATION FACTOR DEFICIENCY

In 2007, Vanakker *et al*[1,47] described a new autosomal recessive disorder which was closely related to PXE and coined it the PXE-like syndrome with multiple coagulation factor deficiency (OMIM#610842). To date, the disorder has been described in 12 patients, 8 of which had molecular confirmation of the clinical suspicion[47,48,66-68].

Clinical characteristics

The initial presentation of the PXE-like syndrome is nearly identical to that of PXE, making it often difficult to distinguish the two diseases in young adults. The natural evolution of the PXE-like disorder is however completely different and characterized by severe cutaneous symptoms with the development of thick and redundant skin folds, not restricted to flexural areas but variably expanding toward limbs and abdomen (Figure 3), a mild retinopathy and a deficiency of the VK-dependent coagulation factors (coagulation factors Ⅱ, Ⅶ, Ⅸ, Ⅹ)[1,47,48]. Furthermore, subclinical atherosclerosis and cerebral aneurysms have been described[47].

Pathogenesis

Biallelic mutations have been described in the *GGCX* gene, encoding a gamma-carboxylase enzyme which performs an essential post-translational modification step of a number of so-called VK-dependent proteins, including clotting factors and mineralization inhibitors (Figure 1). It was shown that the PXE-like mutations result in a reduced activity of the enzyme, thus leading to inadequate carboxylation (or activation) of these VK-dependent proteins. This causes a deficiency of coagulation factors and creates an environment which favors ectopic mineralization[1].

Diagnosis

The diagnosis of PXE-like syndrome is relatively straightforward when typical skin lesions are seen in combination with a deficiency in the VK-dependent clotting factors. Biochemically, a prolonged prothrombin time can be found, though the coagulation factor deficiency can be very mild[47]. In young individuals, the diagnosis should be considered in every patient suspected of having PXE in whom no *ABCC6* mutations are found. Histopathology shows fragmentation and calcification of the mid-dermal elastic fibers, being located in the periphery of the fiber[69]. Light microscopy will not allow differentiating with PXE, but on electron microscopy the elastic fibers are more ragged and the calcification is located in the periphery of the fibers

Table 3 Differential diagnosis of the pseudoxanthoma elasticum-like syndrome[19,47]

Disease	Distinct differences with PXE-like syndrome
PXE (*AR*; *ABCC6* gene)	More severe CV and ophthalmological manifestations Skin lesions are less severe and restricted to flexural areas No coagulation factor deficiency associated EM: mineralization in the core of the EF
Cutis laxa	No retinopathy No deficiency of coagulation factors Atherosclerosis and cerebral aneurysm are infrequent Histopathology: scarce and mottled elastic fibers, no mineralization

ABCC6: Adenosine triphosphate-binding cassette, subfamily C, member 6; AR: Autosomal recessive; CV: Cardiovascular; EF: Elastic fiber; EM: Electron microscopy; PXE: Pseudoxanthoma elasticum.

(compared to fiber core mineralization in PXE). The diagnosis can be confirmed by *GGCX* sequencing[47].

Differential diagnosis

The differential diagnosis of PXE-syndrome is summarized in Table 3.

Management

The management of PXE-like patients is similar to that of patients with classic PXE. In most, treatment of the coagulation deficiency is not necessary, though the use of anticoagulants is not advised. In rare cases, supplementation with VK may be useful[47].

GENERALIZED ARTERIAL CALCIFICATION OF INFANCY

Generalized arterial calcification of infancy (GACI; OMIM#20800) is an early-onset, autosomal recessive disorder, which has only been described in approximately 100 mostly Caucasian patients[1,70]. The disease typically affects infants of less than 6 mo of age[71,72].

Clinical characteristics

GACI is characterized by arterial stenosis, resulting from myointimal proliferation of muscular arteries, and early-onset severe myocardial ischemia due to extensive deposition of hydroxyapatite in the inner elastic lamina of medium- and large-sized arteries[1,70,73]. Complications include myocardial infarction, hypertension and congestive heart failure, leading to early demise[1]. Other possible manifestations include dermatological and ophthalmological findings typical of PXE, extravascular (mostly periarticular) calcifications, hearing loss and development of hypophosphatemic rickets after infancy[49,70,71,74-77]. The majority of patients die before the age of 1, with the highest fatality rate in the first six months of life, most commonly due to myocardial infarction, congestive heart failure, multiple organ failure or persistent arterial hypertension[71,72]. Recently, Rutsch *et al*[71] reported a mortality rate of 55%, with a marked decrease in the mortality of patients, which survived the first 6 mo. In some of these, spontaneous resolution of the mineralization was seen[78,79].

Pathogenesis

GACI is caused by inactivating mutations in the *ENPP1* gene, which encodes ectonucleotide pyrophosphatase/phosphodiesterase 1. Under normal conditions, ENPP1 is associated with the outer plasma membrane of VSMCs in arteries and generates extracellular PPi through hydrolysis of ATP to adenosine monophosphate (AMP)[2]. PPi is a potent calcification inhibitor, which was already shown to hinder mineral crystal growth by binding to the crystal surface in osteoblast cultures[1,2,17].

Diagnosis

Neonates with GACI can present with rather aspecific symptoms, such as poor feeding and respiratory distress. Consequently the diagnosis is often only established by detecting arterial calcification using plain radiography, ultrasound or computed tomography. Typically, diffuse vascular and periarticular ectopic mineralization is found. GACI should be considered antenatally when ultrasonographic anomalies include arterial calcifications, hydrops, abnormal cardiac contractility and/or hyperechoic kidneys[80]. Confirmation of the diagnosis is possible through molecular analysis of the *ENPP1* gene which detects mutations in approximately 70% of cases[49,71,81,82]. When no mutations can be found in *ENPP1*, *ABCC6* sequencing should be performed, due to an overlap in the phenotypes of both diseases[70,73]. An arterial biopsy shows fragmentation in the integral elastic lamina with calcium deposition and fibrointimal hyperplasia causing luminal narrowing, which can occur in places devoid of mineralization[72,83]. Conversely, mineralization can occur without luminal narrowing[84]. Apart from calcium, the depositions also contain iron and mucopolysaccharides[84,85]. The lesions are surrounded by a giant cell reaction[86].

Differential diagnosis

The differential diagnosis of GACI is summarized in Table 4.

Management

The treatment options in GACI are limited and rely mostly on the use of bisphosphonates, such as etidronate and pamidronate, which are analogs of PPi. These bisphosphonates possibly act through decreasing bone turnover, inhibiting further growth of mineralized crystals and/or providing an alternative form of PPi that may influence the regulation of mineralization[91]. Vascular calcifications have been reported to disappear under bisphosphonate therapy within a variable time period (2, 5 wk to 2 years). Calcifications do not tend to reappear after cessation of the therapy, although arterial stenosis persists[92,93]. Since prolonged etidronate use in GACI

Table 4 Differential diagnosis of generalized arterial calcification of infancy[73,78,87-90]

Disease	Distinct differences with GACI
PXE (AR; *ABCC6*)	GACI-like phenotype possible, however infrequent CV phenotype usually less severe No onset in infancy Dermatological and ophthalmological phenotypes more prominent
Singleton-Merten Calcification (AD; unknown causal gene)	Dental anomalies (delayed eruption and early loss of permanent teeth, alveolar bone erosion) Osteopenia Acroosteolysis
Metastatic calcification due to hypervitaminosis D, hyperparathyroidism or end-stage renal disease	Different distribution of extravascular calcification (renal tubules, bronchial walls and basal mucosa and muscularis mucosae of the stomach) Microscopic vascular changes in media instead of intima
Congenital syphilis	Only calcification of the (ascending) aorta Diagnosed mainly in adults Hutchinson teeth, interstitial keratitis, saber tibiae, saddle-shaped nose Histopathology: endarteritis obliterans of vasa vasorum with perivascular plasma cells, lymphocytic cuffing and adventitial fibrosis
Iliac artery calcification in healthy infants	Only calcification in the common and internal iliac arteries

ABCC6: Adenosine triphosphate-binding cassette, subfamily C, member 6; AD: Autosomal dominant; AR: Autosomal recessive; CV: Cardiovascular; GACI: Generalized arterial calcification of infancy; PXE: Pseudoxanthoma elasticum.

patients has been linked to severe skeletal toxicity, bisphosphonate therapy should be closely monitored and according to some, should be stopped as soon as the calcifications have disappeared[94]. Nevertheless, the prognosis of patients remains poor with only few long-term survivors, the oldest GACI patient being 25[1,71,79,95]. Recently, Towler *et al*[96] suggested that restoring PPi levels by inhibition of alkaline phosphatase (ALP) and/or upregulation of vascular ENPP1 or ANKH-mediated secretion of intracellular PPi may serve as possibilities to limit vascular calcification.

KEUTEL SYNDROME

Since its first identification by Keutel *et al*[97] in 1971, approximately 30 cases have been described of Keutel syndrome (OMIM#245150), which is an autosomal recessive multisystem disease with an age of onset in childhood (5-15 years)[97,98].

Clinical characteristics

Keutel syndrome is mainly characterized by peripheral pulmonary stenosis, abnormal cartilage ossification or calcification of typically (para)tracheal, bronchial and rib cartilages as well as auricular and nose cartilage[99]. Less frequently soft tissue calcification, *i.e.*, of blood vessels, brain and kidneys, occurs[1]. Other clinical features include CV (ventricular septal defect, pulmonary artery hypoplasia, hypertension) respiratory (recurrent respiratory infections), skeletal (brachytelephalangism, typically sparing the 5th distal phalanx, height below the 25th percentile), neurological symptoms (subnormal intelligence quotient (IQ) in multiple cases) and recurrent otitis media causing inner ear or mixed deafness. Patients have a typical facial gestalt with mild midface hypoplasia, a depressed nasal bridge, small alae nasi and a deep philtrum[100-104]. A long-term follow-up of 4 sisters with Keutel syndrome showed that all clinical manifestations were progressive. Further, these patients developed skin lesions, *i.e.*, multiple erythematous, irregularly bordered macular lesions without induration, typically after the age of 30. Skin biopsy of these lesions failed to show calcification or ossification and loss of elastic fibers was only seen in the papillary dermis[99]. Nevertheless, the prognosis of Keutel syndrome is good in the majority of patients, with life expectancy mainly depending on the severity of the pulmonary complications[99].

Pathogenesis

Keutel syndrome is caused by loss-of-function mutations in the *MGP* gene, encoding matrix gla protein[1]. MGP is an inhibitor of the pro-osteogenic BMP2-Smad-RUNX2 pathway, by inhibiting BMP2 to bind to its receptor. Consequently, MGP, expressed in chondrocytes, functions as a local mineralization inhibitor under physiological conditions (Figure 1)[105,106]. Impairment of its inhibitory function favors pro-mineralizing signaling, leading to ectopic mineralization[98]. Moreover, Cranenburg *et al*[107] reported a patient in whom the levels of carboxylated/uncarboxlated MGP were very low, unresponsive to VK supplementation, but in whom high levels of phosphorylated MGP were found. Phosphorylation is a VK-independent posttranslational modification of MGP which may allow binding of calcium crystals in the absence of optimal carboxylation. It was hypothesized that this phosphorylation-dependent residual MGP activity might be sufficient to prevent development of arterial calcification[108].

Diagnosis

The majority of Keutel syndrome patients are diagnosed during childhood based on clinical presentation and radiographic examinations, with abnormal cartilage calcification and brachytelephalangism as major signs. The clinical diagnosis can be confirmed by sequencing of the *MGP* gene, in which to date 7 loss-of-function mutations have been reported[98,104,109].

Differential diagnosis

The differential diagnosis of Keutel syndrome is summarized in Table 5.

Management

No etiologic treatment exists for Keutel syndrome, hence management is merely symptomatic, including

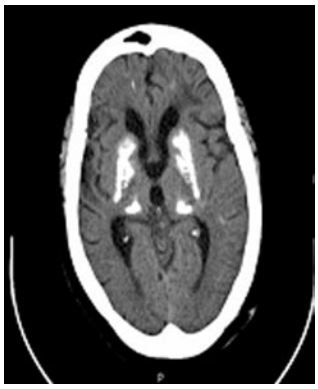

Figure 4 Transverse computed tomography of the brain displaying symmetrical bilateral ganglia calcification in an idiopathic basal ganglia calcification patient.

(angiographic) dilatation of peripheral artery stenosis and bronchodilating agents for respiratory symptoms (dyspnea and wheezing); the latter however can be inefficient in certain patients[99,108]. Most patients develop hypertension before the age of 20, which can be treated with antihypertensive medication such as perindopril, amlodipine or nifedipine[99].

IDIOPATHIC BASAL GANGLIA CALCIFICATION

Idiopathic basal ganglia calcification (IBGC) is a rare neurodegenerative disorder with unknown prevalence. The disease is sometimes referred to as Fahr's disease, although the the patient Fahr described primarily had mineralization in blood vessels of the white matter of the brain[113]. IBGC affects young to middle aged adults, with an average onset in the 3rd or 4th life decade; however the disease has also been described in childhood[114-116].

Clinical characteristics

IBGC is characterized by bilateral and (almost) symmetrical basal ganglia calcifications (Figure 4)[116]. Ectopic mineralization may also occur in other brain regions, including the nucleus dentatus, thalamus, cerebral cortex and centrum semiovale[116,117]. Neurological symptoms include neuropsychiatric (cognitive impairment, depression, hallucinations, delusions, manic symptoms, anxiety, schizophrenia-like psychosis, personality changes) and movement disorders (*a.o.* parkinsonism, ataxia due to cerebellar involvement, tremor and paresis), as well as headache, vertigo, stroke-like events, orthostatic hypotension, dysarthria, seizures and papilledema due to raised intracranial pressure[116,118]. Both sporadic and familial IBGC cases have been reported, the latter predominantly with autosomal dominant inheritance[116].

Pathogenesis

To date, mutations in 3 genes have been associated with IBGC, *i.e.,* solute carrier family 20 (phosphate transporter), member 2 (*SLC20A2*; OMIM*158378), the beta polypeptide of platelet-derived growth factor (*PDGFB*; OMIM*190040) and platelet-derived growth factor receptor, beta (*PDGFRB*; OMIM*173410). So far, no genotype-phenotype correlation has been found[119]. The *SLC20A2* gene, encoding a Pi transporter (also known as PiT2 which belongs to the type III sodium-dependent phosphate transporter family), is expressed abundantly in a variety of tissues and likely plays a housekeeping role in cellular phosphate uptake (Figure 1)[119,120]. Mutations in the gene have been described in more than 40 IBGC families worldwide and *in vitro* resulted in impaired Pi transport, leading to accumulation of this pro-mineralizing factor[119,121,122].

More recently, a few IBGC patients were reported harboring mutations in *PDGFB* or *PDGFRB*[123-128]. In animal models, *Pdgfrb* has been identified as an essential mediator in the development of pericytes in brain vessels, which have a key role in the maintenance of the blood-brain barrier (BBB). The BBB is hypothesized to be defective in IBGC[123]. Moreover, Villa-Bellosta *et al*[129] found that the PDGFB-PDGFRB pathway seems to be involved in phosphate-induced calcifications in VSMCs by downregulating SLC20A2. All these data suggest that cerebral phosphate homeostasis plays a role in the development of vascular mineralization[129]. The mineralization generally develops within the vessel wall and in the perivascular space, ultimately extending to the neuron. Upon progression, the calcifications start to compress the vessel lumen, which causes impaired blood flow, starting off a vicious circle with further neural tissue damage and mineral deposition. The mineral depositions tend to vary in composition according to

Table 5 Differential diagnosis of Keutel syndrome[98,110-112]

Disease	Distinct differences with Keutel Syndrome
X-linked chondrodysplasia punctata (XL; *ARSE* gene)	Ichtyosis Cataracts Microcephaly, intellectual disability ASD, VSD, PDA Failure to thrive in infancy Age at diagnosis: usually infancy
Warfarin embryopathy	Pectus carinatum Congenital heart defects different from those seen in Keutel syndrome (ASD, PDA, ventriculomegaly)
Combined Vitamin K-dependent coagulation factor deficiency	Easy bruising, mucocutaneous bleeding Osteoporosis with normal serum markers
Relapsing polychondritis	Age at diagnosis: 40-60 yr Cartilage inflammation, possibly progressing to destruction Aortic or mitral valvular disease Facies: saddle nose deformity, multifocal, tender chondritis, including variably floppy or calcified auricles Cranial neuropathies, hemiplegia

ARSE: Arylsulfatase E; ASD: Atrial septal defect; PDA: Patent ductus arteriosus; VSD: Ventricular septal defect; XL: X-linked.

Table 6 Differential diagnosis of idiopathic basal ganglia calcification[116,140-143]

Disease	Distinct differences with IBGC
Basal ganglia calcification as incidental finding on CT scans/ aging	In 1% of CT scans Usually benign No clear etiology, especially when in older patients Asymptomatic
Hypoparathyroidism	Early onset: childhood/adolescence Hypoparathyroidism, hypocalcemia, hyperphosphatemia Alopecia, dry hair Dental dysplasia, caries Moniliasis Albright osteodystrophy symptoms (short stature, round facies, obesity, short metacarpals/metatarsals)
Pseudohypoparathyroidism (AD/maternal imprinting; *GNAS*, *GNASAS1* and *STX1A* gene)	Early onset: childhood/adolescence Hyperparathyroidism, hypocalcemia, hyperphosphatemia Baseline cAMP in urine low; after Ellsworth Howard test subnormal Intellectual disability Albright osteodystrophy symptoms
Pseudo-pseudohypoparathyroidism (AD/paternal imprinting; *GNAS* gene)	Similar phenotype as pseudohypoparathyroidism Normal serum PTH, calcium and phosphorus Intellectual disability (more obvious than in PHP)
Kenny-Caffey syndrome, type 1 (AR; *TBCE* gene)	Growth delay Cortical thickening of long bones Hypocalcemia, hypoparathyroidism
PKAN (AR; *PANK2* gene)	Early onset (10% > 10 yr) Pigmentary retinopathy
DRPLA (AD; CAG expansion in *DRPLA* gene)	Phenotype similar to IBGC
Neuroferritinopathy (AD; *FTL* gene)	Dysphagia
PLOSL (AR; *TYROBP* and TREM2 gene)	Radiography: polycystic osseous lesions Frontal lobe syndrome
Cockayne syndrome; Aicardi-Goutières syndrome	Onset in infancy/early childhood

AD: Autosomal dominant; AR: Autosomal recessive; CAG: Cytosine, adenine, guanine; cAMP: 3'-5'-cyclic adenosine monophosphate; CT: Computed tomography; DRPLA: Dentatorubropallidoluysian atrophy; *FTL*: Ferritin light chain; *GNAS*: GNAS complex locus; *GNASAS1*: GNAS complex locus, antisense transcript 1; IBGC: Idiopathic basal ganglia calcification; PANK2: Panthothenate kinase 2; PHP: Pseudohypoparathyroidism; *PKAN*: Panthothenate kinase-associated neurodegeneration; PLOSL: Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy); PTH: Parathyroid hormone; STX1A: syntaxin 1A; *TBCE*: Tubulin-specific chaperone E; TREM2: Triggering receptor expressed on myeloid cells 2; *TYROBP*: Tyro protein tyrosine kinase-binding protein.

their anatomical site and the proximity to vasculature calcifications, containing components such as calcium phosphate and carbonate; other compounds including glyconate, mucopolysaccharide and metals (iron, copper, magnesium, zinc, aluminum, silver and cobalt) may also be found[116]. Abnormal iron metabolism in IBGC has been described in a single case, showing elevated serum ferritin, reduced levels of serum iron and iron-binding capacity. At autopsy iron depositions were found in the liver, the spleen, the bone marrow and the brain[130]. More recent reports confirm abnormalities in metal metabolism (iron, copper, zinc), although there is no consensus whether the metal levels are elevated (cerebrospinal fluid) or reduced (hair) in IBGC patients[131,132].

Diagnosis

IBGC diagnosis is supported by the following criteria: (1) bilateral calcification of basal ganglia; (2) progressive neurologic dysfunction; (3) absence of biochemical abnormalities; (4) absence of infectious, traumatic or toxic cause; and (5) a significant family history (although sporadic IBGC cases have also been described)[116].

However, the diagnosis can only be established by obtaining a computed tomography (CT) or magnetic resonance imaging (MRI) scan of the brain, showing bilateral, almost symmetric calcifications of one or more of the affected brain regions, and ruling out other abnormalities (showing bilateral basal ganglia calcifications, and developmental defects)[116,133-136]. Other possible investigations, which are typically normal in IBGC patients, include blood and urine testing for hematologic and biochemical (ALP, serum creatinine, osteocalcin, serum lactic acid at rest and after exercise, 1,25-dihydroxyvitamin D_3, serum calcium, phosphorus, magnesium, calcitonin, heavy metals and parathyroid hormone (PTH)) parameters and an Ellsworth Howard test (showing a 10-20 fold increase of urinary 3'-5'-cyclic AMP (cAMP) after stimulation with 200 U of PTH)[116,137-139]. Neurological tests are usually normal (electroencephalography, nerve conduction studies, pattern shift visual-evoked potential studies) or show mild abnormalities (brainstem auditory-evoked potentials)[116].

Genetic testing can confirm the IBGC diagnosis. Sequencing of *SLC20A2* is the first choice, as well as deletion/duplication analysis if no mutation is found, with a mutation detection rate of 40%. If no mutations are found, *PDGFRB* and *PDGFB* sequencing can be performed; the precise mutation detection rate is currently unknown. If no molecular confirmation can be obtained, other (genetic) causes of brain calcification should be considered (Table 6), before establishing a clinical diagnosis of IBGC[116].

Differential diagnosis

Symmetrical calcifications of the basal ganglia are not specific to IBGC and a variety of familial and non-familial conditions should be considered. It should be noted that these calcifications can also be incidental findings on CT scan, especially in older individuals (Table 6)[116,140].

Treatment

Since no etiologic treatment is available, management and treatment options focus on symptomatic relief[116,117,120]. Pharmacologic treatment (*e.g.*, anxiolytics

and antidepressants) for the psychiatric and movement symptoms can be attempted[117,120]. Possibly, an early causative treatment may reverse the calcification process, causing complete recovery of mental functions, which was already described in hypoparathyroidism, another basal ganglia causing disorder, provided that an intervention target can be identified[116].

ARTERIAL CALCIFICATION DUE TO CD73 DEFICIENCY

Arterial calcification due to CD73 deficiency (ACDC), also referred to as calcifications of joints and arteries, is an autosomal recessive disease, which usually takes an onset in young adulthood[16,144].

Clinical presentation

ACDC is mainly characterized by prominent and often symptomatic calcification of the large arteries of the lower extremities (iliac, femoropopliteal and tibial arteries), typically sparing the coronary circulation[16,144]. Furthermore, periarticular calcifications of (large and smaller) joints of the lower extremities have been described[144]. Typical symptoms include claudication, hemodynamically significant peripheral obstructive artery disease of the lower limbs, joint swelling and pain[144]. The disease seems relatively rare, being only reported in 3 Caucasian families[144,145].

Pathogenesis

To elucidate the molecular etiology of ACDC, genome-wide homozygosity mapping was performed in three families, revealing homozygous and compound heterozygous loss-of-function mutations in the *NT5E* gene[144,145]. *NT5E* encodes the glycosyl phosphatidylinositol (GPI)-linked plasma membrane CD73 ecto-enzyme, which has 5' ectonucleotidase activity and thus converts AMP to extracellular adenosine and Pi[146]. The enzyme is located on the plasma membrane of vascular cells, supplying adenosine to cell surface receptors[145]. Adenosine is produced immediately downstream of ENPP1 in the extracellular ATP-degradation pathway on the surface of vascular cells, and a lower adenosine level leads to impaired inhibition of TNAP[16,144]. St Hilaire *et al*[144] hypothesized that increased TNAP activity reduces PPi levels, allowing calcification to occur (Figure 1). Since the vascular calcification in ACDC seems to be limited to the lower extremities, it is likely that members of other ectonucleotidase families, such as ectonucleoside triphosphate diphosphohydrolase 1 or CD39 (*ENTPD1*; OMIM*601752) and its isoforms or cardiac ectonucleoside triphosphate diphosphohydrolase 6 or CD39L2 (*ENTPD6*; OMIM*603160) (members of the ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) family), may compensate NT5E activity in other vascular beds[16,147]. An alternative explanation for this predilection may be the particular distribution of adenosine receptors in these vascular beds[148].

Diagnosis

An ACDC diagnosis can be established based on clinical presentation and a full radiographic workup of the patients, as well as determination of the ankle-brachial index, which should be reduced. Plain radiography can visualize the vessel calcifications; magnetic resonance angiography and especially CT angiography can show diffuse and gross calcification of obstructing lesions. Biochemical indices, including serum electrolytes, cholesterol and vitamin D-levels, PTH, C-reactive protein, rheumatoid factor and erythrocyte sedimentation rate should all be normal. The clinical suspicion can be confirmed by *NT5E* sequencing[144].

Differential diagnosis

Other - often not - hereditary causes of vascular calcification have to be excluded, *e.g.,* diabetes mellitus type 2 and impaired renal function[144]. Since joint swelling and pain was present in all three described families, rheumatologic diseases should also be excluded[144].

Management

Because of the rarity of the disease, no treatment guidelines are available. Bisphosphonates, which were proven to be successful in GACI, possibly by restoring PPi levels, may also be a good treatment option in ACDC[91,144]. Adenosine deficiency could be addressed using dipyridamole, which inhibits its cellular reuptake *in vitro* and *in vivo*. Other possible therapeutic options include adenosine receptor agonists or direct TNAP inhibitors (*e.g.,* lansoprazole), all of which need to be further investigated[144].

HYPERPHOSPHATEMIC FAMILIAL TUMORAL CALCINOSIS

Contrary to the disorders described above, autosomal recessive hyperphosphatemic familial tumoral calcinosis (HFTC; OMIM#211900), is characterized by metastatic mineralization[1,149]. Patients usually show first signs in the first or second decade of life[150].

Clinical characteristics

The most prominent clinical manifestation of HFTC is periarticular mineralization of the skin and subcutaneous tissue, mainly affecting the upper limbs and hip regions, although involvement of other localizations (spine, temporomandibular joints, metacarpals/metatarsals and popliteal space) have also been reported[151]. The calcium salt depositions usually present as firm painful tumorlike masses, which may gradually enlarge over a period of years, causing functional problems including restricted joint mobility[149,151]. Complications of the overlying skin, including pain, infection and ulceration, can cause scarring and deformity[1,149,151]. Other possible manifestations of the disorder are dental abnormalities and retinal AS[152].

Pathogenesis

HFTC can be caused by mutations in UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 3 (*GALNT3*; OMIM*601756), fibroblast growth factor 23 (*FGF23*; OMIM*605380) or klotho (*KL*; OMIM + 604824), all of which are key regulators of the phosphate metabolism[1]. GALNT3 protects intact FGF23, a phosphaturia-causing protein, from proteolytic processing by O-glycosylation of Threonine residue 178 in a subtilisin-like proprotein convertase (SPC) recognition sequence motif. In this way, FGF23 is activated and enabled to secrete from the cell, while this glycosilation also competitively inhibits proteolytic FGF23 cleavage by proteases. Hence, this glycosylation step is proposed to be a post-translational regulatory model. In the presence of (nonsense/missense/splice-site) *GALNT3* mutations, intact FGF23 is cleaved prior to secretion which leads to an accumulation of fragmented FGF23 and a reduced amount of active FGF23, causing hyperphosphatemia[153,154]. In physiological conditions FGF23 binds to the FGF receptor 1, of which KL, a β-glucuronidase, is an important co-receptor, inducing high affinity interaction between FGF23 and its receptor. This activates the further downstream effects of this pathway, including the maintenance of serum phosphate levels within the normal range by increasing renal phosphate excretion and both a reduction of synthesis rate and acceleration of the degradation of 1,25-dihydroxyvitamin D_3 to reduce intestinal phosphate absorption (Figure 1)[155,156]. Moreover, KL works independently from FGF23 as an enzymatic inhibitor of renal NaPi-2a (sodium/phosphate cotransporter) transporter activity - which requires glucuronidase activity, subsequent proteolytic degradation and possibly internalization of the transporter - eventually leading to reduced renal expression of the transporter[157]. FGF23 fulfills its biological functions in a tissue-specific way, which is likely to be regulated by the limited local distribution of KL[155]. Inactivating mutations in FGF23 as well as missense mutations in KL cause FGF23 deficiency. Consequently renal phosphate reabsorption and 1,25-dihydroxyvitamin D_3 synthesis is increased, leading to elevated serum concentrations of phosphate, 1,25-dihydroxyvitamin D_3 and calcium and ectopic mineralization[155].

Diagnosis

Next to clinical examination and family history, the diagnosis of HFTC is mainly based on a full radiographic workup: (1) Plain radiographs show the typical appearance of periarticular amorphous, multilobulated and cystic calcifications[158]; (2) CT, showing cystic loculi with fluid-fluid levels caused by calcium layering; (3) MRI imaging, showing lesions of inhomogeneous intensity, help to document the extent and interconnectivity of individual lesions and can help to determine possible surgical approaches; (4) scintigraphy, using a phosphate compound radiolabel (technetium-99m methylene diphosphonate) is helpful in determining the activity level of the disease; and (5) ultrasonography can help to localize fluid collections[149]. A typical feature of HFTC is the absence of erosion/bone destruction by adjacent soft-tissue masses[149]. Biochemically, hyperphosphatemia with normocalcemia, normal or slightly elevated 1,25-dihydroxyvitamin D_3, hypoparathyroidism and low intact FGF23 proteins levels can be found[152]. A biopsy should be avoided, because of the risk of an infection and should only be done as a last resort. Histopathology shows that mineralization depositions fill up the cystic loculi (active stage), which causes them to become encapsulated by fibrous tissue, eventually ending the mineralization process (relative latent stage)[150].

Differential diagnosis

The differential diagnosis of HFTC is summarized in Table 7.

Management

HFTC should be treated according to the location, size of the lesion and its relations to its environment. A first treatment option is medically reducing hyperphosphatemia through phosphate depletion, by dietary phosphorus restriction and/or the administration of phosphate binding chelating agents such as aluminum hydroxide. This method has a variable success rate, both in normo- and hyperphosphatemic cases. When combined with acetazolamide, which induces phosphaturia, a synergistic effect occurs, especially in the hyperphosphatemic form of familial tumoral calcinosis[149].

A second treatment option is early surgical resection of the lesions; however a considerable recurrence rate of the lesions, which have the tendency of growing faster, poses a major problem. Therefore it is very important that resections contain the lesion, and preferably should contain a wider perilesional area/band, including the hypervascular region beyond the periphery of the lesion. Broad resections can cause problems in case of voluminous lesions, which may require extensive reconstructive surgery[149]. Surgery is indicated when lesions are painful, recurrently infected, ulcerated or when functional impairment occurs[149]. Surgical complications include: (1) prolonged drainage of the wound, possibly leading to delayed wound healing and sinus tract formation; (2) secondary infections due to chronic wound problems, especially when the disease is extensive or when resection is incomplete; and (3) recurrence, which is more frequently seen after incomplete resection[167].

During the active stage of the disease, primary medical treatment of HFTC is justified and may even be recommended, because the postresection recurrence rate of lesions is even higher in this stage. In the relative latent stage, encapsulation occurs which hinders ion exchange, thus making surgery more advantageous[149]. Alternative treatment options, including steroids,

Table 7 Differential diagnosis of hyperphosphatemic familial tumoral calcinosis[149,151,158-166]

Disease	Distinct differences with HFTC
Calcinosis universalis	Calcium depositions in tendons and muscle tissues Normophosphatemia High hemosedimentation Microcytic and hypochromic anemia
Calcinosis circumscripta	Adult onset Local calcinosis Fingers symmetrically affected
Calcific tendinitis	Adult onset Calcification limited to tendons
Synovial chondromatosis	Lesions arising from synovial tissue Widespread throughout the body Not all lesions are calcified
Osteosarcoma	Long bone malignant tumor 2nd life decade or late adulthood No subcutaneous/skin lesions
Fibrodysplasia ossificans progressiva (AD; *ACVR1* gene)	Hallux valgus, monophalangism and/or malformed first metatarsal Sporadic episodes of painful soft tissue swellings (flare-ups) in 1st life decade
Tophaceous gout	Severe form of gout Severe joint deformity, chronic pain and functional decline More prominent in Asian population (slow metabolizers) and in young men with strong genetic predisposition
Calcific myonecrosis	Post-traumatic (time interval of several years possible) Lower limbs only
NFTC (AR; *SAMD9* gene)	Reddish-to-hyperpigmented skin lesions during the first year of life (preceding calcified nodules) Severe conjunctivitis and gingivitis Normophosphatemia
Secondary tumoral calcinosis	Renal insufficiency, hyperparathyroidism, or hypervitaminosis D
Rheumatological diseases	Usually normophosphatemia and - calcemia Possibly positive results in antinuclear, anti-Smith, anti-centromere and anti-scleroderma antibodies, which should all be negative

ACVR1: Activin A receptor, type 1; AD: Autosomal dominant; AR: autosomal recessive; HFTC: Hyperphosphatemic familial tumoral calcinosis; NFTC: Normophosphatemic familial tumoral calcinosis; SAMD9: Sterile alpha motif domain-containing protein 9.

bisphosphonates, calcitonin and radiotherapy, have not been proven to be effective[149].

CONCLUSION

Ectopic mineralization disorders comprise a wide range of heterogeneous diseases, which can affect a variety of tissues, causing important health problems. Insights in the mechanisms that cause these diseases have led to the observation that many - if not all - are closely related to one another from a mechanistic point of view. The considerable differences in clinical presentation and natural course however suggest that our current knowledge is merely the proverbial tip of the iceberg and that the subtle mechanisms which render each disease to be unique are still largely to be uncovered. Nevertheless, the pathophysiological knowledge to date has already led to several successful treatment options and a number of promising targets for the future. As such, ectopic mineralization diseases are a fine example for the interaction between the variome and the phenome.

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REVIEW

Role of quorum sensing in bacterial infections

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Abstract

Quorum sensing (QS) is cell communication that is widely used by bacterial pathogens to coordinate the expression of several collective traits, including the production of multiple virulence factors, biofilm formation, and swarming motility once a population threshold is reached. Several lines of evidence indicate that QS enhances virulence of bacterial pathogens in animal models as well as in human infections; however, its relative importance for bacterial pathogenesis is still incomplete. In this review, we discuss the present evidence from *in vitro* and *in vivo* experiments in animal models, as well as from clinical studies, that link QS

systems with human infections. We focus on two major QS bacterial models, the opportunistic Gram negative bacteria *Pseudomonas aeruginosa* and the Gram positive *Staphylococcus aureus*, which are also two of the main agents responsible of nosocomial and wound infections. In addition, QS communication systems in other bacterial, eukaryotic pathogens, and even immune and cancer cells are also reviewed, and finally, the new approaches proposed to combat bacterial infections by the attenuation of their QS communication systems and virulence are also discussed.

Key words: Quorum sensing; Virulence; Infections; *Pseudomonas aeruginosa*; *Staphylococcus aureus*; Animal models

Core tip: In this manuscript we discuss the basics aspects of quorum sensing (QS) and its relationship with human infections, focusing in two major QS bacterial models, the opportunistic Gram negative bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

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INTRODUCTION

Several important bacterial pathogens, like *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), and *Vibrio cholerae,* utilize quorum sensing (QS) cell communication to coordinate the expression of multiple virulence factors and associated behaviors such as swarming and biofilm formation, once a population size threshold is reached. QS systems consist of an enzyme that catalyzes the synthesis of the signal (such as acyl-homoserine lactones or cyclic peptides) and a receptor that binds the signal and reprograms the expression of several genes, including those encoding the enzyme that produces the signal, creating a positive feedback loop. In bacterial pathogens, most of the QS controlled genes codify several different virulence factors, such as proteases, toxins, and adhesins[1]. The expression of QS controlled phenotypes is energetically costly to the cells and only provides an advantage if it is expressed when cells reach high densities[2,3]; hence, in the context of bacterial infections, the expression of QS regulated virulence factors is delayed until a sufficient bacterial load is achieved and once this threshold is reached, bacteria coordinate their attack against the host, which maximizes the probability of establishing the infections and disseminating them, hence increasing the pathogen fitness. In fact, QS along with subversion of the immune system are the main factors that determine the bacterial infectious doses. Hence those bacterial pathogens that need small doses to infect, generally lack QS systems but are very effective at inactivating the immune response by killing professional phagocytes. In contrast, those bacterial pathogens that need high infectious doses rely in QS for the coordination of the expression of virulence[4]. In this review, the current knowledge about QS control of virulence factors in two main model bacterial pathogens, *P. aeruginosa* and *S. aureus* (which are also responsible for nosocomial and wound infections), will be discussed along with the relationship of their QS systems, its virulence in animal infection models, and the data available from human infections. Furthermore, the role of QS in other important infections and the role of QS in immune and cancer cells are discussed. Finally, proposed novel approaches of blocking QS/virulence as an alternative in fighting recalcitrant bacterial infections are also reviewed.

QS-CONTROLS OF THE EXPRESSION OF VIRULENCE FACTORS *IN VITRO*

P. aeruginosa

P. aeruginosa possesses at least three functional QS circuits; two of them are mediated by N-acyl homoserine lactones (HSL) signals and the other mediated by quinolones (Figure 1). The HSL-QS systems were first described and they were named after the virulence factors that were first identified under their control; hence, the Las system was discovered as a positive regulator for elastase production through the expression of the structural elastase gene lasB[5]. This system (by LasI HSL-acyl-synthase) produces the 3-oxo-C12-homoserine lactone (3-oxo-C12-HSL), that binds its receptor LasR which then dimerizes and binds promoters that contain *las* boxes, turning on the expression of several genes, including lasI, which then in a positive feedback loop increases the production of *3*-oxo-*C*12-HSL, the other HSL mediated QS system was named Rhl since it controls the expression of the biosurfactant rhamnolipids[6]. This system (RhlI) produces N-butyryl-L-homoserine lactone that is sensed by RhlR and also shows positive autoregulation[7]. The third QS system is mediated by different kinds of signals, alkyl quinolones, specifically 2-heptyl-3-hydroxy-4-quinolone(Pseudomonas quinolone signal or PQS) which is synthesized from anthranilate by the products of *pqsABCDEH* genes and sensed by PqsR (MvfR)[8,9]. The three systems are interconnected and function in a hierarchical way[10]; the Las system is the first to become activated, and it in turn it stimulates the Rhl and PQS systems[11,12], while PQS activates Rhl[13] and RhL inhibits PQS[11,14]. Moreover, 3-oxo-*C*12-HSL, the Las signal, is able to bind functional

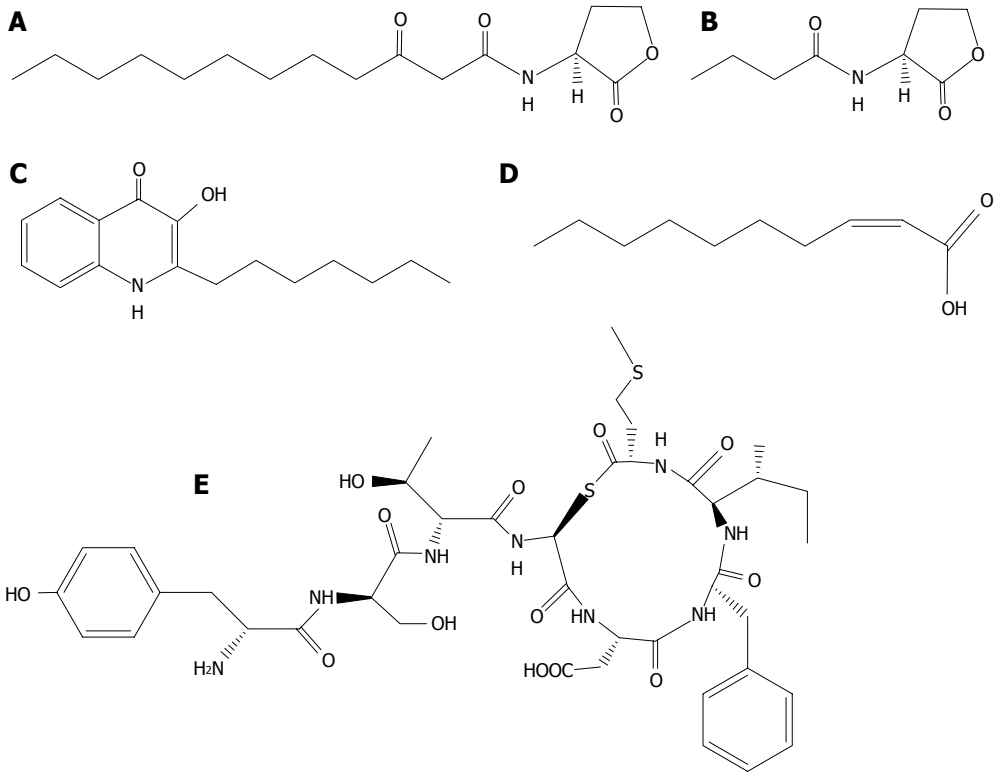


Figure 1 Structures of representative quorum sensing signal molecules of *Pseudomonas aeruginosa*. A: 3-oxo-C12-homoserine lactone; B: N-butyryl-L-homoserine lactone; C: 2-heptyl-3-hydroxy-4-quinolone; D: DSF-like fatty acids, cis-2-decenoic acid) and *S. aureus* (E: AIP group I).

RhlR dimmers, promoting their dissociation and inactivation[15]. In addition to control *lasB* elastase, the Las system also controls the expression of *lasA* elastase, exotoxin A (PA1148), and alkaline protease (PA1246)[16], and the Rhl also controls the expression of the phenazine pyocyanin a pigment able to cause oxidative damage to the eucaryotic host, promoting the production of reactive oxygen species and depleting the host antioxidant defense mechanisms[17], while the PQS system increases the expression of *lasB* elastase and pyocyanin[9]. In fact, the regulation of virulence factors by these 3 QS systems is complex and often overlaps[18]; for example, RhlR is apparently enough to compensate the absence of LasR at least in stationary phase cells in which it promotes the production of exoproteases, pyocyanin, PQS, and the 3-oxo-*C*12-HSL[18,19]. To add even more complexity, recently the role of environmental signals, such as the availability of iron and phosphate in influencing QS systems has been beginning to be explored[20]. In addition, other ions such as calcium strongly influence the production of QS modulated virulence factors such as pyocyanin, and proteases[21] and in fact there is solid evidence that indicates that the chemical composition of the sputum in cystic fibrosis patients promotes the use of the PQS system for communication, preferentially over the HSL systems[22]. Moreover the presence of metabolites like 2,3-butanediol (end product of bacterial fermentation from species that coexist with *P. aeruginosa* in the lung of cystic fibrosis patients) enhance the production of QS controlled virulence factors (phenazines and exotoxin) and improve biofilm formation *via* the Las QS system[23]; hence, the expression of QS-virulence factors *in vivo* is likely influenced by several variables, related with the state of the host as well as the presence or absence of other bacterial species. Indeed, the simultaneous utilization of several QS systems in bacteria, may serve different purposes like identifying community composition[24] or distinguish phases in population development[25], and a recent study shows that the concomitant utilization of Las and Rhl systems allows *P. aeruginosa* to simultaneously assess their population density and the presence of nutrients by combinatorial communication. Therefore, the secretion of QS controlled factors is subjected to "AND-gate" like responses to multiple signal inputs, allowing effective expression of secreted factors in high-density and low mass-transfer environments[26]. Another important role of QS systems in regulating bacterial physiology is that they are implicated in the tolerance against stress[27-29] that allow them to maximize their chances to effectively contend and survive the immune system attack[30], which may be a major determinant for the establishment and progression of *P. aeruginosa* and other pathogens infections.

S. aureus

S. aureus produces several virulence factors and many

of them are regulated by QS. In Gram positive bacteria, regulation by QS is generally mediated by autoinducing cyclic peptides. Specifically for *S. aureus,* QS controls the expression of virulence factors such as hemolysins, leukocidins, cell surface adhesins, exoenzymes, and biofilm formation *via* the Agr system, which relies on the autoinducing peptide (AIP) (Figure 1E). AIPis encoded by *agrD* and consists of 7-9 amino acids, and has a 5-membered thiolactone ring[31-33]; this peptide is secreted by the membrane protein AgrB and activated by the AgrC sensor kinase[1]. The Agr system regulates the expression of several genes by the production of two regulatory RNAs, RNAII and RNAIII[34], which are produced from promoters P2 and P3 respectively[34,35]. Transcription from the *agr* operon (*agrA*, *agrB*, *agrC* and *agrD*) is regulated by a phosphorylated AgrA homodimer from P2[36], while RNAIII is produced by AgrA from P3. RNAIII, which is the effector of the system, upregulates *α*-haemolysin, and increases the production of proteases, toxins, and the synthesis of capsule, while it repress protein A (which allows *S. aureus* to evade opzonization), and the expression of surface adhesions[1,31,34,35,37,38]. Such modulation of the expression of several virulence factors by the Agr system allows *S. aureus* to express a different repertoire of those determinants according to the kind of disease and the environmental conditions including the host status. Noteworthy is that *in vitro* the appearance of clones with diminished QS had been observed; these clones are apparently social cheaters which exploit cooperative individuals without contributing with the production of virulence factors. The presence of cheaters during infections may be very relevant for disease progression, since in controlled experiments, the ratio between cheaters and cooperating individuals strongly affects the mortality rate and extent of infection; *i.e.*, the severity of the infections are inversely proportional to the percentage of cheaters in the population[39]. Among the QS-controlled virulence factors in *S. aureus*, RNAIII is very important since it regulates biofilm formation, and resistance to antibiotics as well as the establishment of chronic infections is intimately related to the biofilm formation abilities of pathogens[40]. However, the *in vivo* biofilms in which *S. aureus* exists can be very complex environments, due the presence of several other bacterial species and their multiple interactions with each other and with the host, hence *in vitro* models for studying *S. aureus* virulence may have the disadvantage of not revealing the real expression of virulence factors. This hypothesis is supported by significant differences in the expression of several virulence factors in *S. aureus* grown in calf serum compared with those grown in defined CDM medium, since in serum the expression of hemolysins, enterotoxins, proteases, iron acquisition factors, and of RNAIII is significantly higher than in standard growth medium, and such differences are partially due the low iron concentration in serum[41,42].

QS-CONTROLS OF THE EXPRESSION OF VIRULENCE FACTORS *IN VIVO P. AERUGINOSA* QS AND VIRULENCE IN ANIMAL MODELS

A number of animal models including the nematode (*Caenorhabditis elegans*), fruit fly (*Drosophila melanogaster*), zebrafish (*Danio rerio*) and mouse (*Mus musculus*), have been used to identify and define the role of virulence determinants in the pathogenesis of *P. aeruginosa*[43,44]. The attenuation of its QS is achieved by two basic strategies: (1) the utilization of mutant strains with QS genes disrupted; and (2) the quenching of QS by treatments that interfere with it; these methods have shown that QS systems as well as QS-independent virulence determinants are required for *P. aeruginosa* infections in animals.

The main animal model that was used to discover the relationship between QS and virulence of *P. aeruginosa* is the nematode *C. elegans*. In 1999 Tan and coworkers, first described conditions to test the role of QS in virulence using this model, showing that the reference strain PA14 kills the nematode either after days (slow killing) or quickly after a few hours (fast killing)[45]. Their evidence indicate that fast and slow killing occur by distinct mechanisms; the slow killing involves an infection-like process and correlates with accumulation of PA14 within worm intestines, while the fast killing is mediated by the production of phenazines(regulated by QS); that increase active oxygen species[45,46]. A third mode by which *P. aeruginosa* can kill *C. elegans is lethal paralysis; this mechanism is mediated by QS since* Darby and coworkers, using QS-less mutant strains of *P. aeruginosa*, found that the lethal effect is associated with a rapid neuromuscular paralysis, caused by the action of diffusible unidentified factors whose production requires the *las* and *rhl* genes, since the infection with a *lasR* mutant and with a *rhlR* reduces the paralysis (by 28%-100% and 100% respectively)[47]. A potential target of these diffusible factors is the EGL-9 worm protein, which is expressed in the neuronal muscle tissues[47]. In a recent study, a reduction of 83% in the death of the nematodes by the double mutant (PA14*rhlRlasR*) was reported; however, the analysis of individual mutants, revealed that only the *rhlR* mutant reduced death 69%, implying that the RhlR system is crucial for infection under their experimental conditions[48]. In addition to lethal paralysis and slow and fast killing, a fourth kind of *C. elegans* death induced by *P. aeruginosa* is the "red death", characterized by the formation of red precipitate (PQS + Fe^{3+} complex) within the intestine of the nematodes. This mode of death is mediated by the quinolone dependant QS system Mvfr-PQS in coordination with the PhoB phosphate sensor and the pyoverdine iron acquisition system[49]. The role of QS in *P. aeruginosa* infectivity and virulence in *C.*

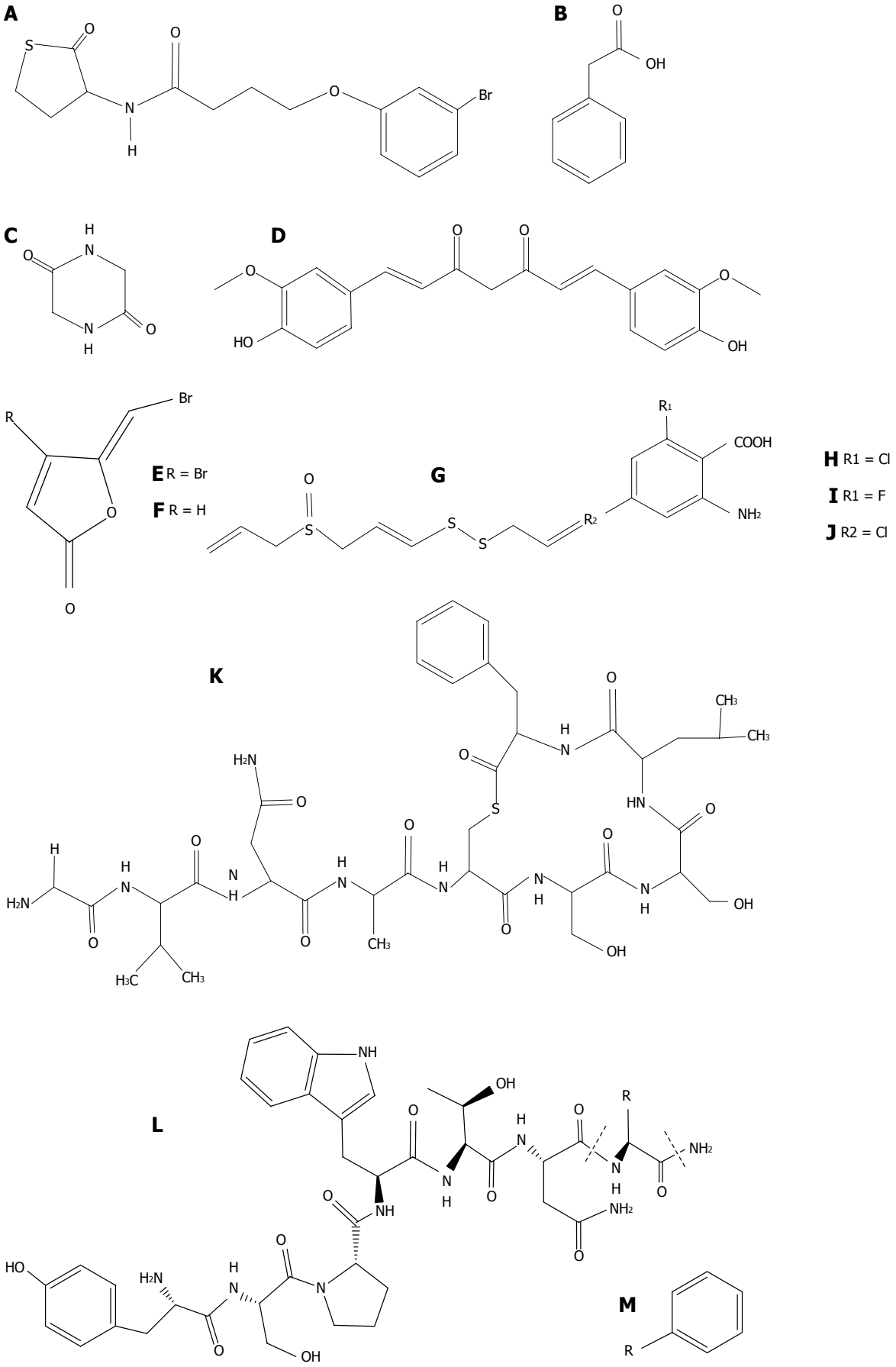


Figure 2 Structures of quorum sensing inhibitors evaluated in animal models. A: Meta-bromo-thiolactone; B: Phenylacetic acid; C: 2,5-piperazinedione; D: Cucurmin; E: Furanone C-30; F: Furanone C-56; G: Ajoene 4,5,9,-trithiadodeca-1,6,11-triene-9-oxide; H: 2-amino-6-chlorobenzoic acid; I: 2-amino-6-fluorobenzoic acid; J: 2-amino-4-chlorobenzoic acid; K: AIP-II; L: RIP (H-Tyr-Ser-Pro-Trp-Thr-Asn-Phe-NH_2); M: FS8 (H-Tyr-Ser-Pro-Trp-Thr-Asn-Ala-NH_2).

elegans is also evidenced by the effect of QS inhibitors, since a synthetic analog of HSL, meta-bromo-thiolactone (Figure 2A) that partially inhibits *in vitro* the LasR and RhrlR systems also reduces the death of worms infected with PA14, to 60% at 24 h. Interestingly, the *in vivo* action of the quencher in the worm model occurs mainly through the RhrlR system[48]. Moreover, phenylacetic acid (Figure 2B), which is a byproduct of the degradation of antibiotics such as penicillin G and cephalosporin Gby G acylase[50], increases the survival of PAO1 infected nematodes by 53%, while untreated worms die within 72 h. This protective activity is perhaps a consequence of interfering with the LasR and RhrlR systems, due to the structural similarity of phenylacetic acid with salicylic acid, a quorum quencher[51]. Another compound,2,5-piperazinedione (Figure 2C), increases the survival of worms by 66%, compared to untreated ones, and it was shown by molecular docking that it interacts with an amino acid residue (E145) in LasR, which is required for correct binding of the natural HSL ligand[52]. Similarly cucurmin (Figure 2D), a secondary metabolite from *Curcuma longa,* increases the survival of worms by 28%; this compound decreases the expression of genes involved in biofilm formation and attenuates HSL production in PAO1. Thus, it was suggested that it may act as a quorum quencher, delaying the synthesis of HSL molecules or by impairing autoinducers perception[53]. Moreover, various enzymes that degrade natural autoinducers are able to decrease the pathogenicity of *P. aeruginosa*; for example, adding the purified acylase PvdQ to *C. elegans* infected with *P. aeruginosa* PAO1, strongly reduces their pathogenicity and increases the nematodes life span[54]. Although the utilization of *C. elegans* as a model for studying *P. aeruginosa* infections has been very fruitful, recently it was proposed to use the fruit fly (*D. melanogaster*) as an animal model for the study of the *P. aeruginosa* pathogenesis, since the fly has a higher similarity to human[55-57]. The importance of both *P. aeruginosa* HSL QS pathways for infection was also demonstrated in *D. melanogaster* using the feeding assay, in which the bacteria are ingested and a local infection type is established in the intestine. In this assay the PA103 (*las*R), PDO100 (*rhl*I), PDO111 (*rhl*R), PAOR1 (*las*R) and PAOJP2 (*las*I/*rhl*I) mutant strains were avirulent with respect to wild-type PAO1 whose infected flies were killed at 14 d post-infection. Similarly, using the nicking assay (needle pricking), in which an injury is produced in the dorsum of the flies and *P. aeruginosa* is added to the wound, all mutant strains showed a lower death rate than wild-type, including the PDO100 mutant (*rhlI*) with 50% survival of the flies compared to 90% death for the PAO1 wild-type 35 d post-infection[57]. However, in contrast to the work with *C. elegans,* to date the effect of quorum quenching in *P. aeruginosa* virulence in the fly was not yet evaluated. With regard of these two infection models, Clatworthy and colleagues pointed out that a drawback to study *P. aeruginosa* infections using invertebrate hosts are the differences between their immune response and the one of vertebrates. For example, *C. elegans* and *D. melanogaster* do not have an adaptive immunity, or complex multilineage immune cells, such as those present in vertebrates[58]. Thus it is important to analyze the participation of QS in the pathogenesis of *P. aeruginosa* in vertebrate animal models, like zebrafish and mice. Specifically for zebrafish, the microinjection of PA14 QS$^-$ mutant strains (*lasR* and *mvfR*) during two different stages of fish development [28 and 50 h post-fertilization (hpf)], revealed that the participation of these two transcriptional activators during the infections is different and is influenced by the maturity of the immune system at different stages of the embryo development, since for the *lasR* mutant, only a 40% decrease in the death of the embryos at 50 hpf (a developmental stage when both macrophages and neutrophils are present) was recorded, whereas the *mvfR* mutant showed a moderate effect by decreasing death by 20% to 28 hpf (an stage in which only macrophages are present), but a higher effect of 60% decrease at 50 hpf[58].

For murine models, different protocols have been used to determine the participation of QS in *P. aeruginosa* pathogenicity[43,44]. The thermal induced injury model is frequently used and consists of producing a burn of second or third degree on the dorsal side of the mouse using water at 90 ℃ and subsequent inoculation of *P. aeruginosa.* Several experiments using this model have linked QS and virulence; for example, a PAO1-R1 (Δ*las*R) mutant has a diminished ability to spread systemically, as well as lower dispersion through the lesion at early stages[59,60]. Also, mice infected with PA14 *pqsA* show a 75% survival rate in contrast to 10% survival with wild-type PA14[61]. Similarly, virulence is reduced in PAO1 *lasR, lasI*, and *rhlI* mutants, with the greatest effect seen for the double mutant *lasI-rhlI* that decreased the mortality of animals by approximately 88%, significantly reduced the number of c.f.u in the lesion, liver and spleen, and delayed the spread of the bacteria from the lesion[59]. In agreement, similar results were found using the pneumonia model in neonatal mice, in which *lasR* mutants showed reduced virulence and are unable to replicate efficiently in the lung tissue; as a consequence, less damage occurs and the bacterial infection does not spread[62]. A third kind of experimental infection, the foreign-body infection model, consists in introducing a fragment of *P. aeruginosa* infected silicone into the peritoneal cavity of mice. This model was successfully used to determine the participation of QS in biofilm formation[43]. In this system, the mutant strain *lasR-rhlR* disappears from the silicone fragments during the first 7 d of infection, in contrast with wild-type PAO1 cells which remain in the silicon implant for at least 14 and up to 21 d. Critically, the establishment of the PAO1 infection in the implants depends in the mouse strain that is used, since for Balb/c, bacterial counts in the implants decayed constantly from day one and several of the implants were completely cleared after 21 d, while for the NMRI strain, bacterial counts initially decreased

(day 1 to 4), then remained constant and finally increased at levels similar to the initials at day 15[63].

Regarding studies testing the quorum quenching effect on virulence, by using the foreign body model, it was found that the intraperitoneal addition of furanone C-30 greatly increased the bacterial clearance rate (Figure 2E)[63]. In agreement a similar effect was observed with the lung infection model in mice in which a related compound, C-furanone 56 (Figure 2F) accelerated the bacterial clearance from the lungs, reducing the severity of the damage and significantly increasing mice survival[64]. Another quorum quencher, the synthetic molecule ajoene (ajoene 4,5,9,-trithiadodeca-1,6,11-triene-9-oxide) (Figure 2G), is able to attenuate the production of various *P. aeruginosa* QS-controlled virulence factors *in vitro*, while in the pulmonary infection model in mice infected with PAO1, its prophylactic administration from two days before the infection and during its course, reduced the bacterial c.f.u. in the lungs 500-fold relative to the non-treated mice[65]. Moreover, in the burn mice model, the intravenous administration of three related quorum quenchers, the anthranilic acid analogues: 2-amino-6-chlorobenzoic acid, 2-amino-6-fluorobenzoic acid, and 2-amino-4-chlorobenzoic acid (Figure 2H-J), that inhibit the biosynthesis of quinolone signals and disrupt the MvfR-dependant gene expression, restrict the systemic spread of the PA14 strain and decreases the animals death by 30% to 50%[61].

QS IN *P. AERUGINOSA* HUMAN INFECTIONS

The importance of the QS systems of *P. aeruginosa* in human infections is highlighted by their presence in most clinical strains that were isolated during the moment of the infection. This was demonstrated in 2004 by Schaber *et al*[66], by screening 200 isolates from patients with urinary tract, lower respiratory tract, and wound infections. Of those isolates, 97.5% (195 isolates) had robust functional HSL-QS communication systems and hence were able to produce elastase (codified by the genes *lasB* and *lasA,* which expression is QS dependent through LasR) and high levels of both HSL autoinducers while only 5 isolates failed to satisfy those criteria; however, 2 isolates were identified as being the same bacteria, but isolated at two different times from the same patient, and for one isolate there was no clinical data available to support that it was implicated in an infective process[66]. Hence only approximately 1% of the isolates that were implicated in infections appeared to be QS deficient. Critically, one of these isolates had no *lasR* and *rhlR* functional genes. In addition the authors demonstrated that those isolates deficient in HSL-QS systems produced high levels of non-QS controlled virulence factors, such as the ExoS and ExoT proteins that are components of the type three secretion system. Hence, perhaps this was an adaptive response that potentially could compensate for the decrease in virulence caused by QS deficiency., Nevertheless, the production of those proteins in the QS proficient isolates was not evaluated, and the virulence of the isolates was not tested using infection models. Other studies have reported similar results;, for example, the characterization of 442 *P. aeruginosa* isolates colonizing the respiratory tract of 13 intubated patients identified 9 genotypically different strains and of these, 6 strains produced both HSL-autoinducers and the virulence factors: elastase, exoproteases, rhamnolipid, hydrogen cyanide, and pyocyanin *in vitro*, and two of them had mutations in both *lasR* and *rhlR* genes, while the third had a mutant *lasR* gene[67]. Another study performed with 100 isolates from patients with respiratory infections that were collected from sputum, tracheal aspirate, and bronchoalveolar lavage identified 11 HSL-QS deficient isolates, six of them with absent *QS* genes (one isolate negative for *rhlR*, two isolates negative for *rhlI* and *rhlR,* and three isolates were negative for *rhlI*, *rhlR*, *lasI* and *lasR*). Interestingly, this study found a negative correlation between the expression of QS controlled virulence factors and antibiotic resistance[68]. Furthermore, the analysis of 82 *P. aeruginosa* clinical strains isolated from urinary tract infections identified 6 isolates deficient in the production of both HSL autoinducers, biofilm, rhamnolipids, and elastase, correlating with the absence of the *lasR* gene in one isolate and the absence of *lasI, lasR, rhlR* in another isolate, while the other 4 isolates harbored point mutations that probably inactivated their *lasI, lasR, rhlR*, and *rhlI* genes[69].

Taken together, these independent studies indicate that about 90% of *P. aeruginosa* isolates that cause infection generally preserve active HSL-QS systems, although clearly a small percentage of the isolates have those systems impaired by mutations or loss of the important QS regulatory genes, nevertheless, in all these studies, the third QS system of *P. aeruginosa*, the quinolone dependent system, was not evaluated; hence, it is not reliable to conclude that these isolates were indeed 100% QS deficient. In addition, the existence of cell communication systems not yet described in this organism cannot be ruled out, and indeed in the reference strain PAO1, cell communication by fatty acids was recently discovered (DSF-like fatty acids, cis-2-decenoic acid) (Figure 1D)[70]. Another possibility that may explain the isolation of QS deficient strains from infections is the presence of multiple *P. aeruginosa* strains in the infection site and that the QS deficient isolates coexist with QS proficient strains; this was demonstrated recently in 8 patients with cystic fibrosis (CF), in which a complex mixture of QS-proficient and deficient isolates were found. Interestingly, among all the patients, the deficiency of the isolates in individual QS regulated phenotypes (LasA and LasB elastase, rhamnolipids, growth in adenosine, and HSL signals) ranged from 0 to approximately 90% and no single

patient with 100% QS deficient isolates was found.

Such high diversity in isolates from the same patient likely is the result of a complex and multifactorial selective process, perhaps including social components like the advantages accrued by QS-deficient clones that use the resources made by the QS positive strains (siderophores, proteases, *etc.*), without contributing to the generation of the public goods; these bacteria are termed social cheaters[71]. Regarding the importance of QS for infections, these results indicate that at the population level, QS may be essential for CF infections; however, more studies increasing the number of CF patients and including other kinds of infections are necessary to better understand the importance of *P. aeruginosa* QS in the infective process. In addition, the elucidation of factors that shape the mosaic-like composition of isolates in patients or in animal models need to be determined in order to design better anti-QS therapies since the current ones are focused on laboratory strains with QS-proficient systems rather than clinical strains recently isolated from infections[72,73]. Although such factors are still unknown, some variables like: the severity and progression of the infection, the nutritional, health, and immunological status of the patients, the exposure of the susceptible individuals to only one, a few, or several strains and the bacterial loads during the infections could be involved. In this sense, animal models would be useful to evaluate the role of these and other valuables in the colonization diversity in the patients, for example experiments comparing the colonization of well feed animals and animals with a deficient nutrition, immune competent animals and immunosuppressed ones, or healthy animals compared to animals harboring important disorders such as the alpha-1-antitrypsin deficiency that promotes major pulmonary inflammation, degradation of lung tissue, and eventually manifestations of pulmonary emphysema, *etc*. using several bacterial strains (QS proficient and QS deficient) alone or in combination could be very valuable to determine the factors involved in the *in vivo* bacterial ecology in infections.

In addition, although *P. aeruginosa* virulence is multifactorial[74], the individual importance of QS controlled virulence factors in different kinds of infections is a current research area, and the role of molecules from the HSL autoinducers themselves, to extracellular factors like rhamnolipids, elastase, pyocyanin, *etc*. have been established. For example several independent studies have shown that the main *P. aeruginosa* autoinducer N-(3-oxododecanoyl)-HSL is readily detected in sputum samples collected from patients with cystic fibrosis[75-77], which correlates with a QS dependent gene expression during the infections[78-80]. However, besides its role as a signal, the autoinducer is also able to inhibit lymphocyte proliferation as well as secretion of tumor necrosis alpha by macrophages and interferon gamma by T-cells[27]. Moreover QS controlled secreted factors such as alkaline protease can interfere with the classical and the lectin pathway-mediated complement activation *via* cleavage of C2, blocking phagocytosis and killing of *P. aeruginosa* by neutrophils[81]. Also elastase, by cleaving the pulmonary surfactant protein-A, can contribute to phagocytosis evasion[82]. Furthermore, rhamnolipids are able to disrupt calcium-regulated pathways and protein kinase C activation, preventing the induction of human beta-defensin-2 in keratinocytes[83]. Remarkably, the production of rhamnolipids in mechanically ventilated patients is associated with the development of life-threatening ventilator-associated pneumonia (VAP), while elastase production and QS independent production of the cytotoxins ExoU and ExoS are not[84]. Another QS controlled virulence factor, the polysaccharide alginate, protects *P. aeruginosa* biofilm cells from IFN-gamma-mediated macrophage killing[85]. Surprisingly, the importance of pyocyanin, a blue redox-active compound which is one of the main *P. aeruginosa* virulence factors studied *in vitro* and one of the more notorious in infections (present in large quantities in sputum from patients with cystic fibrosis infected by *P. aeruginosa)* during clinical infection is still underexplored[86].

S. AUREUS QS AND VIRULENCE IN ANIMAL MODELS

The participation of SarA and Agr *S.aureus* QS systems in pathogenicity has been evaluated using numerous animal models, in which the bacteria induce diseases such as osteomyelitis, septic arthritis, endocarditis, endophthalmitis and soft tissue abscesses. By using the mutant strains *agr* and *sarA,* as well as QS inhibition, the participation of these systems in the infectivity of the bacterium and the damage of tissues has been proved. Intravenous inoculation of the bacteria in mice induces the development of septic arthritis. In this model, *agr* mutants showed a reduced ability to induce the pathology since it is produced in only approximately 10% of the animals while the wild-type strain produces it in approximately 60% of the inoculated mice. Furthermore, in mice infected with the mutant strain, the arthritis severity is less, and only a few developed erosive arthropathy in contrast to those infected with wild-type[87]. Similarly in the endophthalmitis-rabbit model, which is established by the intraocular injection of the bacteria, *agr* mutants produced a smaller loss of neuroretinal function during the first 3 d of the infection, with respect to the wild-type strain. In addition, those infected with the mutant strain had normal eye histology, whereas those infected with the wild-type strain showed focal retinal destruction and mild vitritis[88]. In a subsequent study employing the same animal model, no significant differences in the rabbits eyes infected with the mutant strain (*sarA*) and with the wild-type were found; however, the simultaneous deletion of genes *agr* and *sarA* resulted in a near to complete attenuation of virulence[89]. Moreover, employing the model of endocarditis in rabbits, which consists in introducing a

catheter into the ventricle and subsequently colonizing it by intravenous administration of the bacteria, it was observed that single mutations (*sarA* and *agr*) diminish the bacterial ability to induce the pathology, while a *agr*/*sarA* double mutant was incapable of inducing endocarditis in 100% of the animals inoculated with 10^3 or 10^4 c.f.u.[90]. Another infection model in mammals is the murine brain abscess model in which lesions are produced by embedding bacteria in agarose beads that are later inoculated in the cranial cavity. In this model, the *agr*/*sarA* double mutant, but not the single mutants, had reduced virulence, lower proliferation in the brain and poorly developed abscesses that were drastically smaller than those produced by the wild-type strain. Furthermore, the double mutation attenuates the expression of pro-inflammatory cytokines and chemokines[91]. Similarly invertebrate models such as the nematode *C. elegans*, which is killed by feeding on *S. aureus,* showed similar outcomes, since mutating *sarA* or *agr* increased the survival of the worms with respect to the wild-type strain[92].

Regarding the effect of quorum quenching in *S. aureus* animal infections, several inhibitory peptides have been evaluated; for example, in the murine subcutaneous abscess model, the administration of the synthetic autoinducer analog of AIP-Ⅱ peptide (Figure 2K) in a single dose was able to decrease the formation of abscesses, and although AIP-Ⅱ prevents expression of the *S. aureus agr* QS regulon for only a short time period, this transient inhibition is sufficient to achieve significant effects[93]. Other QS inhibitory peptides such as the RNAⅢ-inhibiting peptide (RIP) and its analogues (Figure 2K and L), that inhibit the phosphorylation of a target protein called "target of RNAⅢ-activating protein" (TRAP), leading to the suppression of virulence factor production *in vitro*[94,95], are also effective *in vivo.* For example, in the vascular-graft rat model, the administration of RIP (Figure 2L), both locally and systemically, is able to completely inhibit the formation of biofilms in graft and in polymethylmethacrylate beads infected with methicillin-susceptible and resistant *S. aureus*. Similarly, in the mouse sepsis model, administering RIP significantly reduced the bacterial load and mice mortality; this effect is potentiated by co-administration of antibiotics like cefazolin, imipenem, or vancomycin[96]. In addition, using the graft rat model, a RIP treatment increases its effectiveness in combination with antibiotics rifampin and temporin, and the complete elimination of infection is achieved by combining it with temporin A[97]. The same phenomenon has been documented with a derivative of RIP, termed FS3, which contains a substitution of alanine in the second position, since FS3 in combination with daptomycin has higher efficiency than single compounds, in the rat model of vascular graft staphylococcal infection[98]. Also in this model, a similar effect was obtained, by combining tigecycline and the RIP analogue called FS8 which contains a terminal alanine (Figure 2M)[99]. Taken together these extensive studies demonstrate the participation of *S. aureus* QS systems in its pathogenicity and indicate that QS inhibition in combination with antibiotics is a promising new strategy that may be effective to treat the infections produced by this important pathogen.

QS IN *S. AUREUS* HUMAN INFECTIONS

Although it is not always pathogenic to humans, the Gram (+) bacterium *S. aureus,* is frequently found in the human respiratory tract and on the skin and it is considered as a transient member of the human microbial flora[32], it is able to cause several kind of infections with a plethora of clinical manifestations. There are risk factors that complicate the infection caused by *S. aureus*, including the presence of prosthetic material and immunosuppression[100], and it is considered one of the three main causes of nosocomial bacterial infections. Among the many kinds of *S. aureus* infections, skin ones are very common; for instance, in children it is the main cause of impetigo, a superficial skin infection that according to its clinical manifestations is divided into non-bullous and bullous impetigo, the non-bullous is the most common form, the lesions begin as papules that progress to vesicles with erythema on its periphery. These become pustules that later form adherent crusts with a golden appearance and can be accompanied by regional lymphadenitis, although systemic symptoms are usually absent. Bullous impetigo is seen in young children in which the vesicles enlarge to form flaccid blisters with clear yellow liquid, which later becomes darker and turbid, leaving a thin brown crust[3,4]. Other common infections produced by *S. aureus* are hair follicle infection or folliculitis, furunculosis, and cellulitis. In addition to skin infections, *S. aureus* also causes respiratory tract infections such as nosocomial and septic pneumonia, septic pulmonary emboli and post viral empyema. It also infects the apocrine glands, causes musculoskeletal infections, produces bacteremia and its complications like sepsis, septic shock, and infective endocarditis and is able to produce toxic shock syndrome and food poisoning. Therefore, *S. aureus* is a major health concern worldwide.

Although the role of QS in regulating the expression of several virulence determinants including toxin production, and biofilm formation have been extensively studied *in vitro* and in animal models, its importance in actual human infections is yet under studied; nevertheless, it is known that as in the case of *P. aeruginosa* the great majority of *S. aureus* clinical isolates implicated in human infections possess active QS systems (agr^+). Although some agr^- strains are also commonly found in *S. aureus* infections[101-103], the presence of both kind of strains during infection indicates that agr^+ and agr^- variants may have a cooperative interaction[103] and also raises the possibility that social interactions like QS cheating may exist during the

infections[39]. In addition, the link of QS and biofilm formation in *S. aureus* strongly suggests that this QS is important for the development and establishment of its chronic infections[32]; however, further work in this area is needed to define the importance and the specifics of QS in regulating *S. aureus* virulence in human infections.

QS ANTIVIRULENCE DRUGS

P. aeruginosa

To date, there are a large number of quorum quenching (QQ) compounds reported. In general, the three types of QQ compounds are degraders of AHL autoinducers, synthase inhibitors, and receptor inhibitors. Here the QQ compounds used against *P. aeruginosa* are sorted into seven categories.

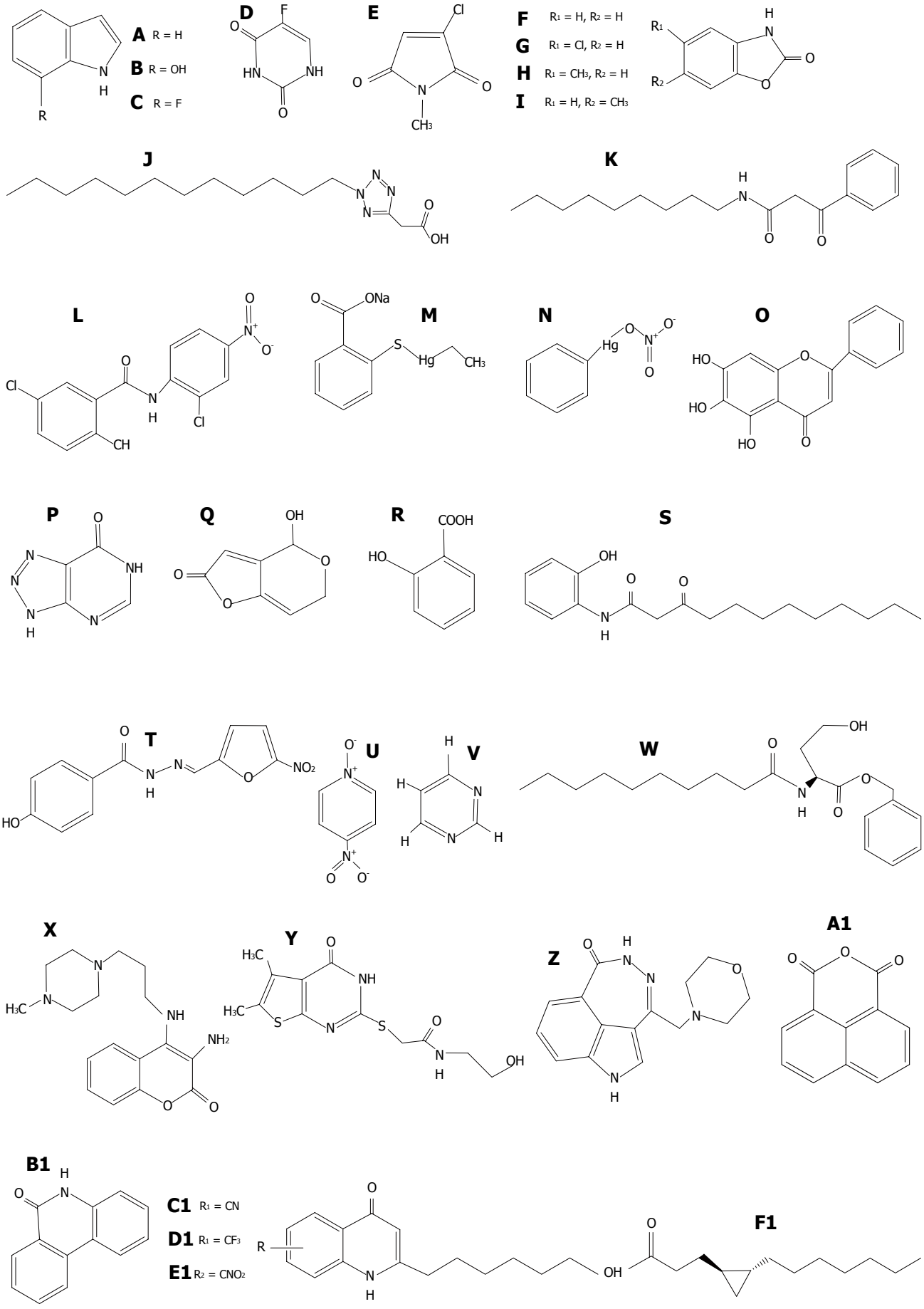
Halogenated compounds

One of the best characterized QQ compounds is the synthetic brominated furanone 4-bromo-5-(bromomethylene)-2(5*H*)-furanone known as C-30 (Figure 2E)[104]. This compound was synthetically modified from the natural brominated furanone (5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(5*H*)-furanone of the algae *Delisea pulchra*. Another furanone compound is 5-(bromomethylene)-2(5*H*)-furanone, also called furanone C-56 (Figure 2F) which is a derivative of the secondary metabolites produced by the algae[105]. Interestingly, although C-30 is effective for the attenuation of several QS-dependent virulence factors *in vitro* and in animal models, resistance against this compound has been found both in laboratory PA14 derived mutants and in clinical isolates; to date, the only resistance described mechanism is the active efflux of this compound by the MexAB-OmpR pump but the existence of other mechanisms cannot be ruled out[72,106,107]. Furanone C-56 affects the processes of biofilm formation and dispersal although it does not influence initial attachment to abiotic substrata. In addition, indole (Figure 3A) produced from L-tryptophan by a variety of bacteria and 7-hydroxy indole (7HI) (Figure 3B), an oxidized compound of indole created by bacterial oxygenases, are extracellular signals that attenuate the production of biofilm and virulence factors in *P. aeruginosa*[108]. In addition, among 31 natural and synthetic indole analogs, 7-fluoroindole (7FI) (Figure 3C) was identified to be a QQ compound capable of reducing the production of virulence factors such as 2-heptyl-3-hydroxy-4(1*H*)-quinolone, pyocyanin, rhamnolipid, pyoverdin, and pyochelin[109]. 7FI shows higher inhibition toward biofilm formation than indole or 7HI.As another fluorine compound which shows the QQ ability, 5-fluorouracil (5-FU) (Figure 3D), an anticancer uracil analog, also is a potent inhibitor of *P. aeruginosa* virulence[110]. Since 5-FU is basically used for clinical purposes as a chemotherapeutic approach in patients with cancer, it holds promise as a QQ compound for clinical use. However, clinical strains resistant against this compound have been identified[72,107].

Based on the previous report that chlorolactone (CL) is an inhibitor to the QQ receptor[111], three other synthetic CL analogs were tested for QQ effects. As a result, meta-bromo-thiolactone (mBTL) (Figure 2A) was the most effective QQ compound since pyocyanin production and biofilm formation were inhibited in the presence of mBTL, in addition, mBTL moderately protected *C. elegans* and human lung epithelical cells from killing by *P. aeruginosa*[48]. Other halogenated QQ compounds include the derivatives of anthranilic acid which is the primary precursor of 4-hydroxy-2-alkylquinolines (Figure 2H-J)[61]. The halogenated anthranilic acid analogs inhibit quinoline biosynthesis and the expression of QS-related genes. Beyond that, halogenated maleimide analogs also are QQ compounds; in particular, bromo- and iodo-substituted maleimides decrease bacterial attachment and biofilm formation whereas chloro-N-methyl-maleimide has bacteriocidal action rather a QQ effect[112]. In addition to this, 5-chloro-1,3-benzoxazol-2(3H)-one[113] also called chlorzoxazone[114] are QQ compounds that contain a halogen group (Figure 3G).

Lactonases and acylases

Degrading enzymes such as lactonases and acylases are another class of QQ compounds. Their effect on QQ is due to the degradation of AHL-based autoinducers. To date, some unique lactonases have been characterized; for example, the halotolerant lactonases derived from *Bacillus* spp[115] and a thermally-stable lactonase from *Bacillus weihenstephanensis* P65[116], which may be useful for future applications. Lactonase itself or in combination with ciprofloxacin prevented systemic spread of the bacteria in murine burn wounds infected with *P. aeruginosa*, while for the combination mice mortality was completely abolished and skin regeneration was promoted[117]. In addition, immobilized esterases and acylases embeded on medical plastic materials inhibit biofilm formation[118]. Another unique approach using a lactonase is to utilize an engineered *Lactobacillus plantarum* strain expressing the lactonase AiiA from *Bacillus thuringiensis* 4A3[119]. Extracellular virulence factors such as pyocyanin, protease, elastase, and rhamnolipids of multi-drug resistant clinical isolates of *P. aeruginosa* were inhibited and the attachment to uroepithelial cells was reduced by co-culturing *P. aeruginosa* with the engineered strain. The original trial was performed using a *P. aeruginosa* strain capable of expressing a lactonase derived from *Microbacterium testaceum*, which led to reduced production of virulence factors and attenuated cytotoxicity against human lung epithelial cells[120]. A new trial using genetic engineering has been recently reported; this is by an engineered T7 bacteriophage expressing a lactonase with activity for a broad-range of bacterial hosts[121]. The engineered T7 bacteriophage was able to inhibit the biofilm formation of a consortium of *P. aeruginosa* and *Escherichia coli*.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C R = F
D
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F R1 = H, R2 = H
G R1 = Cl, R2 = H
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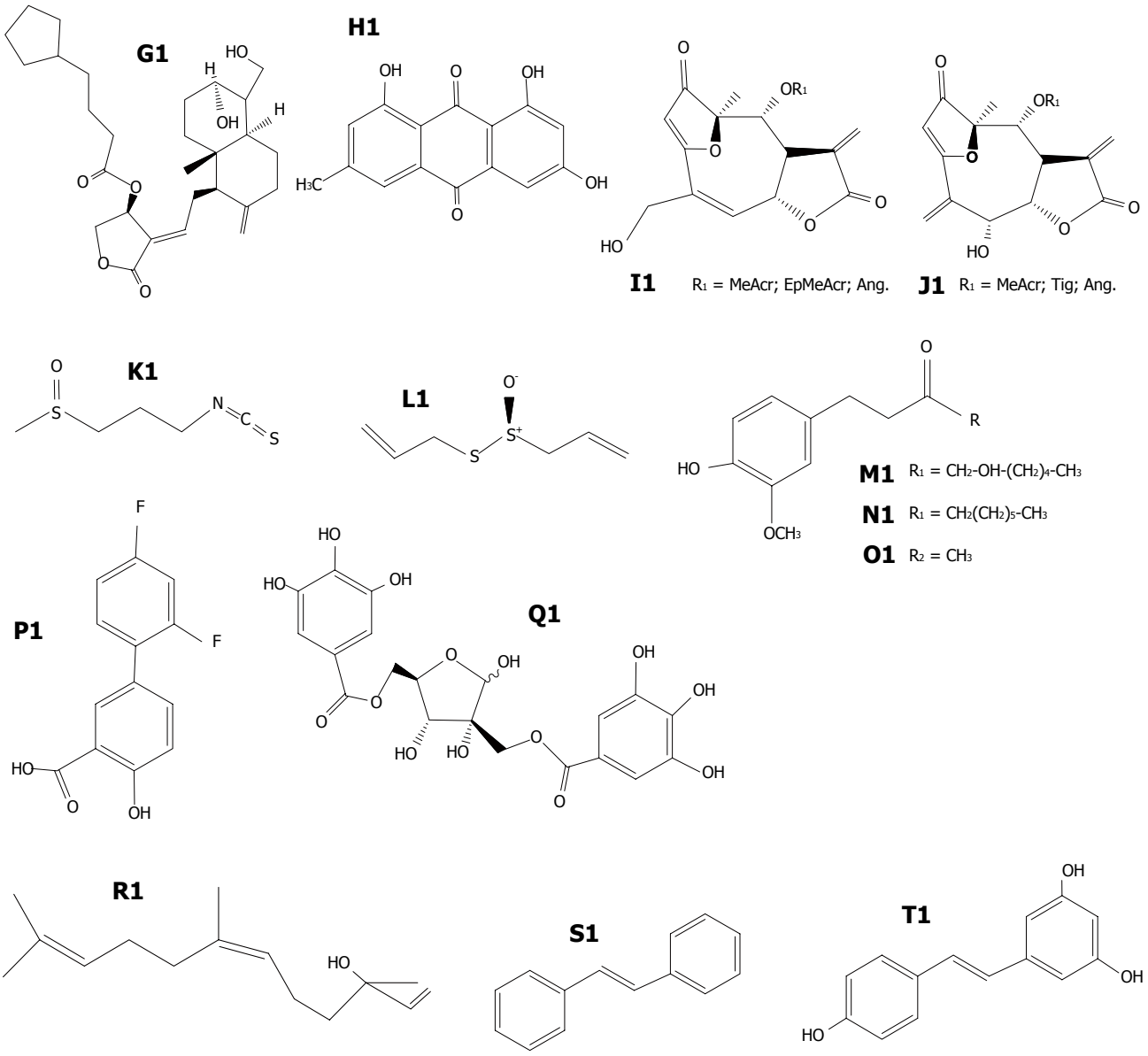

Figure 3 Structures of representative quorum quenching molecules of *Pseudomonas aeruginosa*. A: Indole; B: 7-hidroxy indole; C: 7-fluoroindole; D: 5-fluorouracil; E: 2-chloro-N-methyl-maleimide; F: 1,3-benzoxazol-2(3H)-one; G: 5-cloro-1,3-benzoxazol-2(3H)-one (clorzoxazone); H: 5-methyl-1,3-benzoxazol-2(3H)-one; I: 6-methyl-1,3-benzoxazol-2(3H)-one; J: PD12; K: V-06-018; L: Niclosamide; M: Thimerosal; N: Phenylmercuric nitrate; O: Baicalein; P: 5-imino-4,6-dihydro-3H-1,2,3-triazolo[5,4-*d*]pyrimidin-7-one; Q: Patulin; R: Salicylic acid; S: 3-oxo-C12-(2-aminophenol); T: Nifuroxazide; U: 4-nitropyridine-N-oxide; V: Pyrimidine; W: N-decanoyl-L-homoserine benzyl ester; X: V23; Y: V30; Z: P1; A1: NAP; B1: PJ97A; C1: 6-CN; D1: 6-CF3; E1: 6-NO2; F1: Lyngbyoic acid; G1: Andrographolide 14-(5-cyclopentylvaleryl); H1: Emodin; I1: Goyazensolide-type; J1: Isogoyazensolide-type; K1: Iberin; L1: Allicin; M1: [6]-gingerol; N1: [6]-shogaol; O1: Zingerone and *S. aureus*; P1: Diflunisal; Q1: Hamamelitannin; R1: Cis-nerolidol; S1: Trans-stilbene; T1: Resveratrol.

QQ compounds found by several screening approaches

For searching for novel QQ compounds, structure-based computational screens and high-throughput screens have been conducted. An ultra-high-throughput, cell-based assay to screen a library of approximately 200000 compounds was used to find an inhibitor which can decrease the gene expression regulated by the Las system[122]. As a result, PD12 (Figure 3J), a tetrazole with a 12-carbon alkyl tail and V-06-018, a phenyl ring with a 12-carbon alkyl tail (Figure 3K), which have both similarity with the structure of 3OC12-HSL, were identified as QQ compounds.In addition, a compound having QQ ability was also found among a series of 1,3-benzoxazol-2(3H)-one derivatives[113]; thereby, 1,3-benzoxazol-2(3H)-one (Figure 3F), 5-chloro-1,3-benzoxazol-2(3H)-one (Figure 3G), 6-methyl-1,3-benzoxazol-2(3H)-one (Figure 3I), and 5-methyl-1,3-benzoxazol-2(3H)-one (Figure 3H) have QQ ability. As another approach for clinical application of QQ compounds, the thousands of drugs clinically used in the treatment of different diseases were screened to find drugs with QQ properties which can be applicable to humans. By the screening, it was found that an anthelmintic drug, niclosamide (Figure 3L) strongly inhibits the QS response by *P. aeruginosa*[123], although the active compound was demonstrated to be 5-FU

which was already described as a QQ agent[107,110]. Moreover, since antibiotics are also robust compounds for clinical use, inhibition of QS by antibiotics was surveyed. As a result, it was found that low concentrations of azithromycin, ceftazidime, and ciprofloxacin inhibit QS in *P. aeruginosa*[124]. In addition, QS in a *P. aeruginosa* environmental isolate was inhibited at sub-inhibitory concentrations of tobramycin[125] although other studies demonstrated that a low concentration of tobramycin induces biofilm formation[126].

Screening using a computational approach and molecular docking analysis has also been useful for evaluating the binding capacity of QQ compounds to receptor proteins; thereby, new potential QQ compounds were identified. Pharmacophore modeling and *in silico* screening to find an antagonist for QS in *P. aeruginosa* indicated that a compound with tetravalent lead has QQ ability[127]. Another two compounds thimerosal (Figure 3M) and phenyl mercuric nitrate (Figure 3N) were selected as QQ compounds based on their similarity to the Pb-QQ compound. Also, the automated docking program by which the docking capability of a ligand to a receptor can be analyzed identified 5 potential new QQ compounds; among the candidates, baicalein (Figure 3O) has the strongest QQ ability as it inhibits biofilm formation of *P. aeruginosa* and the QQ effect by baicalein increases synergistically in the presence of ampicillin[128]. Also, another 5 compounds were identified to be QQ by using a structure-based virtual screening approach targeting the QS receptor LasR; of the 5 compounds, the most promising was 5-imino-4,6-dihydro-3H-1,2,3-triazolo[5,4-d]pyrimidin-7-one also called G1 (Figure 3P)[129].

Other AHL antagonists

Some of QQ compounds described above are antagonists of AHL molecules; hence their QS inhibition effect is triggered by interrupting the binding (interaction) between AHL molecules and receptors. To date, there are a large number of AHL antagonists; for example, patulin (Figure 3Q)[130], salicylic acid (Figure 3R)[114], 3-oxo-C12-(2-aminophenol) (Figure 3S)[131], and nifuroxazide (Figure 3T)[114] as well as C-30 (Figure 2E)[104]. In addition, 4-nitropyridine-N-oxide (Figure 3U) is a QQ compound[132], which also reduces bacterial adhesion to silica-coated surfaces[133]. Other QQ compounds are pyrimidine (Figure 3V)[134], N-decanoyl-L-homoserine benzyl ester (C2) (Figure 3W)[135], 2,5-piperazinedione (Figure 2C)[52], and phenylacetic acid (Figure 2B)[51]. The bacterial sensitivities to several antibiotics (tobramycin, gentamycin, cefepime, and meropenem) in the presence of C2 were higher than those without C2[135]. This may be due to the synergistic interactions between C2 and the antibiotics. In addition, QQ by the cyclic dipeptide 2,5-piperazinedione (Figure 2C) might be due to interference with the binding of the natural ligand 3-oxo-C12-HSL to its receptor protein based on the molecular docking analysis[52]. Phenylacetic acid (Figure 2B), which is similar to salicylic acid, has been reported to be a QQ compound[51].

Inhibitors with different QS targets

There are some reports on inhibitors with QS targets different than AHLs and receptors.A new class of antivirulence compounds was reported by Shouldice *et al*[136]; the QQ compounds interact with the bacterial periplasmic protein DsbA, which is essential for the folding and function of exported virulence factors. Another target of QQ compounds is mono-ADP-ribosyltransferase which functions as a bacterial toxin[137]. Some newly-identified QQ compounds were found by using a virtual screen of commercially available compounds combined with a directed poly(ADP-ribose) polymerase; thereby, V23 (Figure 3X), V30 (Figure 3Y), and P1 (Figure 3Z) compounds as well as NAP (Figure 3A1)[137] and PJ97A (Figure 3B1)[138] were identified as inhibitors of toxin production[137]. Other antagonists are a series of compounds targeting PqsR, the receptor of the *pqs* system[139]. Among the analogs of 2-heptyl-4-hydroxyquinoline (HHQ) synthesized, three HHQ analog with 6-CN (Figure 3C1), 6-CF_3 (Figure 3D1), or 6-NO_2 (Figure 3E1) along with n-C_7H_{15} are the best competitors[139], which are promising starting compounds for further drug design.

Cell extracts and secretion products from isolated microorganisms

Based on the concept that microbial interaction (inhibition, repression, acceleration, and dependence) is a complex phenomenon due the large numbers of microbes, a new approach to isolate unique microorganisms with QQ ability and to utilize cell extracts and secretion products has been recently reported. Among the 46 marine bacterial isolates, 11 extracts from *Bacillus*, *Marinobacter*, *Halobacillus*, *Staphylococcus*, or *Ferrimonas* species showed antibiofilm activity against *P. aeruginosa*[140]. The partially-purified antibiofilm compound from S6-15 (similarity with *Bacillus pumilus*) is stable up to 60 ℃ and under neutral and alkaline conditions. In addition, its QQ ability was inactivated by the treatment by enzymes such as proteinase K, trypsin and lysozyme[140]. Also, bacteria able to utilize AHL molecules as a sole source of carbon and nitrogen have been isolated and characterized as AHL-degrading bacteria[141]. Among 41 isolates which retained QQ activity after heat treatment, some of the isolates showed impaired QS inhibition after the treatment by proteinase K whereas the other isolates remained active. In addition, actinomycetes with QQ activity were also isolated from marine sponge. In this study, methanol extracts of 12 actinomycetes had an inhibitory effect on the production of QS-mediated virulence factors[142]; in particular, of the three strains which showed very good anti-QS activity, the most promising strain is NIO 10068 (*Streptomyces* sp.) that secretes cinnamic acid and/or linear Pro-Gly dipeptide which may be QQ compounds. Further bacteria capable of having QQ ability were also isolated from healthy coral species[143]; of 120 bacterial

isolates, up to 24% of the isolates showed anti-QS activity. In particular, a *Favia* sp. coral isolate inhibits the biofilm formation of *P. aeruginosa* by secreting a low-molecular mass compound which is not inactivated by heat and proteinase K[143]. Also, a cell-free lysate of endophytic bacteria isolated from *Pterocarpus santalinus* Linn. also showed QQ activity[144]. *Bacillus firmus* PT18 and *Enterobacter asburiae* PT39 isolated as the endophytic bacteria exhibit potent AHL degrading ability by inhibiting about 80% violacein production in a biosensor strain. QQ activity by the cell lysate was effective against biofilm formation rather than to planktonic cells, and the QQ activity was due to the presence of AHL lactonase in cell-free lysate[144].

Moreover, a small cyclopropane-containing fatty acid, lyngbyoic acid (Figure 3F1), a major metabolite produced by the marine cyanobacterium, *Lyngbya* cf. *majuscule* has been identified to be a QQ compound capable of strongly inhibiting Las-QS system[145]. In addition, the biosurfactant, lunasan produced by *Candida sphaerica* UCP 0995 is also a QQ compound[146]. Recently, it was discovered that a conditioned high density lipoprotein is also a QQ compound capable of reducing the virulence of *P. aeruginosa* by influencing *las*-and *rhl*-QS systems as well as biofilm formation[147]. Furthermore, ultra-small solid lipid nanoparticles for the pulmonary delivery, which are prepared by using various pharmaceutical lipids, are fabricated to deliver QQ compounds to a target site without any penetrable cellular barrier[148]. In this study, plain small solid lipid nanoparticles exhibited anti-virulence properties themselves.

QS inhibitors from food and plant sources

Since biocompatibility of QQ compounds to higher organisms is one of the important requirements for clinical use, there are a lot of trials to find QS inhibitors from food and plants. The anti-QS activity of aqueous extracts from edible plants and fruits, like pineapple, plantain, and sapodilla, was evaluated; most of these extracts showed QQ activity without inhibiting bacterial growth in *P. aeruginosa*[149]. Also, analogs from a natural bicyclic diterpeniod lactone, andrographolide which is the main phytoconstituent from *Andrographis paniculata* Nees (herb), were screened to evaluate QQ activity[150]. An andrographolide-based compound, 14-(5-cyclopentylvaleryl) andrographolide (compound 11b) (Figure 3G1) had the best QQ activity among all the new compounds. In addition, some QQ compounds were found from traditional Chinese medicine by using a molecular docking analysis and QS assays[151]. As a result, emodin (Figure 3H1) had a certain antibiofilm activity as well as the ability to increase the activity of ampicillin against *P. aeruginosa*.

Furthermore, five sesquiterpene lactones of the goyazensolide (Figure 3I1)and isogoyazensolide-type (Figure 3J1)isolated from the Argentine herb *Centratherum punctatum*[152], iberin (Figure 3K1)from horseradish[153], allicin (Figure 3L1)from garlic[154], and phenolic components {([6]-gingerol (Figure 3M1),[6]-shogaol (Figure 3N1), zingerone (Figure 3O1)} from ginger[155] and anacardic acids[156] are QQ compounds, which might be suitable for further development of antivirulence and antibacterial agents.

S. aureus

Since QS regulates the expression of multiple *S. aureus* virulence determinants, and since the frequency of drug resistant clinical strains causing infections is rising (like methicillin-resistant *S.aureus* "MRSA"), several compounds aiming to disrupt these regulatory interactions have been identified; among them, perhaps the best characterized is the QS inhibitor RIP (Figure 2L), an endogenous *S. aureus* peptide that is able to decrease the damage of *S. aureus* in several animal models as discussed before[94,157]. At the molecular level, the production of several toxins is activated in a cell density manner by the RNAIII-activating protein (RAP) and by the autoinducing peptide (AIP), and is inhibited by RIP and by inhibitory AIPs; RAP participation in the pathogenesis consists in inducing the phosphorylation of a 21-kDa protein (known as target of RAP or TRAP). While RIP inhibits its phosphorylation, the phosphorylation of TRAP is essential to create the autoinducing loop since it leads to the activation of RNAIII synthesis[94]. In addition to decreasing the damage of *S. aureus* during infection, RIP treatment also is able to prevent its adhesion to human kidney cells and its biofilm formation on dialysis catheters[158]. Other effective peptides analogues to RIP are FS3 and FS8 (Figure 2M) discussed previously[98,99]. Moreover, recently four AIP non-functional peptide analogues were identified; these peptides have an ample spectrum since they can repress many AgrC receptors (type I-IV) and have a very high affinity. For example, treatment with the peptides block hemolysis (at picomolar concentrations)and attenuate the production of toxic shock syndrome toxin-1 by 80% at nanomolar concentrations; hence, these compounds are the most potent synthetic inhibitors of QS in *S. aureus* to date[159].

Beyond the QS inhibitory peptides, several other interesting molecules able to block the expression of *S. aureus* virulence factors have been discovered, among them, the small molecule biaryl compounds in which the aromatic rings either are either fused or separated by a short linker. This result is particularly interesting, since they are able to inhibit the production of the alpha-hemolysin and the modulin-α toxin in a dose-dependent manner without inhibiting bacterial growth, since they are effective against methicillin-resistant *S. aureus*, and since one of the effective compounds is diflunisal (Figure 3P1), an Federal Drug Administration-approved nonsteroidal anti-inflammatory drug[160]. Diflunisal has the clear advantage that it could be easily repurposed for treating *S. aureus* infections or could be used to coat catheters and other medical devices just as was recently done for 5-FU (Figure 3D)[161], which has QS inhibitory activity against *P. aeruginosa*[110], *Escherichia coli* (*E. coli*)[162] and perhaps several other

pathogens. In addition, some natural products with QS inhibition activity against *S. aureus* like 2,5-di-*O*-galloyl-dhamamelose (hamamelitannin) (Figure 3Q1), a non-peptide analog of RIP found in the bark of the plant *Hamamelis virginiana* had been identified. This compound is effective *in vitro* to inhibit virulence without affecting growth, and *in vivo* in a rat graft model, preventing device-associated infections[163]. Moreover, recently the screening of 83 essential oils led to the identification that black pepper, cananga, and myrrh oils and their common constituent cis-nerolidol (Figure 3R1) strongly attenuate *S. aureus* biofilm formation, its hemolytic activity, and protect *C. elegans* against its infection. Although their mechanism is not fully understood yet, transcriptional analyses showed that at least black pepper oil treatment inhibited the expression of the α-toxin gene (*hla*), nuclease genes, and QS regulatory genes[164]; similar effects can be observed with treatments with trans-stilbene (Figure 3S1)and resveratrol (Figure 3T1)[165].

QS SYSTEMS ARE PRESENT IN SEVERAL OTHER IMPORTANT BACTERIAL PATHOGENS

In addition to *P. aeruginosa* and *S. aureus,* several other bacterial pathogens utilize QS systems to control the expression of multiple virulence factors during infection. Among the more relevant for human health are *Vibrio* spp, *Acinetobacter* spp**,** *Burkholderia cepacea,* and enteric bacteria like *Escherichia* spp. and *Salmonella typhimurium*. The following section is an overview of their known QS systems and their relationship with virulence.

Vibrio spp.

Vibrio is a genus of facultative anaerobic Gram-negative bacteria possessing a curved rod shape (comma shape) typically found in saltwater. Several species are pathogenic to animals including humans and are responsible for food borne infections that are usually associated with eating contaminated food or water. In addition, they also cause wound infections and septicemia. The first QS system was described in the bioluminescent marine bacterium *Vibrio fischeri*, considered the paradigm for QS found in most Gram-negative bacteria. *Vibrio fischeri* colonizes the light-emitting organs of the squid *Euprymna scalopes*, in which it multiplies and reaches a high population density and induces the expression of luminescence genes. This gene expression occurs in a coordinated fashion[166]. The squid uses the light conferred by the bacteria to hide its own shadow in shallow waters and thus avoid predators[167]. To date several QS systems have been described in *Vibrio* spp.

In Vibrio harveyi, the following three QS systems are known: (1) LuxM (synthase), LuxN (receptor) and 3OHC4HSL (signal); (2) LuxS (synthase), LuxP (receptor) and AI-2 (signal); and (3) CqsA (synthase), CqsS (receptor) and CAI-1 (signal).

These systems are associated with bioluminescence, siderophores, protease and extracellular polysaccharide (EPS) production, and other virulence factors[168-170].

In Vibrio cholerae, two QS systems have been described: (1) LuxS (synthase), LuxP (receptor) and AI-2 (signal); (2) CqsA (synthase), CqsS (receptor) and CAI-1 (signal).

These systems have been associated with biofilm formation, EPS production, and other virulence factors[169].

Finally, in *Vibrio fischeri* three QS systems are known: (1) LuxI (synthase), LuxR (receptor) and 3OC6HSL (signal); (2) AinS (synthase), AinR (receptor) and C8HSL (signal); and (3) LuxS (synthase), LuxP (receptor) and AI-2 (signal).

These systems are associated with bioluminescence, host colonization, and motility[168,170]. Other QS systems found in various *Vibrio* spp. and in *Legionella pneumophila* utilize hydroxyketones (AHKs) as signalling molecules[170].

Acinetobacter spp.

Acinetobacter is a genus of aerobic, non-motile Gram-negative bacteria that are widely distributed in nature, commonly occurring in soil. Among them, some species like *Acinetobacter baumannii* (*A. baumannii*) are frequently isolated in nosocomial infections, especially in intensive care units, since they attack debilitated and immunocompromised patients; in addition they have a high tolerance against antibiotics and an inherent ability to acquire antibiotic resistance genes, being therefore a serious emergent health problem. Their QS systems consist of homologues of the LuxR and LuxI proteins of *Vibrio fischeri* known as AbaR (receptor) and AbaI (synthase) and play a role in biofilm formation and motility in *Acinetobacter* spp[171] and in *Acinetobacter baumannii*[172]. This QS system is an important virulence factor responsible for the outstanding antibiotic resistance and survival properties in the latter species[173]. However, the role of QS systems in the regulation of other virulence factors implicated in the development of infection has not yet been established[174].

Synthesis of N-(3-hydroxydodecanoyl)-L-HSL (3-hydroxy-C12-HSL) is catalyzed by AbaI from *Acinetobacter* strain M2 (initially characterized as *Acinetobacter baumannii*, although genomic sequencing studies have distinguished this strain as *Acinetobacter nosocomiales*[174,175]). The completed genome sequence of *A. baumannii* strain ATCC 17978 indicates that autoinducer synthase AbaI (gene *A1S_110*) and acyltransferases may be the sole participants in the synthesis of AHL signals of variable chain length by the organism[176]. Many strains of *Acinetobacter* (63%) produce more than one AHL. However, none of the AHL signals can be specifically assigned to a particular species of the genus[177]. *Acinetobacter* quorum signals are not homogenously distributed, and therefore

distinction between virulent and non-virulent strains on the basis of QS signals is difficult. Communication between bacteria with respect to cell density is integral to the maturation of *Acinetobacter* spp. Biofilm[176,178]. Mutation of *abaI*, which produces the acyl-homoserine lactone molecule, resulted in a 30%-40% reduction in biofilm production relative to that of the isogenic parental strain[173]. Exogenous addition of purified *Acinetobacter* acyl homoserine lactone restored biofilm maturation in the *abaI* mutant[176].

Burkhordelia cepacea

The *Burkholderia cepacia* complex is a group of Gram-negative bacteria composed of at least 18 different species; they are important human pathogens which produce pneumonia in immunocompromised individuals that are affected by lung diseases such as cystic fibrosis. All *Burkhordelia cepacea* complex members encode at least one QS system that consists of homologues of the LuxR and LuxI proteins of *Vibrio fischeri* [CepI(synthase), CepR (receptor), and AHLs C8-HSL and C6-HSL (signal)]. AHL production in the *Burkhordelia cepacea* complex is strain-dependent with respect to both the quantity and type of AHL molecules[179,180]. Another QS system in the *Burkhordelia cepacea* complex is the CciIR system [CciI (synthase), CciR (receptor), and/AHLs C8-HSL and C6-HSL (signal)][181]. Phenotypic assays and global transcript and protein analysis with *cepIR* and *cciIR* mutant strains have shown that AHL-mediated QS controls various functions, including swarming motility, biofilm formation and the production of virulence factors, such as proteases (*e.g.,* the metal proteases ZmpA and ZmpB), siderophores, toxins and antifungal agents[179].

In 2008, Boon *et al*[182] reported the identification of a novel fatty acid signal molecule that is produced by several *B. cenocepacia* strains. The structure of the molecule synthesized by *B. cenocepacia* J2315 was identified as *cis*-2-dodecenoic acid, referred to as BDSF (*Burkholderia* diffusible signal factor). BDSF is structurally related to DSF (diffusible signal factor, *cis*-11-methyl-2-dodecenoic acid), which was first isolated from supernatants of *Xanthomonas campestris* pv *campestris.* The BDSF-regulated QS system is involved in the control of several functions. Mutation of *rpfFBc* resulted in decreased motility, reduced adherence to porcine mucin, diminished exopolysaccharide (EPS) production and lowered protease activity. In addition, the BDSF mutant strains were found to be more susceptible to antimicrobial agents, and their ability to form biofilms was shown to be strongly reduced[179].

Escherichia spp./Salmonella typhimurium

E. coli and *S. typhimurium* are related enteric Gram-negative, facultative anaerobic bacteria. Although most *E. coli* strains are commensal for warm-blooded organisms, such as mammals, some serotypes cause serious food poisoning and other kinds of infections like urinary tract infections and neonatal meningitis, while *S. typhimurium* and other *Salmonella* pathogenic serovars are responsible for Salmonellosis, an infection that causes diarrhea, fever, vomiting, and abdominal cramps. Although usually the illness resolves after four to seven days without medical treatment, several million people are infected by this bacterium each year. In *E. coli* and *S. typhimurium*, three QS systems have been described: (1) Unknown (synthase), SdiA (receptor) and 3OC8HSL (signal). This system has been associated with motility and acid resistance[183]; (2) LuxS (synthase), LsrB (receptor) and AI-2 (signal). Lsr operon expression (AI-2 uptake)[184]; and (3) Unknown (synthase), QseC (receptor) and AI-3 (signal). This system has been implicated in virulence, motility and biofilm formation[185].

QS SYSTEMS BEYOND BACTERIA

QS systems have been extensively studied in bacteria and are of great interest for understanding the development of clinically-significant infections, but whether eukaryotes have cell signaling systems similar to bacterial QS mechanisms is a question that has recently drawn the attention of research worldwide. In this section, we will discuss some examples of eukaryotic microorganisms and human cells that use QS for the development of certain biological functions. The first report of a QS system in eukaryotes was carried out more than 40 years ago, when it was observed that dense cultures of the fungi *Candida albicans* show a reduced tendency towards the morphological transition from yeast to hypha, which is considered a key virulence factor for this opportunistic fungal pathogen[186]. To date, several compounds have been identified as responsible for this phenomenon, such as 2-phenylethanol, tryptophol, farnesol, farnesoic acid, and tyrosol[187]; these QS molecules are secreted by *C. albicans* and when they accumulate over a threshold level, they trigger changes in: (1) fungal dimorphisms[186]; (2) biofilm formation[188]; and (3) expression of virulence genes[189]. In addition, in other dimorphic fungi (*Mucor rouxii, Histoplasma capsulatum, Ceratocystis ulmi*), the "inoculum size effect" is usually observed; however, QS autoinducers in these organisms have not been identified[187]. In 1997, it was discovered that in the protozoan parasite of humans *Trypanosoma cruzi,* the differentiation of replicating and slender forms to non-dividing and stumpy ones is also a density-dependent (quorum) response that limits the population size[190]. This phenomenon is mediated by the soluble factor SIF (stumpy induction factor) that is released by trypanosomes, and a recent study revealed that the QS signaling in *T. cruzi* shares components with the quiescence pathways of mammalian stem cells, providing novel therapeutic targets *via* QS interference[191]. Like those parasitic species described thus far, in 2006 it was demonstrated that the budding yeast *Saccharomyces cerevisiae* endure morphological transition from the yeast form to

Table 1 Quorum sensing systems and quorum sensing-virulence associated phenotypes in the reviewed organisms

Organism	QS systems	Regulated phenotypes
Gram (-) bacteria		
Pseudomonas aeruginosa	(1) LasI (S)-LasR (R)-HSL (s) (2) HSL RhlI-RhlR (3) Alkyl quinolones (PQS)	Expression of several virulence factors including: Pyocyanin, pyoverdine, elastase, alkaline protease, HCN, rhamnolipids and biofilm formation
Vibrio harveyi	(1) LuxM (S)-LuxN (R)- 3OHC4HSL (s) (2) LuxS (S)-LuxP (R)-AI-2 (s) (3) CqsA (S)-CqsS (R)-CAI-1 (s)	Expression of bioluminescence genes and several virulence factors including: Siderophores, protease, EPS production
Vibrio cholerae	(1) LuxS (S)-LuxP (R)-AI-2 (s) (2) CqsA (S)-CqsS (R)-CAI-1 (s)	Expression of several virulence factors including: Biofilm formation and EPS production.
Vibrio fischeri	(1) LuxI (S)-LuxR (R)-3OC6HSL (s) (2) AinS (S)-AinR (R)-C8HSL (s) (3) LuxS (S)-LuxP (R)-AI-2 (s)	Expression of bioluminescence, host colonization and motility genes
Acinetobacter spp	(1) Abal (S)-AbaR (R)-3OHC12HSL (s)	Expression of virulence factors including biofilm formation.
Burkhordelia cepacea	(1) Cep1 (S)-CepR (R)-C8HSL,C6HSL (s) (2) Ccil (S)-CciR (R)-C8HSL,C6HSL (s)	Expression of swarm motility genes and several virulence factors including: proteases, siderophores, toxins, antifungal agents and biofilm formation
Escherichia coli *Salmonella typhimurium*	(1) Unknown (S)-SdiA (R)-3OC8HSL (s) (2) LuxS (S)-LsrB (R)-AI-2 (s) (3) Unknown (S)-QseC (R)-AI-3 (s)	Expression of motility genes, acid resistance and virulence factors including biofilm formation
Gram (+) bacteria		
Staphylococcus aureus	(1)AgrB (S)-AgrC (R)-AIP(s)	Expression of several virulence factors including: hemolysins, leukocidins, cell surface adhesins, exoenzymes, and biofilm formation
Fungi		
Candida albicans	2-phenylethanol, tryptophol, farnesol, farnesoic acid, and tyrosol as (s)	Fungal dimorphism, biofilm formation and expression of virulence genes
Mucor rouxii, Histoplasma capsulatum, Ceratocystis ulmi	QS (s) unknown	Fungal dimorphism
Saccharomyces cerevisiae	Phenylethanol and tryptophol as (s)	Transition from yeast to filamentous form
Protozoa		
Trypanosoma cruzi	SIF soluble factor as (s)	Differentiation of replicating to non-dividing forms
Mammal cells		
$CD4^+$T cells	IL-2 (s) and IL-2Rα (R)	Regulation of the $CD4^+$T cells population
Cancer cells	Multiple (S) and (R) and paracrine factors as (s)	Regulation of the metastatic process

IL-2: Interleukin 2; SIF: Soluble inhibitory factor; QS: Quorum sensing; PQS: Pseudomonas quinolone signal; EPS: Exopolysaccharide.

a filamentous form in response to both cell density and the nutritional state of the environment. This induction is mediated by the phenylethanol and tryptophol auto signaling molecules, that regulate the transcription of a set of approximately 150 genes and which include *FLO11*, an essential gene for filamentous growth as well as several others genes that may play a role in the transition from the exponential to the stationary growth phase[192,193]. However, not only parasitic infections or traits present in unicellular eukaryotes are controlled by QS, since surprisingly in our body the number of cells of the immune system is maintained throughout a similar mechanism, where IL-2 is produced and secreted by activated $CD4^+$ T cells and sensed with high affinity (IL-2Rα) by a population of $CD4^+$ Treg, which in turn can regulate the number of total $CD4^+$ T population[194] by competition for the IL-2 factor[195]. Failure of QS due to the absence of IL-2 or by defects on the sensor IL-2Rα leads to lymphoid hyperplasia and autoimmune diseases[196]. Furthermore, in 2009 Hickson *et al*[197] proposed that cancer cells may use QS mechanisms to operate as communities and regulate different multicellular functions as the metastatic process resembles bacterial biofilm formation and dispersion. This idea emerged based on several lines of evidence that suggested a close relationship between high cancer cell densities and high metastatic ability[197]. This relationship can be possibly explained by the fact that the cells secrete paracrine factors or autoinducers that increase their metastatic efficiency; these observations have been made since the 90's[198] and recently by combining mathematical modeling with experimental evidence, the presence of QS systems in cancer was confirmed[199]. Such interesting findings are now opening new areas of study, including the development of future clinical applications (a summary of all the QS reviewed is provided in Table 1).

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REVIEW

Epidemiology, management, and economic evaluation of screening of gallstone disease among type 2 diabetics: A systematic review

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Abstract

The knowledge of gallstone disease (GSD) is crucial to manage this condition when organizing screening and preventive strategies and identifying the appropriated clinical therapies. Although cholecystectomy still be the gold standard treatment for patients with symptomatic GSD, expectant management could be viewed as a valid therapeutic method for this disorder. If early treatment of GSD decreases the morbidity or avoids further cholecystectomy, it may save clinical care costs in later disease periods sufficiently to offset the screening and early treatment costs. In addition, whether routine screening for GSD is worthwhile depends on whether patients are willing to pay the ultrasonography screening cost that would reduce the risk of cholecystectomy. In this review we discuss the epidemiology, management, and economic evaluation of screening of GSD among type 2 diabetics.

Key words: Gallstone disease; Epidemiology; Management; Economic evaluation; Type 2 diabetes

Core tip: According to the willingness-to-pay viewpoint, this review indicated that from the societal perspective but not from consumer viewpoint, it is worthwhile to organize a routine ultrasonography screening for gallstone disease in diabetic population for further cholecystectomy prevention.

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INTRODUCTION

Gallstone disease (GSD) is a common gastrointestinal condition with crystalline deposits in the gallbladder and impaired excretion of bile into the intestine throughout the world[1]. GSD yields a relative lower mortality rate, however, a relative higher risk of mortality in GSD patients is not totally explained by the high mortality rate of related cancer. This high morbidity of GSD substantially affects the economy and public health[2]. The increasing incidence of GSD over the past several decades is due to parallel modifications in personal dietary habits and physical activity associated with the Western lifestyle[3]. In the absence of an organized screening program for symptomatic GSD, treating GSD and related complications yields substantial medical burden[1].

Based on the Wilson criteria, GSD is needed to screen due to it is one of essential health issues; the disease natural course should be known; there should be a recognizable latent or early symptomatic state; a screening process is easy to do and interpret, accurate, acceptable, reliable, and has good sensitivity and specificity; there should be an acceptable treatment recognized for this disorder; treatment is much better if began early; there should be a clinical policy on who should be treated; both diagnosis and treatment have good efficacy; and this condition should be a continuous disease process[4]. Both obesity and the metabolic syndrome have been viewed as risk factors related to GSD formation[3,5,6]. Epidemiologic evidence suggested that people with diabetes mellitus were at higher risk of stone formation[3,7]. Academic studies indicated an increased morbidity of GSD in diabetic patients[7-9]. In addition, hypertriglyceridemia, hyperinsulinemia, and autonomic neuropathy (leading to gallbladder hypomotility and biliary stasis) were also revealed as associated factors to the incident GSD diabetic population[7,8]. This implies that GSD formation and diabetes development may share pathophysiologic pathways[3]. However, how diabetes predisposes to GSD is still not well known[8].

The choice of ultrasound scanning in GSD evaluation is ideal as it is cheap, non-invasive, safe, and repeatable without known adverse effect on the patients in clinical scenarios[10]. For symptomatic GSD subjects, expectant management may also indicate a valid clinical therapy although cholecystectomy still represents the gold standard[11]. From the viewpoint of preventive medicine, early detection of GSD by routine ultrasonography screening followed by appropriate therapy could avoid the further cholecystectomy. This review aims to explore the epidemiology, management, and economic evaluation of screening of GSD among type 2 diabetics.

THE CLINICAL DIAGNOSIS OF GSD

It is often a diagnostic challenge to determine which abdominal symptoms are related to GSD. Typically, GSD pain occurs in the right upper quadrant of the abdomen, but pain is not specific in this area[12]. Fewer than 50% of those with GSD actually have clinical symptoms, and fewer than 10% further develop potentially life-threatening complications[13]. The physicians must depend on the patient's description of the pain and results of laboratory examinations and diagnostic imaging to decide a appropriate diagnosis[12]. The physical examination also may show mild epigastric or right upper quadrant tenderness, but most patients do not have significant physical characteristics[13]. The majority of asymptomatic GSD will remain asymptomatic for a long time period.

Mechanisms underlying GSD formation include supersaturation of bile with cholesterol, consequent sedimentation, crystallization, and stone formation and abnormal gallbladder motor function with resultant delayed emptying and stasis of bile[10]. The availability of ultrasonography as viewed a valid tool for GSD diagnosis has allowed the evaluation of GSD morbidity[2]. It is safe, fast, and not expensive and involves no radiation exposure[13]. Positive findings include single or multiple stones, a positive Murphy sign on contact with the ultrasonographic probe, thickening of the gallbladder wall, and pericholecystic fluid[14]. Patients are usually left feeling unwell for as much as one or two days. If obstruction persists, it worsens movement and palpation, is associated with fever, and is localized to the right upper guardant part of abdomen, with the pain becoming sharp, which will result in acute cholecystitis[12,15]. Clinical studies showed higher positive (0.99-1.00) and negative (0.90-0.96) predictive values regarding the diagnostic efficacy, indicating that ultrasonography is a reliable technology for GSD screening[1,16]. However, a drawback is that its accuracy is dependent on the people who perform and interpret it[13].

Biliary pain occurs when the neck of the gallbladder is hindered by a gallbladder or stone pressure rises, producing a visceral foregut pain[15]. Factors that relate to choledocholithiasis include tests of abnormal liver function, common bile duct dilation of eight mm or more, and common bile duct stones identified by ultrasonography[17]. In addition, the abdominal plain radiography or computer tomography (CT) scan should also exclude the presence of calcified stones[18-20].

THE MORBIDITY OF GSD

Epidemiological studies in both Eastern and Western countries showed that ultrasonography is an reliable diagnostic tool for GSD morbidity[2,21-23]. The mechanisms of GSD have been implicated in type 2 diabetes. Some controversy exists regarding the association between diabetes and GSD, although population-based epidemiologic studies have demonstrated a positive relationship between type 2 diabetes and increased morbidity of GSD[1,24-26]. The possible pathogenic mechanism for this is that type 2 diabetic population

Table 1 Prevalence of gallstone disease in various populations

Ref.	Study year	Screened number	Setting	Prevalence of gallstone disease	Associated factors
Elmehdaw *et al*[8]	2009	327	Benghazi, Libya	DM: 9.75% Non-DM: 17.5%	Age, obesity
Pradhan *et al*[28]	2009	80	Nepal		Non-vegetarian
Acalovschi *et al*[29]	2009	1332	Romania	19% with chronic hepatitis C; 17% controls	Abdominal obesity, steatosis
Khan *et al*[30]	2009	9175 (5050 males, 4125 females)	England	Male fell from 20.2% to 19.1%, females fell from 30.4% to 29.0%	Diabetes, not for CHD, BMI to females, elderly
Friedrich *et al*[31]	2009	9206 (5559 from Danish, 3647 of German)	Denmark, Northeast Germany		Higher BMI, unfavorable lipid levels, higher prevalence of diabetes
Walcher *et al*[32]	2010	2147	Germany	8%	Protective effect: alcohol consumption
Ruhl *et al*[33]	2011	14228	United States	7.10%	Cardiovascular disease, cancer
Al-Bayati *et al*[34]	2012	200	Iraqi	33% of diabetics, 17% of non-diabetics	BMI > 25 kg/m^2, increased duration of DM, increased HbA1C, multiparous females
Jiang *et al*[35]	2013	1270	Shanghai, China	CAD (+): 19.5% CAD (-): 11.3%	CAD
Agunloye *et al*[10]	2013	400	Ibadan, Nigeria	17.5%	Age, BMI, DM, duration of the disease
Yilmaz *et al*[36]	2014	441	Turkey	12.2%	Age, BMI, Gender, metabolic syndrome
Shen *et al*[37]	2014	6511	Taiwan	13.2%	Age, Gender, metabolic syndrome
Ibitoye *et al*[38]	2014	1283	Nigerian	2.9%	

BMI: Body mass index; CHD: Coronary heart disease.

with GSD may cause acute cholecystitis more obvious and make higher likelihood of progression to septicemia compared with non-diabetic subjects, who exhibit functioning gallbladders normally. Type 2 diabetic patients may show a higher lithogenic bile index compared with non-diabetics after adjustment for sex and age[9]. The association between type 2 diabetes and GSD is stronger among patients who have a history of treated diabetes mellitus than it is among those with a single disease history of diabetes, that is, hyperglycemia may affect gallbladder motility[21]. The linkage between obesity, diabetes, and GSD most likely originate from metabolic syndrome[16,27]. In addition, diabetic patients represent cases of hyperglycemia that reflect relevant effects on gallbladder motility[9].

Tables 1 and 2 indicate that many evidence-based studies of the prevalence, incidence, and risk factors for GSD have been conducted. However, it is difficult to appropriately compare the results of some studies because the heterogeneous nature of these studies (for example, patient selection), which varied significantly. The prevalence of overall GSD was higher than the general Chinese population in Taiwan when using the same methodology of GSD assessment[8,42]. Previous population based studies had resulted in disparate findings on diabetes mellitus and GSD[24,25]. In Italy, the estimated prevalence of GSD is significantly higher in diabetic patients than in the general population (24.8% *vs* 13.8%)[43]. In New Zealand, the prevalence of GSD in diabetics was estimated to be 32.7% as compared to 20.8% in the control group[44]. An epidemiological study in Nigeria concluded that 17.5% of the diabetic patients had GSD on ultrasound[10]. The study about the prevalence of GSD in Chinese type 2 diabetics is rare or lack of appropriate statistical methods. The overall prevalence of GSD among type 2 diabetics in Kinmen was 14.4%, including single stone 8.0%, multiple stones 3.2%, and cholecystectomy 3.2%[42]. Further, the overall prevalence among elderly type 2 diabetics was 17.1% (men: 14.5%, women: 19.0%), which included the presence of single stone, 9.1%; multiple stones, 4.4%; and cholecystectomy 3.7%[45]. Upon international comparison, the prevalence of any type of GSD falls within the range of 10%-32% in type 2 diabetics and is higher than that in non-diabetic patients[44,46-49].

Cross-sectional study designs only reveal useful information of disease prevalence, but reveal nothing about the incidence or temporality in the study population. To explore the incidence and causal relationships between predictive factors and disease, the population needs to be re-examined regarding follow-up time. The morbidity of GSD increases as age increases, noticeably elevating in people aged 40 years and older and becoming from 4- to 10-fold more likely[4,16]. The incidence of GSD appears to vary among test diabetic populations and differs among studies conducted in disparate countries[1]. In the general and elderly Chinese type 2 diabetes population, the incidence of GSD was 3.56% per year (95%CI: 1.78%-6.24% per year) and 4.17% per year (95%CI: 2.22%-7.05% per year), respectively[9,26]. Previous epidemiologic studies showed that the annual incidence of overall GSD in type 2 diabetics was higher than that in other general population-based studies[9,22]. In addition, evidence-based studies exploring GSD in the elderly sub-population have focused almost entirely on the consequences of

Table 2 Incidence of gallstone disease in various populations

Ref.	Study year	Screened number	Setting	Incidence of gallstone	Associated factors
Festi *et al*[2]	2008	9611	Italy	0.67% (0.66% in males, 0.81% in females)	Risk factors: In men: increasing age, high BMI, history of diabetes, peptic ulcer and angina, and low cholesterol and high triglyceride levels; In females: increasing age and high BMI Predictors: In men: increasing age and pain in the right hypochondrium In females: increasing age
Halldestam *et al*[39]	2009	621	Sweden	1.39 per 100 person-years	Length of follow-up and LDL-cholesterol levels Inversely: alcohol consumption
Jonas *et al*[40]	2010	8901	Sweden	Surgical group: 122.2/10000 person-years controls: 22.2/10000 person-years	After antiobesity surgery (A fivefold increased risk)
Liu *et al*[25]	2012	108850 (60734 diabetic patients and 48116 control patients)	Taiwan	0.632% per year	Risk factor: increased age Associated: high body mass index, elevated fasting plasma glucose levels, and nonalcoholic fatty liver disease
Chen *et al*[1]	2014	1296	Taiwan	0.632%	High body mass index, elevated fasting plasma glucose levels, nonalcoholic fatty liver disease
Heida *et al*[41]	2014	288	Dutch	5.9%	BMI

BMI: Body mass index; LDL: Low density lipoprotein.

interventions such as percutaneous cholecystostomy and endoscopic retrograde cholangiopancreatography, or on the management of elderly patients with symptomatic biliary disease at hospitals[50,51]. The annual incidence of GSD in elderly type 2 diabetics was also higher than that in younger diabetic patients or the general population using the same methodology of GSD assessments[2,26]. To explore the incidence and risk factors for GSD is essential to prevent its development and avoid the further cholecystectomy caused by complications, which is often insidious in nature.

Gallstone formation is multifactorial, and involves constitutional and environmental factors. People with GSD have increased mortality, overall mortality, and mortality from cardiovascular disease and cancer. This relationship exists for ultrasound diagnosed GSD and cholecystectomy[33]. GSD with complications, especially cholecystitis and cholangitis, in the elderly is related to higher morbidity and mortality rates[52].

THE NATURAL COURSE OF GSD

The natural course of GSD is usually not malignant, but complications contribute substantially to medical care costs and may even be life threatening[40]. One of the essential advantages of early detection of GSD is that ultrasonography could diagnose asymptomatic stages, which incurs early treatment and the prevention of major complications such as acute pancreatitis or gallbladder cancer[53,54]. The increasing magnitude and epidemiologic shifts in the natural history of GSD worldwide qualify for the need of research in different geographical areas, and also to explore the predictive factors[55,56]. This is particularly because the majority of risk factors associated with GSD are potentially modifiable[41]. In addition, cholecystectomy could be used to treat GSD, that is, the estimated utility value in subjects with GSD will be a 0.09 increase from this therapy. Thus, the number of quality-adjusted life years obtained from cholecystectomy would be 1.8 (0.09 × 20) if subjects had a life expectancy of 20 years[57]. Screening regimes for GSD depend on the incidence and progression rates as well as the risk factors that change these rates. An understanding of the disease progression of GSD would appropriately determine the benefits of prevention strategies.

A chronic disease model according to the epidemiologic information of GSD is necessary to allow the benefits of intervention to be modeled. Since GSD may only persist a short duration before cholecystectomy, a shorter desirable interscreening interval may be warranted. The disease progression of GSD affects the decision of a screening interval for the surveillance of this patient population. In addition, the effectiveness of screening strategy for GSD is determined by the progression of GSD[58]. Since the natural history of GSD may not be homogeneous across study countries, assumptions of disease progression parameters could not be directly compared from previous results[11,59]. Several evidence-based studies on the natural history of GSD also have been conducted[11,58]. A clearer understanding of the risk factors associated with GSD may help us to identify cases and to reduce the risk in some patients[60].

For the disease natural course of GSD, the four-state Markov chains model following the pathway of

proliferative phase is showed as follows:

$$\underset{\text{(State 1)}}{\text{No GSD}} \xrightarrow{\lambda_{12}} \underset{\text{(State 2)}}{\text{single stone}} \xrightarrow{\lambda_{23}} \underset{\text{(State 3)}}{\text{multiple stones}} \xrightarrow{\lambda_{34}} \underset{\text{(State 4)}}{\text{cholecystectomy}}$$

To estimate the progression rates, let λ_{12}, λ_{23}, and λ_{34} indicate the annual progression rate from state 1 to state 2, from state 2 to state 3, and from state 3 to state 4, respectively. The annual progression rates from single stone to multiple stones and from multiple stones to cholecystectomy are estimated as 0.114 (95%CI: 0.015-0.173) and 0.148 (95%CI: 0.101-0.242), respectively. Corresponding average durations in single stone state and multiple stones stage are 8.77 (95%CI: 5.78-66.67) years and 6.76 (95%CI: 4.13-9.90) years, respectively. The application of parameters to the annual transition probabilities from single stone state to multiple stones state and from multiple stones state to cholecystectomy state are 10.00% and 13.76%, respectively. An annual screening program could reduce cholecystectomy by 82.9% (95%CI: 75.7%-90.4%) compared with the non-screening group. Comparatively, biennial screening, 3-year screening, 4-year screening, and 5-year screening could reduce cholecystectomy by 71.6% (95%CI: 57.0%-88.8%), 64.8% (95%CI: 46.1%-81.5%), 49.6% (95%CI: 23.9%-75.3%), and 32.1% (95%CI: -2.8%-66.7%), respectively[58]. However, one problem is in four-state Markov chains model, we should be aware that single stone might not always consequentially develop into multiple stone.

Many factors such as obesity and type 2 diabetes have been indicated to be significant risk factors related to GSD[1,9,11], and the transition state is probably unstable over time. The screening efficacy of preventing cholecystectomy associated with GSD depends on early diagnosis. To choose the frequency of sonographic check-ups as well as sensitivity and specificity, it is helpful to know the disease scenario and progression from the asymptomatic state to the symptomatic state. This characteristic will provide early diagnosis and therapeutic strategies for GSD[57].

THE MANAGEMENT OF GSD

The treatment options for GSD are according to few crucial steps such as typical symptoms, further complications, and gallbladder function, as well as size and composition of GSD[18]. Cholecystectomy remains the reliable operation for patients with symptomatic GSD. It is safe because the lowest risk of recurrence and more than 90% of patients with complete biliary pain relief[12]. Currently, it is also under argumentation if cholecystectomy may be also used for pre-symptomatic GSD. It is generally presumed that surgical procedures are not suggested routinely in symptom-free subjects due to the low rate of complications[18].

Statins used could relieve hepatic cholesterol biosynthesis and may reduce biliary cholesterol secretion, consequently causing decreased cholesterol concentration in bile[61]. A larger observational study showed academic evidence that long-term use of statins is related to a decreased rate of diagnosed GSD requiring advanced cholecystectomy[62]. Another population-based case-control study also indicated that long-term sustained statin use decreases incident GSD in both men and women[63]. The results may be one of clinical relevancies given that GSD represents a major burden for medical care systems[62]. In addition, a previous study showed that ezetimibe could not only prevent cholesterol GSD through obstructing intestinal cholesterol absorption so that biliary cholesterol secretion is decreased, and gallbladder motility function is reserved by desaturating bile in gallstone-susceptible C57L mice disputed to the lithogenic diet, but also promote the dissolution of cholesterol GSD through a greater capacity to develop an abundance of unsaturated micelles[64]. For both prevention and treatment of cholesterol GSD, ezetimibe is viewed as a novel and potential cholelitholytic agent[65].

Oral bile acids have successfully dissolved GSD in an extremely limited patient population for the nonsurgical treatment of GSD[12]. The clinical effectiveness of bile acid therapy was determined in symptomatic GSD smaller than 15 mm within a functioning gallbladder[12]. Oral bile acids could be only selected in symptomatic GSD patients who are unsuitable for cholecystectomy and have small, uncalcified, and cholesterol-enriched stones with a patent cystic duct in a functioning gallbladder[18,66]. Currently, bile acid therapy is revealed only for patients unsuitable or unwilling to receive cholecystectomy[12,67].

A previous study showed that acute cholecystitis grows in up to 10% of symptomatic GSD patients and leads to the entire obstruction of the cystic duct[68]. Once acute cholecystitis is found, patients should be revived with intravenous fluids, accompanying medical conditions should be stabilized, and surgery should be performed at the earliest time[12]. In addition, GSD could migrate from their primary site in the gallbladder through the cystic duct and into the common bile duct[12]. An essential treatment for choledocholithiasis includes gallbladder removal and clearance of retained common bile duct stones. The findings of a prospective, multi-center, randomized controlled trial comparing single-stage laparoscopic cholecystectomy (LC) and laparoscopic stone extraction with preoperative endoscopic retrograde cholangiopancreatography followed by LC indicated that the procedures were equally effective in the clearance of common bile duct stones[69].

THE ECONOMIC EVALUATION OF SCREENING OF GSD

Based on the welfare economic theory, the maximum value of individual's willingness-to-pay (WTP) is defined as the benefit to an individual receiving medical service or intervention[70]. For the WTP perspective, the payment vehicle not only refers to the means of payment by

a patient, but also is assumed as total cost of both copayment for health insurance scheme and out-of-pocket money which is not covered by health insurance benefit[71]. An evidenced-based study indicated that 24.4% of subjects would not like to pay a screening cost for GSD detection, implying that they did not think GSD status would influence their daily quality of life. The WTP values were significantly higher in individuals with more advanced GSD than in those with mild GSD. This also suggests that GSD associates with impaired quality of life and thus such patients would pay more to reduce the sequelae of further cholecystectomy[70].

Economic evaluations are criticized commonly by decision makers for ignoring budget impacts, about which decision makers are desperately concerned. Payers could get into financial difficulty if they adopt too many cost-effectiveness interventions and affordability, which depends on the overall volume of patients, is therefore a prime concern[11,72]. Few well-organized population-based studies have been conducted to explore the cost and effectiveness of GSD screening regimes. The cost-benefit analysis was used in one study to discuss whether a GSD screening regime compared with non-screening group is worthwhile in Taiwan from different viewpoint. The findings revealed that indirect costs play a main role in the routine GSD screening regime. Annual screening program could save the most discounted indirect costs per case (NTD220345) (US$6995.1; 31.0NTD = 1USD) compared with non-screening group. Based on the health care payer's viewpoint, annual screening discounted net cost was NTD24893 (US$790.3) per case. This implied that from health care payer's viewpoint, the clinical efficacy from the routine annual screening regime could not exceed the cost incurred in the GSD screening strategy. To consider the indirect cost, the NTD245238 (US$7785.3) net saving per case indicates that from the societal perspective, the annual screening program is rather constructive ($P < 0.0001$)[72]. In Chile, a screening program for GSD in a high risk sub-population reveals significant cost-effectiveness. The incremental cost-effectiveness ratio of universal screening compared with elective intervention, high risk intervention, and selective screening programs were estimated US$180, US$147, and US$481, respectively[73]. Preventive strategies aimed at GSD screening incur both substantial medical budgetary savings and highly cost-effective clinical care.

METHODOLOGICAL CONSIDERATION

Since GSD has a more complicated clinical aspect, it is not the rule for people with GSD definitely to progress towards more serious complications. In clinic, 60% of the patients with GSD are asymptomatic throughout their life. In these people, early detection may not help them to avoid possible healthy problem. In addition, up to now, there has been no effective early treatment for GSD that could prevent the resulting cholecystectomy. What we could and we should do now for GSD patients is to find the patients more likely to have a serious outcome in the future and perform an early cholecystectomy to avoid the secondary common bile duct stone, gallstone pancreatitis and possible cancerization. That is why some asymptomatic patients are indicated for cholecystectomy. Also, some asymptomatic patients with diabetes and cardio-cerebrovascular complications should be treated by cholecystectomy in a stable condition to avoid unpredictable attack of GSD. That is currently the aim for GSD screening. Furthermore, since the natural history of GSD may be heterogeneous and more complicated, according to current knowledge, there is no relationship between the number of gallstones and cholecystectomy. We still have no clear idea about how the gallstone produces and what is the progression factors for GSD.

CONCLUSION

In conclusion, GSD is an escalating major health problem and involves constitutional and environmental factors. Considering the fact that oral bile acids could be only selected in an extremely limited patient population (symptomatic gallbladder disease patients who are unsuitable for surgery and have small, uncalcified, and cholesterol-enriched stones), the majority of patients with or without complications will need surgery. Without suitable screening programs for symptomatic GSD, treating GSD and related complications yields substantial medical care costs. Whether routine screening for GSD is worthwhile depends on whether subjects are willing to pay the ultrasonography screening costs that would reduce the risk of further cholecystectomy.

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MINIREVIEWS

How to use magnetic resonance imaging following neoadjuvant chemotherapy in locally advanced breast cancer

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Abstract

Magnetic resonance imaging (MRI) is highly sensitive in identifying residual breast cancer following neoadjuvant chemotherapy (NAC), and consequently is a commonly used imaging modality in locally advanced breast cancer patients. In these patients, tumor response is an important prognostic indicator. However, discrepancies between MRI findings and surgical pathology are well documented. Overestimation of residual disease by MRI may result in greater surgery than is actually required while underestimation may result in insufficient surgery. Thus, it is important to understand when MRI findings are reliable and when they are less accurate. MRI most accurately predicts pathology in triple negative, Her2 positive and hormone receptor negative tumors, especially if they are of a solid imaging phenotype. In these cases, post-NAC MRI is highly reliable for surgical planning. Hormone receptor positive cancers and those demonstrating non mass enhancement show lower concordance with surgical pathology, making surgical guidance more nebulous in these cases. Radiologists and surgeons must assess MRI response to NAC in the context of tumor subtype. Indiscriminate interpretations will prevent MRI from achieving its maximum potential in the pre-operative setting.

Key words: Breast; Magnetic resonance imaging; Neoadjuvant chemotherapy; Biomarkers; Phenotypes

Core tip: Following neoadjuvant chemotherapy, breast magnetic resonance imaging (MRI) most accurately predicts surgical pathology in triple negative, Her2 positive and hormone receptor negative tumors, especially if they are of a solid imaging phenotype. In these cases, post-neoadjuvant chemotherapy (NAC) MRI is highly reliable for surgical planning. Hormone receptor positive cancers and those demonstrating non mass enhancement show lower concordance with surgical pathology, making surgical guidance more nebulous in

these cases. Radiologists and surgeons must assess MRI response to NAC in the context of tumor subtype.

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INTRODUCTION

Breast cancer is a heterogeneous disease consisting of many different tumor subtypes, each with its own biology, prognosis, and treatment options. These subtypes are characterized by distinct molecular profiles, proliferation rates, and tumor receptors, including estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). In today's paradigm of personalized medicine, biomarker profiles allow tailoring treatment strategies to the individual tumor. Current treatment of locally advanced breast cancers includes chemotherapy, hormone therapy [if hormone receptor (HR) positive] and surgical resection. Increasingly, chemotherapy is given prior to surgery. Neoadjuvant chemotherapy (NAC) offers advantages in terms of adding prognostic information and improving surgical options. Tumor response to NAC is an important prognostic indicator. Patients who have a pathologic complete response (pCR) following NAC have improved overall survival, disease-free survival and recurrence-free survival[1-6]. NAC can also facilitate breast-conserving surgery in patients whose initial presentation may have warranted mastectomy[7-9]. Even if patients still have residual disease, especially if they need radiation, breast conservation will have fewer complications than mastectomy and radiation. As treatments improve and responses to NAC become more common, a new challenge arises - accurately determining the extent of surgical resection needed to excise residual tumor. Magnetic resonance imaging (MRI) is highly sensitive in identifying residual disease following NAC, with multiple studies demonstrating it to be more accurate than mammography, ultrasound or physical examination[10-16]. Consequently, MRI is a commonly used imaging modality in locally advanced breast cancer patients.

In these patients, pre-operative MRI is an important addition to the decision-making armamentarium. The appearance of breast cancer on MRI can be classified by its morphology into phenotypic categories[17], which are associated with response to NAC and ability to offer breast-conserving surgery[17,18]. Overall, MRI has been shown to be the most sensitive imaging modality by which to follow a patient's response to NAC and to be more sensitive than clinical examination[11-16,19-23]. While an excellent test, MRI is far from perfect. Discrepancies between MRI findings and surgical pathology are well documented. Overestimation of residual disease by MRI may result in greater surgery than is actually required (larger lumpectomies, wider margins, mastectomy)[1,24]. Underestimation may result in insufficient surgery, resulting in positive margins and re-excisions[1]. Thus, it is important to understand when MRI findings [particularly radiologic complete responses (rCR)] are reliable and when they are less accurate.

The general question of the accuracy of an rCR to predict a pCR may be overly broad - accuracy needs to be considered in the context of tumor subtype. Literature has shown that the accuracy of post-NAC MRI is related to tumor subtype, with the strongest evidence arising from multi-institutional trials like I-SPY[18] and Translational Breast Cancer Research Consortium Trial 017[25], as well as additional support from multiple single-institution studies[26-29]. A smaller literature base suggests that MRI phenotype is also related to the accuracy of MRI in the post-NAC, pre-operative setting.

In this manuscript, we review the evidence for accuracy of post-NAC MRI findings and focus on how best to use MRI in this setting, specifically for the evaluation of extent of disease and pCR. In particular, this review will evaluate the association between the diagnostic performance of MRI in the post-NAC setting and the biomarker profile of the tumor, as well as the association between pre-NAC phenotypic tumor appearance on MRI and diagnostic accuracy. A clear understanding of these relationships can be valuable in setting appropriate treatment goals and expectations[18]. In much the same way each breast cancer requires a tailored treatment strategy, a strategy for tailored imaging interpretation should also be employed and would enable more accurate recommendations to be made for individual patients.

ASSOCIATIONS WITH MRI PHENOTYPE

The relationship between phenotypic MRI appearance of breast cancers and response to NAC has been studied[17,29]. Although phenotypic categorizations vary slightly, in general, phenotypes tend to focus on the separation of solid and well-contained unifocal (Figure 1A) and multifocal masses from more diffuse and infiltrative non-mass enhancement (NME) (Figure 1B)[17,18,29]. These phenotypes impact NAC response, with well-defined mass phenotypes more likely to have a response sufficient to allow for breast conserving surgery[17,18]. Well-defined masses also show higher concordance between MRI and surgical pathology, with an rCR in the setting of solid phenotypes (particularly hormone-negative tumors) predictive of a corresponding pCR at surgery[18]. On the other hand, MRI is less accurate in predicting pCR in tumors presenting as non-mass/diffuse enhancement, with larger discrepancies between post-NAC MRI and surgical pathology[18].

Studies have also suggested that these differing phenotypic appearances have particular patterns of

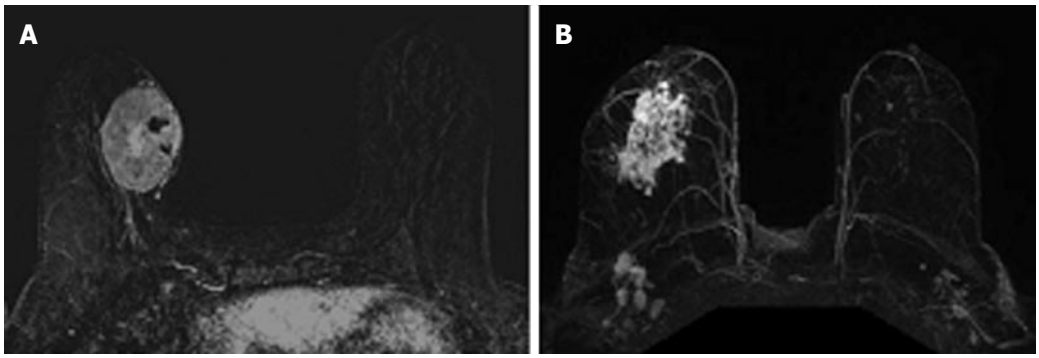

Figure 1 Magnetic resonance imaging phenotypes solid unifocal mass (A) and more diffuse non-mass enhancement (B).

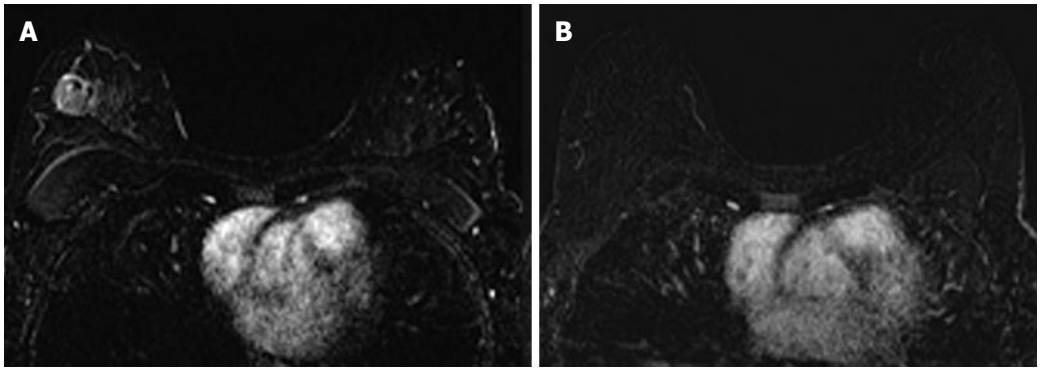

Figure 2 Forty-two years old woman with triple negative right breast cancer. A: Baseline axial T1-weighted post-gadolinium fat-saturated magnetic resonance image demonstrates a 3.6 cm unifocal mass in the upper outer quadrant; B: Post-neoadjuvant chemotherapy magnetic resonance imaging demonstrates complete resolution of the mass seen previously. Surgical pathology demonstrates biopsy site changes and expected changes related to chemotherapy with no evidence of residual cancer.

response to NAC[17,24,29-31]. Locally advanced malignancies presenting as a mass lesion often shrink in a concentric pattern to a smaller mass. Following NAC, NME often diminishes to a scattered pattern of residual disease that can extend throughout the original area of involvement, though as small foci that are difficult to detect on MRI. Residual infiltrating single cells will likely not be visible on MRI.

The associations between MRI accuracy and phenotype are likely confounded by tumor biomarker status. Comparisons of MRI phenotypes relative to tumor biomarker profiles[18,24,28,29,32,33] have shown a number of trends, with an association between unifocal mass presentation and triple negative tumors (TN: ER negative, PR negative, Her2 negative) (Figure 2). Multifocal mass presentation is more common in HER2+ (and questionably in HR positive) tumors. Although they do not have a characteristic phenotypic presentation, HR positive cases, especially ER positive tumors, are more likely to present as non mass/diffuse enhancement compared to other subtypes (Figures 3 and 4). Although these relationships have been demonstrated, all phenotypes are seen in all biomarker profiles[18].

ASSOCIATIONS WITH TUMOR BIOMARKERS

Extent of disease evaluation

The impact of tumor biomarkers on the accuracy of MRI for detecting the extent of disease must be considered when interpreting post-NAC MRI in anticipation of surgical resection. In addition to the individual status of receptors, biomarkers can categorize tumors into different subtypes. Tumor subtypes include luminal (ER/PR positive, Her2 negative), Her2 positive, and basal (ER/PR/Her2 negative; analogous to TN.) Multiple studies have demonstrated that in the post-NAC setting, the MRI assessment of extent of residual disease is most accurate in tumors that are either TN (Figure 2) or Her2 positive.

McGuire *et al*[26] retrospectively reported their institutional experience and found that MRI was most accurate in estimating pathologic size of residual disease in the Her2 positive and TN subtypes. Additionally, they found that MRI was more likely to underestimate the amount of residual disease in the luminal subtype (ER/PR positive, Her2 negative) when compared with TN or Her2 positive tumors. In a study done by Loo *et al*[29], MRI findings correlated well with the pathologic findings in the TN and Her2 positive breast cancers, but not with ER positive or Her2 negative breast cancers. Kuzucan *et al*[24] evaluated only Her2 negative cancers, and report similar findings-higher concordance between post-NAC tumor size on MRI and pathologic size in HR negative tumors compared to HR positive tumors. In Kuzucan's study, MRI accuracy was also increased in tumors expressing high levels of the proliferation marker Ki-67 (defined as > 40% positive). A study by

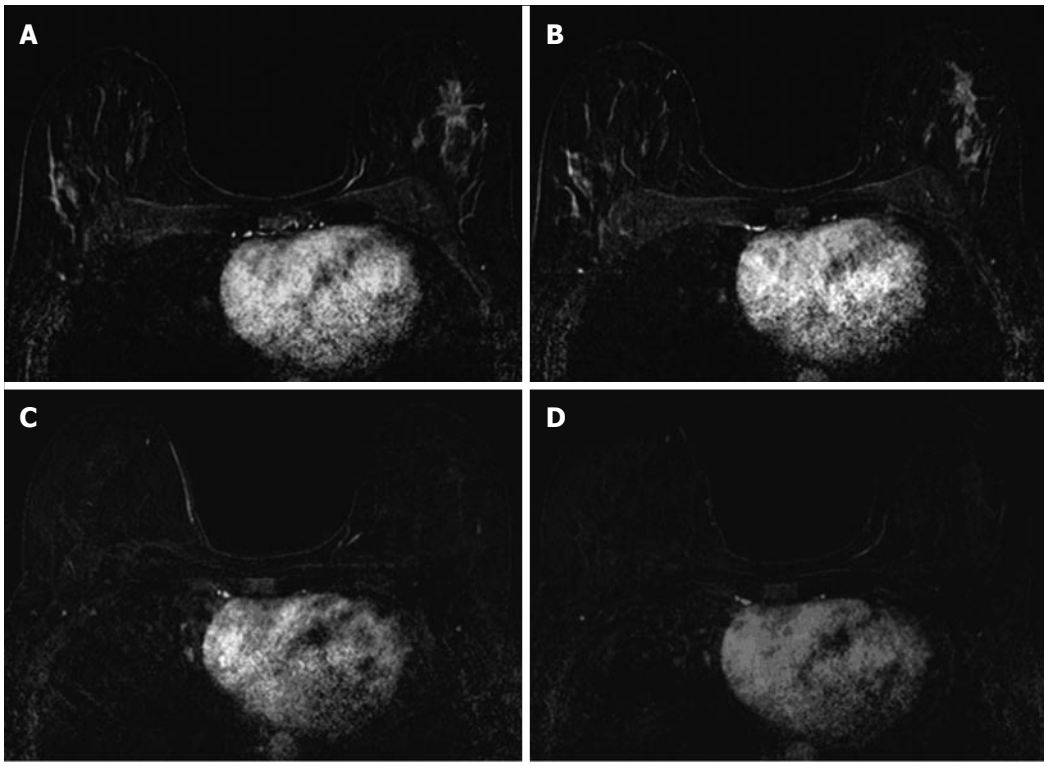

Figure 3 Thirty-seven years old woman with HR+ left breast cancer. A and B: Baseline axial T1-weighted post-gadolinium fat-saturated magnetic resonance image demonstrates a speculated mass and contiguous non-mass enhancement extending posteriorly for a total of 7 cm of disease in the upper outer breast; C and D: Post-neoadjuvant chemotherapy magnetic resonance imaging demonstrates decrease in size and degree of enhancement of prior findings. Surgical pathology demonstrates 6.9 cm of invasive ductal carcinoma.

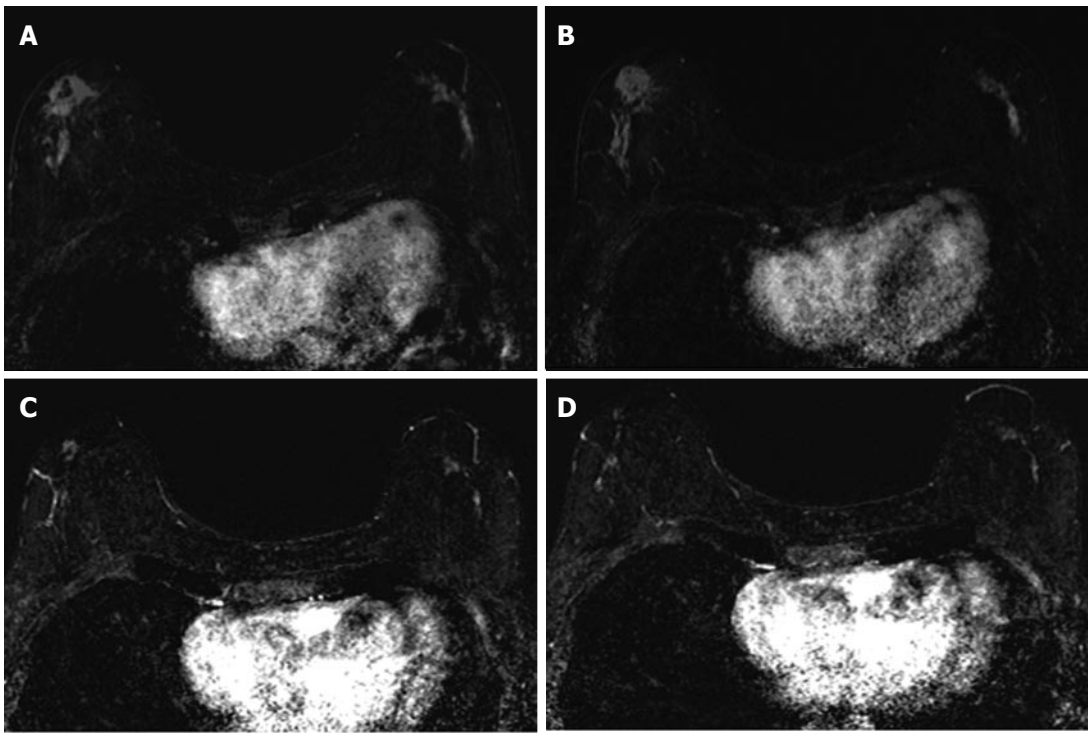

Figure 4 Sixty-four years old woman with bilateral HR+ breast cancer. A and B: Baseline axial T1-weighted post-gadolinium fat-saturated magnetic resonance image demonstrates 3.2 cm irregular mass and contiguous non-mass enhancement (NME), spanning up to 7.2 cm, in the right central outer breast and 3.5 cm of clumped linear NME in the central outer left breast; C and D: Post-neoadjuvant chemotherapy magnetic resonance imaging demonstrates decrease in size of the right breast mass and NME. NME in the left breast demonstrates only mild improvement. Surgical pathology demonstrates 4.3 cm of residual disease on the right and 3.7 cm of disease on the left.

Kim *et al*[34], which investigated TN cancer, also found that Ki-67 affects the diagnostic accuracy of MRI, with higher correlation between MRI and residual tumor size at surgery in Ki-67 positive patients.

In I-SPY, a multicenter neoadjuvant trial with serial MRIs over the course of therapy, there were the fewest discrepancies between the post-NAC MRI tumor size and pathologic size in Her2 positive, HR negative, and TN tumors[18]. Overall, 38% of patients analyzed had a size discrepancy of at least 2 cm between MRI and surgical pathology, with two thirds of these discrepancies being an overestimation of disease on MRI. These size discrepancies were significantly more common in HR+/Her2- tumor subtypes. Additionally, size discrepancies differed by MRI phenotype; among the solid phenotypes, underestimation of disease by 1.5 cm or more was rare. These Her2+, HR-, and TN tumors were also the tumor subtypes most likely to have a substantial response to NAC. The experience at our institution is in accordance with other published reports. In cases of Her2 positive, HR negative, and TN tumors, if there is residual disease on MRI, it is highly likely that there will be residual disease in the surgical specimen. Underestimation of disease in these subtypes is rare, particularly in the triple negative group where no false negative MRI's were seen.

pCR evaluation

Apart from measuring residual disease for the purposes of surgical planning, the ability of MRI to predict a pathologic complete response (pCR), a surrogate for improved outcome, is of particular importance in breast cancer management. Attaining pCR gives prognostic information that can be used for decision making, including the type of surgical procedure and/or reconstruction to recommend, and is also used as an immediate endpoint in evaluating the efficacy of NAC. Data show that pCR is associated with improved outcomes, and is more predictive when assessed by individual tumor subtype than for all subtypes combined[1,35]. A non-invasive method to accurately determine whether or not a pCR had been achieved would potentially change how trials are designed, and could eventually change surgical management of breast cancer.

While MRI accuracy depends on both its positive predictive value (PPV), and its negative predictive value (NPV), the NPV becomes the most important variable if the goal is to spare a patient invasive treatment in the setting of an rCR. That is, one must be able to trust that a negative MRI is a true negative in order to safely omit surgical resection or other treatment. In the reported papers looking at the accuracy of MRI for predicting pCR in the post-NAC setting, one must note that relatively high accuracy is possible with a low NPV. This can occur when MRI has a very high PPV, ultimately leading to high accuracy despite low NPV. In tumor subtypes that are less likely to respond to NAC, such as luminal tumors, the likelihood of residual disease is high, resulting in high PPV. However, the chance of a false negative is also highest in this group, so despite high apparent accuracy (driven by PPV), an rCR should be interpreted with caution (Figures 3 and 4).

Just as the accuracy of MRI in predicting extent of disease differs by tumor subtype, it appears that the ability of MRI to accurately predict pCR also differs by tumor subtype[24]. The NPV of MRI for predicting pCR differs by tumor subtype, highest in HR negative/Her2 positive tumors and triple negative (Figure 2) tumors[18,24-26,36]. In our report of I-SPY patients, when the post-NAC MRI underestimated residual disease (which occurred 4.3% of the time), all the discordant cases were either HR positive (Figures 3 and 4) or had diffuse phenotypes (Figure 4)[18].

Recently, several groups have reported on the accuracy of post-NAC MRI for correctly identifying pCR. Of note, some groups define pCR as the absence of any invasive tumor cells (the preferred definition of the FDA)[37], while others require the absence of both invasive and *in situ* disease - this definition must be noted when interpreting study findings, as residual *in situ* disease may lead to higher local recurrence rates[38].

One retrospective multicenter study of 746 women undergoing NAC found overall NPV for MRI of 47% and accuracy of 74% for predicting pCR[25]. The NPV for MRI varied by tumor subtype, and was highest amongst HR-/Her2+ tumors (62%) and TN tumors (60%). The overall accuracy was highest for HR+/Her2 negative tumors, likely because the PPV in this group was 91%. This likely reflects the fact that because this subtype is the least likely to respond to NAC, the pretest probability for having residual disease is higher.

Single institution studies have shown similar results. Chen *et al*[27] demonstrated the vast difference in MRI accuracy by tumor subtype, with accurate prediction of pCR in 95% of Her2 positive tumors, but only in 50% of Her2 negative tumors. Kim *et al*[34] found MRI accurately predicted pCR in 91% of TN cases. Kuzucan *et al*[24] focused on Her2 negative patients, and also found higher accuracy in HR negative tumors, with a PPV of 88% and NPV of 88%. In HR positive tumors, MRI had a PPV of 100% but an NPV of only 56%. The authors noted that the higher NPV in the HR negative tumors may have been related to a higher prevalence of solid tumor phenotypes, acknowledging that tumor phenotype impacts MRI accuracy and response to NAC[24]. In Ko *et al*[28]'s 2013 report, overall PPV was 89.6% and NPV was 83.8%. Of the five false negative MRI's in their study, 3 were ER positive, 2 were Her2 positive, and 3 initially appeared as non mass enhancement. The most recent report, by Bufi *et al*[36] in 2014, shows the highest NPV rates to date. In the TN tumor subtypes, they report NPV of 100%. In the Her2 positive subtype, they report NPV of 100% using diffusion weighted imaging, suggesting that newer advanced MRI techniques may improve accuracy of MRI in different subtypes[36].

CONCLUSION

MRI most accurately predicts pathology in TN, Her2 positive and HR negative tumors, especially if they are of a solid imaging phenotype. In these cases, post-NAC MRI is highly reliable for surgical planning. Hormone receptor positive cancers and those demonstrating NME demonstrate lower concordance with surgical pathology, making surgical guidance more nebulous in these cases.

While MRI may not yet meet the necessary NPV threshold to safely allow for omission of surgical treatment, this may be feasible for specific tumor subtypes in the future. It is unclear whether or not the differential accuracy of MRI by tumor subtype is mediated by tumor phenotype, tumor response to NAC, or biological differences that affect imaging, or possibly by all of these factors. Regardless, it is clear at this point that radiologists and surgeons must assess MRI response to NAC in the context of tumor subtype. If imaging interpretations are not made in this context, pre-operative MRI will continue to be limited by both overestimation and underestimation of residual disease. The same way each breast cancer requires a tailored treatment strategy, tailored interpretation strategies should also be employed. Future work on redefining thresholds for enhancement interpretation based on tumor biology and on the development of receptor subtype-based imaging protocols may improve accuracy in the future.

With the understanding that pCR predicts recurrence free survival, if an rCR can confidently predict pCR (as in TN and Her2 positive tumors), then an rCR can predict recurrence free survival. As an imaging predictor for such important outcomes, MRI interpreted in the context of tumor subtype would be a tremendous asset in decision-making and patient counseling.

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MINIREVIEWS

Cervical cancer screening: A never-ending developing program

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Abstract

With the term "oncological screening", we define the overall performances made to detect early onset of tumors. These tests are conducted on a population that does not have any signs or symptoms related to a neoplasm. The whole population above a certain age, only one sex, only subjects with a high risk of developing cancer due to genetic, professional, discretionary reasons may be involved. Screening campaigns should be associated, when risk factors that can be avoided are known, with campaigns for the prevention of cancer by means of suitable behavior. The goal of cancer screening cannot however be limited to the diagnosis of a greater number of neoplasms. Screening will be useful only if it leads to a reduction in overall mortality or at least in mortality related to the tumor. Screening should then allow the diagnosis of the disease at a stage when there is a possibility of healing, possibility that is instead difficult when the disease is diagnosed at the appearance of signs or symptoms. This is the reason why not all campaigns of cancer screening have the same effectiveness. In Italy, every year there are about 150000 deaths due to cancer. Some of these tumors can be cured with a very high percentage of success if diagnosed in time. Cervical cancer can be diagnosed with non-invasive tests. The screening test used all over the world is Papanicolaou (Pap) test. This test may be carried out over the entire healthy population potentially exposed to the risk of contracting cancer. Public health has begun the screening campaigns in the hope of saving many of the approximately 270000 new cases of cancer reported each year. Screening is done following protocols that guarantee quality at the national level: these protocols are subject to change over time to reflect new realities or to correct any errors in the system. A simplified sketch of a possible route of cancer screening is as follows: (1) after selecting the target population, for example all women between 25 and 64 years (in the case of monitoring of cervical cancer), an invitation letter with the date and time of the appointment, planned according to the acceptance capacity of the hospital, is sent to all individuals; (2) an examination, which depending on the individual and the type of cancer to be monitored, for example, can

be a Pap smear, is performed and the patient can go home; (3) once available the results of examinations, if negative, they shall be communicated to the person concerned that will be notified by mail and will be recalled for a second test at a few years of distance, in the case of non-negativity, instead, the patient is contacted by telephone and informed of the need to carry out further examinations: it is said that the patient is in the "phase two" of the screening pathway; (4) in phase two, reached by only a small portion of the interested parties (usually less than 3%-5%), more in-depth tests are carried out, which, depending on the individual and the type of cancer, can be: cytological and colposcopic examinations, the removal of a fragment of tissue (biopsy) and subsequent histological examination, additional tests such as ultrasound, radiography, or others such as computerized tomography, magnetic resonance imaging, positron emission tomography, *etc.*, in case of negativity, the concerned person will be called for new control tests at a a few years of distance, in case of non-negativity, it will be proposed instead an oncologic therapeutic plan and/or surgery to treat the diagnosed tumor; and (5) once the treatment plan is completed, the individual enters the follow-up protocol, which is monitored over time to see if the tumor has been completely removed or if instead it is still developing. Cervical cancer is undoubtedly the most successful example of a cancer screening campaign. Paradoxically, its effectiveness is one of the strongest reasons to criticize the usefulness of vaccination against human papillomavirus (HPV) in countries where the screening service with Pap test is organized in an efficient manner. Cervical cancer screening protocols are directed to sexually active women aged 25-64 years: they provide the Pap test performed by examining under a microscope or by staining with a specific "thin prep" the material taken from the cervix with a small spatula and a brush. It is recommended to repeat the test every two or three years. It is important to emphasize that women vaccinated against HPV must continue the screening with Pap test. Although some screening programs (*e.g.*, Pap smears) have had remarkable success in reducing mortality from a specific cancer, any kind of screening is free from inherent limitations. The screening methods are in fact applied to large parts of the apparently healthy population. In particular, the limits for certain cancers may be as obvious as to prohibit the introduction of an organized screening program. Potential limitations of organized screenings are basically of two types: organizational and medical. The limits of organizational type relate to the ability of a program to recruit the whole target population. Although well organized, a screening program will hardly be able to exceed a coverage of 70%-80% of the target population, and in fact the results of the current programs are often much smaller. The limits of medical type are represented by the possibility of reducing the overall mortality, or specific mortality, using a specific screening campaign.

Key words: Cervical cancer; Screening; Papanicolaou test; Human papillomavirus test; Vaccination

Core tip: Most cases of cervical cancer are preventable and, if caught early, highly curable. Despite this, cervical cancer is the second most common cause of cancer death and a leading cause of morbidity in women worldwide. Unfortunately, cure is less likely when the disease is diagnosed at an advanced stage. Although the human papillomavirus is considered the major causative agent of cervical cancer, yet the viral infection alone is not sufficient for cancer progression.

Comparetto C, Borruto F. Cervical cancer screening: A never-ending developing program. *World J Clin Cases* 2015; 3(7): 614-624 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i7/614.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i7.614

INTRODUCTION

Human papillomavirus (HPV) belongs to the diverse group of sexually transmitted viruses that manifest affinity to the squamous epithelia of the skin and mucous membranes. It has been proved that types 16 and 18 in particular could lead to cervical cancer. High-risk strains of HPV (HR-HPV) types have been found in cervical cancer worldwide[1]. The Papanicolaou (Pap) smear was the mainstay of screening in women for over 60 years[2]. All current guidelines recommend colposcopy for women with high-grade squamous intraepithelial lesions (H-SIL), with a view to performing a biopsy or conization. Randomized controlled trials and retrospective comparisons much more strongly suggest that regular well-organized smear testing prevents a number of deaths due to cervical cancer. It should be remembered that many cellular atypia found on cervical smears never progress to cancer. The frequency of overdiagnosis has not been studied. Smear-based screening appears to have very few serious adverse effects. In practice, despite the lack of solid evidence, it seems unreasonable not to recommend screening for cervical cancer. Organized screening is preferable to opportunistic screening performed without quality controls and without research to optimize screening strategy[3,4]. The available technology for prevention and its developments allows real opportunities for cervical cancer elimination in defined populations to be foreseen[5-8].

PHASE ONE: CYTOLOGY AND HPV DNA TESTING

Two quality metrics for gynecologic cytology are

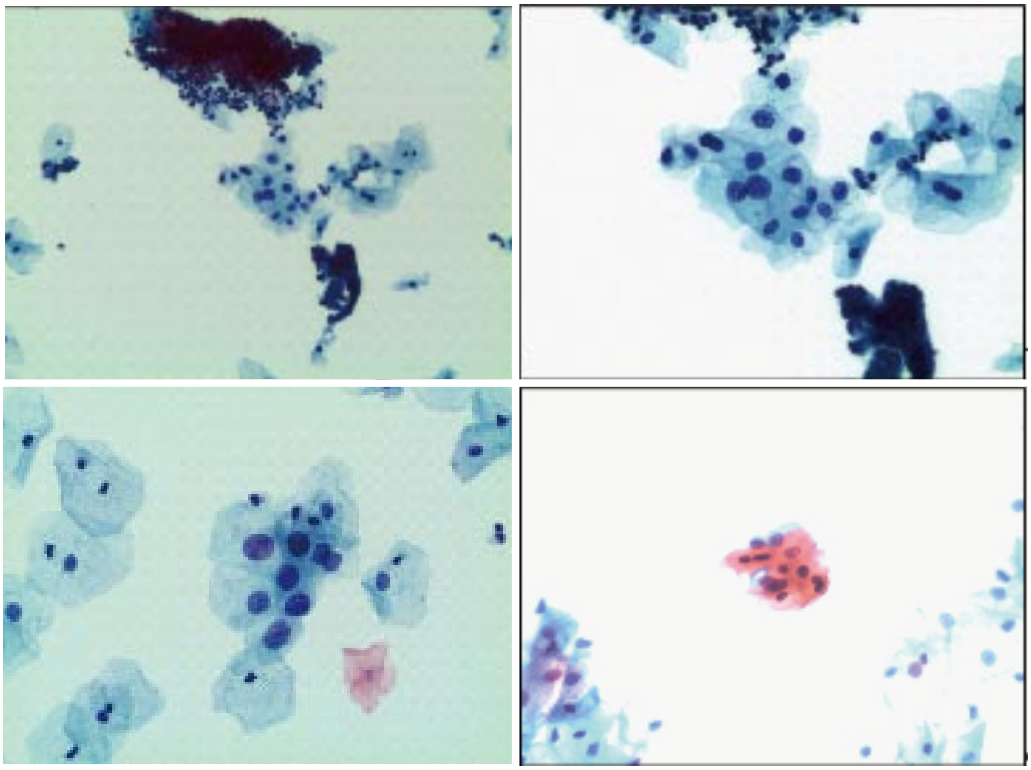

Figure 1 Atypical squamous cells of undetermined significance.

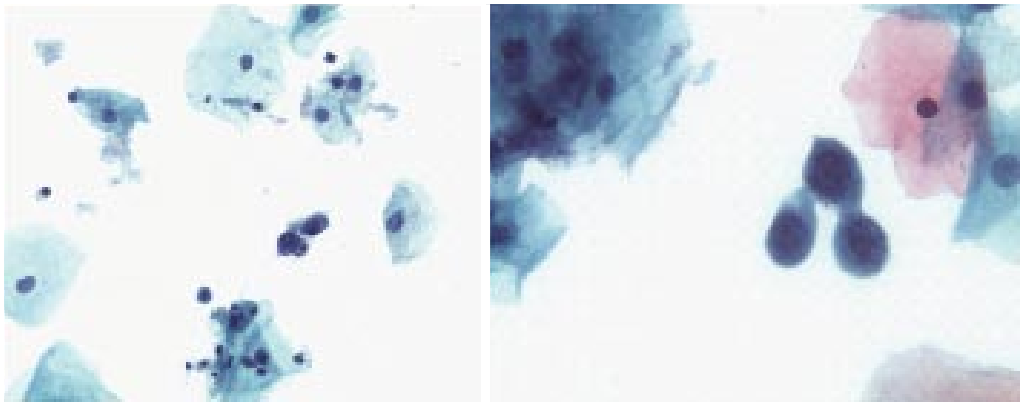

Figure 2 Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion.

available: "prospective rescreening" and "retrospective rescreening". Most laboratories (> 85%) prospectively rescreen more than 10% of Pap tests interpreted as negative for intraepithelial lesion or malignancy. Most (72%) report inclusion of less than 20% high-risk cases. Most laboratories use multiple measures to define "high risk". Most laboratories (96.2%) retrospectively rescreen Pap tests from the preceding 5 years only. In most laboratories (71.4%), only Pap test results with H-SIL or worse prompt retrospective review. Upgraded diagnoses from negative for intraepithelial lesion or malignancy to atypical squamous cells (ASC), cannot exclude H-SIL (ASC-H), should be monitored (Figures 1-5)[9-18].

PHASE TWO: COLPOSCOPY AND HISTOLOGY

Though in the 1980s colposcopically-directed biopsy excluded over 90% of cervical intraepithelial neoplasia (CIN) 3 or worse (CIN3+), recent reviews found sensitivity of colposcopically-directed biopsy for CIN3+ of 50%-65%. Studies from China showed that the sensitivity of colposcopically-directed biopsy for CIN3+ is higher for large CIN3+ than for small CIN3+ and higher for associated high-grade cervical cytology than for low-grade cervical cytology. Colposcopically-directed biopsy excluded over 90% of CIN3+ in the 1980s because colposcopy clinics in the 1980s evaluated women with

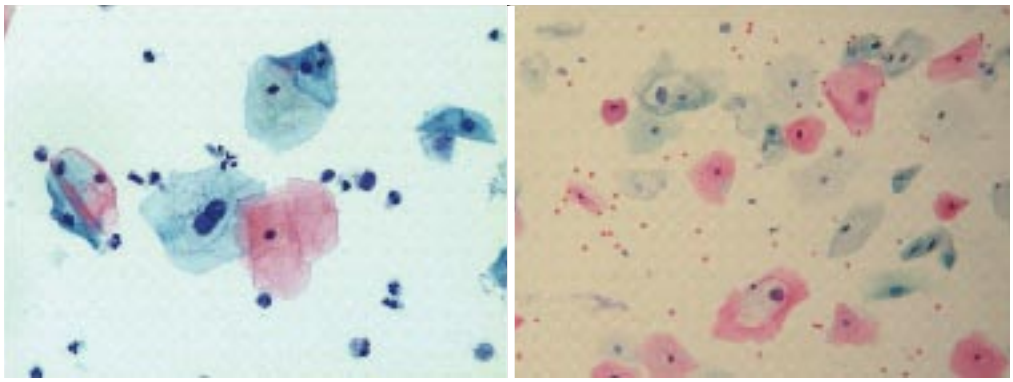

Figure 3 Low-grade squamous intraepithelial lesion.

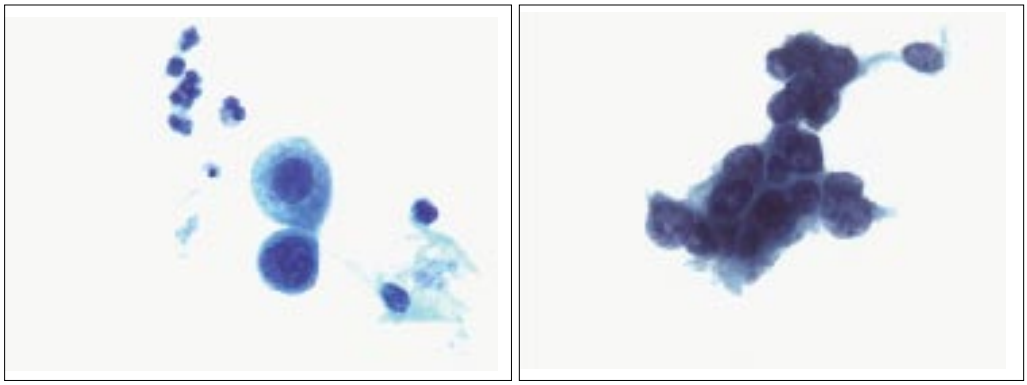

Figure 4 High-grade squamous intraepithelial lesion.

Figure 5 Atypical glandular cells.

high-grade cytology that had large CIN3+. It no longer excludes CIN3+ well because current colposcopy clinics evaluate women with low-grade cytology that have small CIN3+. When colposcopically-directed biopsy is used to exclude CIN3+, our understanding of the natural history of CIN is skewed, errors occur in defining appropriate screening practice, and inaccurate diagnosis results in incorrect treatment. The impression that CIN is more common on the anterior lip of the cervix is an artifact introduced by the inaccuracy of colposcopy. An unjustified enthusiasm for screening with visual inspection with acetic acid (VIA) occurred when the sensitivity of VIA for CIN3+ was inflated by screening studies using colposcopically-directed biopsy as the gold-standard for CIN3+. As the diagnosis of CIN3+ solely by endocervical curettage (ECC) is uncommon in women under age 25, the ECC may be omitted in women under age 25 years. If multiple cervical biopsies are performed, to limit discomfort, a bronchoscopy biopsy instrument which obtains 2-mm biopsies should be used (Figures 6-17)[19,20]. A novel technique uses a high-resolution microendoscope (HRME) to diagnose cervical dysplasia. HRME imaging reduces the limitations of existing cervical cancer screening methods currently in use in low-resource settings and so has the potential to contribute to cervical cancer prevention in the developing world[21,22].

PHASE THREE: TREATMENT AND FOLLOW-UP

Pre-cancerous lesions of cervix (CIN) are usually treated with excisional or ablative procedures. In the United Kingdom, the National Health Service cervical screening guidelines suggest that over 80% of treatments should be performed in an outpatient setting (colposcopy clinics). Treatment methods commonly used for precancerous lesions are conization,

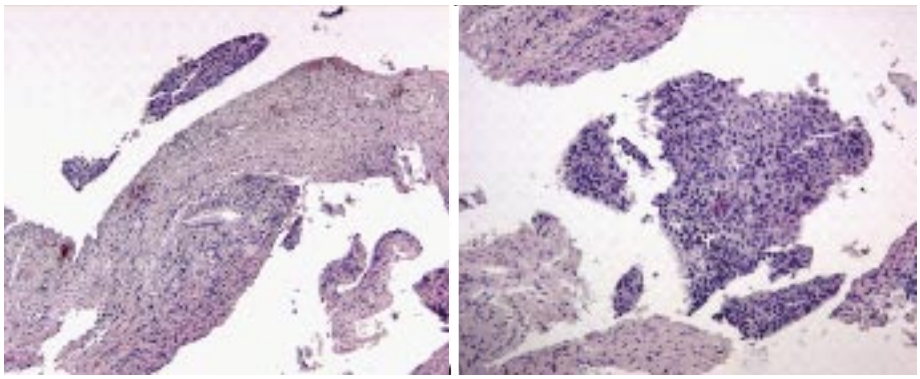

Figure 6 Dysplasia histologic samples.

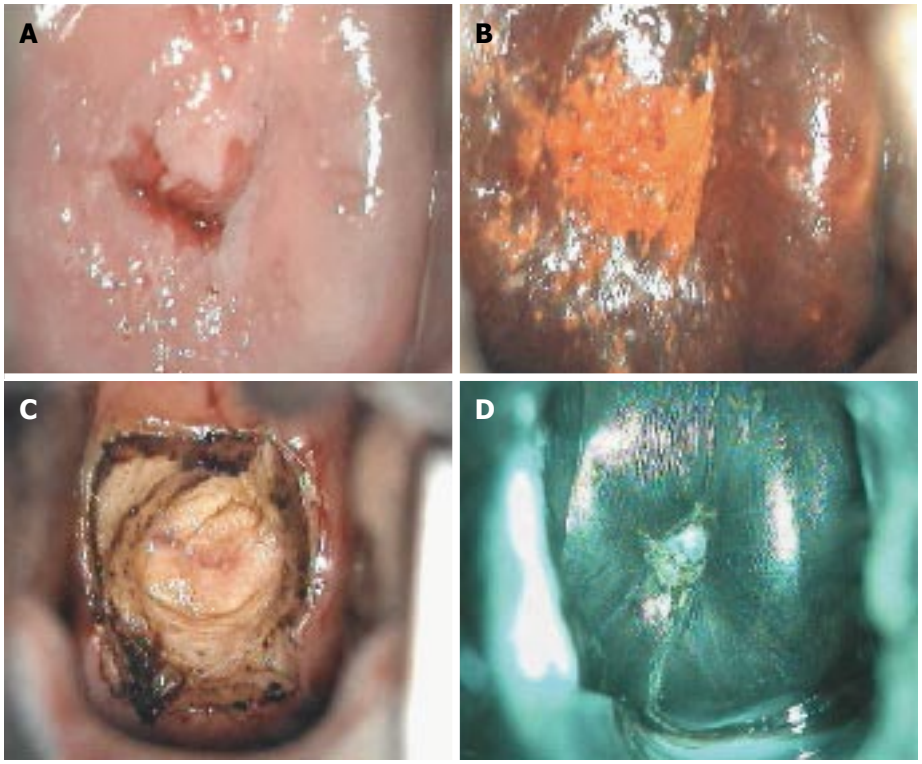

Figure 7 Cervical intraepithelial neoplasia grade 1 colposcopic appearance: (A) after acetic acid application; (B) after Lugol's iodine solution application; (C) after loop electrosurgical excision procedure; and (D) at 6-mo follow-up.

loop electrosurgical excision procedure (LEEP), laser ablation, and cryotherapy. Recently, outpatient LEEP has replaced cryotherapy in many countries. However, a greater awareness of the importance of cervical cancer in the developing world and a greater awareness of the long-term consequences of LEEP like cervical insufficiency, have renewed interest in cryotherapy. Among the trials, cure rates ranged from 56.8% to 96.6% in prospective controlled studies and from 70% to 95.5% in observational trials. Cryotherapy has very low rates of complication and serious complications that require medical therapy or affect the reproductive future results are extremely rare. Side effects include vaginal discharge and cramping which are temporary, usually self-limited, and well tolerated after preventive patient counseling. When surveyed, women highly accept cryotherapy. Compared to other methods of treatment, cryotherapy is very affordable and feasible to integrate screening programs and treatment for cervical cancer[23]. Often, due to improper judgment of interventional indications for cervical lesions, overtreatment to various degrees takes place, influencing patients' health and lives[24,25].

GUIDELINES

Cytopathology experts, interested stakeholders, and representatives from the College of American Pathologists (CAP), the Centers for Disease Control and Prevention, the American Society of Cytopathology

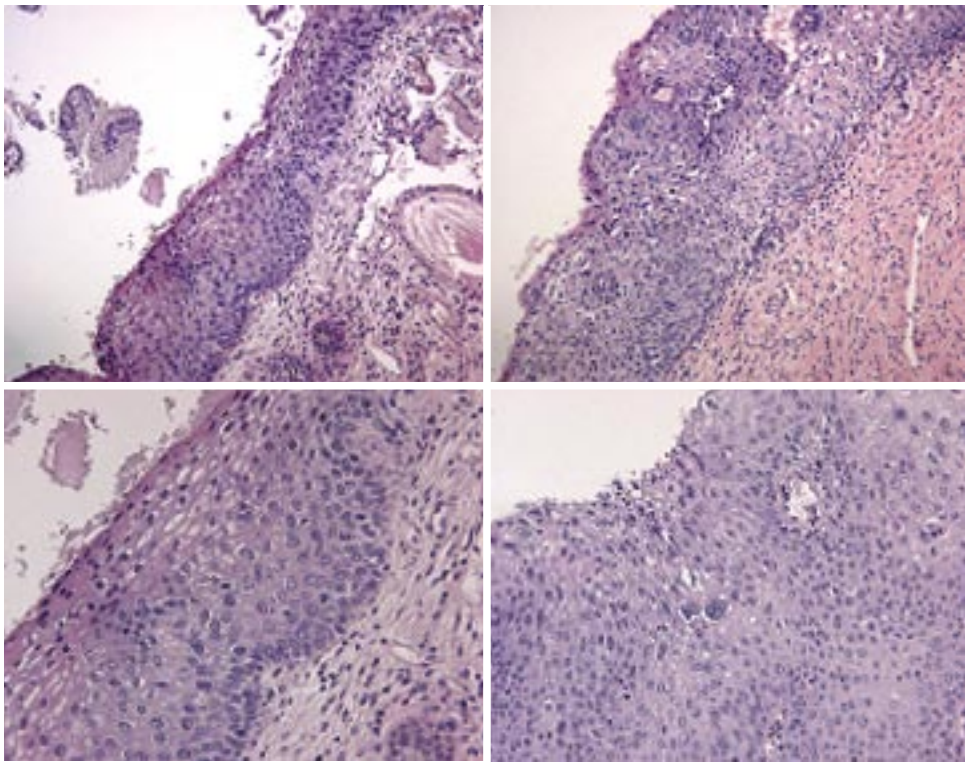

Figure 8 Cervical intraepithelial neoplasia grade 1 histologic samples.

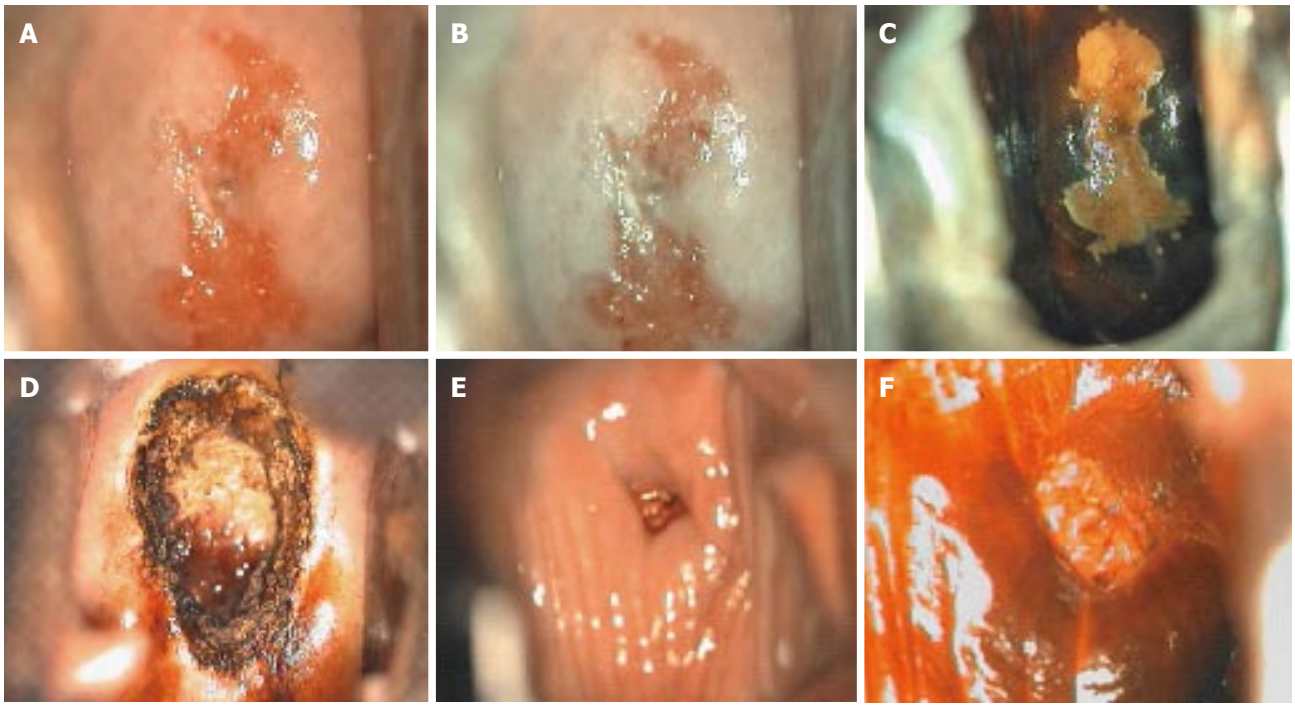

Figure 9 Cervical intraepithelial neoplasia grade 2 colposcopic appearance: (A) before application of any solution; (B) after acetic acid application; (C) after Lugol's iodine solution application; (D) after loop electrosurgical excision procedure; (E) at 6-mo follow-up before application of any solution; and (F) at 6-mo follow-up after acetic acid application.

(ASC), the Papanicolaou Society of Cytopathology, the American Society for Clinical Pathology, and the American Society of Cytotechnology convened the Gynecologic Cytopathology Quality Consensus Conference to present preliminary consensus statements developed by working groups, including the Cytologic-Histologic Correlations Working Group 4, using results from surveys and literature review. Conference

Table 1 The Bethesda system

Specimen type:
Conventional smear (Pap smear)
Liquid-based preparation
Other
Specimen adequacy:
Satisfactory for evaluation (describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g. partially obscuring blood, inflammation, *etc.*)
Unsatisfactory for evaluation… (specify reason)
Specimen rejected/not processed (specify reason)
Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)
General categorization (optional):
Negative for intraepithelial lesion or malignancy conventional smear (Pap smear)
Other: see interpretation/result (*e.g.*, endometrial cells in a woman ⩾ 40 yr of age)
Epithelial cell abnormality: see interpretation/result (specify "squamous" or "glandular" as appropriate)
Interpretation/result:
Negative for intraepithelial lesion or malignancy: when there is no cellular evidence of neoplasia, state this in the general categorization above and/or in the interpretation/result section of the report, whether or not there are organisms or other non-neoplastic findings
Organisms:
Trichomonas vaginalis
Fungal organisms morphologically consistent with Candida spp.
Shift in flora suggestive of bacterial vaginosis
Bacteria morphologically consistent with Actinomyces spp.
Cellular changes consistent with HSV
Other non neoplastic findings (optional to report; list not inclusive):
Reactive cellular changes associated with:
Inflammation (includes typical repair)
Radiation
IUD
Glandular cells status post hysterectomy
Atrophy
Other:
Endometrial cells (in a woman ⩾ 40 yr of age): specify if "negative for SIL"
Epithelial cell abnormalities:
Squamous cell:
ASC:
Of undetermined significance (ASC-US)
Cannot exclude H-SIL (ASC-H)
Low-grade SIL (L-SIL) (encompassing: HPV/mild dysplasia/CIN1)
High-grade SIL (H-SIL) (encompassing: moderate and severe dysplasia, CIS/CIN2 and CIN3):
With features suspicious for invasion (if invasion is suspected)
SCC
Glandular cell:
Atypical:
Endocervical cells (NOS or specify in comments)
Endometrial cells (NOS or specify in comments)
Glandular cells (NOS or specify in comments)
Atypical
Endocervical cells, favor neoplastic
Glandular cells, favor neoplastic
Endocervical adenocarcinoma in situ
Adenocarcinoma:
Endocervical
Endometrial
Extrauterine
NOS
Other malignant neoplasms (specify)
Ancillary testing: provide a brief description of the test methods and report the result so that it is easily understood by the clinician
Automated review: if case examined by automated device, specify device and result
Educational notes and suggestions (optional): suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included)

Adapted from: International Agency for Research on Cancer (IARC), 2013. AIS: Adenocarcinoma *in situ*; NOS: Not otherwise specified; SCC: Squamous cell carcinoma; CIN: Cervical intraepithelial neoplasia; CIS: Carcinoma *in situ*; IUD: Intrauterine contraceptive device; SIL: Squamous intraepithelial lesion; ASC: Atypical squamous cells.

participants voted on statements, suggested changes where consensus was not achieved, and voted on proposed changes. To document existing practices in gynecologic cytologic-histologic correlation (see

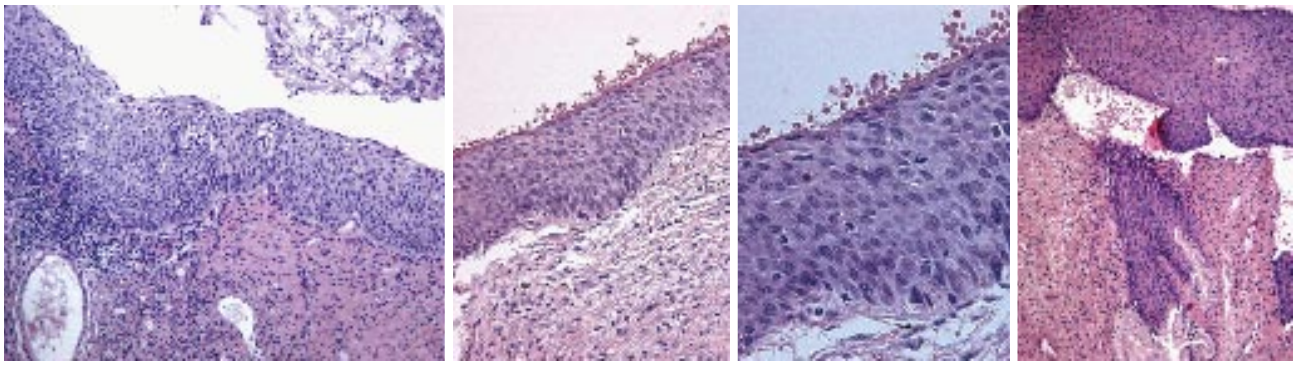

Figure 10 Cervical intraepithelial neoplasia grade 2 histologic samples.

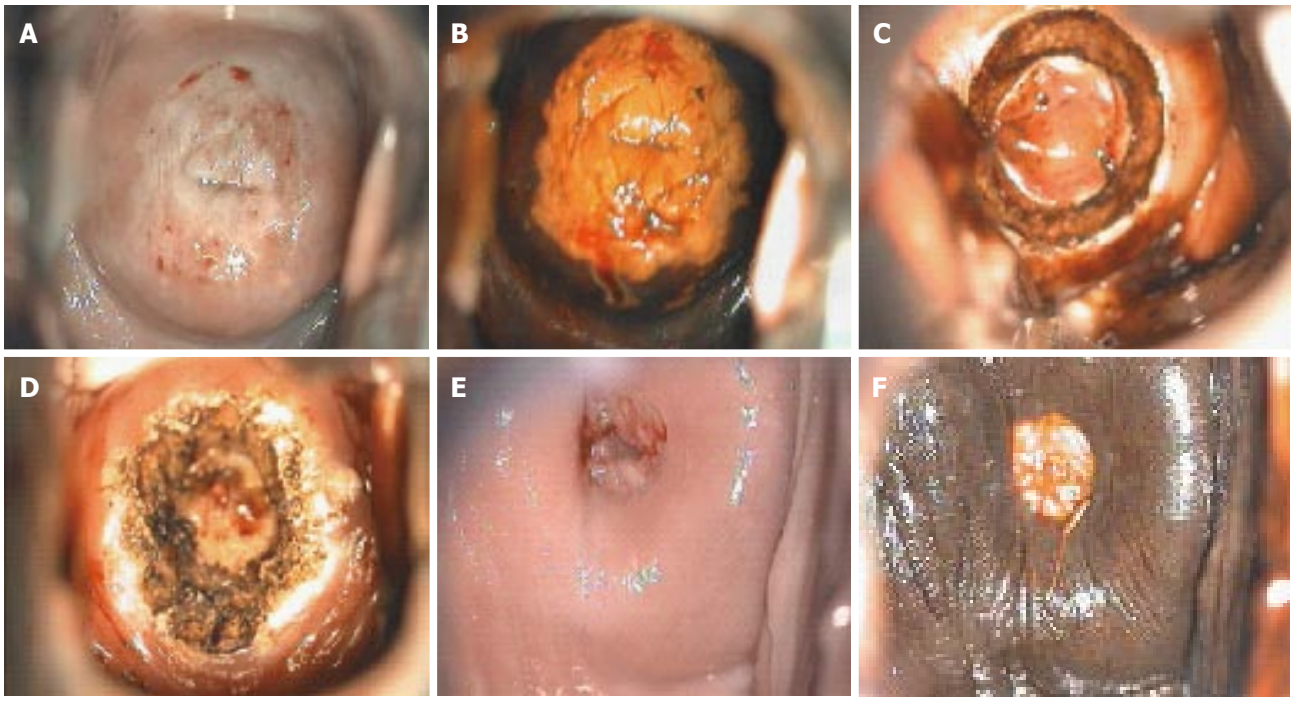

Figure 11 Cervical intraepithelial neoplasia grade 3 colposcopic appearance: (A) after acetic acid application; (B) after Lugol's iodine solution application; (C) during loop electrosurgical excision procedure; (D) after loop electrosurgical excision procedure; (E) at 6-mo follow-up after acetic acid application; and (F) at 6-mo follow-up after Lugol's iodine solution application.

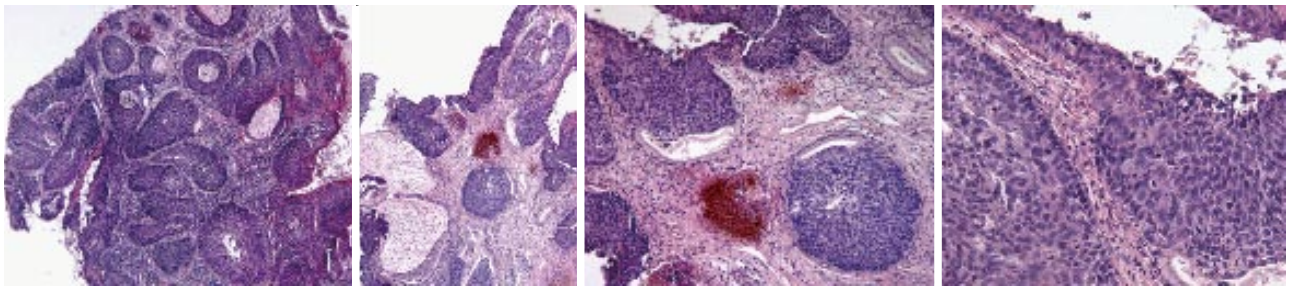

Figure 12 Cervical intraepithelial neoplasia grade 3 histologic samples.

the Bethesda System cytologic reports in Table 1), to develop consensus statements on appropriate practices, to explore standardization, and to suggest improvement in these practices, the material is based on survey results from US laboratories, review of the literature, and the CAP Web site for consensus comments and

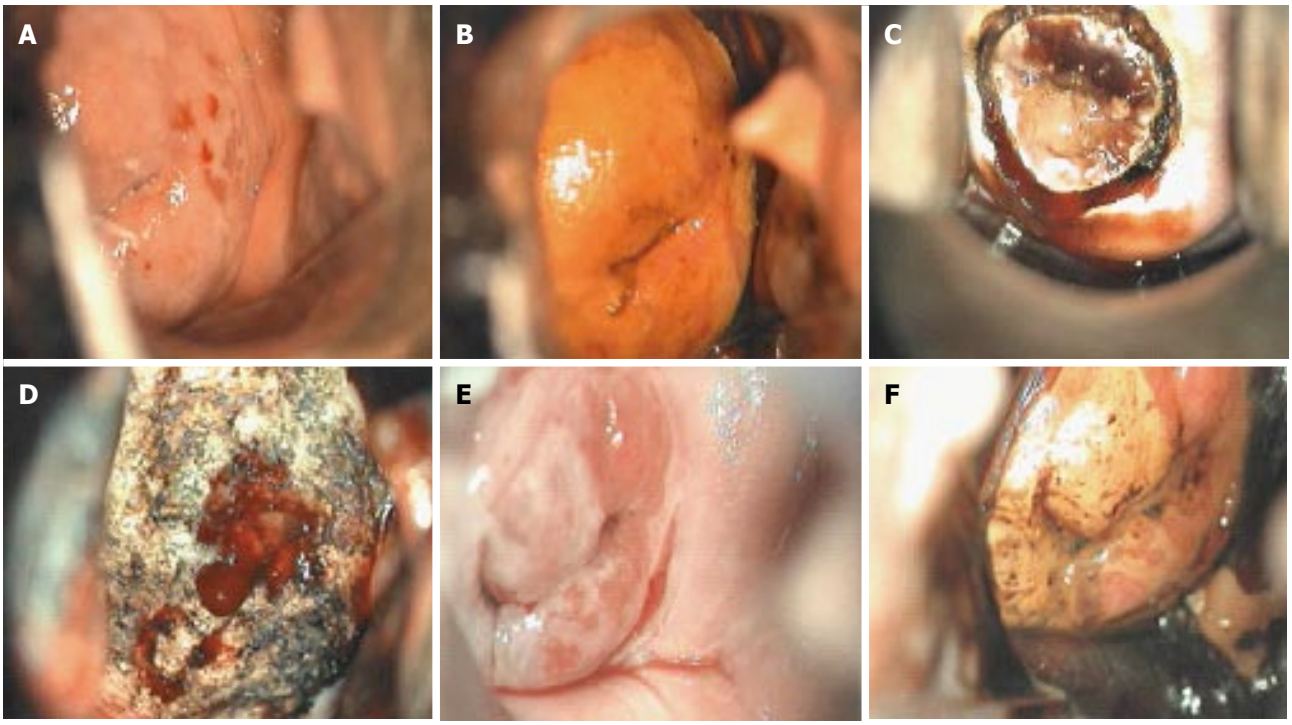

Figure 13 Carcinoma *in situ* colposcopic appearance: (A) after acetic acid application; (B) after Lugol's iodine solution application; (C) during loop electrosurgical excision procedure; (D) after loop electrosurgical excision procedure; (E) at 6-mo follow-up after acetic acid application; and (F) at 6-mo follow-up after Lugol's iodine solution application.

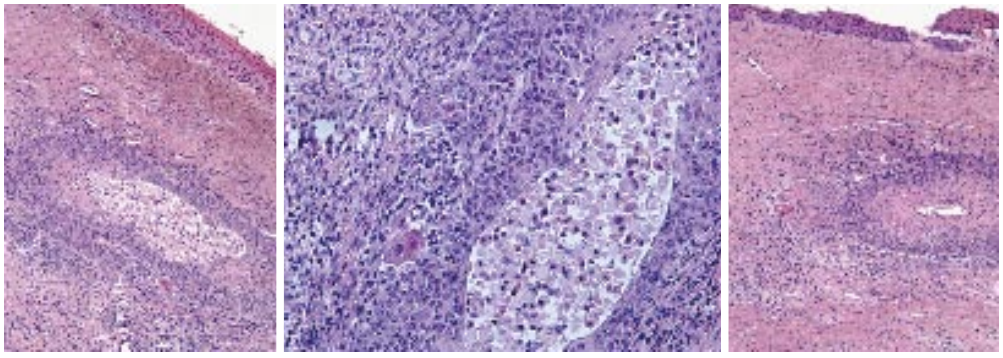

Figure 14 Microinvasive carcinoma histologic samples.

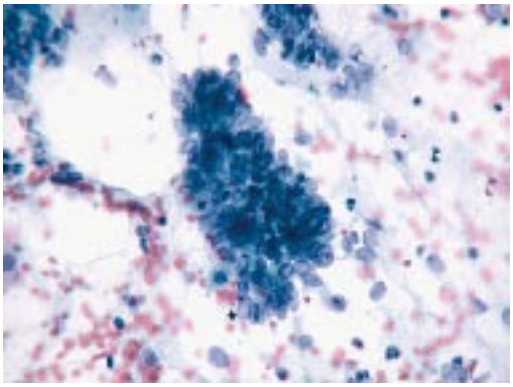

Figure 15 Adenocarcinoma.

additional survey questions[26-30].

CONCLUSION

Vaccination against HPV is expected to decrease the incidence of cervical cancer in most countries. However, it is also expected to influence the effectiveness of screening. In the future, maintaining Pap test as the primary test for cervical screening may become too expensive. As the prevalence of cervical dysplasia decreases, the positive predictive value of citology also decrease, and consequently, more women will undergo unnecessary diagnostic procedures and follow-up. The HPV deoxy ribonucleic acid (DNA) test has recently emerged as the best tool to replace cytology as primary screening. It is less subjected to human errors and much more sensitive than the Pap test in detecting high-grade cervical lesions. By incorporating

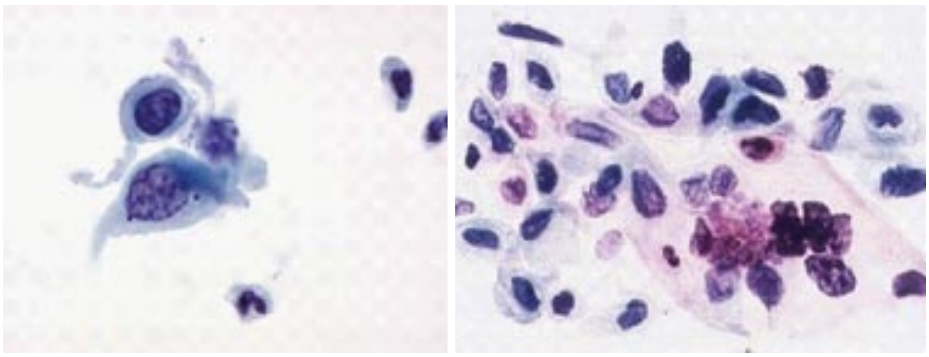

Figure 16 Squamous cells carcinoma.

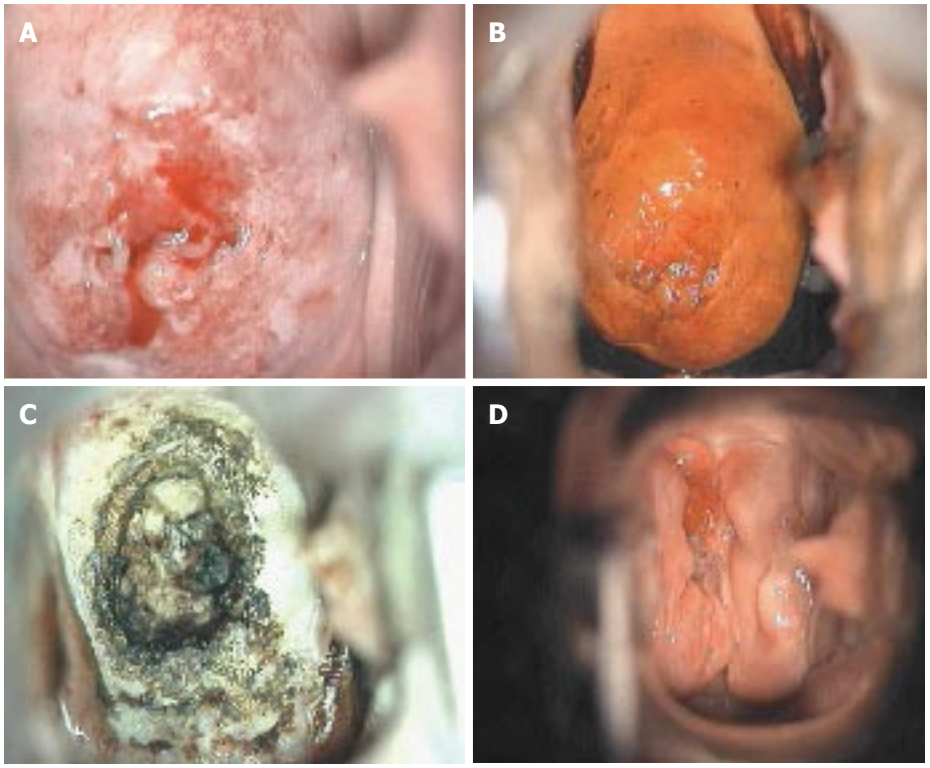

Figure 17 Adenocarcinoma *in situ* colposcopic appearance: (A) after acetic acid application; (B) after Lugol's iodine solution application; (C) after loop electrosurgical excision procedure; and (D) at 6-mo follow-up.

this test the overall quality of screening programs will improve and will allow spacing out the screening tests, while maintaining safety and reducing costs. Although HPV testing is less specific than Pap test, this problem could be solved by reserving the latter for triaging cases of HPV positivity. Since most HPV-positive smears contain significant anomalies, Pap cytology is expected to perform with sufficient accuracy in these cases. Pap triage of HPV-positive patients would also provide a low-cost strategy to monitor the effectiveness of the vaccine in the long term. Although HPV typing could be implemented as a screening tool for the population, further research is needed to determine the optimal age to begin screening, the role of the HPV test and other markers of disease progression, and adequate follow-up procedures for the HPV-positive and smear-negative women[31-33].

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MINIREVIEWS

Bayesian methods in reporting and managing Australian clinical indicators

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Abstract

Sustained clinical improvement is unlikely without appropriate measuring and reporting techniques. Clinical indicators are tools to help assess whether a standard of care is being met. They are used to evaluate the potential to improve the care provided by healthcare organisations (HCOs). The analysis and reporting of these indicators for the Australian Council on Healthcare Standards have used a methodology which estimates, for each of the 338 clinical indicators, the gains in the system that would result from shifting the mean proportion to the 20th centile. The results are used to provide a relative measure to help prioritise quality improvement activity within clinical areas, rather than simply focus on "poorer performing" HCOs. The method draws attention to clinical areas exhibiting larger between-HCO variation and affecting larger numbers of patients. HCOs report data in six-month periods, resulting in estimated clinical indicator proportions which may be affected by small samples and sampling variation. Failing to address such issues would result in HCOs exhibiting extremely small and large estimated proportions and inflated estimates of the potential gains in the system. This paper describes the 20th centile method of calculating potential gains for the healthcare system by using Bayesian hierarchical models and shrinkage estimators to correct for the effects of sampling variation, and provides an example case in Emergency Medicine as well as example expert commentary from colleges based upon the reports. The application of these Bayesian methods enables

all collated data to be used, irrespective of an HCO's size, and facilitates more realistic estimates of potential system gains.

Key words: Clinical indicators; Improvement; System gains; Bayesian; Statistical models

Core tip: The article's purpose is to bring attention to the increasing use of Bayesian methods in the clinical field to overcome shortcomings of previous analyses, and provide an application of how such methods are used in clinical management in Australia; in particular, on how to best report and use clinical indicator data for system improvement. The paper identifies flaws associated with traditional clinical indicator reporting techniques which are still often-used; describes part of current Australian clinical indicator reporting methods; and demonstrates how and why Bayesian methods are fundamental to the improved methods overcoming issues that would otherwise arise with such data.

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INTRODUCTION

Healthcare accreditation systems and quality measurement systems are internationally used for the purposes of improving clinical care and organisational outcomes. Accreditation in healthcare reflects the systematic assessment of hospitals against explicit predetermined standards[1,2] and consists of multiple means of assessment such as self-appraisal, peer-reviewed interviews, scrutiny of documentation, checking of equipment and investigation of key clinical and organisational data[3]. These systems involve considerable levels of resources from the participating agencies and healthcare organizations (HCOs) and are believed to facilitate improved levels of quality in healthcare[4-7].

The Australian Council on Healthcare Standards (ACHS) has a well-established national healthcare accreditation program. It provides robust support for Australian healthcare and is one of the four most commonly cited national healthcare accreditation programs in the world[1,8,9]. In addition to providing a national accreditation scheme, the ACHS supports HCOs by providing sets of clinical indicators (CIs) which HCOs may opt to utilize. HCOs may simply collect their own data, or they may additionally submit their data *via* the ACHS's online performance indicator reporting tool for analysis and reporting[9-13]. CIs measure performance in a clinical setting; the reporting of CIs in HCOs aims to detect suboptimal care either in structure, process or outcome, and can be treated as a tool to assess whether a standard in patient care is being met. They may provide evidence for accreditation purposes and guide the process of quality improvement in healthcare[14].

There is world-wide interest in how to integrate clinical indicators within the accreditation process and mechanisms for their collection differ across countries[9]. A comparison of the four most often referenced national accreditation programs internationally[1,8,9] identified the following key points: (1) the Joint Commission (JC) in the United States and Accreditation Canada are examples of accreditation bodies that have integrated the mandatory requirement that hospitals provide core indicators as part of the accreditation process in order to help focus on-site survey evaluation activities in accreditation[9,15-22]. The JC has done so through its ORYX® program and through its integration of measurement data into its Priority Focus Process for the on-site survey[16,17]. Accreditation Canada has done so through its Qmentum program and combining indicator data with their "instrument" data obtained through questionnaires completed by representative samples of clients, staff, leadership and/or other key stakeholders[9,22]; (2) Haute Autorité de Santé, France, has mandatory accreditation for all its hospitals and has connected many of its accreditation standards to indicators. There are 13 criteria that must be satisfied to achieve certification, of which four are linked to indicators. In total, there are 14 indicators connected with accreditation criteria[9,23,24]; and (3) the ACHS, Australia, provides 6-monthly and trend reports to hospitals which have elected to submit their CI data. The contribution of these reports to a hospital's self-evaluation and quality improvement efforts are relied upon for instigating the CI data collection within hospitals and thus their inclusion in the accreditation process[9].

Taiwan was the fourth country, following the United States, Canada, and Australia, to implement a healthcare accreditation project and the first country in Asia to do so. The reporting of CIs is now required by law for hospitals in Taiwan, and many internal and several nationwide clinical indicator systems have been launched, including three nationwide quality measurement systems: Taiwan Healthcare Indicator Series, Taiwan Clinical Performance Indicators, and Taiwan Community Hospital Association indicators[25]. These three clinical indicator systems are optional for hospitals to utilize. Their target participants are varied and with the diversity of indicators collected there has been difficulty integrating CIs into Taiwan's accreditation process[26], which is governed by Taiwan's Joint Commission on Hospital Accreditation. Taiwan's Ministry of Health and Welfare is currently assessing how best to integrate the CIs from the varied agencies and government departments for enhancing the efficiency and effectiveness of CIs on quality improvement in healthcare.

Since 1993, Australian HCOs preparing for accreditation have submitted data on sets of CIs. The ACHS routinely collates the data in six-month periods and generates reports which are provided to HCOs, along with de-identified reports which are provided to accreditation surveyors, national medical colleges and government bodies. In 2012 the ACHS received data from 670 Australian and New Zealand HCOs on 338 CIs across 22 specialties, or clinical indicator sets[13]. This is the largest source of CI data in the world. The ACHS clinical indicators are not mandatory for any organisation to submit. HCOs select CIs that are relevant to them at that time and where there is a need within that clinical area; for example, high cost procedures, high patient thoroughfare, or a new clinical area to that HCO.

For a given CI, the i^{th} HCO provides the observed number of patients who incur the "event of interest" (O_i) and the number of patients at risk of the event (D_i). Traditional methods of analysis and reporting of such data have been flawed, failing to account for sampling variation and focusing on comparing individual HCO proportions with the mean proportion across all HCOs or with an externally set benchmark value determined by experts, with the primary intention of identifying "outliers". The approach employed in the reporting of the ACHS CIs, as part of the ACHS's Clinical Indicator Program, shifts the focus towards the potential benefits from system-wide improvements of clinical areas rather than simply comparing individual HCO performances within a clinical area which occurs with other traditional approaches. Further, the new approach has required the application of Bayesian hierarchical models to address issues of small samples, which arise in six-monthly data collection, and to preclude overestimation of the potential system improvements. Accounting for sampling variation through Bayesian hierarchical models additionally reduces HCOs' concerns of being misrepresented as extreme as a consequence of a small sample size in a given period.

This paper outlines flaws associated with traditional reporting techniques which are still often used elsewhere; describes part of the current ACHS CI reporting methods; provides examples of annual clinical comments and perspectives based on the reports; and demonstrates how and why Bayesian methods have been fundamental to the improved reporting methods overcoming issues that would otherwise arise with such data.

PROBLEMS WITH TRADITIONAL METHODS OF REPORTING CIS

The implementation of league tables which rank CIs within and across HCOs is a common practice which aims to establish an increased level of accountability and competition, and thus provoke individual strategies towards improved performance[27-29]. Deming's philosophy and systems theory identifies, however, how co-operation rather than competition is required to foster genuine quality improvement and how the system's components and the interdependencies of these components must be foremost in one's mind during the improvement cycle[29,30]. The increased focus upon "competition" between HCOs that occurs as a result of publishing league tables can lead to perverse incentives being created[31]. HCOs may, for example, be motivated towards manipulating their data or taking patients that are considered a "low risk" in order to improve their perceived performance, even if this is at the expense of other HCOs in the system[27,31-33].

There is limited, if any, value reporting league tables of HCO performances. Such presentations are likely to mislead[31,32,34,35] even when statistical techniques have been utilised that adjust for differences that arise due to varying sample sizes, as there will inevitably be a top-ranked HCO and bottom-ranked HCO even if all HCOs were providing outstanding service. Whilst confidence intervals are often introduced to determine where statistically significant differences in the ranks exist, the calculation of multiple intervals will increase the risk of identifying differences due to chance. Employing a conservative significance level to compensate will increase the confidence intervals' widths. In some cases the intervals for HCOs ranked first and last overlap rendering the publication of such tables meaningless[10,32,36,37].

Further, any variations in rank that may be observed with time may be a result of the "regression to the mean" phenomenon[38] rather than reflecting fundamental change in quality. Andersson *et al*[34] produced a measure of the "…expected change in the rank order if one were to repeat the study" to assess the validity of ranking and demonstrated the "…tremendous uncertainty in the ordering…"[34].

Fundamentally, the league table approach is flawed as it focusses attention on individual HCOs and, in particular, those deemed to be poorer performers requiring improvement rather than addressing issues that may help bring systemic advances to the system of HCOs[27,39]. Thus the analysis of clinical indicators must report more than a simple proportion and rank.

Setting thresholds and performing significance tests using p-values is also commonly practiced, and was previously employed by the ACHS. Comparing individual HCO proportions with a nominal threshold value provides minimal assistance to the HCO system as a whole since most HCOs will be within the tolerance level, potentially reducing motivation to undertake improvement, and HCOs with larger volumes of patients (larger sample sizes) are more likely to yield proportions, or rates, which are statistically significant. Consequently, the principal result of such analyses is the classification of individual HCOs as either satisfactory or not[10,27], rather than highlighting system variation or focusing attention on necessary system-wide improvements.

AN IMPROVED APPROACH FOR ANALYSING AND REPORTING CLINICAL INDICATORS

Focusing upon the system of HCOs helps with identifying clinical areas where investigation and improvement activity may produce the most benefit. An approach was applied that uses the data arising from the system of HCOs to identify a potentially achievable mean proportion and identifies clinical areas with large "potential gains" resulting from achieving such a mean proportion. The ACHS reports achieved this by introducing, for each CI, a measure of the gains (or reduction in the number of undesirable events) that could be achieved if the mean proportion was shifted to the 20th centile. The calculation of these potential gains is based on the amount of variation in the system (represented by the difference in the mean proportion, π, and 20th centile proportion, p_{20}, across all HCOs) and the impact upon the system, or volume effect, (represented by the summed D_i, where D_i represents the number of patients at risk of the event at the i^{th} HCO, across all n HCOs providing data for the CI) as shown in equation (1).

$$\text{Potential Gains} = (\pi - p_{20}) \sum_{i=1}^{n} D_i \quad (1)$$

The estimated potential gains facilitates and motivates scientific investigation within clinical areas by providing a relative measure between CIs of the potential improvement. Smaller variation and smaller potential for system impact [in terms of potential for events occurring, represented by ΣD_i in equation (1)] are reflected in a smaller value for the potential gains. Reported as part of the annual Australasian Clinical Indicator reports, this measure enables comparisons of clinical areas for improvement activity rather than allocating responsibility solely to individual HCOs[11].

The use of the 20th centile to calculate potential gains in the system has great appeal as the estimated gains don't rely upon a subjective target but instead is influenced by the system and the data it has produced; the estimated gains are being guided by the existing between-HCO level of variation in proportions. The 20th centile is approximately one standard deviation from the overall mean proportion and may be considered a practicable goal[10]. Since the distribution of proportions is often not symmetric, using standard errors is less useful.

The potential gains as a measure considers the HCOs as part of an holistic system that may have potential for improvement rather than focusing on individual HCOs' performances. Such an approach enables healthcare professionals and governing bodies "…to determine those clinical areas where there are potentially greater gains and hence funding for quality improvement activity would be of a higher priority"[10].

In the case of CIs where higher proportions are desired, potential gains are calculated using the 80th centile, p_{80}, as $(p_{80} - \pi) \Sigma D_i$. This represents the number of additional events that would occur if the mean proportion were equal to p_{80}.

The 20th (or 80th) centile and ensuing calculation of the potential gains, however, should not be obtained simply by using the observed proportions (O_i/D_i) since they are affected by sampling variation. Further, since the HCOs report their data across six-month periods the observed proportions will be based on large and small sample sizes, affecting the precision and reliability of the estimated proportions[10]. Consider for example an HCO that reports only three individuals at risk of a particular event in a given period. A difference of one in the number incurring the event of interest would correspond in a change in the estimated proportion of 33%. Additionally, it would be more likely that HCOs with such small D_i will report the extreme proportions (0% and 100%) despite this most likely not reflecting the true underlying proportion at the HCO.

Rather than exclude HCOs with small data, and thus lose data, a statistical technique known as Bayesian hierarchical modelling and the associated empirical Bayesian shrinkage estimator have been used to better estimate the proportions for the HCOs in a given six-month period. The approach essentially utilizes and combines an individual HCO's proportion and the summary results from the system of HCOs to produce a better estimate of the individual HCO's true underlying proportion.

Empirical Bayes models

"Medical research applications often involve hierarchical data structures as data are collected on random samples of patients nested within each hospital"[10]. When data are collected from many HCOs there is usually substantial variability among the HCOs in addition to variability within the HCOs due to sampling.

Many readers would be more familiar with the non-Bayesian approach to analyses, known as the frequentist or classical approach, which uses only the information provided by the sample data to make wider inference. In contrast, the Bayesian methodology uses additional prior knowledge or belief, presented in the form of probability distributions, in making wider inference. Bayesian methods essentially assess the appropriateness of the prior knowledge given the new data and quantifies this in the form of probability statements or density functions for the values we are wishing to estimate[40,41]. The "Bayesian methodology has been shown to be particularly useful in both the clinical setting and the area of public health policy when the results of a study must subsequently be used to facilitate a decision"[10]. In the Bayesian paradigm, a two-stage hierarchical model representing the nesting of the patients within HCOs may be used to make inferences[42-48], with the first stage representing the distribution of the HCO-specific proportions (CI proportions among HCOs) and the

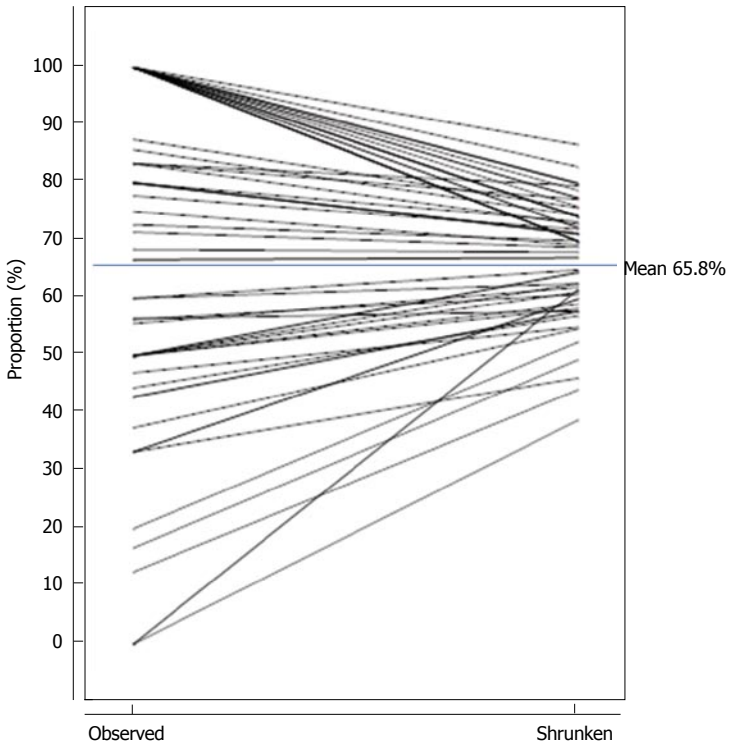

Figure 1 Shrinkage Plot showing Observed and Shrunken Proportions for 62 Healthcare Organisations.

second stage modelling the additional sampling variation associated with what we observe for the patients within HCOs.

The approach borrows strength from the ensemble[49] of HCOs to estimate any individual HCO's true proportion better than an individual HCO's data alone. Essentially the approach uses statistical models for: (1) the conditional probability of the observed counts of events given the unknown true CI proportions (we are attempting to estimate the latter); and (2) a *prior* distribution for the true CI proportions given their overall mean and variance (the latter may be estimated from the data, in which case the approach is referred to as an *empirical* Bayes approach). The two statistical models combine *via* Bayes' rule[45,50] to produce the probability distribution for the unknown true CI proportions given the observed counts and overall mean and variance; this is known as the posterior distribution.

The expectation of this posterior distribution is the empirical Bayes shrinkage estimator for an HCO's CI proportion. The estimated proportion for a given HCO is effectively a weighted average of the individual HCO's observed proportion and the overall mean proportion across all HCOs. The weighting depends upon the systematic variation between HCOs and sample size of the individual HCO, as well as the particular two-stage model's family of distributions.

To reflect the between-HCO and within-HCO sources of variation in the CI proportions, the ACHS reports use the gamma-Poisson hierarchical model. This model was applied since the gamma distribution could represent the distribution of the ratios of observed to expected numbers of events[10]. Further, using the gamma-Poisson model instead of a beta-binomial model for proportions was shown to result in more conservative estimates of the 20th centile-based potential gains due to a relatively greater shrinkage using the former rather than the latter model[10].

The gamma-Poisson model assumes that the O_i follow a Poisson distribution with mean $\lambda_i E_i$, where E_i is the expected number of events at the i^{th} HCO obtained by multiplying D_i by the mean proportion, π, and the true ratios of observed and expected numbers of events, λ_i, are obtained from a gamma distribution with mean, μ, and variance, S_r^2. That is, we have $O_i \sim Poisson\ (\lambda_i E_i)$ and $\lambda_i \sim Gamma\ (\mu, S_r^2)$ which combine using Bayes' rule[45,50] to provide the estimated ratios of an HCO, which are interpretable as proportions by multiplying a ratio by the mean proportion.

The effects of empirical Bayesian shrinkage estimators

Figure 1 visually demonstrates the changes to the individual HCOs' estimated proportions and the distribution of proportions following "shrinkage" for a particular clinical indicator having 62 HCO submissions of data; for this indicator a high proportion was desirable. Each HCO returned data enabling the observed proportion (O_i/D_i) to be obtained and a shrunken proportion was calculated by the afore-mentioned two-stage models and Bayesian methods and multiplying by π (the overall mean proportion). Figure 1 joins each HCO's corresponding observed and shrunken proportions. Several HCOs had common observed and shrunken proportions, hence Figure 1 does not show 62 distinct lines.

The D_i for the CI ranged from 1 to 78. The observed proportions ranged from 0% to 100%. There were 20

Table 1 Report showing Statistics and Estimated Gains for clinical indicators in Emergency Medicine[13]

CI	Desired level	Number HCOs	20th centile (%)	Mean proportion (%)	80th centile (%)	Numerator	Denominator	Potential Gains	Stratum Gains	Outlier Gains
1.1	High	309	99.2	99.1	99.9	26344	26577	219	-	177
1.2	High	323	75.6	79.8	93.7	342984	429896	59829	34972	15420
1.3	High	323	61.0	63.7	93.1	943806	1482555	436635	164974	96017
1.4	High	323	64.6	69.9	96.1	1308074	1870202	488979	77692	100353
1.5	High	317	85.1	87.9	98.6	355355	404382	43560	-	14029
2.1	High	62	58.8	65.8	74.4	338	514	44	29	-
3.1	Low	102	9.9	28.3	47.3	199881	706869	129557	94225	53779
3.2	Low	39	30.1	60.6	82.0	6451	10639	3246	861	1119
3.3	Low	40	19.2	48.5	70.7	7518	15502	4546	2826	2032
4.1	Low	7	0.0	0.1	0.2	938	6742	646	-	241
4.2	Low	6	0.0	0.1	0.1	261	5024	259	-	119
5.1	Low	2	23.9	23.9	23.9	358	15	-	-	-
5.2	Low	3	54.5	54.5	54.5	61	112	-	-	-
6.1	High	7	66.4	71.5	92.7	10276	14363	3033	-	1143
6.2	High	4	20.0	37.0	46.0	2783	7519	678	-	290
7.1	High	10	26.4	44.4	91.8	3395	7653	3630	-	1509
7.2	High	7	21.9	51.4	84.8	183	356	119	-	38
7.3	High	5	21.9	17.7	33.9	854	4818	778	-	-
7.4	High	4	40.8	83.6	99.8	244	292	48	-	33
8.1	Low	28	0.9	4.1	8.8	1547	37888	1203	600	606
8.2	Low	53	1.7	4.9	6.9	49389	1008385	31885	-	10066

CI: Clinical indicator; HCO: Healthcare Organisation.

HCO submissions with observed proportions equaling 100%; the D_i for these HCOs ranged from 1 to 7. There were 3 HCO submissions with observed proportions equaling 0%; the D_i for these HCOs ranged from 1 to 8. The 20th and 80th centiles of the observed proportions were 43.8% and 100% respectively. The 20th and 80th centiles of the shrunken proportions were 58.6% and 74.9% respectively. The mean proportion was 65.8%.

The key points to observe for this example are: (1) HCOs with extreme observed proportions (0% and 100%) are shrunken more greatly towards the mean; a consequence of having smaller D_i; (2) not all HCOs having the same observed proportion will have the same shrunken proportion; a consequence of having differing D_i; (3) the spread of the distribution of shrunken proportions is far less than the spread of the distribution of observed proportions, reflecting the spread of the true underlying proportions; and (4) the 20th and 80th centiles of the shrunken proportions are closer to the mean proportion than the respective centiles of the observed proportions and hence facilitate better estimates of the potential gains.

The potential gains for a CI are then calculable using the shrunken proportions and 20th or 80th centiles using equation (1), or its equivalent for the 80th centile, and are presented along with other summary information for CIs. Table 1 presents an example of such a report for the clinical area Emergency Medicine.

The potential gains provided in Table 1 provide a measure to help prioritise investigation and improvement activity. Consider CIs 1.2 and 2.1; each are desired to have a high proportion. CI 1.2 has a mean proportion of 79.8% whilst 2.1 has a mean proportion of 65.8%. On this alone, it may seem 2.1 should be the priority for improvement; however, the potential gains incorporates the variation between HCOs as well as the size of the potential impact upon the system represented by "Denominator" which is the total number at risk of an event across all HCOs. When this information is considered we observe potential gains for 1.2 that is some 1360 times the potential gains for 2.1, reprioritising where investigation and improvement activity may be best undertaken.

Other estimated gains and funnel plots

In addition to the 20th centile-based potential gains, the ACHS reports present estimates of stratum gains and outlier gains, as shown in Table 1. Stratum gains represent the gains that would be achieved from moving the mean proportions of the poorer performing strata to the mean proportion of the best performing stratum. The strata for the HCOs are: public or private; metropolitan or non-metropolitan; and State (region of Australia).

Outlier gains present the gains that may be achieved from improving the outlier HCO proportions to equal the overall mean proportion. Outlier HCOs have differences in observed and expected numbers of events exceeding three times the standard error of the difference, after shrinkage. A funnel plot, as shown in Figure 2, plots for a given CI the HCOs' differences in observed and expected counts, or *Excess* count, ordered by D_i. Doing so allows a visual check for any patterns that would suggest a volume effect (a pattern due to D_i). In this particular case there were no outliers or an effect due to sample size. Whilst traditional methods may have resulted in little more than the reporting of no outliers,

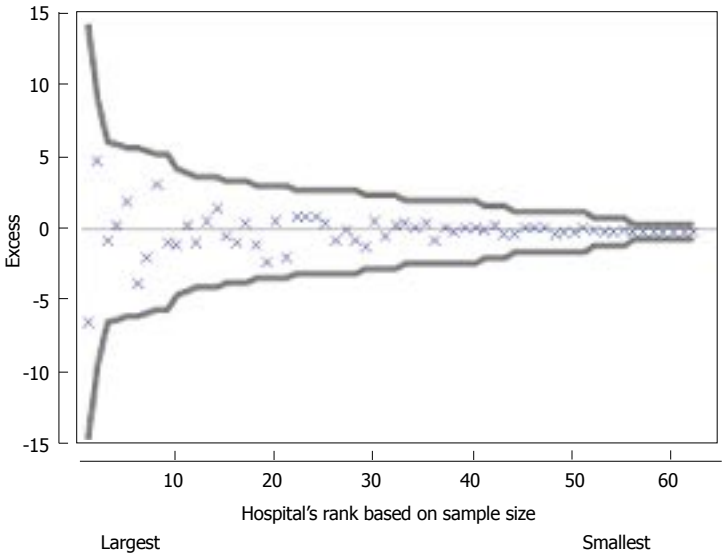

Figure 2 Funnel Plot of Shrunken Estimates of Excess (difference in Observed and Expected) counts for 62 healthcare organisation submissions for clinical indicator 2.1.

the 20th centile gains and stratum gains presented in the ACHS reports focus attention on potential for improvement in the system.

How the reporting system is used

The annual reports described in this paper are part of a two-tiered reporting system. In addition to these annual reports, HCOs receive individual six-monthly reports which identify the individual HCO's performance (rate) compared with both the entire system's rate and themselves based on trend analysis of their six-monthly rates. The latter reports are used by individual HCOs to self-assess whilst the annual report is provided to the relevant Colleges before being published and the Colleges are invited to comment on their set of CIs. In earlier editions of this report the response was less than hoped for, but the annual edition, currently in its 15th year, has more recently received and incorporated clinical comments and perspectives on all results.

Vignettes of the types of comments are provided below[12]. Importantly, Colleges and State Governments are reading reports and thus engaging more with the data.

Example 1: Response from the College of Nursing to the CI representing falls for those aged 65 years or more: "There is no significant change to the data reported in 2013 compared to 2012, despite the fact that the HCO population is aging, with higher numbers of complex and higher acuity patients–particularly within the public HCOs….HCOs with outlier rates within this CI need to review their falls management protocols and falls prevention education, to reduce the current rate of falls–which is nearly double the fitted rate for all reporting HCOs"[12].

Example 2: "The Australian Faculty of Rehabilitation Medicine (AFRM) and the Australasian Rehabilitation Outcomes Centre (AROC) are proud of the continued high standard of compliance with the ACHS CIs by all participating HCOs. The AFRM has included the ACHS CIs in the AROC dataset to encourage HCOs to participate in this important collection and thereby promote continued improvement in these processes and outcomes. The quality of the data collected is of a high standard, with well-established, nationally consistent education programs *in situ*. On that basis, the AFRM and the AROC are confident about the results reported here. HCOs are encouraged to continue reviewing their CI collections to help inform processes and practices in order to maintain the high rates achieved in previous years"[12].

Example 3: Expert commentary from Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) on outcome of selected primipara: "There has been a small increase in the number of spontaneous vaginal births in the selected primipara (CI 1.1), but it remains at around 45.0%. There are several reasons why the number of spontaneous vaginal births will be expected to continue to lessen: (1) women becoming more risk averse and therefore more often requesting obstetric procedures in order to minimize risk. This applies to all women, but particularly in relation to common issues such as how long to tolerate pregnancy progressing beyond the due date; (2) increasing maternal age; and (3) Reducing maternal parity with the consequential reduced morbidity from caesarean section in subsequent pregnancies.

Stratum differences were demonstrated in relation to private and public HCOs (36.4% *vs* 50.8% respectively). This is expected as the above factors are more prevalent in the private sector than public sector"[12].

Example 4: Expert commentary from RANZCOG on Intrauterine growth restriction: "The rate of CI 8.1 has been steadily improving, but this appears to have plateaued at around 1.64%. Failure to diagnose intrauterine growth restriction remains the most obvious

preventable factor in perinatal mortality at term. It has been rewarding to see this statistic fall over the 5 year period and it could be suggested that introducing this CI is partly responsible for this highly desirable improvement"[12].

Example 5: Expert commentary from the Australian and New Zealand College of Ophthalmologists on Cataract Surgery: "Unplanned overnight admission rates following cataract surgery were lower in 2013 with a reduction in the annual rate (CI 1.3). There are known factors such as older age groups, medical co-morbidities and surgery in the latter part of the day that may be contributing. In 2013, there were eleven outlier submissions from eight different HCOs. The outlier organisations can consider a grading/points system based on case complexity to identify cases that should be done in the morning or by an experienced surgeon"[12].

Example 6: Expert commentary from the Australian College of Midwives: General Comment, "The number of services reporting for each CI indicates the ease or difficulty of recording and reporting data defined and possibly the usefulness of the measure to local monitoring. Comparison of local results with the mean and stratified groups would enable health services and professionals to determine areas for policy, practice and research attention"[12].

DISCUSSION

The ACHS CIs aim to get refined data, controlling for casemix, *e.g.*, infection control for each of coronary-artery bypass, hip replacement, knee surgery, *etc.* Since there are 22 specialties (sets of indicators) representing the main Colleges in Australia, and hence some 338 CIs, an individual HCO has to select those sets, or subsets of CIs, that are appropriate to their needs. For example, sets based upon paediatrics, obstetrics, oncology, gynaecology or day only procedures or patients will not be relevant to many HCOs. Even for sets that are relevant some of the CIs within the set may not be collected due to costs of obtaining the data from the medical records, or the CI may not be seen as important to the individual HCO. The non-mandatory nature of the CIs is consistent with the non-punitive and non-invasive nature of the reporting methods described. The data collection and reporting method is designed to assist the healthcare system, not place greater burdens upon the system or HCOs unable to sustain the costs of widespread data collection. Whilst the CIs provide a means for HCOs to more easily provide necessary evidence to warrant accreditation, the aforementioned reasons along with nuances that exist within any HCO warrants the optional nature of their use. This further attracts some HCOs towards the ACHS accreditation process and to provide data honestly rather than be forced to provide data and be motivated towards manipulating their data or taking patients that are considered a "low risk" in order to improve their perceived performance, even if this is at the expense of other HCOs in the system[27,31-33].

The simplistic ranking of HCOs does not quantify the gains that could be achieved and has many disadvantages. The reporting of indicators which measure clinical and healthcare processes should quantify the potential gains to encourage action. Estimating the gains across many indicators enables the comparison and identification of areas with greater potential improvement and thus prioritisation of resources for investigation and improvement efforts. The required tools and resources to investigate and address those areas with the greatest gains must then be provided.

Bayesian hierarchical models and empirical Bayes shrinkage estimators borrow strength from the collection of HCOs to estimate any individual HCO's true proportion better than an individual HCO's data alone, accounting for sampling variation and addressing issues surrounding small sample sizes[49]. Shrinkage estimators are more beneficial in situations where denominators vary in size and some are small[10,51], as is the case for the CI data.

The shrinkage estimator effectively modifies an individual HCO's observed proportion by drawing it closer to the prior mean. The amount of modification (shrinkage) is less for those HCOs having larger sample sizes (reflecting the increased information being reported by those individual HCOs reducing the effects of sampling variation) and for systems exhibiting large systematic, or between-HCO, variation (reflecting lower strength of knowledge about the prior mean since the prior variance is large in such cases). Thus HCOs having smaller denominators will have their observed proportions shifted more closely towards the overall mean than HCOs having larger denominators. The implementation of the shrinkage estimators not only provides better estimates for each HCO, in particular those that would otherwise be identified as extreme due to small sample sizes, but additionally enables all HCOs to be included in the reports irrespective of size. Further, the approach is reflecting the reality that there is a level of dependence between the HCOs as they are all part of the one system.

The use of CIs as flags for required investigation towards system improvement is a valuable area of research. The development and application of appropriate statistical methods for analysing and reporting CIs is important and should focus on improving the healthcare system. Estimating the potential gains achievable through investigation and quality improvement that reduces the mean proportion to the 20th centile focusses efforts on system-wide improvements rather than assigning blame and onus on individual HCOs. In combination with the empirical Bayesian shrinkage estimators, the estimated potential gains support practicable reports on CIs for healthcare providers.

CIs are screening tools, so just as positive blood tests or breast cancer screening result in review and

further investigation so too positive results from the CI analysis must result in further investigation. There are three types of positive results, namely, large variation between HCOs, large variation between strata and outlier HCOs (large variation from expected). This paper has described the use of the difference between the mean and 20th centile proportions to estimate the impact of between HCO variation and stratum and outlier gains which estimate the impact of between stratum variation and variation from expected for individual HCOs. These gains are reported by the ACHS for all 338 CIs[13]. Bayesian methods play a key role in ensuring such measures are not overestimated.

The presented potential gains quantify reductions, or increases for certain CIs, in the numbers of "events of interest". Whilst it is possible to attribute monetary costs to each event in order to estimate potential financial changes, the problem remains of comparing the vast range of outcomes such as delays due to intensive care unit (ICU) access block, readmissions and out of hours discharges from ICU, adverse events related to medication errors, wound infections and failure to administer venous thromboembolism prophylaxis. Whilst the measures may be converted to indicate potential costs to the healthcare system doing so would take a restricted view of these measures of quality. Further, there remains the issue of how one may compare losses of life with additional waiting times. The simplest approach involves identifying CIs where significant numbers of patients were not receiving the standard of care implied by the CI as being acceptable; however, this remains an area of ongoing research.

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MINIREVIEWS

Scourge of intra-partum foetal death in Sub-Saharan Africa

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Abstract

Intra-partum foetal death has been variously defined. However, a definition adopted at a technical consultation in 2006 is employed in this review. The quality of intra-partum care is a crucial factor for pregnancy outcome for both mothers and new-borns. Intra-partum stillbirth is defined as late foetal death during labour, which clinically presents as fresh stillbirth. The largest proportion of the world's stillbirths occurs in the late preterm, term and intra-partum periods. The Western Pacific region has the greatest reduction in stillbirth with a 3.8% annual decline between 1995 and 2009; however, the annual decline in the African region is less than 1%. Caesarean delivery is still uncommon, especially in rural areas: 1% of births in rural Sub-Saharan Africa and 5% in rural South Asia are by caesarean delivery; 62% of stillbirths occurred during the intra-partum period; 61.4% of stillbirths are attributable to obstetrical complications. Preventive measures aimed at reducing the incidence of intra-partum foetal death entail all measures aimed at improving quality antenatal care and preventing intra-partum asphyxia. This review discusses intra-partum foetal deaths from a Sub-Saharan African perspective. It explores the contribution of research within the region to identifying its impact on new-born health and potential cost-effective policy interventions.

Key words: Intra-partum; Foetal; Death; Sub-Saharan; Africa

Core tip: Intra-partum foetal death includes intra-uterine deaths that occur within 12 h of delivery of a new-born weighing more than 1000 g, or that had more than 28 wk of gestation, but could not be resuscitated. Sub-Saharan Africa has the lowest recorded decline of intra-partum foetal deaths; however, this region recorded a doubling of her annual rate of reduction to 3.1% during 2000-2011, from 1.5% during 1990-2000. Impacts of research within the region towards improved new-born health and cost-effective policy interventions are examined.

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INTRODUCTION

Intra-partum foetal death is a subset of perinatal mortality: macerated stillbirths; lethally and congenitally malformed neonates as well as new-borns that died after the first 24 h of life are excluded. It basically refers to intra-uterine foetal deaths that occurred within 12 h of delivery of a new-born whose weight is more than 1000 g or which has gone beyond 28 wk of gestation[1]. It is a health indicator which measures the quality of obstetric care on one hand, and the association between maternal and neonatal health on the other hand; it is a determinant of the quality of intra-partum care.

According to the International Classification of Diseases, Revision 10, a stillbirth or late foetal death is that which occurs after 22 wk of gestation, or when the crown-heel length is 25 cm or more, and the weight is at least 500 g[2]. For the purpose of international comparison, stillbirth is better described as foetal death at a gestational age of 28 completed weeks or a crown-heel length of 35 cm or more and birth weight is at least 1000 g[3]. Gestational age measures are not as reliably documented as birth weight, especially in the low resource countries.

The greatest risk to life for the mother and baby is noted during childbirth[4]. Intra-partum related death accounts for over 2/5 of the world's annual maternal deaths; the death of 1.02 million babies during labour, and 904000 neonatal deaths around delivery are interrelated[1,4,5].

The quality of intra-partum care is therefore a crucial factor for the pregnancy outcome for both mothers and new-borns. Timely and appropriate care rendered by skilled attendants in an atmosphere that is conducive will prevent or at least reduce morbidity for both mothers and newborns.

The foetal mortality rate for gestations of at least 20 wk (6.2 foetal deaths per 1000 live births)[6] and infant mortality rate (6.9 infant deaths per 1000 live births)[7] in the United States in the year 2005 were similar. Depending on the definition used, 40% to 60% of perinatal mortality is due to foetal mortality[8]. According to the World Health Organization (WHO)[9], 8 out of every 1000 babies die during labour worldwide. In 2000, intra-partum mortality rate was estimated at 15 per 1000 births in Middle and Western Africa, while it was only 0.6 per 1000 births in developed countries[9].

Annually, there are 287000 maternal deaths[10] and over 3 million stillbirths, of which about a million die in the course of labour and about four million neonatal deaths, half of which happen on the day of birth[11].

Although stillbirths are underreported in developing countries, 97% of the cases occur in the region, and it accounts for 50% of worldwide perinatal deaths. It is hoped that the problem of stillbirths will be given due attention in these regions with a view to reducing the effect on the society[9,12]. A Nigeria based study posited that of the recorded perinatal deaths, 51.2% were fresh stillborns, while 39.1% were macerated[13]. This finding conforms to the Wigglesworth classification of perinatal death (Table 1). The high proportion of intra-partum foetal death is a reflection of poor-quality intra-partum care in the study-country, however, another study conducted in Nigeria recorded 73% macerated and 27% fresh stillbirths[14], which reflects variations noted from one centre to the other, even within the same country.

The neonatal period is a crucial part of the infant's life, as up to 40% of deaths of children younger than 5 years occurred within the period; also, neonatal death was observed to increase in relation to a rapid fall in postnatal deaths[1,15]. The need for concerted efforts from health practitioners and policy makers can never be overemphasized.

Twenty-five percent of neonatal deaths in low income countries and 8% of all deaths among children younger than 5 years during the 4-year period of 2000 to 2003, were attributed to birth asphyxia by the WHO[16]. A major focus on birth asphyxia is important in reducing child mortality; this will immensely contribute to the attainment of the Millennium Development Goal[17]. Tracking of stillbirths, however, is often incomplete and variable.

The global burden of disease literature gives insight into the prevalence of death during the peri-partum period[17]. The relatively insufficient resource allocation to the health sector, especially in Africa, which is identified as a bane, was addressed by the World bank in 1993; the bank issued a guide on resource allocation in the health sector to assist the developing countries in that perspective.

Intra-partum related neonatal death deserves prominence in global health programming and policy because it has a significant contribution to the under-5 child mortality rate. Early neonatal deaths are intertwined with maternal health, therefore, effective maternal cum neonatal health services are key to reversing the poor outcomes. The reduction of intra-partum-related neonatal deaths is a daunting challenge and success will depend on provision of effective and efficient care delivery[3,11,16].

Vital registration systems are weak in developing countries where more than 97% of neonatal deaths occur[12], therefore little is known about the causes of most of these deaths. Furthermore, autopsies on the dead foetuses, as well as placenta histological studies are rarely carried out in the region. This review discusses intra-partum foetal deaths from a Sub-Saharan African perspective. It explores the contribution of research within the region to identifying its impact on new-born health and potential cost-effective policy interventions.

Table 1 Perinatal deaths by Wiggles worth classification[a]

Birth weight (g)	No. of perinatal deaths					Total (%)
	Macerated stillbirths	Congenital malformations	Immaturity	Asphyxia	Other	
1000 (n = 34)	18	2	14	0	0	34 (5.2)
1001-1500 (n = 42)	16	7	16	3	0	42 (6.4)
1501-2000 (n = 65)	35	4	18	8	0	65 (9.9)
2001-2500 (n = 55)	20	7	11	16	1	55 (8.3)
N2500 (n = 464)	151	5	49	250	9	464 (70.3)
Total (%)	240 (36.4)	25 (3.8)	108 (16.4)	277 (41.9)	10 (1.5)	660 (100.0)

[a]Birth weight data missing for 61 neonates. Adapted from Fawole *et al*[13], Determinant of Perinatal Mortality in Nigeria.

PATHOLOGIC CONSIDERATIONS AND TERMINOLOGIES

Most foetal deaths, intra-partum or immediately post-partum, are caused by birth asphyxia, which is mainly due to mismanaged obstetric conditions. The causes of foetal deaths are: obstructed labour, infections, asphyxia, maternal haemorrhage, severe pre-eclampsia and eclampsia, maternal/foetal malnutrition, congenital anomalies and umbilical cord complications[18,19].

Congenital anomalies, diabetes, and infections associated with preterm birth and post-term pregnancy, which are preventable causes of stillbirth, contribute immensely to a high foetal death rate, however, the causes have been virtually eliminated in high income countries (HIC)[20]; the contributions from these important causes in the Sub-Saharan Africa region are scarcely documented.

"Birth asphyxia" according to WHO in 1997, is a condition in new-borns who had breathing abnormality at birth[21]. Non-specific terminologies such as foetal distress, Apgar score and foetal acidosis are not favoured; these terminologies are not predictive of outcome on the long run[22].

Post–asphyxia encephalopathy, birth asphyxia, hypoxic-ischaemic encephalopathy, foetal distress or perinatal asphyxia should not be used loosely, except when there is evidence of acute intra-partum causation[22-24]. Intra-partum stillbirth presents as fresh stillbirth when it is due to intra-partum hypoxic injury[25].

Intra-partum neonatal death is the death of a baby born alive within 28 d of life from evidenced intra-partum injury, with or without neonatal encephalopathy[3,11,20,21]; acute hypoxia superimposed on chronic hypoxia in a growth restricted foetus could easily lead to foetal death[22]. There is a limit to the ability of the foetus to tolerate excessive reduction in oxygen partial pressure, though a healthy foetus is conditioned to physiological hypoxia that regulates foetal circulation through unhindered oxidative metabolism[26].

The immediate response to acute hypoxia by the foetus and the new-born is shallow breathing followed by cessation of respiration, called primary apnoea, which leads to deep gasping and irregular respirations. It progresses to terminal apnoea when all respiratory efforts cease[26].

In the course of apnoea, the heart rate progressively decreases to a halt few minutes after the onset of secondary apnoea. Primary and secondary apnoea presents with bradycardia and dyspnoea[26].

Prompt tactile stimulation and/or assisted ventilation always lead to a restoration of the heart rate to normalcy; any delay in resuscitation is accompanied by slow recovery. The importance of institutionalization of the concept of emergency obstetric care (EMOC), increased skilled birth attendance and neonatal intensive care in all facilities charged with pregnancy care cannot be over-emphasized.

DEMOGRAPHIC TRENDS

Over 1 million stillbirths occur during labour[23]; a number of studies from some low income countries showed that up to 70% of stillbirths occur in the intra-partum period and are due to obstetric emergencies[4,22], while in advanced countries, half of all stillbirths occur in non-anomalous babies at > 28 wk gestation[20]. A WHO review of vital registration showed that the estimated number of global stillbirths was 2.6 million in 2009 and 3.0 million in 1995[27]. The worldwide stillbirth rate has declined by 14% between 1995 and 2007[27].

The Western Pacific region recorded the greatest reduction in stillbirths, with a 3.8% annual decline between 1995 and 2009, while the African region posted only an annual decline of less than 1% (0.7%)[24]. There is a wide variation in stillbirth rates among countries; South-East Asia and Africa had two-thirds of all stillbirths.

In advanced countries, the third-trimester stillbirth rate is less than 4 per 1000 total births, and this amounts to 25% of the worldwide average and 11% of the average in South Asia and Sub-Saharan Africa[25]. Finland has the lowest reported rate of 2.0 per 1000 total births, while Nigeria reported 41.9 per 1000 total births and Pakistan at 46.1 per 1000 total births, had the highest rates[24].

Sub-Saharan Africa registered a 39% decline in the under-five mortality rate. However, the region had an annual rate of reduction to 3.1% during 2000-2011, from 1.5% during 1990-2000[26]. There was a dramatic acceleration in the rate of decline in Eastern and Southern Africa; this coincided with a recorded sub-

stantial improvement in effective interventions to combat major diseases, especially HIV, and also measles and malaria[27].

RISK FACTORS

Utilization of emergency obstetrics care where available, remained low and even worse in the remote areas; 5% of births in rural South Asia and 1% in rural Sub-Saharan Africa are by caesarean delivery[28]. Sixty-two percent of stillbirths occurred in the intra-partum period; obstetrical complications accounted for 61.4% of stillbirths. The following significant maternal risk factors were documented: un-booked status, illiteracy, age of 35 years and above in pregnancy, extremes of reproductive age groups, multiple pregnancies and prolonged labour; the foetal risk factors associated with stillbirth are mal-presentation, decreased foetal movement, foetal distress, prematurity, small for gestational age, and neonatal infection[28-30]. The following independent risk factors were identified: congenital malformations, true knot of cord, meconium stained amniotic fluid, oligo-hydramnios, poly-hydramnios, previous adverse perinatal outcome, placental abruption, advanced maternal age, and hypertensive disorders[30]. Jewish ethnicity, gestational diabetes, previous caesarean section, and recurrent abortions were negatively associated with intra-uterine foetal death[31].

An Africa based study identified the following risk factors for perinatal death: un-booked status, lack of prenatal care, duration of schooling, maternal age above 35 years, asphyxia, and prematurity[12]. However, the researchers found that when maternal and neonatal factors were considered together, the following are the determinants of perinatal mortality: un-booked status, free maternity service, mother's level of education, mother's age within the range of 26 and 30 years, and mothers older than 40 years, prematurity, asphyxia, lack of prenatal care, mode of delivery and so on[12]. Some of the risk factors peculiar to the Sub-Saharan African society are lack of prenatal care, un-booked status, and lack of quality care to mention a few.

The most common patient-related avoidable factors found in a South African study were un-booked patients, patients booked late in pregnancy, patients who delayed before seeking medical assistance, and patients with inappropriate responses to poor foetal movements[32]; whereas the most common avoidable factors related to medical care were underestimation of foetal size and lack of response by staff to patients with poor obstetric histories. However, the common administration-related avoidable factors were unavailable operating theatres and lack of transportation between institutions[32].

In a number of countries in the Sub-Saharan Africa, a considerable percentage of deliveries are not supervised by skilled workers, there is a dearth of nurses and midwives, the database in these places is weak for setting priorities, and political willingness to address the issue is suboptimal.

It is implied by these identified risk factors that developing an algorithm for the management of such conditions, especially for use in the Sub-Saharan African countries, will be a good step towards reducing the scourge.

PREVENTION

Preventive measures aimed at reducing the incidence of intra-partum foetal death entail all measures aimed at improving quality antenatal care and preventing intra-partum asphyxia.

Appropriate obstetric care in the prenatal and intra-partum periods is vital. Also, close monitoring with readily available appropriate care during labour to enable obstetrical providers recognize conditions such as prolonged labour, placental abruption, placental previa, foetal mal-position, and foetal distress, will allow for rapid intervention through caesarean section to further reduce the rate of intra-partum foetal deaths.

Every death counts; a version of mortality audit in South Africa focuses on saving mothers, babies and children[33]. Replication of the exercise in all African countries, with the intention of putting to use lessons learnt from the exercise, will help stem the tide of the repugnant scourge.

Intermittent auscultation for monitoring foetal heart rate in labour is preferred and should be promoted in the low and medium income countries, rather than continuous foetal heart rate monitoring devices which might appeal to policy makers in such climes. This recommendation is based on the outcome of a Dublin based study, which concluded that there is no difference in intra-partum stillbirth rates, as well as long term outcome between the intermittent auscultation group and the continuous foetal heart rate monitored group[33].

CONCLUSION

The high rates of intra-partum foetal death in Sub-Saharan Africa should be a cause for concern for all stakeholders.

Urgent and effective steps are needed to promote equitable distribution of health facilities, providing maternal and newborn health care with optimal capacity for EMOC.

Initiatives that seek to increase rates of facility births in Sub-Saharan Africa must address the issues of quality of maternity care and socio-cultural determinants of access to health care.

It is important that health systems identify the causes of intra-partum foetal deaths peculiar to their location and endeavour to audit all stillbirths with a view to improving pregnancy outcomes for both mothers and new-borns.

Increased research attention focusing on this subject in the region is also advocated.

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ORIGINAL ARTICLE

Observational Study

In vitro differentiation of human umbilical cord Wharton's jelly mesenchymal stromal cells to insulin producing clusters

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at Dryad repository, who will provide a permanent, citable and open-access home for the dataset.

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Abstract

AIM: To investigate the differentiation of human Wharton's jelly derived mesenchymal stromal cells (WJ-MSCs) to insulin producing clusters (IPC) this study was conducted.

METHODS: The umbilical cords samples were collected from full term caesarian section mothers and the WJ-MSCs were cultured from tissue explants in High glucose-Dulbecco's Modified Eagle Medium (H-DMEM); H-DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics. The expression of CD90, CD44, CD105, CD34 and CD133 as well as osteogenic and adipogenic differentiation of cells in appropriate medium were also evaluated. The cells were differentiated toward IPC with changing the culture medium and adding the small molecules such as nicotinic acid, epidermal growth factor, and exendin-4 during 3 wk period. The gene expression of *PDX1*, *NGN3*, *Glut2*, insulin was monitored by reveres transcription polymerase chain reaction method. The differentiated clusters were stained with Dithizone (DTZ) which confirms the presence of insulin granules. The insulin challenge test (low and high glucose concentration in Krebs-Ringer HEPES buffer) was also used to evaluate the functional properties of differentiated clusters.

RESULTS: WJ-MSCs were positive for mesenchymal surface markers (CD90, CD44, CD105), and negative for CD34 and CD133. The accumulation of lipid vacuoles and deposition of calcium mineral in cells were considered as adipogenic and osteogenic potential of WJ-MSCs. The cells also expressed the transcriptional factors such as Nanog and OCT4. During this three step differentiation, the WJ-MSCs morphology was gradually changed from spindle shaped cells in to epithelioid cells and eventually to three dimensional clusters. The clusters expressed PDX1, NGN3, Glut2, and insulin. The cells became bright red color when stained with DTZ and the insulin secretion was also confirmed. In glucose challenge test a significant increase in insulin secretion from 0.91 ± 0.04 μIu/mL (2.8 mmol/L glucose) to to 8.34 ± 0.45 μIu/mL (16.7 mmol/L glucose) was recorded ($P < 0.05$). The insulin secretion of undifferentiated *WJ-MSCs* was not changed in this challenge test.

CONCLUSION: WJ-MSCs have the ability to differentiate in to islet-like cells *in vitro*. However, this process needs further optimization in order to generate efficient and functional IPCs.

Key words: Mesenchymal stromal cells; Umbilical cord; Beta cell; Islet

Core tip: Diabetes is a major chronic metabolic disorder in the world. Mesenchymal stromal cells (MSCs) has the ability to differentiate in to functional insulin producing cells.In this study, human Wharton's jelly derived MSCs (The clusters expressed *PDX1*, *NGN3*, *Glut2*, and insulin. The cells became red color when stained with DTZ and the insulin secretion was confirmed Wharton's jelly derived MSCs were differentiated to insulin producing clusters (IPCs). More efficient differentiation protocoles for generation of functional IPCs will be a potential new source for cell transplantation in diabetic patients.

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INTRODUCTION

Diabetes mellitus is characterized by an absolute or relative lack of blood insulin and associated the impaired metabolism of carbohydrates, fats and proteins. Recent study reported that 382 million people had diabetes in the world; and by 2035, this number is expected to be increased to 592 million[1]. Pancreas or islet cell transplantation is considered as an effective therapy for diabetic patients[2]. However, the limited number of cadaveric donors and immunological rejection are two major obstacles to achieve effective long term results[3].

Human embryonic stem cell (ESC) is a good promising source for treating diabetes[4]. However, ethical concerns about the use of human embryos and the risk of tumourigenicity are problems regarding the use of these cells for clinical use[4]. Other studies have made efforts to differentiate pancreatic beta cells from other sources such as induced pleuripotent stem cells (IPSs)[5,6], bone marrow-derived mesenchymal stromal cells[7,8], or umbilical cord blood cells[9].

Mesenchymal stromal cells (MSCs) are isolated from the Wharton's jelly or umbilical cord, are easily obtained and processed compared to ESCs and bone marrow derived MSCs[10]. The cells have differentiated into adipogenic, chondrogenic, and osteogenic lineage and also have expressed the CD105, CD44 and CD90 and negative for hematopoietic and vascular markers such as: CD33, CD56, CD31, CD34, CD45[10]. Wharton's jelly derived MSCs (WJ-MSCs), have high proliferation capacity and do not induce teratomas after transplantation[10].

The potential of these postnatal stem cells to differentiate toward insulin-producing cells has been evaluated previously. However, the current protocols are not optimized for efficient transdifferentiation. Here, we describe the three step modified protocol for direct differentiation of WJ-MSCs into insulin producing clusters (IPC). These clusters produce insulin in response to different glucose concentration.

MATERIALS AND METHODS

Preparation of human umbilical cord

Human umbilical cord samples were aseptically collected from full-term delivery by cesarean section at the Hafez Hospital affiliated to Shiraz University of Medical Sciences. Informed consent was received from mothers and the study design was approved by ethical committee of our university.

Preparation of WJ-MSCs

Umbilical cord Wharton's jelly was processed within 2 h after aseptic collection and cut into pieces of 0.5-1 mm^2. These pieces were placed in 10 cm plates and cultured in High glucose-Dulbecco's Modified Eagle Medium; H-DMEM supplemented with 10% fetal bovine serum (FBS) and penicillin 100 U/mL, streptomycin 100 μg/mL. The plates were placed in an incubator with saturated humidity at 37 ℃ containing 5% CO_2. The medium was changed every three days; the cell migrated from the margins of explants. After reaching 70%-80% confluence, the adherent cells were harvested with 0.05% trypsin-Ethylene diamine tetra acetic acid (EDTA), (Gibco, Germany) and the cell suspension was used for subsequent experiments. The presence of transcription factors that regulate maintenance of pluripotent

state in ESC (OCT4, Nanog) were studied by reveres transcription polymerase chain reaction (RT-PCR).

Flow cytometry analysis

The WJ-MSCs was stained with monoclonal antibodies: CD90, CD44, CD105, CD34 and CD133 (BioLegend, San Diego, Calif., United States) and analyzed using the FACS Calibur flow cytometer (Becton Dickinson, NJ, United States).

In vitro osteogenic and adipogenic differentiation

To investigate their capacity for mesodermal differentiation adipogenic and osteogenic differentiation was carried out by culturing cells with appropriate differentiation medium. For adipocytes differentiation, the WJ-MSCs were cultured in L-DMEM supplemented with 10% FBS, 10 nmol/L dexamethasone, 0.5 μg/mL insulin, 2 mmol/L glutamine for 15 d. Then the cells were fixed with 0.4% paraformaldehyde (PFA) and stained with oil-red-O (Sigma). For osteoblastic differentiation, the WJ-MSCs were cultured L-DMEM supplemented with 10% FBS, 10 nmol/L dexamethasone (Sigma), 10 mmol/L b-glycerol phosphate (Sigma) and 50 mg/mL ascorbic acid-2 phosphate (Sigma). After fixation, the differentiated cells were stained with alizarin red stain and calcium deposition was confirmed.

In vitro differentiation of WJ-MSCs to insulin producing clusters

The cultured WJ-MSCs from 4th passage with 80% to 90% confluency were induced to differentiate into IPC in three separate stages.

In first sage, the MSC monolayer was treated for 24 h with high glucose Dulbecco modified Eagle medium-F12 (DMEM-F12) supplemented with10% FBS, 10^{-6} mol/L of retinoic acid (RA; Sigma-Aldrich) and 1% antibiotic; then the medium was switched to DMEM-F12 containing only 10% FBS for 2 d.

In second stage, the cells were detached with 0.5% trypsin-EDTA and seeded in 0.1% gelatin (Sigma-Aldrich) coated plates. The medium was switched to DMEM-F12, supplemented with 10% FBS, 10 mmol/L nicotinic acid (Sigma-Aldrich), and 20 ng/mL epidermal growth factor (EGF, Sigma-Aldrich) for one week.

In third stage, in order to mature the insulin-producing cells, the DMEM-F12 medium was supplemented with 10% FBS and exendin-4 (Sigma-Aldrich) for one week. Glucagon-Like Peptide-1 or Exendin-4 is an insulin secretagogue, with glucoregulatory effects.

The formation of islet like clusters was daily monitored and the DTZ staining confirmed the presence of insulin granules. The genes expression of pancreatic endocrine cell development was monitored in the end of each step. The cells which were cultured in L-DMEM containing only 10% FBS was considered as a control group.

RT-PCR

The total RNA was extracted from WJ-MSCs s before and after third stage of differentiation by using Mini-RNease RNA extraction kit (Cinnagen, Iran). The cDNA was synthesized using MMULV reverse transcriptase (Cinnagen, Iran). The reaction condition for cDNA synthesis was: pre-heating at 42 ℃ for 90 min; followed by annealing at 85 ℃ for 5 s. The synthesized cDNA was used to examine the expression of pancreatic specific transcription factors as listed in Table 1. Amplification used a fluorescence-quantitative PCR instrument (ABI Prism7500, step one plus) by using SYBER Green (TAKARA, Japan) following the manufacturer's instructions. Amplification was carried out as follows: denaturing at 95 ℃ for 5 min; followed by 40 cycle denaturation at 95 ℃ for 1 min; annealing for 1 min. The final stage was run to generate a melting curve for verification of amplification product specificity. Beta actin gene was used as a control. The products were also visualized by gel electrophoresis.

Dithizone staining

Diphenylthiocarbazone, Dithizone (DTZ), (Sigma, United States) is a zinc-chelating substance which is used for identification of insulin granules in pancreatic beta-cells as bright crimson red. The Acinar cells remain unstained.

In order to evaluation insulin production in IPC at the end of 3rd week, the differentiated clusters were examined.

Insulin secretion

The clusters, were rinsed twice in Krebs-Ringer HEPES (KRH) buffer (125 mmol/L NaCl, 4.74 mmol/L KCL, 1 mmol/L $CaCl_2$, 1.2 mmol/L KH_2PO_4, 1.2 mmol/L $MgSO_4$, 5 mmol/L $NaHCO_3$, 25 mmol/L HEPES, 0.1 mmol/L 0.1%BSA, pH = 7.4) containing 2.8 mmol/L glucose (Low glucose concentration) (Sigma-Aldrich, United States).

After 1 h incubation, the medium was collected for insulin assay and the cells were stimulated with KRH buffer containing 16.7 mmol/L glucose for 1 h (High glucose concentration). The insulin level was determined by Enzyme linked Immunoassay kit and the results were compared with undifferentiated MSC cells as control group.

Statistical analysis

The data were presented as means ± SD. Each experiment was repeated 3 times. Data was assessed by one-way ANOVA followed by Tukey's test for pair wise comparison. $P < 0.05$ was considered as statistically significant. The statistical analysis was performed using Graph Pad Prism 5 software.

RESULTS

Cell morphology and Immunophenotyping of WJ-MSCs

WJ-MSCs formed a homogenous monolayer of adherent spindle shaped cells (Figure 1). Flow cytometry ana-

Table 1 Primer pairs for amplification of transcription factors

Names	Forward (F) 5´-3´	Reverse(R) 5´-3´
Insulin	GCAGCCTTTGTGAACCAACA	TTCCCCGCACACTAGGTAGAGA
PDX1	GGATGAAGTCTACCAAAGCTCACGC	CCAGATCTTGATGTGTCTCTCGGTC
NGN3	CAATCGAATGCACAACCTCA	GGGAGACTGGGGAGTAGAGG
GLUT2	AGGACTTCTGTGGACCTTATGTG	GTTCATGTCAAAAAGCAGGG
NANOG	CAGAAGGCCTCAGCACCTAC	ATTGTTCCAGGTCTGGTTGC
Oct-4	CAGTGCCCGAAACCCACAC	GGAGACCCAGCAGCCTCAAA
β-actin	GATCGGCGGCTCCATCCTG	GACTCGTCATACTCCTGCTTGC

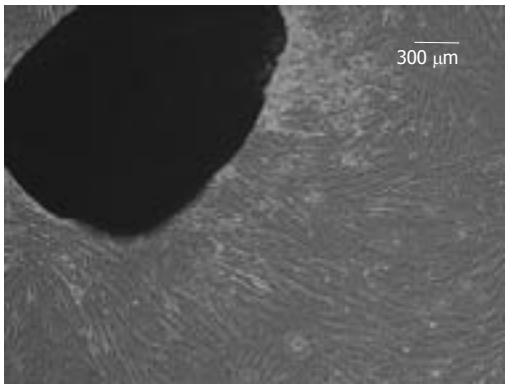

Figure 1 The Wharton's Jelly mesenchymal stromal cells were grown from the edge of tissue explants (× 100).

lyses showed that the WJ-MSCs were positive for mesenchymal markers (CD90, CD44, CD105), and negative for CD34 and CD133 (Figure 2). The ESCs transcriptional factors such as Nanog and OCT4, also expressed.

In vitro osteogenic and adipogenic differentiation of WJ-MSCs

The accumulation of lipid vacuoles (Stained red color in Oil Red O) in cells was considered as adipogenic differentiation. Deposition of calcium minerals (stained as orange red color with Alizarin Red) was also considered as osteogenic potential of these cells (Figures 3 and 4).

Morphological Changes before and after differentiation of WJ-MSCs

In passage 4 (P4), the WJ-MSCs were step wise differentiated toward insulin producing cells.

In stage 1, the cells have shown spindle-shaped morphology (Figure 5A). Cell morphology was gradually changed into round epithelioid cells and by addition of beta cell maturation factor such as nicotinamide in stage 3, three dimensional clusters were formed (Figure 5B-D). These clusters were stained red color with DTZ (Figure 6).

Gene expression analysis

After differentiation, mRNA expression of pancreatic development transcription factors and beta cell specific gene such as *PDX1*, *NGN3*, *Glut2* and insulin were detected. Expressions of the mentioned genes led to *in vitro* production of functional IPCs. Results represent three separate experiments (Figure 7A and B).

Insulin secretion after glucose challenge test

In respect of insulin secretion, a significant increase in insulin secretion from 0.91 ± 0.04 μIu/mL (2.8 mmol/L glucose) to to 8.34 ± 0.45 μIu/mL (16.7 mmol/L glucose) was recorded ($P < 0.05$). The insulin secretion was detected in undifferentiated *WJ-MSCs* (0.3 ± 0.03 μIu/mL) which was not changed in glucose challenge test.

DISCUSSION

Type 1 diabetes mellitus is caused by an autoimmune destruction of pancreatic beta cells. Although the insulin therapy remains the routine treatment for diabetes, but whole pancrease organ transplant or transplantation of pancreatic islets of Langerhans provides a cure for this disorder[2,3]. Various types of stem cells such as ESCs, induced pleuripotent stem cells (IPs) and mesenchymal stromal cells have been differentiated into IPCs by genetic modification and/or modification in culture conditions[7,8,11,12]. Using of ESCs has several limitations such as ethical problem, immune rejection, and risk of tumorigenesis.

To overcome these problems, human IPS is ideal for personalized therapy. However, epigenetic changes, and chromosomal instability during reprogramming remain the obstacle[6] .

Generation of IPCs from MSCs from a variety of tissues such as bone marrow, umbilical cord, and adipose tissue represents an alternative therapy. MSCs can also be used as a cellular vehicle for the expression of human insulin[6,13-15].

The MSC numbers in bone marrow and umbilical cord blood are low and require multiple *ex vivo* expansion. Extra-embryonic tissue such as fetal membrane and umbilical cord Wharton's jelly has the stemness phenotype, immunoprivileged properties, and faster proliferation than adult MSCs and is considered as unlimited source for tissue engineering and regenerative medicine[13]. The WJ-MSCs express HLA-G6 isoform, the unique ability which is important in immune modulation. Therefore, these cells are particularly suitable for cell based therapy. These tissues are normally discarded

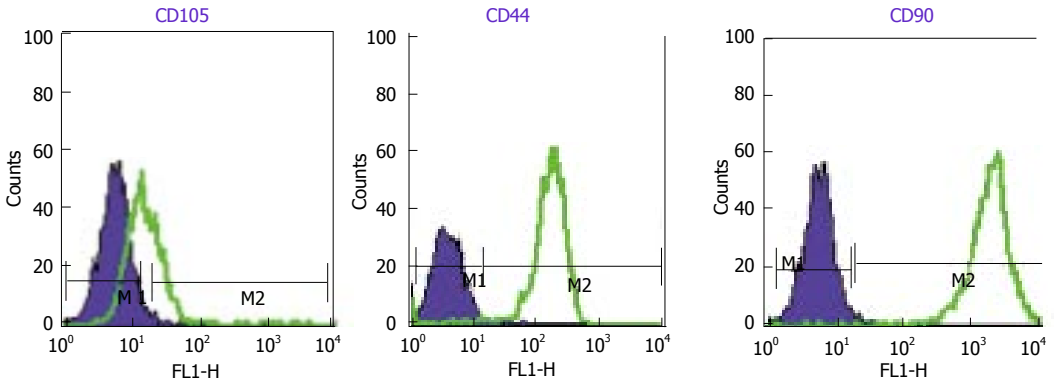

Figure 2 Flow cytometry histogram of Wharton's jelly derived mesenchymal stromal cells (CD90, CD105, CD44). WJ-MSCs are positive for these markers. Dark Blue lines indicate background fluorescence obtained with isotype control. WJ-MSCs: Wharton's jelly derived mesenchymal stromal cells.

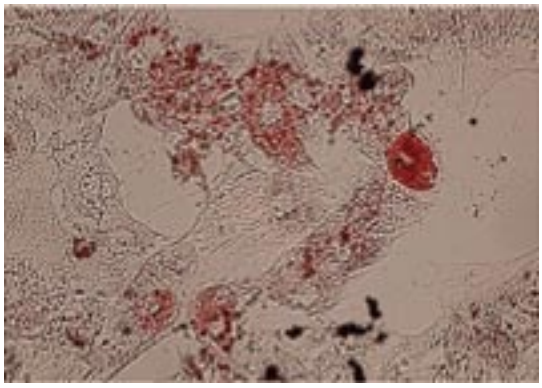

Figure 3 Fat droplets confirmed the adipogenic potential of Wharton's jelly derived mesenchymal stromal cells (Oil Red O staining × 400).

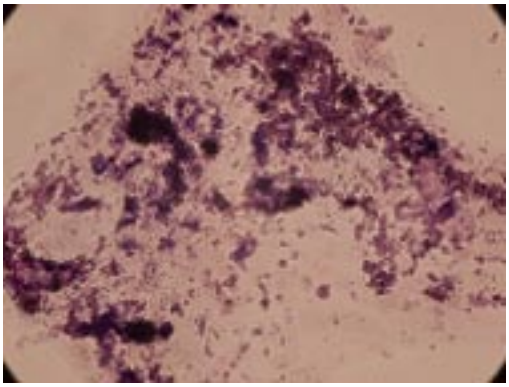

Figure 4 Calcium deposition confirmed the osteogenic potential of Wharton's jelly derived mesenchymal stromal cells (Alizarin Red staining × 100).

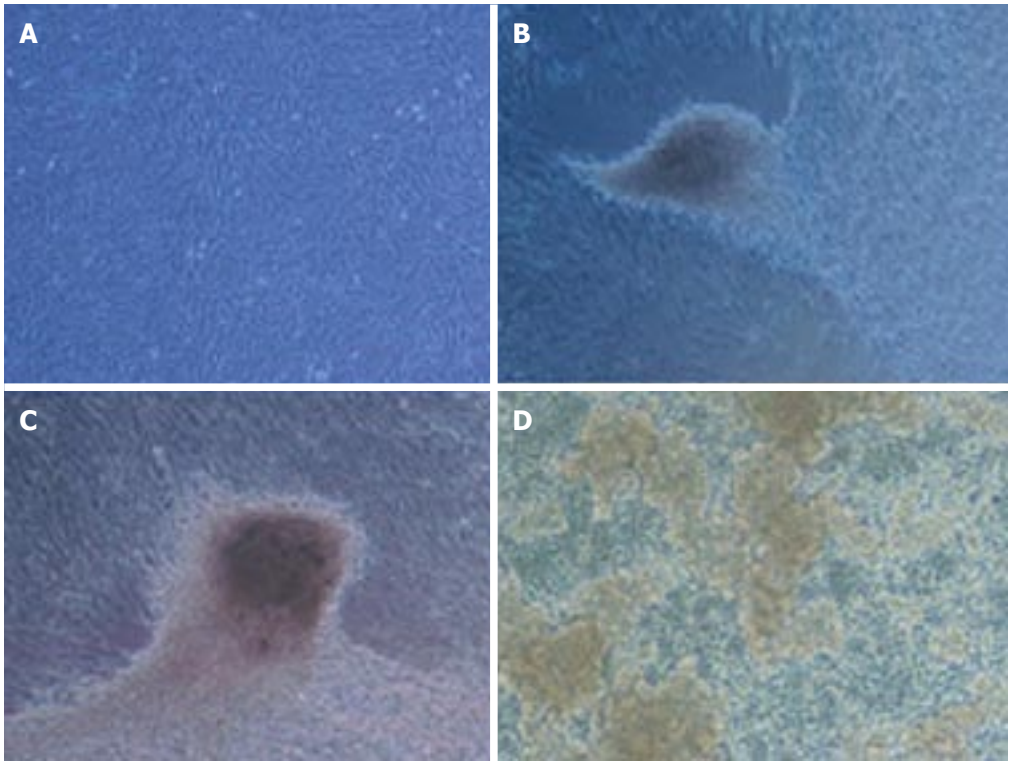

Figure 5 Morphological differentiation of insulin producing clusters from Wharton's jelly derived mesenchymal stromal cells in different stages (A-D × 100). A: WJ-MSCs were typically an adherent spindle shape; B and C: The cells gradually formed clusters in the medium 14 d after differentiation; D: At the end of final stage clusters were floated in the medium. WJ-MSCs: Wharton's jelly derived mesenchymal stromal cells.

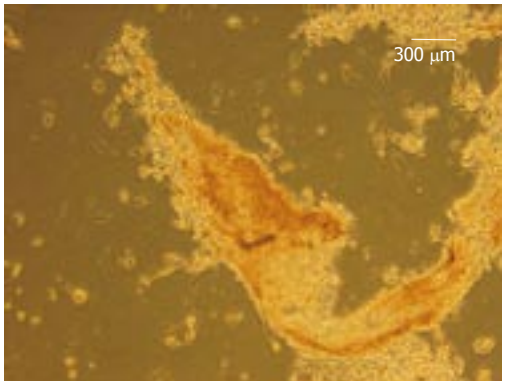

Figure 6 Dithizone staining. The differentiated cells stained as red color (× 100).

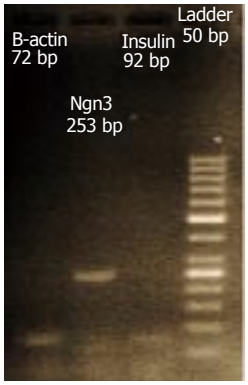

Figure 7 Expression of *Ngn3*, Insulin, *PDX1*, *Oct4* and Nanog transcription factors were confirmed by electrophoresis.

Table 2 Comparison between different methods of islet like cells clusters differentiation from different stem cell sources

Stem cell sources	Differentiation protocol	Efficiency (generation of insulin producing cell)	Ref.
Placenta-derived Mesenchymal stem cells	(α-MEM + 1% BSA + 1 × ITS + 0.3 mmol/L taurine) 3 d (α-MEM + 1.5% BSA + 1 × ITS + 3 mmol/L taurine + GLP-1 + nicotinamide) 7 d (α-MEM + 1.5% BSA + 1 × ITS + 3 mmol/L taurine + GLP-1 + nicotinamide) 10 d	65%-70% ILCs (represents 20%-25% beta-cells per islet)	Kadam *et al*[31]
Human embryonic stem cells	(DMEM-F12, 20% SR, 2 mmol/L GlutaMAX, 1% NEAA and 0.1 mmol β-mercaptoethanol) 7 d (DMEM-F12, 1% ITS, 2 mmol/L GlutaMAX, 5 μg/mL Fibronectin) 7 d (DMEM-F12, 1% N2, 2% B27, 2 mmol/L GlutaMAX, 10 ng/mL bFGF) 7 d (DMEM-F12, 1% N2, 2% B27, 2 mmol/L GlutaMAX, and 10 mmol/L nicotinamide) for 7-9 d	61.7% ± 9.5% insulin positive cells	Wei *et al*[32]
Human embryonic stem cells	(DMEM-F12, 100 ng/mL activin A, 1 μmol/L wortmannin, 1% N2, 1% B27) 4 d (IMDM/F12, 2 μmol/L retinoic acid, 20 ng/mL FGF7, 50 ng/mL Noggin, 0.25 μmol/L KAAD-cyclopamine, 1% B27) 4 d (DMEM, 50 ng/mL EGF, 1% ITS, 1% N2) 5 d (DMEM-F12, 1% ITS, 10 ng/mL bFGF, 10 mM nicotinamide, 50 ng/mL exendin-4) 7-9 d	41.6% ± 11.8% insulin positive cells	Wei *et al*[32]
hESC lines YT1 and YT2	(RPMI1640, 0.2% FBS, 0.56 N2, 0.56 B27, 100 ng/mL activin A, 1 mmol/L wortmannin) 4 d (RPMI1640, 0.5% FBS, 0.5% ITS, 0.56 B27, 2 mM retinoic acid, 20 ng/mL FGF-7, 50 ng/mL Noggin) 4 d (DMEM, 0.5% FBS, 1% ITS, 16 N2, 50 ng/mL EGF) 5 d (DMEM/F12, 1% ITS, 10 ng/mL bFGF, 10 mmol/L nicotinamide, 50 ng/mL exendin-4, 10 ng/mL BMP4) until maturation	17.1% insulin positive cells	Hua *et al*[33]
Human embryonic stem cells	(MCDB-LG, 100 ng/mL GDF8, 2.5 mmol/L MCX-928, 100 ng/mL) 1 d (MCDB-LG, 100 ng/mL GDF8) 2-4 d (MCDB-LG, 50 ng/mL FGF7) 2 d (MCDB-HG, 50 ng/mL FGF7, 20 ng/mL ActivinA, 0.25 μmol/L SANT-1, 2 μmol/L Retinoic Acid, 200 nmol/L LDN193189) 4 d (MCDB-HG, 0.25 μmol/L SANT-1, 200 nmol/L LDN193189, 500 nmol/L TBP, 100 nmol/L CYP26A inhibitor) 3 d (MCDB-HG, 200 nmol/L LDN193189, 1 μmol/L ALK5i, 100 nmol/L CYP26A inhibitor) 3 d (MCDB-HG, 200 nmol/L LDN193189, 1 μmol/L ALK5i) 3 d (MCDB-HG, 200 nmol/L LDN193189, 1 μmol/L ALK5i, 100 nmol/L VitaminA) 7-14 d	6%-10% insulin positive cells	Bruin *et al*[34]
Human embryonic stem cells	(EB formation) 2 d (DMEM-F12, KSR, ActivinA, Retinoic acid) 6 d (DMEM-F12, KSR, bFGF, Noggin) 12 d (DMEM-F12, N2, B27, Laminin, bFGF, Nicotinamid, GLP-1) 12 d (CMRL1066, Nicotinamid, ITS, Zn2SO4, Glutamax, HEPES, KSR, GLP-1, Exendin1, HGF) 10 d	24.5% insulin producing cell	Bose *et al*[35]

Bone marrow mesenchymal stem cells	(Human BMSCs were transfected with adenovirus carrying PDX1 or VEGF) 2 d	50% of cells was differentiated to beta cell	Milanesi *et al*[36]
Human bone marrow-derived stem cells	(RPMI 1640, 5.5 mmol/L glucose, 5% FCS, 10 mmol/L nicotinamide, 10 nmol/L exendin 4) 5 d	20% insulin producing cell	Tang *et al*[37]
Human bone marrow-derived mesenchymal stem cells	(DMEM, 0.5 mmol/L β-mercaptoethanol) 2 d (DMEM, 1% non-essential amino acids, 20 ng/mL bFGF, 20 ng/mL EGF, 2% B27, 2 mmol/L L-glutamine) 8 d (DMEM, 10 ng/mL betacellulin, 10 ng/mL activin-A, 2% B27, 10 mmol/L nicotinamide) 8 d	5% insulin producing cell	Gabr *et al*[38]
Human labia minor dermis-derived fibroblasts	(DMEM/F12, ITS) 7 d (DMEM, 10% FBS, 100 U/mL penicillin/streptomycin, ITS, 10 mmol/L nicotinamide) 7 d	50% insulin positive cell 2×10^6 cells generated around 400–600 ICAs	Kim *et al*[39]
Human adipose tissue derived adult stem cells	(DMEM/F12, 17.5 mmol/L glucose, 1% BSA, 1 × ITS, 4 nmol/L activin A, 1 mmol/L sodium butyrate, 50 mmol/L 2-mercaptoethanol, 2 ng/mL FGF) 2 d (DMEM/F12, 17.5 mmol/L glucose, 1% BSA, ITS, 0.3 mmol/L Taurine) 2 d (DMEM/F12, 17.5 mmol/L glucose, 1.5% BSA, ITS, 3 mmol/L Taurine, 100 nmol/L GLP-1, 1 mmol/L nicotinamide and 1× non-essential amino acids) 12-14 d		Chandra *et al*[40]
Human Adult Fibroblast-Like Limbal Stem Cells	(RPMI-1640, 100 ng/mL Activin A) 2-3 d (RPMI-1640, 2% FBS, 50 ng/mL bFGF) 3-4 d (DMEM, 10% FBS, 1% B27, 2% N2, 1 mmol/L nicotinamide) 3-4 d (DMEM, 10% FBS, 1% B27, 2% N2, 1 mmol/L nicotinamide, 50 ng/mL exendin-4) 3-4 d (RPMI-1640, 100 ng/mL Activin A) 2-3 d (RPMI-1640, 2% FBS, 50 ng/mL FGF 10 μmol/L) 3-4 d (DMEM, 1% B27, 50 ng/mL FGF 10.2 μmol/L retinoic acid) 3-4 d (DMEM, 1% B27, 50 ng/mL exendin-4) 3-4 d	70% to 77% insulin producing cell	Criscimanna *et al*[41]
Dental pulp stem cells	(DMEM-KO, 1% BSA, 1x ITS, 4 nmol/L activin A, 1 mM sodium butyrate, 50 μmol/L 2-mercaptoethanol) 2 d (DMEM-KO, 1% BSA, ITS, 0.3 mmol/L taurine) 2 d (DMEM-KO, 1.5% BSA, ITS, 3 mmol/L taurine, 100 nmol/L GLP-1, 1 mmol/L nicotinamide, 1x non-essential amino acids) 5 d	2×10^6 (Number of cells generated from around 156 ± 23 ILCs)	Govindasamy *et al*[42]
Stem cells from human exfoliated deciduous teeth (SHED)	(KO-DMEM, 1% BSA, 1 × ITS, 4 nmol/L activinA, 1 mmol/L sodium butyrate) 2 d (KO-DMEM, 1% BSA, ITS, 0.3 mmol/L taurine) 1 d	231 ± 21 number of generated ILCs from initial cell seeding density 2×10^6	Kanafi *et al*[43]
Dental pulp stem cells	(KO-DMEM, 1.5% BSA, ITS, 3 mmol/L taurine, 100 nmol/L glucagon-like peptide-1, 1 mmol/L nicotinamide) 7 d	112 ± 2 number of generated ILCs from initial cell seeding density 2×10^6	Kanafi *et al*[43]

MEM: Minimum Essential Medium Eagle; BSA: Bovine serum albumin; ITS: Insulin transferin selenium; ILCs: Islet like clusters; GlutaMAX: Glutamin; NEAA: Non-essential amino acids; DMEM: Dulbecco's Modified Eagle Medium; bFGF: Basic Fibroblast growth factor; FGF7: Fibroblast growth factor 7; Cyclopamine-KAAD: A Smoothened (Smo) antagonist; EGF: Epidermal growth factor; BMP4: Bone morphogenetic protein 4; MCDP: Name of medium; LG: Low glucose; HG: High glucose; SANT-1: (4-Benzyl-piperazin-1-yl)-(3,5-dimethyl-1-phenyl-1H-pyrazol-4-ylmethylene)-amine; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; KSR: Name of medium for embryonic stem cell culture; GLP-1: Glucagon-like peptide-1; HGF: Hepatocyte growth factor; KO-DMEM: Knockout DMEM.

after birth and using them is not associated with ethical problem[16,17].

In this study, WJ-MSCs were isolated and their differentiation into adipocytes, and osteocytes confirmed their multilineage potentials. The retinoic acid we used to start the differentiation process. RA is an essential molecule for dorsal pancreas development in mouse. The effects of RA are achieved through RA binding and activation of retinoic acid receptors such as RARα, RARβ, and RARγ. Over expression of RARβ was detected in early stage of pancreases differentiation and absence of RARβ impaired the terminal differentiation of α and β-cells[18,19]. In vitamin-A deficiency, pancreatic islet function was Impaired. RA directly induces Pdx1 expression in ESCs and Pdx1 is an important transcription factor in the early development of pancreatic progenitors and bud expansion[20,21]. The retinoic acid response element is located at upstream of the transcription start site of Pdx1. Retinoie Acid Receptor (RARβ) expression is depended on epigenetic regulation[18,19]. Hyper methylation of the RARβ2 promoter was reported in pancreatic cancer, and diabetes. Therefore, it is postulated that the epigenetic silencing of RARβ, combined with vitamin A deficiency, may play a role in pathogenesis of diabetes[18,19]. Glucose is considered as a growth factor for β-cells replication both *in vitro* and *in*

vivo[22]. Subsequently, using of EGF was associated with proinsulin biosynthesis and 3H-thymidine incorporation in experimental model[23,24]. Nicotinamide was used during the second stage of differentiation. Various published protocols reported this substance as an effective inducer in pancreatic differentiation. Nicotinamide preserve islet viability through poly-ADP-ribose polymerase[25].

Glucagon-like peptide 1 (GLP-1) and its long acting mimetic exendin-4 are usually used for treatment of type 2 diabetes. Exendin 4 simultaneously stimulates beta cell secretory capacity as well as maintains insulin stores by translational control of proinsulin biosynthesis. Exendin-4 can also stimulate b-cell replication in human islets from young donors[26-28].

In our study, at the end of *in vitro* differentiation protocols, pancreatic endocrine genes and insulin were expressed. However, the insulin secretion after glucose challenge test was very low. As mentioned previously, incomplete beta cell phenotype and consequently poor insulin release in response to glucose challenge test might explain this problem[29,30].

In the literature, various sources of mesenchymal stem were differentiated into insulin secreting cells with different efficacy[31-43] (Table 2). However, the insulin secretion capacity of the cells was variable. The insulin challenge test was done with different protocols; the concentration of insulin was measured with different assays and reference range. Therefore, the comparison of data was not easily performed.

In our experience, differentiation of WJ-MSCs to form IPCs needs further optimization for clinical practice. To overcome this problem, addition of growth factors, extracellular matrix and/or culturing the cells in three dimensional environments are suggestive.

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COMMENTS

Background

Different types of stem cells such as embryonic stem cells (ESCs), induced pleuripotent stem cells and mesenchymal stromal cells have been differentiated into insulin producing cells by modification in cell culture conditions and addition of small molecules. Umbilical cord Wharton's jelly derived stromal cells has the stemness phenotype, with faster proliferation than adult mesenchymal stromal cells (MSCs) and is considered as a good source for generation of insulin producing cells.

Research frontiers

In this research, insulin producing clusters (IPC) was differentiated from Wharton's jelly MSCs (WJ-MSCs) without any genetic manipulation.

Innovations and breakthroughs

The IPCs was differentiated from WJ-MSCs by changing the cell culture medium and growth factors without genetic manipulation. However, the insulin release in response to glucose challenge test was not sufficient. Similar studies used various sources of mesenchymal stem and differentiated into insulin secreting cells with different efficacy and variable insulin secretion capacity. The insulin challenge test was done with different protocols; the concentration of insulin was measured with different assays and reference range. Therefore, the comparison of data was not easily performed.

Applications

Differentiation of WJ-MSCs to form IPCs needs further optimization for clinical practice.

Peer-review

The authors have performed a good study, the manuscript is interesting.

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CASE REPORT

Perifollicular granulomas with IgG4 plasmacytosis: A case report and review of literature

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Author contributions: Liang L and Chen L designed the report, collected the patient's clinical data and wrote the paper; Zhou J collected the patient's clinical data and revised the manuscript.

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Abstract

Perifollicular granuloma is a unique histologic feature and whether it is associated with immunoglobulin G4 (IgG4)-related disease is controversial. We report a case of a 38-year-old man who presented with worsening left eye pain, proptosis, tearing, gritty sensation, blurred vision and multiple lymphadenopathy. An axillary lymph node resection showed reactive follicular and interfollicular lymph node hyperplasia, and increased eosinophils and plasma cells (at least 80% of IgG^+ plasma cells were positive for IgG4). A distinct feature was the presence of multifocal, perifollicular histiocytic granulomas, which formed a wreath around the entire follicles. The human herpes virus 8 was not detected by immunohistochemistry. In addition, an extensive panel of special stains, immunohistochemistry, and flow cytometry was negative for lymphoma, fungal, or mycobacterial infection. The findings were suggestive of IgG4-related sclerosing disease-associated lymphadenopathy. Further laboratory testing showed a significant increase of serum immunoglobulin E (> 23000 IU/mL) and slight increase of total IgG, but normal serum IgG4. Even though perifollicular granuloma is a nonspecific histopathologic feature and can be seen in other diseases, such as nodular lymphocyte predominant Hodgkin lymphoma, IgG4-related lymphadenopathy should be listed in the differential diagnoses of benign reactive lymph nodes, especially when perifollicular granuloma and plasmacytosis coexist.

Key words: Immunoglobulin G4-related disease; Lymphadenopathy; Plasma cells; Granuloma

Core tip: We report a case of a 38-year-old man who

presented with worsening left eye pain and multiple lymphadenopathy. An axillary lymph node resection showed increased eosinophils and plasma cells (at least 80% of immunoglobulin (Ig)G$^+$ plasma cells were positive for IgG4 and the presence of multifocal, perifollicular histiocytic granulomas, which formed a wreath around the entire follicles. An extensive workup was negative for lymphoma, fungal, or mycobacterial infection. The findings were suggestive of IgG4-related sclerosing disease-associated lymphadenopathy. Thus, IgG4-related lymphadenopathy should be listed in the differential diagnoses of benign reactive lymph nodes, especially when perifollicular granuloma and plasmacytosis coexist.

Liang L, Zhou J, Chen L. Perifollicular granulomas with IgG4 plasmacytosis: A case report and review of literature. *World J Clin Cases* 2015; 3(7): 650-654 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i7/650.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i7.650

INTRODUCTION

Immunoglobulin G4 (IgG4)-related disease is a recently recognized fibro-inflammatory condition that can involve multiple organs and cause tumor-like enlargement[1,2]. It is characterized by a lymphoplasmacytic infiltrate enriched in IgG4-positive plasma cells, while elevated serum concentrations of IgG4 are found in 60% to 70% of patients[3]. IgG4-related disease has a male predilection (male to female ratio 8:1)[4].

According to the consensus statement from a multinational, multidisciplinary group of experts, the major histopathological features to make the diagnosis of IgG4-related disease include a dense lymphoplasmacytic infiltrate, plasma cells, storiform fibrosis, and obliterative phlebitis[5]. However, these features are usually uncommonly seen in certain organs, such as lymph nodes.

We describe a case of a 38-year-old man with swelling of soft tissue surround the eye and multiple lymphadenopathy and an axillary lymph node resection showed reactive follicular and interfollicular lymph node hyperplasia, increased eosinophils and plasma cells (at least 80% of immunoglobulin IgG$^+$ plasma cells were positive for IgG4) and multifocal, perifollicular histiocytic granulomas, which formed a wreath around the entire follicles. Review of literature also found our findings may add to the knowledge of IgG4-related disease.

CASE REPORT

Clinical history

We report a case of a 38-year-old man who presented with worsening left eye pain, proptosis, tearing, gritty sensation, and blurred vision. Magnetic resonance imaging of orbits confirmed enlargement of the left medial rectus, superior oblique and inferior rectus muscle, and enhancing soft tissue signal encasing the left optic nerve sheath. Computed tomography scan of chest and abdomen showed multiple lymphadenopathy. Clinically, lymphoma was suspected. Patient's rheumatology work up was negative, including anti-neutrophil cytoplasmic antibodies, anti-double stranded DNA, anti-nuclear antibodies, anti-Smith, anti-ribonucleoprotein, anti-complement C3 and C4. Complete blood count shown mild eosinophilia, otherwise, it was unremarkable.

Microscopic and immunohistochemical features

An axillary lymph node resection showed reactive follicular and interfollicular lymph node hyperplasia, and increased eosinophils and plasma cells (at least 80% of IgG$^+$ plasma cells were positive for IgG4). A distinct feature was the presence of multifocal, perifollicular histiocytic granulomas, which formed a wreath around the entire follicles (Figure 1A). Increased plasma cells were marked by CD138 immunohistochemical stain (Figure 1B). IgG4$^+$ plasma cells are markedly increased (Figure 1D), compared with total IgG stain (Figure 1C). EBER (EBV encoded small RNA by *in situ* hybridization) was negative. The human herpes virus 8 was not detected by immunohistochemistry. In addition, an extensive panel of special stains, immunohistochemistry, and flow cytometry was negative for lymphoma, fungal, or mycobacterial infection. The findings were suggestive of IgG4-related sclerosing disease-associated lymphadenopathy.

Follow-up

Further laboratory testing showed a significant increase of serum IgE (> 23000 IU/mL) and slight increase of total IgG (1802 mg/dL), but normal serum IgG4 (27 mg/dL). The patient was started on prednisone and methotrexate with reduction in proptosis and in the size of orbital mass by computerized tomography (CT) scan. While patient was maintained with methotrexate and tapering on steroid, he was noted to have left eye redness and itching. Rituximab was added and methotrexate was discontinued. The patient's symptom subsided.

DISCUSSION

According to the consensus statement from a multinational, multidisciplinary group of experts, the major histopathological features to make the diagnosis of IgG4-related disease include a dense lymphoplasmacytic infiltrate, plasma cells, storiform fibrosis, and obliterative phlebitis[5]. However, these features are usually not seen in certain organs, such as lymph nodes. Fibrosis and obliterative phlebitis are usually not present in lymph nodes.

Lymph nodes in IgG4-related disease may show variable histopathologic features. Cheuk *et al*[4] divided it into five different categories, including multicentric

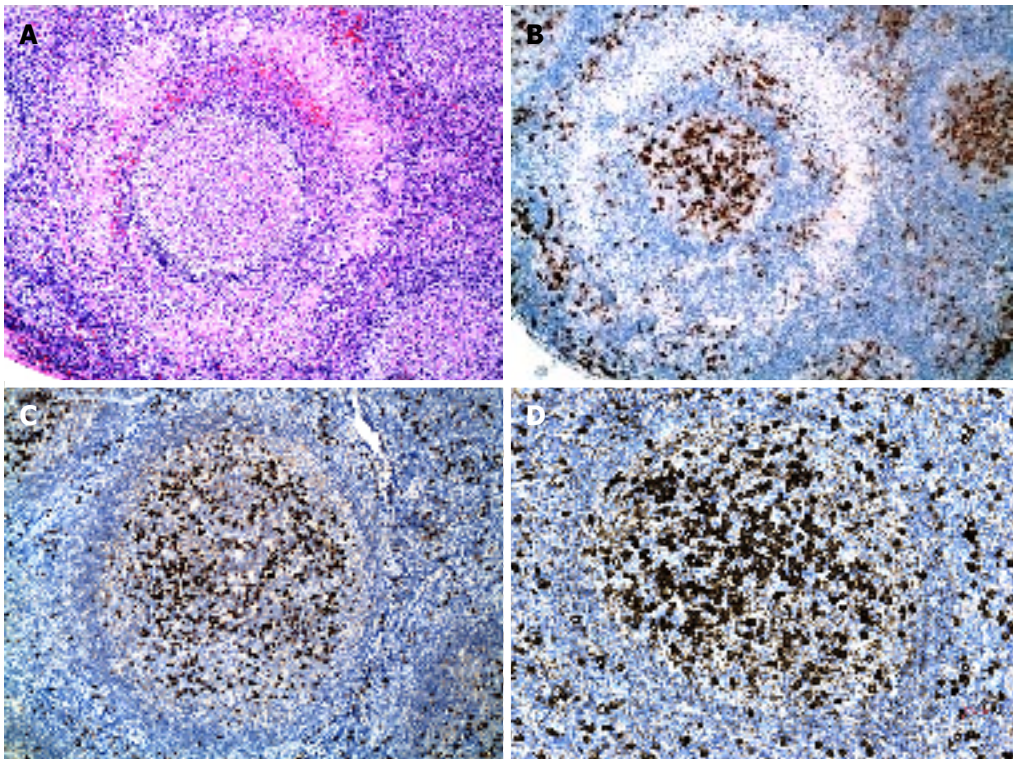

Figure 1 Perifollicular histiocytic granulomas that formed a wreath around the entire follicle. A: Hematoxylin and eosin stain; B: CD138 immunohistochemical stain highlights the plasma cells; C: Immunoglobulin G (IgG) immunohistochemical stain; D: IgG4 immunohistochemical stain demonstrated that more than 80% of IgG^+ cells were positive for IgG4.

Castleman disease-like (type Ⅰ), follicular hyperplasia (type Ⅱ), interfollicular expansion (type Ⅲ), progressive transformation of germinal centers (type Ⅳ), and inflammatory pseudotumor-like (type Ⅴ). Nevertheless, an increase in $IgG4^+$ plasma cells with an IgG4/IgG plasma cell ratio exceeding 0.4, and/or an absolute number of $IgG4^+$ plasma cells of more than 50/high-power field (hpf) are the currently accepted cutoff for IgG4-related disease. However, presence of $IgG4^+$ plasma cells in isolated reactive lymphadenopathy is not exclusively specific for IgG4-related disease[6]. Martinez *et al*[6] reported seven of the 55 solitary reactive lymph nodes with increased IgG4/IgG plasma cell ratio of more than 0.4, and six of them showed more than 50 $IgG4^+$ plasma cells per high power field, but none of these patients had history of IgG4-related disease. On the other hand, Uehara *et al*[7] reported that presence of fibrosis in lymph nodes, together with increased IgG4 ratio and other features of IgG4-related disease, may suggest the diagnosis of IgG4-related lymphadenopathy.

Even though epithelioid cell granulomas is usually not considered a feature of IgG4-related disease at extranodal sites, it has been described in lymph nodes. Siddiqi *et al*[8] described seven cases with perifollicular granuloma in a concentric or crescent-like arrangement encircling lymphoid follicles and associated with a marked elevation of intra-germinal center $IgG4^+$ plasma cells. However, the specificity of these findings were debated by Cheuk *et al*[4]. Grimm *et al*[9] reported histiocytic proliferation in 11 of 29 cases of lymphadenopathy with increased IgG4 plasma cells, and a prominent ringing of follicles by epithelioid histiocytes in 3 patients (Table 1). In addition, Takahashi *et al*[10] reported a case of IgG4-related lymphadenopathy with prominent granulomatous inflammation, most likely due to reactivation of Epstein-Barr virus. Takeuchi *et al*[11] performed Epstein-Barr virus (EBV)-encoded RNA (EBER) in situ hybridization and identified EBER-positive cells in 18 of 31 cases (58%) of IgG4-related lymphadenopathy, significantly higher rate than non-IgG4-related reactive lymphoid hyperplasia. However, EBER was negative in our case, and either negative or rarely positive in the two cases with EBER performed in Grimm group's study (Table 1). Further study is needed to determine whether there is a causal relationship.

IgG4-related disease is a great mimicker. One of the differential diagnoses is multicentric Castleman's disease. However, $IgG4^+/IgG^+$ plasma cell ratio is usually less than 0.4 in multicentric Castleman disease. Furthermore, elevated serum levels of interleukin-6 and vascular endothelial growth factor favor the diagnosis of multicentric Castleman's disease[12]. Rosai-Dorfman disease can also show increased IgG4-positive plasma cells, as well as other autoimmune diseases including rheumatoid lymphadenopathy, are also in the differential diagnosis[13,14]. Moreover, bacterial, viral, fungal and parasitic infections have to be carefully ruled out. In our case, special stains and cultures were performed and the results were negative. The patient

Table 1 Perifollicular granulomatous inflammation and immunoglobulin G4-related disease

Case	Ref.	Age (yr)	Gender	Location	IgG4/IgG ratio	Eosinophils	Fibrosis	EBER
1	Siddiqi *et al*[8]	47	M	Cervical	0.7	Mild	Marked	NA
2	Siddiqi *et al*[8]	63	M	Axillary	0.7	None	None	NA
3	Siddiqi *et al*[8]	50	F	Cervical	0.5	Minimal	Mild	NA
4	Siddiqi *et al*[8]	34	M	Cervical	0.6	None	None	NA
5	Siddiqi *et al*[8]	12	M	Cervical	0.7	Mild	None	NA
6	Siddiqi *et al*[8]	58	M	Unknown	0.7	None	None	NA
7	Siddiqi *et al*[8]	83	M	Axillary	0.7	Mild	Mild	NA
8	Grimm *et al*[9]	47	M	Cervical	> 0.4	NA	NA	NA
9	Grimm *et al*[9]	58	F	NA	> 0.4	NA	NA	Negative
10	Grimm *et al*[9]	83	M	Axillary	> 0.4	NA	Present	Rarely positive
11	Current case	38	M	Axillary	> 0.8	Increased	None	NA

NA: Not available; EBER: EBV encoded small RNA by *in situ* hybridization; M: Male; F: Female.

recovered quickly after immunosuppressive therapy, which was not consistent with infectious lymphadenitis. Other diseases with perifollicular granuloma have been reported, including reactive lymph nodes of unknown etiology, progressive transformation of germinal centers, and nodular lymphocyte predominance Hodgkin lymphoma[4]. However, in our case, morphologic features, immunohistochemistry and flow cytometry results were not consistent with lymphoma.

In our case, the pathologic evaluation is based on lymph node resection specimen. Even though biopsy of the orbital lesion was considered in initial evaluation, it wasn't performed based on the decision of multidisciplinary team, because this invasive procedures carried a significant risk. In addition, our patient had a normal serum level of IgG4. It is a not uncommon finding and is seen in up to 40% of patients with IgG4-related disease[3]. Our patient was treated with prednisone, methotrexate and Rituximab. His eye symptoms subsided dramatically and a post-treatment imaging study showed shrinking of the orbital mass. Thus, clinical presentations and histopathologic findings in this case supported of a diagnosis of IgG4-related disease.

According to the most recently published International consensus guidance statement, the first line treatment of active IgG4-related disease are glucocorticoids, but a combination of glucocorticoids and a steroid-sparing immunosuppressive agent (*e.g.*, azathioprine, methotrexate or mycophenolate) may be beneficial for some patients[15]. In addition, Rituximab has been reported to be an effective drug to treat patients with IgG4-related ophthalmopathy, especially those intolerant of steroid or with recurrent or refractory disease[16].

In summary, we described a case of IgG4-related disease with distinct features of perifollicular granulomas and literature reviews. This may expand our knowledge of pathologic findings of IgG4-related disease in a lymph node, although clinical history, lab results and radiological findings must be taken into consideration when making the diagnosis.

COMMENTS

Case characteristics

A 38-year-old man who presented with worsening left eye pain, proptosis, tearing, gritty sensation and blurred vision.

Clinical diagnosis

Multiple lymphadenopathy was identified.

Differential diagnosis

Lymphoma, Castleman's disease, fungal or mycobacterial infection, autoimmune disorders, *etc*.

Laboratory diagnosis

Serum immunoglobulin E (> 23000 IU/mL) and slight increase of total immunoglobulin G (IgG), but normal serum IgG4. An extensive panel of special stains, immunohistochemistry, and flow cytometry was negative for lymphoma, fungal, or mycobacterial infection.

Imaging diagnosis

Magnetic resonance imaging of orbits showed enlargement of the left medial rectus, superior oblique and inferior rectus muscle, and enhancing soft tissue signal encasing the left optic nerve sheath. Computed tomography scan of chest and abdomen showed multiple lymphadenopathy.

Pathological diagnosis

An axillary lymph node resection showed reactive follicular and interfollicular lymph node hyperplasia, and increased eosinophils and plasma cells (at least 80% of IgG^+ plasma cells were positive for IgG4). A distinct feature was the presence of multifocal, perifollicular histiocytic granulomas, which formed a wreath around the entire follicles.

Treatment

The patient was started on prednisone and methotrexate with reduction in proptosis and in the size of orbital mass by computerized tomography scan. While patient was maintained with methotrexate and tapering on steroid, he was noted to have left eye redness and itching. Rituximab was added and methotrexate was discontinued. The patient's symptom subsided.

Related reports

Perifollicular granuloma is a unique histologic feature and whether it is associated with IgG4-related disease is controversial. Very few cases have been reported in the English literature.

Term explanation

IgG4-related disease is a recently recognized fibro-inflammatory condition that can involve multiple organs and cause tumor-like enlargement, which is characterized

by a lymphoplasmacytic infiltrate enriched in IgG4-positive plasma cells.

Experiences and lessons

IgG4-related lymphadenopathy should be listed in the differential diagnoses of benign reactive lymph nodes, especially when perifollicular granuloma and plasmacytosis coexist.

Peer-review

The authors have performed a good study, the manuscript is interesting.

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CASE REPORT

Reversible postural orthostatic tachycardia syndrome

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Author contributions: Abdulla A made the diagnosis, performed the test to confirm the diagnosis and finalised the writing; Rajeevan T performed literature search and preliminary writing.

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Informed consent statement: Patient involved in this case report provided informed written consent.

Conflict-of-interest statement: None.

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Abstract

Postural orthostatic tachycardia syndrome (POTS) is a relatively rare syndrome recognised since 1940. It is a heterogenous condition with orthostatic intolerance due to dysautonomia and is characterised by rise in heart rate above 30 bpm from base line or to more than 120 bpm within 5-10 min of standing with or without change in blood pressure which returns to base line on resuming supine position. This condition present with various disabling symptoms such as light headedness, near syncope, fatigue, nausea, vomiting, tremor, palpitations and mental clouding, *etc*. However there are no identifiable signs on clinical examination and patients are often diagnosed to have anxiety disorder. The condition predominantly affects young female between the ages of 15-50 but is rarely described in older people. We describe an older patient who developed POTS which recovered over 12 mo. Recognising this condition is important as there are treatment options available to alleviate the disabling symptoms.

Key words: Postural; Orthostatic; Tachycardia; Dysautonomia; Hypotension; Postural tachycardia syndrome; Older person

Core tip: This is a short report and literature review on postural orthostatic tachycardia syndrome (POTS). POTS commonly affects younger patients and is rarely reversible. Here we describe an older patient who presented with disabling POTS which was reversed. Although rare, it is now being recognised in older people and increasing awareness among geriatricians is important as early diagnosis and treatment may alleviate the disabling symptoms. Reviewing the literature we argue whether hypotension should be considered as a feature of POTS.

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INTRODUCTION

Postural orthostatic tachycardia syndrome (POTS) is an orthostatic intolerance due to dysautonomia. This heterogeneous group of syndromes is characterised by rise in heart rate above 30 bpm from base line or to more than 120 bpm within 5-10 min of standing with or without change in blood pressure. This condition present with various disabling symptoms such as light headedness, near syncope, fatigue, nausea, vomiting, tremor, palpitations and mental clouding, *etc*. Various pathophysiological mechanisms have been recognised. It commonly affects younger patients and is rarely reversible. However it has been recognised in older people too. Recognising this condition is important as there are treatment options available to alleviate the disabling symptoms.

CASE REPORT

A 70-year-old woman presented with an 8 mo history of dizziness on standing and unsteadiness on her feet. More recently she experienced a fall and several near falls. She complained of nausea resistant to antiemetics and weight loss. She felt generally weak and had become dependent for all her activities of daily living. She had a brief hospital admission 10 mo prior to the current presentation and was investigated for the persistent nausea and vomiting. Upper gastrointestinal endoscopy, barium swallow and computed tomography scan of abdomen, pelvis and chest showed no identifiable pathology.

She had past medical history of polymyalgia rheumatica, hypertension and anxiety. She was on maintenance dose of prednisolone 1 mg, ramipril 2.5 mg and diazepam at night. She lived with her husband, previously independent for her activities of daily living, was a non smoker and consumed alcohol occasionally.

On assessment she appeared anxious and was lethargic. Her heart rate was 80 bpm regular. Blood pressure was 179/85 mmHg sitting and 119/73 mmHg on standing associated with rise in heart rate to120 bpm. She complained of dizziness on standing. Systemic examination including cardiovascular and neurological examinations was unremarkable.

Routine blood tests including thyroid function tests and random cortisol were normal. Twenty-four hours urine collections on 4 consecutive days for 5-hydroxyindoleacetic acid (5HIAA), cortisol, dopamine, epinephrine, norepinephrine and sodium were sent. 5HIAA excretion was elevated on 2 occasions to 49 and 99 micromol/d (normal 10-42) as was excretion of epinephrine to 190 nmol/d (normal 0-144). Elevation of catecholamines was possibly related to hyperadrenergic condition and significance of 5HIAA elevation was unexplained as it returned to normal in subsequent assays. Twelve lead electrocardiogram (ECG) and 24 h tape showed sinus rhythm. An echocardiogram showed signs of left ventricular hypertrophy. She underwent tilt table test which showed a rise in heart rate above 30 bpm from baseline associated with fall in blood pressure (Figure 1).

She was commenced on propranolol 10 mg tds and fludrocortisone at a starting dose of 50 mcg daily which was increased to 150 mcg. She responded remarkably well; her postural tachycardia resolved with improvement in symptoms of dizziness and nausea. She gradually regained her mobility and was able to resume her personal activities.

All the endocrine tests were repeated after 2 mo of her recovery of symptoms. All her baseline endocrine tests were normal at this point.

On follow up at 4 mo her blood pressure was 150/70 mmHg. She was slowly weaned off fludrocortisone with no recurrence of symptoms. Head up tilt test was repeated at 15 mo (Figure 2) which showed complete recovery and no change in heart rate or blood pressure with postural change.

DISCUSSION

POTS is a relatively rare syndrome recognised since 1940[1,2]. It is most often seen in women of child bearing age (between the ages of 15 and 50), nevertheless it may appear at any age. This is rarely described in older people and pathophysiology can be significantly different in older group of people. The mechanism depends on underlying pathology. It is not always easy to identify the mechanism therefore treatment can be difficult.

Standing results in approximately 0.5 to 1.0 L of blood pooling in the lower extremities and splanchnic circulation. A normal hemodynamic response to postural change requires normal function of the cardiovascular and autonomic nervous systems. An increase in sympathetic outflow, increases peripheral vascular resistance, venous return, and cardiac output, limiting the decrease in blood pressure. Normal compensatory mechanisms result in a decrease in systolic blood pressure (5 to 10 mmHg), an increase in diastolic blood pressure (5 to 10 mmHg), and an increase in pulse rate (10 to 25 beats per minute). Orthostatic hypotension is defined as a decrease in systolic blood pressure of 20 mmHg or a decrease in diastolic blood pressure of 10 mmHg within three minutes of standing compared with blood pressure from the sitting or supine position.

POTS is an orthostatic intolerance due to dysautonomia and is divided into primary and secondary types. It is important to identify the type to facilitate treatment. By definition the primary form occurs in the absence of an underlying condition. In contrast the secondary form of POTS is due to chronic conditions such as diabetes mellitus, sarcoidosis, connective tissue disorders, *etc*[3]. The condition runs a remitting and relapsing course.

This heterogeneous group of syndromes is characterised by rise in heart rate above 30 bpm from base line or to more than 120 bpm within 5-10 min of standing with or without change in blood pressure. In

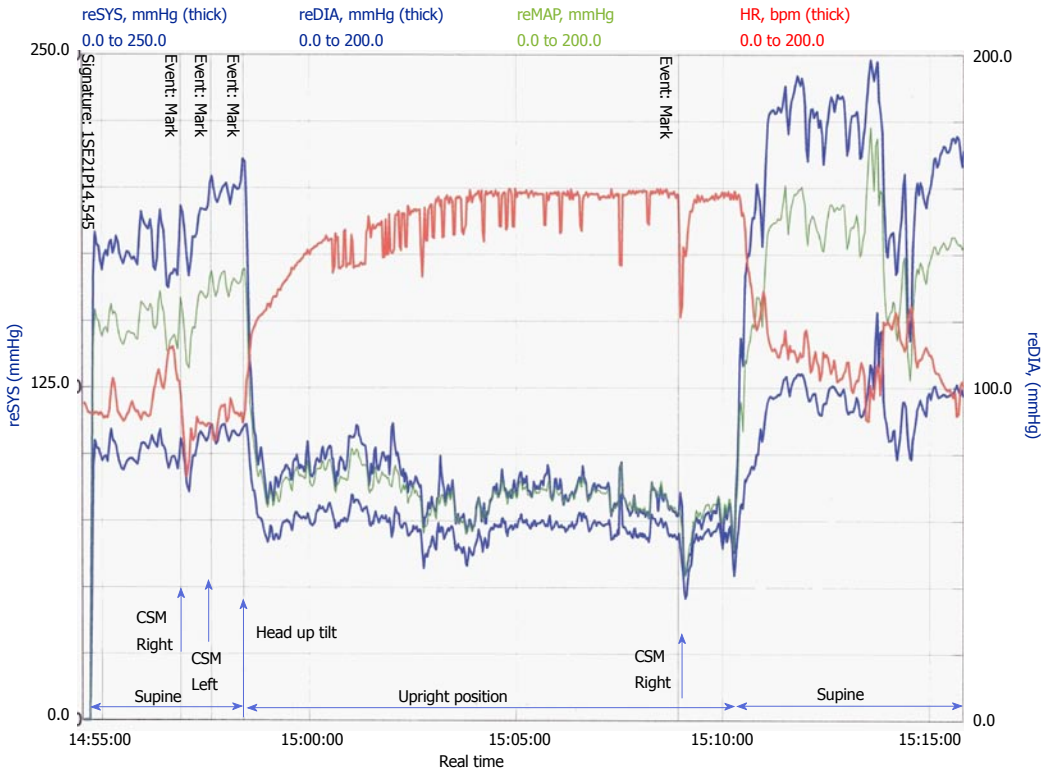

Figure 1 The significant increase in heart rate on head-up tilt (red line) associated with drop in systolic and diastolic blood pressure (in blue). Note the return of both heart rate and blood pressure on returning to supine position. MAP: Mean arterial pressure; reSYS (right Y-axis): Systolic blood pressure; reDIAS (left Y-axis): Diastolic blood pressure; HR: Heart rate; CSM: Carotid sinus massage.

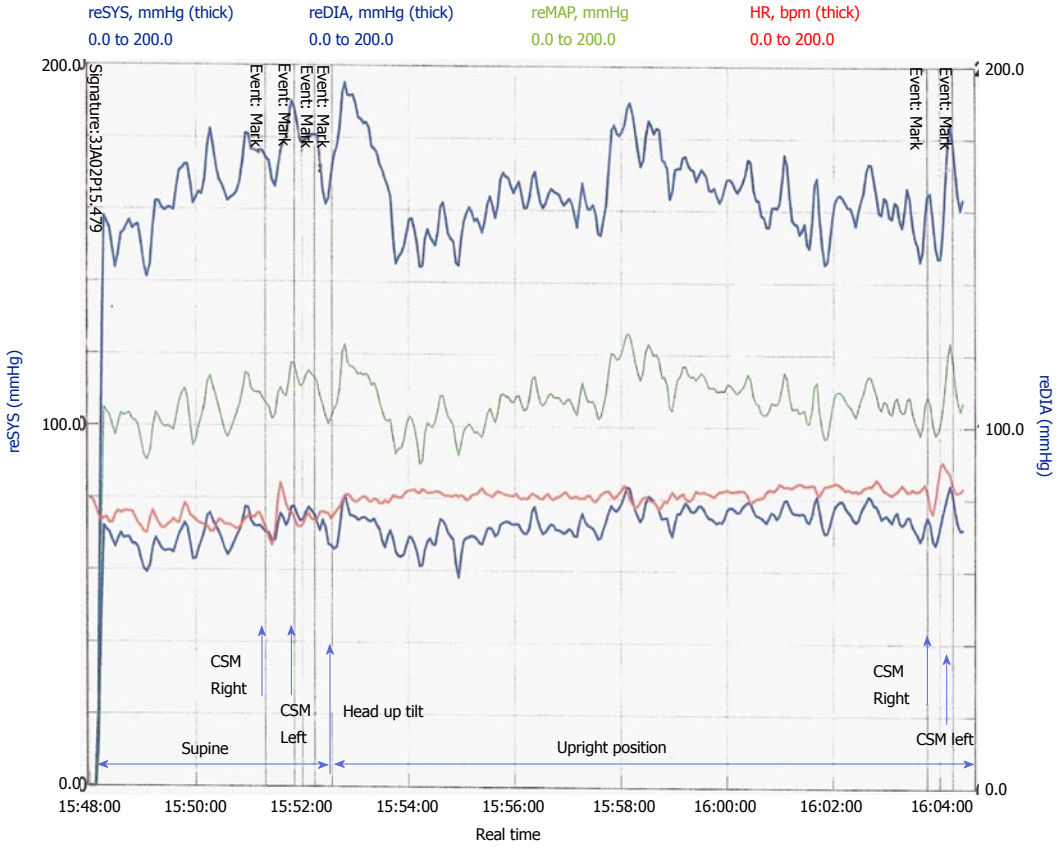

Figure 2 An essentially normal response with minimal change in heart rate on head-up tilt (red line) and non-significant alteration in blood pressure (in blue). CSM: Carotid sinus massage; reSYS (right Y-axis): Systolic blood pressure.

Table 1 Differentiating partial dysautonomic and hyperadrenergic forms

	Partial dysautonomia	Hyperadrenergic form
Frequency	Most common primary form	Less common primary form
Mechanism	Inability of peripheral vessels to constrict	Tachycardia due to elevated catecholamine
Onset	Abrupt	Gradual
Pathophysiology	Autoimmune mediated	Familial–single point mutation
Investigation	Serum acetylcholine receptor antibodies	Standing catecholamine level

fact some authors require no change in blood pressure on standing as a pre-requisite for the diagnosis of POTS but this is controversial[2,4-7]. Many of the mechanisms described in POTS such as vasodilatation, blunted response to angiotensin Ⅱ, low blood volume and red cell volume, abnormal vascular structure and venous capacitance can potentially drop the blood pressure. The drop in blood pressure due to above mechanisms may be compensated to a certain degree in POTS patients by disproportionately elevated heart rate though at times the compensation may be incomplete.

Pathophysiology

The various pathophysiological mechanisms involved in POTS are: (1) high level of standing norepinephrine level (due to reduced norepinephrine transporter expression resulting increased systemic norepinephrine spill over); (2) presence of ganglionic acetylcholine receptor antibodies; (3) alpha 1 adrenergic receptor denervation or hyposensitivity; (4) beta adrenergic super sensitivity; (5) peripheral autonomic denervation with preserved cardiac and cerebral innervations; (6) Partial renal sympathetic denervation leading to reduced renin/Aldosterone[8]; (7) increased angiotensin Ⅱ level with blunted responsiveness of receptors to angiotensin Ⅱ[9]; (8) Low blood volume and Red cell volume; (9) Abnormal vascular structure with impaired venous capacitance; and (10) Increased capillary permeability.

Not all of these mechanisms present in any one patient and treatment should be tailored accordingly. Symptoms are most likely due to cerebral hypoperfusion[10].

Types of POTS

As mentioned earlier POTS patients present with varying clinical features depending on the underlying pathology. Unifying feature is orthostatic intolerance which improves on lying or sitting down. The primary form is not associated with or caused by other chronic disorders. Here the onset is usually abrupt particularly when it occurs following viral illness, immunisation, pregnancy or surgery. An exception is the developmental form which occurs following a period of rapid growth[3]. In this case it runs a slow progress to reach a peak within 2 years.

Two major types of primary forms are identified. They are partial dysautonomic and hyperadrenergic forms (Table 1). Partial dysautonomic form is due to peripheral autonomic neuropathy which results in excessive pooling of blood in blood vessels of lower limbs and mesenteric circulation with the reflex tachycardia. Antibodies to ganglionic acetylcholine receptors are often found in patients with post viral autonomic neuropathy[3,11]. In contrast the hyperadrenergic form is usually a familial condition where there is rise in norepinephrine level on standing which causes the orthostatic tachycardia and orthostatic intolerance[12]. The rise in norepinephrine is due to reduced clearance secondary to poorly functioning reuptake transporter protein[6]. These patients suffer from profuse sweating, anxiety, tremulousness, tachycardia and hypertension[12,13].

Secondary forms are mainly due to chronic disorders such as diabetes mellitus, joint hyper mobility syndrome, sarcoidosis, systemic lupus syndrome, heavy metal poisoning and chemotherapies which affect the nervous system.

Symptoms

Various symptoms are described such as light headedness, near syncope, fatigue, nausea, vomiting, tremor, palpitations, mental clouding, *etc*. Symptoms can be brought on minimal exertion or activities such as eating, showering and walking short distance[2,5,14]. Both symptoms and tachycardia resolve with sitting or lying down. Patients are often diagnosed to have anxiety disorder[1,5].

Investigations

Head up tilt (HUT) is the investigation of choice although some studies suggest that standing haemodynamics is more specific[2,15]. It is important to rule out other conditions which cause tachycardia such as phaeochromocytoma, carcinoid, thyrotoxicosis, cardiac arrhythmia, *etc*. Tachycardia in these conditions is not related to change in posture.

Important investigations are blood tests which should include full blood count, renal function, thyroid function, calcium level, glucose, catecholamines on standing from supine position. Twenty-four hours urine collection for 5HIAA, catecholamines, sodium level are relevant investigations in POTS to rule out other causes of tachycardia and aim treatment options[12]. Routinely an ECG should be performed and further investigations such as 24 h tape and echocardiogram are carried out if indicated. The fact that this syndrome is not often recognised by clinicians, it leads to unnecessary investigations before the diagnosis is made especially in older people.

Management

Conservative: Review of medications which can aggravate POTS and appropriately stopping these medications; these include: (1) drugs that enhance vasodilatation-alpha adrenoreceptor blockers, angiotensin converting enzyme inhibitor (ACEI), calcium channel blockers and nitrates; (2) drugs that enhance tachycardia-beta adrenoreceptor stimulants, tricyclic antidepressants; and (3) drugs that worsens volume depletion-diuretics and ACEI.

Exercise - Aerobic exercise and lower limb resistance training will help pumping the blood. The intensity and duration of exercise should be built up gradually and also depend on patient's age.

Avoid salt and fluid depletion - Increasing salt and fluid take have great impact on reducing severity of symptoms. Fluid intake of 2 L/d and salt intake of 3-5 g/d is recommended. Performing 24 h urinary collection for urinary sodium level would help to identify the patients who would benefit from salt supplements. Studies show that patients with urinary excretion < 124 mmol/d is an indicator of good response to salt treatment[16].

Pharmacological treatment: Vasoconstrictors: Fludrocortisone is the most commonly used drug in orthostatic intolerance. Its action is mediated by improving peripheral sensitivity of alpha adrenoceptors, fluid and salt retention. Midodrine is an alpha-1 adrenoreceptor agonist not only increases the peripheral vascular resistance but also helps orthostatic intolerance by having an effect on heart rate. Other vasoconstrictors used with variable results are: (1) methylphenidate - increases vasoconstriction by increasing catecholamine release and inhibiting monoamine oxidase; (2) erythropoietin: increases the sensitivity of angiotensin II; (3) clonidine is a central sympatholytic and increases peripheral vascular resistance; and (4) octreotide: somatostatin analogue is potent vasoconstrictor.

Heart rate limiting drugs: Beta blockers are the main group of drugs and among them propranolol is favoured by clinicians. There are limited studies with regards to the dosage at which it is effective in treating POTS. Moderate dose of propranolol (20 mg) not only reduces heart rate but also improves symptoms, whereas higher dose (80 mg) is effective in reducing heart rate but does not improve symptoms. In fact it has been reported to worsen symptoms[17]. Other drugs which can reduce heart rate and alleviate symptoms are selective serotonin reuptake inhibitors (SSRI) and selective noradrenalin reuptake inhibitors. SSRI have been used for cardiogenic syncope and orthostatic hypotension. Serotonin plays and important role in central control of heart rate.

Ivabradine has effect on reducing the heart rate and symptom control.

Volume expanders: As mentioned earlier fludrocortisone is a mineralocorticoid and enhances the fluid and salt retention. Erythropoietin stimulates red cell production and increase the red cell mass and blood volume. Treatment with Erythropoietin is reserved for people with refractory symptoms in spite of other medications. Cost and administration by subcutaneous injection are the limiting factors for its use. Desmopressin increases the reabsorption of fluid from kidney, but its use in POTS has not been adequately studied.

Other medications: Pyridostigmine is an acetylcholine esterase inhibitor and is a very promising drug particularly for POTS following viral illness and POTS secondary to autoimmune process and paraneoplastic syndrome.

In conclusion, POTS is a condition characterised by orthostatic intolerance with excessive increase in heart rate due to disturbances in autonomic control. Patients with this condition suffer from numerous disabling symptoms with no specific abnormalities on clinical examination. Head up tilt test is the choice of investigation. Identifying the subtypes is the key to achieve successful management.

Our patient is a rare case of POTS in older people with full recovery.

Her symptoms were severe enough to warrant hospital admission. Medications were commenced once diagnosis was established with HUT and she made good recovery with her mobility and other symptoms.

COMMENTS

Case characteristics

A 70-year-old woman presented with dizziness and unsteadiness on feet.

Clinical diagnosis

Heart rate increased to more than 30 bpm from baseline on standing associated with fall in blood pressure.

Differential diagnosis

Postural hypotension and other conditions which causes tachycardia such as pheochromocytoma, thyrotoxicosis carcinoid syndrome and cardiac arrhythmia.

Laboratory diagnosis

Head up tilt test showed rise in heart rate from 80 to 120 bpm on standing with drop in blood pressure.

Imaging diagnosis

Chest X-ray and computerised tomography of abdomen, pelvis and chest were normal.

Treatment

The patient was treated with fludrocortisone and propranolol.

Related reports

Postural orthostatic tachycardia syndrome (POTS) is described predominantly in young females and to our knowledge this is the first case reported in older person.

Term explanation

Head up tilt test is the tilt-table test involves placing a patient on a flat table with a foot support, then tilting the table upward for a period of time to observe

changes in blood pressure and heart rate.

Experiences and lessons

POTS is a rare disabling condition; but is treatable when diagnosis is established which needs high index of suspicion.

Peer-review

This is an interesting case report.

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CASE REPORT

Arteriovenous malformation of the vestibulocochlear nerve

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Author contributions: Tucker A and Tsuji M contributed to conception and design; Tucker A contributed to acquisition of data; Tucker A, Tsuji M and Yamada Y contributed to analysis and interpretation of data; Tucker A contributed to drafting the article; Tucker A and Yamada Y contributed to critically revising the article; all authors reviewed submitted version of the article; Tucker A approved the final version of the manuscript on behalf of all authors.

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Abstract

We describe a rare case of an arteriovenous malformation (AVM) embedded in the vestibulocochlear nerve presenting with subarachnoid hemorrhage (SAH) treated by microsurgical elimination of the main feeding artery and partial nidus volume reduction with no permanent deficits. This 70-year-old woman was incidentally diagnosed 4 years previously with two small unruptured tandem aneurysms (ANs) on the right anterior inferior cerebral artery feeding a small right cerebellopontine angle AVM. The patient was followed conservatively until she developed sudden headache, nausea and vomiting and presented to our outpatient clinic after several days. Magnetic resonance imaging demonstrated findings suggestive of early subacute SAH in the quadrigeminal cistern. A microsurgical flow reduction technique *via* clipping between the two ANs and partial electrocoagulation of the nidus buried within the eighth cranial nerve provided radiographical devascularization of the ANs with residual AVM shunt flow and no major deficits during the 2.5 year follow-up. This is only the second report of an auditory nerve AVM. In the event of recurrence, reoperation or application of alternative therapies may be considered.

Key words: Arteriovenous malformation; Flow reduction; Microsurgery; Subarachnoid hemorrhage; Vestibulocochlear nerve

Core tip: Arteriovenous malformations (AVMs) originating within or impinging on cranial nerves are extremely rare, and there is an increased risk of hemorrhage in

AVMs associated with aneurysms. The authors describe the second report of a patient with a vestibulocochlear nerve AVM who presented with subarachnoid hemorrhage. A discussion of the delicate diagnostic and therapeutic implications is presented.

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INTRODUCTION

Arteriovenous malformations (AVMs) originating within or impinging on cranial nerves are extremely rare, most of which have been identified in the the optic chiasma[1-6] or trigeminal nerve[7-24]. Posterior fossa AVMs account for only 7%-15% of all intracranial AVMs, however these lesions tend to involve greater morbidity and mortality and recent studies have shown an independent association between infratentorial AVM location and hemorrhagic presentation[25]. Moreover, there is an increased risk of hemorrhage in AVMs accompanied by aneurysms (ANs)[26].

Based on a search of the available literature, the authors found only one report of an intrinsic auditory nerve AVM[27], however the patient suffered a total hearing deficit. Herein we describe a rare case of an AVM embedded in the vestibulocochlear nerve presenting with subarachnoid hemorrhage (SAH) treated initially by microsurgical elimination of the main feeding artery and partial nidus volume reduction with no permanent deficits.

CASE REPORT

This 70-year-old right-handed woman with an unremarkable past medical history was incidentally diagnosed 4 years prior with two small tandem ANs on the right anterior inferior cerebral artery (AICA) feeding a small right cerebellopontine angle AVM. The patient was referred to our hospital for further evaluation and treatment and despite informed consent regarding the potential risks of subsequent hemorrhage and recommendations for microsurgery, Gamma Knife stereotactic radiosurgery, or endovascular embolization, or other interventions, she and her family chose conservative outpatient observation.

Digital subtraction angiography (DSA) was recommended for primary evaluation, however based on the invasiveness, potential complications, and inconvenience, she declined this modality in favor of three-dimensional computed tomography angiography (3D-CTA) and magnetic resonance angiography (MRA) for initial diagnostic evaluation followed by biannual and thereafter annual serial magnetic resonance imaging (MRI) (Figure 1). Dedicated informed consent was routinely offered to the patient and family including an explanation of the cumulative bleeding risks and recommendations for aggressive surgical or alternative treatment options. However the patient declined treatment and was followed on an outpatient basis until she presented to our outpatient clinic with a complaint of severe headache, nausea and vomiting of several days duration. The neurological exam was unremarkable with no apparent cranial nerve deficits. Head computed tomography (CT) did not demonstrate any evidence of bleeding, however, high intensity signals only in the quadrigeminal cistern on T1-weighted MRI were observed, suggesting early subacute SAH (Figure 2). Fluid attenuated inversion recovery imaging was not performed. MRA source imaging and magnetic resonance cisternography demonstrated a small (11.4 mm × 4.2 mm) relatively compact nidus located in the cerebellopontine angle around the cisternal portion of the facial and vestibulocochlear nerves (cranial nerves Ⅶ and Ⅷ, respectively) with extension into the internal auditory canal (Figure 3). On the arterial phase of DSA the right AICA was identified as the predominant feeder with drainage into the right petrosal vein, superior petrosal sinus, and cavernous sinus (Figure 4). Based on the lack of any significant cranial nerve deficits, the occurrence of either minor AVM leakage, hypertensive venous bleeding, or subarachnoid hemorrhage from AN rupture was postulated. Because of the symptomatic nature of this Spetzler-Martin grade Ⅲ AVM (S1V1E1) with two feeding artery aneurysms, and after a comprehensive explanation of the risks and benefits of the various treatment options, including the risks of microsurgical neurological sequelae, the persistent delayed risk of bleeding following radiation therapy, and the possible risk of recurrence or other complications following endovascular embolization, the patient and family agreed to a recommendation of microsurgical treatment.

Surgical procedure

The patient was placed in the right lateral oblique position and a right retrosigmoid craniotomy was performed. After exposing the right lateral cistern and cerebellopontine angle, the right facial and vestibulocochlear nerves (cranial nerves Ⅶ and Ⅷ, respectively) were identified followed by confirmation of the right lower cranial nerves (cranial nerves Ⅸ, Ⅹ, and Ⅺ), the right AICA, proximal basilar-AICA bifurcation, and surrounding trigeminal and abducens nerves (cranial nerves Ⅴ and Ⅵ, respectively). The right AICA was determined to be the predominant feeder supplying a small peripheral nidus buried within the right cranial nerve Ⅷ at the meatus of the internal auditory canal with venous drainage into an engorged red petrosal vein (Figure 5A and B). Close inspection revealed a mid-sized fusiform AN at the proximal AICA

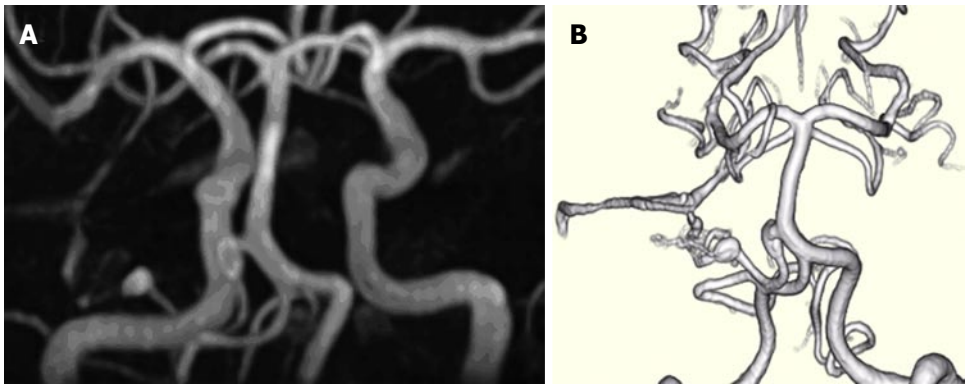

Figure 1 Three-dimensional time-of-flight magnetic resonance angiography. A: Three-dimensional time-of-flight magnetic resonance angiography (3D-TOF-MRA) four years prior to admission; B: Volume rendering 3D-TOF-MRA study 1 year prior to admission, demonstrating two unruptured prenidal feeder artery aneurysms, one 8 mm × 3 mm fusiform proximal anterior inferior cerebral artery (AICA) aneurysm near the takeoff of the basilar artery and one 4.5 mm × 3 mm elliptical distal pedicle AICA aneurysm located at the entrance to the main inflow area of a small inconspicuous vascular nidus.

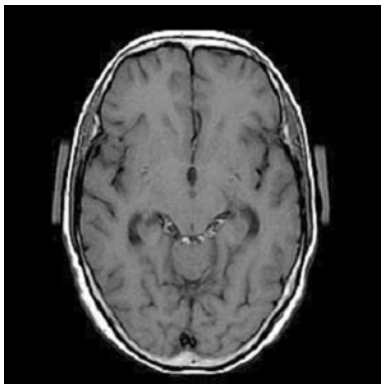

Figure 2 On admission, this 70-year-old woman presented with a severe headache, nausea and vomiting of several days duration. T1-weighted magnetic resonance imaging demonstrated high intensity signals only in the quadrigeminal cistern, suggestive of early subacute subarachnoid hemorrhage.

near the takeoff from the basilar artery and a small distal pedicle feeding artery AN at the inflow area of the nidus. Xanthochromic cerebrospinal fluid was observed with a slight predominance near the proximal AICA AN, however there was no clear evidence of a rupture point from either of the ANs, nidus, or venous drainers. Based on the preoperative imaging studies, the nidus was suspected to extend into the auditory canal, and complete resection was not attempted because of the anticipated high risk of auditory nerve injury. Instead a feeder clip was applied to the AICA which demonstrated normalization of color from red to blue in the right petrosal vein. However, intraoperative microvascular doppler ultrasonography and indocyanine green video angiography confirmed the presence of persistent flow within the AVM despite temporary clipping, suggesting the existence of collateral supply from the external carotid artery system or other sources. Definitive clipping between the proximal and distal AICA ANs followed by partial electrocoagulation of both the nidus and the predominant entry area to the nidus were performed (Figure 5C) with the intention of alleviating hemodynamic stress on the remaining nidus and proximal AN. Figure 6 shows an overall intraoperative schematic illustration. Although auditory brainstem response monitoring remained normal throughout all procedures, preventative intravenous corticosteroids were administered postoperatively.

Postoperative course

The patient had an uneventful postoperative course with follow-up clinical and head CT examinations every 2 to 3 mo for a period of 2.5 years. Biannual follow-up outpatient MRI and MRA source imaging during that period showed clip artifact at the entrance to the internal auditory canal with apparent disappearance of proximal and distal right AICA aneurysms, and a prominent right superior petrosal sinus, indicative of residual shunt flow (Figures 7 and 8 compare preoperative and postoperative changes). The patient experienced a transient right hearing deficit, diplopia, and vertigo which almost completely resolved on discharge. More extensive diagnostic imaging and treatment options including Gamma Knife radiosurgery were under consideration pending the onset of radiological regrowth or symptomatic recurrence.

DISCUSSION

Intrinsic AVMs limited exclusively to the cranial nerves are extremely uncommon although there have been many reports of AVM rupture resulting in damage to nearby cranial nerves or indirect feeder compression of cranial nerves with associated symptoms manifesting in various conditions such as optic nerve apoplexy[1-6], oculomotor nerve ophthalmoplegia[28], trigeminal neuralgia[7,8,10-18,20,21,23,24], hemifacial spasm[29,30], glossopharyngeal neuralgia[31], and hypoglossal nerve paresis[32]. Several rare reports of AVMs originating from within the trochlear nerve[33] and trigeminal nerve[9,19,22] have also been documented. Maher *et al*[19] proposed an embryological basis for explaining the occurrence of AVMs arising in cranial nerves such as the trigeminal nerve, whereby normally migrating fetal axons carry

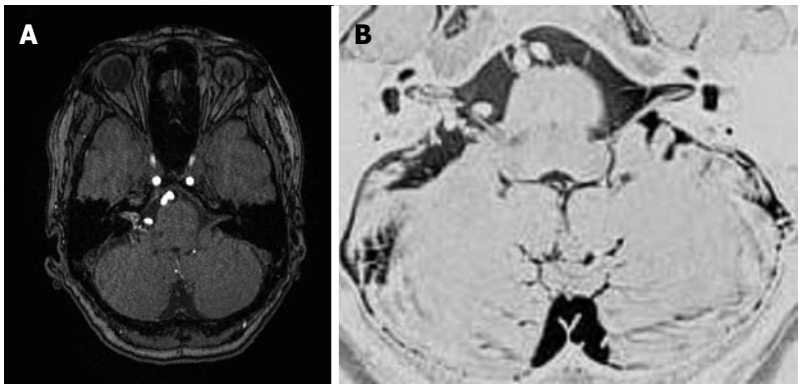

Figure 3 Magnetic resonance angiographic source imaging (A) and magnetic resonance cisternography (B) reveal a small (11.4 mm × 4.2 mm) relatively compact nidus located in the cerebellopontine angle at the cisternal portion of the cranial nerves VII and VIII with extension into the internal auditory canal.

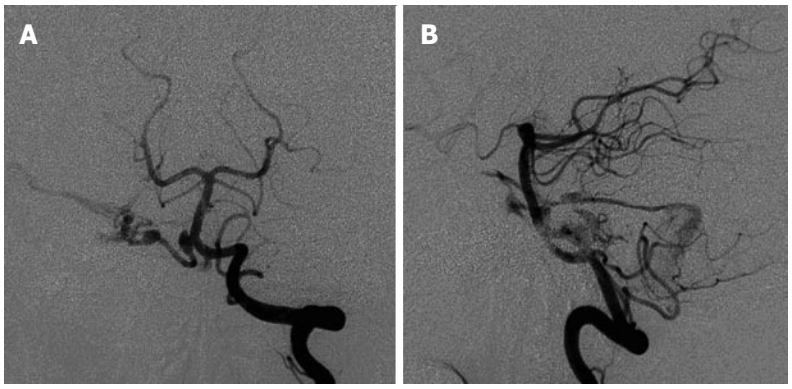

Figure 4 Anterioposterior (A) and lateral (B) views of cerebral angiogram for this patient showing the right anterior inferior cerebral artery as the predominant feeder supplying a small nidus with drainage into the right petrosal vein, superior petrosal sinus, and cavernous sinus [Spetzler-Martin grade III (S1V1E1)].

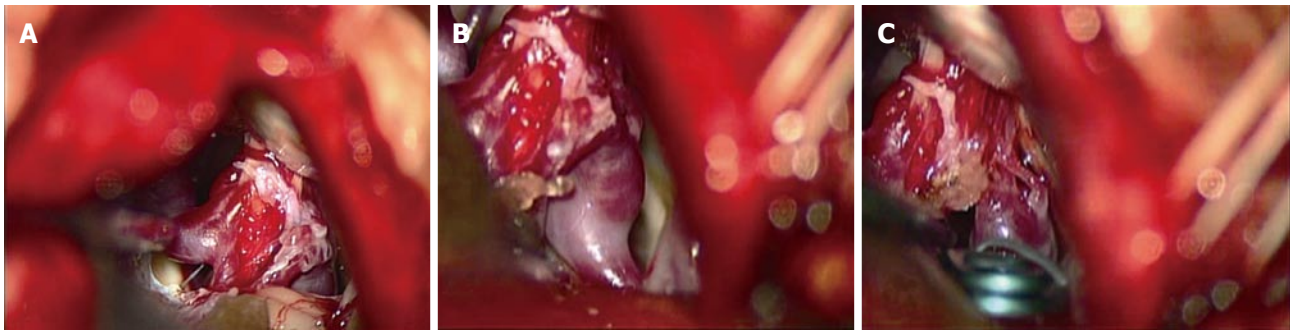

Figure 5 Intraoperative microscopic view. A: Intraoperative microscopic view showing a right retrosigmoid craniotomy in the a right lateral oblique position which exposed a small nidus buried within the right cranial nerve VIII at the meatus of the internal auditory canal; B: View showing distal right anterior inferior cerebral artery feeding aneurysm, nidus-VIII cranial nerve complex, and draining petrous vein; C: Definitive clip application proximal to the aneurysm and partial electrocoagulation of the nidus and the inflow area just proximal to the nidus was performed.

precursor AVM cells toward an ectopic cranial nuclei location during the 5th to 6th weeks of embryogenesis.

To date, there has been only one case report of an AVM located within the auditory nerve bundle which presented with SAH yet this case involved complete sensorineuronal hearing loss[27]. Because of the severe functional deficit from onset in that patient, total removal of both the nidus and nerve remnant were performed. Interestingly, in many of the cases of intrinsic trigeminal or trochlear AVMs as well as in our case, the patients presented with symptoms unrelated to cranial nerve dysfunction from direct AVM rupture[19,33], or symptoms were not caused by the nidus itself but by neural compression from surrounding vessels such as the feeding superior cerebellar artery[17,18,20,22] or impingement from other vessels at the trigeminal nerve

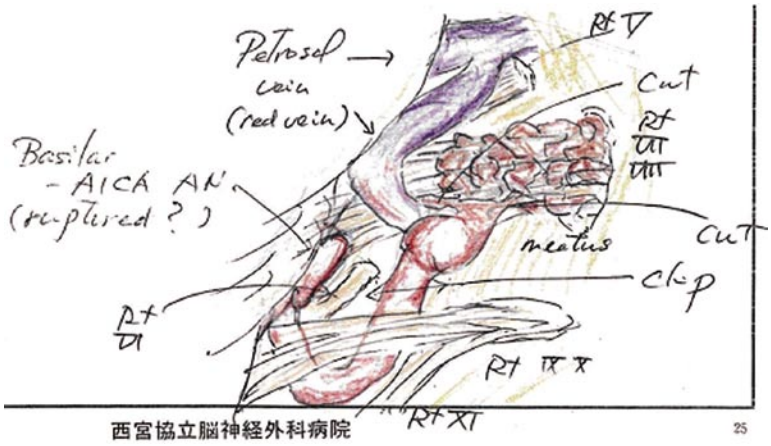

Figure 6 Intraoperative schematic illustration depicting anatomical structures and definitive clipping between the proximal and distal feeding anterior inferior cerebral artery aneurysms and partial electrocoagulation of both the nidus and the main inflow area to the nidus.

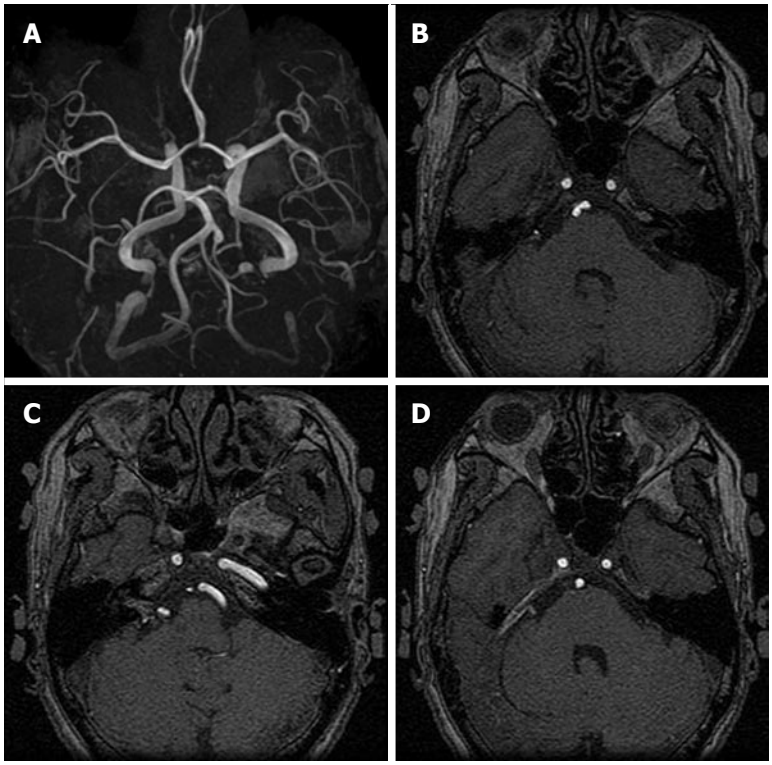

Figure 7 Preoperative three-dimensional time-of-flight magnetic resonance angiography showing proximal and distal right anterior inferior cerebral artery aneurysms (A), and magnetic resonance angiographic source imaging showing proximal right anterior inferior cerebral artery aneurysm (B), distal right anterior inferior cerebral artery aneurysm with relatively strong internal auditory canal nidus signal intensity (C), and right superior petrosal sinus venous shunt (D).

root entry zone[15,16]. In a study by Sumioka *et al*[22] a review of over 600 cases of trigeminal neuralgia were identified to be caused by vascular compression while only a small minority showed no evidence of vascular involvement. In these non-vascular cases, symptoms of trigeminal neuralgia were postulated to be derived from either the twisting or "tilting" by mechanical compressive forces, thickened arachnoid, or the direct effects of embedded AVMs.

In addition, many of the cases of chiasmal or optic nerve apoplexy due to intrinsic AVMs (including cavernous malformations) were typically diagnostically labeled as angiographically occult and only after performing high-resolution MRI were these cryptic micro-AVMs identified[3]. MRI has also been considered to offer a considerable advantage over standard angiography both for reducing complications and procedural related morbidity as well as providing detailed delineation of neurovascular structural relationships crucial for making treatment decisions during the preoperative and postoperative periods[34]. Moreover, in a recent report of the largest surgical series of radiological and clinical follow-up of AVM recurrence after surgery, MRI with and without contrast administration was

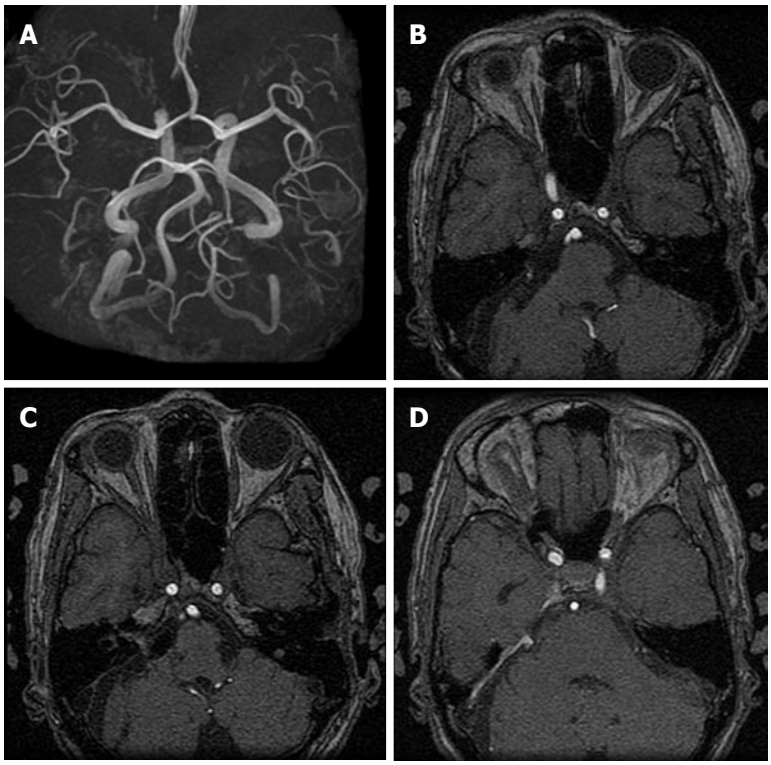

Figure 8 Postoperative three-dimensional time-of-flight magnetic resonance angiography (3D-TOF-MRA) and magnetic resonance angiographic source imaging showing anterior inferior cerebral artery (AICA) stump with disappearance of proximal and distal right AICA aneurysms (A, B), decreased intensity of right AICA and seemingly disappearance of proximal aneurysm, clip artifact at the entrance to the internal auditory canal and decreased intensity of intracanicular nidus (C), and persistence of prominent right superior petrosal sinus venous shunt (D).

recommended as the initial method for monitoring recurrence, with subsequent performance of digital subtraction angiography only in the event of suspected recurrence[35].

Likewise, a review of the recent literature seems to indicate a more palliative, minimally invasive trend for the treatment of cranial nerve AVMs. Sumioka *et al*[22] have suggested that the goal of treating intrinsic AVMs of the trigeminal nerve is generally symptomatic treatment, with prevention of rebleeding and preservation of cranial nerve function. Although treatment of cranial nerve cavernous malformations has been shown to be relatively successful with a lack of major associated complications to the surrounding neurovascular structures, direct microsurgical AVM resection in and around the optic nerve as well as other cranial nerves has typically been associated with significant damage to the nerve itself as well as surrounding microvasculature structures[9,18,22,23]. Indeed Sasagawa *et al*[5] have suggested that curative surgical treatment of optic nerve AVMs is considered nearly impossible, while other reports have shown that microsurgery can be used diagnostically as well as theraputically *via* limited surgical treatment such as optic nerve decompression or by subtotal operation for prevention of AVM growth or rebleeding[1]. As an example, an 8-year-old boy who presented with optic nerve apoplexy was found to have an AVM of the optic nerve and chiasm, but was treated by a diagnostic craniotomy alone without resection[5]. In another case of trigeminal neuralgia caused by an intrinsic trigeminal nerve AVM, complete resection of the AVM was judged to be too risky and only partial microsurgical nidus coagulation, transposition of the offending superior cerebellar artery, and subsequent Gamma Knife radiosurgery was performed for eventual complete radiological obliteration[22].

In our case the choice of treatment took into consideration both the angioarchitectural and clinical features as well as patient preference for a less aggressive option involving minimal risk. Due to the presence of symptomatic SAH presumed to be related to AN rupture and based on the high risk of nerve injury from complete AVM extirpation, a more palliative flow reduction strategy of feeder artery electrocoagulation at the inflow area of the nidus, effective feeder AN trapping, and partial electrocoagulation of the nidus was performed. Although intraoperative findings showed a slight predominance of xanthochromic cerebrospinal fluid near the proximal AICA AN, which was suggestive of having a higher likelihood of being the causative source of bleeding, because of the risk of damaging the abundant small perforators in the basilar-AICA takeoff area, a more distal obliteration point closer to the main inflow region of the AVM was chosen. Despite the seemingly unconventional nature of this method, in principle, we preliminarily followed standard microsurgical AVM

treatment protocol including coagulation of the major superficial feeding artery at a location as close as possible to the point of feeder entry into the nidus[34]. And according to current recommendations for treatment of coexisting intracranial ANs, we attempted to diminish the hemodynamic stress by obliteration of high flow shunting, a variation on a technique which has been shown to result in either regression or disappearance of the prenidal AN[34,36]. However, by placing a clip distal to the proximal AN, there could be a risk of subsequent increased hemodynamic stress and hemorrhage for this aneurysm, especially due to limited distal outflow. Careful follow-up diagnostic studies and consideration of endovascular or other treatment modalities would be recommended. Finally, the observation of a temporary postoperative hearing disturbance may possibly be explained by intraoperative manipulation of the cochlear nerve. However, the possibility of vascular compromise with compensatory extracarotid flow could explain the recovery in hearing, yet this mechanism would be expected to occur over a longer period of time.

Classically there has been caution regarding the potential increased risks of bleeding from incomplete, partial, or subtotal AVM resection[8], as well as recent concerns of bleeding after palliative embolization[34]. In contrast, embolization designed to reduce flow and decrease risk of rupture has been indicated in various conditions, including treatment of associated ANs and large inoperable AVMs, or "inoperable" vascular lesions with recurrent hemorrhage, or locations in eloquent regions, where multimodality approaches of preoperative embolization combined with microsurgical extirpation have been considered. However, there is no evidence in support of such empirical methods and ultimately treatment must be individualized[34,37]. Recently, the Barrow vascular group has found moderate success for treatment of vertebrobasilar aneurysms *via* low-flow bypass and flow reduction *via* complete or partial basilar artery vessel occlusion or distal vertebral artery occlusion[38]. Although our procedure as well as the angioarchitecture of the lesions differed in many ways from the technique used in that study, both lesions resided near critical neurovascular structures and in principle, both involved application to some degree of a procedure designed to reduce flow to the inflow vessel of the aneurysm. Another study from the same institution achieved satisfactory long-term outcome using a technique of subtotal nidus resection for effective devascularization of glomus spinal AVMs (lesions known to have similar angioarchitectural features as found in intracranial AVMs)[39], a technique similar in some ways to our technique of partial nidus electrocoagulation. And a recent pooled analysis of treatment outcomes for spinal glomus AVMs has shown that hemorrhagic risk can be significantly reduced by partial endovascular treatment[40]. In this sense, although the ultimate goal of surgical treatment is AN elimination and AVM extripation, care can be tailored according to the individual lesion, whereby a less invasive, more palliative treatment option can be employed as the initial preemptive strategy.

Radiation therapy as a primary treatment for asymptomatic brain AVMs or as an adjunctive therapy for recurrent AVMs has been advocated for treatment of lesions smaller than 3 cm in diameter and in deep or eloquent brain areas depending on size, angioarchitecture, and other factors, greater than 1- to 3-year obliteration rates range from 60% to 90%[34,41,42]. Moreover, radiosurgery for brainstem AVMs can attain up to 88% complete obliteration[43] and other reports claim complete obliteration rates in approximately two thirds of patients with a 1.7% annual hemorrhage rate for the first 3 years[44]. However, well known drawbacks include a 2.5% to 10% annual hemorrhage risk until complete obliteration, the potential for delayed hemorrhage after angiographically proven obliteration, and depending on location, there is a reported risk of clinically significant complications directly from radiosurgery for AVMs in excess of 3% to 6%, especially in cases of brainstem and other deep seated AVMs[34,43-45]. Radiosurgery for AVMs of the optic nerve and surrounding areas have been reported to cause optic neuropathy and injury to nearby neurovascular structures and consequently recommendations for maximal doses have been limited to less than 8 Gy[46]. And a study of Gamma Knife surgery for pituitary adenomas reported a 4.1% incidence of new visual dysfunction[47]. However, stereotactic radiosurgery for trigeminal neuroma presenting with trigeminal neuralgia has been shown to achieve symptomatic control without additional deficits[48]. Finally, there have been recommendations for other alternative management strategies including endovascular embolization for preoperative reduction of arterial inflow, occlusion of deep feeders, and prenidal aneurysm treatment, however, embolization in general has been associated with relatively high rates of morbidity and mortality (0% to 27%), mostly because of inadvertent embolization of feeding arteries or draining veins in normal surrounding neurovascular structures or post-embolization bleeding due to changes in flow dynamics[34]. Recently, primary embolization treatment with Onyx has shown total AVM obliteration rates of approximately 50%[49].

Major limitations of our study include a relatively brief 2.5 year follow-up in a single case report. Furthermore, we did not perform confirmatory follow-up DSA for verifying persistent patency, degree of alternative feeders, or other evidence of recurrence, however, this was a well informed decision in respect of the patient's ongoing preference for less invasive imaging modalities such as MRI which as mentioned, has been shown to be useful for therapeutic decision making during both the preoperative and postoperative periods as well as for identification of cryptic angiographically occult AVMs. However, as in our case, follow-up MRI showed diminished AICA and nidal flow, with persistence of venous shunt flow. Indeed, mere MRI or MRA confirm-

ation may only show relative flow reduction or stasis in flow and may not accurately represent actual residual or persistent vascular supply. In addition, clip artifact on MRI may prevent accurate assessment, while other testing such as 3D-CTA or DSA, may provide a more definitive understanding of the angioarchitecture. Standard follow-up DSA and application of additional therapy were offered to the patient, but the patient declined and ultimately application of such modalities were considered to be implemented on an as-needed basis in the event of suspected development of recurrence. We surmised that either thrombosis or diminished flow resulted in apparent radiological devascularization of the ANs and no associated clinical recurrence, however, the persistence of AVM shunt flow on follow-up MRI indicates that there is a continued risk of bleeding, although perhaps much less risk than without feeder artery and AN clipping. Furthermore, persistent external carotid artery or other feeding sources, or the potential for added changes in flow dynamics from our treatment could lead to subsequent AVM regrowth or bleeding, bleeding from the proximal basilar-AICA takeoff AN, or other *de novo* AN development. Currently the patient is symptom free and continues to express no interest in invasive testing or further treatment with Gamma Knife radiosurgery or other modalities.

ACKNOWLEDGMENTS

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COMMENTS

Case characteristics

A 70-year-old woman with a history of two incidental aneurysms (ANs) on the right anterior inferior cerebral artery (AICA) feeding a small right cerebellopontine angle arteriovenous malformation (AVM) presented with sudden headache, nausea and vomiting.

Clinical diagnosis

The neurological exam was unremarkable with no apparent cranial nerve deficits.

Differential diagnosis

Although admission head computed tomography did not demonstrate any evidence of bleeding, T1-weighted magnetic resonance imaging showed high intensity signals only in the quadrigeminal cistern, suggesting early subacute subarachnoid hemorrhage, and based on the lack of any significant cranial nerve deficits, the occurrence of either minor AVM leakage, hypertensive venous bleeding, or subarachnoid hemorrhage (SAH) from AN rupture was postulated.

Intraoperative diagnosis

The AICA was determined to be the predominant feeder supplying a small peripheral nidus buried within the right cranial nerve VIII (vestibulocochlear nerve) at the meatus of the internal auditory canal with a mid-sized fusiform AN at the proximal AICA near the takeoff from the basilar artery and a small distal pedicle feeding artery AN at the inflow area of the nidus, and although xanthochromic cerebrospinal fluid was observed, there was no clear evidence of a rupture point from either of the ANs, nidus, or venous drainers.

Treatment

The patient was treated initially by microsurgical elimination of the main feeding artery via clipping between the proximal and distal AICA ANs, followed by partial electrocoagulation of both the nidus and the predominant entry area to the nidus with the intention of alleviating hemodynamic stress on the remaining nidus and proximal AN.

Related reports

The authors describe the second report of a patient with a vestibulocochlear nerve AVM who presented with subarachnoid hemorrhage. Curative treatment of AVMs arising within delicate cranial nerve structures is difficult and fraught with a paucity of surgical evidence.

Term explanation

Flow reduction is an indirect less definitive treatment typically performed by proximal occlusion of the parent artery designed to alleviate hemodynamic stress on distally located ANs or AVMs and other potential hemorrhagic lesions.

Experiences and lessons

After dedicated informed consent, the patient in this case report chose less invasive testing and flow reduction treatment which resulted in no symptoms or permanent deficits throughout the 2.5 year follow-up, however despite radiographical devascularization of the ANs, residual AVM shunt flow and the potential for added changes in flow dynamics could lead to subsequent AVM or AN regrowth or bleeding.

Peer-review

The authors report on a rare case of an arteriovenous malformation of the vestibulocochlear nerve. The case was managed with microsurgery with only transient deficits noted.

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CASE REPORT

Conservative management of type 2 gallbladder perforation in a child

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Abstract

Gallbladder perforation (GBP) is a rare but serious complication of cholecystitis and needs to be managed promptly. Acalculus cholecystitis leading to GBP is frequently associated with enteric fever and found in critically ill patients, and a surgical approach is not always feasible in such patients. Use of percutaneous tube cholecystostomy (PTC) in such patients is a known entity but it is usually followed by interval cholecystectomy. Here we report a case of perforated gallbladder in a child managed conservatively and successfully with PTC as the definitive treatment wherein cholecystectomy was avoided. The functionality of the gallbladder was confirmed by a Tc99m-HIDA scan.

Key words: Spontaneous; Gallbladder; Perforation; Percutaneous tube cholecystostomy

Core tip: Percutaneous cholecystostomy for selected patients with gallbladder perforation or distended gallbladder with symptoms is a good technique to tide over the acute crisis and may even avert the need for cholecystectomy.

Dikshit V, Gupta R, Kothari P, Gupta A, Kamble R, Kesan K. Conservative management of type 2 gallbladder perforation in a child. *World J Clin Cases* 2015; 3(7): 671-674 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i7/671.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i7.671

INTRODUCTION

Gallbladder perforation (GBP) is rare in children and is seen as a complication of cholecystitis. Gallbladder stone disease is the most frequent cause of acute cholecystitis and acalculus cholecystitis is seen in only 5%-10% of cases[1]. The majority of the reported cases of GBP are associated with enteric fever. High level of

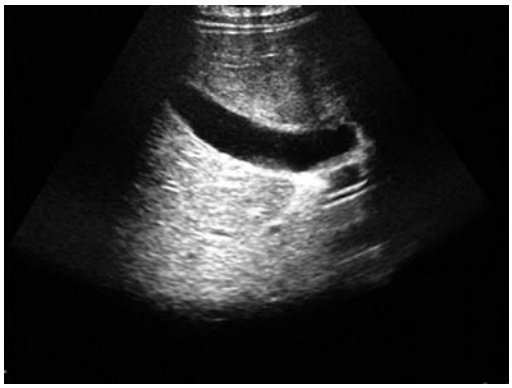

Figure 1 Over-distended gallbladder.

suspicion, early diagnosis and prompt management are of paramount importance in dealing with this entity.

CASE REPORT

A 12-year-old male patient admitted on the medical side was referred to us with complaints of pain in the abdomen for 12 d. The pain was dull aching in nature, more in the right hypochondrium. He had a history of 7-8 episodes of vomiting, gastric in nature with mild fever and headache.

The child was febrile and had tachycardia. There was no icterus or features of liver failure. The abdomen was distended and there was guarding in the right hypochondrium without any rigidity. The liver was palpable 2 cm below the costal margin and the gallbladder was felt as a firm, globular and tender mass.

Complete haemogram, bilirubin, liver enzymes, prothrombin time and renal function tests, were within normal limits. Widal test and blood culture were negative. An erect X-ray of the abdomen was suggestive of gaseous distention of bowel loops. Ultrasonography (USG) on admission was suggestive of a hugely distended gallbladder with echogenic sludge within and mild hepatosplenomegaly. The patient was being treated conservatively and a follow-up USG was done 2 d later for persistence of pain. This time sonography revealed an over-distended (13 cm) gallbladder with perforation at the tip with a narrow tract and small volume of sealed-off collection with duodenal wall thickening and edema (Figure 1).

With these findings the patient was transferred to us and underwent a contrast-enhanced computed tomography (CT) scan of the abdomen which confirmed the USG findings (8.5 mm sized rent seen in the gallbladder wall with mild peri-gallbladder collection 4.2 cm × 2.3 cm × 2.2 cm suspected of sealed-off gallbladder perforation. No free fluid was noted in the abdomen) (Figure 2). USG guided percutaneous cholecystostomy using an 8-Fr pigtail catheter was done and around 150 mL of bile was drained (Figure 3).

Bile culture showed Enterobacter species sensitive to piperacillin and amikacin. The patient received intravenous piperacillin at 250 mg/kg per day for 10 d and amikacin at 15 mg/kg per day for 5 d. Daily bile output through the cholecystostomy tube was around 120 to 140 mL. There was improvement in the patients' general condition with resolution of fever and abdominal pain.

One week after the procedure bile culture showed no growth and liver function tests (LFTs) were normal. Tube cholecystogram showed free passage of contrast into the bowels (Figure 4), hence, intermittent clamping of the pigtail was started with monitoring for fever, pain and change in LFTs. Clamping trial after 4 d was successful and the pigtail was removed on post procedure day 13.

The patient was discharged symptom free and a Tc-99m Mebrofinin (HIDA) scan done 1 mo later showed morphologically normal liver with normal hepatic function and hepatobilliary drainage (Figure 5). At follow-up after 1 year of the episode, the patient remained to be asymptomatic.

DISCUSSION

GBP is a rare complication of acute cholecystitis (2%-11%)[1,2] and is more often seen in patients having critical illness like severe trauma, burns and cardiovascular surgeries. Compared with adult population GBP is even rarer in children and is mainly due to acalculus cholecystitis, trauma, enteric fever, gallbladder wall necrosis due to sepsis or sometimes it may occur spontaneously[3,4].

The most common part of the gallbladder to perforate is the fundus followed by the body, with the reason being attributed to poor blood supply[1]. The majority of the cases of cholecystitis followed by perforation are seen in gallbladder stone disease where the cystic duct often gets occluded, leading to retention of secretions and rise in the intraluminal pressure. Acalculus cholecystitis is seen in 5%-10% of patients with acute cholecystitis[1] and may lead to perforation as seen in our case.

GBP may be traumatic, iatrogenic or idiopathic (spontaneous) and was classified by Niemeier[5] into 3 types. Type 1 (acute) is associated with generalized biliary peritonitis; type 2 (subacute) consists of localized fluid collection at the site of perforation, pericholecystic abscess and localized peritonitis; and type 3 (chronic) has an internal or external fistula formation.

Most recent studies have cited highest rates of type 2 GBP and the same was the case in our patient.

Historically GBP has been associated with a high mortality rate which ranges from 11% to 26%[6] and great care must be taken to diagnose the condition as early as possible. Generally GBP mimics bowel perforation and many cases are diagnosed intraoperatively. Modalities useful for this condition are USG and CT scan, with the latter being more sensitive[1]. Abdominal X-ray may not always show free gas in the

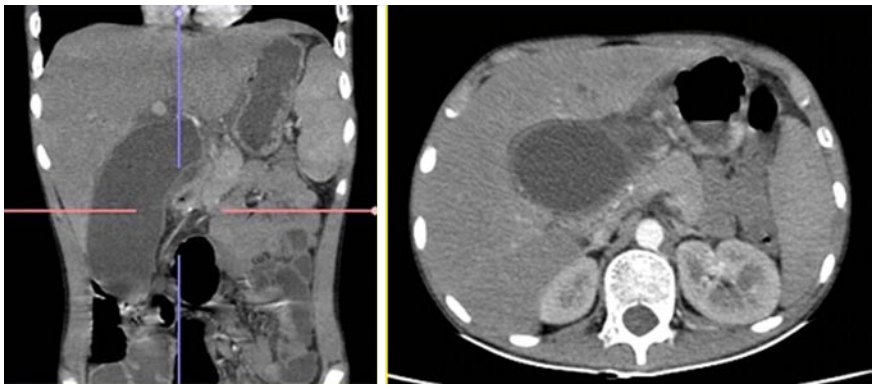

Figure 2 Computed tomography images.

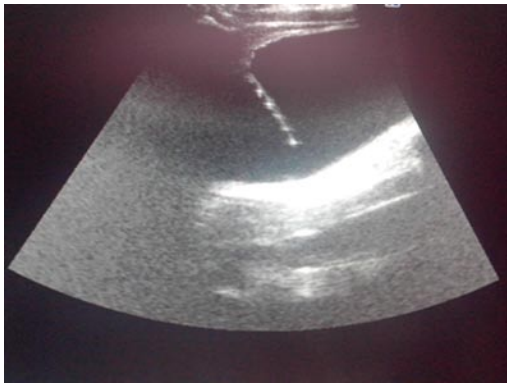

Figure 3 Percutaneous cholecystostomy.

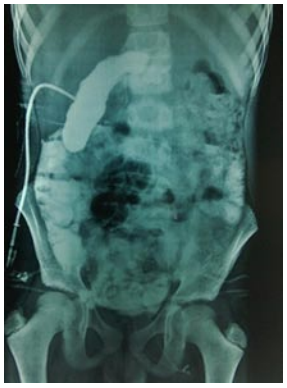

Figure 4 Tube cholecystogram.

peritoneum. HIDA scan, retrograde cholangiography and peritoneal lavage may also be used[7].

Reported complications include bile peritonitis, intrahepatic abscess formation (possible mechanisms include direct extension, subcapsular extension and hematogenous dissemination *via* the portal vein), subhepatic abscess formation, pelvic abscess formation, pneumonia, pancreatitis and acute renal failure[8].

Once diagnosed GBP mandates early intervention, and cholecystectomy with peritoneal lavage is considered sufficient. Laproscopic approach may also be used[2]. In our patient USG and CT scan showed a sealed-off

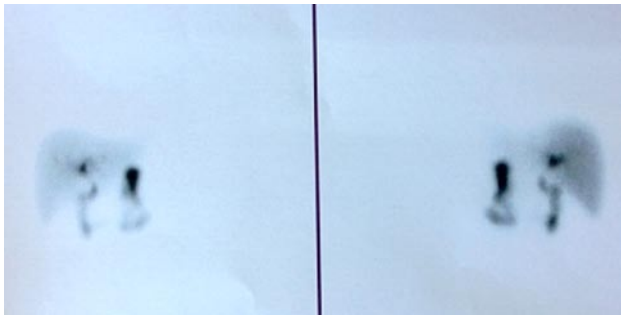

Figure 5 HIDA scan.

perforation without any free fluid in the peritoneum but a distended gallbladder which was persistent even on post prandial scans. Hence, a tube cholecystostomy was done under USG guidance by which the patient improved dramatically and a cholecystectomy was avoided. In many reports tube cholecystostomy is followed by interval cholecystectomy, but in our case we successfully avoided the procedure, thus preserving the gallbladder. Similar management in children has been reported by Mirza B[4] and Alghamdi[9]. The patient also underwent a Tc99m-HIDA scan to confirm the integrity and functionality of the biliary outflow tract, which did well at one-year follow-up.

It should also be mentioned that one must be very vigilant regarding the complications of GBP like persistent bile leak, persistent peritonitis, gallbladder necrosis, *etc.*[4] as they may warrant surgical exploration if not responding to percutaneous cholecystostomy.

In conclusion, we would like to highlight that GBP is a rare condition but demands a high level of suspicion, early diagnosis and prompt management. Using a percutaneous tube cholecystostomy in selected cases may help in avoiding cholecystectomy.

COMMENTS

Case characteristics

The patient was a 12-year-old boy who came with pain in the abdomen, fever and vomiting. He had abdominal distention and guarding in the right hypochondrium.

Clinical diagnosis
The initial suspicion was bowel perforation.

Differential diagnosis
Gallbladder perforation, even though rare, was kept as a differential diagnosis due to guarding localized to right hypochondrium.

Laboratory diagnosis
Complete hemogram, bilirubin, liver enzymes, prothrombin time and renal function tests, were within normal limits. Widal test and blood culture were negative.

Imaging diagnosis
An erect X-ray of the abdomen was unremarkable except gaseous distention of bowel loops. Ultrasonography revealed a perforated gallbladder which was confirmed by a computed tomography scan.

Treatment
As the gallbladder remained persistently over distended and the patient continued to have fever, a tube cholecystostomy was performed which led to resolution of the symptoms. Tube cholecystogram showed free flow of bile into the bowel without any leak.

Related reports
Similar technique of tiding over the acute phase of cholecystitis has been used before but is usually followed by interval cholecystectomy. In the case authors performed a HIDA scan at one month interval to confirm the functionality of the gallbladder and the billiary outflow system and found it satisfactory, thus cholecystectomy was successfully avoided.

Experiences and lessons
Gallbladder perforation is a rare but serious complication of cholecystitis and needs to be managed promptly. Percutaneous cholecystostomy is a good technique to tide over such crisis and can even be used as the definitive treatment, thus avoiding cholecystectomy altogether.

Peer-review
This is an interesting case history.

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EDITORIAL

New skin closure system facilitates wound healing after cardiovascular implantable electronic device surgery

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Abstract

The manuscript describes the efficacy of a new skin closure system (ZipLine™) for wound closure after pacemaker/implantable cardioverter defibrillator surgery. The system is particularly useful when wound healing is difficult with traditional methods and in patients at high risk for surgical site infections (SSIs). This skin closure option is easy and quick to apply and remove, and produces excellent cosmetic results. Although it is associated with a minimal expense upcharge, the benefits, including the potential for decrease in SSI, make it attractive and worth considering for skin closure in device patients, particularly those at increased risk of complications.

Key words: Cardiovascular implantable electronic device infection; Surgical wound; Skin closure system

Core tip: A new skin closure system (ZipLine™) is available for wound closure after pacemaker/implantable cardioverter defibrillator surgery. The system is particularly useful when wound healing is difficult with traditional methods and in patients at high risk for surgical site infections (SSIs). This skin closure option is easy and quick to apply and remove. The benefits, including the potential for decrease in SSI, make it attractive and worth considering for skin closure in device patients, particularly those at increased risk of complications.

De Maria E. New skin closure system facilitates wound healing after cardiovascular implantable electronic device surgery. *World J Clin Cases* 2015; 3(8): 675-677 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i8/675.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i8.675

TEXT

Cardiovascular implantable electronic device (CIED) infections are dramatically growing; estimated incidence is between 1% and 2% of all procedures, with a higher

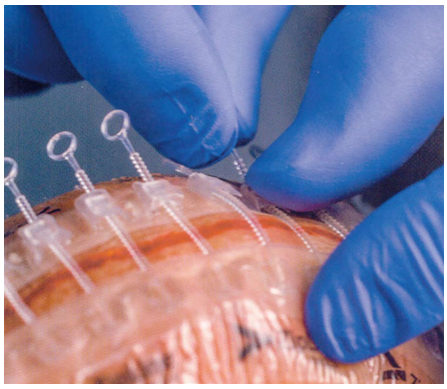

Figure 1 Example of a Zip™ device placement. Reproduced with permission from www.ziplinemedical.com MA00XX REVA.

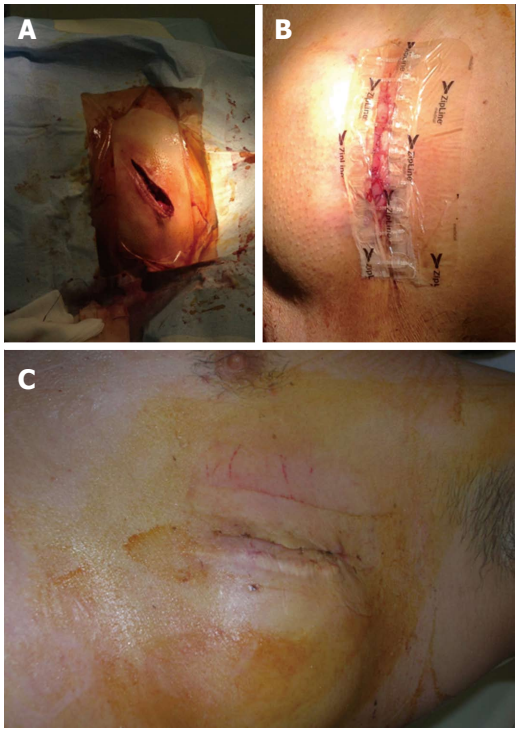

Figure 2 Zip™ applied on the wound after subcutaneous defibrillator replacement in the left anterior axillary area (A and B), wound healing after 14 d (C).

risk for implantable defibrillators and biventricular systems. Prevention is of the utmost importance and should be approached with the same strategy for surgical site infections (SSIs)[1]. The method of skin closure may be a contributing factor toward avoiding such complications; insufficient and/or delayed closure of the wound is a well recognized risk factor for subsequent infections[1]. Options for skin closure include subcuticular reabsorbable suture (braided versus monofilament), surgical "glue", and staples[2]. Closing surgical incisions with traditional sutures and staples increases the risk of SSIs compared to tape-based closure[3,4]. Sutures and staples hold the skin where they pass through it, creating high stress points that can cause ischemia, pain, irritation, edema, slow wound

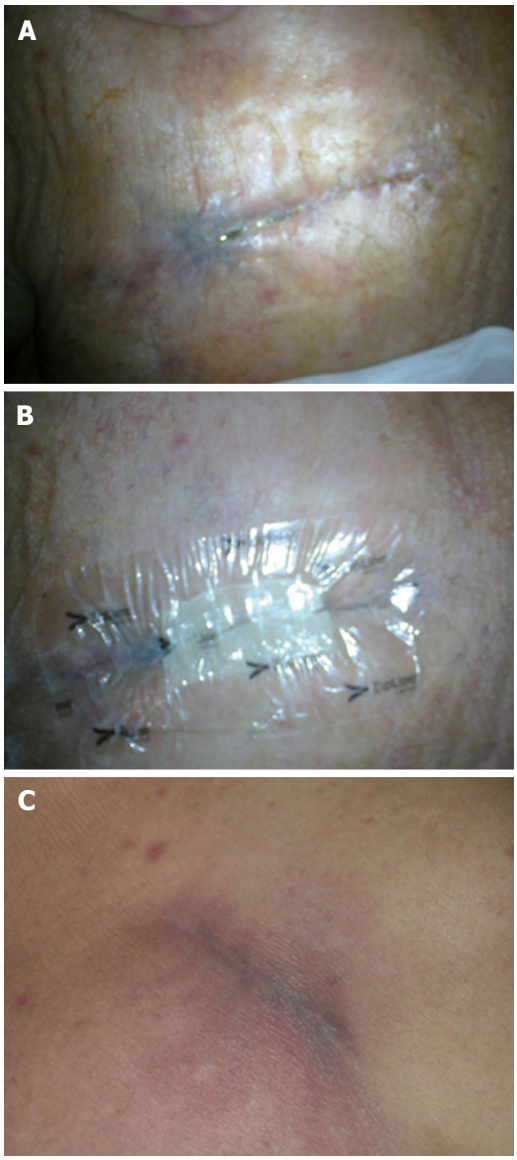

Figure 3 Zip™ applied on a wound with incomplete healing after transvenous implantable cardioverter defibrillator placement in a high risk patient (obese, diabetic, on hemodialysis) (A and B) and wound appearance after removal of the device (C).

healing and promote scar formation.

Recently a new "non invasive" device for skin closure (Zip™ Surgical Skin Closure, ZipLine Medical, Inc., Campbell, CA, United States) was approved for low tension, non-infected, surgical wounds. It is a sterile single-use system with two self-adhesive hydrocolloid pressure-sensitive strips linked with individually adjustable self-locking fasteners (Figure 1). This device increases tensile strength of a wound faster than sutures; the non-uniform distribution of closure forces associated with sutures allows immature collagen fibers to remain in a disorganized arrangement for a longer time, while in a wound closed with an adhesive tape-based closure, stress is uniformly applied to the collagen fibers which cross the wound causing rapid fiber orientation and increased tensile strength[2,3]. In addition, necrosis of tissue, needle puncture marks

and suture scarring are eliminated, leading to better cosmetic results and reduced closure time, potentially reducing SSI risk[5].

Here we present two cases of CIED surgery wounds treated with Zip™ system. In the first case the system was placed as first-line option after the replacement of a subcutaneous defibrillator [S-implantable cardioverter defibrillator (ICD)] due to battery depletion; the pocket was localized near the left anterior axillary line, a zone at risk for suboptimal wound closure (Figure 2). The second patient was an obese diabetic on hemodialysis who had an incomplete wound healing three weeks after a transvenous ICD placement (pocket in the right subclavicular area): the skin remained partially "opened" after having used all "traditional" methods for closure, so a Zip™ device was placed as a "rescue" (Figure 3). In both cases subcutaneous tissues had already been closed with monofilament suture for pocket and subcuticular layer, leaving a < 5 mm incision gap (importantly the device does not replace subcutaneous or other deep, tension-reducing sutures). After 14 d ZipLine was removed obtaining a complete wound healing without further problems in both patients, even at 6 mo follow up.

Although this system is associated with a minimal expense upcharge, the benefits (including the potential for decrease in SSI) make it attractive and worth considering for skin closure in device patients, particularly those at increased risk of complications and delayed healing of the wound: patients with heart failure, diabetes, renal insufficiency, cancer, immunodeficiencies, long-term corticosteroid use, high bleeding risk, longer and more complex procedures. Future studies will be needed to determine if and to what percentage this new device will reduce the incidence of SSI.

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EDITORIAL

Fractional flow reserve: Current applications and overview of the available data

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Abstract

Flow fractional reserve (FFR) allows to evaluate the functional significance of coronary artery lesions, through the ratio of the mean coronary artery pressure after the stenosis to the mean aortic pressure during maximum hyperemia. The actual widely accepted cut-off value is 0.80. Below this value a coronary lesion is considered significant and therefore it requires invasive revascularization. Several studies [in particular Fractional Flow Reserve *vs* Angiography for Multivessel Evaluation 1 (FAME-1) and FAME-2] have shown the relationship between FFR measurement and hard end-points (death, myocardial infarction, and urgent revascularization). Consequently, FFR evaluation represents the cornerstone in the decision-making in intermediate coronary lesions. Recent studies paved the way for further applications of FFR evaluation in complex and tricky clinical settings. In this paper, we perform an overview of the data regarding contemporary application of FFR. In particular, we review the use of FFR in: left main intermediate stenoses, serial stenoses, evaluation after stenting, guidance in coronary artery bypass surgery, and acute coronary syndrome. All the data presented in our overview confirm the essential role of FFR assessment in the daily clinical practice. The shift from "operator-dependent" to "FFR-dependent" evaluation in intermediate coronary artery stenosis is of paramount importance in order to improve the prognosis of our patients, through the discrimination of the functional role of every single coronary stenosis.

Key words: Intermediate coronary lesion; Fractional flow reserve; Coronary artery bypass surgery; Left main; Acute coronary syndrome; Serial stenoses

Core tip: Fractional flow reserve (FFR) evaluation is well validated in intermediate coronary lesions. Still, there are several clinical settings in which its use is debated. In this paper, we perform an overview on the available data regarding FFR and complex clinical settings, as left main intermediate stenoses, serial stenoses, evaluation after stenting, guidance in coronary artery bypass surgery, and acute coronary syndromes.

Tebaldi M, Campo G, Biscaglia S. Fractional flow reserve: Current applications and overview of the available data. *World J Clin Cases* 2015; 3(8): 678-681 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i8/678.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i8.678

INTRODUCTION

Fractional flow reserve (FFR) is an objective method to assess the functional significance of coronary artery lesions. FFR is defined as the ratio of maximal achievable blood flow in coronary artery to the hypothetical maximal achievable blood flow in the same artery in the absence of stenosis. It is derived from the ratio of the mean distal coronary artery pressure to the mean aortic pressure during the period of maximum hyperemia. Initial studies suggested that the cut-off value of 0.75 was reliable in the identification of ischemia-producing lesions. Afterwards, several outcome studies[1,2] validated the cut-off value of 0.80, which is actually widely accepted.

CLINICAL STUDIES

The randomized clinical trial FFR to Determine the Appropriateness of Angioplasty in Moderate Coronary Stenoses (DEFER) first evaluated the clinical benefit of FFR-guided revascularization. This study enrolled 325 patients with intermediate coronary stenosis. Patients with FFR value < 0.75 underwent percutaneous coronary intervention (PCI) (reference group, n = 144), whereas patients with an FFR value $\geqslant$ 0.75 were randomly allocated to PCI (perform group, n = 90) *vs* medical therapy (DEFER group, n = 91). At a median follow-up of 5 years, the prognosis of "non-ischemic" stenosis (FFR $\geqslant$ 0.75) was excellent even without the placement of a stent[3,4]. In the Fractional Flow Reserve *vs* Angiography for Multivessel Evaluation (FAME) study, 1005 patients with multivessel coronary artery disease were randomly assigned to PCI with drug-eluting stents guided by angiography alone or guided by FFR. Patients randomized to receive PCI, underwent stenting of all lesions; whereas those randomized to receive a functional assessment of coronary stenosis by FFR, underwent PCI only if FFR value was $\leqslant$ 0.80. The study showed a statistically significant reduction of the primary end point at 1 year (a composite of death, MI and repeat revascularization) in favor of the procedure guided by FFR (P = 0.02)[5]. In the subsequent FAME-2 trial, patients with stable coronary artery disease and at least one stenosis with FFR $\leqslant$ 0.80, were randomly allocated to medical therapy alone or to medical therapy plus PCI. The trial was stopped prematurely due to a highly significant difference in the incidence of the primary endpoint (a composite of death, myocardial infarction, and urgent revascularization) in favor of FFR-guided PCI, entirely driven by lower incidence of urgent revascularization. There were no statistically significant differences with regard to death or MI between the two groups[2,6]. In summary, FAME-2 showed that FFR-guided angioplasty reduces the incidence of urgent revascularization when compared to medical therapy.

FFR AND LEFT MAIN

In a study published in 2009, Hamilos *et al*[7] randomized 213 patients with angiographically equivocal left main coronary artery stenosis. All patients underwent FFR evaluation on left main. When the value was $\geqslant$ 0.80, patients were treated with medical therapy alone (non surgical group), while when FFR was < 0.80, coronary artery bypass grafting was performed (surgical group). The follow-up performed at 5 years did not demonstrate statistically significant differences between the two groups of patients, with regard to survival and event-free survival. Percent diameter stenosis at quantitative coronary angiography correlated significantly with FFR (r = -0.38, P < 0.001), but a very large scatter was observed. An important evidence emerging from this analysis is that 23% of lesions judged less than 50% at angiographic analysis, resulted critical after FFR evaluation. It follows that in left main intermediate lesions, angiography alone is unable to determine whether a stenosis is critical or not, tending to underestimate its functional significance. In this very important scenario, FFR plays a central role in the decision-making. In a worthy editorial by Kern[8], the author investigated a particularly complicated angiographic scenario, where stenosis in the left main (LM) is associated with a stenosis of the left anterior descending (LAD). In clinical practice, the sum of FFR across both lesions (LM + LAD) determines the need to treat, while the pressure pullback recording determines which lesion needs to be treated. In fact, the lesion with the largest pressure drop (ΔP, not FFR) is treated first. Then, FFR evaluation is repeated across the remaining lesion in order to decide whether even the second lesion needs to be treated. Such a method can be used to assess serial LM - LAD disease, but this approach engenders a downside: acceptance of stenting an unprotected LM after LAD treatment, if FFR remains < 0.80. In this particular case, both anatomical and physiopathological variables can determine a high number of errors. In order to overcome these limitations, intravascular ultrasound is frequently used to assess the minimum luminal area in the LM. Although a minimal luminal area > 6 mm^2 is an oft-quoted threshold, it represents a conservative approximation of true physiology, best indicating a lack of functional significance rather than a minimal luminal area < 6 mm^2 being an indication to treat[8,9].

FFR AND SERIAL STENOSES

Kim *et al*[10] investigated the treatment of intermediate coronary stenoses in series on the same coronary artery

using FFR pullback pressure tracings. If FFR result was < 0.80, the stenosis with the largest pressure step-up was treated with stenting first. Subsequently, the patients were divided into two groups (FFR < 0.8 *vs* FFR > 0.8), according to FFR value after PCI. There were no events related to deferral of lesion treatment. The evaluation by conventional FFR of a single coronary artery stenosis in a vessel where there are several intermediate lesions, is underestimated ("apparent FFR"). Indeed, in this scenario, the assessment with FFR tends to ignore the real contribution of each stenosis to the ischemic burden. The real value of the FFR ("true FFR") can be calculated only after the treatment of the lesion which gave the largest pressure step-up using FFR pullback pressure tracings or by using a more complex method described by De Bruyne *et al*[11] which also considers the coronary wedge pressure[11,12]. In conclusion, FFR-guided revascularization strategy using pullback pressure tracing in serial stenoses is safe and effective[10].

FFR AFTER STENT

In a study published in 2011, Nam *et al*[13] assessed the impact of FFR value after stent on MACE (myocardial infarction, death, ischemia-driven target vessel revascularization) at one year. Patients were divided into two groups according to the value of FFR detected after PCI (FFR < 90: low-FFR group; FFR > 90: high-FFR group). The study showed a statistically significant MACE reduction in the high-FFR group; for that reason the 0.90 value it is considered the cut-off reference to obtain after PCI.

FFR AND CORONARY ARTERY BYPASS SURGERY

Myocardial revascularization is recommended when a large territory of reversible myocardial ischemia is present. Of note, patients undergoing cardiac surgery usually have a very complex coronary anatomy. In such situation, non-invasive functional testing has shown many limitations. On the contrary, as described previously, FFR is safe and accurate even in patients with multivessel disease with multiple stenosis. The usefulness of FFR in patients undergoing coronary artery bypass surgery was confirmed by the results of a registry by Toth *et al*[14]. In this registry, authors evaluated patients with at least one intermediate coronary stenosis. These patients were divided into two groups: the angiography-guided group, in which patients underwent coronary artery bypass graft surgery (CABG) solely on the basis of coronary angiography; and the FFR-guided group, in which patients underwent CABG if FFR was ⩽ 0.80, and were treated conservatively if FFR was > 0.80. The registry showed that FFR-guided coronary artery bypass graft surgery was associated with a lower number of graft anastomoses, a lower rate of on-pump surgery and a lower rate of angina in the absence of an increase of events during follow-up[14].

FFR AND ACUTE CORONARY SYNDROME

During the acute phase of acute coronary syndrome (ACS), we deal with a non-permanent microvascular dysfunction, which both changes during hours and is affected by multiple factors (embolization, changes in filling pressures, duration and intensity of ischaemia, changes in systemic or local vasoconstrictors, *etc.*)[15]. The microvascular dysfunction is a key aspect for the evaluation with FFR: a reduced ability to respond to vasodilation through adenosine by the microcirculation, may cause an underestimation of coronary stenosis. At present, the main study evaluating the relationship between FFR and outcome in ACS patients is the FAMOUS-NSTEMI trial[16]. In this trial, 350 patients with no ST-segment elevation MI were enrolled. The evaluation using FFR resulted in a reduction of patients undergoing PCI without increase of events at 1 year follow-up.

CONCLUSION

All available studies suggest that FFR assessment should be considered an essential tool in daily clinical practice. Therefore, the operator-dependent evaluation of intermediate stenosis should now represent just an old memory in the history of interventional cardiology. FFR value permits to better discriminate the functional role of intermediate stenosis significantly improving the prognosis of our patients.

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REVIEW

Depressive symptoms in neurodegenerative diseases

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Abstract

Depressive symptoms are very common in chronic conditions. This is true so for neurodegenerative diseases. A number of patients with cognitive decline and dementia due to Alzheimer's disease and related conditions like Parkinson's disease, Lewy body disease, vascular dementia, frontotemporal degeneration amongst other entities, experience depressive symptoms in greater or lesser grade at some point during the course of the illness. Depressive symptoms have a particular significance in neurological disorders, specially in neurodegenerative diseases, because brain, mind, behavior and mood relationship. A number of patients may develop depressive symptoms in early stages of the neurologic disease, occurring without clear presence of cognitive decline with only mild cognitive deterioration. Classically, depression constitutes a reliable diagnostic challenge in this setting. However, actually we can recognize and evaluate depressive, cognitive or motor symptoms of neurodegenerative disease in order to establish their clinical significance and to plan some therapeutic strategies. Depressive symptoms can appear also lately, when the neurodegenerative disease is fully developed. The presence of depression and other neuropsychiatric symptoms have a negative impact on the quality-of-life of patients and caregivers. Besides, patients with depressive symptoms also tend to further decrease function and reduce cognitive abilities and also uses to present more affected clinical status, compared with patients without depression. Depressive symptoms are treatable. Early detection of depressive symptoms is very important in patients with neurodegenerative disorders, in order to initiate the most adequate treatment. We review in this paper the main neurodegenerative diseases, focusing in depressive symptoms of each other entities and current recommendations of management and treatment.

Key words: Neurodegenerative diseases; Alzheimer; Depressive symptoms; Frontotemporal degeneration; Vascular dementia; Lewy body disease; Depression; Dementia

Core tip: Neurodegenerative diseases commonly associate depressive symptoms. Depressive symptoms of neurodegeneration occur both in the beginning and in the main course of neurodegenerative diseases. They can dominate the clinical picture mostly in the first stage of disease. Besides, depressive symptoms decrease quality of life of patient and relatives in every

stage of disease. This is certainly an usual condition in Alzheimer's disease, by far the main cause of dementia worldwide. Such a situation often happens in neurodegenerative diseases. Depressive symptoms are treatable and its treatment can improve perceived health status and welfare of patients and relatives.

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INTRODUCTION

Depressive symptoms are very common in general medical practice and its frequency is remarkable in neurological diseases. Really, depressive symptoms are usual in chronic diseases; every kind of chronic or limiting condition is frequently associated with mood disorders[1]. However, from the very first glance it is apparent that the association between mood disorders and brain disorders is clearly more complex than the association between depressive symptoms and other group of diseases. The mean reason of this complexity is simple: the diseases of the brain have the potential to modify the mood of the affected person as brain is the ultimate controller of the behavior.

In the present paper, we review the relation between depressive symptoms and neurodegenerative disorders from a clinical point of view, focusing on the depressive symptoms described in main neurodegenerative diseases. Specially in neurodegenerative disease, depression may appear as an early symptom and depression may be the main manifestation, more often but no only in the early stages of degenerative brain processes. These depressive symptoms are relevant in medical practice as they can be the more important demand noted by patient or caregivers. Besides, they have an impact on the quality-of-life of patients and have been associated with increased caregiver burden, more rapid progression of disability and functional decline and earlier institutionalization and mortality[2]. However, although the importance of depressive symptoms, they have little or no interest regarding on diagnosis of neurological diseases; so, the features that clearly define the neurodegenerative disease are cognitive or motor symptoms, and not mood disturbances.

DEPRESSION, DEPRESSIVE SYMPTOMS AND COGNITION

Following the DSM-V classification[3] (Table 1) depression is defined as a mood disorder which expresses itself as a combination of symptoms with predominance of affective ones (sadness, desperation, apathy, anhedonia and subjective sensation of discomfort), also associating cognitive and physic phenomenology, causing a marked decreased interest in daily life activities.

In presence of any neurodegenerative disease, the depression diagnosis may be difficult. Frequently, depressive symptoms are masked by cognitive decline. Often cognitive symptoms and mood disorders mix in such a way that it's difficult to determine what group of symptoms are the most relevant to the patient. Neurological patients have difficulty to express typical feelings of sadness and hopelessness. Instead of sadness, prominent symptoms in neurodegenerative diseases may be anhedonia, anxiety, panic, motor disturbances and also lack of concentration. Lack of concentration or indecisiveness is a symptom that can be characteristic of cognitive decline caused by neurodegenerative diseases[4,5] but its specificity is not elevated. Weight loss and sleep disorders, often valuable symptoms of depression, can appear in neurological diseases with or without any associated mood disturbance. On the other hand, patients with neurodegenerative diseases use to manifest apathy[6]. This mood symptom is easily mistaken as anhedonia, that marked decrease in interest or pleasure with different activities to be considered as a main symptom of depression. Also, some particular neurological symptoms complicate the diagnosis because different reasons; as an example, the existence of a language disorder provokes difficulty of patient to express feelings. Another condition like pseudobulbar palsy may be misdiagnosed of depression as result of misunderstanding the significance of pathological crying or emotional lability.

From the clinical point of view, often depression and dementia are combined and their clinical phenomenology can be coincident and considered as strongly linked. From the epidemiological point of view, late-onset depression itself may be considered a risk factor or an early symptom of develop dementia[7]. So, certainly, this risk factor relation as explanation about the epidemiologic link between late-onset depression and dementia is not the only possibility, and other ones will be mentioned soon. It has been proposed that neurodegenerative disease may express as depressive symptoms in the early stages. That explanation is supported amongst other data by neuropathological evidences[8]. Thus, there would be common neuropathological hallmarks found in cognitive impairment that also are associated to depressive symptoms. So, depressive symptoms may be an early manifestation of diseases that later will cause dementia, not really a "risk factor" for dementia. On the other hand, psychopathology experts have argued that the depressive symptoms may be a consequence of self-perception of cognitive deterioration by patient[9].

From the opposite point of view, patients with depression commonly present cognitive disturbances. Cognitive disturbances, specially attention, short term memory, psychomotor speed, and executive function are often reported by depressive patients[10,11]. In fact, it has been observed that functional impairment in depression is closely related to severity of depression and cognitive

Table 1 Criteria for major depressive episode: DSM 5

Five (or more) of the following symptoms have been present during the same 2-wk period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood; or (2) loss of interest or pleasure
Depressed mood most of the day, nearly every day, as indicated by either subjective report (*e.g.*, feels sad or empty) or observation made by others (*e.g.*, appears tearful)
Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation made by others)
Significant weight loss when not dieting or weight gain (*e.g.*, a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day
Insomnia or hypersomnia nearly every day
Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)
Fatigue or loss of energy nearly every day
Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide
The symptoms cause clinically significant distress or impairment in social, occupational or other important areas of functioning
The symptoms are not due to the direct physiological effects of a substance (*e.g.*, a drug of abuse, a medication) or a general medical condition (*e.g.*, hypothyroidism)

disturbance[12]. Interestingly, attentional deficits are part of the current diagnostic criteria of major depression and are commonly found in clinical practice[13]. In addition, several studies have shown the improvement of cognitive functions in patients with major depression treated with selective serotonin reuptake inhibitors[14] (SSRI) or dual serotonergic-noradrenergic reuptake inhibitors.

However, cognitive impairment in depression may produce some added difficulties. On one hand assessment of cognitive impairment may be difficult because of severity of depression. On the other hand, cognitive impairment can remain after antidepressant treatment despite of remission of depressive symptoms[15-17]. Thus, it may be recommended to continue the pharmacological and non-pharmacological treatment in presence of cognitive deficits[18], even though neurotransmission and other biological pathways and mechanisms involved in the association of cognitive deficits and major depression remain not clearly understood.

Several risk factors to develop dementia after a depressive episode have been described: mainly, high cultural level, depression severity and failure of treatment with antidepressive drugs[19]. The role of other risk factors, such as stress, depression severity and/or treatment with psychotropic drugs itself continues unclear[20].

So, it is essential to distinguish between depressive symptoms that can be the very first symptom of a neurodegenerative process and those ones what are not linked to this group of brain diseases.

DEPRESSIVE SYMPTOMS IN THE DIFFERENT NEURODEGENERATIVES DISEASES

Alzheimer's disease

Alzheimer's disease is the paradigmatic dementia's cause. As it is, provokes progressive memory and other cognitive functions impairment and causes marked decline in activities of daily living and variable behavioral changes. Neuropathologically it is characterized by neuronal loss with associated accumulation of neurofibrillary tangles and amyloid plaques. Currently, Alzheimer's disease is the most frequent cause of dementia all over the world as a whole and in most, if not all, population subgroups.

Most of the patients suffering from Alzheimer's associate behavioral and psychological symptoms, so called "non cognitive" symptoms, at some point of the evolution of the disease[21,22]. The prevalence of these symptoms is found to oscillate between 60% to 90% of cases, depending on both defined population and methodology of the study[23-26]. These neuropsychiatric symptoms are not included within the diagnostic criteria; in contrast, they contribute to develop a great disability and mortality and represent the main reason for patient institutionalization[27].

Early detection of neuropsychiatric symptoms is very important because they are the main cause of caregiver burden and also they cause acceleration of cognitive decline. In fact, when this symptomatology is observed and correctly identified, it may be treated with pharmacological and non-pharmacological treatment with improvement of the quality of life of patients and caregivers[28]. When these neuropsychiatric symptoms are identified, they can be prevented to recur too. Frequently, neuropsychiatric symptoms may fluctuate during the course of the disease and they disappear when cognition is severely impaired[29,30]. Depressive symptoms are included within this category of neuropsychiatric symptoms and are specially common in early stages of disease when lack of concentration and inattention are commonly found[31].

Depressive symptoms are usual in Alzheimer's disease patients according to different studies[32]. Based on descriptive population studies, about 80% of Alzheimer's patients can develop depressive symptoms to a greater or lesser degree in the whole course of the disease[33]. Depressive symptoms may vary and disappear, in contrast to cognitive symptoms that remain steady and invariably progress with the course of disease. In most cases, depression may be less intense than the depression found in neurologically healthy people or depression in another subtypes of brain diseases like cognitive impairment due to brain vascular disease, so

Table 2 The cornell scale for depression in dementia

Mood-related signs
Anxiety: Anxious expression, rumination, worrying
Sadness: Sad expression, sad voice, tearfulness
Lack of reaction to present events
Irritability: Annoyed, short tempered
Behavioral disturbance
Agitation: Restlessness, hand writing, hair pulling
Retardation: Slow movements, slow speech, slow reactions
Multiple physical complaints (score 0 if gastrointestinal symptoms only)
Loss of interest: Less involved in usual activities (score only if change occurred acutely, *i.e.*, in less than one months)
Physical signs
Appetite loss: Eating less than usual
Weight loss: (score 2 if greater than 5 pounds in one month)
Lack of energy: Fatigues easily, unable to sustain activities
Cyclic function
Diurnal variation of mood: Symptoms worse in the morning
Difficulty falling asleep: Later than usual for this individual
Multiple awakening during sleep
Early morning awakening: Earlier than usual for this individual
Ideational disturbance
Suicidal: Feels like is not worthy living
Poor self-steem: Self-blame, self-depreciation, feelings of failure
Pessimism: Anticipation of the worst
Mood congruent delusions: Delusions of poverty, illness or loss
Scoring system
A= Unable to evaluate; 0 = Absent; 1 = Mild to intermittent; 2 = Severe score greater than; 12 = Probable depression

Table 3 Provisional diagnostic criteria for depression in Alzheimer's disease

Three or more of the following criteria over the same 2-wk period, representing a change from previous functioning:
Depressed mood (sad, hopeless, discouraged, tearful)
Decreased positive affect or pleasure in response to social contacts and activities
Social isolation or withdrawal
Disruption in appetite
Disruption in sleep
Psychomotor agitation or retardation
Irritability
Fatigue or loss of energy
Worthlessness, hopelessness or excessive guilt
Recurrent thoughts of death or suicidal ideation
All criteria are met for dementia of the Alzheimer's type
Symptoms cause distress or disruption in functioning
Symptoms do not occur exclusively during delirium
Symptoms are not due to substances (medications or drugs of abuse)

called, when intense, vascular dementia[34,35].

The recognition of depression in Alzheimer's patients may be a challenge for different reasons: first of all, the absence of a validated questionnaire to detect and quantify the disorder. Second, dementia symptoms themselves like apathy can be confounded with typical features of depression such as sadness or anhedonia, masking the depressive disorder. Finally, the cognitive impairment of these patients supposes difficulties in the expression of sadness, hopelessness and other common affective feelings.

Numerous instruments have been proposed for assessing mood disorders and other neuropsychiatric symptoms in patients suffering from dementia. In 1994 the group of Cummings published the Neuropsychiatric Inventory[36] (NPI). The NPI has been used to characterize neuropsychiatric symptoms in several neurological diseases and is currently the most used scale for this purpose. NPI largely correlates with increasing disability in activities of daily living and increasing cognitive impairment. It has shown to be able to demonstrate the improvement on behavioral symptoms in Alzheimer's disease and other dementias after appropriate treatment[37]. In the initial version of the NPI, this scale evalued ten neuropsychiatric symptoms[38]: delusions, hallucinations, dysphoria, anxiety, agitation, euphoria, apathy, irritability, disinhibition and aberrant motor behaviour. Later, two more items, sleep and eating disorders, were added.

NPI is passed as a structured interview driven by the professional and answered by the caregiver, focusing on the presence or absence of neuropsychiatric symptoms and their intensity. A form to be self-administered by the caregiver[39] (NPI-Q) and another one to be used in nurse home settings[40] (NPI-NH) have been developed later. Different translations of the NPI in its distinct forms are validated in a great number of languages[41-43].

Another more specific instruments to describe and quantify mood disorders in patients with dementia also has been developed: The Dementia Mood Assessment Scale[44] and the Cornell Scale for Depression in Dementia (CSDD)[45]. Particularly, the CSDD is widely used and it allows to differentiate between cognitive and mood symptoms (Table 2). It also may be useful to measure response to treatment and it's commonly used in clinical trials on this purpose.

Finally, specific provisional diagnostic criteria for depression in Alzheimer's disease (PDC-dAD) were proposed in 2002[46] (Table 3). PDC-dAD have shown to provide higher prevalence rates of depression than generic diagnostic criteria[47] such as ICD-10, CAMDEX or DSM-IV. The PDC-dAD are similar to standard depression diagnosis but reduces the importance on verbal expression and in contrast includes irritability and social isolation. Patients must have a diagnosis of Alzheimer's disease and three or more listed symptoms during two weeks. The symptoms must include low mood or decreased pleasure in daily living activities.

Together with depression, apathy is the most common symptom in Alzheimer's disease[48]. Both depression and apathy have a negative impact on evolution of the disease. Frequently, apathy is difficult to separate from depression. In fact, it's often a symptom observed in depression[49]. However, apathy can exist isolated without depression and it is not rare to find isolated apathy. Some paper have addressed the situation that both apathy and depression occur simultaneously in Alzheimer's disease and when both apathy and depression occur it has been shown that they are clinically and anatomically independent[50,51]. In fact, several neurophysiological studies focus on prevalence and clinical features of apathy have been

able to characterize this symptom and formulate some differences in relation to depression[52-54].

Certainly, the significance of depression or the significance of apathy in patients with neurodegenerative disease are different. First, apathy increases the risk of being diagnosed of dementia in patients with mild cognitive impairment and apathy do it more frequently that isolated depression in mild cognitive impairment[55]. Also, apathy tends to be more prevalent as cognitive function declines, in contrast to prevalence of depression that it's reduced in advanced stages of dementia[56]. On the other hand, apathy do not respond to antidepressive treatment, actually, antidepressive treatment have been reported even to increase the intensity of apathy in some cases[57].

As stated, depressive symptoms are important in patients with mild cognitive impairment. Mild cognitive impairment is characterized by cognitive symptoms and demonstrated impairment in neuropsychological testing but no significant functional decline, so patients with MCI do not fulfill dementia diagnostic criteria[58]. Its most common etiology is Alzheimer's disease and constitutes a high risk group to develop dementia at an annual rate of 10% to 15%[59-61].

Behavioral abnormalities are reported in 35%-75% of mild cognitive impairment patients[62]. As in Alzheimer's disease, neuropsychiatric symptoms in mild cognitive impairment are associated with cognitive decline and disability[63]. The most common behavioral symptoms are apathy, anxiety, depression, irritability and agitation[64,65]. Less common symptoms are euphoria, hallucinations, disinhibition and aberrant motor behavior. As previously commented, coexistence of depression and apathy or the presence of isolated apathy have shown to increase the risk of later conversion to Alzheimer's disease[66].

Depressive symptoms have been described up to 30% of the patients with mild cognitive impairment and in most studies depression was the most common neuropsychiatric symptom followed by apathy and irritability[67-69].

Frontotemporal degeneration

Frontotemporal degeneration or frontotemporal dementia (FTD) is clinically characterized by progressive behavioural changes such as disinhibition, compulsion, hyperorality or dietary changes. Patients also show social interpersonal dysfunction. Involvement of memory and other cognitive functions[70,71] is later than behavioural alterations. All these symptoms are due to degeneration of frontal and temporal lobes.

Nowadays, it's commonly accepted that frontotemporal degeneration is expressed with any of three main clinical variants: the more common behavioural variant FTD that have been forementioned and the language variants semantic dementia and progressive non-fluent aphasia[72]. There is also an overlap of FTD with motor neuron disease (FTD-MND or FTD-ALS), as well as another overlap exists with the parkinsonian syndromes progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS)[73]. Characteristicly, a certain grade of parkinsonism tends to be present in all syndromes.

Depression is quite common in FTD (40% of cases in the study by Levy *et al*[74]), although generally with mild or moderate intensity. When depressive symptoms are present, they usually do not manifest as typical features of major depression. Indeed, patients experience mainly apathy and decreased energy, hyperphagia and inappropriately preserved self-esteem, feature that it's extremely uncommon in usual depression.

The diagnostic challenge of FTD is the predominance of clashing behavioral symptoms. Consequently, it's difficult to make an adequate diagnose in initial stages of the process. Often patients may be misdiagnosed with psychiatric disorders conditioning a delay in the diagnosis of neurodegenerative disease. Psychopharmacological treatment with antipsychotics can cause more prominent motor symptoms and thus another confounding factor may contribute to misdiagnosis and failure to provide appropriate treatment.

Lewy body disease

Lewy body disease is another of the most frequent primary causes of degenerative dementia behind Alzheimer's disease. Both Lewy body disease and Parkinson's disease with or without dementia have been proposed to constitute a group of disorders called α-synucleinopathies. This proposal takes the fact that both entities' neuropathological handmark is the presence of Lewy bodies in different regions of the brain, mainly limbic, paralimbic and neocortical regions, and Lewy bodies are constituted mainly by the protein α-synuclein. Lewy body disease is clinically expressed with the presence of dementia associated with visual hallucinations, parkinsonism and a remarkable fluctuation of symptoms. Severe neuroleptic sensitivity is also typical of this disease[75]. Although these evident clinical features would seem to easily distinguish Lewy body dementia from Alzheimer's disease, in the common practice such a distinction is difficult to be made, specially in the early stages. The presence of visual hallucinations becomes relevant in differential diagnosis to Alzheimer's disease[76,77].

Depression in Dementia with Lewy bodies is similar to depression in Alzheimer's disease. Several studies have found higher rate of depression specially in early stages. Also depressive symptoms seems to be more severe[78]. First guidelines for diagnosis of Body Lewy's disease were described in 1996 and laterly in 1999. Current reviewed diagnostic criteria since 2005[75] includes depression as supportive feature of the disease (Table 4).

Corticobasal degeneration

Corticobasal degeneration (CBD) is histopathologically characterized by focal cortical neuronal loss and gliosis. It has been included into the spectrum of frontotemporal lobar degeneration as well as PSP and Pick's disease. All of these entities are biologically included into the group of tauopathies because tau protein is the main

Table 4 Diagnostic criteria for Lewy bodies disease

Central feature
Progressive dementia-deficits in attention and executive function are typical
Prominent memory impairment may not be evident in the early stages
Core features
Fluctuating cognition with pronounced variations in attention and alertness
Recurrent complex visual hallucinations
Spontaneous features of parkinsonism
Suggestive features
REM sleep behavior disorder which can appear years before the onset of dementia and parkinsonism
Severe intensity to neuroleptics occurs in up to 50% of LBD patients who take them
Low dopamine transporter uptake in the brain's basal ganglia as seen on SPECT an PET imaging scans
Supportive features
Repeated falls and syncope (fainting)
Transient, unexplained loss of consciousness
Autonomic dysfunction
Hallucinations of other modalities
Visuospatial abnormalities like depth perception, object orientation, directional sense and illusions
Other psychiatric disturbances like systematized delusions, aggression and depression
A probable LBD diagnosis require either
Dementia plus two or more core features, or
Dementia plus one core features and one or more suggestive features

LBD: Lewy body dementia; PET: Positron emission tomography; SPECT: Single photon emission computed tomography.

component of different microscopic alterations to be found in these diseases. CBD presents in a sporadic pattern without familial aggregation. The common clinical presentation of CBD is the CBS associated to progressive asymmetric rigidity, limb apraxia, alien limb phenomenon, cortical sensory loss, myoclonus and dystonia. However, neither CBS patients have always CBD neuropathology when their brain is studied, nor corticobasal histopathology itself produces always CBS.

In fact, CBS is associated commonly with Alzheimer's disease histopathology. On the other hand, corticobasal histopathology has been associated to different clinical presentations like PSP, FTD or nonfluent/agrammatic primary progressive aphasia.

Depression is common in CBD and it has been described in up to 70% of these patients[79]. Conversely to the findings of similar studies in other neurodegenerative disease, Litvan *et al*[80] found in CBD patients a high prevalence of depression (73%) superior to the prevalence of apathy (40%). As it occurs specially in patients with FTD and another neurodegenerative diseases with prominent neuropsychiatric symptoms, the clinical predominance of depressive symptoms may explain that this entity can be misdiagnosed as a primary psychiatric disorder[81].

Huntington's disease

Huntington's disease is a highly penetrant autosomal dominant disease caused by a mutant protein - huntingtin - that results from an expanded CAG repetition. The progressive neurodegenerative disorder caused by Huntington's disease typically includes chorea and dystonia, incoordination, cognitive decline, and behavioural disturbances.

Classical and recent studies have shown that apathy, aggression and disinhibition are common. Suicide rates in Huntington's disease patients are over four times those of the general population[82,83]. Depression is diagnosed up to 40% of cases[84,85].

Parkinson's disease

Parkinson's disease manifests mainly with motor disturbances. Typically it causes asymmetric bradykinesia, resting tremor, rigidity and in later stages postural instability. Pathologically is characterized by depigmentation of substantia nigra due to loss of melanin-laden dopaminergic neurons containing eosinophilic cytoplasmic inclusions called Lewy bodies and mainly composed of α-synuclein, as previously mentioned.

Apart from motor symptoms that constitute the main clinical features, a wide range of nonmotor symptoms exists since early stages of the disease. These nonmotor symptoms are olfactive disturbances, depression, dementia, sleep disorders, fatigue, apathy and autonomic symptoms. Such a symptoms and other ones like dementia, a late complication of typical Parkinson's disease, are recognized as a major cause of disability and decline of quality of life in patients suffering from Parkinson's disease, especially in the more advanced stages[86]. Characteristically, depressive symptoms may experience fluctuation in the same way as motor symptoms, being often severe in off-periods[87,88]. They may appear in all stages of Parkinson's disease, and also precede motor symptoms[89]. Although sometimes is difficult to identify depressive symptoms in this patients, several risk factors have been described for developing depression: severity of cognitive impairment, female sex, onset of parkinsonian symptoms before age 40 and history of depression prior to diagnosis of Parkinson's disease[90].

Prevalence of depressive symptomatology varies from 20% to 50% in Parkinson's disease. Depressive symptoms are frequently associated with greater disability, rapid progression of motor symptoms and cognitive impairment[91-93]. In fact, depression is the main negative factor that impacts quality of life in Parkinson's disease and it may precede motor symptoms for years[94].

Depression in Parkinson's disease is different in some aspects from major depression: on one hand, guilty or worthlessness and suicidal ideation are not common[95]. Furthermore, only a small percentage of patients have major depression (2%-7%) and most of cases experience minor depression or mild depressive symptoms.

However, despite of frequency and importance of depression in Parkinson's disease, there are not any defined diagnostic criteria for depressive disorder in Parkinson's disease. The current gold standard

for establishing the diagnosis of depression in these patients remains the DSM criteria[96,97].

PSP

PSP is a rare neurodegenerative disorder clinically characterized by symmetrical parkinsonism, postural instability and falls, slowing of vertical saccades and frontal lobe symptoms. It's no so rare degeneration. Although classically grouped into the so-called Parkinson-plus syndromes, nowadays PSP is considered into the Frontotemporal Degeneration Complex. Histopathologically PSP presents cellular inclusions composed by aggregated tau protein that accumulate in prefrontal cortex, globus pallidus, substantia nigra and subthalamic nucleus.

Behavioral abnormalities are often observed in PSP patients, more than half experiencing apathy, depression, and sleeping problems[98]. In fact, the most common feature of mood disorder is apathy, found in more than 90% of PSP patients[99].

Vascular dementia

Cerebrovascular disease is the second most common cause of acquired cognitive impairment. Vascular cognitive impairment and vascular dementia are within the spectrum of cognitive impairment occurring as a result of cerebrovascular disease. The current definition of vascular dementia includes the hereditary vascular dementias, multi-infarct dementia, post-stroke dementia, subcortical ischemic vascular disease and atherosclerotic dementia dementia[100].

Referring to behavioural and psychological symptoms of vascular cognitive impairment, depression and apathy are the commonest symptoms found in most of studies[101-103]. Emotional lability is frequently reported as a classic feature of pseudobulbar palsy[104].

In comparison to Alzheimer's disease, prevalence of depressive symptoms in several studies has shown different results. Some studies showed higher prevalence and severity of depression in vascular dementia[105-107], but other publications has not been found significant differences in the prevalence of neuropsychiatric symptoms between Alzheimer's disease and vascular dementia patients[108-111].

In relation to the affected lobe, patients with posterior circulation lesions have shown a significantly lower rate of depression than patients with middle cerebral artery lesions. Moreover, depression following posterior circulation infarcts was of significantly shorter duration than depression following carotid strokes. Patients develop more severe depressive symptoms according to severity of stroke[112]. In addition, depression may be commoner in subcortical strokes[113] (lacunar state).

THERAPEUTIC APPROACH AND RECOMMENDATIONS

A wide variety of treatments have been used to improve neuropsychiatric symptoms in neurologic diseases including antipsychotics, antidepressive drugs or anti-convulsant ones. Non-pharmacological interventions like supportive psychotherapy or psychological counseling are recommended either complimentary or alternative to drug treatment.

Regarding primary dementias, cholinesterase inhibitors have shown a mild but consistent effect on behavioral symptoms in Alzheimer's disease[114]. They reduce behavioral changes and delay cognitive and functional decline and should be initiated earlier than others pharmacological treatments.

Neuropsychiatric symptoms like apathy, depression, and aberrant motor behavior are the most likely to improve[115,116]. Memantine tends to improve specially agitation and irritability more than mood symptoms, apathy, and aberrant motor behavior. Combination therapy with cholinesterase inhibitors may have advantages in patients with multiple neuropsychiatric symptoms[117]. Current evidence also recommends to use cholinesterase inhibitors in patients with Parkinson's disease with a positive effect on cognitive function and behavioral disturbances. The effect on body Lewy disease remains unclear but usually it's similar at the effect on Alzheimer's disease. Neither cholinesterase inhibitors nor memantine have shown effectiveness in frontotemporal dementias.

Besides cholinesterase inhibitors and memantine, other psychopharmacological treatments should be used individually considering the presence of comorbidities and associated medications. In most of patients, useful treatments are SSRIS, specially sertraline and citalopram[118-123]. Paroxetine has been proposed specifically to fontotemporal dementia[124,125] and Parkinson's disease[126]. However, paroxetine has been associated to further impairment of motor symptoms in some patients[127].

In case of Parkinson's disease patients, is important to determine if depressive symptoms appear in off-periods. In this case, adjustment of antiparkinsonian medication usually allows to obtain an improvement of depression.

Generally, classical drugs as tricyclic antidepressants are poorly tolerated; worsening of mental status is a common secondary effect of its use in this group of patients. By its anticholinergic effect, tricyclic antidepressants tend to worse cognition and also generate orthostatic hypotension, specially in patients with advanced disease[128]. Fluoxetine and fluvoxamine are less used because of potential interactions. Other antidepressive drugs, such as mirtazapine, trazodone, duloxetine and venlafaxine may be used but possibly their use may be restricted to cases of very limited or no response to initial treatment with SSRI[117]. Atypical antipsychotics should be used with extreme caution: their side effects are frequent in dementia patients and easily overcome the possible therapeutic effect. An increase of death rates have been found with the use

of every antipsychotic drug, more marked with typical ones but also present with atypical ones; so these drugs are commonly restricted to old age patients by administrative normatives in most of countries.

The commonly proposed therapeutic strategy to establish the effective dosage of antidepressive and antipsychotic drugs is that of "start low and go slow". That means to start with low doses and progressively increase dosage with caution to minimize side effects. Clinical evolution should be closely observed and clinician must evaluate the possibility of modifying or stopping the pharmacological treatment according to the intensity of symptoms and the potential harm of drug treatment.

Specially in elderly, depressive symptoms may be first manifestation of a neurodegenerative disease. Although clinical patterns of neurodegenerative diseases are different, the presence of depression may be insufficient to distinguish between them. Thus, in most cases it is required to observe closely the clinical evolution to lead the accurate diagnosis. Specific complementary test like neuroimaging or lumbar punction may be useful and can provide data to ascertain a correct diagnosis.

In conclusion, neuropsychiatric symptoms and specially depression are frequent in dementia stages and in most of neurodegenerative diseases. Depressive symptoms contribute significantly to increase disability, morbidity, caregiver burden and illness costs. To limit their extent, adequate identification and evaluation of these symptoms is essential to initiate appropriate early non-pharmacological and pharmacological treatment.

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REVIEW

Targeting chronic lymphocytic leukemia cells in the tumor microenviroment: A review of the *in vitro* and clinical trials to date

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Abstract

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the western world. Despite significant advances in therapy over the last decade CLL remains incurable. Current front-line therapy often consists of chemoimmunotherapy-based regimens, most commonly the fludarabine, cyclophosphamide plus rituximab combination, but rates of relapse and refractory disease are high among these patients. Several key signaling pathways are now known to mediate the survival and proliferation of CLL cells *in vivo*, the most notable of which are the pathways mediated by the B-cell receptor (BCR) and cytokine receptors. A better understanding of the pathogenesis of the disease, the underlying biology of the CLL-cell and the roles of the tumour microenvironment has provided the rationale for trials of a range of novel, more targeted therapeutic agents. In particular, clinical trials of ibrutinib and idelalisib, which target the Brutons tyrosine kinase and the delta isoform of phosphoinositol-3 kinase components of the BCR signaling pathway respectively, have shown extremely promising results. Here we review the current literature on the key signaling pathways and interactions of CLL cells that mediate the survival and proliferation of the leukemic cells. For each we describe the results of the recent clinical trials and *in vitro* studies of novel therapeutic agents.

Key words: Chronic lymphocytic leukemia; Therapy; Microenvironment; Leukemia; Novel

Core tip: The treatment of chronic lymphocytic leukemia (CLL) is in a period of unprecedented revolution. A better understanding of the mechanisms that drive the survival and proliferation of CLL cells has led to the development of novel therapeutic strategies. This review article is a timely summary of the results of many of the recent key clinical and pre-clinical studies of novel therapeutic agents for CLL.

Crassini K, Mulligan SP, Best OG. Targeting chronic lymphocytic leukemia cells in the tumor microenviroment: A review of the *in vitro* and clinical trials to date. *World J Clin Cases* 2015; 3(8): 694-704 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i8/694.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i8.694

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is characterized by the proliferation and accumulation of CD5/CD19 positive monoclonal B-lymphocytes in the peripheral blood, bone marrow and lymphoid organs. The introduction of the fludarabine (F), cyclophosphamide (C), rituximab (R) regimen[1,2] represented a major advance in the clinical management of the disease with durable remissions beyond 10 years being achievable in a proportion of patients[3]. However, novel treatment strategies are still required for the significant proportion of patients that do not respond to or relapse following FCR treatment.

In recent years it has become increasingly apparent that successful treatment of CLL must target the proliferative compartment of the disease that resides in proliferation centres within the lymph nodes and marrow. Interaction of CLL cells with the tumour microenvironment is believed to be a major contributing factor to resistance and relapse following treatment with the more conventional regimens, including FCR[4]. A better understanding of the role of the microenvironment in CLL-cell survival, proliferation and in drug-resistance has provided the rationale for the clinical trials discussed in this review.

B-CELL RECEPTOR PATHWAY

Given the importance of B-cell receptor (BCR)-mediated signaling in the survival and proliferation of CLL cells[5] much of the recent work on novel therapeutic agents in CLL has focused on inhibitors which target specific components downstream of the receptor. Most notably, clinical trials of ibrutinib and idelalisib, which target the Brutons tyrosine kinase (Btk) and delta isoform of phosphoinositol-3 kinase (PI3-kinase δ respectively, have shown extremely promising results in recent clinical trials.

Ibrutinib

Ibrutinib has been shown to induce apoptosis of CLL cells *in vitro* against both cells in media alone and cells cultured with micro-environmental mimics such as stromal contact or culture with soluble factors such as CD40L, interleukin 4 (IL-4) and BAFF[6]. Ibrutinib interferes with CLL-cell adhesion and migration, which is believed to be important in its mechanism of action[7,8].

RESONATE, a randomized trial comparing ibrutinib to the CD20 monoclonal antibody ofatumumab as single agents in previously treated patients demonstrated significantly better overall response (OR) and survival (OS) rates in the ibrutinib arm of 42.6% *vs* 4.1% and 90% *vs* 81% respectively[9]. The findings of the RESONATE trial have recently been updated showing that these effects are seen regardless of genetic mutation and previous treatment, furthermore an improved haematologic function has also been shown[9,10]. In patients treated with ibrutinib, the phenomenon of treatment-induced lymphocytosis occurs, which is thought to be due to the redistribution of leukemic cells from the tissue microenvironments into the circulation[11], highlights the mechanisms that CLL cells rely on to populate the lymph node and marrow environments. In a phase Ⅱ trial combining ibrutinib with rituximab in 40 high-risk patients (defined as patients with deletion of 17p, mutation of *TP53* or deletions of 11q with disease relapse) the OR rate was 95% of which 8% of patients achieved a complete remission (CR)[12]. It is worth noting that the treatment-induced lymphocytosis in this trial resolved faster than in patients treated with single agent ibrutinib, supporting the rationale for combining inhibitors of Btk with agents that target CLL cells liberated from the lymph nodes and marrow. Investigations into ibrutinib-containing regimens are continuing with a recent dose escalation trial that investigated ibrutinib in combination with lenalidomide. With only 11 patients enrolled and evaluable data on 9 the results of this trial are currently limited, but have shown an OR rate of 100% and do suggest that the combination is well tolerated[13].

Idelalisib

Idelalisib specifically targets the - isoform of PI3-kinase, which in turn decreases phosphorylation of Akt and induces caspase-dependent apoptosis. Idelalisib induces apoptosis of primary CLL cells *in vitro* cultured either in media alone or in combination with factors such as CD40L and tumor necrosis factors (TNF)-α. As well as its cytotoxic effects, idelalisib inhibits the interaction of the leukemic cells with the tumour microenvironment[14,15].

Several phase Ⅰ, Ⅱ and Ⅲ studies of idelalisib as a single agent or in combination with the CD20 antibodies ofatumumab or rituximab and bendamustine have been conducted or are on-going for relapsed/refractory or previously untreated elderly patients. In a phase I trial of idelalisib as a single agent in 54 patients with poor risk characteristics the OR rate was 72%, with 81% of patients demonstrating a nodal response[16].

A phase Ⅰ trial of idelalisib in combination with ofatumumab or rituximab demonstrated that these combinations are well tolerated. Among 40 patients the OR rate was 83% with 3% of patients achieving a CR. Notably, among the 11 patients with deletion or mutation of *TP53* the OR rate was 73%[17]. More recently, the results of a phase Ⅲ trial comparing idelalisib plus rituximab with placebo plus rituximab in 220 relapsed, co-morbid patients demonstrated a significant improvement in OR rate and survival in those patients treated with idelalisib in combination with rituximab; OR

rates were 81% *vs* 13% in the idelalisib and placebo arms respectively with OS rates of 92% *vs* 80%[18]. A phase Ⅱ trial of idelalisib in combination with rituximab in 50 previously untreated elderly (> 65 years) CLL or small lymphocytic leukemia (SLL) patients demonstrated an OR rate of 96% with a progression-free survival at 24 mo of 91%[19], supporting the use of the PI3-kinase inhibitor as potential first-line therapy. An extension to this trial is now underway investigating the use of idelalisib as a mono-therapy in elderly CLL/SLL patients; to date 37 patients have been enrolled and 27 evaluated with an OR rate of 81%[20].

A phase Ⅰ trial of idelalisib in combination with bendamustine and/or rituximab, fludarabine or chlorambucil and/or rituximab in relapsed refractory disease has proven these combinations are also highly active with an OR rate of 82% and a CR rate of 10%[21,22]. Phase Ⅲ trials of idelalisib in combination with rituximab are on-going with promising results[23].

Fostamatanib

The tyrosine kinases SYK and Lyn are also key components of the BCR signaling cascade and as such have been proposed as therapeutic targets. Suljagic *et al*[24] established a rationale for clinical trials of the SYK inhibitor fostamatanib by showing that the drug inhibits signaling downstream of the BCR and increases the survival of Eμ-TCL1 transgenic mice, which represent an *in vivo* model of CLL disease.

A phase Ⅱ trial of fostamatanib (R788) demonstrated activity against relapsed/refractory CLL/SLL disease, with 6 of 11 patients achieving an objective response[25]. Interestingly, 9 of the 11 patients had evidence of treatment-induced lymphocytosis, similar to the effects observed with both ibrutinib and idelalisib. Although there appear to be no plans to pursue clinical trials of fostamatanib for CLL, studies of the next generation of SYK inhibitors, PRT318 and P505-15, have shown that both compounds induce apoptosis under *in vitro* conditions that mimic the microenvironment, inhibit CLL cell migration and chemokine secretion and prevent BCR-induced activity of mitogen activated protein kinase (MAPK)-extracellular regulated kinase (ERK)1/2[26].

Dasatinib

Dasatinib is a broad-spectrum inhibitor of Src-kinases and Abl and in CLL patients appears to function mainly through inhibition of Lyn. Dasatinib has been shown to inhibit the phosphorylation of Akt, ERK1/2 and p38 and induce apoptosis of CLL cells *in vitro*[27].

In a phase Ⅱ trial of dasatinib, among 15 relapsed/refractory CLL patients, 3 (20%) achieved a partial response (PR), 5 a nodal response and 1 had a reduction in node size and lymphocyte count[28]. A similar PR rate (16.7%) was observed in a more recent phase Ⅱ trial of dasatinib in combination with fludarabine[29], suggesting that either as mono-therapy or in combination with fludarabine, dasatinib has, at best, modest effects against relapsed/refractory, fludarabine-resistant CLL disease.

Novel BCR-pathway inhibitors in pre-clinical development

The clinical trial data of idelalisib discussed above highlight the potential of PI3-kinase targeted therapies for CLL and provide the rationale for investigations of novel agents that target multiple isoforms of PI3-kinase. SAR245409 and duvelisib (IPI-145) are inhibitors of the α/δ and δ/γ isoforms of PI3-kinase respectively. Thijssen *et al*[30] have shown that unlike SAR245409 neither BYL719, a specific α isoform inhibitor, nor idelalisib, completely block the phosphorylation of mTOR and that SAR245409 is more cytotoxic *in vitro* than idelalisib. Similarly, Dong *et al*[31] have shown that duvelisib is cytotoxic against CLL-B cells *in vitro* with little effect on normal B cells and that duvelisib prevents the spontaneous development of leukemia and delays leukemic cell engraftment in the Eμ-TCL1 mouse model. Early results of a phase Ⅰ clinical trial of duvelisib for relapsed/refractory CLL disease suggests that the drug is well tolerated with an OR rate of 55% among 54 patients. Pharmacodynamic studies within this trial suggest that duvelisib modulates chemokine and cytokine levels, cell proliferation and the activity of Akt[32]. Direct inhibition of Akt has also been proposed as a therapeutic option for CLL. In a recent study, Ding *et al*[33] demonstrated that the Akt inhibitor MK2006 induces apoptosis of CLL irrespective of poor prognostic characteristics.

MEK1/2 is a key component of the MAPK, ERK pathway, which promotes the survival and proliferation of multiple forms of cancer cell. In B-CLL cells MAPK-ERK signaling is activated in response to BCR ligation and as such is believed to play a role in promoting CLL-cell survival. However, we recently demonstrated that MEK1/2 inhibition by Binimetinib (MEK162, Novartis) has little effect against CLL cells in the absence of factors that mimic the tumor microenvironment[34]. The efficacy of Binimetinib only under *in vitro* conditions that mimic tonic BCR stimulation highlights the importance of considering the interaction of leukemic cells with the tumour microenvironment as part of similar *in vitro* studies[34]. Similar studies have also recently been described for the MEK1/2 inhibitor trametinib. Apollonio *et al*[35] described the efficacy of this MEK1/2 inhibitor against primary CLL cells and in a mouse xenograft model of CLL. In this study trametinib had a marked effect on the viability of primary CLL cells in which ERK1/2 was constitutively phosphorylated, an indication of CLL cell anergy, and inhibited tumour growth and delayed leukemic cell dissemination in the mouse model.

In normal B-cells the Raf-1/MEK/MAPK-ERK1/2 pathway is negatively regulated by the Raf kinase inhibitory protein (RKIP). However, in CLL-cells dysfunction of RKIP may lead to over activity of the Raf-1 and Akt-mediated pathways. Our recent studies suggest that the RKIP inhibitor locostatin induces apoptosis in CLL cells cultured in media and on a CD40L fibroblast layer *via* a

mechanism that involves the down-regulation of both ERK1/2 and Akt, highlighting the potential of RKIP as a therapeutic target in CLL[36].

CC-115 is a novel dual inhibitor of both the mammalian target of rapamycin (mTOR) and DNA protein kinase (DNA-PK). Signals downstream of PI3-kinase and Akt are mediated by a complex consisting of mTOR1 and 2, while DNA repair and genomic stability rely on the function of DNA-PK. DNA repair pathways are of interest in CLL as mutations in the DNA repair machinery, particularly in the *ATM* and *TP53* genes, are associated with poor prognosis. Thijssen *et al*[37] demonstrate that CC-115 is cytotoxic against primary CLL cells irrespective of *ATM* mutational status.

The clinical efficacy of fostamatanib discussed above and several recent *in vitro* studies suggest that CLL cells are sensitive to SYK inhibition. A study by Purroy *et al*[38] suggests CLL cells may be more sensitive to SYK inhibition by TAK-659 than to fostamatanib in a co-culture model of the CLL tumour microenvironment and that this agent may be effective in synergy with fludarabine, ibrutinib and idelalisib. Based on promising results in acute myeloid leukemia, Dielschneider *et al*[39] investigated the efficacy of gefitinib against CLL cells. The drug was effective particularly against CLL cells expressing ZAP-70, which represents a poor prognostic sub-group. Similar studies of the SYK inhibitor GS-9973, also demonstrate cytotoxicity against primary CLL cells *in vitro* and synergy with idelalisib[40].

Finally, the constitutive activity of protein kinase C (PKC) in CLL cells and its role in nuclear factor-kappa B (NF-κB)-mediated cell survival[41] suggest that it may also represent a therapeutic target in CLL[42]. Enzastaurin and sotrastaurin (AEB071) are PKC inhibitors in pre-clinical and early clinical trials for a range of malignancies including CLL. El-Gamal *et al*[43] have shown that sotrastaurin is cytotoxic against CLL cells in both *in vitro* and *in vivo* pre-clinical trials. While the results of trials of enzastaurin (reviewed in[44]) suggest that targeting PKC may well have well have some efficacy in B-cell malignancies, including CLL, as the authors suggest understanding the mechanisms that account for the limited therapeutic actions of enzastaurin may lead to the development of novel PKC-targeted agents or novel combinations.

CELL TO CELL INTERACTIONS WITHIN THE CLL TUMOUR MICROENVIRONMENT

Successful treatment of CLL relies on targeting the proliferative pool of CLL cells that populate the lymph node and bone marrow microenvironments. Pre-clinical models of the tumour microenvironment involving co-culture of primary CLL cells with nurse-like, mesenchymal, stromal or T-cells significantly reduce the spontaneous apoptosis rate of the leukemic cells through mechanisms that likely depend on direct cell to cell contact and the production of cytokines and growth factors. The association between CLL and stromal cells is also likely to facilitate the interaction of CLL cells with T-cells.

It is now widely acknowledged that the proportions and function of each of the T-cell subsets is abnormal in CLL; a high proportion of T-regulatory cells and the limited cytotoxic capacity of $CD8^+$ T-cells may suppress the anti-tumour functions of $CD4^+$ T-cells and allow CLL cell proliferation to proceed unchecked[45]. Recent studies suggest that the formation of the immunological synapse between T-cells and antigen presenting cells is defective in CLL due to dysfunction of the actin cytoskeleton in the T-cells[46]. Data from the same study, involving culture of CLL cells with allogeneic T-cells from healthy individuals, suggested that the CLL cells induce these defects in the cytoskeleton of the T-cells enabling the tumour cells to escape normal immune surveillance.

Lenalidomide

On the strength of trials in other B-cell malignancies lenalidomide was initially trialed in a cohort of CLL patients with relapsed or refractory CLL, with OR and CR rates of 47% and 9% respectively[47]. While the exact mechanisms of action of lenalidomide are not clear a recent study employing lenalidomide to consolidate first line therapy with pentostatin, cyclophosphamide, rituximab (PCR), identified a long-term improvement in anti-tumour T-cell synapse formation and overall response to chemo-immunotherapy[48], suggesting that as well as direct tumor activity through altered activity of an E3 ubiquitin-ligase[49] lenalidomide has immuno-modulatory properties and may at least partially correct the T-cell defect and improve immune surveillance in CLL. Lenalidomide also has marked anti-inflammatory effects that will be discussed in more detail later.

Clinical trials of lenalidomide as a single agent have demonstrated its efficacy against relapsed/refractory disease[50] and in the treatment of elderly patients[51] and more recently as a frontline therapy in combination with rituximab[52,53]. Lenalidomide has also proven effective in combination with rituximab as a salvage therapy for relapsed/refractory disease[54]. Several trials using lenalidomide as maintenance therapy following first and second-line FCR therapy are in progress (CONTINUUM study, clinicaltrials.gov NCT00774345, ALLG Residuum and DCLLSG)[55,56].

CAR-T cells

Studies suggest that the *ex vivo* manipulation of T-cells from CLL patients to produce T-cells with chimeric antigen receptors (CAR T-cells) also represents a method of overcoming the defect in T-cell surveillance of the leukemic clone[57]. In CLL, trials of CAR T-cells expressing a receptor to the CD19 antigen (CTL019) have proven successful in the treatment of relapsed/refractory disease or as consolidation following first-line treatment of high-risk patients (defined as those patients with un-mutated *IgVH* genes, or deletion

of 17p- or 11q-) with PCR. In a trial of 14 relapsed/refractory patients, 4 (29%) achieved a CR and 4 (29%) a PR with an OR rate of 57%[58]. Of the 6 patients enrolled on the phase Ⅰ consolidation trial 2 patients who were in PR after therapy with PCR achieved a CR following the infusion of the CAR-T cells, suggesting that CAR-T cells may be useful in increasing CR rates[59].

The study by Porter *et al*[58] suggests that the advantages of this form of cellular therapy include the provision of ongoing tumor surveillance through the *in vivo* expansion of the CAR T-cells and the specificity of the therapy for B-cells, although the effects on normal B-cells may result in B-cell aplasia. The long-term activity of CAR T-cells is illustrated by the delayed onset of tumour lysis syndrome[60]. Porter *et al*[61] have now commenced a phase Ⅱ trial of CTL019 cells in patients with relapsed or refractory CLL, to 23 patients have been evaluated with a CR rate of 22% and a PR rate of 17%.

Lucatumumab

Targeted blockade of CD40-mediated signaling in CLL cells represents a rational approach to interfering with the interaction between CLL and T cells, since CD40-mediated signaling promotes CLL-cell survival and proliferation[62]. Lucatumumab, a humanized anti-CD40 agonist antibody was shown to inhibit B-cell growth and induce antibody-dependent cellular cytotoxicity *in vitro*[63]. However, the results of a phase Ⅰ trial of lucatumumab (HCD122) were disappointing; of the 26 patients on trial, 17 had stable disease for a mean duration of 76 d but only 1 patient achieved a PR. The authors concluded that further trials of lucatumumab should focus on incorporating the antibody in combination therapies[64].

Bevacizumab

Despite data highlighting the important angiogenic role of vascular endothelial growth factor (VEGF) in CLL proliferation centres within the secondary lymphoid structures[65] and promising pre-clinical data[66,67] the results of a phase Ⅱ trial of the VEGF inhibitor bevacizumab in CLL proved disappointing. No clinical efficacy of bevacizumab was demonstrated among 13 patients with relapsed/refractory disease resulting in early closure of this trial[68].

Novel inhibitors of cell-to-cell interactions in pre-clinical development

CC-122 is a next generation immunomodulatory drug that is believed to have mechanisms of action similar to lenalidomide. Blocksidge *et al*[69] demonstrate that the anti-proliferative effects of CC-122 against primary CLL cells cultured in a CD154/IL-21 tumour model were superior to those of lenalidomide.

C6-ceramide is a nanoliposomal molecule and a member of the ceramide family of lipids. Ceramides are "tumor suppressor" lipids that induce anti-proliferative and anti-apoptotic responses in a variety of malignant cells[70]. Doshi *et al*[71] have shown that C6-ceramide induces tumour regression in an *in vivo* mouse model of CLL and that these effects are likely mediated by its effects on STAT3 phosphorylation and signaling.

BI 836826 is an anti-CD37 monoclonal antibody that mediates its effects on CLL cells through both antigen dependent cell-mediated cytotoxicity and by directly inducing apoptosis[72]. Stephens *et al*[73] investigated the effects of BI 836826 in combination with established BCR pathway inhibitors and suggest that BI 836826 may be effective in combination with idelalisib for CLL, particularly for those patients with *TP53* mutations.

The immunomodulatory drug dimethyl fumarate (DMF) is believed to exert its effects *via* down-regulation of NF-κB activity and TNF signaling. DMF is currently in clinical use for the treatment of psoriasis[74]. In keeping with the role of NF-κB in the survival of CLL cells[75], Wu *et al*[76] demonstrated that DMF is cytotoxic against CLL cells *in vitro* and is synergistic with ibrutinib, *via* a mechanism of action that involves down-regulation of Wnt signaling.

INFLAMMATORY PATHWAYS

Cytokines and chemokines play a significant role in the survival, proliferation and homing of CLL cells to the tumour microenvironment. Studies suggest many of these soluble factors are elevated in the sera of CLL patients compared to normal individuals[77], while *in vitro* studies involving the addition of cytokines to primary CLL cells in culture highlight their role in promoting cell survival, proliferation and migration[78]. Levels of cytokine expression have been linked with the disease course of CLL[77]. However, it remains to be elucidated whether many of these factors are derived from the leukemic cells or accessory cells within the tumour microenvironment.

Targeting the mechanisms that enable CLL cells to home to lymph nodes and to the marrow or interfering with the soluble factors that promote CLL cell survival have been proposed as being effective methods of limiting their longevity and proliferation.

Lenalidomide

In addition to the anti-leukemic effects discussed earlier there is also evidence suggesting lenalidomide, as with its analogue thalidomide, may have potent anti-inflammatory activity through suppression of cytokine and TNF-α production[79].

Plerixafor (AMD3100)

Data from a preclinical study of the CXCR4 inhibitor plerixafor suggests that the agent effectively blocks the capacity of CLL cells to home to the tumour microenvironment, overcomes the protective effect of *in vitro* models of the microenvironment and may represent a means of mobilizing tumor cells to increase the efficacy

of chemotherapies[80]. In a phase Ⅰ dose-escalation trial of 14 patients with previously treated disease plerixafor in combination with rituximab was well tolerated and was associated with a marked increase in lymphocyte count, suggesting successful mobilization of CLL cells from the lymph nodes and marrow[81].

PRO-APOPTOTIC/CELL-CYCLE INHIBITION

CLL-cell survival and proliferation relies on the over-expression of the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2)[82] and the activity of cyclin-dependent kinases (CDKs)[83]. Redressing the balance of expression of the Bcl-2 family proteins towards a pro-apoptotic profile and inhibition of CDKs has been a focus of several recent clinical trials in CLL.

ABT-199

ABT-199 is the most clinically advanced compound among several developed known as BH3 mimetics, so-called after the binding domain that is common to the BH3-family of proteins, which includes Bcl-2. Tumour lysis syndrome appears to be a complication associated with treatment with ABT-199, particularly in the earlier trial cohorts; Souers *et al*[84] reported tumour lysis in three of their relapsed/refractory CLL patients on trial. In a recent phase Ⅰ clinical trial, ABT-199 was highly active against relapsed/refractory, FCR-refractory or 17p-deleted disease; in 56 relapsed/refractory CLL or SLL patients the OR rate was 84%, of which 20% achieved a CR[85]. Within this same trial those patients with deletion of 17p or fludarabine-refractory disease displayed similar OR rates of 82% and 78% respectively. Phase Ⅱ trials of ABT-199 as a single agent or in combination with rituximab (ClinicalTrials.gov NCT01682616) or obinutuzumab (ClinicalTrials.gov NCT01685892) for 17p-deleted or relapsed/refractory CLL patients are ongoing. To date, 49 patients have been enrolled in a trial combining ABT-199 and rituximab, which has had an OR rate of 86% and CR rate of 31%[86]. A trial of ABT-199 and obinutuzumab has recruited 9 participants to date but has yet to report any response or remission rates. Findings of these latter trials suggest the adjusted regimens and novel combinations of ABT-199 are well tolerated with tumour lysis syndrome having only been observed in 1 study participant[87].

Dinaciclib

Pre-clinical studies of flavopiridol provide a strong rationale for CDK inhibition as a therapeutic option in CLL, with a mechanism of action that includes both cytostatic and pro-apoptotic effects. While flavopiridol is undoubtedly the most thoroughly studied CDK inhibitor in CLL, its narrow therapeutic window and toxicity has prompted development of newer, more specific inhibitors, including dinaciclib. While *in vitro* studies suggest that dinaciclib induces apoptosis in CLL cells irrespective of poor-risk indications, including 17p deletion, it fails to overcome the protective effects of CLL-cell co-culture with a stromal layer[88]. These data suggest that the clinical efficacy of dinaciclib may require its incorporation into combination therapies. The results of a phase Ⅲ trial of dinaciclib are yet to be presented but recent data from an update on a phase Ⅰ dose escalation trial of 52 relapsed/refractory patients suggests that dinaciclib is clinically active and well tolerated in this setting. Of the 48 patients assessed, the overall response rate was 58%. Importantly, 57% of the patients with deletion of 17p13 and 63% with deletion of 11q23 achieved at least a PR[89]. A phase 1b/2 study of dinaciclib and ofatumumab has also recently commenced and to date 36 patients have been enrolled with a PR rate of 33% and stable disease in 56% of patients[90].

Novel pro-apoptotic/cell cycle inhibitors in pre-clinical development

MLN4924 is an inhibitor of the NEDD8-activating enzyme. It is believed that MLN4924 induces DNA damage and cell cycle arrest through its effects on Cdt1[91]. MLN4924 has been shown to induce apoptosis in primary CLL cells stimulated with CD40L and IL-21 and, although the exact mechanisms of action remain unclear, Cdt1 accumulation was also demonstrated in two further studies[92,93].

It is well established that mutations of *TP53* and *ATM* are associated with unchecked DNA repair in cancer and poor risk disease in CLL[94]. Inhibition of DNA repair which triggers accumulation of DNA damage has been proposed as a method for overcoming resistance to genotoxic agents. AZD6738 is one such drug which targets the Ataxia Telangiectasia and Rad3 related (ATR) protein and inhibits ATR-mediated DNA repair. Stankovic *et al*[95] and Kwok *et al*[96] have shown that AZD6738 is cytotoxic against CLL cells *via* a mechanism of action that involves the accumulation of DNA damage. Furthermore, studies suggest AZD6738 is synergistic with the DNA-damaging agents chlorambucil, fludarabine, bendamustine and cyclophosphamide and the PARP inhibitor, olaparib.

TARGETED THERAPY RESISTANCE MECHANISMS AND COMBINATION THERAPIES

There is emerging evidence that a subset of patients develop resistance to BCR-pathway targeted therapy. In data presented at the recent European Haematology Association meeting late disease progression while on ibrutinib was found to be associated with the acquisition of mutations in *BTK* and *PLCγ2*[97].

In a recent report that utilised an *in vitro* model of the CLL tumour microenvironment, prolonged CD40

stimulation resulted in resistance to ABT-199, due to induction of Bcl-XL, Mcl-1 and Bfl-1. Interestingly, these CD40-mediated effects could be blocked by the broad-spectrum kinase inhibitor dasatinib[98]. Using interaction proteomics, Abl and Btk were identified as dominant targets of dasatinib in primary CLL cells. Like dasatinib, the Abl inhibitor imatinib, but not ibrutinib, can overcome resistance to the BH3-mimetics. Conversely, BCR and chemokine-mediated adhesion can be abolished by dasatinib and ibrutinib, but not by imatinib[98]. These reports highlight the complexity of potential resistance mechanisms and the potential of drug combinations for overcoming resistance to these agents. They also highlight the need for ongoing research into signal pathways, their interactions and the relationship between CLL cells and the microenvironment.

CONCLUSION

The treatment of CLL is in a period of extraordinary revolution with the advent of multiple novel, targeted therapies. Unprecedented response rates among relapsed/refractory, high-risk and elderly patients suggest that there may very soon be more effective treatment options available for these patients. While the trials of treatment naïve patients support the use of the novel agents in the frontline setting, this would currently be difficult to justify in patients other than the 17p-deleted subgroup, given the high response rates to FCR. Extended follow-up data from on-going and future trials will provide the key to determining the response duration or curative potential of the novel agents reviewed here.

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REVIEW

Ventricular repolarization markers for predicting malignant arrhythmias in clinical practice

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Abstract

Malignant cardiac arrhythmias which result in sudden cardiac death may be present in individuals apparently healthy or be associated with other medical conditions. The way to predict their appearance represents a challenge for the medical community due to the tragic outcomes in most cases. In the last two decades some ventricular repolarization (VR) markers have been found to be useful to predict malignant cardiac arrhythmias in several clinical conditions. The corrected QT, QT dispersion, Tpeak-Tend, Tpeak-Tend dispersion and Tp-e/QT have been studied and implemented in clinical practice for this purpose. These markers are obtained from 12 lead surface electrocardiogram. In this review we discuss how these markers have demonstrated to be effective to predict malignant arrhythmias in medical conditions such as long and short QT syndromes, Brugada syndrome, early repolarization syndrome, acute myocardial ischemia, heart failure, hypertension, diabetes mellitus, obesity and highly trained athletes. Also the main pathophysiological mechanisms that explain the arrhythmogenic predisposition in these diseases and the basis for the VR markers are discussed. However, the same results have not been found in all conditions. Further studies are needed to reach a global consensus in order to incorporate these VR parameters in risk stratification of these patients.

Key words: Electrocardiographic predictor; Ventricular repolarization markers; Ventricular fibrillation; Sudden cardiac death; QT interval; Corrected QT interval; QT dispersion; Tpeak-Tend interval; Tpeak-Tend QT ratio

Core tip: Malignant ventricular arrhythmias are a common cause of sudden cardiac death in clinical

practice. They may present in individuals apparently healthy or be associated with other medical conditions. It is possible to predict the appearances of ventricular arrhythmias by analysing ventricular repolarization markers through surface 12 leads electrocardiogram. It may represent a tool to evaluate the risk stratification and for achieving a better medical management of our patients.

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INTRODUCTION

An electrocardiogram (ECG) represents one of the most common medical tools used by physicians in clinical practice. Its adequate interpretation gives the possibility for diagnosing and predicting multiple cardiac diseases. These features accompanied by its relative low cost allow that ECG interpretation needs to be known by medical doctors of several specialities including internists, cardiologists, anaesthesiologists, family physicians, and all that have a direct contact with patients.

Sudden cardiac death (SCD) causes approximately 800000 deaths each year in the world[1]. It is often produced by malignant ventricular arrhythmias (MVA). In most cases it is derived from ventricular fibrillation, or less frequently, by monomorphic or polymorphic ventricular tachycardia and Torsades de Pointes[2]. MVA which may result in SCD frequently occur in sick hearts but around 15%-20% occur in healthy hearts[3].

Many people that develop MVA have a previous disease that may be the cause of this condition. By ECG analysis it is often possible to predict the development of ventricular cardiac arrhythmias in these patients. That is feasible by analysing several ventricular repolarization (VR) markers. Some of the most explored predictors in clinical practice are: QT interval and its correction by heart rate (QTc)[4], QT interval dispersion (QTd)[5] and other recently published markers like Tpeak-Tend (Tp-e)[6], Tp-e dispersion (Tp-ed)[7] and Tp-e/QT ratio[8]. These markers have demonstrated a high usefulness to indicate patients with a high risk to develop cardiac arrhythmias in multiple clinical conditions (Table 1).

The methods to measure these VR markers are not difficult. These VR markers are useful tools for evaluation of patients' risk to develop MVA. They help us make better medical decisions in the management of patients with various medical conditions.

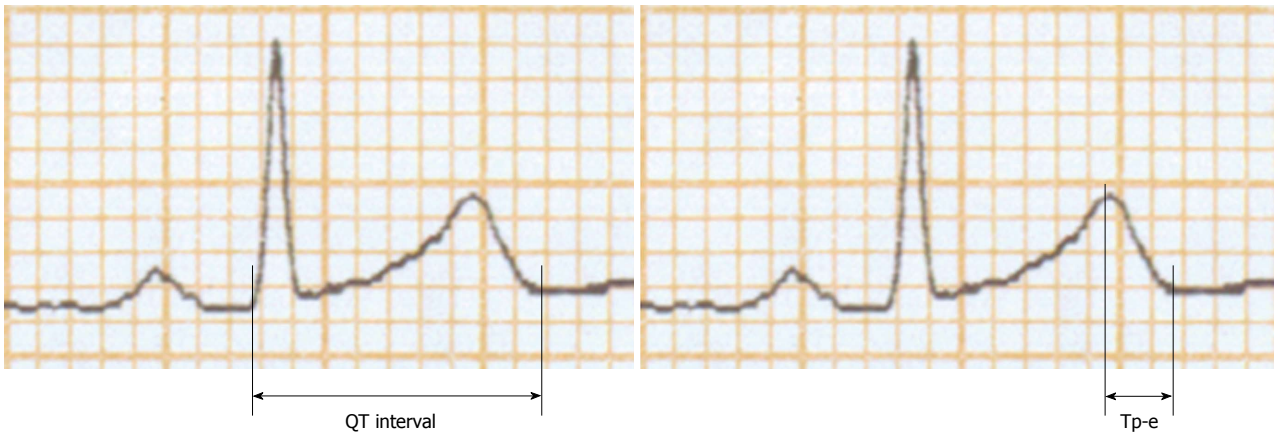
The aim of this manuscript is to review the usefulness of electrocardiographic VR markers to predict SCD in several conditions such as long and short QT syndromes, Brugada syndrome, early repolarization syndrome, acute myocardial ischemia, heart failure, hypertension, diabetes mellitus, obesity and high trained athletes and the possible mechanisms involved in order to encourage their clinical use to improve patients' risk evaluation.

QT, QTc and QTd

Measurement of the QT interval from surface 12 lead ECG was proposed at the beginning of last century[9]. It was primarily used to identify patients with arrhythmogenic syndromes which are common causes of SCD. These syndromes are long and short QT syndromes. However, its usefulness has been extended in multiple clinical conditions.

The QT interval is measured from the onset of the QRS complex to the end of the T wave (Figure 1). It should be recorded in Ⅱ or V5 leads where it has demonstrated the most predictive capacity. The QT interval may be modified by heart rate. Because of this, formulas have been proposed with the aim to reduce the heart rate influence. QT interval corrected by heart rate is known as QTc. Around 20 formulas to measure the QTc have been suggested. However, the most commonly used in clinical practice are the QTc by Bazzet's method [QTc = QT/(R-R)$^{1/2}$] and by Fridericia's method [QTc = QT/(R-R)$^{1/2}$] both expressed in seconds (Table 2)[10]. Also it is recommended to consider gender in QRS prolongation. Abnormal proposed values of QTcfor adults are $\geqslant$ 450 ms in men and $\geqslant$ 460 ms in women, while a QTc $\leqslant$ 390 ms is considered short[11]. Proposed normal values of QTcin children between 1 and 15 years are < 440 ms, with a range between 440 to 460 ms and abnormal values > 460 ms[12]. However, other authors have established that the 98th percentile limit for rate-adjusted QT is approximately 450 ms in children younger than 12 years of age[11].

Although QT interval and its correction (QTc) have been satisfactorily used to predict cardiac arrhythmias, another marker which evaluates its dispersion has been created and implemented in clinical practice. This marker is the QTd, which is defined as the difference between the maximum and minimum QT interval in surface 12 lead ECG[13]. QTd represents the heterogeneity state of VR. An increase in ventricular heterogeneity augments the vulnerable period time in the heart and predisposes to ventricular arrhythmias[14]. It was implemented in clinical practice by Day *et al*[15], and currently has been suitably evaluated as a marker to predict MVA. The values of QTd in normal subjects and general population are controversial. Reports from several studies reveal values of 33.4 ± 20.0 ms, with a range from 10.5 ± 10.0 ms to 71 ± 7.0 ms and a median in 37 ms. There are not statically significant differences between the genders while by other studies were found age-related differences < 10 ms[5]. Recently published data show that in healthy individuals a QTd > 58 ms increase 3.2-fold the risk of cardiovascular mortality[16] and those with a QTd $\geqslant$ 80 ms have 4-fold risk for cardiac death compared to patients with QTd



QTc = See different formulas to measure QTc
QTd = Maximun QT interval-Minimun QT interval on 12 leads ECG

Tp-ed = Maximun Tp-e-Minimun Tp-e on precordial leads ECG
Tp-e/QT ratio = Values of these markers on the specific lead

Figure 1 Methods to measure the different markers on electrocardiogram.

Table 1 Medical condition where have been investigated the ventricular repolarization markers

Long QT syndrome	Heart failure
Short QT syndrome	Hypertension
Brugada syndrome	Diabetes mellitus
Early repolarization syndrome	Overweight/obesity
Acute myocardial ischemia	Highly trained athletes
Kawasaki disease	Duchenne muscular dystrophy
Systemic lupus erythematosus	Liver transplantiation
Rheumatoid arthritis	Rheumatoid arthritis
Chronic renal failure	Rheumatic fever
Scleroderma	Chagas disease
Ankylosing spondylitis	Chronic hepatitis B
Obstructive sleep apnea	β-thalassemia
Spinal injury	Polycythemia vera

values < 30 ms[4].

Tp-e and Tp-ed

An increase in dispersion of repolarization and modifications in normal pattern of ventricular recovery are mechanisms associated with predisposition to develop cardiac arrhythmias[17]. Tp-e is the interval between the peak of the T wave and the end of the T wave (Figure 1). Commonly it is considered a reflection of the transmural cardiac repolarization expressed through surface 12 lead ECG. It has been proposed to indicate patients at an increased risk for ventricular arrhythmias[18,19]. Recent investigation examined Tp-e interval using a computer model of the rabbit heart ventricles. The authors concluded that Tp-e corresponds with global dispersion of repolarization[20].

Tp-e should be measured in precordial leads where it has been demonstrated to be more specific[21]. It has been found that in healthy men subjects Tp-e has a mean value in V5 lead of 94 ± 10 ms in men and 92 ± 11 ms in women[22]. However, there is not a consensus about Tp-e normal values and further investigations are needed to define them. Tp-e has been evaluated in several clinical conditions. It has been considered more useful to predict cardiac arrhythmias than QTc and its dispersion in some clinical conditions[23].

Table 2 Formulas to calculate the QTc

Name	Formula
Bazzet modificated by Taran and Szilagyi	$QTc = QT/(R\text{-}R)^{1/2}$
Fridericia	$QTc = QT/(R\text{-}R)^{1/3}$
Framingham	$QTc = QT + 0.154 \times (1 - RR)$
Hodges	$QTc = QT + 1.75 \times (HR - 60)$
Sarma	$QTc = QT - B1\ Exp(-k1 \times RR)$
	$QTc = QT\ [1 - Exp(-k2 \times RR)]$
	$QTc = QT\ (RR)^{1/2} + B3$
	$QTc = QT\ (RR)^{1/2}$
Ecuación de fuerza	$QTc = 453.65 \times RR1/3.02\ (R2 = 0.41)$
Van de Water	$QTc = QT - 0.087\ (RR - 1000)$
Matsunaga	$QTc = \log(600)\ QT/(\log RR)$
Kawataki	$QTc = QT/RR \times 0.25$
Mayeda	$QTc = QT/RR \times 0.604$
Larsen y Skulason	$QTc = QT + 0.125 \times (1 - RR)$
Schlamowitz	$QTc = QT + 0.205 \times (1 - RR)$
Wohlfart	$QTc = QT + 1.23 \times (HR - 60)$
Boudolas	$QTc = QT + 2.0 \times (HR - 60)$
Sagie	$QTc = QT + 0.154 \times (1 - RR)$
Malik	$QTc = QT/RR \times 0.371$
Lecocq	$QTc = QT/RR\ (0.314)$

This table was taken and adapted with permisson of Editor in Chief from Chávez González[10]. B and K are regression parameters. Exp: Exponential function with base e = 2.718; HR: Heart rate; RR: RR distance; QT: QT interval; QTc: Corrected QT interval.

Tp-ed was proposed by Castro Hevia *et al*[7]. Using this marker, they found an increased risk for ventricular arrhythmias in patients with Brugada syndrome than healthy controls. It has been examined in other diseases demonstrating its usefulness to predict malignant arrhythmias and SCD. However, limited information has been published about it and it should be studied in further investigations.

Tp-e/QT ratio

Tp-e/QT ratio is a novel index to predict cardiac arrhythmias[8]. It includes the values of transmural dispersion

(Tp-e) and spatial dispersion (QT) of VR (Figure 1). It has a substantial advantage than other markers because does not need to be corrected by heart rate. Tp-e/QT ratio almost has no variations between 60-100 beats/min. Tp-e/QT measured in healthy populations in precordial lead V6 which best reflects the trasmural axis of left ventricle has a mean value of 0.21 ± 0.03 and a range of value from 0.15 to 0.25[24].

Brief electrophysiological considerations

Heterogeneity of VR is associated with MVA[25]. QT interval is an index of VR and its variations represents its heterogeneity, commonly measured by QTd. An increase in VR dispersion increased the vulnerable period in the heart and predisposes to MVA. Regional differences in the potential action (inhomogeneity) may be found in several heart parts, but the transmural (ventricular wall) is the most important[14]. Ventricular myocardium is comprised by endocardial, epicardial and myocardial (M) cells. These cells are structurally similar, but have different electrophysiological properties. M cells have a longer action potential than cells located in epicardium and endocardium[26,27]. This property is noted in response to slowing of heart rate or agents than prolong the action potential. The prolonged action potential of M cells is due to a delay or blockage of K^+ and a rise in Na^+ currents[14]. In normal conditions, the physiological differences among these cells are minimized by electronic influences from well coupled myocytes[28]. The interplay between these opposing transmural forces among these cells determines the height and width of the T wave[29]. A rise in transmural repolarization determines a prolongation of T wave and subsequently the Tp-e. Multiple conditions determine pathophysiological changes with alteration in normal pattern of repolarization, increasing heart heterogeneity and the risk of MVA.

Application of QT, Tp-e, its dispersions and Tp-e/QT ratio to predict MVA in some diseases is discussed below.

Long QT syndrome

Long QT syndrome is an arrhythmogenic channelopathy characterized by severe alterations in VR. Long QT syndrome can be congenital or acquired.

The congenital long QT syndrome is caused by hereditary defects of molecular structure in ion channel proteins. Mutations in genes that codify to sodium and potassium channels cause a prolongation of VR. These alterations predispose to the development of Torsade de Pointes. This arrhythmia can result in ventricular fibrillation and SCD[30,31]. Long QT syndrome is often an autosomal dominant disease. It has been found mutations in 13 genes in long QT syndrome, determining 13 different types of this condition. However, the most known are long QT syndrome 1, long QT syndrome 2 and long QT syndrome 3, which are related with *KCNQ1*, *KCNH2* and *SCN5A* genes respectively[32].

The acquired form of long QT syndrome is a prolongation of QT interval that also predisposes to the patient to develop Torsade de Point and SCD. It may be caused by drugs[33], electrolyte abnormalities[34], hypothermia[35], toxic substances[36] and central nervous system injury[37].

Patients with QTc $\geqslant$ 500 ms have a high risk for developing MVA. Sauer *et al*[38] studied patients with long QT syndrome and the utility of QTc to predict SCD in these cases. Those with QTc duration between 500 and 549 ms were associated with a greater risk. In this same work, a QTc $\geqslant$ 550 ms was related with a 6.3-fold increase in the risk. Another investigation showed that patients with QTc duration $\geqslant$ 530 ms had a higher predisposition to develop cardiac arrest and SCD than those with shorter QTc values[39]. Recently, the risk for life-threating cardiac events was evaluated in 403 patients with long QT syndrome. Patients with multiple gene mutations had longer QTc than those with a single mutation (506 ± 72 ms *vs* 480 ± 56 ms respectively; $P = 0.003$) and a higher rate of life-threating cardiac events[40]. This study demonstrates the complexity of this syndrome, and how the gene mutations may modify the values of QTc and maybe other markers. The phenotypes derived from the gene mutations may express several clinical forms of this condition and make the interpretation of the results difficult.

In these studies, higher QTc values were associated with SCD. However, it has been demonstrated that the risk stratification in patients with congenital long QT syndrome is beyond QTc values. Also, it has been demonstrated that it is necessary to evaluate other variables as the gender, specific gene mutation/s and family history[41].

QTd is another marker evaluated in these cases. It has been found prolonged in patients with idiopathic long QT syndrome and represents a marker for therapeutic efficacy[42]. Yamaguchi *et al*[43] evaluated 27 patients with long QT syndrome. These patients were divided into two groups. The group A ($n = 27$) were patients with Torsades de Pointes and group B ($n = 15$) without this. The measurement of QTd in the first group was 112 ± 64 ms and in the second 70 ± 40 ms; $P = 0.0456$. These authors also evaluated the Tp-e. Values of Tp-e in V5 were 185 ± 46 ms in group A and 84 ± 18 ms in group B; $P < 0.0001$. Tp-e showed more significant differences than QTd between patients with and without Torsades de Pointes. Moreover, Tp-e has been found to be prolonged and to be an arrhythmogenic index in patients with long QT syndrome in other studies[44,45]. In this same investigation by Yamaguchi *et al*[43], Tp-e/QT ratio in V5exceeding 0.28 was also associated with the risk to develop Torsades de Pointes.

Short QT syndrome

Congenital short QT syndrome is a rare chanelopathy which increases the incidence of paroxysmal atrial fibrillation, ventricular tachycardia and/or fibrillation[46-48]. The diagnostic criteria are in debate and not yet been established. Most investigators use a grey area for the

QTc between 370 and 330 ms[49]. Recently data have been published which reveal an estimated prevalence of 0.7 per 100000 persons using a QTc ⩽ 300 ms as diagnostic criteria[50]. It has been proposed values of QTc values of 350 ms for men and 360 ms for women derived considering a cutoff values of ⩽ 2 SD from the mean value of general population[51]. Currently a QTc < 320 ms is accepted as an abnormal QTc value[52]. However, the risk to develop MVA in patients with abnormal short QTc values is undetermined. In patients with a prior history of atrial or ventricular fibrillation a QT interval less than 340 ms or a QTc less than 345 ms is usually sufficient[53]. Due to the non-existence of an international consensus of QT values for the diagnosis of short QT syndrome it has been proposed diagnostic criteria. These include the values of QTc, personal clinical history, family history and genotype[49].

Several mutations in potassium channels (KCNH2, KCNQ1 and KCNJ2) and calcium channels (CACNA1C, CACNB2B, CACNA2D1) have been identified in these patients[54].

Anttonen *et al*[55] studied patients with symptomatic short QT syndrome and evaluated some VR markers. They found that in the group of patients with short QT syndrome values of Tp-e/QT ratio were more prolonged than in the control group (0.30 ± 0.04 *vs* 0.24 ± 0.04, $P = 0.001$). Tp-e/QT ratio in the normal population is around 0.21. In this case both patients' groups had values over this point, but it seems that there is a cut off value which increases the risk of malignant arrhythmias which is unknown and should be explored by researchers and physicians. Similar results were found in another study. In this case after a follow-up with a 24-h ECG recording patients with short QT syndrome the Tp-e/QT ratio was 0.28 ± 0.03 and 0.21 ± 0.02 in control subjects, $P = 0.01$[56]. Also Tp-e has been demonstrated to be prolonged and correlated with transmural dispersion repolarization in an experimental model of short QT syndrome[57].

Short QT syndrome should be studied further like other conditions which are associated with genetic mutations and several clinical presentation forms. There are limited studies to conclude about the usefulness of VR markers to evaluate the risk of patients with short QT syndrome to develop MVA. There are points which should be analysed such as the absence of a consensus about diagnostic criteria, the low prevalence of this condition, the genetic heterogeneity and possible differences among age, gender and races. An advance in this field has been with the exploration of Tp-e/QT ratio with encouraging results.

Brugada syndrome

An alteration of VR markers has been observed in other clinical conditions. Brugada syndrome was established in 1992. It is defined by a J point and ST-segment elevation 2 mm or greater followed by a negative T wave in the ECG right precordial leads[58]. Recently, a consensus report from the International Society for Holter and Noninvasive Electrophysiology has established 2 Brugada patterns[59]. They combined the type 2 and 3 patterns outlined in a previous report[60]. This syndrome has been linked to *SCN5A* gene mutations which affect sodium channel function[61,62]. Its prevalence is around 1 in 2000 people[63] and may explain 20% of SCD in patients without structural cardiac disease[60]. Patients with Brugada syndrome have an increased risk to develop malignant cardiac arrhythmias. They have a high incidence of ventricular tachycardia and/or ventricular fibrillation in structurally normal hearts[64-67] and are asymptomatic in most cases[68].

A prolongation of QTc in right precordial lead has been found in patients with Brugada syndrome[69]. A mechanism to explain QTc prolongation in patients with Brugada syndrome may be by the rise of the action potential notch in the right than in the left ventricular epicardium observed in these subjects.

Castro Hevia *et al*[7] studied 29 patients with Brugada syndrome and 29 healthy controls. Patients were followed by a mean of 42.65 ± 24.42 mo. QTc > 460 ms in V2 lead was a risk factor for arrhythmias recurrence. Maximum Tp-e (measured in precordial leads) and Tp-ed were associated with more recurrence of life-threatening cardiac events in Brugada syndrome patients compared with controls. Also, all Brugada syndrome patients with Tp-e values ⩾ 100 ms or Tp-ed values > 20 ms had events during 60 mo of follow-up. As was established by the authors, this work gives a novel way to analyze the risk stratification in Brugada syndrome.

Recently 23 individuals (spontaneous $n = 10$ or drug-induced $n = 13$) with type 1 ECG pattern of Brugada syndrome received a programmed ventricular stimulation. Tp-e in leads V2 and Tp-e/QT ratio in leads V6 were significantly increased in patients who developed ventricular tachycardia/ventricular fibrillation. However, QTc (by Bazzet's method) and QTd did not show significant differences[70].

Several mechanisms have been formulated to explain arrhythmogenic risk in patients with Brugada syndrome. One of the most accepted establishes that the decrease of the depolarizing in sodium channels presented in this condition leads to a functional predominance of the repolarizing Ito current. These differences result in an increase in epicardial action potential notch. These changes produce an increase in the transmural heterogeneity of repolarization and a predisposition to MVA[71-73]. It has been proved that phase 2 reentry might appear under these conditions[74]. Another theory is based on depolarization abnormalities. It has been observed that there are regional differences between activation of the cells located in right ventricular tract outflow myocardium and the rest of right ventricle. These alterations modify cell interactions and increase the risk of reentry arrhythmias[72,73,75]. Also it has been proposed discontinuous conduction and malfunctioning channels as hypotheses to explain arrhythmogenic risk in these patients[72].

Brugada syndrome is a very complex condition with several genetic subtypes, arrhythmic pathophysiological mechanisms, electrocardiographic patterns and clinical presentations. Thus, it is possible to understand the multiple variants described in clinical practice. These characteristics also may influence the ability of VR markers to detect patients at high risk of malignant cardiac arrhythmias in this condition. In order to elevate clinical sensitivity of these markers in these cases investigators should be take into account these factors and to design clinical trials to achieve better conclusions.

Early repolarization syndrome

Early repolarization pattern consists in a J wave or J point elevation, a notch or slur of the terminal part of the QRS and an ST-segment elevation on surface 12 lead ECG[76-78]. It has prevalence from 6% to 24% in the general population[79] and is higher in trained athletes[80]. Early repolarization pattern was primarily considered a benign electrocardiographic finding[81,82]. However, recent studies have demonstrated an increased risk for malignant cardiac arrhythmias in these patients[83-85]. Early repolarization pattern adopts the name early repolarization syndrome when it is associated with cardiac arrhythmias and SCD[86]. Current studies have determinated three patterns of early repolarization and the risk to develop arrhythmic events of each of them[87].

The predisposition to have malignant ventricular arrhythmia in these patients was evaluated by Letsas *et al*[88]. They found increased values of Tp-e in leads V2, Tp-ed in precordial leads and Tp-e/QT ratio in leads V2 in patients with early repolarization pattern compared with those without it. There were not significant differences regarding markers studied in localization of early repolarization pattern (lateral *vs* infero-lateral). Previously, another investigation demonstrated that QTc was useful to predict primary end points in these individuals[89]. The use of these markers to indicate patients at risk to suffer SCD by MVA in this setting should be explored further.

The arrhythmogenic substrate of early repolarization syndrome has been proposed to be the outward current of Ito. Ito current is more prominent in epicardium than endocardium. This gradient voltage has been associated with the beginning of cardiac arrhythmias[90,91]. Furthermore, an investigational group demonstrated that there are abnormal large spatial repolarizations gradients in early repolarization that predispose to reentry cardiac arrhythmias[77]. Previous investigations demonstrated a high incidence of malignant arrhythmias in patients with early repolarization pattern in inferior/inferiolateral leads or in inferior, lateral and right precordial leads than patients with only in the lateral precordial leads[87]. These differences provide additional data and increase the complexity of this condition. As the arrhythmogenic index may be variable depending of ECG topography, VR markers should be explored independently to reach trusty results.

Acute myocardial ischemia

Cardiovascular diseases are the most common cause of mortality in developed countries[92]. Acute coronary syndrome is often associated with SCD[93]. In patients with acute coronary syndrome an increased risk for MVA with a worse prognosis has been observed[94]. QT interval has been used to estimate the arrhythmogenic risk after an acute coronary syndrome. Schwartz *et al*[95] demonstrated the clinical value of QT interval many years ago. They showed that patients with Q wave acute myocardial infarction and prolonged values of QT interval had a high probability to develop SCD. Another study documented that exercise after an acute myocardial infarction may induce changes in QTc. In this case, the prolongation of QTc interval predisposes to develop SCD and was useful to differentiate patients at high risk for SCD and those at low risk for it[96]. These results show the benefit of using the QTc for identifying patients with exercise-induced ischemia who are at risk of SCD. In addition, a correlation between QTc prolongation and SCD in patients with non-ST elevation acute coronary syndrome has been observed[97,98]. These studies support previous results about the utility of QTc in acute coronary syndrome and additionally provide new elements from QTc applicability in risk stratification for SCD.

Other VR markers have been explored with the purpose to increase the sensibility to predict SCD in patients with acute myocardial ischemia. Ciolli *et al*[99] studied 101 patients with acute myocardial infarction and a control group of 97 healthy patients. After 10 d follow-up, QTd was significantly more prolonged in subjects who developed severe ventricular arrhythmias than control (125.8 ± 68.5 *vs* 80.8 ± 38.9, $P < 0.0005$). Similar results were found by other researchers. In this case, QTd was found to be increased in patients with ventricular tachycardia or ventricular fibrillation compared those with only ventricular premature beats (96.25 ± 15.97 ms *vs* 80 ± 15.04 ms, $P < 0.01$)[100]. Moreover, QTd represented a predictor to death by ventricular arrhythmias. It was a very interesting work about QTd utility in patients with acute myocardial infarction. As can been seen, it was not only prolonged in patients with ventricular arrhythmias but was a predictor of death. This study provides relevant data to continue our investigation about QTd importance in this field.

Tp-e also has been found to be useful to predict cardiac arrhythmias in patients after myocardial infarction. Seventy-six patients with previous myocardial infarction were followed during 23 ± 19 mo. Tp-e was longer in patients who developed ventricular arrhythmias than those without (116 ± 26 ms *vs* 102 ± 20 ms, $P = 0.01$). Additionally, Tp-e was found to be an independent predictor of ventricular arrhythmias when adjusted for age, ejection fraction and QRS duration[101]. Also, QTc has been found to be prolonged in patients with unstable angina with an increased risk for cardiac arrhythmias[102].

Underlying mechanisms to explain modification of these indicators in acute myocardial ischemia include

an expression of M cells properties. Activation of M cells determines an increase in the action potential in the heart and subsequently a QT interval and Tp-eprolongation[14,26-29]. Other proposed mechanisms are the reduction in epicardium temperature[103], acidosis[104] and changes in sodium and potassium currents[105]. These processes increase myocardium heterogeneity and predispose to malignant cardiac arrhythmias.

Reperfusion therapies (fibrinolysis and primary percutaneous coronary intervention) are the primary goal in ST-elevation myocardial infarction for improving clinical outcomes. They have demonstrated to be useful reducing the incidence of cardiac arrhythmias, including ventricular tachycardia and/or ventricular fibrillation[106,107]. There are studies demonstrating the usefulness of VR markers to predict malignant cardiac arrhythmias in this setting. QTc[108], QTd[109-111], Tp-e[112,113] and Tp-e/QT[114] have been found to be useful to indicate effectiveness of thrombolytic therapy and primary percutaneous coronary intervention in patients with acute ST-elevation myocardial infarction. These results indicate patients with reduced risk to develop MVA giving the physician another tool to evaluate the patients' risk in this setting. However, these markers need further study in other situation commonly present in patients treated with reperfusion methods. Patients with ST-elevation acute myocardial infarction develop with relative frequency a transient ST-elevation on ECG when undergoing treatment with fibrinolysis or primary percutaneous coronary intervention. This phenomenon is named "reperfusion peak" and it is followed by a complete ST-resolution[115]. The real significance of this event is not clear. In some studies it has been related with negative cardiac outcomes[116,117]. Application of VR markers in this setting may be important to detect patients at an increased risk for malignant arrhythmias and SCD when presenting with this phenomenon.

Heart failure

Patients with heart failure frequently have ventricular arrhythmias. Ventricular tachycardia and premature ventricular beats are seen with increased incidence in individuals with a dilated left ventricle and reduced ejection fraction[118]. Taking this into account, several studies have been designed to explore arrhythmia markers and to determine the arrhythmogenic risk in these patients.

Davey *et al*[119] found that QTc was significantly longer in heart failure patients than in controls or in subjects with left ventricular hypertrophy (471 ± 10 ms, 421 ± 6 ms, 420 ± 6 ms, $P < 0.05$ respectively). Tp-e also has been found to be useful in this setting. Its prolongation in V1 lead has correlated with increased incidence of SCD in chronic heart failure patients[120]. Tp-e has been evaluated in patients with left ventricular ejection fraction $\leqslant$ 35% and therapy with implantable cardioverter-defibrillator. It demonstrated to be effective predicting ventricular tachycardia and overall mortality[121]. Heart failure is defined as a syndrome derived from multiple cardiac and non-cardiac conditions. It has a complex pathophysiology and mechanisms proposed for the development of MVA are diverse and include: ischaemia, infarction, cardiomyopathy, myocarditis, hypokalaemia, hypomagnesaemia and digitalis overdose[118]. All of these processes may increase the ventricular heterogeneity and predispose to MVA. Some factors which could be associated are age, aetiology, drugs and comorbidities. As heart failure may be caused by multiple conditions, to analyse the risk for MVA can be very difficult.

Hypertension

Hypertension is a very common condition worldwide. Its prevalence is around 30%-45% in the general population[122]. Hypertensive patients have an increased risk for cardiac arrhythmias due to an increase in VR dispersion[123]. Left ventricular hypertrophy often develops in hypertension and it increases the risk of developing cardiac arrhythmias[124,125]. Carmona Puerta *et al*[126] observed that QTd and Tp-e were more prolonged in patients with left ventricular hypertrophy than those without it. Also, a linear correlation among QTd, Tp-e and duration of hypertension was observed ($r = 0.453$, $P = 0.001$ and $r = 0.306$, $P = 0.034$ respectively). Mozos *et al*[127] support these findings with similar results. In another investigation, QTc and Tp-e were associated with systolic blood pressure, body mass index, and left ventricular mass in resistant hypertensive patients[128]. The prognosis of patients with resistant hypertension is probably worse compared with those that have easily controlled hypertension. However, it has not been specifically evaluated. It may be influenced by some risk factors or associated conditions[129]. This investigation may give us a new alternative to evaluate the prognoses of resistant hypertensive patients by the analyses of VR markers. However, prospective studies would be required to achieve definite conclusions.

Recently, an investigational group demonstrated that in hypertensive patients without ischemic heart disease the global cardiovascular risk is related to some electrocardiographic markers for cardiac arrhythmias. QTc and Tp-e showed the most significant correlation ($P = 0.010$ and $P = 0.000$ respectively)[130]. Global cardiovascular risk may be examined by several methods. In this study the risk score proposed in the 2007 European Guidelines for the Management of Hypertension was used. The use of global cardiovascular risk scores associated with VR markers could help to identify patients at high risk of malignant cardiac arrhythmias. It could represent a novel tool for physicians in the future for better patients' management.

Further studies should be conducted to explore other arrhythmias markers and global cardiovascular risk scores.

There are several pathophysiological mechanisms to explain the predisposition to develop MVA in hypertensive patients. Left ventricular hypertrophy is found often in these cases and is recognized as the most important factor. It correlates with malignant arrhythmias and

SCD[131]. Left ventricular hypertrophy causes early after-depolarization and favors the occurrence of ventricular arrhythmias[132]. It may also cause myocardial ischemia by an unbalance between blood supply to the myocardium and oxygen consumption. Moreover, in this condition it may cause an increase in subendocardial ischemia due to a reduction in diastolic blood flow to this region[133]. These processes may be more intense in long-term hypertensive patients' in which left ventricular hypertrophy is more frequent. In fact, QTd has been found to be increased in elderly hypertensive individuals with the presence of left ventricular hypertrophy and myocardial ischaemia on ECG[134]. Myocardial ischemia increases cardiac heterogeneity and the predisposition to SCD.

Other changes in the heart of hypertensive patients include arise of collagen deposits as part of ventricular remodeling. Ventricular remodeling favors ventricular heterogeneity and ventricular re-entry arrhythmias[135]. In hypertensive patients there is often an increase in sympathetic nervous system activity, and activation of this system has been associated with increased cardiac arrhythmias[136]. Additionally, alterations in gap-junctions in myocardial cells and an elevated risk to develop ventricular arrhythmias in hypertensive animal models with hypokalaemia has been observed[137].

Diabetes mellitus

Diabetes mellitus had a prevalence of 360 million people worldwide in 2011. This prevalence will increase to 552 million people with diabetes by 2030[138]. SCD is often observed in diabetic patients and may be associated with ventricular arrhythmias[139,140]. Ventricular arrhythmias predictors in these patients have been evaluated in several studies.

Patients with type 1 diabetes mellitus and autonomic dysfunction have shown significantly higher values of QTd than patients without autonomic dysfunction and controls. After a follow-up by 24 h holter monitoring, individuals with autonomic dysfunction and a prolonged QTd had a higher incidence of ventricular arrhythmias[141].

Clemente *et al*[142] designed an investigation with the aim to study the effects of diabetes mellitus on VR markers. A group of 110 diabetic patients and a group of 110 controls were selected. Maximum QTc was significantly greater in diabetic patients than in controls (413.70 ± 28.10 ms *vs* 395.31 ± 16.28 ms, $P < 0.001$). Diabetics had a significantly higher mean QTd than controls (27.49 ± 10.10 ms *vs* 15.73 ± 4.18 ms, $P < 0.001$). Similar results were found with the measurement of QTd corrected by heart rate (29.92 ± 10.57 ms *vs* 16.68 ± 4.48 ms, $P < 0.001$).

More recently an investigational group found that QTc and QTd are prolonged in newborns of diabetic mothers. QTd prolongation was associated with inter-ventricular septal thickness at end diastole ($r = 0.514$, $P = 0.042$). The authors concluded that elevated values of these markers represent risk factors for the development of arrhythmias in these patients[143].

The results of these studies are encouraging, but further studies are needed to know with more precision the risk of diabetic patients to develop malignant cardiac arrhythmias. It should be important to evaluate other markers to know whether they may be added to the risk estimation in these patients.

The proposed mechanisms explain predisposition to develop cardiac arrhythmias in diabetic patients are diverse. They have a high incidence of coronary atherosclerosis, microvascular disease and autonomic neuropathy. The arrhythmogenic substrate, may be in part, due to compensatory hypertrophy in non-infarcted myocardium, progressive ventricular remodeling and neurohormonal abnormalities[144,145]. These processes increase VR heterogeneity and elevate the risk of re-entry arrhythmias.

Obesity

Obesity is a common condition worldwide. It is present in developed and non-developed countries and affects peoples of all ages. People with obesity have an increased risk to develop several diseases which worsens significantly their prognosis[146,147]. Several studies have demonstrated alterations in VR markers in overweight or obese people which appear to increase risk for MVA in these cases. Mshui *et al*[148] evaluated maximum and minimum QTc in obese patients before and after therapeutic weight reduction. Both QTc values were significantly longer in obese patients than controls before weight reduction. With weight reduction these markers were reduced significantly in the obese group. The authors concluded that obesity is a cause of prolongation of this marker and that it may be modified by weight reduction. Positive effects on reduction in QTc values with a low calorie diet followed by a weight reduction were observed in a previous study[149] supporting the benefits of weight loss to improve the QTc. Continuing on this topic, Seyfeli *et al*[150] studied both QTc and QTd in obese women and controls with m (B) index = 40 ± 3 kg/m^2 *vs* 22 ± 1 kg/m^2, $P < 0.001$ respectively. A significant correlation was found with m (B) index, maximum QTc and QTd ($r = 0.410$, $P < 0.001$ and $r = 0.429$, $P < 0.001$ respectively). Another work, found similar outcomes, but failed to find a statistically significant association among uncomplicated obesity and overweight with QTd, Tp-e, Tp-ed and Tp-e/QT[151].

Minimal myocardial dysfunction may be detected in obese patients even in the absence of any apparent symptoms[152]. These primary heart changes may increase the cardiac heterogeneity and could explain prolongation of these markers observed in uncomplicated obesity. For other hand, some conditions such as hyperinsulinemia, glucose intolerance and autonomic dysfunction may affect the values of these markers increasing the risk of ventricular arrhythmias[150]. Heterogeneity of samples involved in these studies and possible association of obesity/overweight with other comorbidities are factors which may modify and explain these results. These investigations show that QTc

appears to be the most useful marker in these patients, but to achieve definite conclusions further studies should be performed.

Highly trained athletes

SCD has been observed with higher incidence in athletes than the general population[153]. It is the main cause of death during exercise. MVA may play an important role in these cases. Several studies have examined the arrhythmogenic risk in these patients. QTc has been found more prolonged in athletes than non-athletes. It was observed by Lengyel *et al*[154]. They studied 76 professional soccer players and 76 controls with age mean 22.0 ± 0.61 and 22.0 ± 0.54 respectively. The maximum QTc by Fridericia and Hodges formulas were significantly longer in athletes than controls. Lawan *et al*[155] studied variations of QTd in 100 dynamic athletes, 50 static athletes and 100-matched controls. Results showed that both groups of athletes had an increased prolongation of QTd and that it significantly related to duration of physical activity. In other investigations it was found that maximum QTc, QTd and Tp-e were more prolonged inelite female water polo players than controls[156].

Currently the results derived from application of these markers to predict malignant cardiac arrhythmias in elite athletes are inconsistent. Maximum QTc has demonstrated to be shorter in professional soccer player than controls 413.9 ms *vs* 445.3 ms, $P < 0.001$ respectively)[157]. Similar results have been obtained by other researchers[158-160]. There are some points which should be analyzed in future studies and could represent possible explanations to contradictory outcomes after application of these markers in several setting of high trained physical activity: (1) Lack of identification of associated comorbidities; (2) Cardiac modifications presented in these cases (*e.g.,* left ventricular hypertrophy; (3) Exercise type depending on each sport characteristics; (4) Duration and intensity of physical activity; and (5) Recovery after highly trained activity.

These elements may act alone or combined to explain why VR markers are prolonged in athletes in some studies but not in others. The development of left ventricular hypertrophy has been widely studied and commonly associated with no prolongation of these parameters. In this case it could be a shield factor against the appearance of malignant cardiac arrhythmias and for that these markers are reduced compared with non-athletes. It has been observed that patients with left ventricular hypertrophy induced by physical training activity have shorter values of QTd than those with pathological left ventricular hypertrophy by hypertension[159].

As was discussed above, SCD occurs with increased incidence in athletes. Many of these cases are secondary to conditions which predispose to malignant cardiac arrhythmias. For that reason and due to the usefulness of these markers in other clinical conditions there should be continued investigation in this field. Other more expensive tests could be avoided in these patients if ECG through the analysis of these markers demonstrates to be effective in these cases.

Perspectives

The prevalence of malignant cardiac arrhythmias and their association with SCD is relatively frequent. They may occur in healthy individuals without structural cardiac abnormalities or be found in other medical conditions. There is the potential to predict their development and subsequently design strategies to avoid adverse outcomes in our patients through the analysis and interpretation of VR markers. So far, the predictive capacities of VR markers have demonstrated to be useful in some clinical situations but not completely in others. Some of these predictors should be explored further, such as Tp-ed and Tp-e/QT which are relatively new in clinical practice. In order to increase their value, they need to be studied in healthy patients to know normal values of markers by sex, age and maybe among races. For achieve this aim it will be important to design studies with long-term follow up and many patients. However, this approach will be difficult in some conditions which have low incidence in the general population such as long and short QT syndromes and Brugada syndrome. A way to increase the sensitivity of these markers could be to combine several of them during studies in order to reduce the margin of error.

There are other medical conditions not discussed in this manuscript in which markers have demonstrated positive results[161-177] such as Kawasaki disease, systemic lupus eythematosus, rheumatoid arthritis, chronic renal failure, scleroderma, Duchenne muscular dystrophy, liver transplantation, HIV-infected patients, rheumatic fever and Chagas disease among others (Table 1). The interest in these ventricular repolarization markers has grown and has been introduced novel predictors with good results[120,178-181]. The QT variability index has been associated with SCD. A study by Piccirillo *et al*[120] demonstrated that patients with chronic heart failure with left ventricular ejection fraction $\leqslant$ 35% and SCD have higher values of QT variability index than in the group of left ventricular ejection fraction > 35%. The QT/RR and Tp-e/RR slopes have demonstrated to be useful to show loss of rate-dependent property in patients with Brugada syndrome with history of ventricular fibrillation[178]. Another marker commonly studied in patients with ventricular tachycardia is JT interval and its correction by heart rate. A study population of 20 patients with idiopathic ventricular tachycardia and 30 controls demonstrated that JT interval and its correction by heart rate were higher in patients with previous history of malignant arrhythmias JT (272 ± 36 ms *vs* 265 ± 25 ms, $P = 0.01$), JTc (336 ± 28 ms *vs* 318 ± 18 ms, $P=0.01$)[179]. T wave alternant have been proposed has a marker for heart heterogeneity and lead to electrical instability. These phenomenon have often found in patients with long QT syndrome that develop

MVA[180]. Also, it has been assessed the value of T wave alternant in predicting SCD in patients after an acute myocardial infarction[181].

Currently there is a growing evidence to support the use of these markers to evaluate the risk of ventricular arrhythmias. However, it should be necessary to discuss some possible limitations on their use: (1) The manual determination of T wave offset is unreliable, principally when there is a low T wave amplitude and merges of T waves with U and/or P waves. In order to reduce the margin of error it has been developed automatic methods[5]. A precise calculation of some markers as Tp-e could be difficult in clinical practice and a computer method may represent a better choice; (2) For an accurate assessment of QTd is necessary all 12 leads of the ECG to be recorded simultaneously in order to avoid the effect of QT dynamicity[5]. However, not always is possible to achieve this aim due to mainly to technological deficit in non-developed countries; and (3) Although the utility of these markers for predicting malignant arrhythmias has been recognized, there are some conditions such as long and short QT syndromes, Brugada syndrome and early repolarization pattern when the patients' risk stratification must be evaluated taking into account another variables such as gender, age, family history and gene mutations.

These markers extend our alternative for arrhythmias' prediction. It is necessary to continue medical studies in this field with the aim to clarify some aspects discussed above and to achieve a global consensus which would result in better management of our patients.

CONCLUSION

The QTc, QTd, Tp-e, Tp-ed and Tp-e/QT have been found to be useful in predicting malignant cardiac arrhythmias in multiple medical conditions, but further long-term studies are needed. The aim should be to include them as tools to evaluate arrhythmogenic risk and as a way to improve clinical management of patients.

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Intestinal obstruction due to phytobezoars: An update

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Abstract

The term bezoar refers to an intraluminal mass in the gastrointestinal system caused by the accumulation of indigestible ingested materials, such as vegetables, fruits, and hair. Bezoars are responsible for 0.4%-4% of cases of mechanical intestinal obstruction. The clinical findings of bezoar-induced ileus do not differ from those of mechanical intestinal obstruction due to other causes. The appearance and localization of bezoars can be established with various imaging methods. Treatment of choice depends on the localization of the bezoar which makes the clinical findings.

Key words: Phytobezoar; Intestinal obstruction; Ileus

Core tip: Bezoars are conglomerates of indigested foreign material that accumulate in the gastrointestinal tract. They are responsible for 0.4%-4% of cases of mechanical intestinal obstruction although the true incidence is not known. Recent advances in imaging methods have facilitated the diagnosis of intestinal obstruction due to phytobezoars. The most valuable method for determining the location and etiology of intestinal obstructions is contrast-enhanced computed tomography. This review aims to summarize the definition and history, causes of bezoar formation, clinical findings, diagnostic methods, treatment of these rare intestinal obstructions caused by phytobezoars.

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DEFINITION AND HISTORY

The term bezoar refers to an intraluminal mass in the gastrointestinal system caused by the accumulation of indigestible ingested materials, such as vegetables, fruits, and hair[1,2]. Called "panzehr" in Arabic and "padzhar" in Persian, the term means antidote[3]. Although there is written evidence of bezoars identified in animal and human gastrointestinal systems in the 10th century AD, the first scientific definition was made by Baudmant[1,2] with the publication of a case of trichobezoar in 1779. In 1854, Quain named the mass of intragastric food residue found at autopsy a "bezoar". The first preoperative trichobezoar case was reported by Stelzner[4] in 1894.

Bezoars are named according to the material they are made of: a trichobezoar consists of hair; a phytobezoar of vegetable and fruit residues; a lactobezoar is formed from dairy products; a pharmacobezoar is caused by medications; and a polybezoar is caused by ingested foreign bodies[3,4]. In addition, Chintamani *et al*[5] identified biliary bezoars caused by bile stasis following hepatobiliary or gastric diversion surgery. The most common type of bezoar is the phytobezoar, which consists of indigestible food residue, such as cellulose and hemicellulose.

Trichobezoars are generally seen in individuals with trichophagia, a psychiatric disorder that usually occurs during childhood and in young adults. Trichobezoars are generally located in the stomach. However, if trichophagia is not noticed by the parents and if it becomes prolonged, then a condition called Rapunzel Syndrome can develop; it starts with hair in the stomach and the bezoar extends into the small intestine[3,4]. Since a psychiatric disorder underlies trichobezoar, recurrence is inevitable if insufficient psychiatric support is provided after surgical treatment[6]. Pharmacobezoars are usually caused by Kayexalate (sodium polystyrene sulfonate), cholestyramine, and antacid medications[7]. Lactobezoars generally occur in low-birth-weight newborns as a result of concentrated baby formulas[4].

CAUSES OF BEZOAR FORMATION

Bezoars are responsible for 0.4%-4% of cases of mechanical intestinal obstruction, although the true incidence is not known[8-10].

Previous gastric surgery

Gastric motility disorders and hypoacidity after gastric surgery are the basis of bezoar formation. Bezoars located in the stomach can pass through the small intestines easily and cause symptoms of intestinal obstruction, especially in patients with pyloric dysfunction after a pyloroplasty or wide gastrojejunostomy, resulting in a wide gastric outlet[3,11-13]. In a study of 42 diseases, Kement *et al*[14] reported that previous gastric surgery was the most important factor predisposing to bezoar formation, with a rate of 48%. In their series, Krausz *et al*[15] and Bowden *et al*[16] reported rates of 20% to 93%. Bezoar-associated ileus is more common in cases undergoing surgery for ulcer treatment, although this has become more rare with the introduction of proton pump inhibitors[16].

In patients who have had surgery for ulcer treatment, a vagotomy accompanied by a partial gastrectomy is the most important factor predisposing to bezoar formation[9-17]. A vagotomy and partial gastrectomy reduce gastric acidity, negatively affecting peptic activity. Furthermore, the antrum has an important role in the mechanical digestion of ingested food. The pylorus also prevents ingested food from passing through the small intestine as bolus, contributing to digestion. In this regard, the risk of bezoar formation was higher in patients who had a pyloroplasty and antrectomy[14,15]. The time taken for a bezoar to form after gastric surgery ranges from 9 mo to 30 years[15].

Bezoars can also form primarily in the small intestine when a mechanical factor alters the small intestinal passage, such as a diverticulum, stricture, or tumor[14,17]. Primary bezoars of the small intestine almost always cause intestinal obstruction. The most common location of obstruction is the terminal ileum[18].

High-fiber diet

High-fiber foods such as celery, pumpkins, grape skins, prunes, and especially persimmons are a risk factor for bezoar formation[14,15]. Persimmons, which means the "God of fruits" in Greek, are the fruit of plants in the genus *Diospyros*. Immature persimmons contain tannins, which form an adhesive-like substance when they encounter acids and hold other food residues, causing bezoar formation[14]. Krausz *et al*[15] and Erzurumlu *et al*[9] reported that 17% to 91% of bezoars in their series were caused by persimmons.

Other factors

Other factors predisposing to bezoar formation include systemic diseases such as hypothyroidism causing impaired gastrointestinal motility, postoperative adhesions, diabetes mellitus, Guillain-*Barré* syndrome, and myotonic dystrophy. Personal factors such as swallowing a large amount of food without sufficient chewing due to dental problems, especially in the elderly, the use of medications slowing gastrointestinal motility, and renal failure are also predisposing factors[4,19-21]. Erzurumlu *et al*[9] suggested that bezoar formation could occur without any predisposing factors.

CLINICAL FINDINGS

The clinical findings of bezoar-induced ileus do not differ

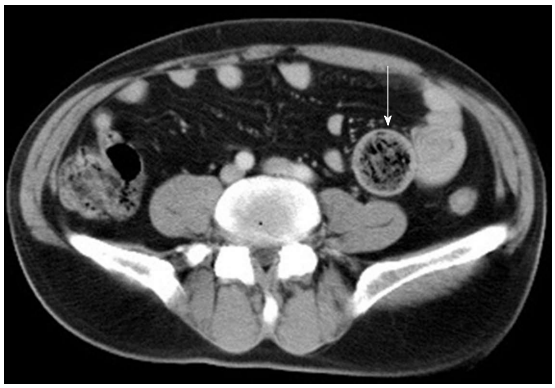

Figure 1 Intraluminal round bezoar and mottled gas pattern were seen in the jejenum segment. Wall thickening due to inflammation were seen at the obstruction site (arrow).

from those of mechanical intestinal obstruction due to other causes. Almost all patients have poorly localized abdominal pain that is similar to ischemic pain. Other symptoms include abdominal distention, vomiting, nausea, a sense of satiety, dysphagia, anorexia, weight loss, gastrointestinal hemorrhage, and constipation[22,23].

It is generally difficult to determine whether bezoars are the clinical cause of ileus. The great majority of patients have a history of abdominal surgery, and adhesions following previous surgery are often responsible for ileus[24,25]. To reduce mortality and morbidity, it is important to consider bezoars in patients with a history of gastric surgery because the treatment of intestinal obstruction suspected of being induced by bezoars is mostly surgical. Prompt treatment can minimize the complications that might develop during medical follow-up.

DIAGNOSTIC METHODS

Recent advances in imaging methods have facilitated the diagnosis of ileus[24-27]. The air-fluid levels associated with mechanical intestinal obstruction can be seen on plain X-rays in most patients, but plain radiographs are not useful for differentiating other causes of ileus[27].

The appearance and localization of bezoars can be established with barium studies, which are effective for differentiating diverticular disease, intraluminal adenomas, primary malignancies of the small intestine causing mechanical obstruction, and bezoars[27,28]. However, these studies are not applicability in an emergency setting, can exacerbate peritonitis in the presence of perforation, and increase symptoms in complete obstruction[28].

Ultrasonography can detect the cause in 88%-93% of bezoar-induced ileus[28,29]. Typically, bezoars create hyperechoic acoustic shading on ultrasonography. However, the place of ultrasonography is controversial, since the examination is operator-dependent and requires experience. Furthermore, the air-fluid levels in the obstructed intestines block the view and ultrasonography has low sensitivity when there are multiple bezoars[30].

The most valuable method for determining the location and etiology of intestinal obstructions is contrast-enhanced computed tomography (CT) (Figure 1). The sensitivity and specificity of abdominal CT for bezoar-induced ileus are 90% and 57%, respectively[29,31]. Abdominal CT is effective for excluding other causes of intestinal obstruction. The advantages of CT are its ability to detect dilatation and edema in the intestinal loops, the presence of intra-abdominal free fluid, and the level of obstruction and development of strangulation[31]. Zissin *et al*[32] reported that a round, mottled intraluminal mass in the area of obstruction with dilated intestinal loops proximally and collapse distally was a pathognomonic CT finding for a bezoar resulting in ileus. Air bubbles might be seen within bezoars. When there are multiple bezoars, intraluminal bezoars distant from the area of obstruction area might go unnoticed if not sought carefully[28,31,32].

Feces in the small bowel can appear similar to a bezoar radiologically and are seen in about 8% of the patients treated for intestinal obstruction[32]. Their radiological differentiation from bezoars is important because the treatment is generally medical. Small bowel feces generally appear in a longer segment than the bezoar and cause sharp-margin dilatation. Zissin *et al*[32] reported that the most evident radiological feature for differentiating a bezoar and small bowel feces was the longer transition zone of the feces-like view in the dilated segments proximal to the obstruction in small bowel feces compared to bezoar.

Preoperative CT assessment in patients with suspected intestinal obstruction induced by bezoars is helpful for determining the timing of surgery. When a bezoar is detected on CT, the surgery is generally performed within 48 h[31]. For small intestinal obstructions thought to result from non-bezoar causes, such as previous surgery, most patients can be treated medically, instead of surgically. A preoperative CT assessment allows the diagnosis of complications, such as perforation, necrosis, and ischemia secondary to bezoar-induced obstruction, and facilitates the planning of the timing of the surgical intervention[32].

TREATMENT

The initial treatment of bezoars causing obstruction is no different from that for obstructions of other etiologies. With intestinal obstruction, fluid deficiency and electrolyte imbalances result from vomiting and fluid accumulation within the intestinal segment. Therefore, the first step in treatment is intestinal decompression and fluid-electrolyte replacement[33].

The most common location of bezoars in the gastrointestinal system is the stomach, although gastric bezoars usually do not cause obstruction. Therefore, endoscopic methods have become the main treatment. Mechanical disintegration (mechanical lithotripter, large polypectomy snare, electrosurgical knife, drilling, laser destruction, and a Dormia basket for extraction) and chemical dissolution (saline solution, hydrochloric acid,

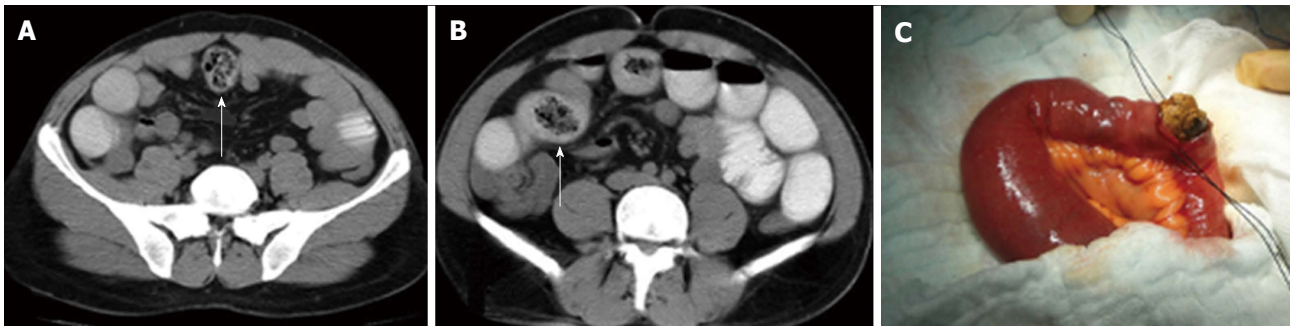

Figure 2 A 62-year-old male patient. Multiple small bowel bezoars which have round or irregular shape and mottled gas pattern were detected on abdominal computed tomography (A and B; arrows). Intraoperative finding (C); an irregular shaped bezoar which caused intestinal obstruction was removed *via* enterotomy, dilated proximal segments and non-dilated distal segments are visible.

sodium bicarbonate, and Coca-Cola lavage) methods have been described[33,34] Ladas *et al*[35] reported a very high success rate (90%) in gastric bezoars using a combination of mechanical and chemical methods. The treatment of choice is surgical for gastric bezoars in which endoscopic treatment failed.

Bezoar-induced intestinal obstructions occur mostly in the distal small intestine[35,36]. The greater width of the colon lumen reduces the possibility of colonic mechanical obstruction due to bezoars, although a few rare cases of colon obstruction have been reported in children[37,38].

Bezoar-induced intestinal obstructions usually occur 50-70 cm proximal to the ileocecal valve[9], because of the reduced lumen diameter towards the distal end, the lower motility in the distal small intestine, and the decreased motility of bezoars due to the increased water absorption at the distal end[36].

When surgical treatment is chosen, open or laparoscopic abdominal exploration may be performed[39]. Laparoscopic exploration is currently used more frequently; however, it requires technical experience because of the presence of dilated intestinal segments. It also requires a good preoperative radiological study to localize the bezoar because there can be multiple bezoars and there are reports of cases requiring surgery for an unnoticed bezoar[40]. The factors to be considered when selecting the laparoscopic method include the size and number of bezoars, the presence of complications (such as perforation and peritonitis), and previous abdominal operations[39,41]. In a study of 24 diseases, Yau *et al*[42] reported that the laparoscopic method had more advantageous outcomes when used in selected patients compared to the open method in terms of operating time, complication rates, and duration of hospital stay. Nirasawa *et al*[43] reported treatment outcomes with the laparoscopic method in patients with gastric bezoars and Son *et al*[44] reported such outcomes in patients with gastric bezoars that could not be treated endoscopically. Fraser and Song emphasized that the laparoscopic technique might be used safely in selected patients with giant gastric bezoars[45,46]. Son *et al*[44] discussed the risk of intra-abdominal contamination and the location of the gastric incision as the points to be considered for laparoscopic gastric bezoar surgery.

Laparoscopic interventions are being performed increasingly; however, open surgery is still the most common method used for the surgical treatment of bezoar-induced intestinal obstructions. The review by Gorter *et al*[47] reported that laparotomy intestinal bezoar treatment was successful in more than 100 studies.

After determining the need for surgical intervention, the issue of what kind of intervention should be performed arises. The decision to perform an enterotomy to remove bezoars varies with experience[9]. Surgeons often use the milking technique, which is used to advance the intestinal contents manually either proximally or distally. However, a laceration in the intestinal serosa or mesentery can occur during this procedure[36,39]. Aysan *et al*[48] reported that rats undergoing milking developed significantly more peritoneal adhesions compared to the control group and that the peritoneal cultures were positive.

The bezoar location in the gastrointestinal system (duodenum, jejunum, or ileum) plays an important role in selecting the surgical method (Figure 2). Oh *et al*[49] reported that an enterotomy was more successful for bezoars located in the proximal small intestine. For the patients with ischemia and perforation caused by bezoars, anastomosis or stoma procedures should be performed with segmental small bowel resection[49-52].

In conclusion, the possibility of bezoars must be considered in patients with mechanical intestinal obstruction, although they are rare. This possibility must be considered especially in regions where there is excess consumption of high-fiber food, which causes bezoar formation, in patients with intestinal obstruction, and in patients who have a history of abdominal surgery for a peptic ulcer. Early preoperative contrast-enhanced CT assessment aids both the diagnosis and decision for early surgical treatment. Surgery is usually chosen for bezoars causing intestinal obstruction. In addition to the physical characteristics of the bezoar, its location should be considered when selecting the surgical procedure.

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CASE REPORT

T-lymphoblastic lymphoma with cutaneous involvement

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Abstract

To study dermatological manifestation of T-lymphoblastic lymphoma and to help clinicians in the diagnosis, we report here the case of a 75-year-old patient who presented with violaceous nodules acquired during the last 4 wk and affecting the scalp and right arm. The diagnosis of systemic lymphoma was suggested upon the appearance of cutaneous tumors, palpable lymph nodes and general symptoms including asthenia and weight-loss. The pathology features: positive immunostaining for CD3 and terminal deoxynucleotidyl transferase (TdT) and staging, led us to the final diagnosis of T-lymphoblastic lymphoma (T-LBL) with cutaneous involvement. He received a CHOP regimen as first-line treatment. Unfortunately, the patient relapsed and died 8 mo after the treatment initiation. T-LBL may be diagnosed by skin lesions. Additional immunostaining including TdT and experienced histopathologists are needed to correctly classify this aggressive disease and discuss the correct management including bone-marrow transplantation where appropriate.

Key words: Cutaneous lymphoma; Lymphoblastic lymphoma; Skin

Core tip: The clinical presentation of our patient with disseminated erythematic patches and infiltrated nodules suggested a diagnosis of cutaneous involvement of T-lymphoblastic lymphoma (T-LBL). Finally, histopathological examination of a skin biopsy with immunohistochemical study established the diagnosis of T-LBL. For accurate diagnosis, experienced histopathologists are needed. We wish to add this case to the current literature of T-LBL with cutaneous involvement,

emphasizing the importance of a correct diagnosis and aggressive treatment.

Ginoux E, Julia F, Balme B, Thomas L, Dalle S. T-lymphoblastic lymphoma with cutaneous involvement. *World J Clin Cases* 2015; 3(8): 727-731 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i8/727.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i8.727

INTRODUCTION

T-lymphoblastic lymphoma (T-LBL) is a rare subtype of adult non-Hodgkin lymphoma (NHL) which represents almost 2% of lymphoid leukemias. This disease arises from precursor T lymphocytes and is associated with a very poor prognosis. Although skin involvement occurs in less than 20% cases it is often the first observable manifestation of the disease and leads to the correct diagnosis. By adding this case, we hope to help clinicians in this diagnosis which needs to be made fast as possible.

CASE REPORT

A 75-year-old patient was referred to our out-patient unit following the rapid onset of several reddish nodules associated with loss of weight and fatigue. His past medical history was marked by type 2 diabetes, sarcoidosis, hypertension, and prostatectomy for prostate cancer 5 years previously.

On physical examination, we observed 2 large cutaneous nodules: the first was located on his right arm (Figure 1) while the second was on the occipital area (Figure 2). The skin lesion on the arm was purplish asymptomatic and measured 10 cm on the larger diameter. The second one was a large purple painful nodule located on the occipital part of the scalp. They were both deeply infiltrated into the sub-cutaneous fat, non-pruriginous and rapidly growing. He also presented several red patches on trunk and back. On general examination we noticed bilateral palpable superficial lymph nodes on the axillary, cervical, and inguinal areas. He complained about extreme fatigue and up to 10% weight loss during the last 2 mo. Additional B-symptoms such as fever and nightly sweats were absent.

Blood samples analysis showed a lymphocytosis at 4.14 G/L, including 20% of atypical lymphocytes with irregular chromatin and a high nucleo-cytoplasmic ratio. By flux cytometry, 93% of the atypical population was CD4 positive, 50% was also CD3 positive while, of the tumoral cells, 17% aberrantly expressed CD4 and CD8.

Histological examination of skin biopsy specimens from the arm lesion revealed that the dermis was infiltrated by monomorphic medium size lymphoid cells. This infiltrate was dense and diffuse infiltrating collagen bundles. The tumoral cells showed a scant cytoplasm, and an irregular prominent nucleus with dispersed chromatin and inconspicuous nucleoli. The epidermis was spared. The high number of mitotic figures rate suggested an aggressive process (Figure 3). Few mitotic figures were seen.

Immunostaining was performed on skin samples. The tumoral infiltrate was positive for CD3, CD5, CD4 (Figure 4), ki67 + (Figure 5) and negative for EMA (Epithelial Membrane Antigen), CD34, CD68, CD10, CD30, CD20, CD79, CD23, CD56, CD8, and myeloperoxidase. Terminal deoxynucleotidyl transferase (TdT) marker, a specialized DNA polymerase expressed in immature, pre-B, pre-T lymphoid cells, and acute lymphoblastic leukemia/lymphoma cells, was found positive on paraffin embedded biopsy (Figure 6). Bone marrow aspiration showed an identical intramedullar lymphocytic T-cell proliferation, thus resulting in a diagnosis of lymphoblastic lymphoma. A total body computed tomography-scan showed multiple tumoral lymph nodes on cervical, axillar, inguinal and deeper areas.

The diagnosis of T-LBL $CD4^+$, $CD56^-$ with cutaneous involvement and leukemic involvement was made. The Ann Arbor stage was Ⅳ B and E.

The patient was then referred to the haematology department. This patient was not eligible for bone marrow transplantation. Because of the aggressiveness of the disease, a CHOP regimen (cyclophosphamide, doxorubicin, vincristin, prednisone) was immediately started as first-line treatment. Despite a short-term partial response, the patient relapsed after the fourth cycle of chemotherapy and was subsequently included in a Holoxan (ifosfamide) Vepeside (etoposide) therapeutic protocol. After 3 cycles of this chemotherapy a clinical remission occurred and because of poor hematologic tolerance (grade 4 leucopenia and thrombopenia) the treatment was discontinued. The patient relapsed and was admitted into a palliative care strategy. He eventually died 8 mo after the initial diagnosis.

DISCUSSION

T-LBL is a rare subtype of adult non-Hodgkin lymphoma with an incidence representing approximately 2% of NHL cases[1]. Lymphoblastic lymphoma predominates in young adults and teenagers with a median age at diagnosis of 20 years. It has a poorer prognosis compared to B-LBL. In adults the overall survival rate at 5 years is 26%[2-6]. Prognostic factors for adult T–LBL have not so far been determined.

This disease is the malignant counterpart of the precursor T-cell lymphocytes. The lymphoblasts infiltrate lymph nodes or extranodal structures, especially the bone marrow, spleen, and central nervous system[3]. It is characterized by male predominance, high rate of lactate dehydrogenase, Ⅲ or Ⅳ Ann Arbor staging and mediastinal involvement[2] (Table 1). Typically, patients with lymphoblastic lymphoma are diagnosed with painless lymphadenopathy, splenomegaly, neurologic deficits or testicular masses[2].

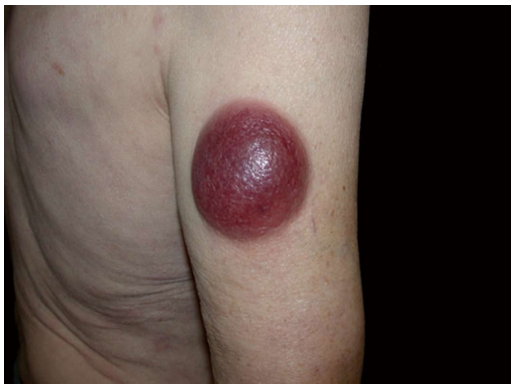

Figure 1 Right arm's nodule, 6 cm × 6 cm in diameter.

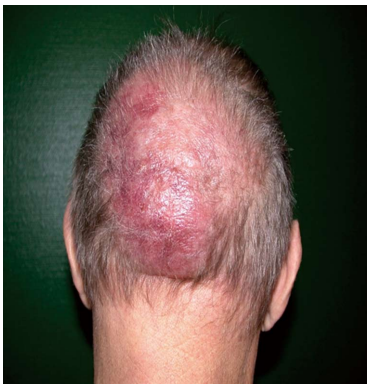

Figure 2 Vertex's nodule, 16 cm × 13 cm in diameter.

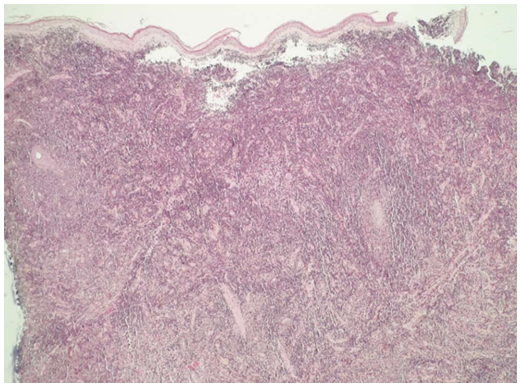

Figure 3 Histological aspect of a skin biopsy of the right arm's nodule (HE).

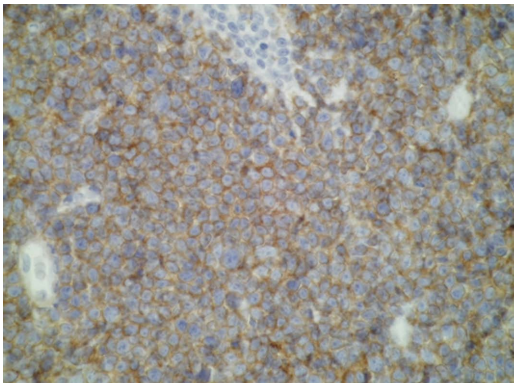

Figure 4 Tumour cells positive for CD4.

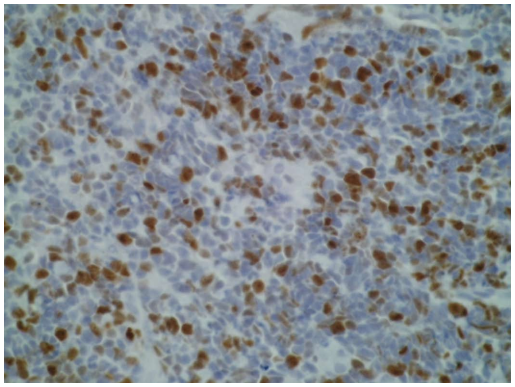

Figure 5 Tumour cells positive for ki67.

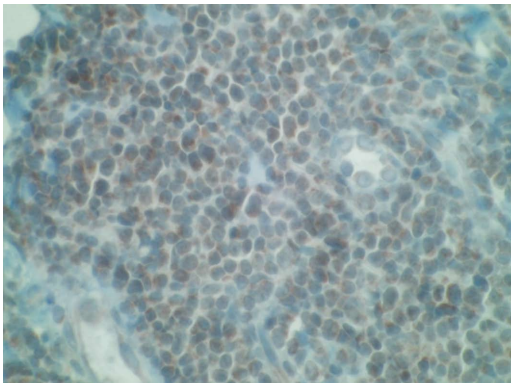

Figure 6 Positive terminal deoxynucleotidyl transferase marker.

Involvement of the skin in T-LBL is rare, although the exact frequency at which this occurs has not been calculated[4]. In the majority of reported cases, the cutaneous involvement is secondary or concomitant to bone marrow or lymph node spreading[6]. Skin involvement is more common in association with B-LBL.

Diagnosis is commonly made thanks to an excisional node biopsy or exploration of extranodal sites. Typically, histology with hematoxylin-eosin coloration shows a monomorphous dermis-infiltrate of small to medium-sized lymphoid cells with scant cytoplasm, moderately condensed to dispersed chromatin and prominent nucleus with inconspicuous nucleoli[5]. Epidermotropism is not present and the grenz zone is located beneath the epidermis. In some areas, the tumor cells can be arranged in a mosaic-like pattern[4]. However, diagnosis is not made by Hematoxylin-Eosin sections alone; immunohistochemical studies are also needed to diagnose T-LBL. T-LBL is confirmed when lineage markers for B-cells (CD20) and NK cells (CD56) are negative, and those for precursor T cells (CD3 and CD5) are positive, whilst expression of ki67 proves malignancy. TdT expression is seen only in lymphoblastic lymphoma (B-LBL and T-LBL) contrary to the mature lymphoma[5]. A recent study based on cytogenetic analysis and gene expression profiling

Table 1 Clinical presentation of the patients with cutaneous T-lymphoblastic lymphoma, cases from the review of literature

Patient	Sex/age	Staging	Cutaneous involvement	Extracutaneous lesions
1[2]	M/16	Stage 4	Subcutaneous nodule on the left side of neck	BM involvement, lymph nodes
2[2]	M/20	Stage 4	Multiple scalps nodules	Prostate involvement
3[2]	M/25	Stage 4	Multiple subcutaneous nodules on left side of face, scalp, and chest wall	BM involvement, lymph nodes, scrotal involvement
4[2]	M/17	Stage 4	Subcutaneous nodules on left side of neck and abdomen	Mediastinal mass, BM involvement, lymph nodes
5[2]	F/25	Stage 4	Multiple subcutaneous nodules on right breast, right side of neck, chest wall, and both legs	BM involvement, lymph nodes
6[2]	M/39	Stage 4	Multiple nodule on scalp	Mediastinal mass, lymph nodes, BM, bone, left kidney, left pleura involvement
7[2]	M/22	Stage 4	Multiple subcutaneous nodules on back	Mediastinal mass, lymph nodes, tonsil, nasopharyngeal involvement
8[7]	F/24	Stage 4	Multiples nodules on malar regions of face, breast, trunk	Lymph nodes, conjunctiva
9[7]	M/25	Stage 4	Nodule on scalp	Mediastinal mass
10[8]	M/8	Stage 1	Nodule on abdominal wall	None
11[4]	M/65	Stage 4	Multiples nodules on lower extremities and abdomen	Mediastinal mass, lymph nodes and BM
12[9]	F/29	Stage 4	Multiples nodule face, breast, thoraco - mammary region, abdomen, tights	Lymph nodes, BM
13 case reported	M/75	Stage 4	Nodules on scalp and right arm and multiples patches on trunk	Lymph nodes, BM

BM: Bone marrow; F: Female; M: Male.

proved that primary cutaneous T-LBL is characterized by a gain of 1p36.33-p22.1 in the early stages and a large number of chromosome losses/gain in the later stage. The gain of 1p36.33-p22.1 could be an interesting marker to perform an earlier diagnosis of primary cutaneous T-LBL[9].

Diagnosis is also made on bone marrow biopsy, with lymphoblasts which may comprise up to 25% of marrow elements. If lymphoblasts involve more than 25% of the marrow, patients are arbitrarily diagnosed with acute lymphoblastic leukemia (ALL).

Indeed, conversely to cutaneous B-LBL which typically presents as a unique lesion located in neck or head area, T-LBL develop multiple skin lesions on back, chest, legs and breasts to the same extent as the head and neck areas[2]. This clinical presentation must be differentiated from the blastic plasmacytoid dendritic cell neoplasm presentation which can be quite similar. It is a disease with frequent skin involvement appearing as widespread, purplish, dermal nodules localized on the trunk, limbs or head. Usually, as in T-LBL, patients progress rapidly with involvement of bone marrow, peripheral blood, lymph nodes and extranodal sites. Diagnosis can be made from the immunohistochemical analysis of tumor cells when TdT and T-cell markers (CD3) are negative and CD4 and CD56 positive[10].

Various treatments have been applied to LBL in adult: protocols for high-grade NHL such as CHOP, CHOEP, or treatments according to a regimen for ALL, and autologous stem cell transplantation (SCT)[1]. The major challenge of local disease control is the effective treatment of mediastinal tumors. It is now believed that ALL chemotherapy regimens are more appropriate for LBL[11]. It has been shown that a hybrid NHL/ALL chemotherapy protocol followed by consolidation with SCT appears superior to the use of chemotherapy alone[11].

The clinical presentation of our patient with disseminated erythematic patches and infiltrated nodules suggested a diagnosis of cutaneous involvement of T-LBL. Finally, histopathological examination of a skin biopsy with immunohistochemical study established the diagnosis of T-LBL. For accurate diagnosis, experienced histopathologists are needed.

We wish to add this case to the current literature of T-LBL with cutaneous involvement, emphasizing the importance of a correct diagnosis and aggressive treatment.

ACKNOWLEDGMENTS

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COMMENTS

Case characteristics

The disease arises from precursor T lymphocytes and has a poor prognosis.

Clinical diagnosis

The clinical presentation of the skin involvement is large purplish skin nodules.

Differential diagnosis

The blastic plasmacytoid dendritic cell neoplasm but the Cd4 and CD56 are positive.

Laboratory diagnosis

Positive terminal deoxynucleotidyl transferase (TdT) marker, positive precursors T cell markers (CD3 and CD5) and histology make the diagnosis.

Pathological diagnosis

The pathological diagnosis of skin involvment of T-lymphoblastic lymphoma (T-LBL) is made by histology with hematoxylin-eosin coloration which commonly shows a monomorphous dermis-infiltrate of small to medium-sized lymphoid cells with scant cytoplasm, moderately condensed to dispersed chromatin and prominent nucleus with inconspicuous nucleoli.

Treatment
Protocols for high-grade non-Hodgkin lymphoma (NHL) like CHOP CHOEP or stem cells transplantation.

Related reports
Some related case has been reported by Lee *et al*[2] (Precursor B- or T-LBL presenting with cutaneous involvement: a series of 13 cases including 7 cases of cutaneous T-LBL).

Term explanation
Tdt marker is a specialized DNA polymerase expressed in immature pre-B, pre-T lymphoid cells and acute lymphoblastic leukemia.

Experiences and lessons
Importance of a correct diagnosis for a fast treatment.

Peer-review
The authors have performed a good study, the manuscript is interesting.

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CASE REPORT

Incarceration of Meckel's diverticulum in a left paraduodenal Treitz' hernia

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Author contributions: Gerdes C, Gerdes A and Gerdes B designed the presentation of the case study, wrote the manuscript; Akkermann O and Krüger V collected clinical data and reviewed the literature.

Institutional review board statement: The authors confirm that the report follows the World Medical Association's Declaration of Helsinki.

Informed consent statement: The patient provided informed written consent for case report in a scientific paper.

Conflict-of-interest statement: All authors exclude a conflict of interest.

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Abstract

Meckel's diverticula incarcerated in a hernia were first described anecdotally by Littré, a French surgeon, in 1700. Meckel, a German anatomist and surgeon, explained the pathophysiology of this disease 100 years later. In addition, a congenital paraduodenal mesocolic hernia, known as a Treitz hernia, is a rare cause of small bowel obstruction. These hernias are caused by an abnormal rotation of the primitive midgut, resulting in a right or left paraduodenal hernia. We treated a patient presenting with pain and diagnosed extraluminal air in the abdomen after a computed tomography examination. We performed a laparotomy and found a combination of these two seldomly occurring congenital diseases, incarceration and perforation of Meckel's diverticulum in a left paraduodenal hernia. We performed a thorough review of the literature, and this report is the first to describe a patient with a combination of these two rare conditions. We considered the case regarding the variety of terminology as well as the treatment options of these conditions.

Key words: Incarceration; Meckel's diverticulum; Perforation; Left paraduodenal hernia; Treitz' hernia; Littré's hernia

Core tip: Meckel's diverticula incarcerated in a hernia were first described anecdotally by Littré, a French surgeon, in 1700. Meckel, a German anatomist and surgeon, explained the pathophysiology of this disease 100 years later. In addition, a congenital paraduodenal mesocolic hernia, known as a Treitz hernia, is a rare cause of small bowel obstruction. We performed a thorough review of the literature, and this report is the first to describe a patient with a combination of these two seldomly occurring congenital diseases,

incarceration and perforation of Meckel's diverticulum in a left paraduodenal hernia.

Gerdes C, Akkermann O, Krüger V, Gerdes A, Gerdes B. Incarceration of Meckel's diverticulum in a left paraduodenal Treitz' hernia. *World J Clin Cases* 2015; 3(8): 732-735 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i8/732.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i8.732

INTRODUCTION

Internal hernias are the protrusion of viscus through an opening in the peritoneal or mesenteric fold[1]; they account for 0.2% to 0.9% of small bowel obstructions[2] and are classified as acquired or congenital. Littré's hernia is a very rare type of hernia. In 1700, a French surgeon, Alexandre de Littré, first described a type of inguinal hernia that was different from known hernias[3]. An antimesenteric sacculation, not the whole bowel's circumference, protruded through a defect in the abdominal wall, which explains why Littré's patient did not die from a bowel obstruction. Littré could not discover a reason for the bowel eversion; however, 100 years later (1809), Meckel, an anatomist and surgeon, theorized that the persistence of the omphalomesenteric duct could be causative[3]. The omphalomesenteric duct, also known as the Ductus vitellinus, joins the midgut to the yolk sac. If the duct does not obliterate, a Meckel's diverticulum remains, which is the most common congenital anomaly of the gastrointestinal tract. Only 4% to 16% of the patients with this true diverticulum experience complications[2] such as bleeding from ectopic gastric mucosa or obstruction caused by intussusceptions or adhesion bands[4].

For several decades, studies have described cases presenting with incarceration of Meckel's diverticulum in different types of hernias, including inguinal, femoral or umbilical hernias[5], as well as in incarcerations in incisional hernias[6].

Another type of hernia, congenital paraduodenal mesocolic hernias, represents 53% of internal hernias[7]. Paraduodenal hernias, known as Treitz hernias, had been described by others before being defined for the first time by Treitz in 1857[1]. The two types of paraduodenal hernias are left and right, and left paraduodenal hernias occur more frequently than right ones[7]. Left paraduodenal hernias are caused by an abnormal rotation of the primitive midgut[8] when the small bowel invaginates into an avascular segment of the left mesocolon[9]. The bowel prolapses through the Landzert's fossa behind the fourth segment, the ascending duodenum. It locates behind the inferior mesenteric vein and left colic artery[10] and becomes trapped between the mesocolon and the posterior abdominal wall in a hernial sac within the leaflets of the left colon mesentery[7]. Bowel loops are present between the stomach and pancreas. Recent studies have reported laparoscopic management of this condition[11].

We performed surgery on a patient with an incarceration of Meckel's diverticulum in a left paraduodenal internal hernia.

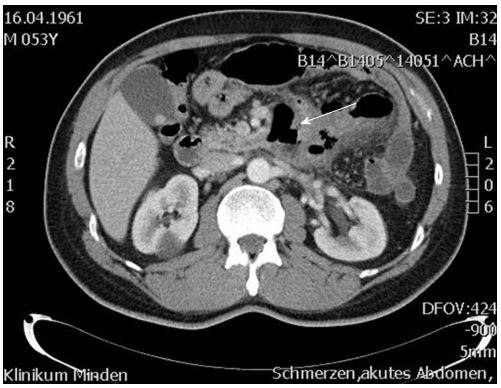

Figure 1 Extraluminal air was detected beside the duodenojejunal flexure (arrow) caused by perforated Meckel's diverticulum.

CASE REPORT

A 53-year-old man reported having unspecific moderate pressure for two weeks before presenting with sudden acute and severe pain in his upper abdomen. The patient consulted the emergency department of our hospital. During the clinical examination, we found moderate abdominal epigastric tenderness. The white blood cell count (16.9 g/L) and C-reactive protein were elevated (203.5 mg/L), and the patient underwent a computed tomography examination of the abdomen (Figure 1). Extraluminal air was detected beside the duodenojejunal flexure. Therefore, we performed surgery. During the laparotomy, we found that the small bowel was fixed adjacent to the duodenojejunal flexure, with the abdomen appearing to be "empty". Small bowel was herniating through a tight, well-formed, fibrous ring. After having mobilized the non-ischemic bowel, we identified a Meckel's diverticulum showing a fibrous ring at the base and perforation at the tip (Figure 2). The small bowel, including a 5-cm Meckel's diverticulum that was located 60 cm oral of the ileocecal valve, was slipped through the hernial orifice of a left paraduodenal hernia. We repositioned the bowel and resected the diverticulum. Histologically, we found venous congestion of the diverticulum with a perforation at the tip and signs of peritonitis. After having opened the bursa omentalis by dividing the gastrocolic ligament, we opened the hernial sac and lavaged and drained the bursa. In the hernial sac, we found local fibrinous peritonitis. We closed the hernial orifice with an omental flap. The postoperative course of the patient was uneventful despite infection with clostridium difficile, which was treated with metronidazole.

DISCUSSION

The terminology in this medical field is highly variable.

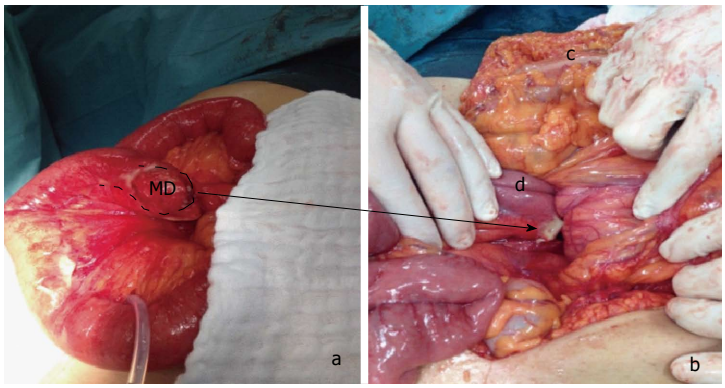

Figure 2 Intraoperative situs. a: Meckel's diverticulum shows a well-formed, fibrous ring where it was assumably incarcerated; b: Hernial orifice (arrow's tip); c: Colon; d: Duodenum. MD: Meckel's diverticulum.

In a scientific paper in 1785, Gottlieb Richter, a professor of medicine in Göttingen, described the diversity of hernias. He counted intestinal wall hernias and Littré's hernias among the small hernias that include all the hernias in which only one side of the bowel is incarcerated[3]. His descriptions are probably the reason that the clinical terminology of these hernias is confusing. All intestinal wall hernias are frequently referred to as Richter-Littré hernias or Littré's hernias, particularly by German speakers. In 1888, Frederic Treves differentiated Littré's hernias from Richter's hernias[3]. A Littré's hernia is present if the content of the hernia exists in a Meckel's diverticulum whereas Richter's hernia applies to all intestinal wall hernias.

Paraduodenal hernias as well as Meckel's diverticula are rare, and their discovery is frequently delayed because they induce diffuse symptoms. The symptoms of Meckel's diverticula could be similar to those of appendicitis. The diagnosis of paraduodenal hernias is challenging because of the lack of explicit symptoms. Paraduodenal hernias are rare causes of bowel obstruction, and if strangulation is present, the mortality rate could approach 50%[12]. There is a discussion of left paraduodenal hernias being identified as congenital mesocolic hernias[13].

To treat our patient, we considered the basic principles of hernia surgery by reduction and assessment of the hernial contents and correction of the defect. First, we repositioned the sac contents, which frequently consist of the majority of the small bowel, so that following the laparotomy, the surgeon accomplishes a lack of small bowel in the abdominal cavity[14], as in our case. After the reduction, we unexpectedly found the perforated Meckel's diverticulum with a fibrous ring at its base. We suspected that the Meckel's diverticulum was incarcerated in an additional pouch of the large hernial sac and that the fibrous ring had to be interpreted as an incarceration ring. This finding was confirmed histologically. We resected the Meckel's diverticulum with a stapler. To prevent a postoperative abscess in the preformed hernial sac caused by contamination resulting from perforation of the Meckel's diverticulum, we opened the bursa omentalis by transection of the gastrocolic ligament, resected the hernial sac and lavaged it. We performed a tension-free closure of the paraduodenal hernial orifice with the use of an omental flap to prevent recurrence of the hernia. To achieve that surgical goal, we used an absorbable running suture, taking care to avoid injury to the nearby mesenteric vessels. Our finding was an incarcerated and perforated Meckel's diverticulum in a Treitz hernia.

In a thorough search of the literature, we could not find a similar case. Developing a hernia induced by a combination of these two seldomly seen congenital defects is a rare coincidence. There were several case reports of patients with a Littré's hernia during the 20th century, as well as other rare conditions including Meckel's diverticulum in an obturator hernia[5]. A recently published case described a patient with a congenital defect of the mesocolon in which an ileal loop with a Meckel's diverticulum was prolapsed[4]. In that case, there was a defect in the right transverse mesocolon; however, the patient presented without a hernial sac, and thus there was no true internal hernia. To the best of our knowledge, this paper is the first report of an incarcerated and perforated Meckel's diverticulum in a confirmed internal paraduodenal hernia.

COMMENTS

Case characteristics

Acute abdominal pain.

Clinical diagnosis

Temperate abdominal epigastric tenderness.

Differential diagnosis

Diseases causing hollow organ perforation in the upper abdomen.

Laboratory diagnosis

White blood cell count: 16.9 g/L; C-reactive protein: 203.5 mg/L.

Imaging diagnosis

Extraluminal air was found in the computed tomography-scan near the ascending duodenum.

Pathological diagnosis
Perforated Meckel's diverticulum with venous congestion and peritonitis in a left paraduodenal hernia.

Treatment
Reduction of the bowel, resection of the Meckel's diverticulum and the hernia sac and closure of the hernial orifice with an omental flap.

Experiences and lessons
This case report presents the clinical characteristics of a perforated Meckel's diverticulum in a Treitz' hernia. This new clinical situation has to be considered in differential diagnosis of acute abdomen.

Peer-review
The authors have described an interesting case.

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CASE REPORT

Rituximab therapy for primary glomerulonephritis: Report on two cases

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Abstract

The evidence in the medical literature on the efficacy and safety of rituximab therapy for primary glomerulonephritis is limited and controversial. We describe two male Caucasian patients with rapidly progressive kidney failure due to primary proliferative glomerulonephritis. Both of them received high-dose intravenous corticosteroids and oral cyclophosphamide with limited benefit. The first patient (hepatitis C virus-negative mixed cryoglobulinemia) underwent plasma-exchange with intravenous immunoglobulins; he showed significant benefit on kidney function (he became dialysis independent with serum creatinine going back to 1.6 mg/dL) after one rituximab pulse even if urinary abnormalities were still present. No improvement in renal function or urinary changes occurred in the second patient. Both these individuals developed sepsis over the follow-up, the first patient died two months after rituximab therapy. This report is in keeping with the occurrence of severe infections after rituximab therapy in patients with renal impairment at baseline and concomitant high-dose steroids.

Key words: Chronic kidney disease; Cryoglobulinemic vasculitis; Membranoproliferative glomerulonephritis; Rituximab

Core tip: A small but growing body of evidence is emerging on the efficacy and safety of rituximab therapy for primary glomerulonephritis. Various authors have claimed that rituximab for glomerular diseases is effective and has minimal adverse effects. We report on two male Caucasian patients who were refractory to conventional immunosuppressive therapy; each of them received one rituximab pulse and developed sepsis over the follow-up, the first patient died two months after rituximab therapy. The risks (and the predictive

factors) of severe infections in kidney patients on rituximab therapy are unclear and appear an area of active research.

Fabrizi F, Cresseri D, Fogazzi GB, Moroni G, Passerini P, Martin P, Messa P. Rituximab therapy for primary glomerulonephritis: Report on two cases. *World J Clin Cases* 2015; 3(8): 736-742 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i8/736.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i8.736

INTRODUCTION

Primary glomerulonephritis (GN) remains an important cause of end-stage kidney disease. Preliminary trials have recently shown the efficacy of rituximab for adult-onset primary GN[1]; rituximab being a genetically chimeric monoclonal antibody directed to CD20 antigen, a B-cell-specific transmembrane found on immature and mature cells, as well as on malignant B cells. Following treatment with rituximab (RTX), B-cells are prevented from proliferating, and undergo apoptosis and lysis through complement-dependent and - independent mechanisms. B-cell depletion usually persists for 6-9 mo in around 80% of patients, but the degree of depletion is greatly variable. Rituximab is currently approved for treating various malignancies including B cell non-Hodgkin's lymphoma and chronic lymphocytic leukemia; also, it has been licensed for refractory rheumatoid arthritis, granulomatosis with polyangiitis (Wegener's granulomatosis) and microscopic polyangiitis. Rituximab was expected to inhibit the production of autoantibodies involved in the pathogenesis of the disease without the toxicity of nonspecific immunosuppression. It has been the first monoclonal employed for the treatment of glomerular diseases and has been initially used for patients with membranous nephropathy but its use has rapidly spread to other glomerular diseases[1]. Membranous nephropathy and membranoproliferative GN are characterized by glomerular deposition of immune complexes; a crucial role of B cells in membranous (MN) and membranoproliferative glomerulonephritis pathogenesis through autoantibody production and antigen presentation has been mentioned. A small but growing body of literature is emerging on the benefits of rituximab in MN and membranoproliferative glomerulonephritis as primary treatment or as treatment of lesions refractory to other immunomodulatory regimens. In this setting, the drug appears to be well tolerated with small adverse events (Table 1)[2-8].

We report here our experience on rituximab use in two patients with progressive kidney failure due to primary proliferative GN. Both of them received conventional immunosuppressive therapy with limited benefit on urinary and biochemical abnormalities; then, they underwent one RTX pulse but developed sepsis over the follow-up. A brief review on the safety and efficacy of rituximab for primary GN has been also added.

CASE REPORT

Patient 1

A 51-year-old Caucasian male patient was admitted to hospital for two-week's duration of abdominal pain with vomiting and diarrhoea. His medical history included arterial hypertension and symptomatic hepatitis C virus (HCV)-negative mixed cryoglobulinemia (since three years) with recurrent purpura and peripheral neuropathy at the lower extremities. Skin biopsy had shown leukocytoclastic vasculitis whereas neurological evaluation had revealed mono-neuritis at the left foot with axonal ischemic damage, probably related to cryoprecipitable immune complexes in the vasa nervorum. He had received low dose oral corticosteroids and azathioprine with partial control of cutaneous and neurological abnormalities. A bone marrow biopsy had reported no evidence of malignant lymphoma, and a small expansion of B lymphocytes (10%-15%).

A physical examination showed bilateral edema and hypertension (180/100 mmHg), purpuric rash with ulcers at the legs (Figure 1); no bowel movements were apparent from the clinical standpoint, this being confirmed by an abdomen X-ray. An ultrasound scan of the abdomen showed normal sized kidneys bilaterally, with normal echotexture. At presentation (Table 1), abnormal laboratory results included serum creatinine level of 2.5 mg/dL, proteinuria, 3.6 g/24 h, and hypoalbuminemia (2 g/L). Other pertinent chemistries were: positive cryoglobulins, with a cryocrit of 3% (polyclonal IgG and monoclonal IgM*k*), elevated rheumatoid factor (148 IU/mL) and hypocomplementemia. Serology was negative for hepatitis B virus (HBV), HCV and human immuno-deficiency virus (HIV) markers, polymerase chain reaction tested negative for HCV RNA. Repeat urine sediment, analyzed by phase-contrast microscopy, showed severe microscopic hematuria (> 50 erythrocytes/microscopic field), many dysmorphic erythrocytes and casts (ialine, granular and red cell casts). Bence Jones proteinuria (kappa type) was positive. The search for anti-neutrophil cytoplasmic antibody (proteinase 3 and myeloperoxidase), anti-glomerular basement membrane antibody, extractable nuclear antigen antibody, antinuclear and anti-double stranded DNA tested negative.

Renal biopsy was not performed due to anatomic reasons, and a diagnosis of essential MC with rapidly progressive renal failure due to nephritic/nephrotic syndrome was made. Treatment was initiated with intravenous methylprednisolone (600 mg/d for three days), oral prednisone (50 mg/d on taper), and oral cyclophosphamide (100 mg daily). The progressive deterioration of kidney function (serum creatinine raised to 6.3 mg/dL, blood urea nitrogen to 279 mg/L) led us to make sequential plasma-exchange (nine sessions) and high-dose intravenous immunoglobulins (five procedures); in addition, hemodialysis was started. We

Table 1 Blood chemistries at presentation and over follow-up (patient 1)

	Admission	Discharge	Middle follow-up	Final follow-up
Creatinine (0.5-1.2, mg/dL)	2.8	1.59	1.76	0.8
Blood urea nitrogen (8-20, mg/dL)	147	104	86	85
AST (5-32, IU/L)	12	24	27	23
ALT (5-31, IU/L)	9	43	39	7
γGT (5-36, IU/L)	12	44	41	69
Cholinesterasis (5300-12900, IU/L)	3833	2160	2920	3173
Total bilirubin (0.2-1.1, mg/dL)	0.2	0.25	0.22	0.23
Direct bilirubin (0-0.3, mg/dL)	0.09	0.07	0.08	0.16
Total protein (6.6-8.7, g/dL)	3.9	4.6	4.6	4.5
Albumin (3.4-4.8, g/dL)	2.4	3	2.5	3
Prothrombin time (0.88-1.16)	1.08	1.07	1.06	1.08
Partial thromboplastin time (0.85-1.18)	1.01	1	1.03	1.19
C_3 (90-180)	20	56	59	99
C_4 (10-40)	0	1	3	2
Cryoglobulins	Present	Absent	Absent	Present
Leucocytes (4.8-10.8, 10^3/mmc)	10670	3440	3400	3730
Hemoglobin (12-16, g/dL)	10.8	9.7	10.2	10.5
Platelets (130-400, 10^3/mmc)	313000	159000	153000	73000

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: Gamma-glutamyl transpeptidase.

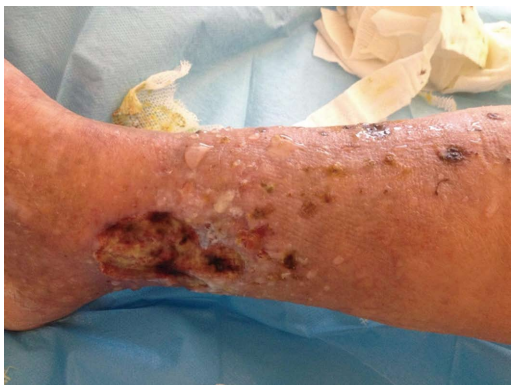

Figure 1 Purpuric rash with ulcers at the leg (patient 1).

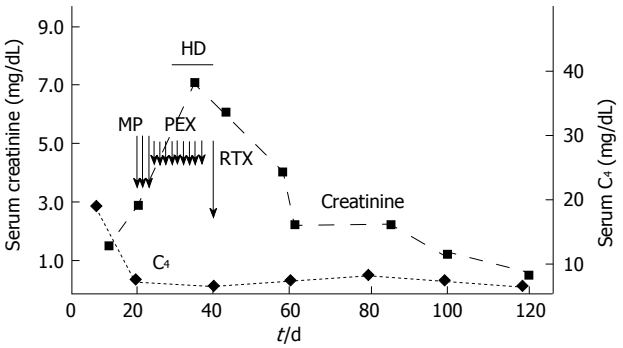

Figure 2 Biochemical/serological response to therapy (patient 1). HD: Haemodialysis; MP: High-dose methylprednisolone; PEX: Plasma-exchange; RTX: Rituximab.

observed healing of skin ulcers and improvement of neuropathic pain; GI disorders disappeared but immunosuppressive therapy was complicated by *Clostridium Difficile*-positive diarrhea, which was successfully treated with oral vancocine. Due to the persistence of severe renal failure (serum creatinine of 4.2 mg/dL), he received one infusion of RTX (375 mg/m^2) off-label (Figure 2); by day 6 of the RTX dose, an improvement of urine output occurred. Since then, serum creatinine went back to 1.6 mg/dL, and blood urea nitrogen to 104 mg/dL. At discharge from the hospital, his medications included oral steroids, rabeprazole, amlodipine, furosemide, calcium carbonate, gabapentine, and darboepoetin.

Two weeks after hospital discharge, serum creatinine was 1.76 mg/dL, blood urea nitrogen, 86 mg/dL, serum albumin 2.5 g/dL; severe and dysmorphic hematuria and non-nephrotic proteinuria (1.43 g/L) being still present. Low white blood cell count after RTX administration (3.4 × 10^3/mmc) occurred. According to flow cytometry, CD20$^+$ B cells were 19% of total peripheral blood lymphocytes (before RTX) and fell to 1% (after RTX, since day 6) with no increase over the following weeks.

One month later, he became pyrexial with a temperature around 38 ℃ and was admitted again to the hospital. Pertinent biochemistry included serum creatinine (0.8 mg/dL), cryoglobulins and rheumatoid factor tested positive. Complete blood count gave the following features: white blood cells 2.67 × 10^3/mmc, erythrocytes 3.57 × 10^6/mmc, platelets 209 × 10^3/mmc. Flow cytometry: lymphocytes 169/mm^3, CD3$^+$ cells 142 (83.9%), CD19$^+$ 0 (0%), natural killer cells 25 (14.9%). Gamma globulins were 0.16 g/dL (3.9%, 11%-18.8%). IgA < 4 mg/dL (70-400), IgG 84 mg/dL (700-1600), IgM 108 mg/dL (40-230). Monoclonal component by serum electrophoresis (IgMk + oligoclonal Ig) was again detected. An active urinary sediment with non-nephrotic proteinuria (1.21 g/d) was still present. Sepsis from *Enterococcus Spp.* was identified whereas the chest radiograph reported multiple pneumoniae. The culture of the broncho-alveolare lavage fluid was positive for *Candida albicans*, thus, we initiated intravenous imipenem and antifungal

medications. Medical and supportive therapy was unsuccessful and the patient ultimately expired due to septic shock (two months after RTX pulse).

Patient 2

A 49-year-old Caucasian male underwent kidney biopsy for evaluation of serum creatinine 1.35 mg/dL [estimated glomerular filtration rate (eGFR) 56 mL/min per 1.73 m^2 by MDRD equation], 3.2 g of proteinuria on 24-h urine collection and active urinary sediment (severe microscopic hematuria with red blood cell casts). Renal biopsy showed global ialinosis in some glomeruli (5 out of 14); the others had intense glomerular hypercellularity (mainly due to mesangial proliferation), a limited number of mesangial immune deposits and segmental thickening of glomerular basement membrane were also present. Final diagnosis was mesangial proliferative GN with immune deposits of unclear significance. Other pertinent chemistries were: negative cryoglobulins, normal rheumatoid factor and complement fractions; serum protein electrophoresis in the normal range. Serology tested negative for HBV, HCV and HIV markers, polymerase chain reaction was negative for HCV RNA. The search for anti-neutrophil cytoplasmic antibody (proteinase 3 and myeloperoxidase), anti-glomerular basement membrane antibody, extractable nuclear antigen antibody, antinuclear and anti-double stranded DNA tested negative. At discharge from the hospital, his medications included oral steroids, rabeprazole, amlodipine, doxazosine, furosemide, calcium carbonate, and darboepoetin. Partial remission of nephritic/nephrotic syndrome with improvement of kidney function (serum creatinine going back to 1.1 mg/dL) was obtained with intravenous methylprednisolone pulses, oral cyclophosphamide, azathioprine, and mycophenolate mofetil in variable associations. Eight years later, he was again admitted to our unit, showing bilateral lower-extremity edema, arterial hypertension and serum creatinine of 2.9 mg/dL (eGFR 23 mL/min per 1.73 m^2 by MDRD equation). Nephrotic proteinuria was demonstrated (proteinuria of 9.2 g/d) with active urinary sediment. A repeat kidney biopsy revealed intracapillary/extracapillary glomerular proliferation with several crescents and fibrinoid necrosis, diffuse arteriolosclerosis, in addition to uniform and diffuse thickening of the glomerular basement membrane. Immunofluorescence demonstrated sporadic and granular deposition of C1q/C3 in the mesangium and capillary walls; fibrinogen in the Bowman space. Renal biopsy was complicated by perirenal hematoma and a few units of red packed cells were given. He received high-dose intravenous diuretic therapy with edema resolution and body weight loss, and intravenous methylprednisolone pulse therapy (500 mg daily intravenous for three alternate days) with low-dose oral steroids was not effective. Thus, one infusion of RTX (375 mg/m^2) off-label was administered. One month after rituximab administration he was again hospitalized (acute pulmonary insufficiency with septic shock); serum creatinine of 4.56 mg/dL (eGFR, 11 mL/min per 1.73 m^2). Active urinary sediment and nephrotic proteinuria persisted. Pulmonary aspergillosis was documented- medical plus supportive therapy was initiated and the patient recovered in a few weeks; however, he developed irreversible kidney failure and initiated dialysis acutely. He is currently doing well on maintenance hemodialysis (thrice weekly) treatment.

DISCUSSION

We report here on two patients with rapidly progressive renal failure due to idiopathic proliferative GN who were resistant to conventional immunosuppressive therapy. Both the patients underwent rituximab treatment in off-label condition, RTX infusion was well tolerated by both the patients but sepsis developed over the follow-up, fatal course occurring in patient 1. Numerous case reports and case series have suggested that the addition of rituximab to standard chemotherapy for malignant lymphoma increases the risk of viral infections such as varicella zoster[9], cytomegalovirus[10], HBV[11], parvovirus[12], and enteroviral encephalitis[13]. The risk of HBV reactivation has been added to the existing Boxed Warning of the rituximab label by the Food and Drug Administration in 2013[14]. Impaired immunity against non-viral pathogen agents such as Pneumocystis jirovecii[15] or cryptococcus[16] after rituximab therapy has been also noted.

A recent systematic review and meta-analysis has shown that rituximab plus standard chemotherapy for malignant lymphoma increases the incidence of severe leucopenia (RR = 1.24; 95%CI: 1.12-1.37) and granulocytopenia (RR = 1.07; 95%CI: 1.02-1.12) even if the overall risk of severe infections has not been increased (RR = 1.0; 95%CI: 0.87-1.14)[17]. We have already reported on a case of cholestatic hepatitis C after rituximab therapy for gastric cancer in a renal transplant recipient[18]. On the other hand, various authors have claimed that rituximab use for glomerular diseases is effective and has minimal adverse effects (Table 2)[1,19,20].

Our first patient presented idiopathic cryoglobulinemic vasculitis which has undefined therapeutic management[21]. There is some evidence on the efficacy and tolerance of RTX in patients with HCV-associated mixed cryoglobulinemia vasculitis who were naïve, resistant or intolerant to antiviral therapy[22-25]. Two randomized controlled trials have compared RTX with conventional immunosuppressive therapy for HCV-related mixed cryoglobulinemia vasculitis[26,27]. As listed in Table 3, evidence in the medical literature on RTX use among patients with non-infectious cryoglobulinemia vasculitis targeting kidneys is extremely limited, and a total of 16 cases were retrieved[28-37]. Patient 1 gives emphasis on the efficacy of RTX, as one RTX pulse made possible the control of renal disease: kidney function normalized, nephrotic syndrome disappeared and only nephritic urinary changes persisted. However, RTX use was complicated by sepsis a few weeks after

Table 2 Literature review: Adverse events during rituximab therapy for primary membranous and membranoproliferative glomerulonephritis

Ref.	*n*	Rituximab treatment dose	Follow-up period	Concomitant therapy	Response to RTX	Side-effects after RTX
Fervenza *et al*[2]	15	1 g × 2, on days 1 and 15	12 mo	ACE-I + ARB	Complete (*n* = 2) or partial remission (*n* = 6)	Nonserious transient AE (*n* = 10) pneumonia (*n* = 1)
Segarra *et al*[3]	13	375 mg/m^2 once weekly × 4	30 mo	Tac (*n* = 10), CyA (*n* = 3), CCS (*n* = 3)	Partial remission (*n* = 13)	None
Fervenza *et al*[4]	20	375 mg/m^2 once weekly × 4	24 mo	ACE-I + ARB	Complete (*n* = 4) or partial remission (*n* = 12)	Nonserious transient AE (*n* = 11) pneumonia (*n* = 1)
Michel *et al*[5]	28	375 mg/m^2 once weekly × 2 or 3 or 4 (*n* = 27) 1 g × 2, on days 1 and 15 (*n* = 1)	12 mo	ACE-I + ARB, CCS (*n* = 1), Tac (*n* = 1)	Complete (*n* = 6) or partial remission (*n* = 13)	Nonserious transient AE (few)
Ruggenenti *et al*[6]	100	375 mg/m^2 once weekly × 4	29 mo	CCS	Complete (*n* = 27) or partial remission (*n* = 38)	Nonserious transient AE (*n* = 28)
Dillon *et al*[7]	6	1 g × 2, on days 1 and 15	12 mo	ACE-I + ARB	Complete (*n* = 2) or partial remission (*n* = 3)	None
Kong *et al*[8]	13	500 mg × 1 (*n* = 6) 500 mg × 2 (*n* = 3) 500 mg × 4 (*n* = 4)	31.5 mo	CCS (os) (*n* = 9) CyA (*n* = 2) CCS (*iv*) (*n* = 2)	Remission (*n* = 19)	Nonserious transient AE (*n* = 8) Pneumonia (*n* = 1)

ACE-I: Angiotensin converting enzyme inhibitors; AE: Adverse events; ARB: Angiotensin receptor blockers; CCS: Corticosteroids [by intravenous (*iv*) or oral (os) route]; CyA: Cyclosporine; MN: Membranous nephropathy; MPGN: Membranoproliferative glomerulonephritis; Tac: Tacrolimus; RTX: Rituximab.

Table 3 Overview of cases with non-viral hepatitis mixed cryoglobulinemia (and kidney involvement) on rituximab

Ref.	*n*	Age (yr)/gender	Treatment prior to RTX	Features	Response to RTX	Side-effects after RTX
Arzoo *et al*[28]	1	71/F	CS	C, N, R	Remission	None
Ghijsels *et al*[29]	1	44/M	CS, CPH, CHL	C, Ca, R	Remission	None
Koukoulaki *et al*[30]	1	48/F	CS, CPH	GI, P, R	Partial remission	None
Bryce *et al*[31]	1	NA	NA	R	No response	None
Ruch *et al*[32]	1	64/M	CS	R	Remission	Cold agglutinine disease, sepsis
Annear *et al*[33]	1	42/F	CS	C, R	Remission	None
Terrier *et al*[34]	7	73 ± 5/M (*n* = 4)	CS (*n* = 4)	C (*n* = 6), N (*n* = 2), A (*n* = 2), R (*n* = 7)	Remission (*n* = 3), partial remission (*n* = 1), NA (*n* = 3)	Severe infections (*n* = 4)
Wink *et al*[35]	1	72/F	CS, Aza	C, P, R	Remission	None
Choudhry *et al*[36]	1	61/F	CS, CPH	C, P, R	Remission	None
Kamel *et al*[37]	1	77/F	CS	C, A, R	Remission	None
Own case	1	51/M	CS, Aza	C, GI, N, R	Remission	Severe infection

A: Arthralgias; Aza: Azathioprine; C: Cutaneous; Ca: Cardiac; CHL: Chlorambucil; CPH: Cyclophosphamide; CS: Corticosteroids; GI: Gastrointestinal; N: Neurological; NA: Not available; P: Pulmonary; R: Renal; RTX: Rituximab.

RTX pulse. On the basis of the evidence reported in Table 3, severe infections after RTX treatment are not uncommon [35% (6/17)].

RTX therapy in patients with nonviral cryoglobulinemia vasculitis or membranoproliferative GN raises various questions such as the role of RTX as first-line or rescue therapy, the efficacy/safety of maintenance therapy with RTX, and the tolerance to RTX. In the absence of randomized controlled trials, such questions remain unanswered; as an example, the poor tolerance of our patients after RTX administration remains unclear. The French multicenter CryoVas survey retrospectively evaluated 242 patients with non-infectious mixed cryoglobulinemia vasculitis, RTX plus corticosteroids had greater therapeutic efficacy compared with corticosteroids alone and corticosteroids plus alkylating agents[38]. However, RTX plus corticosteroids was associated with more frequent infections than corticosteroids alone (HR = 9; 95%CI: 3.1-20, $P < 0.001$). Prospective data from the AIR (AutoImmunity and Rituximab) registry, which includes data on patients treated with rituximab off-label, have shown that among patients (*n* = 23) with nonviral cryoglobulinemia vasculitis on RTX, side-effects occurred in almost half of the patients (*n* = 11), including severe infections[34]. Infectious episodes were mostly reported in a patient subgroup (age > 70 years, essential type Ⅱ MC, GFR < 60 mL/min, and high-dose steroids) and were fatal in many (*n* = 3)[34]. Both our patients had important kidney impairment at baseline and concomitant therapy with intravenous high-dose corticosteroids, among other immunosuppressive agents.

The current study calls for further research on the RTX-based treatment of essential cryoglobulinemic vasculitis or membranoproliferative GN but the low frequency of patients in individual centers would make

randomised controlled trials extremely difficult. Rituximab has surfaced as potential treatment option for some primary glomerular diseases and the HCV KDIGO Study Group[39] had already included rituximab among the recommended drugs (steroids, and cyclophosphamide) for the immunosuppressive treatment of HCV-associated kidney disease. The risks (and the predictive factors) of infections in kidney patients on RTX-therapy are not yet understood and are an area of active research. These patients should be monitored over the follow-up to avoid the occurrence of infectious episodes.

COMMENTS

Case characteristics

Two male Caucasian patients with progressive kidney failure.

Clinical diagnosis

Arterial hypertension, bilateral lower-extremity edema.

Differential diagnosis

Progressive kidney failure due to secondary glomerular disease.

Laboratory diagnosis

At presentation serum creatinine ranged between 2.5 and 2.9 mg/dL and proteinuria 3.6 and 9.2 g/d, microscopic haematuria with dysmorphic erythrocytes and red cell casts.

Imaging diagnosis

Computed tomography scan revealed normal sized kidneys bilaterally with normal echotexture in both the patients.

Pathological diagnosis

Renal biopsy (patient 2) showed intracapillary/extracapillary glomerular proliferation with several crescents and fibrinoid necrosis, in addition to uniform diffuse thickening of the glomerular basement membrane.

Treatment

Both the patients received one infusion of rituximab (375 mg/m^2) off-label.

Related reports

Various authors have claimed that rituximab use for glomerular diseases is effective and has minimal adverse effects.

Term explanation

Phase-contrast microscopy is a microscopy technique to analyze the morphology of urine erythrocytes.

Experiences and lessons

The risks and the predictive factors of severe infections in kidney patients on rituximab therapy are still unclear and appear an area of active research.

Peer-review

It is a good article.

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CASE REPORT

When sepsis affects the heart: A case report and literature review

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Author contributions: Clemente G, Tuttolomondo A and Pinto A designed the report; Colomba D performed transthoracic echocardiogram evaluation; Clemente G, Pecoraro R, Renda C, Della Corte V, Maida C and Simonetta I contributed new reagents or analytic tools; Clemente G and Tuttolomondo A analyzed data; Clemente G and Tuttolomondo A wrote the paper.

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Abstract

A 59-year-old nursing home patient with Down syndrome was brought to the internal medicine department of our hospital due to fever, cough without expectorate, and dyspnea. A thoracic computed tomography revealed the presence of bilateral basal parenchymal opacities. Her condition deteriorated after admission and troponin reached a peak serum concentration of 16.9 ng/mL. The patient was in cardiogenic shock. In addition to fluid resuscitation, vaso-active amine infusion was administered to achieve hemodynamic stabilization. The differential diagnosis investigated possible pulmonary embolism, myocardial infarction, and myocarditis. Furthermore, a second transthoracic echocardiogram suggested Tako-Tsubo syndrome. This is a septic patient. The purpose of this manuscript is to review studies which formerly examined the possible association between high levels of troponin and mortality to see if it can be considered a positive predictive factor of fatal prognosis as the case of thrombocytopenia, already a positive independent predictive factor of multiple organ failure syndrome, and generally to characterize risk profile in a septic patient.

Key words: Sepsis; Shock; Hypotension; Troponin; Myocardial dysfunction

Core tip: The importance of cardiac involvement during sepsis, when occurs, worsens prognosis. However, as myocardial dysfunction is reversible, an early diagnosis and treatment to improve the survival. The awareness of risk profile to develop a severe myocardial dysfunction in a septic patient would be suitable in order to enforce careful resources in this subset of patients. Moreover, other research are needful to perform the best therapeutic strategy of haemodynamic stay which,

sometimes, (*e.g.*, when Tako-Tsubo syndrome occurs) can call for intra-aortic balloon pump counter pulsation.

Clemente G, Tuttolomondo A, Colomba D, Pecoraro R, Renda C, Della Corte V, Maida C, Simonetta I, Pinto A. When sepsis affects the heart: A case report and literature review. *World J Clin Cases* 2015; 3(8): 743-750 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i8/743.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i8.743

INTRODUCTION

Sepsis is a syndrome caused by the inefficiency of the mechanisms of control and containment of the infection. It is characterized by symptoms and signs of systemic inflammatory reaction to infection and manifestations of organ dysfunction resulting from alterations in the microcirculation.

It is the second most common cause of death in non-coronary intensive units, and the tenth in high-income countries, with a mortality rate between 15% and 50%. Approximately 150000 deaths per year are caused by sepsis in Europe. The number of cases is expected to increase at a rate of 1.5% per year from the current prevalence of 3 cases for every 1000 inhabitants[1].

The most common pathogenic Gram positives (whose incidence is progressively increasing) are *Staphylococcus aureus* and *Streptococcus pneumoniae*, whereas among the most frequent Gram negatives it is possible to include *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas aeruginosa*[2].

In a smaller percentage of cases, sepsis can be caused by mycobacteria, mycetes, protozoa (*Plasmodium Falciparum*) and viruses[1].

CASE REPORT

A 59-year-old nursing home patient with Down syndrome had high fever unresponsive to paracetamol, and unproductive cough for 4 d. Accordingly, cefriaxone was administered with some improvement (defervescence and reduction of cough). After the reappearance of fever associated with dyspnea, acrocyanosis, and her deteriorating condition, she was brought to the emergency room, and on initial evaluation she was admitted to the internal medicine department of our hospital. She had a ventricular (pacing), ventricular (sensing), inhibition (response) (and) rate-adaptive holder pacemaker because of a third degree atrioventricular block. Furthermore, her past medical history included chronic cerebrovascular disease due to previous ischemic strokes complicated by vascular dementia and epilepsy. The latter was possibly due to Alzheimer-like disease as is often seen in down syndrome patients.

On arrival in the internal medicine department the patient was drowsy, tachypneic, tachycardic, low blood pressure (80/50 mmHg) and hypoxemic (PaO_2 57 mmHg). The thoracic computed tomography (CT) revealed the presence of bilateral basal parenchymal opacities. The patient was treated empirically with piperacillin/tazobactam, levofloxacin, and vancomycin according to protocol for health care-associated pneumonia. An initial bed-side echocardiogram evaluation revealed severe left ventricular dysfunction with an ejection fraction of 36%, and dilatation of the right ventricle with medium-apical akinesis. In addition to fluid resuscitation, dopamine, dobutamin, and norepinephrine infusion were administred. At times simultaneous administration of two vaso-active amines was necessary to achieve hemodynamic stabilization and adequate diuresis. The first electrocardiogram showed regular activation of the pacemaker and subsequent evaluations revealed repolarization abnormalities of probable hypoxic nature in the inferior wall only (Figure 1).

The results of blood tests are shown in Table 1, reporting thrombocytopenia (80000 $10^3/\mu L$) and the peak serum concentration of troponin I (16.9 ng/mL - reference range < 0.012 ng/mL). Blood and urine cultures showed no growth.

A second transthoracic echocardiogram showed akinesis of medium-apical segments of both ventricles with moderate systolic dysfunction (E.F. 45%). This evidence does not rule out an acute ischemic event, but could be seen as suggesting Tako-Tsubo syndrome.

The differential diagnosis also concerned pulmonary embolism, myocardial infarction and myocarditis. The former was excluded through execution of CT angiography. In relation to myocardial infarction and myocarditis, it was not possible to perform coronary angiography or a myocardial biopsy. However, the absence of persistent regional abnormalities ruled out acute coronary syndrome. The third transthoracic echocardiogram showed complete remission of the regional abnormalities (E.F. 50%). The patient was discharged after gradual weaning from vaso-active amines in adequate clinical condition. Therefore, our patient had survived, in spite of severe cardiac involvement and possible Tako-Tsubo syndrome.

DISCUSSION

In our case there was a significant cardiac involvement associated with sepsis due to pneumonia, up to hearth failure which presented itself as an out-and-out cardiogenic shock. The "fluid resuscitation", the administration of vasoactive amines and early antibiotic therapy were needed to restore the hemodynamic stability, until the complete recovery of cardiac function, as indeed typically happens in Tako-Tsubo syndrome. The latter, in our patient, was induced by septic injury and characterized initially by hypokinesia of intermediate and apical segments of left ventricle and at a later stage by akinesis of the same with hyperkinesis of basal segment that typically characterizes the disease.

Nevertheless, the absence of head trauma, cerebral hemorrhage, pheochromocytoma, hypertrophic cardiomyopathy made the diagnosis of Tako-Tsubo syndrome

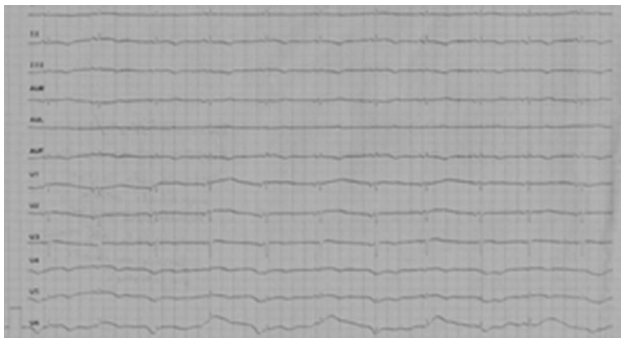

Figure 1 Electrocardiogram at the third day from presentation (showed repolarization abnormalities of probable hypoxic nature in the inferior wall).

plausible. Instead, it was ruled out obstructive atherosclerosis of coronary epicardial artery, since coronary angiography has not been carried out within 48 h, as suggested by Mayo Clinic's diagnostic criteria[3]. However, the disappearance of the alterations of the segmental kinesis at echocardiographic final assessment, allowed us to exclude this diagnosis. Endomyocardial biopsy would have been necessary for ruling out a myocarditis, in which the predominant involvement of right ventricle is quite, as it has been at any rate[4] initially in our case. The clinical presentation, at last, was not suggestive of Guillain-Barrè syndrome[5] nor electrocardiographic monitoring of recurrent ventricular tachycardia[6], conditions in which cases of reversible left ventricular dysfunction have been observed[7]. Therefore, differential diagnosis about Tako-Tsubo syndrome has been ruled out after analyzing anamnesis and clinical presentation. The latter (hypotension, tachycardia, hypoxiemia) also was suggestive of pulmonary embolism, excluded by CT angiography.

The principal cardiovascular manifestation of severe sepsis and septic shock is hypotension and myocardial dysfunction is often associated with them. Myocardial dysfunction does not seem to be caused by myocardial hypoperfusion[8,9] (coronary circulation is maintained or even intensified, although to observe disfunctions in the microcirculation is probable)[10] but rather by the action of depressant factors such as alpha tumor necrosis factor and beta interleukin 1 and does persist despite fluid resuscitation, as Court *et al*[11] have already shown. In addition to the effects of host's immuno-inflammatory responses (*e.g.,* cytokines and mechanisms related to nitric oxide)[12] circulating substances released by pathogens (*e.g.,* endotoxins) also seem to play an important role in provoking myocardial depression. In this sense, the first-line therapy is causal and consists of antibiotic therapy associate with the possible surgical excision of the infectious focus[13]. However, the restoration of hemodynamic stability is an important goal for the survival of the patient. Fluids remain a first-step therapy in clinical management of the cardiovascular failure in sepsis but it is arguable which of them would be the gold standard. Recent results indicate that

Table 1 Results of blood tests on admission and discharge

	Admission	Discharge	Reference range
Aspartate aminotrasferase (U/L)	94	17	< 37
Alanine aminotrasferase (U/L)	66	37	< 41
Calcaemia (mg/dL)	7.5	7.4	8.4-10.2
Gamma-glutamyltranspeptidase (U/L)	155	171	8-61
C-reactive protein (mg/dL)	14.6	3.2	0-0.5
Alkaline phosphatase (U/L)	163	47	40-129
Lactate dehydrogenase (UI/L)	755	511	240-480
Ferritin (ng/mL)	2440		15-150
D-dimer (ng/mL)	478	338	10-250
RB count ($\times 10^6/\mu L$)	3.72	3.53	4.5-5.5
Hemoglobin (g/dL)	12.5	11.7	12-18
Platelet count ($\times 10^3/\mu L$)	92	217	150-450
Myoglobin (ng/mL)	1031	99	0-62
Troponin I (ng/mL)	6.43	1.36	0-0.034

albumin also might be used with advantage in some specific subgroups of patients pending for the results of the ongoing trials on new generation starches[14]. Furthermore, thanks to its electrostatics properties, albumin reduces the endothelial permeability (sealing effect)[15-20]. Its efficacy is still now matter of debate. In patients with severe sepsis, treated with albumin and crystalloids compared with ones treated with crystalloids only, an increase in survival to 28 and 90 d was not observed[21]. As regards the methods of liquids' administration, according to Surviving Sepsis Campaign 2012, an initial fluid challenge in patients with tissue hypoperfusion and suspected hypovolemia, up to achieve $\geqslant$ 30 mL of crystalloids per kilogram of body weight. It would be needed to continue with the fluid-challenge technique until an actual hemodynamic improvement. Yet, a particular attention in balancing the fluids is necessary, inasmuch a positive fluid balance and elevated central venous pressure are associated with increased mortality[22,23].

With ongoing sepsis, advantageous effects, especially as for cardiac output, could be gained with administration of hypertonic saline solutions, as Oliveira *et al*[24] already suggested in their review. For the first time, this kind of therapy was employed in the treatment of hemorrhagic and traumatic shock, with quick restoration of central and peripheral blood flow[25]. Intravenous infusion of hypertonic saline solution summons fluids into vascular compartment and determines a redistribution of blood flow which, as for that matter our team has shown, in refractory heart failure enhances myocardial performance[26]. The proposed mechanism to explain these effects suggests a direct action on myocardial functionality and a decreased sympathetic tone[27]. Hence, infusion of hypertonic saline could be an alternative to early volume resuscitation of a patient with sepsis[28].

Furthermore, in a multicentric trial conducted in a tertiary care setting, protocol-based resuscitation of patients with septic shock diagnosed in the emergency

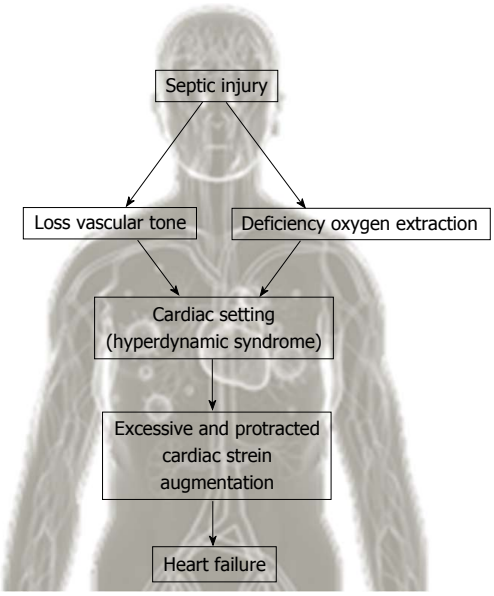

Figure 2 Synopsis of heart failure pathogenesis sepsis.

department, does not improve the outcomes[29].

Even more complex is the pathogenesis of heart failure that could occur during sepsis and which can provoke a significant increase of troponin.

The increase of troponin in sepsis is an event to be rationally expected. Its dosage, therefore, should not be taken for granted. Considering the heart's fundamental cardiovascular adaptation role in sepsis, a significant metabolic-inflammatory impairment can occur with high levels of troponin, associated with severe myocardial dysfunction. Moreover, a meta-analysis in "Intensive Care Medicine" a year ago evaluated the prognostic role of troponin in sepsis, showing that its elevated serum concentration was associated with a subset of patients at higher risk of death. Nonetheless, further studies are needed to determine an optimal troponin cut-off value[30]. B-type natriuretic peptides could also have a role in alerting clinicians to myocardial dysfunction. Their low serum values could exclude severe myocardial impairment. Yet echocardiography is the gold standard method to reveal cardiac dysfunction. Heart rate has also been proposed in the prognostic evaluation of septic patients. A rate of < 106 bpm on presentation suggested a favorable prognosis[31]. Concerning the latter, it is still debatable whether the use of β-blockers in septic tachycardial patients improves the survival. It has been observed that patients being in chronic treatment with β-blockers and later developed sepsis, and were admitted to the intensive care unit (ICU), could have advantages in terms of survival. However, physicians' doubts about using β-blockers in early stages of sepsis are licit[32]. Among other things, it is not still clear enough if in septic shock the increased cardiac rate is pathological or simply an expression of sympathetic hyperactivation. Instead, tachycardia is associated with a worse prognosis. In a prospective observational study in an ICU, esmolol's titrated administration for 24 h, maintaining a cardiac rate between 80 and 94 bpm in selected adult patients in septic shock after 24 h of hemodynamic stabilization, was able to maintain the microvascular blood flow and reduced the demand for epinephrine. However, patients with severe myocardial disfunction had been excluded from the study[33]. Recent results suggest that β-blockers' effects on metabolism, glucidic homeostasis, inflammatory feedback and cardiac function might be advantageous for septic patients[34,35]. In regard to the anti-inflammatory, antioxidant, immunomodulatory and anti-apoptotic actions, statins also might fall within preventing and treating patients with severe sepsis and septic shock[36,37].

However, beyond the value of troponin, B-type natriuretic peptides, and heart rate, the presence of myocardial dysfunction in sepsis is associated with higher mortality. It has been shown that cardiovascular disablement increased mortality from 70% to 90%, compared to 20% in septic patients without myocardial impairment[38].

Therefore, cardiac dysfunction in sepsis has prognostic value and coincides with its severity. Hence it's mandatory to know the pathophysiology of cardiovascular disease in sepsis. Microcirculatory disfunctions and mitochondrial derangement occurring in septic shock reduce the cellular energetic production[39]. Septic injury triggers a reaction in the cardiovascular setting that aims to increase the peripheral availability of oxygen and reduce the cellular effects of oxygen deficiency[40]. The cellular deficiency of oxygen and reduction in systemic vascular resistance give rise to hyperdynamic syndrome (increased stroke volume, heart rate, usage of oxygen)[41] (Figure 2). Alteration of cellular energetic production following to mitochondrial imbalances might be of great relevance in determining tissue injury and sepsis-associated multi organ failure. Future studies should focus on mitochondrial disfunction in order to comprehend the pathophysiological mechanisms of apoptosis and cellular protection to achieve a increasingly accurate treatment[42].

Such a hyperdynamic reaction, favoring adrenergic stimulation, can be hidden by hypovolemia due to insufficient fluid contribution or a mechanism of myocardial impairment[43] (Figure 3). The most frequent occurrence is heart failure with high cardiac index, which is in a phase of unbalance, but it is insufficient to increase metabolic requirements. Hyperdynamic syndrome endows an organism with the possibility to reduce septic injury and survival derives largely from that[44]. Hence the usefulness of administering inotropic positive drugs and to correct hypovolemia because a possible condition of circulatory failure, in the presence of increased oxygen requirements, is linked to a fatal prognosis[45].

The management of myocardial dysfunction sepsis-induced encompasses fluids's administration until the optimization of preload and, among positive inotropic agents, norepinephrine is the first choice[39]. The administration of dopamine should be reserved to carefully selected patients (those with a low risk of

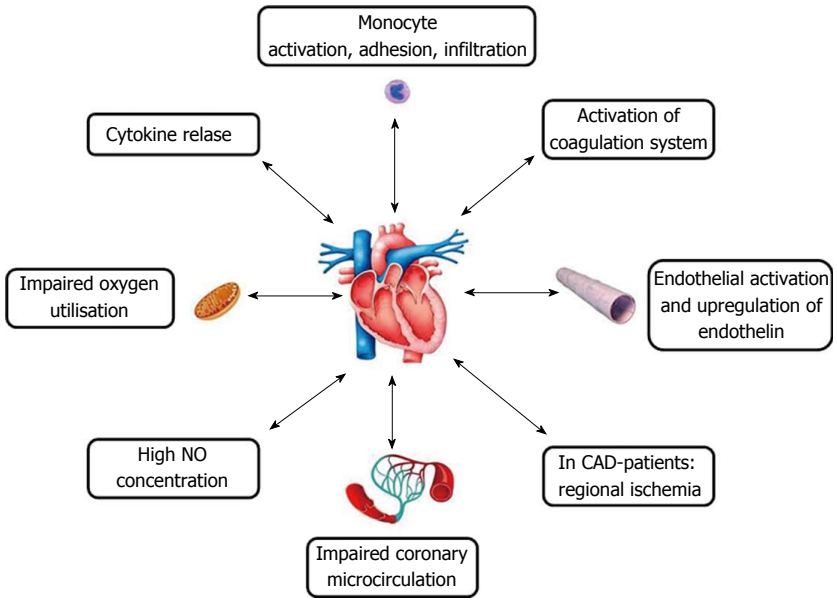

Figure 3 Possible mechanisms of myocardial impairment[43].

arrhythmias and either known grave left ventricular systolic dysfunction or low heart rate). The Surviving Sepsis Campaign guidelines 2012 promote either norepinephrine or dopamine as the first-choice vasopressor agent to maintain adequate perfusion tissue in septic shock[46,47]. Dobutamine also can be used in the early stages of sepsis in order to increase cardiac output. It has a certain selectivity for β_1-receptors[48]. Anyway, β1-agonists can be less effective in case of septic shock. It has been shown that its infusion improves the left ventricular ejection fraction more than 10% in 35% of patients affected by septic shock[49]. Its use requires careful clinical and instrumental monitoring for risk of tachycardia or arrhythmias and hypotension through beta$_2$-adrenergic receptors activation[50]. However, dobutamine is endorsed as the care's fundamental element of sepsis-related cardiovascular failure in international guidelines. Furthermore, it has been demonstrated that dobutamine enhances liver function and hepatic perfusion after experimental hemorrhagic shock[51]. Since one of the mechanisms of sepsis-induced myocardial dysfunction is the alteration of intracellular transport of calcium, a possibility of therapy might be represented by levosimendan[52], inotrope and peripheral vasodilator which is employed in acute congestive heart failure[53]. Levosimendan, acting with a mechanism of calcium-sensibilization in randomized studies comparing it with dobutamine in patients with severe heart failure with low cardiac output, has been observed as emodynamically more effective than dobutamine[54-56].

Another kind of heart failure associated with sepsis is Tako-Tsubo syndrome. For this reason it has been supposed that sepsis-induced systemic inflammation could have a role in starting the pathogenesis of the syndrome[57-59]. Myocardial dysfunction in sepsis could be a consequence of the direct action of different mediators of flogosis (cathecolamines responsible for hyperdynamic syndrome included) and of products of microbial derivation[13]. On the other hand, another pathogenic hypothesis for Tako-Tsubo syndrome is cardiac cathecolamine toxicity, as it could occur in sepsis, which would constitute the trigger[60].

Our case shows that exogenous support of vasoactive amines can be essential in facilitating hyperdynamic syndrome which characterizes sepsis in the pre-clinical phase. As for Tako-Tsubo syndrome, even though β-agonist agents have often been used, the results are conflicting[7], so intra-aortic balloon pump counter pulsation remains the first-line treatment if, after medical therapy (dopamine) and volume resuscitation, hypotension endures[61]. However, the disappearance of segmental kinesis's alterations and complete resolution of myocardial dysfunction, as in our case, if Tako-Tsubo syndrome is actually diagnosed, offer new perspectives that could improve our understanding of the physiopathology of this illness. Randomized clinical trials could demonstrate the possible efficacy of the treatment.

COMMENTS

Case characteristics

A 59-year-old nursing home patient with down syndrome presented fever, cough and dyspnea.

Clinical diagnosis

Main clinical findings were tachypnea, tachycardia and hypotension.

Differential diagnosis

Computed tomography (CT) angiography, thoracic CT, transthoracic echocardiogram were executed and differential diagnosis concerned pulmonary embolism, myocardial infarction, myocarditis, Tako-Tsubo syndrome and sepsi with severe myocardial involvement.

Laboratory diagnosis

The results of blood tests showed alterations of liver function (aspartate aminotrasferase: 94 U/L; alanine aminotrasferase: 66 U/L; gamma-glutamyl-transpeptidase: 155 U/L; alkaline phosphatase: 163 U/L); ferritin: 2240 ng/mL; myoglobin: 1031 ng/mL; C-reactive proteinmg: 14.6 mg/dL; lactate dehydrogenase: 755 UI/L; calcaemia: 75 mg/dL; D-Dimer: 478 ng/mL; thrombocytopenia (92000 × 10^{3}/μL); myoglobin: 1031 ng/mL; troponin: I 643 with peak serum concentration of 169 ng/mL.

Imaging diagnosis

Echocardiogram revealed severe left ventricular dysfunction with an ejection fraction of 36% and dilatation of the right ventricle with medium-apical akinesis.

Pathological diagnosis

The thoracic CT showed the presence of bilateral basal parenchymal opacities but blood cultures showed no growth.

Treatment

The patient was treated with piperacillin/tazobactam, levofloxacin, and vancomycin according to protocol for health care-associated pneumonia in add to fluid resuscitation and infusion of dopamine, dobutamin and norepinephrine.

Related reports

The sepsis-induced systemic inflammatory response syndrome can produce myocardial dysfunction that sometimes defines Tako-Tsubo syndrome.

Term explanation

The dosage of troponin and B-type natriuretic peptides, the monitoring cardiac rate can be helpful to identify setting risk of myocardial dysfunction during sepsis.

Experiences and lessons

This article points out the importance of early haemodinamic support with fluid resuscitation, vaso-active amine and catecholamines in sepsis-induced myocardial dysfunction, trying at the same time to define a risk profile of a septic patient with cardiac involvement whose mortality is high.

Peer-review

In spite of richness of literature about the cardiac involvment during sepsis and management of sepsis-induced myocardial dysfunction, other research to identify the more suitable therapeutic strategy is necessary.

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CASE REPORT

Combined surgical-orthodontic rehabilitation of cleidocranial dysplasia: 5 years follow-up

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Author contributions: Çimen E, Tüzüner-Öncül AM, Özdiler E and Sayan NB designed the research; Çimen E, Tüzüner-Öncül AM, Yazıcıoglu D and Sayan NB performed the surgery; Özdiler E and Şenol A performed orthodontic treatment; Çimen E, Dereci Ö, Özdiler E and Şenol A analyzed the data; Dereci Ö and Çimen E wrote the paper.

Institutional review board statement: The name of our instutional review board is 'Clinical Research Ethical Board of Eskişehir Osmangazi University, Faculty of Medicine.' This study is a case report with a long follow-up. Our Ethical board does not accept case reports due to there is no need for ethical approval for case reports. Therefore, we did not apply for instutional ethical board for approval.

Informed consent statement: Patient presented in this study gave his informed consent prior to study inclusion.

Conflict-of-interest statement: All authors declare that there is no conflict of interest.

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Abstract

Cleidocranial dysplasia (CD) is an autosomal dominant syndrome which is characterized by several skeletal malformations such as non-closed fontanelles, skeletal abnormalities of the maxilla and mandible and absence of clavicles. Mid-facial hypoplasia and mandibular prognathism are mostly seen jaw abnormalities in CD. In this study, the combined orthodontic-surgical treatment of a patient with CD with class Ⅲ malocclusion and multiple unerupted primary and deciduous teeth is presented.

Key words: Cleidocranial dysplasia; Marie-Sainton syndrome; Forced eruption; Prognathism; Orthodontic extrusion

Core tip: Cleidocranial dysplasia is a syndromic disease with distinct craniofacial and maxillofacial manifestations. The satisfactory treatment of dental and

skeletal deformities in this disease can only be possible with combined orthodontic-surgical approach.

Çimen E, Dereci Ö, Tüzüner-Öncül AM, Yazıcıoğlu D, Özdiler E, Şenol A, Sayan NB. Combined surgical-orthodontic rehabilitation of cleidocranial dysplasia: 5 years follow-up. *World J Clin Cases* 2015; 3(8): 751-756 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i8/751.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i8.751

INTRODUCTION

Cleidocranial dysplasia (CD) is a syndromic disease which is also known as cleidocranial dysostosis or Marie-Sainton disease[1]. It is characterized by multiple or solitary supernumerary teeth, non-closed fontanelles, skeletal abnormalities of the maxilla and mandible, absence of clavicles, presence of open skull sutures, widening of pubic symphysis, multiple impacted permanent teeth and miscellaneous skeletal changes[2,3]. Mutations of the transcription factor RUNX2 which is one of the major regulators of bone maturation, are responsible for the occurrence of CD[2].

A multidiscipliner team approach is needed to overcome the dental abnormalities and facial deformities which are well-known complications that cause morbidity in CD patients. Combined treatment with orthognathic surgery and orthodontic therapy is the mostly-accepted treatment modality to correct mid-face hypoplasia and Class Ⅲ malocclusion in the treatment of CD patients.

In this study, the combined orthodontic-surgical treatment of a CD patient with class Ⅲ malocclusion and multiple impacted permanent teeth is presented.

CASE REPORT

Eighteen years old male with CD was referred to the department of orthodontics with complaints of ineffective chewing, biting and facial asymmetry (Figure 1). Orthopantogram, lateral cephalometric radiograph and antero-posterior radiograph were obtained for examination of skeletal and dental abnormalities (Figures 2 and 3). A diagosis of class Ⅲ, division 1 malocclusion was made and cone beam computerized tomography (CBCT) was taken to decide the exact shapes and positions of impacted teeth (Figure 4). Multiple permanent and 3 supernumerary impacted teeth were detected in CBCT sections and after orthognatic and surgical consultation, it was decided to erupt the impacted teeth and perform bimaxillary surgery to correct skeletal deformity.

The teeth 42, 31, 32, 33, 13, 12, 11, 21, 22, 23 were surgically exposed and orthodontic buttons were bonded for orthodontic traction under general anesthesia. Three impacted supernumerary teeth were extracted at the time of the exposure of permanent teeth. Missing teeth of the dentition were erupted in 10 mo (Figure 5). After eruption, orthodontic treatment was sustained and the alignment of dental arch was rendered at the end of 12 mo (Figures 6 and 7).

Bimaxillary orthognathic surgery was planned for correction of skeletal open-bite and class Ⅲ malocclusion. Patient was operated in general anesthesia. Post-operative recovery was non-eventful. Clinical outcome after surgery was satisfying according to the clinical point of view. Patient was undertaken in follow-up period with 6-mo intervals. Facial profile and occlusal appearance were normal and unchanged in 5th year follow-up control (Figure 8).

DISCUSSION

CD is associated with several heterogenous mutations in RUNX2 which is an osteoblast-specific transcription factor and also takes role in the tooth development[4]. The role of RUNX2 in tooth development partly accounts for dental abnormalities such as impacted permanent or supernumerary teeth[1]. However, the abnormal impaction of permanent and supernumerary teeth could not be fully explained since 40% of the CD cases do not have any apparent genetic mutation[2,5]. Most of CD patients are reported to have open sagittal sutures or fontanelles, multiple erupted or impacted supernumerary or primary teeth[5]. These symptoms are considered pathognomonic in the diagnosis of CD[2].

The treatment of CD varies due to the skeletal or dental abnormalities and the condition of impacted normal or supernumerary teeth. Daskalogiannakis *et al*[6] suggested that appropriate treatment of CD should be planned with careful consideration of age of the patient, expected duration of the treatment and patient expectation from the treatment. Dental management in CD is challenging in most cases and aims to achieve functional and esthetic results. Dental prosthesis is frequently used method for the functional rehabilitation of CD patients. With increasing attention to craniofacial anomalies in syndromic patients lately, collaborative orthodontic-surgical treatment of dental and skeletal anomalies of the jaws is suggested[6,7]. Several combined treatment regimens were proposed according to the timing and manner of approach to the impacted permanent teeth[8-12]. In our study, a technique similar to the Belfast-Hamburg[10,12] approach is applied to facilitate the eruption of impacted permanent teeth.

The traction of impacted teeth with elastics is a common method that is used in contemporary orthodontics, especially in syndromic cases such as CD. After surgical exposure of impacted or partially impacted tooth, a button or bracket is bonded and elastics are applied between impacted and opposing permanent erupted teeth[10,11]. If there are no opposing teeth or permanent erupted teeth are not suitable for traction, titanium screws may be used as anchorage

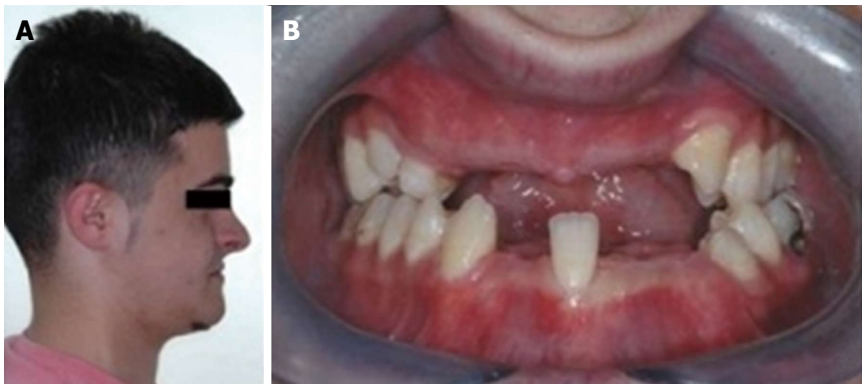

Figure 1 Clinical appearance before orthodontic treatment. A: Profile photo of the patient before orthodontic treatment; B: There were several missing teeth in the upper and lower dental arches.

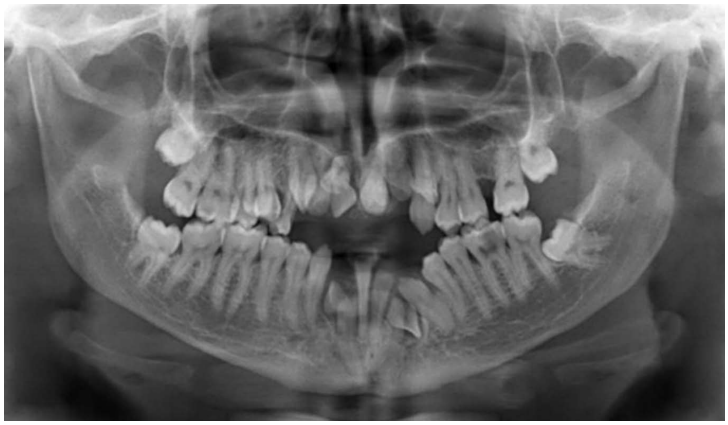

Figure 2 Pre-orthodontic panoromic radiography reveals multiple impacted permanent teeth with impacted supernumerary teeth and retained deciduous teeth. Ten permanent teeth were impacted. There were 2 supernumerary impacted teeth in the mandible and 1 supernumerary impacted tooth in the maxilla.

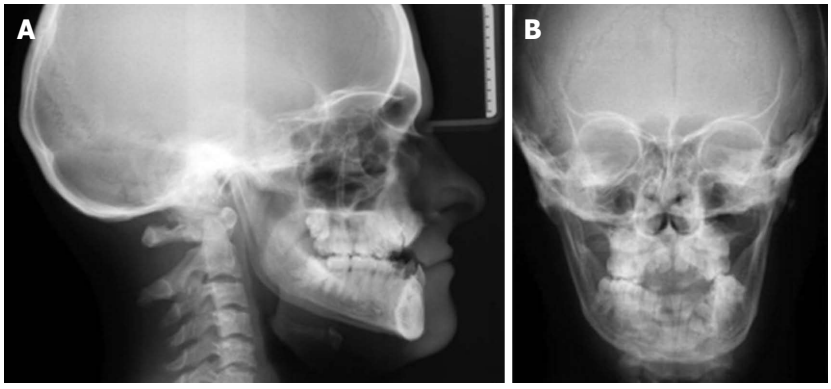

Figure 3 Sephalometric (A) and posterio-anterior radiographies (B) of the patient before orthodontic treatment.

for the application of traction elastics[13]. Rocha *et al*[14] suggested the use of a removable partial prosthesis to apply traction forces. In the current case, elastics were applied between lower and upper surgically exposed unerupted incisors and canines. The impacted teeth behaved as self-anchorage systems and full eruption of all impacted teeth were facilitated.

CD patients mostly have skeletal deformations leading to postural deformities. It is suggested that skeletal morphology may have a role in the development of temporomandibular joint (TMJ) disorders[15]. CD patients have jaw deformities and thus, tend to develop TMJ disorders. Physiotherapy or splint therapy is indicated in CD patients with TMJ disorders. The patient in the current study did not suffered from any myofacial pain or TMJ disorders and did not need any treatment on this matter.

CD is a syndromic disease with a prevalance of 1/1000000[16]. Dental manifestations of the disease is well-documented in the literature. Treatment planning in

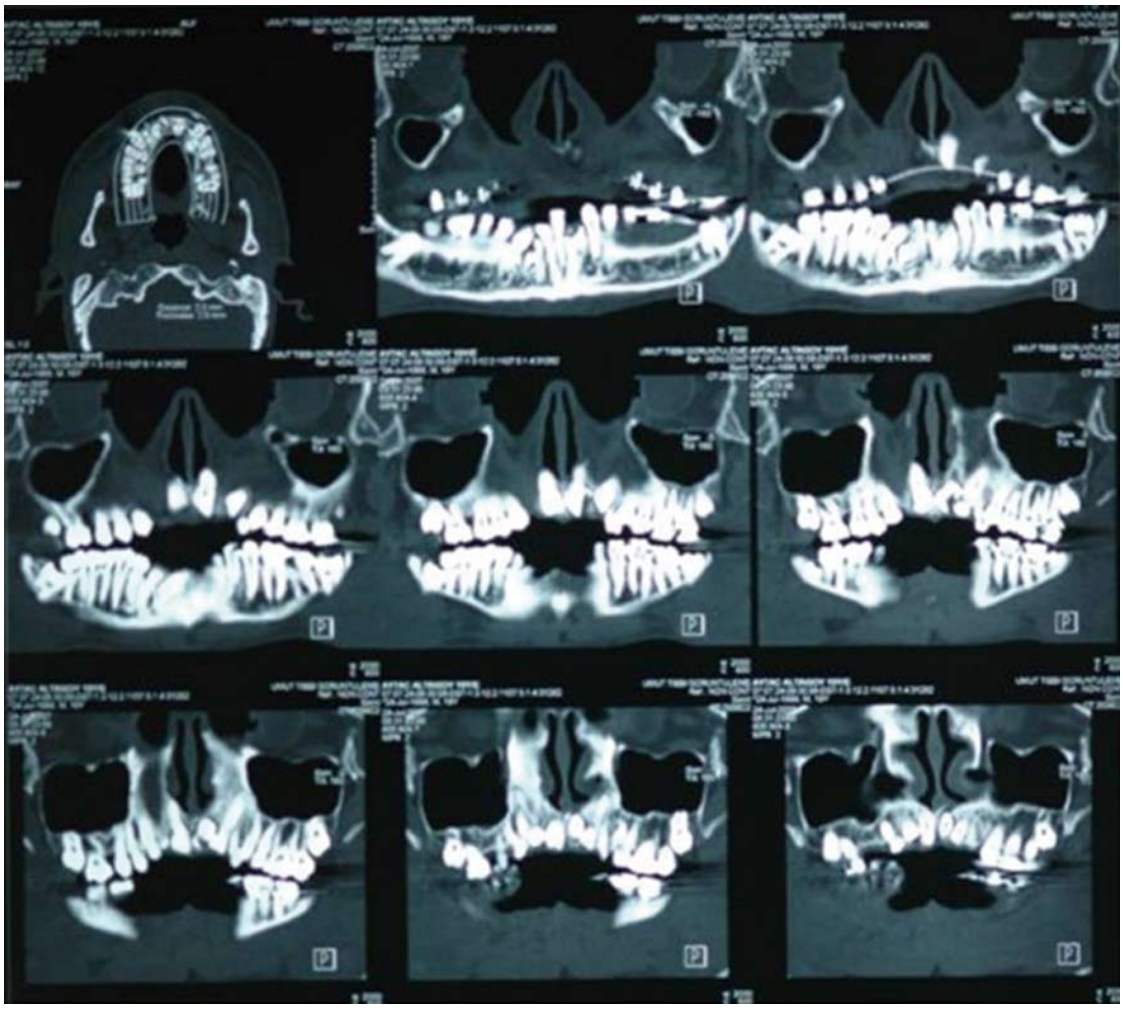

Figure 4 Frontal cone beam computerized tomography sections show impacted permanent and supernumerary teeth in detail.

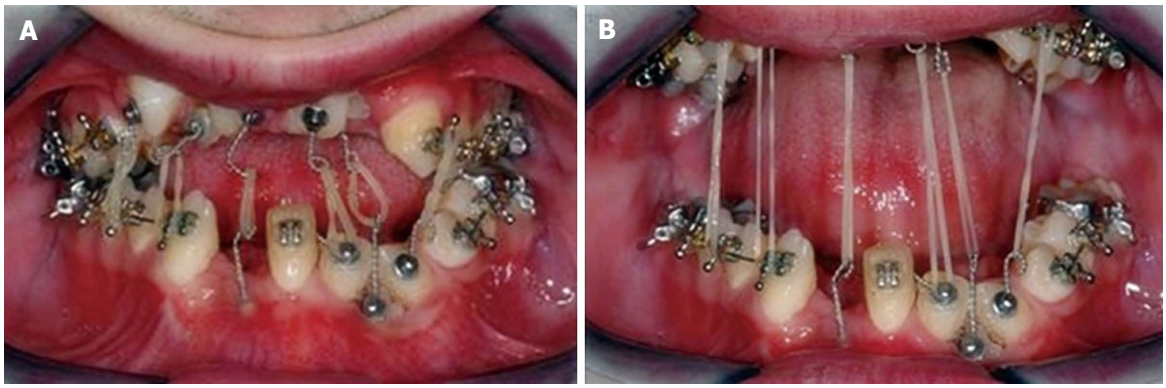

Figure 5 Intraoral elastics were applied between impacted teeth. A: Elastics remained passive when mouth is closed; B: They were activated when patient opened his mouth for daily activity such as speaking or drinking.

the rehabilitation of CD patients varies according to the degree of dental malocclusion, number of missing teeth and patients' expectations. The maintenance of force-aligned permanent teeth with elastic traction is also an important matter if the alignment of dental arches are provided with orthodontic treatment. Patients who are treated with combined surgical-orthodontic treatment should be monitored with continuous follow-up periods during early adulthood as in the current case.

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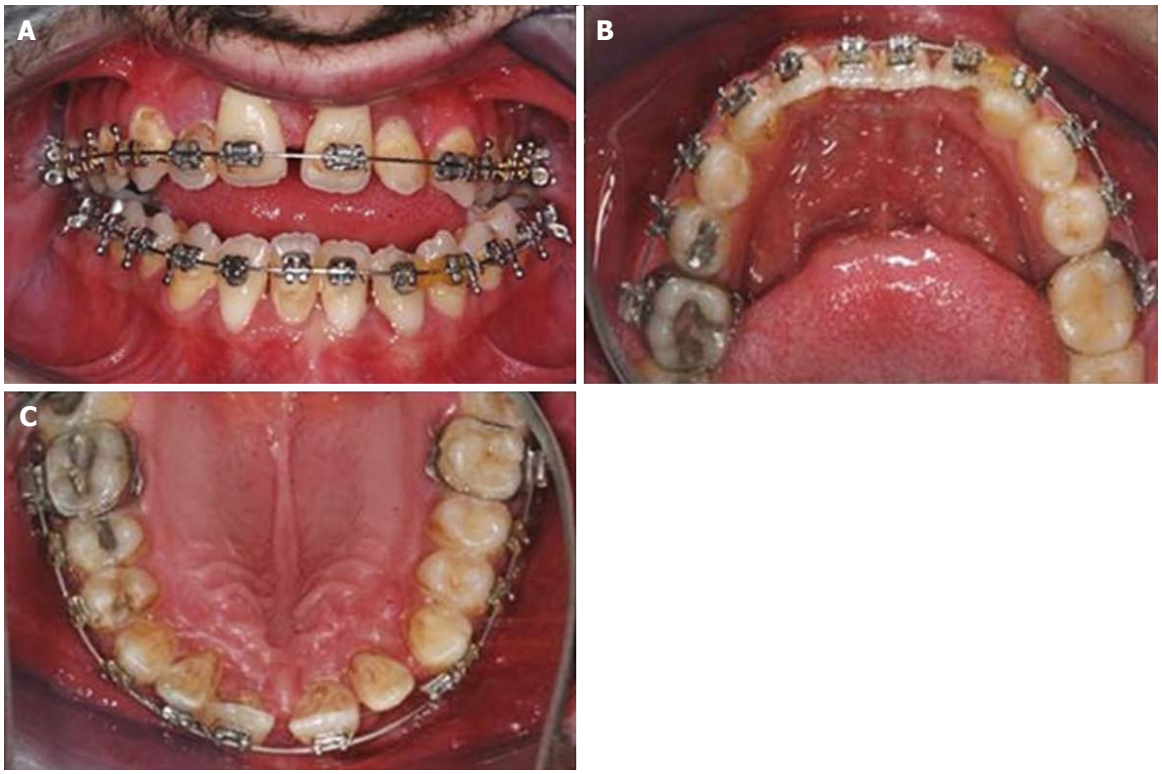

Figure 6 Clinical intraoral appearance after orthodontic treatment. A: The intraoral front-view of upper and lower dental archs after orthodontic traction and leveling treatment; B: Occlusal view of lower dental arch; C: Occlusal view of upper dental arch.

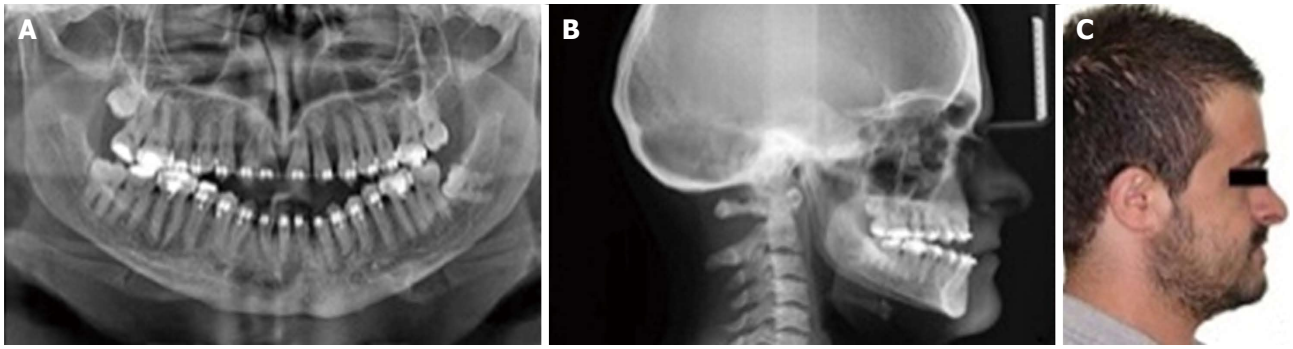

Figure 7 Radiographic and clinical evaluation after orthodontic treatment. A: Panaromic radiography after orthodontic leveling phase; B: Sephalometric radiography shows anterior open-bite malocclusion; C: Profile photograph of the patient after orthodontic leveling phase.

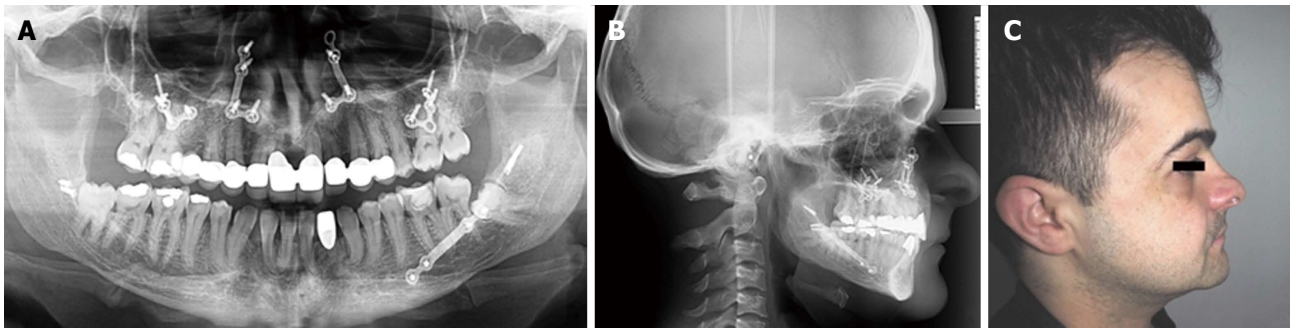

Figure 8 Radiographic and clinical evaluation after 5 years follow-up period. A: Panaromic radiography 5 years after orthognathic surgery reveals preserved and unchanged dental alignment; B: Dental class I occlusal relationship is clearly seen on sephalometric radiography; C: Profile appearance of the patient seems satisfying.

Santiago, Chile.

COMMENTS

Case characteristics

Cleidocranial dysplasia (CD) is a rare, dominantly inherited autosomal disease.

Clinical diagnosis

The main symptoms of CD includes hypoplastic or aplastic clavicles, supernumerary teeth, delayed eruption, impaction of permanent dentition and morphologic abnormalities of the maxilla and mandible.

Differential diagnosis

The differential diagnosis includes Crane-Heise syndrome, mandibuloacral dysplasia and pycnodysostosis.

Laboratory diagnosis

Laboratory testing methods are not needed in the diagnosis of CD.

Imaging diagnosis

Multiple impacted permanent or supernumerary teeth are detected in panoromic radiography or cone-beam computerized tomography sections of patients with CD.

Pathological diagnosis

Pathological examination is not needed in the diagnosis of CD.

Treatment

The treatment of CD includes orthodontics, orthognathic surgery or combined treatment modalities.

Experiences and lessons

The treatment of CD should be handled in a multidisciplinary basis. Follow-up is crucial after combined orthodontic-surgical therapy of cleidocranial dysplasia.

Peer-review

It is a well-written manuscript about a rare case.

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REVIEW

Vitamin K and hepatocellular carcinoma: The basic and clinic

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Abstract

Vitamin K (VK), which was originally identified as a cofactor involved in the production of functional coagulation factors in the liver, has been shown to be involved in various aspects of physiological and pathological events, including bone metabolism, cardiovascular diseases and tumor biology. The mechanisms and roles of VK are gradually becoming clear. Several novel enzymes involved in the VK cycle were identified and have been shown to be linked to tumorigenesis. The VKs have been shown to suppress liver cancer cell growth through multiple signaling pathways *via* the transcription factors and protein kinases. A VK2 analog was applied to the chemoprevention of hepatocellular carcinoma (HCC) recurrence after curative therapy and was shown to have beneficial effects, both in the suppression of HCC recurrence and in patient survival. Although a large scale randomized control study failed to demonstrate the suppression of HCC recurrence, a meta-analysis suggested a beneficial effect on the long-term survival of HCC patients. However, the beneficial effects of VK administration alone were not sufficient to prevent or treat HCC in clinical settings. Thus its combination with other anti-cancer reagents and the development of more potent novel VK derivatives are the focus of ongoing research which seeks to achieve satisfactory therapeutic effects against HCC.

Key words: Hepatocellular carcinoma; Vitamin K; Steroid and xenobiotic receptor; Nuclear factor-kappa B; Protein kinase A; Protein kinase C; Drug repositioning

Core tip: Vitamin K (VK) is essential nutrient initially identified as a cofactor to produce functional coagulation factors. In addition to the roles in hemostasis, pleiotropic effects of VK in bone health, atherosclerotic diseases and cancer have been attracting. VK has been shown to play tumor-suppressive roles in several cancers including hepatocellular carcinoma (HCC) and reported to have beneficial effects in the treatment of HCC although its anti-tumor effects remain to be improved. Currently novel VK derivatives are under developing and will be

applied to cancer treatment in the future.

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INTRODUCTION

Vitamin K (VK) is a well-known lipid-soluble vitamin that includes two natural types: VK1 and VK2, and synthetic types, known as VK3, VK4, and VK5. VK1, also known as phylloquinone, was first accidently identified by Dam[1] and Dam *et al*[2] for its anti-hemorrhagic activities in 1929. VK1 is ubiquitous and abundant in green leafy vegetables because it plays a direct role in photosynthesis. It performs the classic functions of VK that help the production of blood-clotting proteins[3-5]. VK2 is also known as menaquinone (MK), the subtypes of which are mainly synthesized by limited bacteria. They are mainly stored in animal products. The VK2 subtypes, which are characterized by the isoprenoid side chain length. MK-4 differs from the other MKs, which are synthesized by bacteria, such as MK-7, MK-8, and MK-9, in that it is the most common VK2 subtype in animals. It is a unique subtype because it is normally synthesized from VK1 *in vivo*[6-8]. In addition to VK1 and VK2, VK3 is an efficient coagulant[9]. Unlike the safe natural forms and other synthetic forms of VK, VK3 (menadione) is considered to be toxic because large doses have been shown to cause various adverse effects, such as allergic reactions, hemolytic anemia, and cytotoxicity in liver cells[10]. Aside from its clinical use as a hemostasis medicine, VK4 has recently been reported to have inhibitory effects on prostate cancer[11]. VK5 is used in many areas including the pet food industry to inhibit fungal growth[12] and has been shown to mimic the effect of insulin[13].

OVERVIEW OF VK METABOLISM

Dietary VK is absorbed from the small intestine along with dietary fat[14]. The latest findings have demonstrated that a cholesterol transporter, Niemann-Pick C1-like 1, is a key regulator of intestinal VKs absorption[15]. Despite VK's rapid metabolism in tissue, which results in comparatively low body storage[16], primary VK deficiency is rare in healthy adults. In addition to the average diet, which provides plenty of VK, other mechanisms maintain its balance within the human body. The VK cycle plays a critical role in maintaining VK function. The cycle proceeds through the coupled carboxylation and epoxidation carried out by gamma-glutamyl carboxylase (GGCX) and VK epoxide reductase (VKOR)[17,18]. The product, VK epoxide, plays an important role as a cofactor in blood coagulation factor production and is then reconverted to VK by VKOR[19]. It is well-known that warfarin and other 4-hydroxycoumarins block the activity of VKOR to inhibit coagulation[20,21], however, the identification of the *VKOR* gene was a recent finding[22,23]. More recently, it was demonstrated that VKOR deficiency caused early postnatal lethality in a knockout mice model due to severe intracerebral hemorrhage[24]. For decades it was believed that VK1 had the potential to transform into MK-4 endogenously in animals[8,25]. This was proven by mouse experiments[26,27]. Recently, the same group found that UbiA prenyltransferase containing 1 (UBIAD1), a human homologue of prenyltransferase menA, is a human MK-4 biosynthetic enzyme. Furthermore, they demonstrated that UBIAD1 is located in the ER and that it is not suppressed by warfarin[7].

THE INVOLVEMENT OF VK IN CELL AND TUMOR BIOLOGY

In addition to the initially identified role of VK as a cofactor in the production of functional clotting factors through Gla residue formation in the liver, Gla protein was identified in the bone matrix proteins such as osteocalcin in 1975[28], and the involvement of VK in the bone physiology has been studied[29]. Furthermore, since the 1980s researchers have shown the anti-proliferative effects of VK in several cancer cell lines, including (HCC)[30-33]. Although the novel attractive functions of VK were reported, the mechanisms of VK function beyond their role in activating hepatic coagulation factors remained unknown. In 2003, Tabb *et al*[34] identified the steroid and xenobiotic receptor (SXR), also known as PXR, as a ligand of VK2 and showed that SXR mediated gene expression in an osteosarcoma cell line. Interestingly, this research group further demonstrated that SXR is abundantly expressed in the liver and that it reciprocally regulates nuclear factor-kappa B (NF-κB)-regulated gene expression[35].

The anti-tumor effects of VK were attractive to investigators studying cancer biology. Otsuka *et al*[36] reported that VK2 inhibited the growth of HCC cells as well as their invasiveness *via* the activation of protein kinase A (PKA) and the subsequent inhibition of Rho activation. They also demonstrated the activation of the transcription factors AP-2-, USF-1- and CREB in HCC cells by showing the nuclear accumulation of Ser-phosphorylated CREB, although the roles of these factors in the VK2-induced suppression of cell growth and invasion are not known.

We have revealed that VK2 inhibits the growth of human HCC cells by suppressing cyclin D1 expression through the inhibition of NF-κB activation by suppressing IKK activity[37]. The suppression of NF-κB activation by VK2 was also observed in lipopolysaccharide-mediated macrophage activation[38] and in the VK-mediated suppression of the osteoclastogenesis of bone cells through the RANK/RANKL pathway[39,40]. It has been

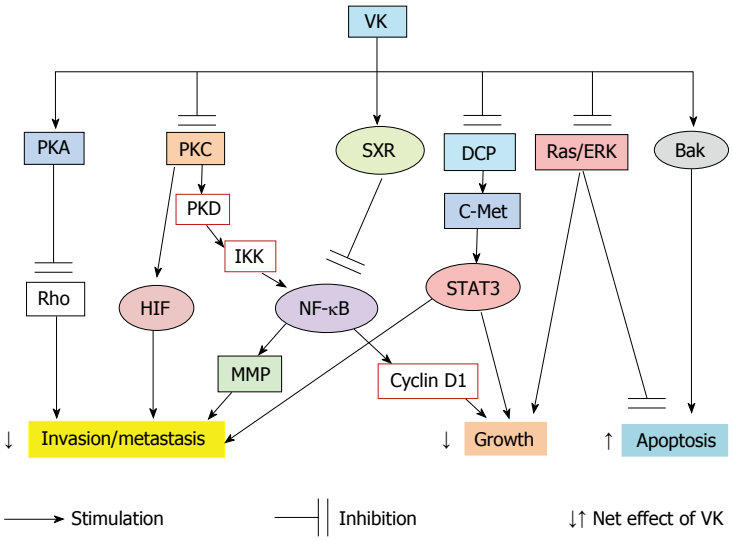

Figure 1 The effects of vitamin K on the multiple signaling pathways and the cellular behavior of liver cancer cells. PKA: Protein kinase A; PKC: Protein kinase C; PKD: Protein kinase D; DCP: Des-gamma-carboxy prothrombin; SXR: Steroid and xenobiotic receptor; ERK: Extracellular signal-regulated kinase; IKK: IκB kinase; NF-κB: Nuclear factor-kappa B; MMP: Matrix metalloproteinase; HIF: Hypoxia-inducible factor; VK: Vitamin K; STAT: Signal transducer and activator of transcription; Bak: Bcl-2 antagonist killer 1.

demonstrated that VK2 inhibits the expression of matrix metalloproteinases that contain NF-κB binding motifs in their promoter region[41], and augments the 5-fluorouracil-induced growth inhibition of HCC cells by inhibiting NF-κB activation[42]. Furthermore, we elucidated that VK2 inhibited the NF-κB activation through the inhibition of protein kinase C (PKC)-alpha and -epsilon kinase activities, as well as through the subsequent inhibition of PKD1 activation[43]. We have recently found that VK2 suppressed hypoxia inducible factor (HIF)-1 alpha activity through the inhibition of PKC by inhibiting the translocation of HIF to the nucleus[44].

Another interesting function of VK in the suppression of tumor development is its ability to induce apoptosis in certain cancer cells. Matsumoto *et al*[45] showed that VK2 induced apoptosis in Hep3B cells through the activation of AP-1. VK2-induced apoptosis is shown to be associated with p53 status in the human HCC cell line[46]. Recently Karasawa *et al*[47] demonstrated that VK2 covalently binds to Bcl-2 antagonist killer 1, a mitochondrial-mediated proapoptotic factor. The enhancement of apoptosis when VK2 was used in combination with acyclic retinoid (ACR) has been reported[48,49]. Kanamori *et al*[49] treated Huh7 cells with the combination of VK2 and ACR and found that VK2 plus ACR synergistically inhibited the growth of Huh7 cells by increasing apoptosis. When combined with ACR, VK2 inhibited Ras activation, followed by the inhibition of ERK phosphorylation. Interestingly, Suzuki *et al*[50] reported that des-gamma-carboxy prothrombin (DCP), also called protein induced by VK absence or antagonist Ⅱ, which is widely used as a tumor marker of HCC, has a binding affinity to c-Met, a hepatocyte growth factor receptor, and that it transmits aberrant STAT3 signaling[50]. They also reported the involvement of variant GGCX mRNA expression in the production of DCP in liver cancer[51]. Furthermore, Ma *et al*[52] showed the DCP-dependent growth advantage of HCC cells.

An interesting topic that has recently been reported in tumor biology is the role of UBIAD1, also called TERE1. UBIAD1 was recently identified as the menaquinone-4 biosynthetic enzyme[7]. UBIAD1 mRNA has been reported to be downregulated in prostate carcinoma cells and the overexpression of UBIAD1 inhibits the proliferation of tumor cell lines. UBIAD1 has therefore been considered to be a tumor suppressor in prostate cancer tumors[53]. Fredericks *et al*[54,55] reported that UBIAD1 controlled SXR-dependent gene expression in prostate cancer cells through several mechanisms. Since SXR transcription factor is a ligand of VK2 and because it has been shown to be involved in HCC cell growth[56,57], UBIAD1 expression might be linked to the effects of VK in HCC cells. More recently, UBIAD1 has been reported to be essential for embryonic development in mice[58] and VK2 has been shown to drive the metabolic maturation of pluripotent stem cells and fetal hepatocytes[59]. These findings suggest a novel role of VK metabolism in stem cells and that VK might be involved in cancer stem cell biology.

Collectively, the possible signal mechanisms of VK are summarized in Figure 1. VKs have been shown to have diverse effects on the phosphorylation states of various proteins[60]. Although the involvement of PKA, PKC, NF-κB, STAT, SXR and MAPK pathways are reported, the mechanisms by which VK2 modulates the protein kinases and/or phosphatases still remain to be elucidated. VK2 may reduce the growth and invasion of cancer cells through the modulation of protein kinases/phosphatases cascades.

VK DEFICIENCY BLEEDING IN NEWBORNS

Before the identification of VK as an essential cofactor for the production of functional coagulation factors, Townsend[61] (1894) reported 50 cases of a generalized bleeding tendency in neonates in a condition that was named the hemorrhagic disease of the newborn (HDN). He described that HDN differed from hemophilia in its earlier presentation, the lack of a family history and in its self-limiting course. Townsend[61] suggested a link between the mother's capacity to breast-feed and the hemostatic capacity of the newborn infant. After the identification of the role of VK in blood coagulation, the disease was shown to be related to VK nutritional deficiency and was renamed as VK deficiency bleeding (VKDB) by the ISTH Pediatric/Perinatal Subcommittee in 1999[62,63]. Although VK deficiency can occur in adults, it is common in newborns because of their limited VK storage, immature gastrointestinal absorption and due to the low placenta transfer of VK. The diagnosis of VKDB can be made in infants younger than 6 mo of age who present spontaneous bleeding, bruising, or intracranial hemorrhage with a prolonged clotting time but with a normal or elevated platelet count. Since the VKDB patients who present with intracranial bleeding are exclusively breastfed, Greer *et al*[64] investigated phylloquinone intakes in exclusively breast-fed infants in a North America and found that the average daily intake was one-tenth of that in healthy adults while formulated milk contained 50-fold higher concentration of phylloquinone than human milk. Although VKDB is rare in most developed countries, the consequences for the small number of patients who develop intracranial hemorrhage are often catastrophic. Nearly all cases of HDN/VKDB reported in the literature occur in infants who did not receive prophylactic VK supplementation in the newborn period. Consequently, many countries have introduced the routine prophylactic administration of VK at the time of birth to prevent hemorrhagic events[65,66].

THE INVOLVEMENT OF VK IN GENERAL HEALTH

Beyond the originally identified function of VK in blood coagulation system, it has been widely reported that VK has possible benefits on bone health and cardiovascular diseases[6,67]. Menatetrenone, a VK2 analog, has been used safely for the treatment of osteoporosis. Several clinical trials have shown it to be effective for treating osteoporosis in postmenopausal women, although the effects of VK2 alone might not be sufficient[68-70]. The potential benefit of VK in reducing cardiovascular disease risk is also reported and it might due to its function as a cofactor in the post-translational modification of the calcification-inhibiting matrix Gla protein[71,72]. An investigation revealed that UBIAD1-generated VK2 played an essential role in maintaining endothelial cell survival and overall vascular homeostasis[73].

A European large cohort study showed that dietary VK intake was associated with the reduced risk of cancer incidence in the prostate and the lung and that the effects were more pronounced in men than in women[74]. Furthermore, a more recent study demonstrated the association between the dietary intake of VK and the reduced risk of cardiovascular disease, cancer, and all-cause mortality in a Mediterranean population[75].

THE CLINICAL ASPECTS OF VK IN LIVER CANCER

In 1984, abnormal des-carboxy prothrombin was specifically detected in the plasma of patients with HCC[76]. It has since been used as specific diagnostic marker of HCC independent of α-fetoprotein[77,78]. Since the administration of VKs to patients with increased DCP levels showed a transient reduction of DCP levels, HCC was considered to exist under a condition of VK deficiency[79,80]. Earlier studies showed that the administration of VKs on cancer cells including HCC *in vitro*, resulted in anti-proliferative effects[30-33]. Thus, the anti-tumor effects of VK on HCC have been expected to be found *in vivo*.

In 2004 Habu *et al*[81] demonstrated that menatetrenone, a VK2 analog, suppressed the development of HCC in women with viral hepatitis-related cirrhosis. Since then, several randomized controlled studies reported the suppressive effects of menatetrenone on the recurrence of HCC after curative ablation therapy and surgical resection of the liver[82-86]. Although several initial reports with small study populations showed the favorable effects of VK2 in inhibiting the recurrence of HCC after treatment and improving tumor recurrence-free survival, a large randomized control trial (RCT) in which VK2 was administered after curative treatment, failed to show the advantage of VK2 administration[87].

Zhong *et al*[88] reviewed six RCTs and one cohort study, with a total of 930 patients and performed a meta-analysis. Although treatment with VK2 did not reduce the 1-year recurrence rate, there was a significant association between VK2 and reduced 2- and 3-year tumor recurrence. VK2 treatment was also associated with a significant improvement of 1-, 2-, and 3-year overall survival. However, the results might be considered to still be preliminary because the large scale RCT was evaluated at only 1 year. Therefore, a longer follow-up will be required to confirm the effects of VK2 on HCC.

FUTURE DIRECTIONS

Although various studies have reported the anti-HCC effects of VK2, the analogs in current use do not appear to exhibit dramatic anti-tumor effects when administered alone. One way to overcome this situation

is with the co-administration of VK and other reagents with anti-cancer properties. Yoshiji *et al*[85] reported the beneficial effects of VK2 combined with ACE inhibitor. Recently acyclic retinoid peretinoin showed beneficial effects on the recurrence and survival of hepatitis C virus-infected HCC patients after curative therapy[89]. VK2 plus ACR synergistically inhibited the growth of Huh7 cells[49]. Currently sorafenib is the only drug approved for the systemic treatment of HCC[90]. It has been shown to extend the survival period of end-stage HCC patients for several months. However, the effect of sorafenib on HCC is not yet satisfactory. Many novel-developed anti-cancer reagents that specifically target the signal transduction pathway of HCC cells have been tested clinically, but most of trials failed to demonstrate their non-inferiority to sorafenib[91,92]. A combination treatment with sorafenib and VK2 was examined *in vitro* and *in vivo* animal models and the studies showed that VK2 enhanced the tumor-suppressive effects of sorafenib[93-95].

Another way to enhance the effects of VK would be to develop a new VK derivative, which may be achieved by modifying the side chains of VK. Several approaches to develop novel VK analogs have been conducted. Since some of the effects of VK are considered to be mediated by SXR transcription factor as a ligand of VK2[34,56], Suhara *et al*[96,97] screened a series of chemically synthesized VK analogs by measuring the SXR-mediated transcriptional activity and found that the modification of the side chain of VK affects the SXR-mediated transcriptional activity. Setoguchi *et al*[98] synthesized a prodrug of an active form of menaquione-4 that is effectively delivered to HCC cells and which showed the enhanced anti-tumor effects on HCC cell growth.

Recently the repositioning (repurposing) of pre-existing drugs that have been safely used for long-term treatment in a clinical setting has been performed with many drugs such as aspirin and metformin[99,100]. Some of these drugs have begun to be used for chemoprevention and/or for the therapeutic purpose of enhancing anti-cancer effects. VKs seem to be one of the successful examples of repositioned drugs. After the discovery of VK as a cofactor of functional coagulation factor production, it has been shown to be beneficial for the maintenance of bone physiology and the prevention of cardiovascular diseases. Beyond these effects, the novel function of VK as an anti-tumor agent has been applied to the prevention and treatment of HCC, however, the beneficial effects of VK on HCC were found to be limited. The recent progress of novel technologies, such as a genome wide association studies and computational analysis, has been the first step to the repositioning of drugs[101,102]. These approaches will lead to novel applications of VKs and the development of novel VK-based reagents, and may be applied to the treatment of HCC in the future.

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MINIREVIEWS

Intraoperative neurophysiological monitoring in spinal surgery

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Abstract

Recently, many surgeons have been using intraoperative neurophysiological monitoring (IOM) in spinal surgery to reduce the incidence of postoperative neurological complications, including level of the spinal cord, cauda equina and nerve root. Several established technologies are available and combined motor and somatosensory evoked potentials are considered mandatory for practical and successful IOM. Spinal cord evoked potentials are elicited compound potentials recorded over the spinal cord. Electrical stimulation is provoked on the dorsal spinal cord from an epidural electrode. Somatosensory evoked potentials assess the functional integrity of sensory pathways from the peripheral nerve through the dorsal column and to the sensory cortex. For identification of the physiological midline, the dorsal column mapping technique can be used. It is helpful for reducing the postoperative morbidity associated with dorsal column dysfunction when distortion of the normal spinal cord anatomy caused by an intramedullary cord lesion results in confusion in localizing the midline for the myelotomy. Motor evoked potentials (MEPs) consist of spinal, neurogenic and muscle MEPs. MEPs allow selective and specific assessment of the functional integrity of descending motor pathways, from the motor cortex to peripheral muscles. Spinal surgeons should understand the concept of the monitoring techniques and interpret monitoring records adequately to use IOM for the decision making during the surgery for safe surgery and a favorable surgical outcome.

Key words: Motor-evoked potentials; Somatosensory-evoked potentials; Intraoperative neurophysiological monitoring; Direct wave; Spinal surgery

Core tip: Recently, many surgeons have used intraoperative neurophysiological monitoring (IOM) in spinal surgery to reduce the incidence of postoperative neurological complications, including level of the spinal cord, cauda equina and nerve root. Several established technologies are available and multimodality combinations are considered necessary for practical and effective

IOM. Spinal surgeons should understand the concept of the monitoring techniques and interpret monitoring records adequately to use IOM for the decision making during the surgery for safe surgery and a favorable surgical outcome. In this review, the authors will review the different IOM techniques to provide a fundamental concept for better comprehension.

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INTRODUCTION

Recently, many surgeons have used intraoperative monitoring (IOM) in spinal surgery to reduce the incidence of postoperative neurological complications, including level of the spinal cord, cauda equina and nerve root. Its importance has been emphasized to prevent an unsuspected and unpleasant neurological deficit after spinal surgery. Surgeons should understand the rationale and clinical basis for IOM to interpret the monitoring alerts and to utilize them for a better surgical outcome. Several established technologies are available and combined motor and somatosensory evoked potentials are considered mandatory for practical and successful IOM. In this review, the authors will review the different IOM techniques to provide a fundamental concept for better comprehension.

MONITORING TECHNIQUES

Spinal cord evoked potentials

This technique was invented in Japan in the 1970s. Electrical stimulation was provoked on the dorsal spinal cord from an epidural electrode[1]. The evoked compound potentials from the stimulated spinal cord are recorded over the spinal cord. The spinal cord evoked potentials (SCEP) correspond to summation of neural activities originating from the ascending and descending tracts and neurons near the recording electrode. The recorded potentials are very vigorous and most likely represent the combined activity of the tracts of the spinal cord, such as dorsal columns, the corticospinal tracts and others[2]. In a practical setting, SCEP cannot provide sufficient information about motor-related function because sensory-related potentials, which are large in amplitude, mask motor-related potentials. The advantages and disadvantages of each modality, including SCEPs, are summarized in Table 1.

Somatosensory evoked potentials

Somatosensory evoked potentials (SEPs) assess the functional integrity of sensory pathways from the peripheral nerve, through the dorsal column and to the sensory cortex. SEPs were first used in the 1970s to monitor the spinal cord function during surgery for scoliosis correction. After stimulation of peripheral nerves, SEPs are recorded both from the spinal cord with an epidural electrode and/or from the cortex. SEPs can be applied for monitoring the peripheral sensory pathways consistently[3]. From a technical point of view, the posterior tibial nerves are stimulated (duration of stimulus, 0.2 ms; frequency, -3 Hz; intensity, -25 mA). SEPs are the result of averaging. The acquisition time is on the order of 1 min[4]. Data are measured to determine latency and amplitude. Latency is a measure of time and is related to distance. Amplitude is a measure of power and characteristically more variable than latency. This potential indicates activities from the dorsal column such as the sensory tract. By shifting the stimulus site, one can identify the laterality of dorsal column lesions. Monitoring the laterality can provide important information during posterior myelotomy for removing intramedullary spinal cord tumors. An increase in latencies greater than 10% and a decrease in amplitudes greater than 50% constitute warning signals. SEPs are altered by the surgical procedure due to a mechanical factor or secondarily by ischemia. SEP alterations can also be related to patient's age, height and length of the limbs, systemic hypotension, hematocrit decrease, hypothermia and anesthesia (volatile agents such as isoflurane, halothane, nitrous oxide attenuate SEPs and should not be used if monitoring is employed). Body temperature is a common factor affecting spinal somatosensory evoked potential (SSEP) latency readings.

SSEP

Monitoring of dorsal column integrity with SSEP is the most commonly used technique in spinal surgery. Large diameter, myelinated and fast conducting cutaneous and muscle afferents carry the peripheral SSEP. SSEP can monitor the dorsal column-medial lemniscus pathway, which mediates tactile discrimination, vibration sensation, form recognition and joint/muscle sensation (conscious proprioception). In SSEP monitoring, stimulation electrodes excite controlled repetitive action potentials that propagate from peripheral nerves to the contralateral sensory cortex through the dorsal roots and the dorsomedial tracts of the spinal cord.

These signals can be recorded at various anatomically accessible locations, such as the peripheral nerve, spinal cord, brainstem and its endpoint and the somatosensory cortex. Platinum subdermal needle electrodes are used for both stimulation and recording. Generally, a 50% decrease in amplitude with an associated 10% increase in latency in comparison to the patient's baseline values constitute a warning signal. A previous study reported that false negative SEP monitoring occurred during surgery in only 0.063% of patients[5]. A large multicenter study has reported that postoperative paraplegia was reduced more than 50%-60% with SEP monitoring[6]. Although SSEP signals are good basic indicators of spinal cord function, they cannot provide much information regarding nerve root function.

Table 1 Summary of advantages and disadvantages of each monitoring technique

	Advantages	Disadvantages
SCEP	Easy to record using very simple hardware	The electrode used to deliver stimulation to the spinal cord should be located in the epidural space and the recording electrode in the intrathecal space
	Provides real-time information because its potentials are large enough without averaging	The malposition of the electrode can occur
		Previous scarring can sometimes impair electrode placement
SEP	Broadly available	Does not directly monitor corticospinal tract. Only assess the functional integrity of spinal cord dorsal columns. In the case of anterior spinal artery syndrome, postoperative paraplegia despite intraoperative SEP preservation has been reported
	Easy to implement	When approaching the intramedullary tumor during the initial dorsal myelotomy, SEPs can completely disappear
	Has no contraindications	SEP recording requires signal averaging, which results in a time delay until data interpretation can generate a response to the surgeon. Therefore, an injury can be irreversible before it is even detected
	Can be combined with other monitoring techniques	
	Allows continuous monitoring throughout case	
	Excellent specificity (approaching 100%)	
Neurogenic	Fast and easy to implement	Their specificity remains relative because they correspond to the joined activation of motor and sensory pathways
MEP	Resistant to most anesthetics	Require curarization
	Sensitive in detecting a lesion	The terminal medullary cone is not monitored
	In case of alert, the lesional level can be determined by displacing the stimulation electrode along the intervertebral spaces	
D waves	Very rapid acquisition	The recording electrode is in the surgical field and its use by the surgeon can produce artifacts
	D waves are specific of motor pathways	Laterality cannot be distinguished
	They can establish a lesional level by displacing the spinal electrode along the intervertebral spaces	D waves cannot be used in small children, generally under 4 yr of age (incomplete maturation of motor path-ways)
	D waves have prognostic value	Cannot be recorded below the level of T12 because there are not enough corticospinal tract fibers
	Correlates most accurately with long-term motor function following intramedullary spinal cord tumor resection	Previous scarring can sometimes impair electrode placement
Muscle MEP	Do not require an averaging. Thus immediate feedback can be available	Require at least partially functional motor pathways
	Preserved sensitivity and sensitivity even after posterior myelotomy	Incompatible with prolonged curarization
		Exceptional adverse effects have been described: tongue or lip laceration, mandibular fracture, cardiac arrhythmia, epileptic seizures, scalp burn and intraoperative awareness
		Often difficult to carry out on patients under the age of 6 yr because of incomplete maturation of motor pathways
Pedicle screw testing	Rapid and easy technique	Sensitive to a large number of anesthetics
	Can be combined with new surgical instruments used during screw placement	Can be distorted by curarization
	High sensitivity for medial pedicle breach	Less sensitive for thoracic pedicle screws than for lumbar pedicle screw
	Useful in minimally invasive surgery where anatomical landmarks may be challenging to visualize	Optimal alarm criteria not firmly established
		Does not directly assess for neurological injury, only provides information regarding pedicle integrity

SCEP: Spinal cord evoked potentials; MEP: Motor evoked potential; SEP: Somatosensory evoked potentials.

Dorsal column mapping

It is important to decide where to perform the myelotomy during intramedullary spinal cord surgery. Anatomic landmarks are often utilized as an indicator for midline intraoperatively. The typical anatomical landmarks for midline of the spinal cord include the dorsal median sulcus between the dorsal columns and the median dorsal sulcal vein which enters the midline raphe. Dorsal column mapping technique can be applied to identify the "physiological midline". It is helpful for reducing the postoperative morbidity associated with dorsal column dysfunction when an intramedullary cord lesion distorts the normal spinal cord anatomy that results in confusion in distinguishing the midline for the myelotomy[7-9]. The multielectrode grid is placed transversely over the dorsal surface of the cord. It has multiple wires and each wire has been stripped of its insulating coating. These recording wires pass parallel to the long axis of the

spinal cord and a reference needle electrode is inserted in a nearby muscle. This multielectrode grid can record the traveling SEP waves from the dorsal surface of the exposed spinal cord very selectively with the amplitude gradient corresponding to the topographic arrangement of the dorsal column. Because of the somatotopic distribution of ascending fibers in the dorsal column, the highest amplitude close to the midline will be recorded after SEP stimulation on the right posterior tibial nerve. By the same reaction from the contralateral side, we can identify "physiological midline" between these two amplitude peaks.

MOTOR EVOKED POTENTIALS

Direct waves (or spinal motor evoked potentials)

D (direct) waves are compound corticospinal action potentials initiated by direct axonal activation with conduction velocity approximately 50 m/s[10], making it possible to monitor the motor pathways from the cortex to the level of the spinal electrode. D waves are obtained by a single transcranial electrical stimulation (intensity, 80-100 mA; duration of stimulus, 0.5-1 ms; frequency, 0.5-2 Hz) recorded from the epidural or subdural space of the spinal cord[11]. It is generated directly by the electrical pulse. Therefore, they are called "single stimulus technique" or "spinal motor evoked potentials (MEPs)". D waves usually do not need averaging, although a few averages improve its quality of recording. This provides clinically real-time feedback. If the D wave amplitude decreases more than 50% of the baseline value or cannot be detectable, there is high probability of severe neurological deficit such as permanent paraplegia.

Neurogenic MEP

Neurogenic MEP is an elicited potential that is electrically stimulated at the spinal cord with epidural electrodes and then recorded from the peripheral nerves. Neurogenic MEPs are recorded by stimulating the spinal cord through electrodes inserted by the surgical team: A flexible spinal electrode inserted into the epidural space proximal to the operating field. The stimulation parameters are the following: intensity, 20-50 mA; duration of stimulation, 1 ms; frequency, 4.1 Hz. Recordings are performed at the internal popliteal sciatic nerves or the posterior tibial nerves. This technique allows monitoring of the overall spinal cord. In 1988, recording neurogenic evoked potentials from peripheral nerves in lower extremities after spinal cord stimulation was suggested[12] to monitor spinal motor pathways. It is now widely revealed that these potentials contain at least a significant antidromical sensory component[13-15]. Neurophysiological collision studies have shown that the biphasic component corresponds to antidromical activation of the sensory pathways, whereas the polyphasic component corresponds to activation of the motor pathways. Neurogenic MEPs provide combined sensory and motor spinal pathway monitoring[16,17].

Muscle MEP (or myogenic MEP)

Motor potentials are evoked with transcranial electrodes which are placed on the scalp over the motor cortex area of the skull. The stimulus points are C3, C4, C1, C2, Cz and a point 6 cm in front of Cz (international 10-20 system)[11]. Muscle MEPs are derived from transcranial electrical stimulation (five to seven pulses, 2-4 ms apart; intensity, 250-750 V; duration of each pulse, 0.5 ms) over the same electrodes as for the D-wave. The technique is called the "multipulse technique" or "train stimulus technique". Compound muscle action potentials (CMAP) are recorded with needle electrodes from target belly muscles in all four limbs. Muscles are selected based on the surgical procedure and spinal levels involved. Typical muscles for arm MEP recordings include the abductor pollicis brevis (thenar muscle); the 1st dorsal interosseous and hypothenar muscles are alternatives. Typical muscles for leg MEP recordings include the tibialis anterior (TA) and abductor hallucis (AH). Other muscles, such as the quadriceps, deltoids, biceps, even the diaphragm, and the external anal sphincter can be selected. Muscle MEPs allow selective and specific analysis of the functional integrity of descending motor tracts, from the motor cortex to muscles. Recordings are lateralized for each limb. Muscle MEPs do not require averaging and can be repeated at a rate of 0.5-2 Hz. A reduction of more than 50% of the baseline amplitude can be an alarm point for postoperative motor deficit. However, even though muscle MEPs are lost, there may be a transient deficit but no permanent postoperative deficit if the D wave is preserved[18]. A temporary postoperative motor deficit can occur when muscle MEP amplitude is lost but D wave amplitude preserved. In this situation, surgeons can continue the resection or stop and wait for recordings to recover, which they often do. Muscle MEP loss without D wave changes or the D wave decrease without muscle MEP changes in muscle MEP occurs. A D wave maintained over 50% of the baseline indicates that the neurons of the corticospinal tract which controls delicate voluntary movements remain. In any event, if the D wave was preserved over 50% of the baseline value, it is considered that the voluntary motor control in the lower extremities are preserved[19]. A decrease of the amplitude of muscle MEP is not always associated with postoperative neurological deficit; however, it is valuable to evaluate the early ischemic or mechanical damage to the spinal cord. Despite several reports that refer the accuracy of D-wave monitoring during surgeries for intramedullary spinal cord tumors, muscle MEP monitoring is maintained as a valuable tool. This is because muscle MEP does not need an epidural electrode, has a higher generation rate of MEP and it is more accurate in monitoring for scoliosis surgery[20].

ANESTHETIC CONSIDERATIONS

Both SSEP and MEPs are affected by various pharmacological and physiological factors. Any drug or

physical parameter that influences electrical conduction along an axon may alter the evoked potential waveform. In general, the longer with more synapses tracts are, the more sensitive they are. Furthermore, a greater number of pulses will be needed for lower extremity recordings compared with upper extremity sites because it is easier to obtain signals from the upper extremity than from the lower extremity. This is because the hand area occupies a larger representation on the motor cortex[21]. Inhaled anesthetics reduce the amplitude and increase latency, while intravenous anesthetics have the same effect but to a lesser degree. Halogenated or nitrous oxide-based agents influence SSEP amplitude and latency. MEPs are generally more sensitive to anesthetics than SSEPs. Total intravenous anesthesia without neuromuscular blockade is material to muscle MEPs to allow CMAP monitoring. Typically, induction with short-acting muscle relaxants, a continuous infusion of propofol and fentanyl and low level nitrous oxide use (not exceeding 50% by volume) are mandatory for MEP monitoring. In our institute, when we need IOM for the surgery, anesthesia is maintained with a continuous infusion of propofol (10 mg/kg per hour) and remifentanil (0.25 mg/kg per minute). At induction, a single bolus of non-depolarizing short acting muscle relaxant (rocuronium) is given to facilitate tracheal intubation and ventilation. The level of neuromuscular block is monitored by recording the CMAP to a train of 4 stimuli.

ELECTROMYOGRAPHY

Spontaneous electromyography

Spontaneous or free-running electromyography (EMG) is widely applied to monitor selective nerve root function during spinal cord surgery. Unlike SEP and SSEP data, EMG is "real-time" recording from peripheral musculature. Spontaneous EMG can help to prevent postoperative radiculopathy during spinal instrumentation surgery, including pedicle screw placement. This technique does not require stimulation and can be recorded continuously from preselected muscle groups based on the nerve roots at risk[22]. One muscle group per nerve is generally considered appropriate. Common EMG recording sites by spinal levels are as follows: C4 - supraspinatus; C5 - deltoid and biceps; C6 - biceps and wrist extensors; C7 - triceps, wrist flexors and finger extensors; C8 - hand intrinsics and finger flexors; T1 - hand intrinsics; T6-12 - rectus abdominis; L1 - iliopsoas; L2 - adductor longus; L3 - adductor longus and vastus medialis; L4 - vastus medialis and vastus lateralis; L5 - TA and extensor hallucis longus; S1 - medial gastrocnemius and peroneus longus; S2-5 - perianal musculature and urethral sphincter. However, in case of cervical spinal surgery, many surgeons favor monitoring 2 muscle groups, deltoid (predominantly C5, also C6) and biceps brachii (predominantly C6, also C5), due to the risk of C5 palsy[23]. At baseline, no muscle activity is recorded from an intact nerve root. Surgical manipulations such as pulling, stretching or compression of nerves provokes spikes or bursts of activity termed neurotonic discharges, resulting in activity in the corresponding innervated muscle(s). Spontaneous EMG is quite sensitive to irritation of the nerve root, such as retraction of spinal cord or nerve root, saline irrigation and manipulation during surgery. However, false spontaneous EMG activation commonly occurs during irrigation with cold water, cauterization and use of a high-speed drill because it is sensitive to temperature changes.

Spontaneous EMG trains are continuous, repetitive EMG firing caused by continuous mechanical stress on the nerve root. Trains of higher frequency and/or amplitude are likely to indicate a high probability of nerve injury unless a prompt corrective management is performed. On the other hand, spontaneous EMG spikes and bursts indicate the proximity of the nerve root.

It is also important to note that EMG signals are interfered with in patients with various neurological disorders, such as myasthenia gravis, botulinum toxin treatments for dystonia and muscular dystrophy.

Pedicle screw testing by triggered EMG

The introduction of triggered EMG for evaluating the integrity of lumbar pedicles during screw placement and the accuracy of pedicle screw placement was first described by Calancie *et al*[24] in 1992 using a porcine model. The concept of triggered EMG is that intact cortical bone should be electrical insulation for a well-placed pedicle screw from the adjacent nerve root. In contrast, the pedicle screw would be relatively poorly insulated when medial pedicle breach occurred[22]. Intraoperative triggered EMG detects root irritation or the post injury condition of the root after medial pedicle breach. An irritated or damaged nerve root causes a decrease in electric threshold followed by sudden appearance of CMAPs of the specific muscles of the myotome after stimulation *via* the screw[25]. Each pedicle screw is electrically stimulated with an increasing intensity from 5 to 30 mA (duration, 0.2 ms; frequency, 0.8 Hz). Recordings are made at the level of the lower limb muscles with or without the rectus abdominis muscles (depending on the root levels to test). The recording of muscle activity at an intensity under 10 mA (the classically accepted threshold) argues in favor of a medial breach (close proximity of the screw to the nerve root).

PATTERNS OF MEP CHANGES DURING SURGICAL PROCEDURES

MEP changes occur most frequently towards the end of the tumor resection when the interface between the tumor and normal tissue comes close. Some of the authors of this study have previously reported that the type of muscle recorded affects the changing patterns and prognostic values of muscle MEP[26]. Muscle MEPs recorded from the AH muscle were relatively resistant to perioperative neural damage, otherwise the

muscle MEPs from the TA were relatively vulnerable to perioperative neural damage at an earlier time and/or more sensitive compared with the single-muscle recordings in the AH. Thus, the loss of muscle MEP in the TA, even though muscle MEPs in the AH were preserved, should be considered a potential early warning sign for possible postoperative neurological deficit. This result is a meaningful point of view in that using a combination of muscle MEPs from different muscles with individual sensitivities and clinical significances will improve intraoperative muscle MEP monitoring interpretations and surgical strategies.

ACTIONS TO RECOVER DETERIORATED MEP

There are some procedures useful for promoting the recovery of deteriorated or lost MEPs during spinal surgery[8,10,11,27,28]. First, transient stopping the surgical manipulations immediately after MEP and observation may recover MEP spontaneously. Second, irrigation of the surgical field with warm saline solution generally removes irritating blood products and metabolites. It is believed that accumulated extracellular potassium which is derived from local tissue damage by surgery acts as a strong axonal blocking agent. Third, local application of papaverine and increasing the mean arterial pressure (MAP) more than 90 mmHg can facilitate local perfusion to resist local ischemia. Sustained hypotension may affect MEPs and may result in an unfavorable postoperative outcome. Temporary moderate elevation of MAP may restore blood flow and may lead to improving both D-waves and muscle MEPs successfully if ischemia due to insufficient MAP is the primary cause. Fourth, when dissection in a particular location results in MEP changes, the resection at a different site can proceed without further aggravation. Fifth, increasing body temperature also may help recover MEPs because deep hypothermia obliterates muscle MEPs. Sixth, when all the previous methods have failed to regain MEP, applied correction is reduced or anchoring hardware removed in case of the correction of the spinal deformity. If there is still no significant signal recovery, a steroid bolus (methylprednisolone or dexamethasone) could be considered. Finally, if there is no noticeable neurophysiological improvement in response to all methods described above, consider removing the spinal implant or discontinuing tumor resection.

IOM IN PRACTICE

For many years, only SEPs were monitored during spinal cord surgeries before MEP techniques were developed. Since the multipulse technique was introduced in the mid-1990s, combined muscle MEP and SEP monitoring have been routinely used intraoperatively in various spinal operations, including from deformity correction to intramedullary cord tumors. Many authors have reported that combined SEP/MEP monitoring provided higher sensitivity and higher positive/negative predictive values than single-modality monitoring techniques and that optimal monitoring requires both SEPs and MEPs[27-30].

The reliability of intraoperative MEP to identify impending motor deficits has been improved, mainly as a result of accumulated knowledge interpreting the MEP changes. Monitoring muscle MEPs became the gold standard due to their increased sensitivity and the importance of motor function. It has been believed most widely that MEP recordings are stable if changes were less than 50% in amplitude and less than 10% in latency. However, there is no definite alarm point. Various different alarm points have been reported. From a surgical viewpoint, the 50% criterion could have the effect that the surgical correction of the deformity is performed incompletely or tumor resection is forsaken too early and the 80% criterion that the operation proceeded too aggressively[30]. The benefit of more sensitive MEP criteria and the risk of stopping resection too early should be always considered in parallel. This is important especially for tumors of which the most important prognostic factor is complete resection, such as spinal cord ependymomas.

Some authors have attempted to establish a more sensitive and specific warning criteria recently[31]. They recommend an alarm point of a 70% decrease in amplitude for routine spinal cord monitoring, particularly during surgery for spinal deformity, ossification of posterior longitudinal ligament and extramedullary spinal cord tumor, but not in cases of intramedullary spinal cord tumor. Different MEP methods and warning criteria still exist (muscle MEP, D-wave, absence/presence criteria, amplitude criteria and morphology criteria, *etc*.) and neither definite monitoring method nor definite warning criteria have been established. Future study should establish a more confident and accurate IOM method or criteria which prosecutes an aggressive tumor removal while preserving function of the spinal cord.

Reliable IOM enables surgeons to perform more difficult and challenging surgical procedures to the spine and spinal cord safely. Its role is to accurately identify the topography of neural structures and to avoid surgical insults by giving real-time alarms to the surgical team so that there is a response immediately. The role of IOM is increasing not only in deformity correction and intramedullary tumors but also in various spinal surgeries. It is well known that a rapid decompression of cervical medulla or cervical cord could result in neurological deficits such as C5 palsy, paresis or even plegia. It is possible to have a stretching of the lumbar roots during the reduction of the high dysplastic lumbar spondylolisthesis. IOM can help the surgical team accomplish a safe and successful outcome in spinal surgery which affects the spinal cord or spinal roots directly or indirectly. Multimodal IOM is a useful method to prevent spinal cord injury during neck positioning in cervical spine surgical procedures. It should not only be

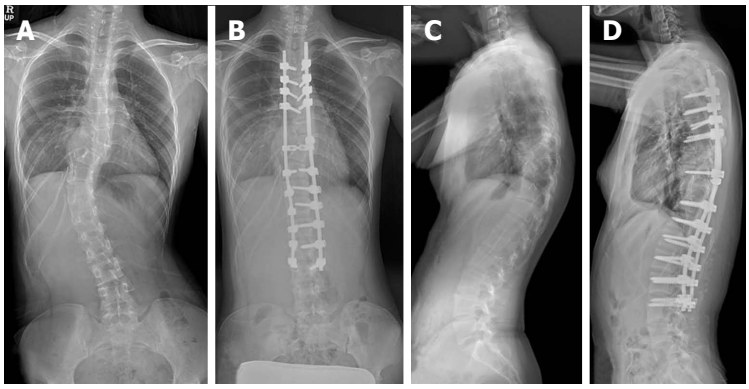

Figure 1 Plain radiography of pre- and postoperative whole spine anterior-posterior view (A and B) and lateral view (C and D).

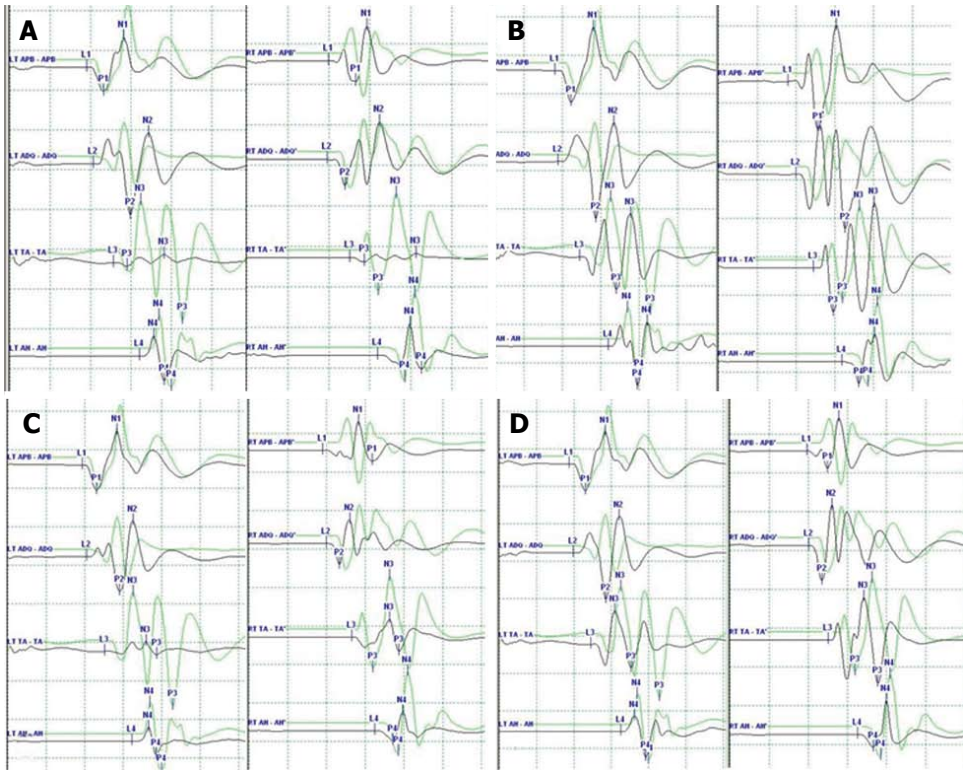

Figure 2 Representative case demonstrating clinical usefulness of intraoperative neuromonitoring in spinal surgery. A: MEP after applying rod to the screw heads using derotation maneuver and cantilever maneuver. The amplitude of MEP (black line) at both lower extremities decreased more than 50% compared with the baseline amplitude (green line); B: The amplitude of MEP recovered after correction release by removal of the rods and set screws; C: The amplitude of MEP re-deteriorated after reassembly of the implants; D: The amplitude of MEP recovered finally after raising MAP and administration of dexamethasone. APB: Abductor pollicis brevis; ADQ: Abductor digiti quinti; TA: Tibialis anterior; AH: Abductor halluces; MEP: Motor evoked potential; MAP: Mean arterial pressure.

considered for detecting intra-operative warnings, but also during positioning[32].

Case illustration

A 17-year-old girl visited our institute with back pain. She was diagnosed as a scoliosis associated with neurofibromatosis (Figure 1). We planned to perform a deformity correction by posterior column osteotomies and posterior segmental spinal instrumented fusion with intraoperative combined MEP and SSEP monitoring. When we applied the rod to the screw heads using a derotation maneuver and cantilever maneuver, the amplitude of MEP at both lower extremities decreased more than 50% compared with the baseline amplitude (Figure 2A). However, SSEP showed no change compared with the baseline amplitude (Figure 3). The amplitude of MEP recovered after correction release by removal of the rods and set screws (Figure 2B). The amplitude of MEP re-deteriorated after reassembly of the implants (Figure 2C). The amplitude of MEP recovered finally after raising MAP and administration of dexamethasone (Figure 2D). After the corrective surgery (Figure 1), she woke up without any postoperative neurological deficit and was discharged from the hospital on foot.

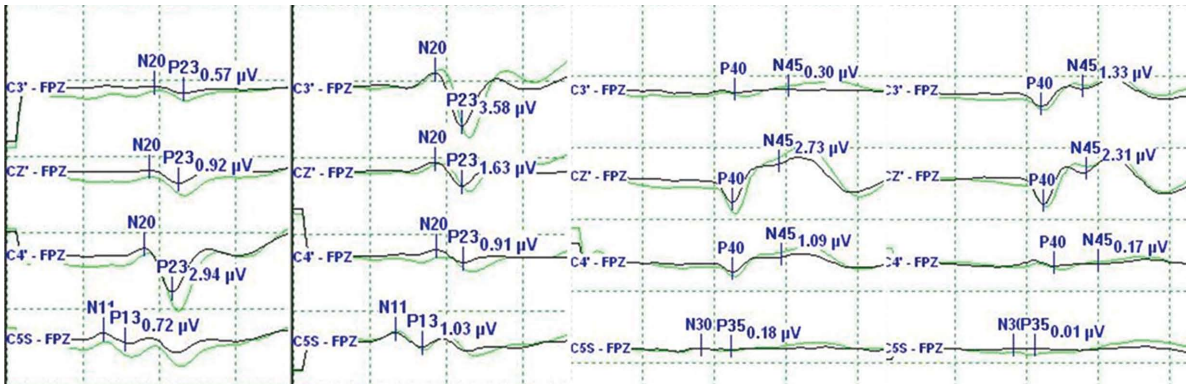

Figure 3 Spinal somatosensory evoked potential showed no change compared with the baseline amplitude (green line).

CONCLUSION

Although we cannot monitor every function of the spinal cord during spinal surgery, the technology of IOM has markedly developed. It is certain that the significance and utilization of IOM during spinal surgery will increase because of medicolegal issues as well as its usefulness. Spinal surgeons should understand the concept of the monitoring techniques and interpret monitoring records adequately to use IOM for decision making during the surgery for safe surgery, favorable surgical outcome and the surgeon's medicolegal safety.

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MINIREVIEWS

Esthesioneuroblastoma: Multimodal management and review of literature

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Abstract

Esthesioneuroblastoma (ENB) is a rare malignant neoplasm arising from the olfactory neuroepithelium. ENB constitutes only 3% of all malignant intranasal neoplasm. Because of the rarity, the number of patients of ENB treated in individual departments is small. Most of these patients presents in locally advanced stages and require multimodality treatment in form of surgery, chemotherapy and radiotherapy. Multimodality approach with a risk-adapted strategy is required to achieve good control rates while minimizing treatment related toxicity.

Key words: Surgery; Radiotherapy; Chemotherapy; Esthesioneuroblastoma

Core tip: This article is a comprehensive review of literature of a rare and aggressive neoplasm. This article outlines the various newer details of diagnosis, staging and treatment aspects of esthesioneuroblastoma (ENB). The importance of multimodality approach in management of ENB is reviewed in detail.

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INTRODUCTION

Esthesioneuroblastoma (ENB) is a rare malignant neoplasm arising from the olfactory neuroepithelium, including the superior one third of the nasal septum, cribriform plate, and superior turbinates, extending to base of the skull and to the intracranial space[1]. ENB was first described by Berger *et al*[2] in 1924. Because of uncertainty regarding the precise histological origin of the tumour, it has been described using various names in the literature, but the commonly used terms in recent publications are ENB and olfactory neuroblastoma. It constitutes only 3% of all intranasal neoplasms and its etiology remains unclear[3]. ENB manifests across all ages, with peaks in the second and sixth decades of

Table 1 Hyams histopathological grading

Grade	Lobular architecture preservation	Mitotic index	Nuclear polymorphism	Fibrillary matrix	Rosettes	Necrosis
Ⅰ	+	Zero	None	Prominent	HW rosettes	None
Ⅱ	+	Low	Low	Present	HW rosettes	None
Ⅲ	+/-	Moderate	Moderate	Low	FW rosettes	Rare
Ⅳ	+/-	High	High	Absent	None	Frequent

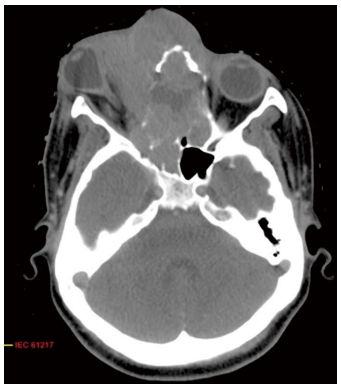

Figure 1 Axial computed tomography scan showing locally advanced esthesioneuroblastoma with extension to paranasal sinus and orbit.

life[4].

CLINICAL PRESENTATION

ENB have varying biological behaviour, ranging from slow indolent growth to a highly aggressive neoplasm with rapid widespread metastasis resulting in poor survival[5]. The most common presentation is unilateral nasal obstruction followed by epistaxis. Other clinical features develops with local spread of tumor like proptosis (infiltration into orbital canal), cranial nerve palsies (infiltration into cranial foramens and brain), swelling in neck (neck nodes) and metastatic symptoms[6].

PATHOLOGY

Light microscopy features consists of homogeneous small cells having uniform round to oval nuclei. Rosette or pseudorosette formation, and eosinophilic fibrillary intercellular background material is seen. However, in undifferentiated tumors, with anaplastic hyperchromatic small cells showing many mitotic figures and scant cytoplasm, differentiation from other small-cell nasal neoplasms is difficult with light microscopy[7]. Hyams Grading system is routinely used for histopathological grading and it consists of four grades (Ⅰ-Ⅳ)[8]. Hyams grading takes into account the architectural pattern, mitosis, nuclear pleomorphism, fibrillary matrix, rosettes and necrosis (Table 1). On immunohistochemistry (IHC), the tumour is positive for neuron specific enolase (NSE), synaptophysin, chromogranin and S-100 protein, but most cases are negative for cytokeratin, vimentin, desmin, actin, glial fibrillary acidic protein, UMB45, and leucocytic common antigen[7]. Electron microscopy is reliable in visualising uniform round nuclei, dense core neurosecretory granules, neuronal processes with microtubules and neurofilaments, and rare synapses[7]. The closest histopathological differential diagnosis is neuroblastoma. The neuroblastoma stains positive for NSE, synaptophysin, Leu7, and neurofilament protein. Elevated serum catecholamines are also suggestive of neuroblastoma.

INVESTIGATIVE WORK-UP

The gold standard of diagnosis is biopsy of the lesion showing characteristic histopathological features along with IHC. Fine needle aspiration cytology of suspected lymph nodes in neck is recommended to rule out nodal spread. Local extension of the tumor is evaluated using contrast enhanced computed tomography (CECT) of the face and neck (Figure 1). Magnetic resonance imaging (MRI) is better in evaluating sinonasal, intraorbital or intracerebral extension (Figure 2). As computed tomography (CT) can better demonstrate bony erosions, both studies are usually required in most of the patients[9]. Chest X ray, Ultrasound abdomen and CECT chest and abdomen is helpful in detecting systemic metastasis. Baseline hemogram, renal function tests and liver function tests are done for further treatment planning. Positron emission tomography is not routinely recommended for staging evaluation but can be used as an adjunct to CT and MRI.

STAGING

Tumor staging is an important guide for prognosis and therapy. Several staging systems, including Hymans, Kadish, and tumor, node, metastasis (TNM) systems, have been proposed as a guide to choosing treatment modalities. Staging for ENB was first proposed by Kadish *et al*[10] and the tumors were staged as three group categories. Group A is limited to tumors of the nasal cavity; group B is extension to the paranasal sinuses; and group C is extension beyond the paranasal sinuses and nasal cavity. Morita *et al*[11] in 1993 published a revised Kadish system that redefined stage C (consisting of local disease spreading beyond the paranasal sinuses) and included a stage D (distant metastasis) (Table 2). In 1992, Dulguerov *et al*[6] proposed TNM staging system based on the TNM system, determined on pre-treatment CT and MRI findings (Table 3). However, modified Kadish staging system is the most widely used staging system.

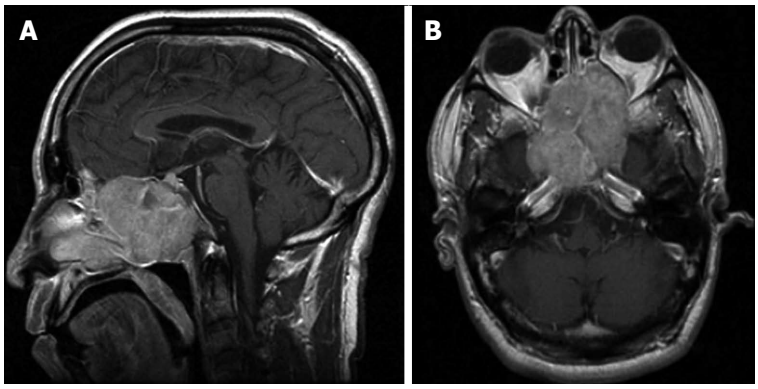

Figure 2 T1 W sagital section (A) and axial section (B) magnetic resonance imaging showing locally advanced esthesioneuroblastoma. Reprinted with permission from medscape drugs and diseases (http://emedicine.medscape.com/), 2015, Available from: URL: http://emedicine.medscape.com/article/250237-overview.

Table 2 Modified kadish staging

Stage A: Tumour limited to the nasal fossa
Stage B: Tumour extension into the paranasal sinuses
Stage C: Tumour extension beyond the paranasal sinuses and nasal cavity
Stage D: Distant metastasis

Table 3 Tumor, node, metastasis staging system

T1	Tumour involving the nasal cavity and/or paranasal sinuses (excluding sphenoid), sparing the most superior ethmoidal cells
T2	Tumour involving the nasal cavity and/or paranasal sinuses (including the sphenoid) with extension to or erosion of the cribriform plate
T3	Tumour extending into the orbit or protruding into the anterior cranial fossa, without dural invasion
T4	Tumour involving the brain
N0	No cervical lymph-node metastasis
N1	Any form of cervical lymph-node metastasis
M0	No metastases
M1	Distant metastasis

TREATMENT MODALITIES

The various treatment modalities used in the management of ENB are surgery, chemotherapy, radiation therapy (RT) and palliative care. Nowadays, the multimodal approach is recommended for improved survival and quality of life of the patients.

Surgery

The mainstay of the treatment is surgery. The advantage of surgery is tumor removal, immediate improvement in compressive symptoms, proper tissue for histopathological and prognostic evaluation. Intracranial extension and close proximity to the cribriform plate and ethmoidal roof requires a combined transfacial and neurosurgical approach[12]. For T1 tumors, craniotomy is not justified when there is clear radiological evidence of a normal cribriform plate and upper ethmoid cells. All other patients should be managed by combined craniofacial approach. Dulguerov *et al*[5] showed 100% local control with craniofacial resection as compared to 40% local control with other surgical resections. Similarly, Spaulding *et al*[13] at the University of Virginia showed reduction of 20% local recurrence rate with the craniofacial resection as compared to non-craniofacial approach. An international collaborative study of 17 centres reported the role of craniofacial resection in ENB in 2012. Five-year overall survival was 78% and 5-year recurrence-free survival was 64%[14]. Craniofacial resection allows *en bloc* resection of the tumour with better assessment of intracranial extension and protection of the brain and optic nerves. The current accepted practice is open or endoscopic craniofacial surgical resection.

RT

Specimens from the nasal cavity and paranasal sinuses, even en bloc, are difficult to orient, and surgical margins are difficult to analyze properly. Due to locally infiltrative nature of the disease, surgically clear margins are difficult to achieve. Thus there is a role of adjuvant RT to minimize the risk of local recurrence[12,15]. Adjuvant RT is indicated for Kadish stage B and C, whereas Kadish A disease can be managed with surgery alone. RT is delivered to the tumor bed and local extension with nodal irradiation reserved for involved nodes. Elective nodal irradiation is not practiced routinely. The RT doses have varied from 50 to 60 Gy in the literature[16,17]. With higher RT doses, there is always a risk of long term neural toxicity. But with advancement in technologies in delivery of RT with Intensity modulated RT (IMRT), Image guided RT and proton therapy, the long term neural toxicities can be minimized. RT is also used in neoadjuvant settings in locally advanced tumors and in palliation in metastatic settings. In small local recurrences, stereotactic radiosurgery and stereotactic radiotherapy can be used even for re-radiation[18].

Chemotherapy

The role of chemotherapy is not very clear in adjuvant settings in early tumors, but in locally advanced and metastatic tumors it has a definitive role. It decreases the chances of systemic failure by acting on systemic micro-

metastasis[19]. In neoadjuvant settings, it decreases the size of tumor, decreases compressive symptoms and helps in further complete removal of the tumour. It can be combined with RT in both neoadjuvant and adjuvant settings for better results[20]. The common drugs used are cisplatin, etoposide, adriamycin, vincristine and cyclophosphamide. Initial literature in support of neoadjuvant CT (NACT) comes from the University of Virginia, where Kadish C stage patients received 2 cycles of NACT with vincristine and cyclophosphamide with or without doxorubicin, followed by RT dose of 50 Gy, followed by craniofacial resection[21]. The 5-year and 10-year actuarial survival was 72% and 60% repectively. Subsequently, Cisplatin-based regimens became the preferred CT regimen in ENB at the Mayo Clinic, Gustave-Roussy Institute and Harvard Medical Institute[22-24]. Presently, the preferred chemotherapy regimen is cisplatin (33 mg/m^2 daily) and etoposide (100 mg/m^2 daily) for 3 Da.

Management of neck

Neck metastases at presentation in seen in 5% of patients[25]. In such patients, neck should be addressed by neck dissection or radiotherapy. Delayed neck metastases has been reported in 16% of patients in older series[25]. But with newer investigative, surgical, CT and RT techniques, these incidences are better managed. Patients with advanced local disease should be evaluated radiologically for neck metastases and regional treatment in form of neck dissection or neck RT should be offered in case of positive neck nodes.

STAGE WISE TREATMENT

Kadish A

Surgery alone with clear margins is sufficient in Kadish A staged tumors. Adjuvant RT is indicated in close and positive margins or with residual disease. No role of adjuvant chemotherapy.

Kadish B

Surgery followed by adjuvant RT is the treatment of choice. Role of adjuvant CT is controversial. Recent reports shows use of adjuvant CT. Neoadjuvant CT or RT can be used in inoperable cases.

Kadish C

Kadish C staged tumors requires all the three modalities. Neoadjuvant approach (CT/RT/concurrent CT-RT) is preferred. Definitive role of adjuvant CT in these settings.

Kadish D

Systemic chemotherapy and palliative RT to local site and metastatic sites are advised. Palliative care should be incorporated for improving the quality of life.

RECURRENCE

Local recurrence and/or distant metastases remain the main problem in the management of ENB. Salvage treatment consists of surgery, surgery and postoperative RT, RT alone, palliative chemotherapy (CCT), or supportive care. The management options depend upon the type of relapse and initial treatment received by the patient. Bachar *et al*[26] reported local recurrence was documented in 30.7%, regional recurrence in 17.9%, and distant metastasis in 7.7% of patients. In a metaanalysis by Dulguerov *et al*[6], local regional, and distant recurrence rates were reported in 29%, 16%, and 17%, respectively.

PROGNOSIS

The most important prognostic factors influencing the outcome reported in ENB are Hyams grade, positive lymph nodes, Kadish stage, extent of resection and postoperative RT with atleast 54 Gy[27-29].

ENB IN CHILDREN

In pediatric population upto 15 years of age, the incidence of ENB is 0.1/100000[30]. In younger patients, the tumors have a more aggressive presentation and advanced disease. Bisogno *et al*[31] reported 9 young patients of ENB managed with combined modality approach. The 5-year progression-free survival and overall survival were 77.8% and 88.9% respectively[31]. El Kababri *et al*[32] reported 11 children and adolescents treated with combined modality approach between 1982 and 2002. The 5-year actuarial disease-free survival and overall survival rate was 91% with ten patients being long term survivors[32]. Thus, pediatric ENB has an aggressive presentation but has good clinical results with combined modality approach.

CONCLUSION

Most of the patients of ENB present in locally advanced stage and the optimal management depends on the cooperation between clinicians, surgeons, radiologists and pathologists from establishing diagnosis to organizing the therapeutic strategy. Novel strategies including combined CCT with RT and/or dose escalation with advanced RT techniques such as IMRT and proton therapy should be prospectively investigated to improve the survival results in ENB.

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MINIREVIEWS

Gingival enlargements: Differential diagnosis and review of literature

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Abstract

Gingival enlargement is one of the frequent features of gingival diseases. However due to their varied presentations, the diagnosis of these entities becomes challenging for the clinician. They can be categorized based on their etiopathogenesis, location, size, extent, *etc*. Based on the existing knowledge and clinical experience, a differential diagnosis can be formulated. Subsequently, after detailed investigation, clinician makes a final diagnosis or diagnosis of exclusion. A perfect diagnosis is critically important, since the management of these lesions and prevention of their recurrence is completely dependent on it. Furthermore, in some cases where gingival enlargement could be the primary sign of potentially lethal systemic diseases, a correct diagnosis of these enlargements could prove life saving for the patient or at least initiate early treatment and improve the quality of life. The purpose of this review article is to highlight significant findings of different types of gingival enlargement which would help clinician to differentiate between them. A detailed decision tree is also designed for the practitioners, which will help them arrive at a diagnosis in a systematic manner. There still could be some lesions which may present in an unusual manner and make the diagnosis challenging. By knowing the existence of common and rare presentations of gingival enlargement, one can keep a broad view when formulating a differential diagnosis of localized (isolated, discrete, regional) or generalized gingival enlargement.

Key words: Differential diagnosis; Gingival hyperplasia; Gingival overgrowth; Gingival diseases; Decision tree

Core tip: In clinical dentistry, patients frequently report with isolated/regional or generalized gingival enlargements, which could fall under varied presentations. The diagnosis of these lesions is essential for their successful management and of the patient as a whole. This article revises the existing knowledge of different types of enlargements and highlights some important diagnostic features. A customized decision tree is designed, which will help clinicians to keep a broad view when formulating a differential diagnosis of localized (isolated, discrete, regional) or generalized gingival enlargement and arrive at a particular diagnosis is an easy and systematic manner.

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INTRODUCTION

Gingival enlargement or gingival overgrowth, a common trait of gingival disease, is characterized by an increase in the size of gingiva. Pertinent management depends on precisely diagnosing the origin of enlargement. However, the skills of a clinician is put to test when arriving at a particular diagnosis among the myriad of gingival enlargements that can be classified according to etiologic factors and pathologic changes, according to location and distribution and/or according to the degree of enlargement. Based on etiopathogenesis, enlargements could be inflammatory, drug influenced, those associated with systemic conditions or diseases, neoplastic or false enlargements. According to location, enlargements could be marginal, papillary or diffuse. Based on distribution they can be localized or generalized. Localized enlargements could be further divided into three sub-types, *viz.*, "isolated, discrete" or "regional". "Isolated" enlargements are those limited to gingiva adjacent to single or two teeth (*e.g.,* gingival/periodontal abscess). "Discrete" lesions are isolated sessile or pedunculated, tumor-like enlargements (*e.g.,* fibroma/pyogenic granuloma). "Regional" enlargements refer to involvement of gingiva around three or more teeth in one or multiple areas of the mouth (*e.g.,* inflammatory enlargement associated with mouth breathing in maxillary and mandibular anterior region). "Generalized" enlargement refers to involvement of gingiva adjacent to almost all the teeth present (*e.g.,* drug influenced gingival overgrowth). Inglés *et al*[1] summarized different methods and presented their clinical index to measure the degree of gingival enlargements.

The purpose of this article is to highlight significant findings of different types of gingival enlargement which would help clinician to differentiate between them. For the purpose of clinical diagnosis, enlargements mentioned in this review are grossly are divided into isolated lesions (epulis) and regional or generalized gingival enlargements. Among these, diagnostic points are discussed for the more commonly occurring lesions and the very rare and unusual presentation are listed as per the category to which they belong.

ISOLATED REACTIVE LESIONS OF THE GINGIVA

Localized enlargement of gingiva, historically termed as edulis[2], refers to any solitary/discrete, pedunculated or sessile swellings of the gingiva with no histologic characterization of a particular lesion. A corrective term "reactive lesion of the gingiva"[3], seems to be more appropriate for these category of swellings. Frequent diagnosis in this category is inflammatory rather than neoplastic and may fall in one of the following group of reactive lesions: fibrous epulis/peripheral fibroma, angiogranuloma/pyogenic granuloma and the peripheral giant cell lesion/granuloma.

Fibrous epulis/peripheral fibroma

In adults, this lesion frequently represents as firm, pink, un-inflammed mass, and it seems to grow from below the free gingival margin/interdental papilla (Figure 1A). Most often the lesion is painless. Pain may be associated due to secondary traumata *via* brushing, flossing or chewing. Histologically, the fibroma may show additional focus of calcification (peripheral calcifying fibroma), foci of cementicles (peripheral cementifying fibroma, Figure 1B) or trabeculae of bone (peripheral ossifying fibroma, Figure 1C).

Angiogranuloma/pyogenic granuloma

Presents in adults as smooth surfaced mass, often ulcerated and grows from beneath the gingival margin. These reddish/bluish color mass are highly vascular, compressible and could bleed readily. Typically they grow rapidly within first few weeks and then slowly. The mass may penetrate interdentally and present as bilobular (buccal and lingual) mass connected through the col area, but bone erosion in uncommon (Figure 2A). Angiogranuloma which appears during pregnancy are termed as pregnancy epulis/tumor or granuloma gravidarum (Figure 2B).

Histologically, the stratified squamous epithelium is thickened, with prominent rete pegs and some degree of intracellular and extracellular edema, prominent intercellular bridges and leukocytic infiltration.

Peripheral giant cell granuloma

They occur particularly in anterior region in young patients or in posterior mouth during mixed dentition phase and in adults. They are very aggressive lesions with significant growth potential. The high vascularity of these lesions can be understood by their purplish-red color and tendency to bleed. They also tend to penetrate interdentally and erosion of adjacent bone along with separation of adjacent teeth is a common occurrence (Figure 3).

Gingival cysts

Gingival cysts are unusual cysts of odontogenic source. They are frequently found in females in their 50's or 60's. A greater number of these are found on the labial attached gingiva of the mandibular anterior teeth. Presence of fluid may give them a bluish hue and they may lead to resorption of the labial bone due to pressure. Radiographically, its radiolucency may sometimes lead to confusion with lateral periodontal cyst. Excisional biopsy is the best management for these lesions[4].

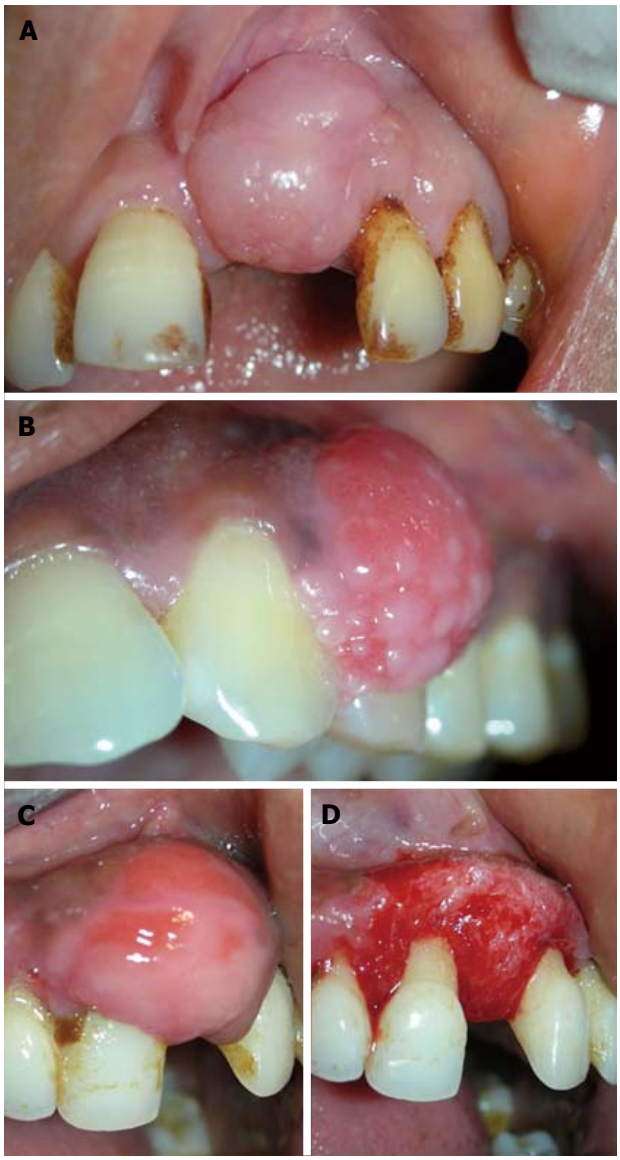

Figure 1 Fibrous epulis and its subtypes. A: Peripheral fibroma, presenting as pink firm, uninflammed mass growing from under the gingiva; B: Peripheral cementifying fibroma, a subcategory of fibroma, shows additional foci of cementicles; C and D: Surgical exposure of the lesion showing extensive bone formation in the core of the lesion. Also presence of bony trabeculae was seen histologically.

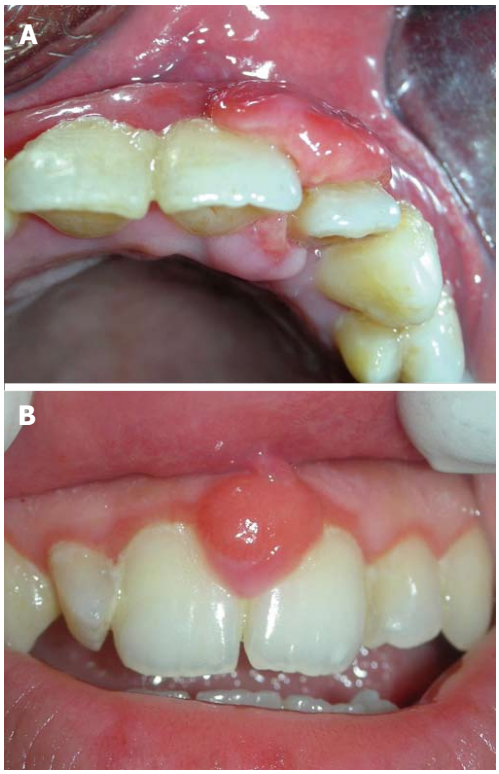

Figure 2 Angiogranulomas may present as: Pyogenic granuloma, a bilobular mass connected through the col area (A), and similar lesion occurring during pregnancy is termed as "pregnancy epulis" (B).

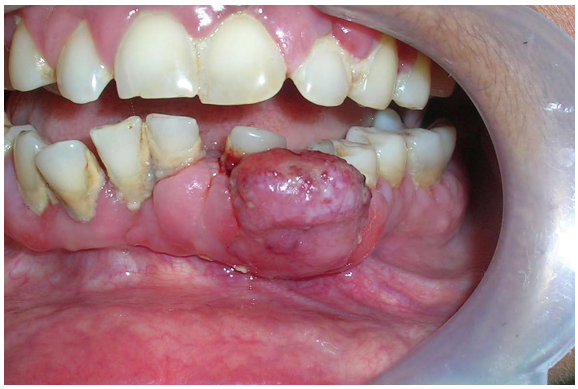

Figure 3 Lesion of peripheral giant cell granuloma. This highly vascular lesion is characterized by purplish red-color and its tendency to bleed.

Neoplastic

The localized epulis like lesions can also be classified as being benign or malignant. Benign masses could be, fibroma, peripheral giant cell granuloma, central giant cell granuloma, papilloma, leukoplakia, nevus[5], myoblastoma[6], hemangioma (Figure 4A)[7,8] neurilemoma[9], neurofibroma[10], ameloblastoma[11]. Malignant tumors could be squamous cell carcinoma or melanoma. Among sarcomas, Kaposi's sarcoma[12] is more common, and fibrosacroma, lymphosarcoma and reticulum cell sarcoma are rarely reported[13]. Other rare lesions (< 2% prevalence) include angioma, osteofibroma, myxoma, fibropapilloma, adenoma and lipoma[14].

Others

Some other localized chronic lesions that can be misdiagnosed as epulis would be palatal mucocele (Figure 4B), a lateral periodontal cyst projecting on labial/lingual surface (Figure 4C and D), *etc.*

Acute

The acute form of isolated gingival enlargement could be various abscesses such as gingival, periodontal, periapical or pericoronal. They can be differentiated from their location and vitality of the associated tooth. It could be located near gingival margin or papilla (gingival abscess) (Figure 5A), or may be diffuse and forms significant portion of attached gingiva (periodontal abscess) (Figure 5B). When the associated tooth is non-vital the lesion may be originated from an endodontic

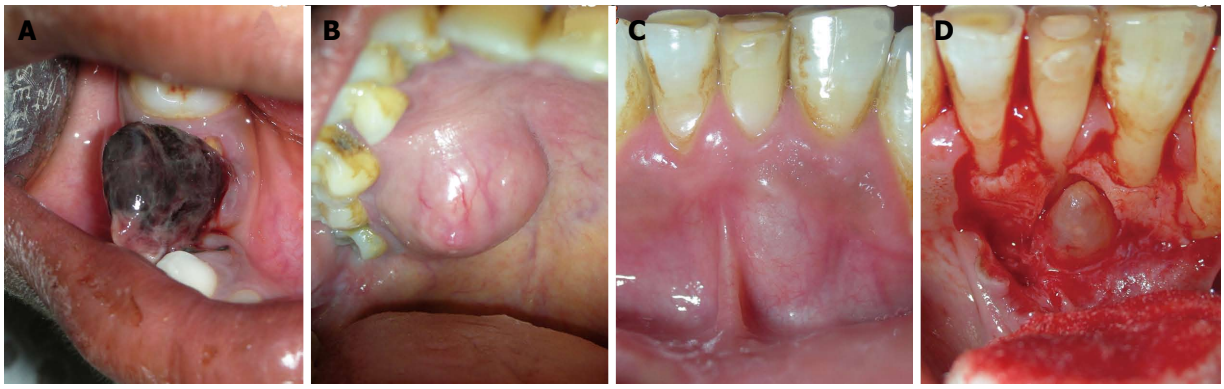

Figure 4 Other uncommon localized gingival enalargements which could be misdiagnosed as epulis. A: Hemangioma located in mandibular right quadrant; B: Mucocele associated with palatal minor salivary gland; C and D: A lateral periodontal cyst projecting labially and causing localized gingival enlargement.

problem (periapical abscess/endo-perio lesion) (Figure 5C). Often the pericoronal flap covering distal most mandibular teeth might become inflamed and swollen. Abscess of these pericoronal flaps could eventually develop if the inflammation persists (Figure 5D).

Histopathological examination of gingival/periodontal/pericoronal abscess may present purulent focus in the connective tissue, surrounded by diffuse infiltration of polymorphonuclear leukocytes, edematous tissue and vascular engorgement. The surface epithelium has varying degrees of intra- and extracellular edema, invasion by leukocytes, and sometimes ulceration.

CHARACTERISTIC FEATURES OF GENERALIZED GINGIVAL ENLARGEMENT

More commonly, gingival disease manifests as regional or generalized gingival enlargement, which might fall into one of the different types.

Inflammatory gingival enlargement

These are inflammatory response to local irritant associated with gingiva. The irritant could be microbial deposits (plaque and calculus) (Figure 6A), fractured tooth, overhanging restorations, ill-fitting prosthesis (Figure 6B), orthodontic brackets (Figure 6C), *etc.* The presentation begins as slight ballooning of the papilla or marginal gingiva, depending upon the location of the irritant. The bulge may progressively increase in size and extent to become generalized. Clinical they may appear bluish or deep red. They are frequently friable and soft with a smooth shiny surface and they usually bleed easily. Occasionally, chronic inflammatory enlargement may also present as firm, resilient, pink and fibrotic enlargement which histologically show abundance of fibroblasts and collagen fibers.

Gingival enlargement in mouth breathers

Although considered as inflammatory, the exact mechanism of enlargement in mouth breathers is not clear. It is thought to be due to alternate wetting and drying of the gingival surface. The gingiva appears red and edematous with diffuse shiny surface. A diagnostic feature of this type of enlargement would be presence of significant enlargement in maxillary and mandibular anterior regions and no involvement of posteriors. In a typical bimaxillary protrusion case, the enlargement will be limited to palatal aspect of maxillary anteriors and labial aspect of mandibular anteriors. Patients present with mouth breathing habit that may be due to short upper lip, hyperactive labii superioris, proclined incisors, rhinitis, *etc.*

Fibrotic

Drug induced gingival enlargement: There are many anticonvulsants, immuno-suppressants and calcium channel blockers that may lead to gingival enlargement in varied presentations (Table 1 and Figure 7). Signs and symptoms related to gingival enlargement are seen within 2-4 mo of initiation of drug intake. Usually at the initial presentation there is no pain. The enlargement starts as beadlike enlargement of the interdental papilla and eventually may involve marginal gingiva. When not involved by secondary inflammation, the enlargement looks like mulberry shape, firm, pink and resilient with minute lobulations and no bleeding on probing. Although it may involve the gingiva around all teeth, it is more prominent in maxillary and mandibular anteriors. It will be absent in edentulous areas and will disappear in areas where teeth are extracted. When infected secondarily, there is increase in the size of existing enlargement and adds characteristic features of inflammatory enlargement. Among commonly encountered drug induced gingival enlargement (DIGO), those due to immunosuppressive agent like cyclosporine, appear more vascularized than phenytoin induced[17].

When patients are in combination therapy, in which two or more drugs are known to cause gingival enlargement, then, which should be attributed to the diagnosis of DIGO, is a puzzle. One way to arrive at a diagnosis in such cases is to consult the patient's physician, request him to substitute/stop one drug at a time, starting with the one that would have least effect on patient's routine schedule. Frequently, patient gives history of related medications (antihypertensives/anticonvulsants/immune-

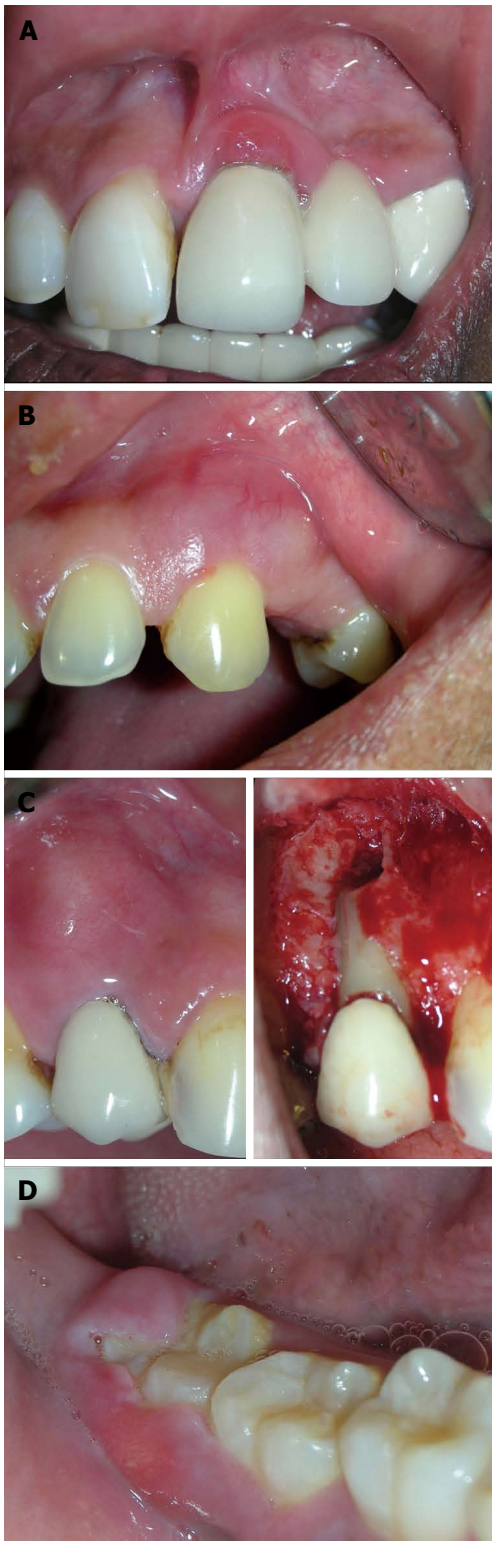

Figure 5 Abscess presenting as localized gingival enlargement. A: Gingival abscess, near gingival margin or papilla; B: In periodontal abscess, the swelling is diffuse; C: Periapical abscess, near apex of concerned tooth; D: Abscess of pericoronal flap.

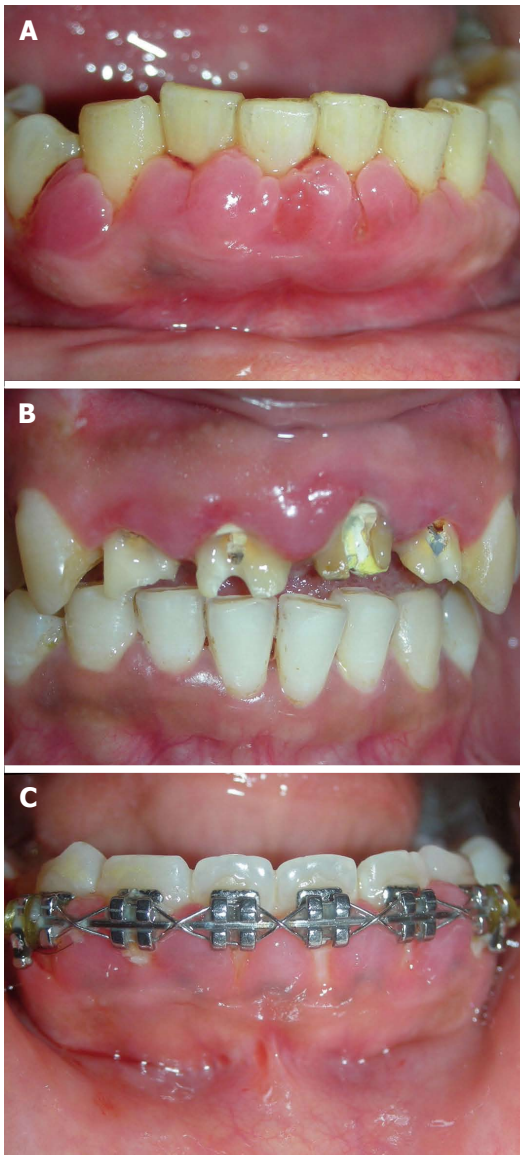

Figure 6 Inflammatory gingival enlargement. A: Plaque and calculus; B: Ill-fitting prosthesis; C: Orthodontic brackets.

Table 1 Different drugs known to predispose to gingival enlargements

Anticonvulsants[15,16]		Immunosuppressants[17]	Calcium channel blockers[15]
Phenytoin	Vigabatrin	Cyclosporine	Nifidipine
Ethotoin	Ethosuximide	Tacrolimus	Diltiazem
Mephenytoin	Topiramate	Sirolimus	Felodipine
Phenobarbital	Pyrimidinone		Nitrendipine
Lamotrigine			Verapamil
			Amlodipine

suppressants) since many years but enlargement was noted since few months. In these cases, it becomes difficult to associate duration of occurrence of enlargement with related drug history. Specific query related to recent change in type/dose of drug will help to associate both.

Genetic disorders associated with gingival enlargement: They can be divided into 4 primary categories based on their etiology, clinical features and histology. Namely, idiopathic gingival enlargement, lysosomal storage disorders, vascular disorders and

Table 2 Gingival enlargement associated with syndromes in different types of genetic disorders

Syndromes associated with heriditary gingival fibromatosis	
Zimmerman-Laband syndrome[18]	Abnormal fingers, nails, nose and ears, splenomegaly, hepatomegaly, hyperextensible metacarpophalangeal joints
Ramon syndrome[19]	Cherubism, seizures, mental deficiency, hypertrichosis, stunted growth, juvenile rheumatoid arthritis
Systemic Hyalinosis[20]	Painful joint contractures, diffuse thickening of the skin with pearly papules and fleshy nodules and failure to thrive
Jones syndrome[21]	Progressive sensorineural deafness
Rutherford syndrome[22]	Corneal opacity, mental retardation and aggressive behavior, failure of tooth eruption
Cross syndrome[23]	Hypopigmentation, mental retardation and writhing movement of hand and legs
Schinzel-Giedion syndrome[24]	Severe mid face retraction, severe mental retardation and congenital heart defect, patient usually die under 10 yr of age
Costello syndrome[25]	Macrostomia, redundant skin of neck, hands and feet, nasal and perioral papillomas, enlargement within first years of life
Syndromes associated with lysosomal storage diseases	
Hurler syndrome[26]	Dwarfism, flexion contractures, hernias, corneal clouding, macroglossia, short mandibular rami, peg-shaped teeth
Maroteaux-Lamy syndrome[27]	Enlargement of skull, corneal opacities, short peg-shaped poorly formed teeth, hypertrophy of alveolar ridges, anterior open bite
Neimann-Pick disease[28]	Thick lips, macroglossia and widely spaced teeth
Anderson-Fabry disease[29,30]	Painful crises on extremities and abdomen, angiokeratomas of skin, labial mucosa and buccal mucosa
Cowden syndrome[31]	Cobblestone papules of gingiva and buccal mucosa, macrocephaly, multiple hamartomas, learning disabilities, autism
Gingival enlargement associated with vascular disorders	
Sturge-Weber syndrome[32]	Unilateral cutaneous nevi, unilateral vascular hyperplasia, neurological manifestations and ocular complications
Klippel-Trenaunay syndrome[33]	Capillary hemangiomas, increased size of lips, tongue, teeth malformations, delayed exfoliation of teeth, calcified roots
Gingival enlargement associated with characteristic dental abnormalities	
Wilson syndrome[34]	Enamel hypoplasia, multiple small red papules of lips, early onset periodontitis and repeated oral candidiasis, signs of cirrhosis
GoltzGorlin syndrome[35]	Partial anodontia, hypoplastic teeth, atrophy and linear pigmentation of skin, herniation of fat, multiple papillomas, digital anomalies

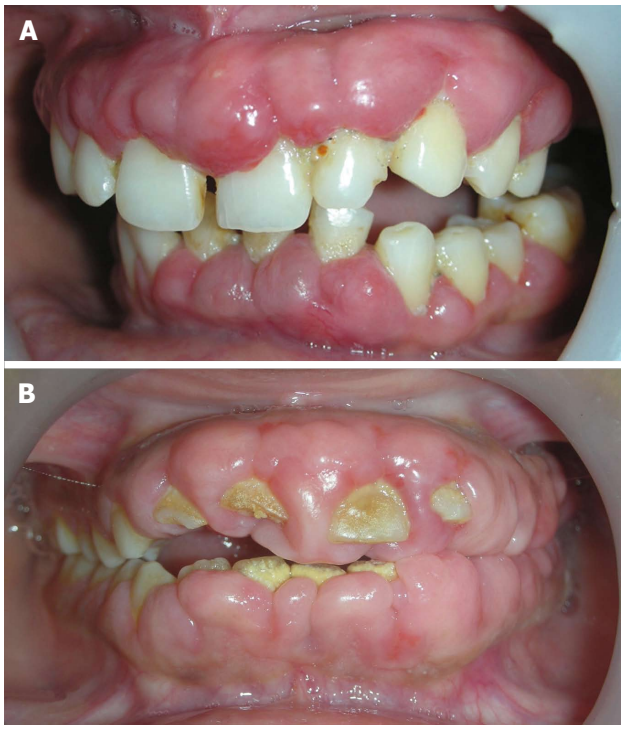

Figure 7 Drug influenced gingival overgrowth. A: Superimposed with secondary inflammation; B: Fibrotic and leathery.

those associated with characteristic dental abnormalities (Table 2). Idiopathic gingival enlargement is also referred to as congenital familial fibromatosis, gingivomatosis, idiopathic fibromatosis, elephantiasis and hereditary gingival hyperplasia. It presents as unusual fibrotic gingival enlargement of localized or generalized extent. It may present as a specific entity or as a part of syndrome. Diagnosis can be made by a positive family history of gingival enlargement. It usually begins with the eruption of the primary or permanent dentition. A frequent finding could be presence of firm bulky enlargement of gingiva restricted to maxillary and mandibular second and third molar areas only. The enlarged mass may be pink or reddish and may be firm/nodular on palpation. Alveolar bone is rarely affected, but presence of pseudo-pockets and difficulty in maintaining oral hygiene may lead to some periodontal problems. Extensive overgrowths can lead to esthetic and functional concerns to the patient (Figure 8).

Conditioned gingival enlargement

Hormonal: Generalized gingival hyperplasia, during pregnancy and puberty, is influenced by hormonal changes that pretentious the response to local irritants. The interproximal gingiva shows more prominent enlargement than the facial and/or lingual surfaces (Figure 9). The enlarged gingiva usually is soft and friable, bright red or magenta, with a smooth, shiny surface. Bleeding may occur extemporaneously or on mild stimulation. The enlargement may reduce spontaneously after the delivery, but complete elimination may require the removal of all local irritants and additional surgical intervention of any fibrotic remnants.

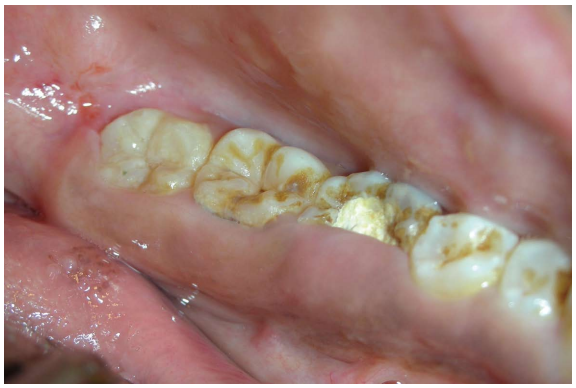

Figure 8 Unusual firm fibrotic gingival enlargements in a patient with hereditary gingival fibromatosis.

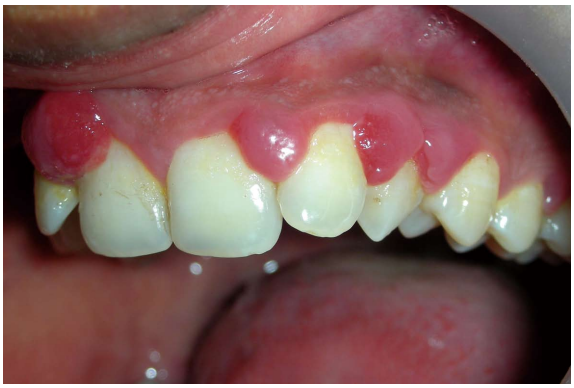

Figure 9 Typical multiple interproximal enlargements in a pregnant patient.

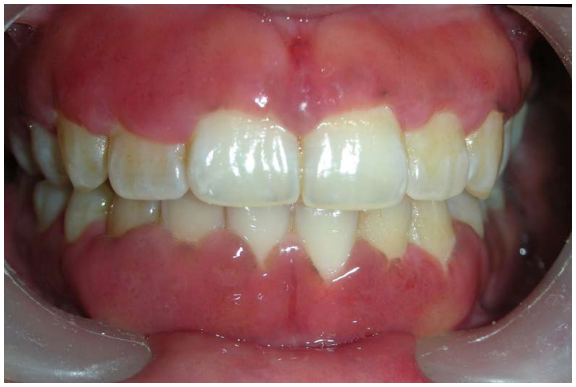

Figure 10 Appearance of gingiva in patient with plasma cell gingival enlargement. The color is reddish and involves almost complete attached gingiva and slightly granular appearance.

Vitamin C deficiency: Deficiency of vitamin C is defined as a serum ascorbic acid level < 2 μg/mL. Diabetes, stress and smoking are the commonly labeled factors leading to mild vitamin C deficiency. The gingiva, of vitamin C deficiency associated enlargement, is bluish red, soft and friable with a smooth, shiny surface. Bleeding may occur spontaneously or on slight irritation. Surface necrosis with pseudomembrane formation are also frequently seen[36]. Kubota *et al*[37] pointed out that high-sensitivity C-reactive protein (hs-CRP) levels were inversely proportionate with serum vitamin C concentration, and therefore, hs-CRP blood level may be elevated in these patients.

Plasma cell gingivitis: The etiology of this entity is difficult to establish, but it is considered to be a hypersensitivity reaction with affluent plasma cells seen histologically. Usual allergens known to be associated with this lesion could be, *e.g.,* toothpaste, khat, food product particularly cinnamon, chewing gum or unknown origin. It might bleed on provocation. Patients usually complain about burning sensation on eating hot and spicy food. Appearance is reddish in color, involves almost complete attached gingiva, slight granular surface appearance is typical (Figure 10).

Gingival enlargement associated with systemic disease

Leukemia: Generalized gingival enlargement associated with leukemia is due to the massive infiltration of leukemic cells in the gingival connective tissue. Clinically it may mimic inflammatory origin. Apart from gingival enlargement other associated features could be oral ulceration, spontaneous gingival bleeding, petechiae, mucosal pallor, herpetic infections and candidiasis. Rarely, uncommon features such numbness in chin and/or tooth pain has been reported[38]. The most serious condition associated with gingival enlargement in this category would be acute myeloid leukemia. It can be associated with signs and symptoms of bone marrow failure, such as ecchymoses, night sweats, recent infections and lethargy. An expeditious diagnosis can be made by a simple full blood count. A rare case of gingival hyperplasia secondary to acute lymphoblastic leukemia has also been reported[39].

Wegener's Granulomatosis: "Strawberry gingivitis", formed by reddish-purple exophytic gingival swelling with patechial haemorrhages, is a characteristic sign of Wegener's granulomatosis. The oral lesions could be of great help in timely diagnosis of this potentially fatal condition, because they persists for a long time before multi-organ involvement occurs (Figure 11)[40,41]. At least two of the following conditions should be fulfilled to diagnose the condition as Wegener's granulomatosis: (1) ulcerative lesions of oral mucosa or nasal bleeding or inflammation; (2) nodules, fixed infiltrates or cavities in chest radiograph; (3) abnormal urinary sediment; and (4) granulomatous inflammation on biopsy[40].

Crohn's disease: The gingiva is pink, firm and almost leathery in consistency, with a characteristic minutely pebbled surface. Signs and symptoms of these conditions must be strenuously trailed in these patients. It is normally associated with swelling of lips, bowel disorders, fever and ulcers. A consultation with gastroenterologist will prove to be helpful.

Sarcoidosis: Sarcoidosis is a multiorgan disorder. The

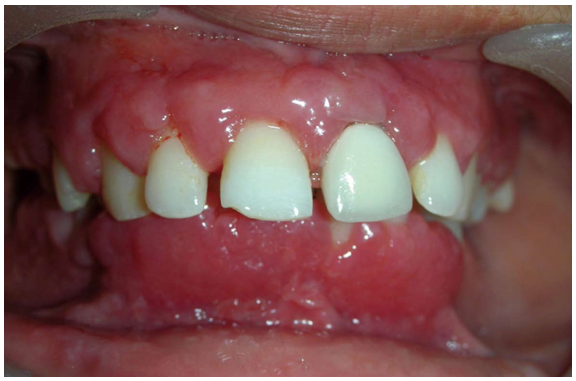

Figure 11 Gingival condition in patient with Wegenersgranulomatosis, presents as reddish purple, exophytic gingival overgrowth.

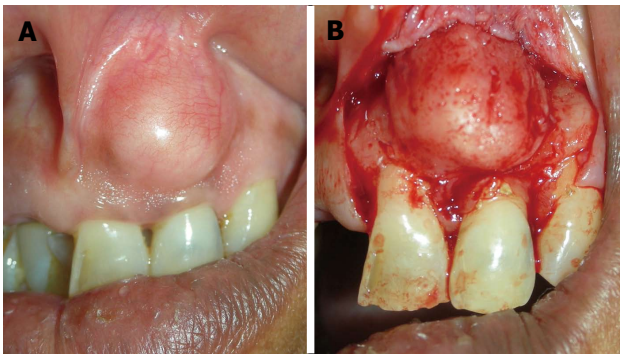

Figure 12 Case of false enlargement wherein. A: The overlying gingiva presents with no abnormal clinical features except the massive increase in size of the area; B: Formed completely by underlying bone.

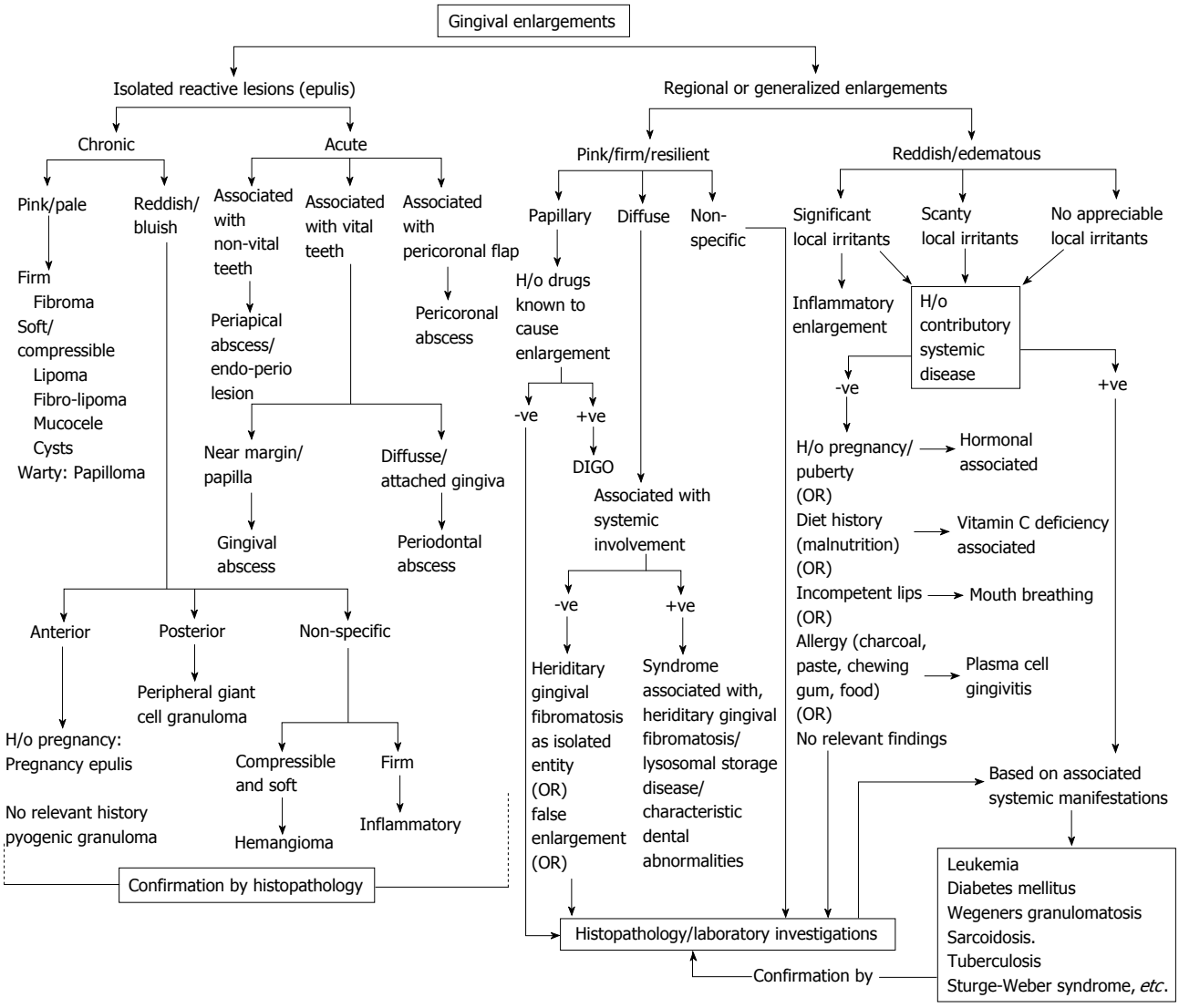

Figure 13 Decision tree for differential diagnosis of isolated, regional and generalized gingival enlargement. DIGO: Drug induced gingival enlargement.

most common presentation consists of pulmonary infiltration and hilar lymphadenopathy, dermal and ocular lesions[42], however oral involvement is uncommon. There are no specific tests for sarcoidosis. Diagnosis of sarcoidosis is mainly based on exclusion of other non-caseating granulomas forming conditions and other laboratory tests[42,43]. Eosinophil count might be significantly increased (normal range 0%-4%), and serum angiotensin converting enzyme levels can also be significantly raised (normal range less than 670 nkat/L).

Chest X-ray might show hilar lymphadenopathy[44].

Tuberculous gingival enlargement: Primary oral tuberculous lesions are very rare, but when they occur they are usually seen in younger age. The lesions themselves remain painless in most cases, however associated caseation of the dependent lymph nodes may be seen[45,46]. Furthermore, primary tuberculosis manifesting solely as gingival enlargement is extremely rare which can be diagnosed based on history of fever, weakness, loss of appetite and weight loss[47]. The diagnosis can be confirmed based on histopathology, complete blood count and polymerase chain reaction[47]. In contrast, secondary oral tuberculosis can be seen in 0.05% to 1.5% of cases and the prevalence is more in older adults[48,49].

Unusual presentations: Generalized gingival enlargement has been rarely reported with amelogenesis imperfecta[50], Hashimoto's thyroiditis[51], I-cell disease[52] and Multiple myeloma[53].

False enlargement: These pseudo-enlargements appears as a result of increase in size of underlying osseous (tori, exostosis, Paget's disease, cherubism, osteoma, *etc.*) or dental tissues (during tooth eruption). The overlying gingiva presents with no abnormal clinical features except the massive increase in size of the area (Figure 12).

Decision tree: Differential diagnosis of gingival enlargement requires thorough dental and medical history, careful evaluation of the type, nature and extent of enlargement and identification of etiologic or predisposing factors. A decision tree (Figure 13) is specially designed to get a broad overview of different possible diagnosis for localized or generalized gingival enlargements. This systematic presentation would be very helpful for the clinicians to arrive at a particular diagnosis. Furthermore, laboratory investigations and/or biopsy specimens may be required to confirm the diagnosis or make a diagnosis of exclusion.

CONCLUSION

Inspite of a myriad of etiology, gingival enlargements can often be diagnosed by a careful history (*e.g.,* drug influenced or hormonal influenced gingival enlargement), by location (*e.g.,* mouth-breathing enlargement around anterior teeth) or by the clinical presentation (*e.g.,* strawberry gingivitis). Presence of local irritants (plaque and calculus) could be primary or associated cause of gingival enlargements. Hence, plaque control is an essential aspect of management in all the patients. An excisional/incisional biopsy and/or hematologic/histologic examination may be needed occasionally to correctly diagnose the uncommon cases of gingival enlargement. The clinician should have an open mind and consider all possibilities before coming to the final diagnosis of the condition at hand.

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MINIREVIEWS

Current trends in laparoscopic groin hernia repair: A review

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Abstract

Hernia is a common problem of the modern world with its incidence more in developing countries. Inguinal hernia is the most common groin hernia repaired worldwide. With advancement in technology operative techniques of repair have also evolved. A PubMed and COCHRANE database search was accomplished in this regard to establish the current status of laparoscopic inguinal hernia repair in view of recent published literature. Published literature support that laparoscopic hernia repair is best suited for recurrent and bilateral inguinal hernia although it may be offered for primary inguinal hernia if expertise is available.

Key words: Laparoscopic hernia repair; Lichtenstein repair; Day care surgery; Open hernia repair; Inguinal groin hernia

Core tip: This review compares the laparoscopic hernia repair to conventional open hernia repair techniques in terms of cost, recurrence, procedure related morbidity and quality of life of the patient. Recent published literature has been included in this regard to focus on if any supremacy exists between the two approaches.

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INTRODUCTION

Hernia is a common problem of the modern world with an incidence ranging from 5%-7%. The prevalence of hernia is far greater in developing countries like India amounting to a major health care burden. Of all groin hernias, around 75% are inguinal hernias[1,2]. The repair of the groin hernia is therefore a commonly performed surgery worldwide.

Operative techniques have evolved continuously over the past decades establishing tension free mesh repair as standard of care for inguinal hernia management. A PubMed and COCHRANE database search was accomplished in this regard to establish the current status of laparoscopic inguinal hernia repair in view of recent published literature.

The groin is a naturally occuring defect in the ante-

rior abdominal wall. This weak muscular area in the inguinal region has been named after surgeon and anatomist Henri Fruchaud. The anatominal extents of this myopectineal orifice are as follows: cranially and medially this is bordered by the conjoined tendon and the rectus abdominis muscle, laterally by the iliopsoas muscle and caudally by the superior ramus of the os-pubis. This area is covered by the fascia transversalis, split in two by the inguinal ligament, and penetrated by the spermatic cord (in men)/round ligament (in women) and femoral vessels. The integrity of the area is hence primarily depends on fascia transversalis, whose failure to sustain the preperitoneal fat and varying intraabdominal pressure is therefore the fundamental cause for formation of congenital or acquired inguinal hernia. Inguinal hernias are treated by repairing the fascial defect in the myopectineal orifice of Fruchaud or by strengthening the weakened fascia transversalis by placement of a prosthesis (mesh).

CONSERVATIVE TRIAL *VS* SURGERY

Inguinal hernia is a disease of benign nature and follows a fixed course but their complications are dramatic and frequent. Surgical repair done under emergency conditions has higher recurrence and is associated with increased morbidity and mortality[3,4]. Hence, a repair in elective setting is always preferred. Open repairs applying principles of Pascal's law include tension free like Lichtenstein repair, which can be done under regional anaesthesia in a safe and economic way[5,6]. Recently with advancement in laparoscopy, endoscopic repairs seems to offer better quality of life, decreasing hospital stay and early return to work. Henceforth every possible attempt should be made for early repair of inguinal hernia if no addded comorbidity is present[7,8]. A few of asymptomatic elderly individual, not fit for surgery can be advised conservative management.

ANAESTHESIA

Till date all anaesthetic techniques have been used to undertake the operative repair safely. General anaesthesia was the most common method used in early days but in recent past it has been replaced by regional anaesthesia[9]. Few benefits of regional anaesthesia include: (1) A conscious patient at the operating table. Patient can cough to increase the intra-abdominal pressure thereby checking the effectiveness or repair; and (2) Lesser time spent in the operating room, lower incidence of nausea, fewer requirements of post operative analgesia and early discharge on a day care basis[10].

However general anaesthesia is still the method of choice for undertaking endoscopic/laparoscopic inguinal hernia repairs.

TYPES OF REPAIR

Herniorrhaphy techniques include: Bassini repair; Shouldice repair; McVay repair.

Hernioplasty techniques include: Anterior (Lichtenstein repair; Plug and patch repairs; Double layer hernia repair); Posterior (pre-peritoneal) repairs {Rieves repair; Stoppa repair; Laparoscopic/endoscopic repair [Total extra peritoneal repair (TEP); Trans abdominal pre peritoneal repair (TAPP)]}.

Among these various methods prosthetic repairs have established their supremacy over repairs withour using prosthesis. A recent metaanalysis published in cochrane has revealed that Shouldice herniorrhaphy is the favoured non prosthetic technique comparing recurrence but it lacks favour on terms of operational time and hosptial stay[11,12]. Concluding, lower recuurence rates have been established for mesh repair techniques compared to tissue repair techniques alone.

Recent European hernia society guidelines state that none of the alternative mesh techniques except for Lichtenstein and endoscopic techniques have received sufficient scientific evaluation to be recommended[13]. American college of surgeons and National Institute of Clinical Excellence consider Lichtenstein repair as gold standard open repair[4,14,15]. However tissue repairs are a viable alternative in females because of the more durable transversalis fascia[16] and in emergency repairs where use of mesh is associated with increased surgical site infections[17,18].

Minimal access surgical repairs also provide very promising results if surgeon has technical expertise. It results in minimum postoperative pain, reduced wound infection and early return to work[19]. A Cochrane review between TEP and TAPP repair found the above said approaches are equal in terms of considering duration of operation, hematoma, length of hospital stay and rate of recurrence[20]. European hernia society guidelines promote TEP as a preferred method of repair to TAPP in the case of minimal access (endoscopic) surgery[13].

LAPAROSCOPIC REPAIR *VS* OPEN SURGERY

In recent times a rousing debate is brewing between open and endoscopic prosthesis repairs for the preferred approach status. Open and minimal access surgcal (laparoscopic/endoscopic) techniques have been compared in a number of studies in published literature. To begin with, cost factor remains a burning issue in pulling down the laparoscopic repairs as thery involve high cost compares to open repair. Hynes *et al*[21] has stated that laparoscopic repair amounts to an average of $638 more compared to open surigcal techniques in North America. Similarly, McCormack *et al*[22] showed that laparoscopic repair is exorbitant to the health service compared to open surgical repair by approximately 300-350 pounds per patient. A Swedish study has demonstrated that the total hospital cost was 710.6 Euro higher for TEP repair which would increase to 795.1 Euro when the added bills due to recurrences and complications within 5 years were acknowledged[23].

Similarly Khajanchee *et al*[24] reported a cost difference of $128.58 for a TEP repair. The cost-minimization analysis, including complications, reoperations and community costs during follow-up of 5 years, in a randomized trial showed that laparoscopic inguinal hernia repair had a small but significant increase in overall costs compared with open repair[23]. Above all financial burden on the patient and high infrastructal cost has been a limiting factor specially in developing countries.

A systematic review by McCormack *et al*[22] comparing laparoscopic and open repairs has revealed no apparent difference in recurrence. Laparoscopy seems to cause less persisting pain and numbness. Return to normal day to day activities is also faster[25]. However, operation time using laparoscopy technique is longer and there appears to be a higher risk of serious complication rate in respect of visceral (especially bladder) and vascular injuries[26].

In the similar systematic review, on further comparing complications of laparoscopic repair to open repair, it was evident that laparoscopic repairs are associated with overall more incidence of seroma formation. On the other hand there are less frequent chances of hematoma formation (more in TEP patients) and wound/superficial infections but there has been a heterogenity in data to deduce a final statement[26]. Other complications related to laparoscopic hernia repair, although in lower frequency, include trocar site hemorrhage and/or herniation, and injury to the epigastric or gonadal vessels[25]. Complications related to use of laparoscopy and less to surgeon technique are hypotension secondary to elevated intra-abdominal pressure, hypercapnia, subcutaneous emphysema, pneumothorax, and increased peak airway pressures[25].

A large number of hernia repairs are still done with open technique as endoscopic repairs have a steep learning curve and requires costlier infrastructure[27]. Despite a few hurdles, endoscopic repair is becoming a preferred approach specially for bilateral and recurrent hernias.

TYPES OF MESH

Types of mesh includes synthetic: Heavy weight (density > 100 g/m^2) [Polypropylene; Polyester; Light weight (density 35-50 g/m^2); Non absorbable (Plain polypropylene; Coated polypropylene; Partially absorbable: Polypropylene + polygalactin; Polypropylene + polyglycaprone)] and Biological.

Use of meshes has decreased the rate of recurrence to a significant extent but complications related to these prostheses have been reported in published literature.

Since mesh is a foreign antigen, theoritical reasoning supports the notion of increased chances of infection but practically this complication is well taken care of. Standard polypropylene mesh is most frequently used because of low cost, easily availability and reasonable strength to avoid recurrence[28].

Foreign body sensation and chronic postoperative pain have discouraged the regular use of established polypropylene mesh. Newer light meshes have been developed to overcome these problems but they are fairly expensive and only reduce the foreign body sensation without significant difference in recurrence rate compared to heavyweight mesh[29-31]. Biologic meshes, on the other hand may gain importance in future as they have been proposed to be advantageous in contaminated areas but they are extremely expensive, not widely available and studies supporting use of biologic meshes is limited which needs further in depth analysis[32,33].

Hence conentional polypropylene mesh is a trustworthy option for inguinal hernioplasty. On the other hand, lightweight meshes may be considered based on patient's affordability and surgeon's discretion.

CONCLUSION

Patients with no atendent comorbidities with asympatomatic inguinal hernia at presentation should be offered hernia repair. Laparoscopic hernia repair is best suited for recurrent and bilateral inguinal hernia although it may be offered for primary inguinal hernia.

Mesh Repair is associated with the lowest recurrence rates with pain being the most common complication of hernia surgery.

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ORIGINAL ARTICLE

Basic Study

Leukocyte function-associated antigen-1 deficiency impairs responses to polymicrobial sepsis

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Abstract

AIM: To determine the role of leukocyte function-associated antigen-1 (LFA-1) in polymicrobial sepsis model in mice.

METHODS: Cecal ligation and puncture model was used to study polymicrobial sepsis in wild type and LFA-1 knockout (KO) (= CD11a KO) mice. Their survivals were examined. Neutrophil recruitment to the abdominal cavity, bacterial tissue load and bacterial killing by neutrophils, tissue cytokine profiles, and serum cytokines were examined. Apoptosis of tissues was assessed using cleaved-caspase 3 and TUNNEL staining. The recruitment of neutrophils to various tissues was assessed using myeloperoxidase staining or measuring myeloperoxidase activity.

RESULTS: LFA-1 deficiency significantly decreased survival (P = 0.0024) with the reduction of neutrophil recruitment to the abdominal cavity and higher bacterial load in blood. It was also associated with increased apoptosis in spleen and more organ injuries probed by interleukin-6 mRNA level. However, the deficiency of LFA-1 did not prevent neutrophil recruitment to lung, liver, spleen or kidney, which suggested the existence of LFA-1 independent recruitment mechanism in these organs.

CONCLUSION: LFA-1 deficiency did not attenuate neutrophil recruitment to various organs to adequately mitigate secondary tissue injury in sepsis. It was associated with decreased neutrophil recruitment to

the abdominal cavity, higher bacterial load, leading to increased mortality in an abdominal, polymicrobial sepsis.

Key words: Leukocyte function-associated antigen-1; Tissue injury; Neutrophil recruitment; Polymicrobial sepsis; Apoptosis

Core tip: We report our result on the role of leukocyte function-associated antigen-1 (LFA-1) in polymicrobial abdominal sepsis model induced by cecal ligation and puncture. LFA-1 is a key player in neutrophil migration, but its role in neutrophil migration to tissues in polymicrobial sepsis is yet to be determined. This study demonstrated that LFA-1 deficiency blocked neutrophil migration to the abdominal cavity, but maintained migration to other organs, and reduced survival in sepsis.

Liu JR, Han X, Soriano SG, Yuki K. Leukocyte function-associated antigen-1 deficiency impairs responses to polymicrobial sepsis. *World J Clin Cases* 2015; 3(9): 793-806 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i9/793.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i9.793

INTRODUCTION

Sepsis remains to be associated with high morbidity and mortality and accounts for a large expenditure in public health. The recent study demonstrated that the mortality was still over 30% in Europe and the United States despite the implementation of sepsis management protocols[1]. This suggests that our understanding of the pathophysiology of sepsis remains quite limited.

Accumulating evidence demonstrated that the innate immune system is activated and works as the first line of defense against sepsis. This inflammatory response is characterized by leukocyte infiltration into the extravascular space. Leukocytes are not only bactericidal but also mediate local tissue damage and alternations in microvascular perfusion. Adhesion molecules present on leukocytes and endothelial cells control leukocyte adhesion and extravasation.

Leukocyte-function associated antigen-1 (LFA-1, CD11aCD18) is an adhesion molecule expressed ubiquitously on the surface of all leukocytes and plays a central role in leukocyte recruitment. LFA-1 is composed of two subunits CD11a and CD18 and binds to their counter ligands, intercellular adhesion molecule-1 and -2 (ICAM-1 and ICAM-2). While LFA-1 does not bind its ligands in a resting state, in inflammation it becomes active and binds them. The interactions between them facilitate leukocyte rolling, arrest on the blood vessels and transmigration to facilitate leukocyte recruitment. This is a critical, immunological process to fight against and reduce infection.

The role of LFA-1 in infection has been examined in several *in vivo* models with mixed results. Deficiency of LFA-1 worsened the outcome of *Streptococcus pneumoniae* infection[2] and *Mycobacterium tuberculosis*[3], but was protective in listeriosis[4] and lipopolysaccharide shock[5]. Specifically neutrophils are innate immune cells for the first-line defense against infection. Their bactericidal properties emanates from phagocytosis and release of proteases and reactive oxygen species. However, these indiscriminant processes can also harm host tissues[6]. Sepsis often results in an exaggerated inflammatory response that leads to secondary tissue injury and organ dysfunction. We hypothesize that the LFA-1 deficiency will attenuate tissue injury and ameliorate bacterial sepsis. To test this hypothesis we used cecal ligation and puncture (CLP) model, an abdominal polymicrobial model which mimics human sepsis physiology[7] and measured the extent of histopathological damage and cytokine response in LFA-1 deficient and wild-type (WT) mice.

MATERIALS AND METHODS

Mice

All the mice were purchased from Jackson laboratory (Bar Harbor, Maine, United States) and inbred in the Boston Children's Hospital animal facility. CD11a knockout (KO) mice (LFA-1 KO mice) have been previously described[8]. Male mice on the C57BL/6J background aged 8-10 wk were used for the current experiments. They were housed under 12 h day and night cycles and specific pathogen free conditions.

CLP model and animal care use

All the experimental protocols were approved by the Review Board in Boston Children's Hospital and followed the Animal Research: Reporting of *in vivo* experiments guidelines. All the appropriate measures were taken to minimize pain and discomfort as described below. Polymicrobial sepsis was induced by cecal ligation and puncture model as described in our previous report for the details[7]. The mice were anesthetized with ketamine 60 mg/kg and xylazine 5 mg/kg. The cecum was punctured through and through using a 18-G needle. Following the completion of CLP surgery, 0.1 mL/kg of warmed saline was given subcutaneously. Postoperative surgery pain was treated with buprenorphine. Activity levels were recorded as follows: 5 = full response and activity (baseline activity), 4 = little less than full response and activity, 3 = responsive, active, but decreased appetite, 2 = responds, but barely reacts, 1 = barely responsive, and 0 = no response. In some of the experiments, LFA-1 blocking antibody (M17/4; BioXcell, West Lebanon, NH, United States) 2 mg/kg was given intravenously prior to CLP surgery.

Peritoneal leukocyte count

Peritoneal lavage samples were collected at 6 and 12 h after CLP as described in our previous report[7]. The

Table 1 Primer sequences

Gene	Forward primer	Reverse primer
TNF-α	CTGTAGCCCACGTCGTAGC	TTGAGATCCATGCCGTTG
IL-1b	TGTAATGAAAGACGGCACACC	TCTTCTTTGGGTATTGCTTGG
IL-6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA
IL-10	CAGAGCCACATGCTCCTAGA	GTCCAGCTGGTCCTTTGTTT
GAPDH	GCACAGTCAAGGCCGAGAAT	GCCTTCTCCATGGTGGTGAA

TNF: Tumor necrosis factor; IL: Interleukin; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

number and differential of peritoneal leukocytes was assessed using TURK blood diluting fluid and Giemsa staining.

Quantitative blood and organ culture

The residual bacterial loads in the organs and blood were determined by loading serially diluted tissue homogenates and blood on agar plates as described in our previous report[7].

Bacterial killing assays

As previously described[9], we isolated neutrophils from mouse bone marrow. We performed bacterial killing assays as described in our previous report[7]. Briefly, 50% diluted plasma from either WT or CD11a KO mice was mixed with 5×10^5 CFU of bacteria derived from tissues after CLP for opsonization. Then 5×10^4 of neutrophils were added and kept for one hour in a shaker at 37 ℃, and then the number of residual bacteria was determined by plating on blood agar.

Complete blood count, serum cytokine level measurement and immunoglobulin level measurement

VetScan HM2 (Abaxis, Union City, CA, United States) was used for complete blood count counts. Levels of various serum cytokines except interleukin-6 (IL-6) were determined by using mouse Th1/Th2 kit (Meso Scale Discovery; Gaithersburg, MD, United States). Serum IL-6 was measured using mouse IL-6 enzyme-linked immunosorbent assay (ELISA) kit (Life Technologies, Grand Island, NY, United States). Serum mouse immunoglobulin levels were measured with a commercially available ELISA kit (BD, Sparks, MD, United States).

Reverse transcription and real time quantitative polymerase chain reaction

Following the harvest, tissues were snap frozen and stored at -80 ℃ for a short period. Total RNA was extracted using TRIzol reagent (Life Technologies) and subject to DNase digestion. 1 μg of RNA were subject to reverse transcription using Superscript Ⅲ RNase reverse transcriptase (life technologies). Real time quantitative polymerase chain reaction (PCR) was performed using SYBR green master mix (Applied Biosystems, South Francisco, CA, United States). As examples of proinflammatory and anti-inflammatory cytokines, we quantitated mRNA level of tumor necrosis factor (TNF)-α, IL-1β, IL-6 and IL-10. glyceraldehyde-3-phosphate dehydrogenase was used as housekeeping gene. The sequences of primers used for this assay were listed in Table 1.

Histology

Mice tissues were fixed using 4% paraformaldehyde. Tissues for histology analysis were prepared in Boston Children's Hospital pathology core.

The determination of tissue apoptosis

The degree of splenic apoptosis was determined by Western blot analysis and immunohistochmistory probed by anti-cleaved caspase-3 antibody and TUNEL staining as we previously described for details[7,10]. For Western blot analysis, the total protein extracted from spleen was used. For immunohistochemistry analysis, anti-cleaved caspase 3 antibody was probed with biotin-labeled anti-rabbit IgG and peroxidase-conjugated streptavidin. 3,3'-diaminobenzidine (DAB) was used for a chromogenic reaction. TUNEL staining was performed according to the company protocol (Millipore, Serological Corporation, Norcross, GA, United States).

Myeloperoxidase staining

The degree of neutrophil recruitment to various organs was assessed by probing myeloperoxidase activity (MPO) in the tissues. Histology sections were deparaffinized as we previously described[7] and probed with anti-myeloperoxidase antibody, which was captured by anti-rabbit IgG-HRP and DAB.

Leukocyte counts in bronchoalveolar lavag fluid

Bronchoalveolar lavag fluid was obtained as described in our previous report[7]. The number and differential of leukocytes was determined with TURK blood diluting fluid and Giemsa staining.

MPO assay of lung

MPO level was measure in lung samples obtained from mice at 12 h after CLP. MPO assay was well described in the past[11]. Briefly, MPO was extracted from tissues into the supernatant by homogenization in 50 mmol/L KPO_4 buffer containing 0.05% hexadecyltrimethlammonium bromide, followed by repeated freeze and thaw, and centrifugation. O-dianisodine and H_2O_2 were added to the supernatant. The wave length of 450 nm was used for absorbance measurement.

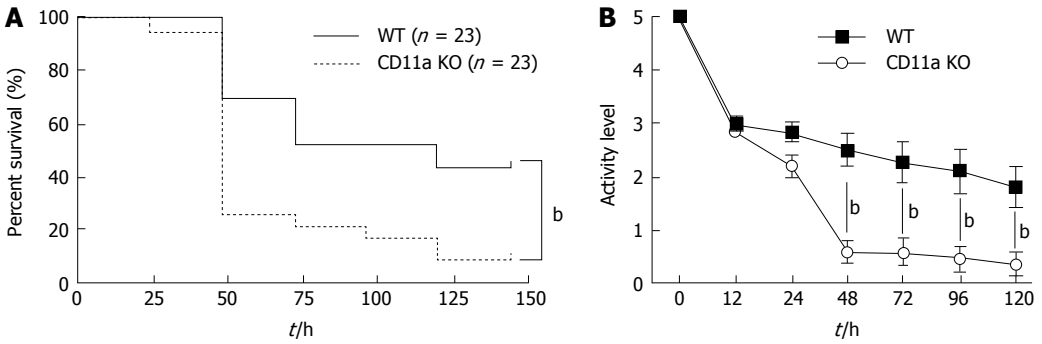

Figure 1 The outcome and activity level of CD11a knockout mice in polymicrobial sepsis. A: The outcome of polymicrobial sepsis in wild-type (WT) and CD11a knockout mice. Statistical significance was evaluated using Log-rank test (P = 0.0024). The activity level of WT and CD11a mice following polymicrobial sepsis is shown; B: Activity was numbered from 0 to 5 based on responsiveness and appetite as described in Methods. Statistical analysis was performed using two-way analysis of variance with Bonferroni post hoc analysis. In both (A) and (B), $^{b}P < 0.01$, respectively. KO: Knockout.

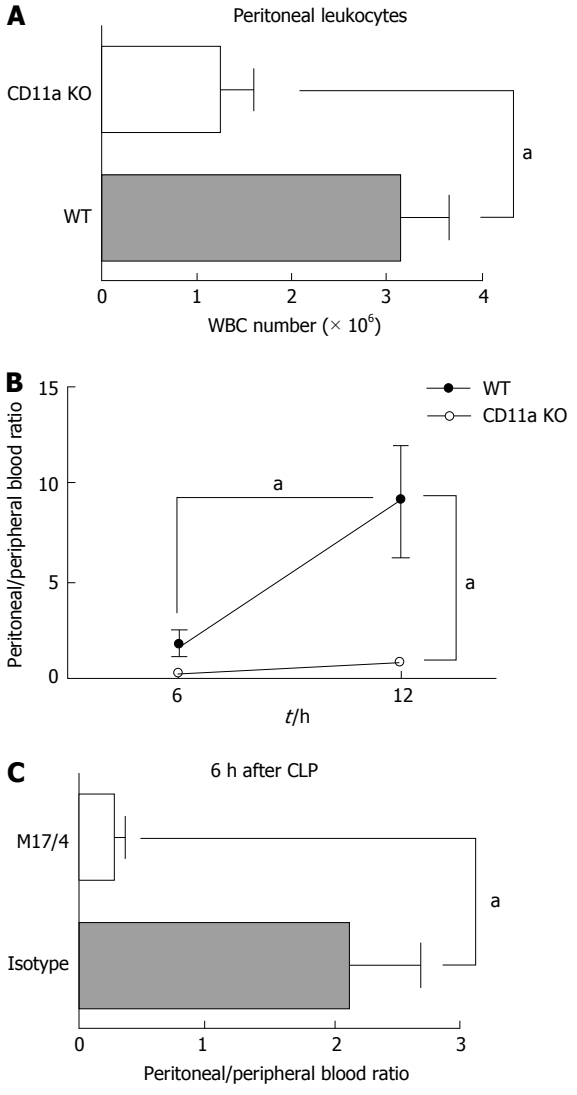

Figure 2 Leukocyte migration to the peritoneal cavity. A: Total numbers of peritoneal leukocytes in both wild-type (WT) and CD11a knockout (KO) mice at 6 h after cecal ligation and puncture (CLP) were compared. Statistical analysis was performed using two-tailed Student's *t*-test; B: The peritoneal emigration ratio defined as in the manuscript were shown at 6 and 12 h after CLP. Data represent mean ± SD of 4 mice. Statistical analysis was performed using one-way analysis of variance with Tukey's post hoc analysis; C: The peritoneal emigration ratio was shown at 6 h after CLP in both isotype-control injected WT group and CD11a blocking antibody M17/4 injected WT group. Data represent mean ± SD of 4 mice. Statistical analysis was performed using two-tailed student's *t*-test. $^{a}P < 0.05$.

Statistical analysis

The data were shown as mean ± SD. Differences were statistically analyzed using PRISM software (GraphPad software, Inc. La, CA, United States). The statistical methods used were detailed in corresponding figure legends. $P < 0.05$ was used for a statistical significance. The statistical methods of this study were reviewed by Amber Hall, MPH (Boston Childrens Hospital).

RESULTS

The outcome of polymicrobial sepsis

In the previous study using less severe CLP model (survival about 80%), administration of LFA-1 blocking antibody did not improve survival despite the attenuation of lung injury[12]. The use of blocking antibody carries the potential problem because (1) in the case of physical approximation of LFA-1 with an other surface receptor, anti-LFA-1 antibody may directly interfere with the receptor, and (2) blocking antibody may activate Fc receptors and induce functional changes in neutrophils[13]. Therefore, we studied using CD11a KO mice here.

Contrary to our hypothesis that LFA-1 deficiency would attenuate sepsis, KO mice had significantly higher mortality (Figure 1A). Their activity level was significantly reduced 48 h after CLP compared to WT mice (Figure 1B), which was in line with the observed pattern of mortality.

Leukocyte infiltration to the abdominal cavity

LFA-1 plays an important role in leukocyte recruitment. Now we evaluated the degree of leukocyte recruitment to the peritoneal space. The total number of peritoneal leukocytes was greater in WT mice (Figure 2A), which were primarily neutrophils (data not shown). Previously, the peritoneal emigration ratio was defined as (peritoneal leukocyte number)/(peripheral leukocyte number) by Prince *et al*[2]. When we compared this parameter, KO mice had much lower peritoneal emigration ratio than WT mice (Figure 2B), suggesting that leukocyte recruitment to the peritoneal cavity was significantly reduced in the former. Similarly, the administration of the blocking antibody was associated with lower peritoneal

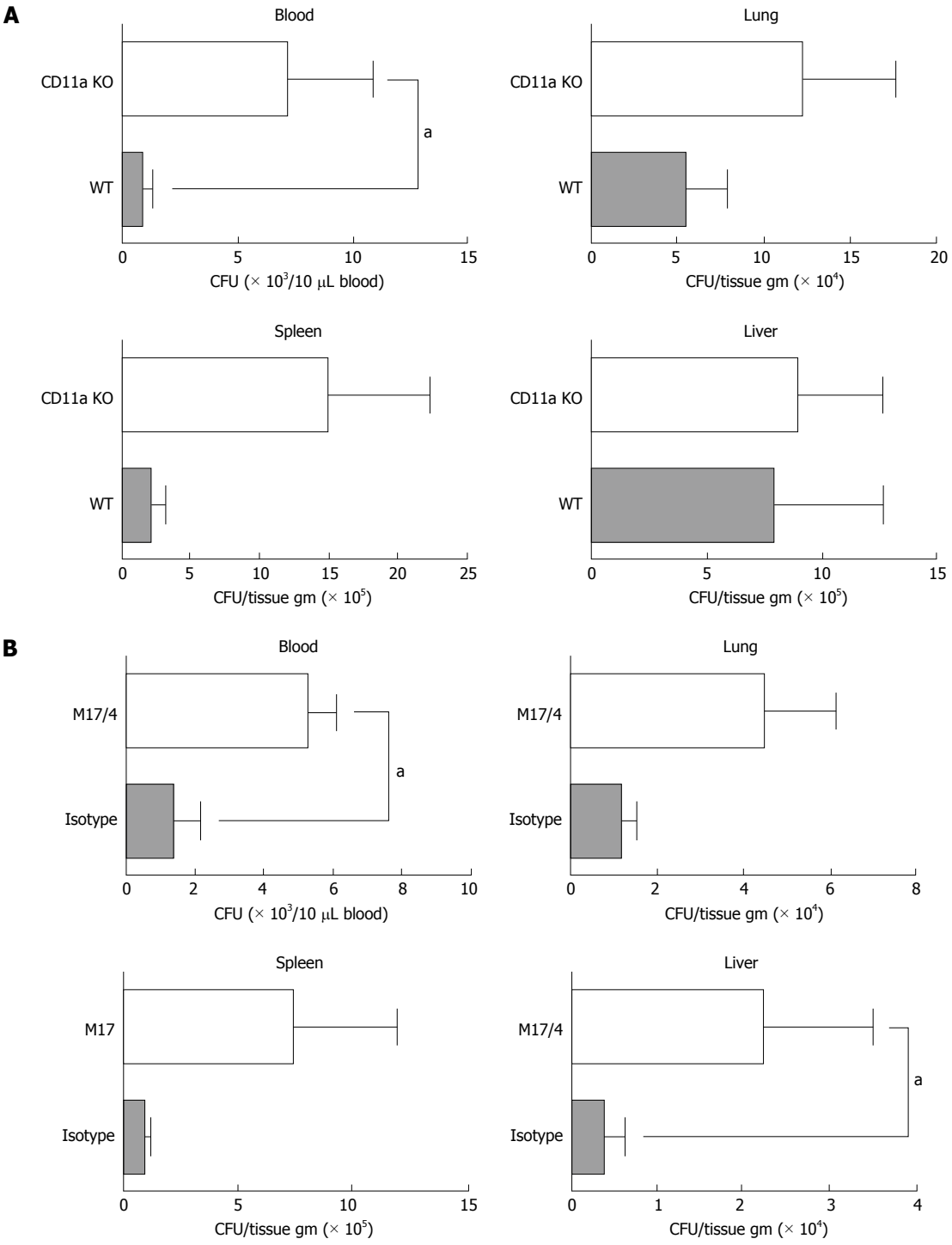

Figure 3 Tissue bacterial load. A: The comparison between wild-type and CD11a knockout (KO) mice. The tissue bacterial load was studied and shown as mean ± SD of 8 mice. Statistical analysis was performed using two-tailed Mann-Whitney test; B: The comparison between isotype-control injected group and CD11a blocking antibody M17/4 injected group. The tissue bacterial load was studied and shown as mean ± SD of 8 mice. Statistical analysis was performed using two-tailed Mann-Whitney test. $^{a}P < 0.05$.

emigration ratio than isotype control (Figure 2C). This observation is compatible with thioglycollate-induced peritonitis model[14] and *Streptococcus pneumoniae* abdominal infection model[2].

Bacterial loads

Since KO mice had significantly reduced leukocyte recruitment to the peritoneal cavity (site of infection), we reasoned that the higher mortality in KO mice might be due to the difference of bacterial clearance between WT and KO mice. Bacterial loads in blood and various tissues were measured. Even at 6 h after CLP surgery, the KO mice had a significantly higher bacterial load in blood than the WT (Figure 3A). Similarly the presence of CD11a blocking antibody M17/4 was associated with higher bacterial load in blood and liver than the

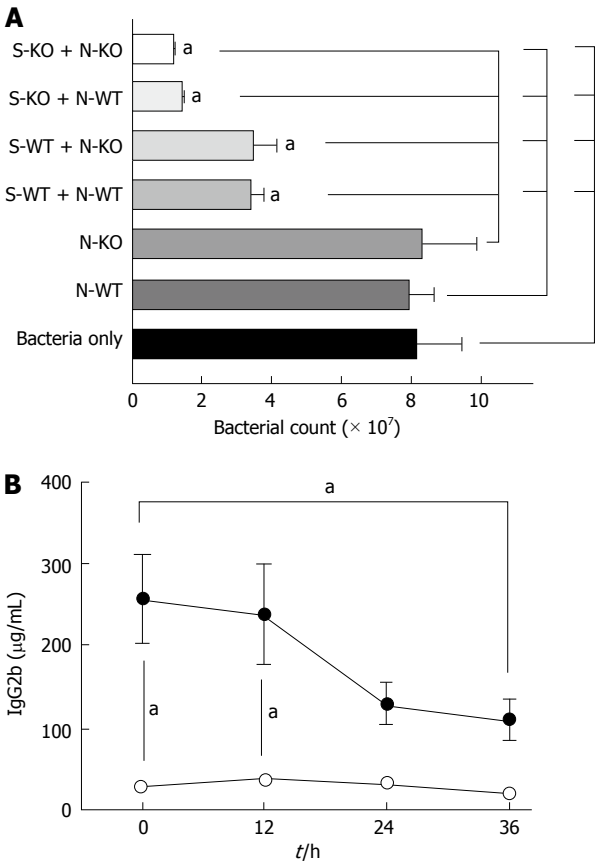

Figure 4 Bacterial killing and IgG2b level. A: Bacterial killing assay was performed using bacteria obtained from cecal culture as described in the methods. Data represent mean ± SD of 3 independent experiments. Statistical analysis was performed using one-way analysis of variance with Tukey post hoc analysis. $^{a}P < 0.05$; B: Serum IgG2b level was shown. Statistical analysis was performed using one-way variance of analysis with Tukey post hoc analysis. $^{a}P < 0.05$, respectively of the comparison between wild-type *vs* CD11a knockout (KO) mice at the same time point, or comparison among wild-type or CD11a KO mice. N-WT: Wild-type neutrophils; N-KO: CD11a KO neutrophils; S-WT: Wild-type serum; S-KO: CD11a KO serum.

presence of isotype-control antibody (Figure 3B).

Bacterial killing assays

Because KO mice had higher bacterial load, we assessed any alternation of bacterial killing in KO mice in addition to the reduced leukocyte recruitment to the abdominal cavity. We performed assays using bacteria derived from the cecal flora. Isolated neutrophils themselves were not bactericidal, but the addition of serum to the neutrophils effectively killed bacteria in both WT and KO mice (Figure 4A). The serum itself killed bacteria and KO serum was more efficient in bacterial killing. Furthermore neutrophils were more bactericidal with KO serum than with WT serum. Therefore, higher bacterial load in KO mice is attributed to the significantly reduced number of leukocytes at the site of infection. The observation that KO serum was more efficient in bacterial killing with and without neutrophils may suggest the possibility that KO serum have more complement activity, or it may be due to significant reduced serum IgG2b level in KO mice (Figure 4B). IgG2b can bind to inhibitory Fc receptor and impair optimal opsonization through binding to antigenic sites on the bacteria[15]. Therefore, the reduced IgG2b level may be in favor for opsonization in KO mice.

Serum cytokines

Systemic activation of the innate immune system plays an important role in the pathophysiology of sepsis. The initial immune response is proinflammatory, but this rapidly shifts to hypoinflammatory (anti-inflammatory) process[16]. The attenuation of proinflammatory cytokines was previously associated with the improved outcomes in this model[17]. Since KO mice had higher mortality, we compared proinflammatory [TNF-α, interferon (IFN)-γ, CXCL1, IL-1β, IL-2, and IL-12] and anti-inflammatory (IL-4, IL-5, and IL-10) cytokines (Figure 5). There was a slight increase of IFN-γ level in KO mice at 12 h after CLP procedure, but there was no difference at 24 and 36 h. IL-1β level of KO mice was significantly higher at 24 h after CLP procedure, but at 36 h no difference was observed. IL-6 is a cytokine with both proinflammatory and anti-inflammatory properties. Previously the serum level of IL-6 was elevated in septic patients[18] and shown to correlate with their outcomes[19,20]. Serum IL-6 level was higher at 12 h in KO mice. At 24 and 36 h, serum IL-6 level in KO mice was not statistically different (Figure 5).

Tissues injury

Next we evaluated local injury to see if there was any difference between WT and KO mice. IL-6 serves as sensitive marker of tissue inflammation (injury)[21]. In both lung and spleen, the level of IL-6 mRNA was significantly higher in KO mice than WT mice (Figure 6), suggesting that there was more tissue injury in KO mice. mRNA levels of TNF-α, IL-1β and IL-10 were not statistically significant (Figure 7).

Apoptosis

Apoptosis in the spleen is linked to the severity of sepsis[22]. Therefore, we evaluated the degree of apoptosis in our model. We first evaluated the cleaved-caspase-3 expression in spleen using western blot. The expression of cleaved caspase-3 was peaked at 12 h following CLP procedure in both WT and CD11a KO mice (Figure 8). Expression was higher in KO mice. Next we evaluated apoptosis using TUNEL stain and cleaved caspase-3 staining on the spleen histology. As predicted, apoptotic cells of spleen were present more in KO mice (Figure 9). Taken together, LFA-1 deficiency contributed to severity in tissue injuries in CLP model.

Neutrophil infiltration in the spleen, liver, kidney and lung

Since KO mice had increased tissue injury, we examined neutrophil recruitment to spleen, liver, kidney and lung. MPO is a peroxidase enzyme that is most abundantly expressed in the granules of neutrophils. We examined the expression of MPO in tissues as a surrogate of

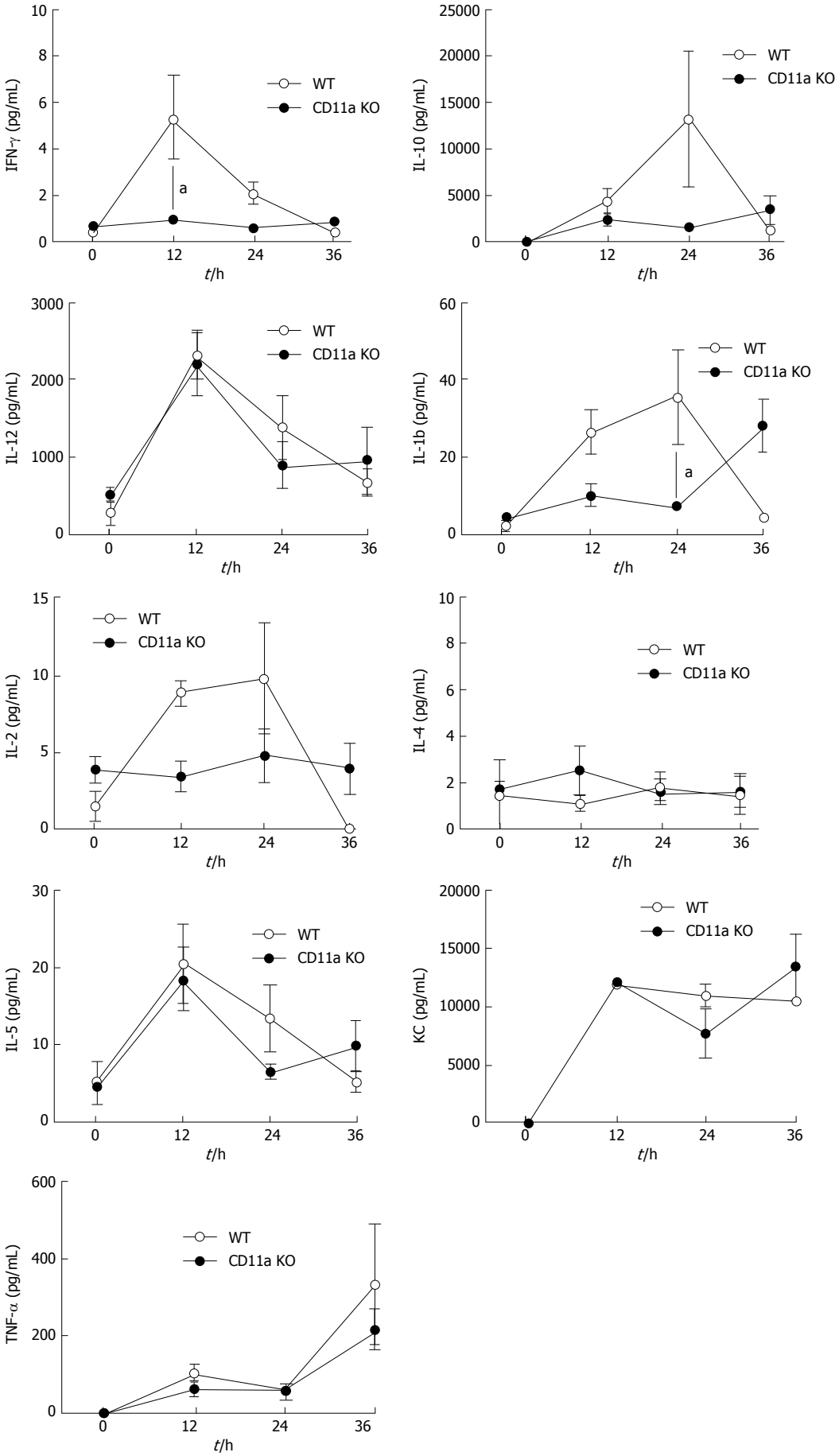

Figure 5 Serum cytokine profiles in polymicrobial sepsis. The cytokine profiles of wild-type (WT) and CD11a knockout mice were compared. The data represents mean ± SD of 8 mice. Statistical analysis was performed using two-way analysis of variance with Tukey post hoc analysis. $^{a}P < 0.05$.

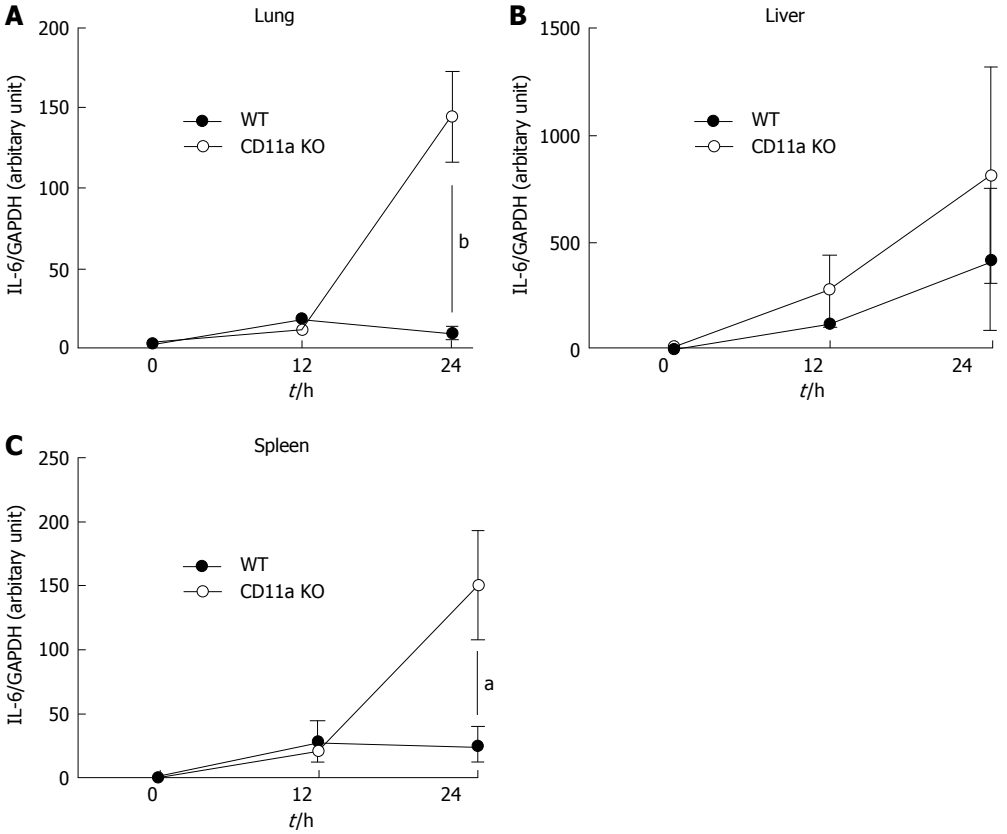

Figure 6 Tissues interleukin-6 mRNA level. Interleukin-6 (IL-6) expression at various tissues was examined using real-time quantitative polymerase chain reaction (A-C). Data represent mean ± SD of 4 independent experiments. Statistical analysis was performed using two-way analysis of variance with Tukey post hoc analysis. $^{a}P < 0.05$ and $^{b}P < 0.01$, respectively.

neutrophil number. There were more MPO positive cells on tissues of KO mice in organs tested after CLP (Figure 10). Activated leukocytes are often trapped in lung in sepsis, causing lung injury. The number of leukocytes in bronchial lavage fluid was not statistically different between WT and KO mice (Figure 11A). The majority of the population was neutrophils (data not shown). However, MPO value was significantly higher in lung tissue in KO mice than in WT mice (Figure 11B), suggesting that neutrophils in KO mice were trapped in lung tissue with less migration out into the alveolar space. Although the baseline neutrophil count was higher in KO mice as previously reported due to the elevated level in G-CSF level[23], there was no difference at 12 h after CLP (Figure 12). This larger reduction in peripheral neutrophil counts could be well explained by more neutrophil recruitment to the tissues in KO mice. In addition, we tested neutrophil recruitment to lung using LFA-1 blocking antibody M17/4. The result demonstrated more neutrophil recruitment to lung in the group that received LFA-1 blocking antibody, consistent with our KO mice data (Figure 11C). These results suggested that neutrophils migrated in a LFA-1 independent fashion to the tissues tested.

DISCUSSION

Here we demonstrated that both LFA-1 blockade and deficiency were detrimental in polymicrobial sepsis. CD11a KO mice demonstrated higher mortality with more bacterial load in blood than WT mice in CLP model. The reduction of available leukocytes at the site of infection (the abdominal cavity) was significant. Contrary to our hypothesis, LFA-1 deficiency did not attenuate neutrophil recruitment to various organs, suggesting that tissue damage by neutrophils was not attenuated. Therefore, we conclude that the loss of LFA-1 did not alleviate organ injury in this experimental model of abdominal sepsis.

Neutrophil phagocytosis of a certain bacteria strain (such as Streptococcus pyogenes) was previously reported to be potentiated by LFA-1/ICAM-1 interaction[24]. The capability of bacteria killing was not different between WT and KO mice, suggesting that neutrophils devoid of LFA-1 could phagocytize bacteria effectively. Reduction of leukocyte recruitment to the abdominal cavity can certainly reduce the efficiency of bacteria killing. Therefore, higher bacteria load in blood may be simply due to the impaired recruitment of neutrophils to the abdominal cavity.

The process of leukocyte (neutrophil) recruitment to organs was previously assumed to uniformly follow an orchestrated progression consisting of initial tethering and rolling by selectins, firm adhesion by integrins such as LFA-1 and macrophage-1 antigen (Mac-1, CD11bCD18) and transmigration. Molecules involved in the recruit-

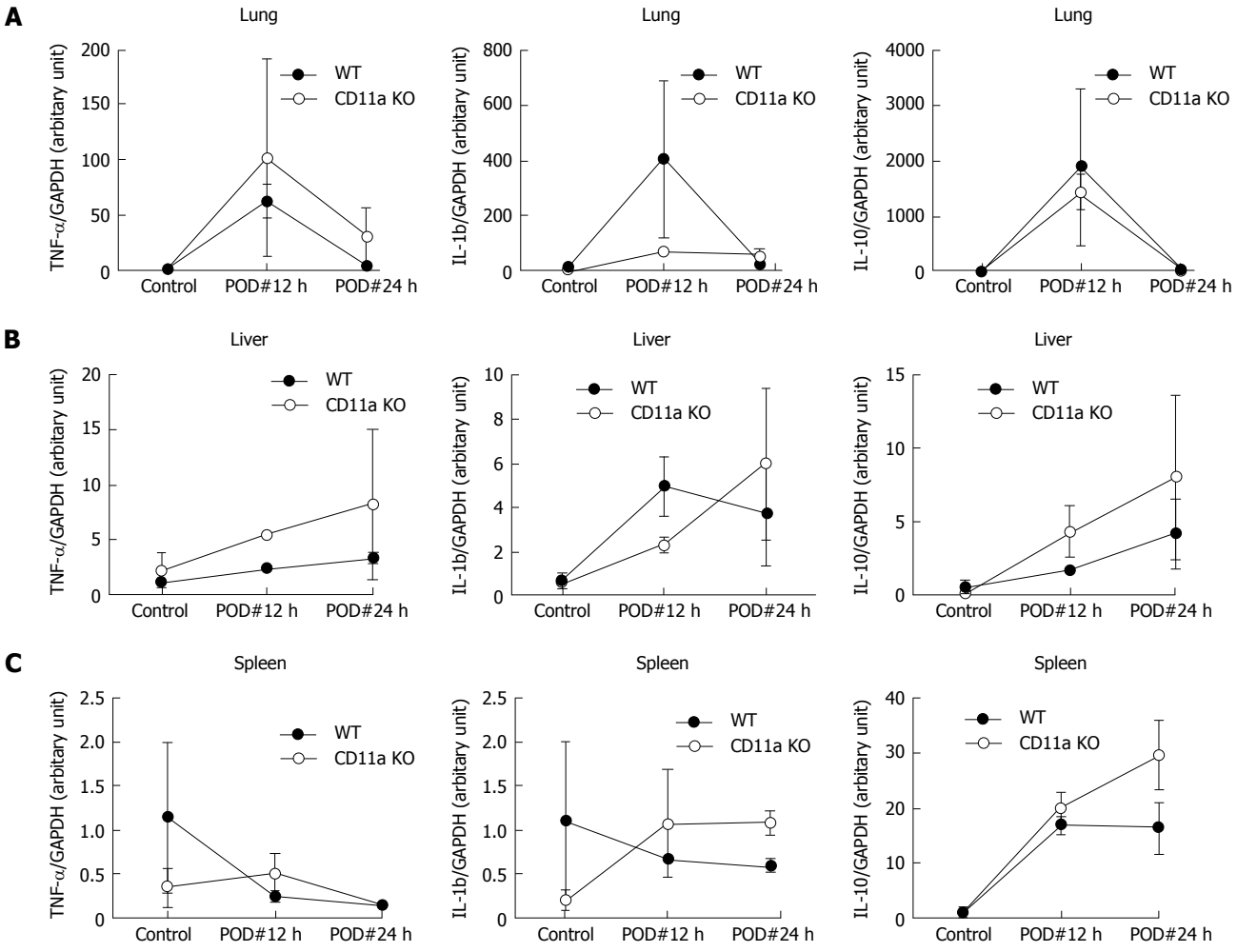

Figure 7 Tissues tumor necrosis factor-α, interleukin-1b and interleukin-10 levels. Various tissues of IL-6 expression were examined using real-time quantitative polymerase chain reaction. Data represent mean ± SD of 4 independent experiments. Statistical analysis was performed using two-way analysis of variance with Tukey post hoc analysis. IL: Interleukin; TNF: Tumor necrosis factor; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

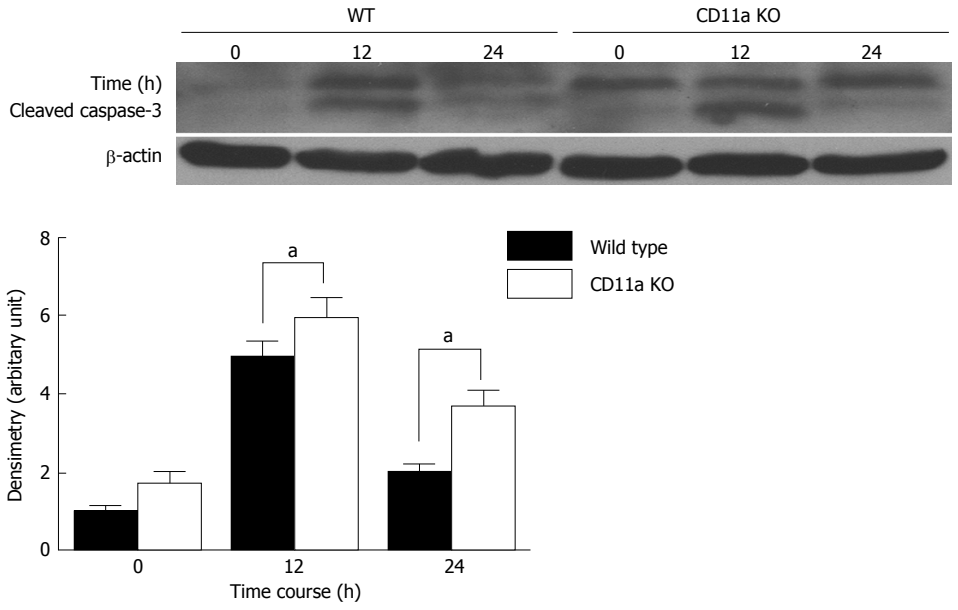

Figure 8 Clevased caspase-3 expression in spleen at different time point. Cleaved caspase-3 and β-actin expression was examined at 12 and 24 h after cecal ligation and puncture procedure. The density of bands was determined three times using Image J and plotted. Statistical analysis was performed using one-way analysis of variance with Tukey post hoc analysis. $^{a}P < 0.05$.

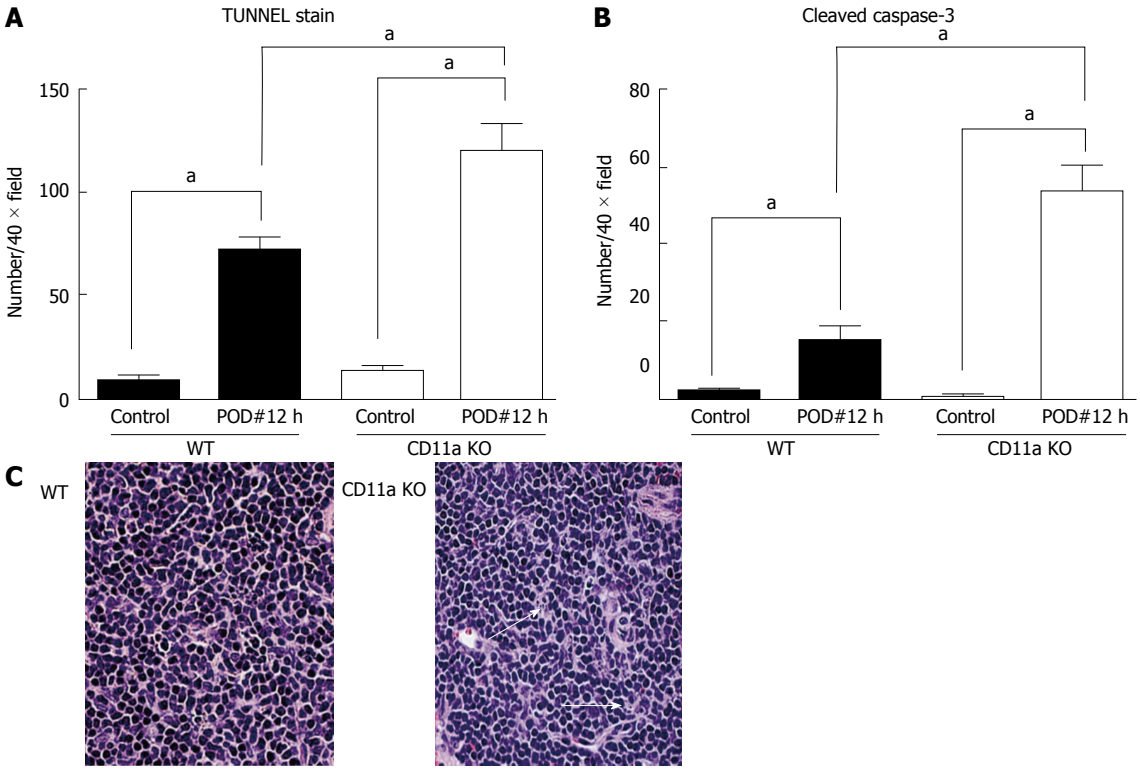

Figure 9 Apoptosis in spleen. A and B: Apoptosis in spleen was examined using (A) tunnel staining and (B) cleaved-caspase-3 staining. The number of positive cells was counted per 40 × magnification field of the same size. Eight different regions were counted. Data show mean ± SD statistical analysis was performed using one-way analysis of variance with Tukey post hoc analysis. $^{a}P < 0.05$; C: In hematoxylin and eosin staining, apoptotic cells are indicated using arrows.

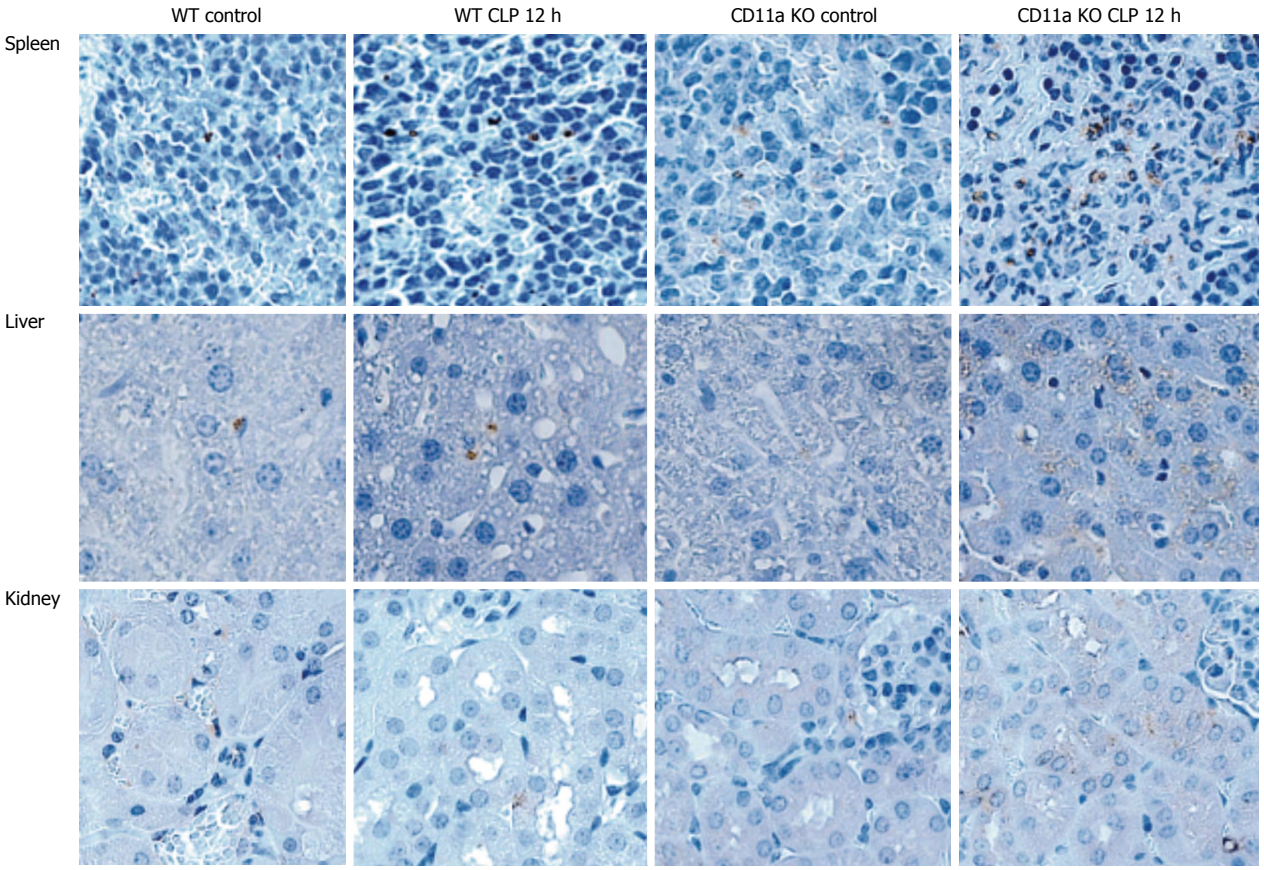

Figure 10 Neutrophil recruitment to tissues. Neutrophil recruitment to tissues was studied by examining myeloperoxidase staining. Cells stained with brown show myeloperoxidase positive cells, largely representing neutrophils. In figures, control means no cecal ligation and puncture surgery, and cecal ligation and puncture (CLP) 12 h is 12 h after cecal ligatation and puncture model.

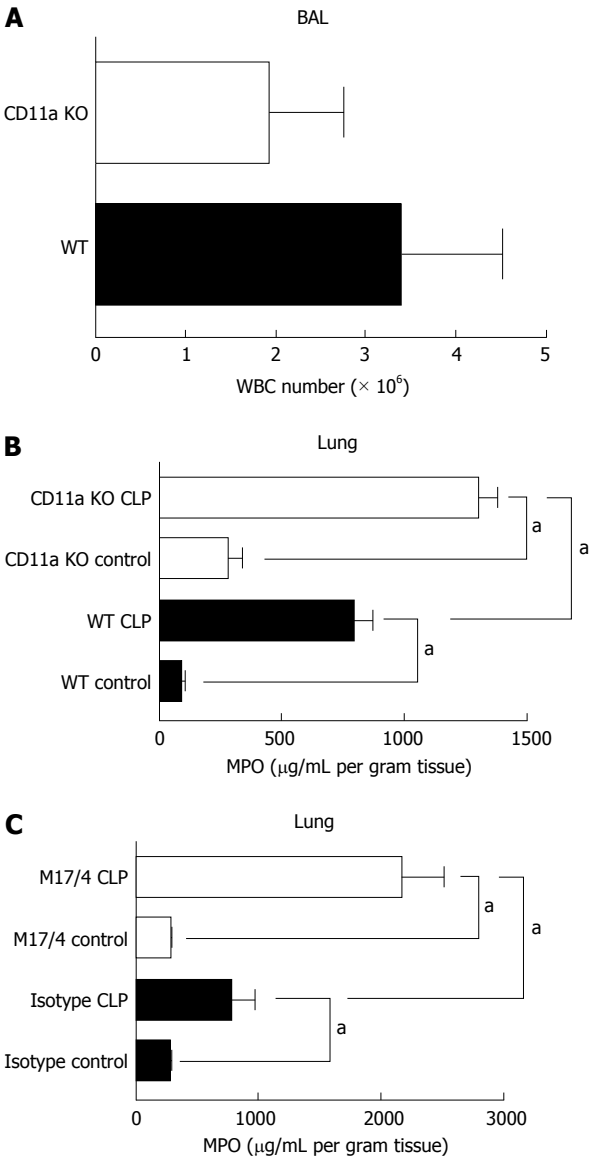

Figure 11 Neutrophil recruitment to the lung. A: Leukocyte recruitment to the alveolar space was examined by BAL. The number of leukocytes in BAL was shown. Statistical analysis was performed using two-tailed Student's *t* test. There was no statistical significance (P = 0.1158); B and C: Neutrophil recruitment to the lung was examined by measuring myeloperoxidase level. In the figure, control means no cecal ligation and puncture surgery, and CLP means cecal ligation and puncture model. Data represent mean ± SD of 4 different samples. Statistical analysis was performed using one-way analysis of variance with Tukey post hoc analysis. $^{a}P < 0.05$. Isotype means isotype control antibody. M17/4 is anti-CD11a blocking antibody. KO: Knockout; CLP: Cecal ligation and puncture; MPO: Myeloperoxidase; WT: Wild type.

ment of leukocytes to the abdominal cavity were examined in thioglycollate-induced inflammation[25]. The study demonstrated that two integrins LFA-1 and α4β1 contributed to neutrophil recruitment. In our study migration of leukocytes to the peritoneal cavity was much less in CD11a KO mice than WT mice, suggesting that LFA-1 played a major role. Microcirculation of peritoneum seems to fit this traditional scheme. Microcirculation in tissues such as mesentery, skeletal muscle, and skin also follow the original scheme[26]. In contrast, LFA-1 deficiency did not have an effect on neutrophil recruitment to liver, kidney, spleen and lung in our model, suggesting that other processes are involved.

Recruited leukocytes are predominantly neutrophils early in sepsis. Neutrophils efficiently kill bacteria *via* various methods, but the secondary damage by neutrophils could be detrimental. The importance of early, enhanced neutrophil recruitment by the chemokines CXCL1 (= KC) and CXCL2 (macrophage inflammatory protein 2; MIP-2) administration in peritonitis-induced sepsis was previously shown to improve bacterial clearance and survival[27]. However, blockade of CXCL2 was associated with the reduced percentage of neutrophils in the peritoneal fluid but with better survival[28]. Furthermore, leukotriene B4 receptor (BLT1) deficiency was associated with reduction of neutrophils migration to the abdominal cavity with a better survival[29]. The impact of these molecules on other effects such as neutrophils recruitment to various tissues and cytokine productions were not fully explored in these studies. However the completely opposite outcomes despite similarly reduced neutrophil recruitment to the abdominal cavity suggested that there are important factors other than local infection control. The function of CXCL2 includes the activation of neutrophils. Blockade of CXCL2 or BLT1 deficiency may possibly reduce neutrophil migration to various organs outside of the abdominal cavity, limiting organ injury. It is important to understand how we modulate the behavior of neutrophils (leukocytes) in the whole body rather than simple control of neutrophils to the site of infection.

In the present report we demonstrated that in CD11a KO mice, neutrophil recruitment seems organ specific. Neutrophil recruitment to liver in lipopolysaccharide shock was previously shown to be independent on LFA-1 and dependent on CD44[5,30]. Interestingly, neutrophil utilizes Mac-1 when fMLP was injected locally in liver, suggesting that main players can change depending on circumstances[30]. Lung microvasculature is a network of thin capillaries. Neutrophils have to deform to pass through these small capillaries, which may make selectins and integrins unnecessary[31]. However, the other report suggests that integrins play a significant role in leukocyte recruitment to lung[12,32]. In the study by Asaduzzaman *et al*[12], the administration of LFA-1 blocking antibody did not change the outcome of mice in mild CLP model (survival around 80% in both vehicle and blocking antibody injected groups) despite the less neutrophil recruitment to lung. In our more severe model, LFA-1 blocking antibody did not attenuate neutrophil recruitment to lung. Whether or not the pattern of neutrophil recruitment to lung varies in different disease processes as well as severity of diseases remains to be clarified. In kidney, Mac-1 is shown to be involved in neutrophil recruitment[33]. Various insights into neutrophil recruitment at various organs start to emerge. However, it is required to have further investigations on clear mechanism of neutrophil recruitment to each organ in the future. Our study highlighted the importance of this approach.

Since LFA-1 works by interacting with its ligands, it

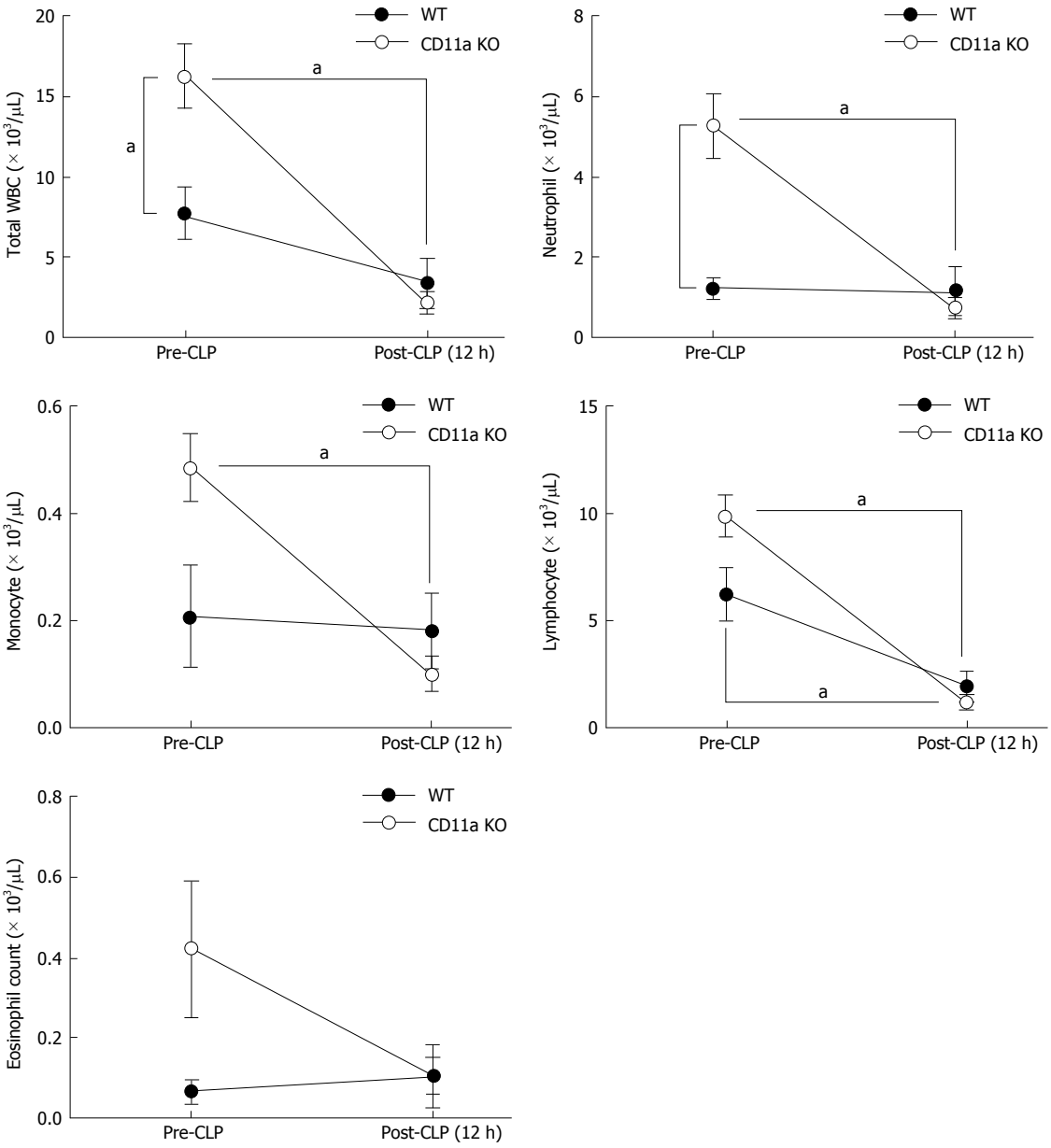

Figure 12 The changes of peripheral leukocyte counts in septic mice. The number of leukocytes was examined. The data represent mean ± SD of 4 independent experiments. Statistical analysis was performed using one-way analysis of variance with Tukey's post hoc analysis. $^{a}P < 0.05$.

is important to study the role of ICAM-1 and ICAM-2, both of which are ligands for LFA-1. They are expressed on leukocytes, epithelial cells, endothelial cells, and fibroblasts. ICAM-1 is uniformly expressed on the surface of resting endothelium at low level and its expression is upregulated by inflammatory cytokines. On the other hand, ICAM-2 shows high expression constitutively, particularly at endothelial cell junctions. The predominant role of ICAM-1 is to establish stable leukocyte adhesion while ICAM-2 mediates trans-migration[34]. The role of ICAM-2 in polymicrobial sepsis has not been studied. ICAM-1 KO mice subjected to CLP surgery had reduction of serum TNF-α, IL-1β, IL-6 and IL-10 levels as well as leukocyte migration to liver and lung, and had significantly higher survival over WT mice in CLP model[35]. The degree of leukocyte migration to the abdominal cavity and bacterial tissue load were not assessed in this study, but it is highly likely that neutrophils recruitment to the abdominal cavity was reduced by disrupted LFA-1/ICAM-1 interaction. The significantly lower cytokine levels and attenuation of leukocyte migration to organs such as liver in ICAM-1 KO mice might reduce their mortality. In our study, we did not see a significant difference of cytokine levels between WT and CD11a KO mice. Because ICAM-1 also binds to Mac-1 and p150,90, the elimination of interaction of ICAM-1 with these targets other than LFA-1 might contribute to this paradoxical result in ICAM-1 KO mice. The recruitment of neutrophils to liver is dependent on Mac-1[31], and the reduction of leukocyte recruitment to liver might be due to the abolishment of Mac-1 interaction. It is interesting to investigate in the future if ICAM-1 KO mice have less neutrophil recruitment to the abdominal cavity as well as other

organs.

We tried to address the degree of tissue injury by measuring IL-6 levels. IL-6 is induced by various signals including IL-1β, TNF-α, IL-2 and endotoxin, and it was reported as a marker of severity in sepsis and correlated with the outcome of patients. Remick *et al*[21] stated it as a sensitive marker for tissue injury. We demonstrated that IL-6 mRNA levels in various tissues were more elevated in CD11a KO mice. This was in line with our result of more neutrophil recruitment to various organs in KO mice. It remains to be studied whether or not other markers suit better for this purpose.

In conclusion, we demonstrated that LFA-1 blockade selectively reduced neutrophil recruitment to the site of infection without attenuating various organ injuries. Blockade of LFA-1 may not have a therapeutic implication in the setting of abdominal polymicrobial sepsis.

COMMENTS

Background

Neutrophils are the double-edge swords in sepsis because they eradicate bacteria as the first-line innate cells, but cause tissue injury. Leukocyte function-associated antigen-1 (LFA-1) is an important adhesion molecule in leukocyte recruitment. The effect of LFA-1 deficiency on leukocyte recruitment and tissue injury has not been well characterized in polymicrobial sepsis model.

Research frontiers

The author aimed to study the role of LFA-1 in neutrophil migration to various organs in polymicrobial sepsis model.

Innovations and breakthroughs

To illustrate the contribution of LFA-1 in neutrophil migration to various tissues so as to understand the impact of LFA-1 deficiency (or blockade) in polymicrobial sepsis model.

Applications

The author hypothesized that LFA-1 deficiency would reduce neutrophil recruitment to various organs, thereby reducing tissue injury and improving the outcome in polymicrobial sepsis model. However, the authors results showed that LFA-1 reduced neutrophil migration to the abdominal cavity, but did not reduce to other organs. The blockade of LFA-1 does not likely improve sepsis outcomes.

Terminology

LFA-1 is an adhesion molecule expressed on neutrophils and binds to its ligands such as intercellular adhesion molecule-1 on the endothelium, allowing neutrophils to stop on the blood vessel. Traditionally, this step is considered to be a critical step in neutrophil migration.

Peer-review

This is a clear cut study using LFA-1 knockout mice. The authors revealed that the absence of LFA-1 results in survival disadvantage in polymicrobial sepsis. The experiments were performed properly, and the paper is well-written with reasonable discussions.

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Role of genetic polymorphisms in hepatitis C virus chronic infection

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Abstract

AIM: To analyze the host genetics factors influencing the clinical course and the response to antiviral treatment in patients with chronic hepatitis C (CHC).

METHODS: We conducted an electronic search on the PubMed and MEDLINE (2000-2014) databases and Cochrane library (2000-2014). A total of 73 articles were retrieved and their data were extensively evaluated and discussed by the authors and then analyzed in this review article.

RESULTS: Several studies associated polymorphisms in the interleukin 28B gene on chromosome 19 (19q13.13) with a spontaneous viral clearance in acute hepatitis C and with the response to pegylated interferon (Peg-IFN)-based treatment in chronic hepatitis C patients. Other investigations demonstrated that inosine triphosphate pyrophosphatase genetic variants protect hepatitis C virus-genotype-1 CHC patients from ribavirin-induced anemia, and other studies that a polymorphism in the patatin-like phospholipase domain-containing protein 3 was associated with hepatic steatosis in CHC patients. Although not conclusive, some investigations suggested that the vitamin D-associated polymorphisms play an important role in the achievement of sustained virologic response in CHC patients treated with Peg-IFN-based antiviral therapy. Several other polymorphisms have been investigated to ascertain their possible impact on the natural history and on the response to treatment in patients with CHC, but the data are preliminary and warrant confirmation.

CONCLUSION: Several genetic polymorphisms seem to influence the clinical course and the response to antiviral treatment in patients with CHC, suggesting individualized follow up and treatment strategies.

Key words: Single nucleotide polymorphism; Hepatitis

C virus infection; Interleukin 28B; Inosine triphosphate pyrophosphatase; Patatin-like phospholipase domain-containing protein 3

Core tip: Some single nucleotide polymorphisms have been associated with the clinical presentation and/or response to antiviral treatment in subjects with chronic hepatitis C (CHC). In this review article the effect of old and new host genetics factors [interleukin 28B, inosine triphosphate pyrophosphatase, patatin-like phospholipase domain, cannabinoid receptor type 2 (CB2-63), vitamin D associated polymorphisms, *etc*.] on the outcome of CHC and the response to antiviral treatment will be presented, analyzed and discussed, to provide some guidance for individualized therapies in clinical practice.

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INTRODUCTION

The World Health Organization estimates that 130-170 million people are infected with hepatitis C virus (HCV) worldwide and that more than 350000 people die of HCV-related liver diseases each year[1]. In addition, HCV chronic infection is recognized as the most common cause of end-stage liver diseases in Western countries[2]. Primary infection causes asymptomatic acute hepatitis C (AHC) in most cases, which, however, progresses to chronicity in about two thirds of the cases, whereas about one third clear the virus spontaneously and recover[3-9]. Patients with chronic hepatitis C (CHC) frequently show the increasing severity of liver fibrosis over time, which leads to liver cirrhosis in nearly a quarter of cases. Hepatocellular carcinoma (HCC) develops in HCV-related liver cirrhosis with a yearly rate around 3%[10-18].

The combination of pegylated interferon (Peg-IFN) and ribavirin (RBV) has been recommended as the treatment of choice for CHC for nearly a decade[19-24]. This treatment provides a sustained clearance of circulating HCV [sustained viral response (SVR)] in nearly half of the patients with CHC due to HCV genotype 1 and in nearly 70% of those with HCV genotype 2 or 3. Several predictors of a favorable/unfavorable response to treatment have been identified. Some viral factors (HCV genotype 1/4 and an on-treatment slow or absent viral clearance) and host factors (male sex, older age, insulin resistance, diabetes, Afro-American ethnicity, presence of cirrhosis and/or steatosis, and high body mass index) have been associated with a poor response to Peg-IFN plus RBV treatment.

More recently, genome-wide association studies (GWAS) investigated the association between single nucleotide polymorphisms (SNPs) and the clinical presentation, course of the disease and response to antiviral treatment. Several studies have associated polymorphisms in the interleukin 28B (*IL28B*) gene on chromosome 19 (19q13.13) with a spontaneous clearance of HCV in AHC and with the response to Peg-IFN plus RBV treatment in CHC[25-28].

Some investigations demonstrated that inosine triphosphate pyrophosphatase (ITPA) genetic variants protect HCV-genotype-1 patients from RBV-induced anemia[29-31] and some other studies that a polymorphism in the patatin-like phospholipase domain (PNPLA3) is associated with hepatic steatosis[32,33].

The addition of a direct-acting antiviral (DAA) NS3 protease inhibitor boceprevir or telaprevir to the traditional combination of Peg-IFN plus RBV has increased the SVR rate in CHC patients with HCV genotype 1% to 70%[34-38], and the replacement of these first generation protease inhibitors with the recently developed second or third generation DAAs to nearly 90%[39-43]. In addition, some IFN-free treatments recently investigated have been shown to eradicate HCV infection in 90% or more of CHC patients. These high rates of HCV eradication should reduce the clinical value of most predictors of response to treatment so far considered, at least in countries where the high cost of second and third generation DAAs will not be an obstacle to their use. Nevertheless, the low toxicity of IFN-free DAA regimens allow the treatment of patients with comorbidities for which IFN-based treatments are contraindicated, those with advanced or decompensated liver cirrhosis and liver transplant recipients, who are all patients who may require individualized treatment possibly based on predictors of a favorable response.

MATERIALS AND METHODS

In this review article we focus on old and new host genetic factors influencing the outcome of CHC and the response to antiviral treatment to offer some guidance for individualized therapy in clinical practice. We conducted an electronic search on the PubMed and MEDLINE (2000-2014) databases and Cochrane library (2000-2014). A total of 73 articles were retrieved and their data were extensively evaluated and discussed by the authors and then analyzed in this review article.

RESULTS

Host IL28B and HCV infection

Several studies performed in the last 4-5 years have demonstrated that two SNPs, the rs12979860C/T and rs8099917T/G, located in the *IL28B* gene in chromosome 19 have a substantial impact on the clinical course of HCV-related liver diseases and on the response

to Peg-IFN-based treatment in CHC. The *IL28B* gene region encodes for an endogenous antiviral cytokine interferon-λ3 involved in both the early stage of the host innate immune response to HCV infection[25,44,45] and, by binding to a cellular class Ⅱ cytokine receptor complex, in the activation of interferon stimulating genes through the JAK-STAT pathway[44,45]. Thus, there is an immunological and virological explanation for the protective effect of the rs12979860CC[25] and rs8099917TT[28] genotypes on the natural course of primary HCV infection and on the response to IFN-based treatment[26-28,46].

IL28B and AHC

AHC has an asymptomatic course in 50%-90% of cases[3], but primary HCV infection becomes chronic in two-third of the cases, more frequently in men[47] and in asymptomatic cases[48]. Viral factors (genotype, subtypes and quasispecies)[49-53] and host factors (route of transmission, presence or absence of symptoms, initial immune response) have been described as playing a role in the natural history of the illness[25,28,46,54-57]. More recently, the rs12979860CC and rs8099917TT SNPs have been described as independently associated with a spontaneous clearance of HCV[25,28,58,59]. A recent meta-analysis of 8 studies on 2460 patients with HCV chronic infection and 1052 with a spontaneous HCV clearance, 7 studies investigating rs12979860 and 3 rs8099917, confirmed that, at least in Caucasian populations, rs12979860CC and rs8099917TT favor a spontaneous HCV clearance[60].

Early short-term IFN treatment prevents the progression to CHC in the majority of cases, whereas the results are less encouraging when treatment is started 6 mo or more after the onset of AHC[61-63]. Two controlled randomized studies recently published showed a frequent favorable response to a short Peg-IFN treatment starting three months after the onset of AHC[64,65]. Although both rs12979860CC and rs8099917TT have not been associated with a treatment-induced HCV clearance in AHC[58], some authors have suggested that treatment should be started immediately for patients with a non-CC genotype, while it can be delayed for those with the CC genotype, since these subjects may clear HCV spontaneously[59,66].

IL28B and the natural course of CHC

The influence of IL28B polymorphisms on the progression of CHC remains unclear. The rs8099917TT genotype was found to be associated with more severe liver neuroinflammation and fibrosis in a study from Japan[67], whereas it was not found to be associated with the more severe stages of liver fibrosis in an Italian prospective study[68]. In addition, an association of IL28B polymorphisms with the development of HCC in cirrhotic patients described in an Italian study[69] was not confirmed in a study from Japan showing similar prevalences of rs8099917TT genotype in 69 patients with HCC and in 442 without[70]. Further studies are needed to afford further clarification.

IL28B and response to antiviral treatment in CHC

The combination of Peg-IFN-alfa-2a or -2b and RBV has been used for nearly a decade to treat patients with CHC. More recently, the first generation DAA NS3 protease inhibitors, boceprevir or telaprevir, have been used in combination with Peg-IFN and RBV to treat patients with HCV genotype 1[34,71-73]. Second and third generation DAAs against HCV have been recently developed[74-76] and interferon-free combinations of these drugs are at present available both in the United States and in some European countries[77].

IL28B and Peg-IFN plus ribavirin treatment in patients with HCV-genotype 1: The IL28B genotype SNPs rs12979860 and rs8099917 are reliable predictors of the course of the disease after Peg-IFN and RBV treatment in CHC patients with HCV genotype 1[26-28,46]. In fact, the rs12979860-CC or rs8099917-TT genotypes are detectable in the majority of patients with a favorable response to treatment and patients with these genotypes have a twofold likelihood of achieving SVR[78-81]. These genotypes are more frequent in Asian (73%) than in Caucasian (41%), Hispanic (25%) and African American populations (13%)[26-28,46,79,80]. This different distribution strongly contributes to the racial differences in the response to therapy[79], since the favorable effects of rs12979860-CC and rs8099917-TT are similar for all ethnic groups.

IL28B and Peg-IFN plus ribavirin treatment in patients with non-1 HCV genotypes: The association between the IL28B polymorphisms and the response to Peg-INF plus RBV treatment in patients with HCV-genotype 2 and 3 has been investigated by few authors. In a recent study, Eslam *et al*[82] confirmed that rs12979860 CC and rs8099917 TT are independent predictors of SVR also in patients with HCV-genotype 2 or 3. In a study by Sarrazin *et al*[83] the rs12979860 CC genotype, HCV genotype 2 and a young age were found to be significantly associated with SVR in HCV genotype 2/3-infected patients, whereas rs8099917 and rs12980275 were not found to be associated. In addition, the achievement of SVR in patients with RVR was associated with the rs12979860 CC genotype, while no association was found for non-RVR subjects. In a recent study on the impact of SNP rs8099917 and of the amino acid substitutions in the NS5A region on the response to Peg-IFN plus RBV treatment in 286 CHC patients with HCV-genotype 2, SVR was achieved with similar rates in patients with rs8099917TT (76%) and those with TG or GG alleles (72%), whereas it was significantly less frequent in patients with the wild-type IFN sensitivity-determining region (ISDR) than in those with the mutant type (65.9% *vs* 83.5%). On multivariate analysis the only factors related to SVR were a younger age of patients and the ISDR, indicating that in patients with HCV genotype 2, the ISDR sequence variations are significantly associated with the response to Peg-IFN plus RBV treatment[84].

The SNPs rs12979860CC and rs8099917TT were found to be strongly associated with SVR in a large number of genotype-3-infected patients recently investigated by Firdaus *et al*[85].

In a retrospective study on 169 patients with genotype 4 treated with Peg-INF and RBV for 48 wk, Boglione *et al*[86] demonstrated that the combination of rs8099917/rs12979860 polymorphisms is useful to identify possible SVR patients, null-responders and relapsers. In fact, these authors achieved an 88.8% SVR in cases with rs8099917/rs12979860 TT/CC or TT/TC genotypes. Moreover, Youssef *et al*[87] underscored that alpha-fetoprotein increased the SVR predictive strength of IL28B rs12979860 CC polymorphism in Egyptian CHC patients with HCV-genotype 4.

IL28B and Peg-IFN plus ribavirin and first generation DAA triple therapy: Triple therapy with Peg-IFN, RBV and a first generation protease inhibitor boceprevir or telaprevir has increased the rates of SVR in HCV-genotype 1 CHC patients to nearly 70%, which reduces the importance of predictors of the response to therapy[71]. Nevertheless, IL28B favorable genotypes may still be useful to identify patients with a greater likelihood of achieving SVR with a first-line, low-cost Peg-IFN and RBV regimen, reserving DAA-based treatment for non-responders and relapsers[72,73], a particularly useful strategy in developing countries.

IL28B and second and third generation DAAs: The introduction of the second and third generation DAAs in IFN-based and IFN-free regimens for CHC patients has strongly reduced the need to determine the IL28B genotypes to predict the response to treatment. In fact, a favorable response was obtained in nearly 90% of patients with HCV-genotype 1 treated with sofosbuvir plus Peg-INF and RBV, this rate being slightly lower in patients with cirrhosis. In CHC patients with HCV-genotype 2, the combination of sofosbuvir and RBV given for 12 wk also resulted in SVR of 90% or more, with a slightly lower efficacy in patients with cirrhosis[76]. Other studies showed an SVR rate of nearly 95% in CHC patients treated with IFN-free regimens[74,75], independently of the IL28B status[74,75]. Guedj *et al*[88] found no effect of IL28B on the viral kinetics in HCV-genotype-1 CHC patients treated with sofosbuvir and GS-0938 given alone and in combination for 14 d.

The combination of daclatasvir plus sofosbuvir, with or without RBV, obtained SVR in 98% of both therapy-naïve or - experienced CHC patients with HCV-genotype 1a or 1b (98% and 100%, respectively), with IL28B CC or non-CC (93% and 98%, respectively) and with RBV included or excluded from combination therapy (94% and 98%, respectively)[77]. The data from the above-mentioned studies strongly indicate that we cannot evaluate the influence of *IL28B* genotypes on the response to second or third generation DAA treatments of CHC, due to the high efficacy of these treatments.

IL28B and HCV recurrence after liver transplantation

Some investigations showed an association between IL28B polymorphism and response to therapy in patients with a recurrence of HCV infection after liver transplantation. In particular, the highest SVR rates were observed when both donor and recipient showed the same rs12979860CC or rs8099917TT genotypes[89,90]. It has also been reported that the recipients with rs12979860TT genotype showed a more severe histological HCV recurrence after liver transplantation[89].

PNPLA3 polymorphism and HCV infection

The *PNPLA3* gene encodes a 481 amino acid protein called adiponutrin, which belongs to the patatin-like phospholipase family. Its progenitor, patatin, was first described in potato tubers and has non-specific lipid acyl-hydrolase activity[91]. The adiponutrin has a molecular mass of 53 kDa and is mainly expressed in both human adipocytes and hepatocytes[92]. The protein presents a sequence similar to that of adipose tissue triglyceride lipase, and has both triglyceride lipase and transcylase activity.

In 2008 two GWAS[32,33] showed a correlation between the rs738409 polymorphism of PNPLA3 and non-alcoholic fatty liver disease. In fact, a C to G mutation causes the substitution of isoleucin at codon 148 with a methionine, whose hydrophobic side-chain inhibits the binding of the substrate to the catalytic site, leading to a reduction in the enzymatic activity of the protein towards glycerolipids. Consequently, triglycerides accumulate, resulting in the development of macrovesicular steatosis.

Hepatic steatosis, frequent in patients with CHC and with the highest rates in those with genotype 3[93], has been associated with a more rapid progression of liver fibrosis[94] and a poor response to IFN-based treatments[95]. Due to these associations several authors investigated the impact of the rs738409 polymorphism of PNPLA3 on the clinical presentation and natural history of CHC (Table 1). The I148M mutation was found to be associated with the degree of steatosis and with the development of cirrhosis in two independent cross-sectional studies investigating, respectively, 537 and 819 patients with CHC[96,97]. These data were confirmed by the Swiss Hepatitis C Cohort Study Group on 626 patients with CHC for all HCV genotypes except genotype 3[98]. Zampino *et al*[99] found a stronger correlation between waist circumference and liver steatosis in homozygous 148M Italian CHC patients carrying non-3 HCV genotypes, but not with carotid atherosclerosis[100]. In addition, the association between another PNPLA3 polymorphism, the rs2896019, and the presence of any degree of steatosis, even severe, was demonstrated in a cross-sectional investigation[101] on 972 patients. Instead, Nakamura *et al*[102] did not find any association between the I148M mutation and the presence of steatosis or cirrhosis development in 260 Japanese patients with CHC; in this study, however, liver

Table 1 Studies on the role of the rs738409PNPLA3 polymorphisms in hepatitis C virus chronic infection

Ref.	No. of patients	Country	Type of study	Liver disease	Outcome (GG *vs* GC + CC)				
					Steatosis	Severe steatosis	Cirrhosis	SVR	HCC
Cai *et al*[98]	626	Switzerland	Cross-sectional	Chronic liver disease	OR = 1.880 (95%CI: 1.571-2.250)[1]	OR = 1.578 (95%CI: 1.331-1.870)[1,2]			
Clark *et al*[101]	972	United States	Cross-sectional	Chronic liver disease	OR = 1.62 (95%CI: 1.22-2.14)[3]	OR = 1.78 (95%CI: 1.40-2.27)[3,4]		No association (P = 0.294)[3]	
Dunn *et al*[103]	101	United States	Cohort	Liver transplantation recipients and donors			HR = 2.53, (95%CI: 1.28-5.02)[5,6]		
Guyot *et al*[105]	253	France	Cohort	Cirrhosis				No association (P = 0.5)[7]	No association (P = 0.5)
Nakamura *et al*[102]	260	Japan	Cross-sectional	37 Cirrhosis 223 Chronic hepatitis	No association (P = 0.935)[8]		No association (P = 0.876)[8]		
Nischalke *et al*[104]	162	Germany	Case-control	Cirrhosis					No association (P = 0.386)
Trépo *et al*[96]	537	Belgium, Germany, France	Cross-sectional	Chronic liver disease		OR = 2.84 (95%CI: 1.22-6.60)[2]	OR = 2.43 (95%CI: 1.24-4.78)[9]		
Valenti *et al*[97]	819	Italy	Cross-sectional/ case-control	548 Chronic hepatitis 215 Cirrhosis 56 HCC	OR = 1.90 (95%CI: 1.39-2.73)	OR = 2.09 (95%CI: 1.62-2.67)[4]	OR = 1.47 (95%CI: 1.15-1.87)	OR = 0.63 (95%CI: 0.44-0.86)[10]	OR = 2.16 (95%CI: 1.33-3.59)[11]
Zampino *et al*[99]	166	Italy	Cross-sectional	Chronic hepatitis	Mean steatosis score GG: 1.94 ± 1.6, CG: 1.25 ± 1.2, CC: 1 ± 1.1, P < 0.05				

[1]GG + GC *vs* CC non-genotype 3; [2]Steatosis > 5%; [3]Rs2896019 GG + GT *vs* TT genotype 1; [4]Steatosis > 32%; [5]GG + GC *vs* CC donors; [6]Occurrence of Ishak staging ⩾ 3, acute cellular rejection, chronic rejection or fibrosing cholestatic hepatitis during a 620-d (IQR 317-975) follow-up; [7]226 patients; [8]GG+GC *vs* CC, United States diagnosis of steatosis and cirrhosis; [9]F3 or F4; [10]470 patients; [11]325 patients. SVR: Sustained viral response; HCC: Hepatocellular carcinoma.

steatosis was detected only by ultrasound. Interestingly, Dunn *et al*[103] found the I148M mutation to be independently associated with fibrosis progression and graft loss in a prospective study on 101 CHC patients who underwent liver transplantation.

An independent association between the I148M mutation and a poor response to IFN-based therapy was described by Valenti *et al*[97] in 470 patients with CHC; in the same paper these Authors described an association between this SNP and the development of hepatocellular carcinoma. An association between the I148M mutation and HCC development was found in a case-control study on 160 German patients with alcohol-related cirrhosis, but not in a group of 162 patients with HCV-related end-stage liver disease[104]. While confirming this association in alcoholic liver disease, Guyot *et al*[105] found no association between the rs738409 polymorphism and HCC occurrence or between this SNP and the SVR rate of IFN-based therapy in a prospective study on 253 patients with HCV-related cirrhosis. However, a recent meta-analysis including 2503 European patients with cirrhosis, particularly HCV - and alcohol-related, indicated that rs738409 exerts a marked influence on hepatocarcinogenesis[106].

Concluding on this point, further studies are needed to confirm the association between the I148M mutation and a poor response to IFN-based therapy and to establish the mechanisms relating to the role of PNAPL3 on the development of HCC.

ITPA polymorphisms and HCV infection

Ribavirin has made a strong contribution to the success of old and new combination treatments to eradicate HCV infection[107,108].

This drug, however, induces a dose-related hemolytic anemia that impairs the patients' quality of life and frequently entails a dosage reduction and lowered SVR rates[109-112]. This adverse reaction has been reported as more frequent during the administration of triple combination therapy with Peg-IFN, RBV and telaprevir or boceprevir[108,113].

Erythrocyte hemolysis is considered the main cause of RBV-induced anemia[114]. By reducing adenosine triphosphate (ATP) levels in human erythrocytes, RBV induces a guanosine triphosphate (GTP) depletion followed by the inhibition of the ATP-dependent oxidative metabolism, membrane damage and premature hemolysis of erythrocytes[115,116].

In patients with reduced ITPA activity, however, inosine triphosphate (ITP) accumulates in erythro-

cytes[117-120], replacing the GTP activity and producing adenosine monophosphate[121], thus avoiding the inhibition of the ATP-dependent oxidative metabolism and erythrocyte hemolysis. Therefore, RBV-induced anemia seems primarily to be due to the reduced levels of ATP in erythrocytes consequent to the effect of the drug on GTP[121], and resistance against RBV-related anemia is due to a reduced ITPA activity[115,116].

The genetic bases of these phenomena were first identified in 2010 by Fellay *et al*[29], who in a GWAS found a strong association between the single nucleotide polymorphism rs6051702 and the quantitative hemoglobin reduction at week 4 of Peg-IFN plus RBV treatment. The association was explained by 2 functional variants in the *ITPA* gene (encoding inosinetriphosphatase-ITPase) on chromosome 20: a missense variant in exon 2 (rs1127354, P32T) and a splice-altering single nucleotide polymorphism in intron 2 (rs7270101). The polymorphisms rs1127354 and rs7270101 were found to be associated with a hemoglobin reduction at week 4 of treatment in 304 genotype-1 CHC patients receiving Peg-IFN plus RBV, while the minor alleles of each variant protected against hemoglobin reduction; in particular, a 3g reduction in hemoglobin levels was a rare occurrence in 22 (2%) patients with a reduction in the ITPA activity of less than 30% and in 45% of 212 with normal enzyme activity[30]. These data were confirmed in other investigations both in HCV-genotype-1 patients[31,122-127] and in those with HCV-non-1 genotypes (Table 2)[116,128-132].

There are contrasting opinions on the impact of ITPA polymorphisms in patients treated with telaprevir-based triple therapy since some studies reported an impact of the ITPA polymorphism similar to that observed in patients receiving Peg-IFN plus RBV double therapy (Table 2)[133-135], whereas a recent study did not find ITPA deficiency useful to predict early anemia in patients with advanced fibrosis receiving telaprevir[135]. No information is so far available on the impact of ITPA polymorphisms in patients with CHC treated with a boceprevir-based triple therapy.

Polymorphisms influencing the vitamin D metabolism and HCV infection

Vitamin D is a steroid hormone exerting its primary role in bone mineral homeostasis. The main source of vitamin D comes from the synthesis of its inactive precursor 7-dehydrocholesterol in the skin during an ultraviolet-B radiation-dependent reaction, whereas only small amounts of vitamin D_2 and D_3 come from food. Vitamin D from both sources undergoes 25-hydroxylation by hepatic microsomal enzymes and, through a series of other enzymatic reactions, 1,25-dihydroxy-vitamin D_3 (calcitriol), the active form of vitamin D, is obtained. A vitamin D receptor (VDR) is expressed in several human cells. It binds to its ligand and plays the role of a transcription factor for numerous target genes. Consequently, vitamin D exerts its effect on several tissues.

An anti-inflammatory and anti-fibrotic role of vitamin D in chronic liver diseases has only recently been hypothesized, mostly on the basis of the observation that nearly two thirds of patients with chronic liver disease present low serum levels of vitamin D[136] associated with a high fibrosis score and low response to Peg-IFN-based therapy[137,138]. A case-control study on 110 patients with CHC showed a significant correlation between the CYP27B1-1260 promoter polymorphism rs10877012 and the SVR rate (Table 3)[139]. Falleti *et al*[140] found a significantly higher likelihood of response to antiviral treatment in patients with a higher "vitamin D pathway functional score", a genetic model they constructed considering for each patient the sum of every functional allele associated with the achievement of SVR, including the rs10877012 and another three polymorphisms, the rs7041 and rs4588 of the *GC* gene and the rs10741657 of CYP2R1. These Authors also demonstrated that the achievement of SVR with Peg-IFN plus RBV treatment in CHC patients with difficult-to-treat HCV genotypes is predicted both by the carriage of the GC-globulin WT isoform and by normal levels of serum vitamin D at the baseline[141]. Baur *et al*[142,143] demonstrated a correlation between the carriage of the *VDR* gene bAt (CCA) genotype, comprising three different polymorphisms of the *VDR* gene, and the SVR rate and cirrhosis development. These data were confirmed in another cross-sectional study, which also showed a relationship between another *VDR* gene polymorphism and the likelihood of response to therapy[144].

Concluding on this point, the studies mentioned above do not allow conclusions to be drawn at present, but they certainly suggest that the vitamin D-associated polymorphisms play an important role in the achievement of SVR with Peg-IFN based treatment in CHC patients.

Other polymorphisms and HCV infection

Several other polymorphisms have been investigated to ascertain their possible impact on the clinical presentation and natural history of CHC (Table 4). Huang *et al*[145] proposed a risk score based on 7 different SNPs that were highly predictive of the development of cirrhosis in two retrospective series of 420 and 154 Caucasian patients (a training and validation cohort, respectively). This score was demonstrated to be effective in these series of patients and in subsequent large prospective[146,147] and retrospective[148] studies carried out in patients with mild or moderate chronic hepatitis, human immunodeficiency virus (HIV)-HCV coinfected patients[149] and liver transplant recipients[150].

Interesting data also come from studies investigating the genes regulating the immune system. Yee *et al*[151] showed that patients with chronic hepatitis C carrying the IL-6 rs1800795 G allele have a reduced chance of achieving SVR when treated with Peg-IFN plus RBV. These data are in disagreement with those of a previous study[152], which, however, enrolled only HIV-HCV coinfected patients. This polymorphism was also associated with the higher degrees of liver necroin-

Table 2 Studies on the role of inosine triphosphate pyrophosphatase polymorphisms in hepatitis C virus chronic infection

Ref.	No. of patients	Country	Type of study	SNPs	Liver disease/ HCV genotype	Therapy	Outcome
Thompson *et al*[30]	304	United States	Retrospective	rs1127354 rs7270101	CHC/1	Peg-IFN-α-2a + RBV	Hb reduction > 3 g/dL at week 4 ITPase deficiency (both SNPs): OR = 0.26, 95%CI: 0.15-0.4; $P = 2.7 \times 10^{-7}$
Eskesen *et al*[128]	457	Norway	Retrospective	rs1127354 rs7270101	CHC/2/3	Peg-IFN-α-2b + RBV	Patients with any degree of reduced ITPAase activity were less likely to have their RBV dose reduced: OR = 0.39, 95%CI: 0.16-0.96, $P = 0.040$
Seto *et al*[129]	60	Hong Kong	Prospective	rs1127354	CHC/6	Peg-IFN + RBV	ITPA rs1127354 CA *vs* CC genotype: lesser degree of anemia throughout therapy $P < 0.05$ for all time points
Hai *et al*[122]	66	Japan	Retrospective	rs1127354	CHC/1	Peg-IFN + RBV	At multiple regression analysis, age < 60 yr, ITPA CA/AA genotype and serum RBV concentration were significant independent predictive factors for SVR
Thompson *et al*[116]	238	United States	Retrospective	rs1127354 rs7270101	CHC/2/3	PegIFN-α-2b + RBV	Hb reduction at week 4 ITPase deficiency (both SNPs): $P = 10(-11)$ There was no association between the ITPA variants and SVR
Ahmed *et al*[123]	102	Egypt	Prospective	rs1127354	CHC 1/4	Peg-IFN + RBV	CC patients had more frequently Hb decline > 3 g/dL than non-CC patients at weeks 8 and 12 (P = 0.024 and 0.038, respectively) Reduction of the amount of the planned RBV dose was significantly higher for CC patients than non-CC patients during the first 12 wk (18% ± 12.1% *vs* 8.5% ± 10.2%, $P = 0.021$)
Azakami *et al*[124]	830	Japan	Retrospective	rs1127354	CHC/1	Peg-IFN + RBV	Cumulative reduction of ribavirin was significantly more frequent in genotype CC patients than non-CC patients (OR = 1.928, $P = 8.6 \times 10^{-8}$)
Kurosaki *et al*[125]	446	Japan	Prospective	rs1127354	CHC/1	Peg-IFN + RBV	ITPA AA/CA had the lowest incidence of anemia (17%)
Matsuura *et al*[126]	309	Japan	Retrospective	rs1127354	CHC/1	Peg-IFN + RBV	The incidence of severe anemia, ⩾ 3 g/dL reduction or < 10 g/dL of Hb up to week 12 was more frequent in patients with CC (65% and 33%) than in those with CA/AA (25%, 6%); $P < 0.0001$)
Rau *et al*[130]	216	Switzerland	Retrospective	rs1127354 rs7270101	CHC Mixed genotype	Peg-IFN + RBV	ITPA SNP rs1127354 was associated with Hb drop ⩾ 3 g/dL during treatment (RR = 2.1, 95%CI: 1.3-3.5)
Clark *et al*[131]	193	Australia	Retrospective	rs1127354 rs7270101	CHC Mixed genotype	Peg-IFN + RBV	More severe ITPA deficiency was associated with a lesser reduction in Hb level ($P < 0.001$), lesser ribavirin dose reduction ($P = 0.005$), lesser EPO use ($P = 0.029$) ITPA deficiency was associated with SVR ($P = 0.041$)
Rembeck *et al*[132]	354	Sweden	Prospective	rs1127354 rs7270101	CHC/2/3	Peg-IFN + RBV	Reduced ITPase activity was associated with a decreased risk of anemia ($P < 0.0001$), increased risk of thrombocytopenia ($P = 0.007$), and lower ribavirin concentrations ($P = 0.02$)
D'Avolio *et al*[127]	167	Italy	Retrospective	rs1127354 rs7270101	CHC/1	Peg-IFN + RBV	Both SNPs were associated with Hb decrease. The carrier of at least one variant in the ITPA was associated with a lower decrease of Hb (-1.1 g/dL), compared to patients without (-2.75 g/dL; P = 4.09 × 10)
Suzuki *et al*[133]	61	Japan	Retrospective cohort study	rs1127354	CHC/1	Peg-IFN + RBV + telaprevir	Decreases in Hb levels were greater in patients with CC than CA/AA genotypes at week 2 (-1.63 ± 0.92 g/dL *vs* -0.48 ± 0.75 g/dL, $P = 0.001$), week 4 (-3.5 ± 1.1 *vs* -2.2 ± 0.96, $P = 0.001$) and at the end of treatment (-2.9 ± 1.1 *vs* -2.0 ± 0.86, $P = 0.013$)
Ogawa *et al*[134]	292	Japan	Prospective, multicenter study	rs1127354	CHC/1	Peg-IFN + RBV + telaprevir	Pretreatment predictors of the development of severe anemia: baseline Hb < 135 g/L (HR = 2.53; $P = 0.0013$), estimated glomerular filtration rate < 80 mL/min per 1.73 m^2 (HR = 1.83; $P = 0.0265$), ITPA CC genotype (rs1127354) (HR = 2.91; $P = 0.0024$)
Aghemo *et al*[135]	69	Italy	Retrospective cohort study	rs1127354 rs7270101	CHC/1	Peg-IFN + RBV + telaprevir	During the first 12 wk of TPV triple therapy: grade 3-4 anemia developed in 81% non-ITPA deficient patients *vs* 67% mildly deficient and 55% moderately deficient patients (P = NS); RBV dose reduction in 60% with no deficiency, 58% with mild, 67% with moderate deficiency (P = NS); Erythropoietin use in 65% with no deficiency, 58% with mild, 56% with moderate (P = NS); need for blood transfusion in 27% with no deficiency, 17% with mild, 33% with moderate (P = NS)

CHC: Chronic hepatitis C; Hb: Hemoglobin; RBV: Ribavirin; Peg-IFN: Peg-Interferon; SNP: Single nucleotide polymorphism; NS: Not significant.

Table 3 Studies on the role of the polymorphisms influencing the vitamin D metabolism in hepatitis C virus chronic infection

Ref.	No. of patients	Country	Type of study	Liver disease	Polymorphism	Outcome		
						Cirrhosis	SVR	HCC
Baur *et al*[142]	155	Switzerland	Cross-sectional	Chronic hepatitis	rs7975232		OR = 2.67 (95%CI: 1.24-5.70)[1,4]	
					rs731236		OR = 6.05 (95%CI: 1.71-21.43)[2,4]	
					rs1544410		No association (P = 0.085)	
					CC/CC/AA[3]		OR = 2.50 (95%CI: 1.07-5.87)[4]	
Baur *et al*[143]	223	Switzerland	Cross-sectional	185 chronic hepatitis	rs7975232	2.67 (95%CI: 1.29-5.51)[1]		
				38 cirrhosis	rs731236	No association		
					rs1544410	No association		
					CC/CC/AA[3]	2.54 (95%CI: 1.07-6.01)		
Falleti *et al*[141]	206	Italy	Cross-sectional	Chronic liver disease	rs7041		OR = 0.164 (95%CI: 0.056-0.482)[5]	
					rs4588			
Falleti *et al*[140]	206	Italy	Cross-sectional	Chronic liver disease	rs10741657		1.778 (95%CI: 1.135-2.788)[6]	
					rs7041		No association (P = 0.679)	
					rs4588		No association(P = 0.458)	
					rs10877012		No association (P = 0.422)	
					VDPFA[7]		OR = 2.30 (95%CI: 1.02-5.22)[8]	
García-Martín *et al*[144]	238	Spain	Cross-sectional	169 chronic hepatitis	rs2228570		0.438 (95%CI: 0.204-0.882)[4,9]	
				33 cirrhosis	CC/CC/AA3		2.743 (95%CI: 1.313-5.731)[4]	
				36 not assessed				
Lange *et al*[139]	110	Germany	Case-control	Chronic liver disease	rs10877012		10/13 AA *vs* 27/41 AC and 24/56 CC (P < 0.05)	
Lange *et al*[162]	5604	Germany, Switzerland, Japan	Case-control/ retrospective cohort	1279 HCC	rs2282679			OR = 1.56 (95%CI: 1.12-2.15)[10]
				4325 chronic liver disease	rs7944926			OR = 1.56 (95%CI: 1.13-1.78)[11]
					rs1993116			No association (P = 0.07)[12]
								HR = 1.81 (95%CI: 1.03-3.13)[13]

[1]CC *vs* CA + AA; [2]AA + AG *vs* GG; [3]CCA haplotype comprises rs1544410 (BsmI) C, rs7975232 (ApaI) C and rs731236 (TaqI) A alleles of *VDR* gene; [4]OR for non-SVR; [5]OR for non-SVR in WT *vs* non-WT; WT were patients carrying ⩾ 3 major alleles (GG/CC, GT/CC and GG/CA); [6]GG + GC *vs* CC; [7]VDPFA (Vitamin D Pathway Functional Alleles) was constructed giving a value of 1 to the functional allele of each gene and a value of 0 to the other alleles. Thus, for each patient a VDPFA value ranging from 0 to 8 was obtained; [8]VPDFA > 5 *vs* ⩽ 5; [9]TT + TC *vs* CC; [10]TT + TG *vs* GG, 2534 patients; [11]TT *vs* TC + CC, 2420 patients; [12]5591 patients; [13]GG *vs* GA + AA, 1657 patients. SVR: Sustained viral response; HCC: Hepatocellular carcinoma.

flammation[153] and fibrosis[154]. In addition, spontaneous and treatment-induced HCV viral clearance have been found to be associated with the rs2069707 G allele of the *IFN-γ* gene[155] and with KIR2DL3 and HLAC1 haplotypes[156,157].

More recently, an association between the polymorphism at codon 63 of the cannabinoid receptor 2 gene (*CB2*) and HCV infection was suggested[158,159]. This polymorphism leads to the substitution of glutamine, Gln (Q), with arginine, Arg (R), causing a different polarization state of the protein. The CB2 variants have been demonstrated to affect differently the ability of the CB2 receptor to exert its inhibitory function[160]. Specifically, *in-vitro* T lymphocytes from CB2-63 RR homozygotes showed an approximately two-fold reduction in the endocannabinoid-induced inhibition of proliferation compared to cells from CB2-63 QQ homozygotes[161]. In a cohort of 169 biopsy-proven CHC patients, the CB2-63 QQ variant was found to be independently associated with more extensive neuroinflammation[160], whereas in 253 patients with HCV chronic infection this variant was found to be independently associated with a persistently normal aminotransferase status identified by the Authors as the end-stage of the necroinflammatory activity[159]. Further investigations are needed to better define the role of the CB2 variants.

DISCUSSION

Several genetic polymorphisms seem to influence the outcome of CHC and the response to antiviral treatment, which allows individualized strategies to be devised for monitoring the course of the disease and for the choice of treatment. The recent introduction of second and third generation DAAs in Peg-IFN-based and IFN-free treatments have certainly reduced the clinical importance of these predictors, which, however, may still be useful with difficult-to-treat patients and in developing countries

Table 4 Role of other single nucleotide polymorphisms in hepatitis C virus infection

Ref.	Gene	SNPs/haplotypes	Important results
	CRS (7 genes):		
Huang *et al*[145]	*AZIN1*	rs62522600	The Cirrhosis Risk Score was evaluated both in retrospective and prospective studies and appeared to be a useful predictor of fibrosis progression in patients with mild chronic hepatitis C, even in special populations (*i.e.*, liver transplant recipients or HIV-HCV coinfected patients)
Marcolongo *et al*[146]	*TLR4*	rs4986791	
Trépo *et al*[148]	*TRMP5*	rs886277	
Curto *et al*[147]	*AP3S2*	rs2290351	
do O *et al*[150]	*B008027*	rs4290029	
Fernández-Rodríguez *et al*[149]	*AQP2*	rs2878771	
	STXBP5	rs17740066	
Nattermann *et al*[152]	*IL-6*	rs1800795	The CC genotype was associated with lower plasma levels of IL-6 and seemed to correlate with higher SVR rate and lower grading and staging, although the data from the literature are discordant, probably due to the heterogeneity of the study populations (*i.e.*, different virological and clinical characteristics, HIV-coinfection, *etc.*)
Yee *et al*[151]			
Falleti *et al*[153]			
Cussigh *et al*[154]			
Khakoo *et al*[156]	*KIR-HLA*	KIR2DL3/HLAC1	The association between KIR2DL3 and HLAC1 appeared to be related to both a spontaneous and treatment-induced resolution of HCV infection
Knapp *et al*[157]			
Huang *et al*[145]	*IFNγ*	rs2069707	The C764G polymorphism seemed to be associated with a higher SVR rate and a more frequent spontaneous viral clearance
Hellier *et al*[163]	*CCR5*	CCR5Δ32	The CCR5Δ32 deletion, which was associated with resistance to HIV infection, seemed to correlate with lower spontaneous clearance of HCV and milder inflammation and fibrosis, although the data from the literature are discordant
Nattermann *et al*[164]			
Goulding *et al*[165]			
Coppola *et al*[158]	*CNR2*	rs35761398	The CB2-65 QQ genotype was associated with the PNALT status in chronic HCV infection, but also with a higher HAI
Coppola *et al*[159]			

PNALT: Persistently normal alanine-amino-transferase; HAI: Histological activity index; SVR: Sustained viral response; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; IL- 6: Interleukin-6; SNPs: Single nucleotide polymorphisms.

where the cost of the new DAAs is at present a serious obstacle to their use.

COMMENTS

Background

Chronic hepatitis C (CHC) is a life-threatening disease since nearly a quarter of patients progress to liver cirrhosis and nearly 3% of hepatitis C virus (HCV) cirrhotic patients per year develop hepatocellular carcinoma.

Research frontiers

Genome-wide association studies have recently shown that some nucleotide polymorphisms may influence the clinical course and the response to antiviral treatment in patients with chronic hepatitis C.

Innovations and breakthroughs

Several studies associated the polymorphisms in the interleukin 28B (*IL28B*) gene on chromosome 19 (19q13.13) with a spontaneous viral clearance in acute hepatitis C and with the response to the pegylated interferon (Peg-IFN)-based treatments in CHC patients. The achievement of sustained virological response in CHC patients treated with Peg-IFN-based antiviral therapy has been also associated with the vitamin D-associated polymorphisms in some preliminary investigations. Other studies demonstrated that inosine triphosphate pyrophosphatase (ITPA) genetic variants protect HCV-genotype-1 CHC patients from ribavirin-induced anemia. Evidence of an association between a polymorphism in the patatin-like phospholipase domain (PNPLA3) with hepatic steatosis in CHC patients has been also given in recent studies. Several other polymorphisms have been investigated to assess their possible impact on the natural history and response to treatment in patients with CHC, but the results are preliminary and further confirmation is needed.

Applications

In this review article the authors focus on old and new host genetic factors influencing the outcome of CHC and the response to antiviral treatment to offer some guidance for individualized follow up and therapy in clinical practice.

Terminology

The *IL28B* gene region encodes for an endogenous antiviral cytokine interferon-λ3 involved in both the early stage of the host innate immune response to HCV infection and, by binding to a cellular class Ⅱ cytokine receptor complex, in the activation of interferon stimulating genes through the JAK-STAT pathway. The *PNPLA3* gene encodes for a 481 amino acid protein called adiponutrin, which belongs to the patatin-like phospholipase family and is mainly expressed in both human adipocytes and hepatocytes. The protein presents a sequence similar to that of adipose tissue triglyceride lipase, and has both triglyceride lipase and transcylase activity. In patients with reduced ITPA activity, inosine triphosphate accumulates in erythrocytes, replacing the GTP activity and producing adenosine monophosphate, thus avoiding the inhibition of the ATP-dependent oxidative metabolism and erythrocyte hemolysis by ribavirin. An anti-inflammatory and anti-fibrotic role of vitamin D in chronic liver diseases has been hypothesized only recently.

Peer-review

Good work, which comprehensive summary of genetic polymorphisms that effect on the CHC.

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Sports hernia and femoroacetabular impingement in athletes: A systematic review

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Abstract

AIM: To investigate the association between sports hernias and femoroacetabular impingement (FAI) in athletes.

METHODS: PubMed, MEDLINE, CINAHL, Embase, Cochrane Controlled Trials Register, and Google Scholar databases were electronically searched for articles relating to sports hernia, athletic pubalgia, groin pain, long-standing adductor-related groin pain, Gilmore groin, adductor pain syndrome, and FAI. The initial search identified 196 studies, of which only articles reporting on the association of sports hernia and FAI or laparoscopic treatment of sports hernia were selected for systematic review. Finally, 24 studies were reviewed to evaluate the prevalence of FAI in cases of sports hernia and examine treatment outcomes and evidence for a common underlying pathogenic mechanism.

RESULTS: FAI has been reported in as few as 12% to as high as 94% of patients with sports hernias, athletic pubalgia or adductor-related groin pain. Cam-type impingement is proposed to lead to increased symphyseal motion with overload on the surrounding extra-articular structures and muscle, which can result in the development of sports hernia and athletic pubalgia. Laparoscopic repair of sports hernias, *via* either the transabdominal preperitoneal or extraperitoneal approach, has a high success rate and earlier recovery of full sports activity compared to open surgery or conservative treatment. For patients with FAI and sports hernia, the surgical management of both pathologies is more effective than sports pubalgia treatment or hip arthroscopy alone (89% *vs* 33% of cases). As sports hernias and FAI are typically treated by general and orthopedic surgeons, respectively, a multidisciplinary approach for diagnosis and treatment is recommended for optimal treatment of patients with these injuries.

CONCLUSION: The restriction in range of motion due to FAI likely contributes to sports hernias; therefore, surgical treatment of both pathologies represents an optimal therapy.

Key words: Athletic pubalgia; Groin pain; Laparoscopic treatment; Femoroacetabular impingement; Sports hernia

Core tip: Sports hernia and femoroacetabular impingement (FAI) are two pathologies frequently reported in athletes, which are independently treated by general surgeons and orthopedists, respectively. An association between these two entities has recently been recognized, and this review was conducted to define the prevalence of FAI in patients with sports hernia and evaluate the proposed pathogenic mechanism connecting them. Although the range of terms used to describe groin pain throughout the literature is varied, there is a high prevalence of FAI with sports hernias, for which the treatment of both pathologies is optimal.

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INTRODUCTION

Groin injuries are a common occurrence in elite-level athletes, representing up to 6% of all athletic injuries[1-5]. These injuries occur as a result of running, kicking, cutting movements, or explosive turns and changes in direction, and thus impact 10%-28% of male soccer players[6-8]. One type of groin injury, called sports hernia, has been reported in 50% of athletes with groin pain lasting longer than 8 wk[9]. The most common operative finding in sports hernia is deficiency of the posterior wall of the inguinal canal[10-12], which is a consequence of underlying conjoint tendon dysfunction. Thus, demonstration of a dynamic bulge of the posterior wall on abdominal straining is a criterion for diagnosis of sports hernia. Real-time ultrasound for dynamic evaluation of the inguinal canal is currently the preferred diagnostic technique and provides good assessment of the conjoint tendon[13-15]. A bulging inguinal wall can also be imaged by dynamic computed tomography[16], though magnetic resonance imaging is more useful for detecting musculotendinous disease, osteitis pubis, and hip pathologies.

Risk factors for sports hernia include muscle imbalance[17,18] and significant limb length discrepancy (> 5 mm)[19]. In addition, labral tears, as well as decreased external and internal rotation of the hip joint may be related[20], which have been detected in pre-season soccer players[21-24], Gaelic football athletes[25], professional Australian Football League players[24], and athletes with chronic groin injury or osteitis pubis[26]. Garvey *et al*[13] found that half of the cases of sports hernia were associated with other conditions, including rectus abdominis wasting, osteitis pubis, conjoint tendinopathy, osteoarthritis, and neuralgia. Indeed, multiple co-existing pathologies have been reported in 27%-95% of cases of sports hernia[9,27-29], including adductor tendinosis[30] and adductor pain associated with either lower abdominal or inguinal pain[13]. Feeley *et al*[31] described the sports hip triad (labral tear, adductor strain, and rectus strain) as a common injury pattern in the elite athlete.

Femoroacetabular impingement (FAI) is a hip pathology where the bones of the hip are abnormally shaped. FAI reportedly occurs in a very high percentage of elite-level athletes examined for chronic groin and/or hip pain[32-34]. This suggests that the presence of FAI may be a predisposing factor for developing groin-related sports injuries or indicate a common underlying pathogenic mechanism. To more comprehensively investigate this, a systematic review was performed. Specifically, the prevalence of the concomitant presentation of FAI and sports hernias and their treatments were assessed.

MATERIALS AND METHODS

PubMed, MEDLINE, CINAHL, Embase, Cochrane Controlled Trials Register, and Google Scholar databases were electronically searched using the following search terms: sports hernia, athletic pubalgia, groin pain, long-standing adductor-related groin pain, Gilmore groin, adductor pain syndrome, and FAI. After reading the titles and abstracts, a total of 196 studies, published before August 2014, were identified as potentially relevant. No formal exclusion criteria were applied but, from these studies, only articles concerning the association of sports hernia and FAI or laparoscopic treatment of sports hernia were selected. Finally, 24 studies were included in this review. The terms athletic pubalgia, long-standing adductor-related groin pain, adductor strain, and adductor pain syndrome were considered synonymous with sports hernia because conjoint and adductor tendinopathies and nerve entrapment are frequently associated with sports hernias and similarly result from pelvic instability; this pattern is frequently identified as "groin disruption injury"[14].

RESULTS

Prevalence

The results of the systematic review demonstrate that the prevalence of FAI and the associated restricted internal rotation varies widely among cases of athletic-related groin pain (Table 1)[13,32,34-38]. Whereas Meyers *et al*[35] found an overlap of athletic pubalgia and hip

Table 1 Prevalence of femoroacetabular impingement in patients with sports hernia and groin disruption

Ref.	Primary pathology	Prevalence (%)
Garvey *et al*[13]	Sports hernia	12[1]
Meyers *et al*[35]	Athletic pubalgia	27
Schilders *et al*[36]	Chronic adductor-related groin pain	34.1
Weir *et al*[32]	Long-standing adductor-related groin pain	94.1
Nepple *et al*[34]	Groin strain, sports hernia, hip flexor or hamstring strain	94.3
Sansone *et al*[37]	Adductor tenotomy for chronic groin pain	43.8
Economopoulos *et al*[38]	Athletic pubalgia	86

[1]Restricted internal rotation and labral tear.

Table 2 Laparoscopic mesh repair treatment for sports hernias

Ref.	Treatment	Results	Recurrence
Diaco *et al*[42]	Preperitoneal approach (n = 96)	Return to active participation within 3-6 wk in 92/96 patients	-
Edelman *et al*[43]	Preperitoneal approach (n = 10)	Return to full activity within 4 wk in 9/10 patients	None
Ingoldby[44]	Laparoscopic *vs* conventional (n = 14 each)	Laparoscopic treatment superior: return to training in 4 wk (13/14 *vs* 9/14), resume full contact (3 wk *vs* 5 wk; $P < 0.05$)	Conventional: hernia, n = 1 after 22 mo; laparoscopic: pain, n = 1 after 5 mo
Susmallian *et al*[10]	Preperitoneal approach (n = 35)	Return to full activity in 34/35 patients	-
Srinivasan *et al*[45]	Extraperitoneal approach (n = 15)	Return to full activity within 4 wk in 13/15 patients	None
Genitsaris *et al*[46]	Transabdominal preperitoneal approach (n = 131)	100% of patients returned to full activity within 2-3 wk	Pain, n = 4; hernia, n = 1 after 7 yr
Paajanen *et al*[47]	Extraperitoneal approach *vs* conventional treatment (n = 30 each)	Laparoscopic treatment superior: return to full activity within 3 mo (27/30 *vs* 8/30)	-
Paajanen *et al*[48]	Extraperitoneal approach (n = 41)	Return to full activity within 4 wk in 39/41 patients	-
van Veen *et al*[49]	Extraperitoneal approach (n = 55)	100% of patients returned to full activity within 3 mo	-
Kluin *et al*[4]	Preperitoneal approach (n = 17)	Return to full activity within 3 mo in 13/17 patients	Minor symptoms, n = 2 after 1 yr
Ziprin *et al*[50]	Transabdominal preperitoneal approach (n = 17)	Return to full activity in 16/17 patients within a median of 42 d	Mild pain, n = 5

pathology in 27% of hockey players, Larson *et al*[39] reported that 90% of collegiate football players with hip and groin pain participating in a National Football League combine showed radiologic signs of cam- or pincer-type FAI. FAI was also found in patients with adductor tendinopathies, and Sansone *et al*[37] reported that, although most (75%) patients were satisfied with the results of tenotomy, the prevalence of FAI was greater in patients who were not satisfied when assessed after a median follow-up of six years.

Pathophysiology

Verrall *et al*[24] postulated that chronic groin injuries result from increased loading and mechanical stress on the pubis symphysis and surrounding structures caused by reduced hip range of motion. Furthermore, Larson *et al*[39,40] hypothesized that the decreased hip range of motion from an underlying FAI promotes compensatory extra-articular patterns, which subsequently lead to osteitis pubis and athletic pubalgia. Birmingham *et al*[41] analyzed the three-dimensional motion of the pubic symphysis in six fresh-frozen human cadaveric pelvises to compare native and simulated cam lesion hips. They found that rotational motion was significantly increased by cam-type lesions due to the repetitive loading of the symphysis, which is one possible precursor to athletic pubalgia.

Treatment

Laparoscopic mesh repair for the treatment of sports hernias can be performed using either a transabdominal preperitoneal or total extraperitoneal approach. Most studies demonstrate a > 90% success rate with these treatments, with return to full activities within 1-3 mo (Table 2)[4,10,42-50].

Only one case report and one retrospective case series reported on combined treatment of FAI and sports hernia. Matsuda[51] reported a case report regarding an endoscopic pubic symphysectomy in a case with bilateral FAI and recalcitrant osteitis pubis with high patient satisfaction, decreased pain, improved function, and resolution of the classic waddling gait at the 12-mo follow-up. In a retrospective study of 31 patients, Larson *et al*[40] found that surgical management of both pathologies leads to a greater rate of return to full sporting activities compared with sports pubalgia surgery or hip arthroscopy alone after a mean follow-up of 29 mo (89% *vs* 33%). In this study, whether the surgeries were performed concurrently, hip arthroscopy was performed first to avoid excessive stress placed on the pubalgia repair during positioning and traction for arthroscopy. There were no differences for return to sports or

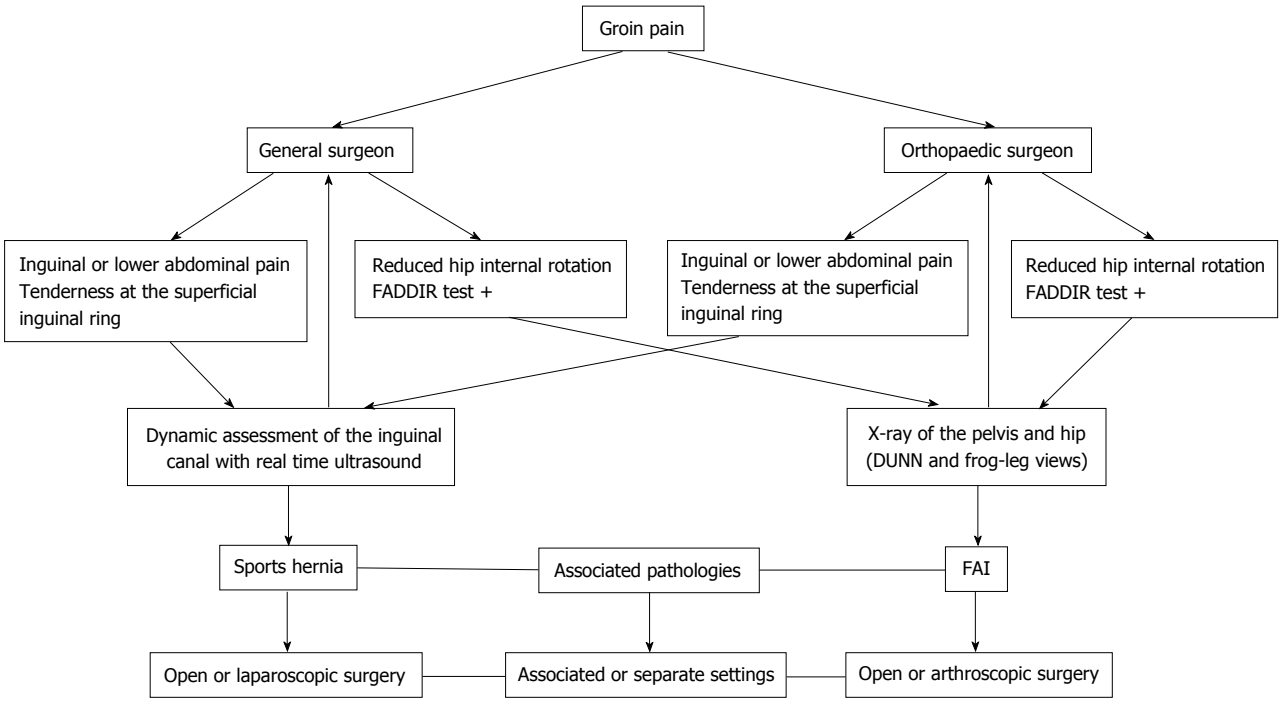

Figure 1 Diagram showing the proposed multidisciplinary approach. FADDIR: Flexion-adduction-internal rotation; FAI: Femoroacetabular impingement.

outcomes scoring for concurrent *vs* separate setting hip arthroscopy and sports hernia surgery. However, hip arthroscopy alone was sufficient to resolve sports pubalgia symptoms in four patients. It was postulated that the improved hip range of motion and function after FAI correction reduced the stress on extra-articular pelvic structures, thus alleviating the symptoms.

The presence of multiple pathologies does not dictate treatment outcome. The importance of treating both pathologies to obtain good and stable results during time is confirmed in a study of Hölmich *et al*[52], which found that active treatment of adductor-related groin pain can be effective even in the presence of morphologic changes to the hip joint. However, clinical outcome decreased over time in patients with angles > 55. A multidisciplinary approach is recommended, such as one involving orthopedic and general surgeons, to detect the presence of both pathologies in order to provide optimal treatment (Figure 1).

DISCUSSION

A wide variety of terms are used throughout the literature to describe groin pain or pubalgia, making it difficult to obtain an accurate assessment of the association between sports hernia and FAI. However, the results of this review indicate that the conditions commonly co-occur, and treatment of the underlying morphologic abnormalities can impact the sports-related symptoms.

The majority of the patients with sports hernias complain of unilateral inguinal pain or lower abdominal pain, often radiating to the pubic tubercle and the inner thigh or across the midline. Although the pain can occur after a specific event, more often the onset is insidious with exacerbation of the symptoms by activity that persists for a day or two and is temporarily relived with rest[14,53,54]. There may be pain and tenderness at the superficial inguinal ring, but without a visible or palpable lump indicative of a classical inguinal hernia. In patients with FAI, the groin pain is accompanied with pain at the greater trochanter, deep posterior buttock, and sacroiliac joint[55]. Whereas 50% of these patients reported an insidious onset, approximately 26% reported acute development of symptoms in the absence of a traumatic event. In the early stage, the pain is exacerbated with prolonged sitting or walking or with athletic activity; however, the pain becomes more constant with the progression of articular damage and osteoarthritic changes.

Upon physical examination, patients with FAI demonstrate pain with combined flexion, adduction, and internal rotation of the hip during the anterior impingement test[55-57], as well as during other tests of joint rotation[58]. These patients typically show restricted internal rotation and/or flexion in the hip range of motion, and restricted abduction or external rotation is also sometimes observed. Diagnosis of FAI is confirmed from radiographic exams showing an increased alpha-angle and/or signs of overcoverage or retroversion of the acetabulum (Figure 2)[56].

The additional stress from restricted rotation can lead to weakening or tearing of the transversalis fascia and surrounding tissues over time, resulting in tendon enthesitis of the adductor longus and/or abdominal muscles and groin pain[1,59,60]. This injury affects the external oblique aponeurosis, which can cause irritation of ilioinguinal or iliohypogastric nerves, as well as the

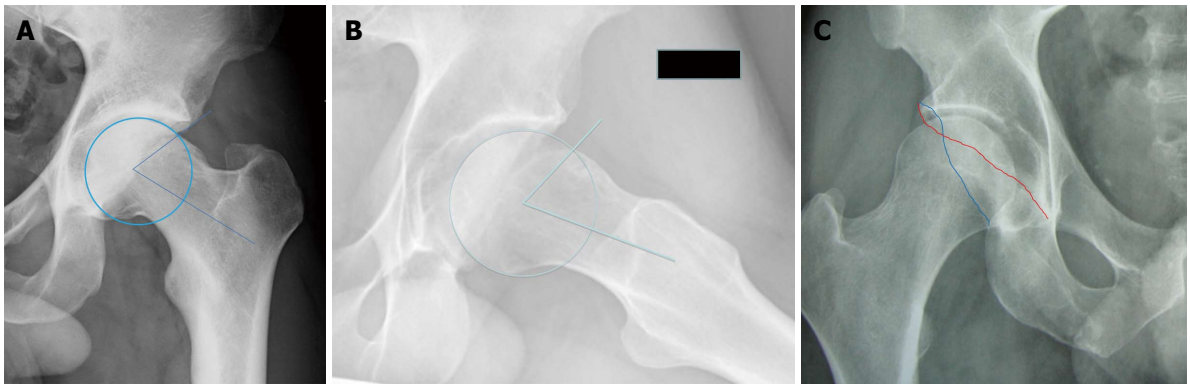

Figure 2 Radiographic findings of femoroacetabular impingement. X-ray scans of a cam-type femoroacetabular impingement (FAI) showing increased alpha-angles with bumps in the A: Superior head-neck junction; B: Anterior head-neck junction (DUNN-view 45°); C: Superior focal acetabular retroversion is observed in pincer-type FAI when the anterior wall (red line) is lateral to the posterior wall (blue line), referred to as the crossover sign.

conjoint tendon and inguinal ligament, resulting in weakness (deficiency) of the lower abdominal wall and occult hernias. Athletes performing rapid acceleration and deceleration movements and repetitive, high-speed twisting and cutting motions are especially vulnerable to these injuries[1,61].

Conservative treatment of sports hernias or FAI can be effective in a variable percentage of patients[62]. However, surgical treatment results in higher success rates, as demonstrated by Paajanen *et al*[48]. Moreover, Polglase *et al*[63] conducted a randomized clinical trial and found that appropriate repair of the posterior wall of the inguinal canal is superior to nonoperative management in athletes, effectively curing 60% of patients and providing improvement to an additional 20%. In a randomized study by Ekstrand *et al*[64], surgical treatment significantly reduced symptoms at six months compared to conservative treatment. For patients with FAI and sports hernias, surgical treatment of both pathologies appears to be the best option[40].

The surgical techniques performed to treat sports hernias are classified into three categories: open sutured repair, open mesh repair, and laparoscopic mesh repair by either transabdominal preperitoneal or extraperitoneal approaches. Sutured repairs are the most commonly performed operations for athletic pubalgia[65-68], with successful return to sport activities in 68%-100% of cases[3,5,30,60,67,69-74] and a recovery time ranging from to four weeks to three months[67,69,71,73,74]. The open anterior mesh repair technique is analogous to the Lichtenstein method of inguinal repair and is designed to reconstruct the posterior inguinal floor in a tension-free fashion. The reported success rate with this method is 77%-100%[75-79], allowing for a return to full activities within 3-4 mo[76,79]. Open or arthroscopic treatment has also been successful for treatment of FAI in athletic patients[80-83]. In addition, Dojčinović *et al*[30] used a shouldice technique and an ilioinguinal nerve neurolysis and resection of the genital branch of the genitofemoral nerve in a patient with untreated FAI.

The results of the present review indicate that newer laparoscopic techniques are as effective or better for successfully treating sports injuries and allowing patients to return to full activities more quickly. Compared to open surgeries, the recovery time was on the order of weeks, rather than months, and with rare incidences of recurrence. The results also suggest that athletes undergoing extraperitoneal repair do well in the postoperative period, even when a macroscopic abnormality is not detected, which is in keeping with the idea that strengthening the posterior wall relieves symptoms. There were few instances of recurrence with these techniques, however, most reports did not include a long-term follow-up.

The wide variety of terms used throughout the literature to describe groin pain and injuries complicates evaluation of the association between sports hernias and FAI. For the purposes of the present review, the terms sports hernia, athletic pubalgia, long-standing adductor-related groin pain, adductor strain, and adductor pain syndrome were considered as synonymous. However, groin pain is a common complaint of athletes with sports hernias and FAI, indicating that they may share an underlying pathogenic mechanism, such as the placement of excessive rotational stress on the pubic symphysis. Thus, surgical repair of both pathologies, likely provides the optimal treatment for elite-level athletes with these injuries.

COMMENTS

Background

Pubalgia and groin pain are common in athletes, and can be caused by sports hernias and femoroacetabular impingement (FAI). In the past, these were considered isolated pathologies and treated by a general or orthopedic surgeon. However, osteoarthritis and reduced hip range of movement have increasingly been observed in athletes with sports hernias, indicating that these pathologies are related.

Research frontiers

Recently, alterations in the pelvic biomechanics due to a FAI have been described. In addition, a case series concerning the combined treatment of sports hernia and FAI was published. However, the association between FAI and sports-related groin injuries is not well defined. Therefore, the objective of this study was to systematically review articles reporting on the incidence, pathophysiology, and treatment of these two pathologies.

Innovations and breakthroughs

Recent studies report a variable association between sports hernia and FAI, ranging from 12% to 94%. To explain the association, it was proposed that increased rotational stress on the symphysis pubis and the surrounding structures from FAI leads to weakness of the posterior inguinal wall, which can result in sports hernia. The optimal treatment of athletes with sports hernias may rely on a multidisciplinary approach, involving repair of both underlying pathologies to ensure a rapid and complete return to sport activities.

Applications

This review highlights the relationship between sports hernias and FAI in athletes with groin pain, and will be useful to promote the knowledge of these pathologies for both orthopedic and general surgeons. A multidisciplinary approach is proposed to optimize the diagnosis and treatment of affected patients.

Terminology

A sports hernia is defined as a dynamic bulge of the posterior wall upon abdominal straining that results from conjoint tendon dysfunction and posterior wall weakness. FAI is a hip pathology where the bones of the hip are abnormally shaped resulting in restricted rang of motion. The asphericity of the femoral head with a bump in the head-neck junction defines the cam-type impingement, whereas a deepened or retroverted acetabulum defines the pincer-type impingement. These bone abnormalities result in repetitive collision between the femoral neck and the acetabular rim, which damages the labrum and surrounding cartilage.

Peer-review

This review describes recent reports concerning FAI in cases of sports hernia. The co-occurrence of the two conditions indicates a common underlying pathology. This article provides a review of studies using laparoscopic techniques to repair sports hernias and indicates that concurrent treatment of FAI is optimal for earlier recovery and return to full activity.

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CASE REPORT

Coumadin ridge: An incidental finding of a left atrial pseudotumor on transthoracic echocardiography

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Abstract

Coumadin ridge is a normal anatomic variant that is occasionally found in the left atrium. It can present as a linear or nodular mass which can undulate with cardiac motion and if particularly prominent, can easily be mistaken for a tumor or thrombus. Careful evaluation and consideration of the common variants discussed in this review can help limit misdiagnosis, as well as unnecessary workup and treatment. We present a case of coumadin ridge that was found on a patient using two-dimensional transthoracic echocardiography.

Key words: Coumadin ridge; Pseudotumor; Warfarin ridge; Left atrial pseudotumor

Core tip: As we improve imaging modalities, normal anatomic variants of the heart are coming to our attention more frequently. Coumadin ridge is an example of such a variant that is occasionally found on various imaging modalities of the heart, including transthoracic echocardiography, transesophageal echocardiography, cardiac magnetic imaging, among others. Coumadin ridge is a term that refers to a part of the left atrium that lies between the left atrial appendage and the left superior pulmonary vein. Since this is a not a common finding, and due to its shape and location, it has the potential of being misdiagnosed as a left atrial myoxma or thrombus.

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INTRODUCTION

As imaging modalities continue to improve in resolution and clarity, several normal anatomic variants of the heart have come to our attention. One of these normal variants is the coumadin ridge, which is found in the left atrium between the left atrial appendage and the left superior pulmonary vein. Although the coumadin ridge is a normal anatomic finding, its shape and location can cause it to be mistaken for a tumor or thrombus, leading to unnecessary, risky, and costly treatments for patients. In this case report, we present a patient in whom a coumadin ridge was found using two-dimensional transthoracic echocardiography with color Doppler.

CASE REPORT

A 58-year-old African American woman with hypertension, morbid obesity, and no remarkable history of heart disease presented to general medicine clinic with complaints of worsening exertional dyspnea with abdominal and lower extremity swelling. She denied chest pain, palpitation, orthopnea, or paroxysmal nocturnal dyspnea. Her medications included aspirin 81 mg, amlodipine 10 mg, hydrochlorothiazide 25 mg, and losartan 50 mg. She weighed 114 kg with a body mass index of 41 kg/m^2. Physical examination revealed a blood pressure of 170/88 mmHg, pulse of 72 bpm, and respiration rate of 18 breaths per minute. Lungs were clear to auscultation. There was no jugular venous distention. Normal heart sounds, no S3 or S4, no murmurs, and no rubs. No hepatomegaly or ascites was present. There was 1+ pitting edema over the dorsum of each foot. An electrocardiography showed sinus rhythm with a heart rate of 63 and left atrial abnormality (Figure 1). Given long-standing history of hypertension, worsening exertional dyspnea and lower extremity edema, a transthoracic echocardiography was performed to evaluate her cardiac anatomy and function, which showed prominent coumadin ridge in the left atrium, mild concentric left ventricular hypertrophy, grade Ⅱ left ventricular diastolic dysfunction with pseudonormalization pattern, normal valves and normal biventricular size and systolic function (Figures 2 and 3). All other workup was negative in assessing the etiology of the patient's edema and worsening exertional dyspnea, including a complete metabolic panel, thyroid stimulating hormone, pulmonary function tests, and urine analysis. The patient's serum albumin level was also within normal limits. The patient's elevated blood pressure was improved by up-titrating her losartan to 100 mg. The patient's edema improved with lifestyle changes such as keeping her feet elevated. Her worsening exertional dyspnea was attributed to her conditioning, and physical therapy was recommended.

DISCUSSION

Over the past few decades, echocardiography has become an increasingly common tool to assess cardiac anatomy and function. As imaging modalities continue to improve in resolution and clarity, we are more likely to come across normal anatomic variants of the heart. Some of these normal variants include the eustachian valve, chiari network, and crista terminalis of the right atrium, lipomatous hypertrophy of the interatrial septum, interatrial aneurysms, pectinate muscles of the right and left atriums, transverse sinus of the pericardium, moderator band of the right ventricle, Lambl's excrescence of the aortic valve, and coumadin ridge of the left atrium[1].

The coumadin ridge is a prominent, muscular ridge of tissue that lies in the left atrium in between the left superior pulmonary vein and the left atrial appendage[2]. It may often appear to be attached to the roof of the left atrial appendage, with a rounded end extending into the left atrium[2]. Due to this rounded end, the coumadin ridge is often referred to as a "Q-tip sign" on echocardiography[2]. It is important to keep in mind that it may not always appear rounded. When viewing it in the parasternal long axis view, it may appear as a linear band within the left atrium (LA)[2]. Also, when several cross sections of heart specimens were looked at along their narrowest point, 75% of the samples were shown to be round, 15% flat, and 10% pointed[3].

In our case, we used two-dimensional transthoracic echocardiography to show the coumadin ridge in its rounded and flattened shape. Due to its rounded edge, the coumadin ridge has often been mistaken for a thrombus, thereby, resulting in unnecessary anticoagulation and hence the name, Coumadin ridge. It may also be mistaken for a myxoma, leading to inappropriate surgeries[3,4]. Correct identification of these variants on cardiac imaging is crucial and will help prevent unnecessary workup and treatment. These inappropriate treatments can have a devastating impact on patients' outcomes, raise healthcare spending, and may often times result in legal repercussions for healthcare providers[4]. Knowledge of its location and features on various imaging modalities is critical in making the correct diagnosis.

Figure 2 shows the patient's coumadin ridge on an apical transthoracic echocardiography view, while Figure 3 shows it on an apical four-chamber transthoracic echocardiography view. In both of these views, the echogenicity of the ridge is similar to the surrounding cardiac tissues, suggesting this is a normal structure of the heart and not a thrombus or myxoma. Its location in the left atrium and its shape are also consistent with other reported cases. In both of the apical transthoracic echocardiography views, the coumadin ridge can be seen as a rounded "Q-tip" shape.

Two-dimensional transthoracic echocardiography can provide adequate clarity of the coumadin ridge. Additionally, fast spin echocardiography techniques with contrast can further help to clarify the diagnosis[5]. If coumadin ridge is seen using transthoracic echocardiography and question still remains about the

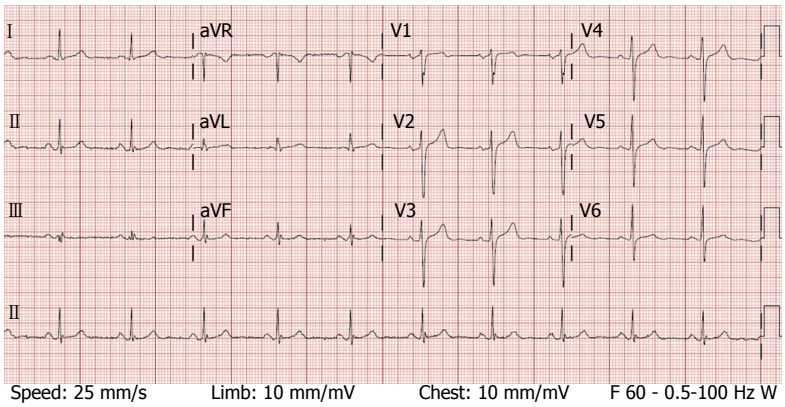

Figure 1 Electrocardiography showed sinus rhythm with a heart rate of 63 and left atrial abnormality.

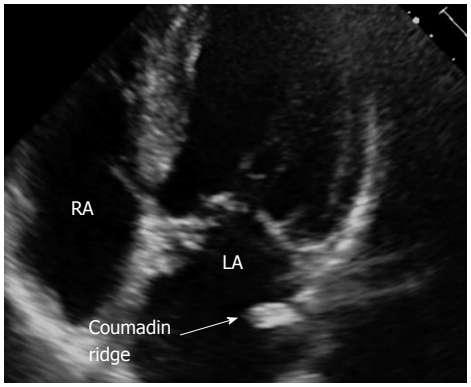

Figure 2 Apical transthoracic echocardiography view of coumadin ridge in the left atrium. RA: Right atrium; LA: Left atrium.

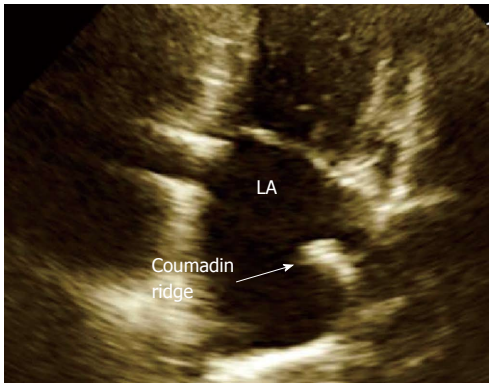

Figure 3 Apical four-chamber transthoracic echocardiography view of coumadin ridge in the left atrium. LA: Left atrium.

diagnosis, full volume three-dimensional transthoracic echocardiography with color Doppler can also provide additional clarity of the surrounding structures[2]. In other cases, transesophageal echocardiography may be needed, as the coumadin ridge is best seen using midesophageal two-chamber view[6]. In cases where it is difficult differentiating coumadin ridge from a thrombus or tumor, cardiac magnetic resonance or computed tomography may help to provide clarity to the diagnosis[4,5]. On both T1 and T2-weighted imaging, the coumadin ridge should have the same signal strength as adjacent cardiac tissue[5]. In addition, late gadolinium enhancement cardiac magnetic resonance may assist in clarifying the diagnosis; the coumadin ridge is unlikely to exhibit late enhancement[5]. Thrombus, however, will typically have low signal-strength surrounded by structures with higher signal strength on late gadolinium enhancement[5].

The usual location of the coumadin ridge is between the left superior pulmonary vein and the left atrial appendage. Along with its location, the lack of mobility and its unique linear structure with a rounded tip help differentiate this normal anatomic variant from other abnormal masses[1,6]. However, if the mass is not imaged correctly, it may appear as a free-floating structure in the left atrium or may even appear to undulate with cardiac motion (LA)[1,5]. In addition, there are a few features that help distinguish intracardiac thrombi from a coumadin ridge. Thrombi will typically have different echogenicity from adjacent tissue. Also, thrombi will have mobility with underlying tissue[1]. Thrombi are often seen in patients with atrial fibrillation, and are much less likely in patients with sinus rhythm[1]. The gold standard in assessing these thrombi is with transesophageal echocardiography using color flow and pulse wave Doppler[1]. Color flow Doppler will help in assessing if there is a low-flow risk for thrombus, which occurs when peak velocity is less than 40 cm/s[1].

Coumadin ridge may also be misinterpreted for a myoxma of the left atrium. Myxoma is the most common benign tumor in adults, and is found in the left atrium 75% of the time[1]. It is typically located on the interatrial septum and has a stalk attached to the fossa ovalis[1]. It has a smooth surface with homogeneous consistency, and can grow very large in size[1]. Midesophageal four-chamber view gives the best indication of the size and location of left atrial myxomas[1].

While there is concern that the coumadin ridge may be misinterpreted as a tumor or thrombus, one should also be careful not to overlook lesions that may be near or attached to the coumadin ridge, as there have been cases of myxomas and fibroelastomas attached to the

tip of the coumadin ridge[7]. These abnormalities can be differentiated on imaging from the coumadin ridge by their different sizes, shapes, echogenic features, and mobility[7]. Although these cases are not common, careful attention needs to be given to rule out other pathology when the diagnosis is not immediately clear. Just like with any study, it is important to evaluate the findings from imaging in context with the patient's clinical evaluation.

As we continue to see more cases of coumadin ridge, we will likely arrive at a better understanding of the implications of this anatomic variant. One such example is its value in patients with atrial fibrillation, where coumadin ridge may serve as a site for catheterization[8]. There are also implications of using the coumadin ridge as a site to stabilize the catheter while ablating adjacent areas in the left atrium[9].

In conclusion, coumadin ridge is a normal variant of the left atrium. We presented a case in which a prominent ridge was found on two-dimensional transthoracic echocardiography. Awareness of its typical location and features on various image modalities are crucial to avoid unnecessary interventions which potentially put patients through unnecessary and risky treatments and surgeries.

COMMENTS

Case characteristics

A 58-year-old African American woman with hypertension, morbid obesity, and no remarkable history of heart disease, presented to general medicine clinic with complaints of worsening exertional dyspnea with abdominal and lower extremity swelling.

Clinical diagnosis

Lungs were clear to auscultation. Normal heart sounds, no S3 or S4, no murmurs, and no rubs. No hepatomegaly or extremity edema.

Imaging diagnosis

Transthoracic echocardiography showed prominent coumadin ridge in the left atrium.

Differential diagnosis

Coumadin ridge, left atrial pseudotumor.

Treatment

Coumadin ridge is a normal variant of the left atrium, which requires no additional intervention.

Related reports

Coumadin ridge may present as a linear or nodular mass, which can undulate with cardiac motion and can easily be mistaken for a tumor or a thrombus.

Experiences and lessons

Awareness of the location and imaging features of coumadin ridge will help prevent misdiagnosis and unnecessary workup.

Peer-review

It is a very interesting, well written paper.

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CASE REPORT

Transhepatic venous approach to permanent pacemaker placement in a patient with limited central venous access

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Author contributions: Siddiqui AM, Harris GS and Movahed A designed the report; Chelu MG, Chiang KS and Nekkanti R performed the procedure.

Institutional review board statement: Please note that case reports consisting of less than three cases are not required to undergo the IRB approval process at our institution (East Carolina University).

Informed consent statement: Please note that case reports consisting of one case are not required to undergo the informed consent statement process at our institution (East Carolina University).

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Abstract

The end-stage renal disease population poses a challenge for obtaining venous access required for life-saving invasive cardiac procedures. In this case report, we describe an adult patient with end-stage renal disease in whom the hepatic vein was the only available access to implant a single-lead permanent cardiac pacemaker. A 63-year-old male with end-stage renal disease on maintenance hemodialysis and permanent atrial fibrillation/atrial flutter presented with symptomatic bradycardia. Imaging studies revealed all traditional central venous access sites to be occluded/non-accessible. With the assistance of vascular interventional radiology, a trans-hepatic venous catheter was placed. This was then used to place a right ventricular pacing lead with close attention to numerous technical aspects. The procedure was completed successfully with placement of a single-lead permanent cardiac pacemaker.

Key words: Trans-hepatic venous access; Permanent cardiac pacemaker; End-stage renal disease

Core tip: End-stage renal disease patients pose a great challenge to establish central venous access. In situations when life-saving cardiac procedures are required in such patients, the clinician must use non-traditional venous access sites to perform these procedures. In our case report, we illustrate the novel

use of the trans-hepatic venous access route to implant a single-lead permanent cardiac pacemaker in a patient with bradycardia and hypotension. Additionally, we describe the technical challenges associated with this procedure.

Siddiqui AM, Harris GS, Movahed A, Chiang KS, Chelu MG, Nekkanti R. Transhepatic venous approach to permanent pacemaker placement in a patient with limited central venous access. *World J Clin Cases* 2015; 3(9): 835-837 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i9/835.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i9.835

INTRODUCTION

The end-stage renal disease population poses a challenge for obtaining venous access required for invasive cardiac procedures due to placement of multiple arteriovenous fistulas for hemodialysis access or venous thrombus formation. The left subclavian vein is the preferred venous access for implantation of a pacing lead, but in patients with limited access alternative approaches include the right subclavian vein[1] and iliac veins[2]. In this case report, we describe an adult patient with end-stage renal disease in whom the hepatic vein was the only available access to implant a single-lead permanent cardiac pacemaker.

CASE REPORT

A 63-year-old African American male with end-stage renal disease on maintenance hemodialysis for 10 years and permanent atrial fibrillation/atrial flutter presented with symptoms of bradycardia including lightheadedness and dizziness. Ventricular rate was between 30 to 40 beats per minute. Hypotension was noted during dialysis sessions with a lack of positive dromotropic response due to atrioventricular node dysfunction. Non-functioning arteriovenous fistulas were present in the left upper and left lower extremities. A right lower extremity arteriovenous fistula was being used for hemodialysis access. Contrast venography of the right chest revealed an occluded right innominate vein (Figure 1A). Vascular interventional radiology placed a 6 French/45 cm Berenstein catheter (Boston Scientific, Inc., Marlborough, MA, United States) *via* the right peripheral hepatic vein terminating in the right atrium (RA). In the cardiac electrophysiology lab this catheter was exchanged over a 0.81 mm wire with placement of a 7 French/25 cm peel away introducer sheath into the RA, Figure 1B. The right ventricular pacing electrode with a soft curved (hockey stick curve) stylet was advanced through the sheath into the right ventricular apex. The stylet was withdrawn and the lead was advanced to provide redundancy in the RA. Sensing and pacing threshold parameters were optimal. The suture sleeve was advanced and 2 sutures were applied around the suture sleeve incorporating the indwelling muscle tissue. A subcutaneous pocket was made at the insertion point of the pacing lead. The procedure was successfully completed with no complications. A final fluoroscopic image is shown in Figure 1C.

DISCUSSION

Our main challenge was to implant a pacing lead in a symptomatic patient with no central venous access in the right and left upper extremities or right and left lower extremities. The trans-hepatic approach has been used in a variety of clinical situations where traditional central access was not possible, such as exhausted hemodialysis options[3]. Current literature states complication rates are < 5% and include line sepsis, catheter migration, thrombosis, and bleeding[4]. However, as the patient population described is so unique, the incidence rate of patients with no central venous access requiring trans-hepatic access is unknown.

One limitation of this approach is the technical aspect of inserting the lead into the right ventricle *via* the inferior vena cava. Additionally, the pacing lead traverses the substance of the liver, and therefore is subject to respirophasic diaphragmatic excursions potentially leading to loss of redundancy and dislodgement. Consequently, it is important to provide more than the usual redundancy in the RA to minimize dislodgement of the lead (Figure 1D).

The trans-hepatic venous approach is feasible for a single-lead permanent pacemaker implantation when all other central venous access options are exhausted.

COMMENTS

Case characteristics

A 63-year-old African American male with a history of end-stage renal disease on renal replacement therapy and permanent atrial fibrillation/flutter.

Clinical diagnosis

Symptomatic bradycardia with hypotension.

Differential diagnosis

Lack of positive dromotropic response due to atrioventricular node dysfunction.

Imaging diagnosis

Contrast venography of the right chest revealed an occluded right innominate vein.

Treatment

A single-lead permanent cardiac pacemaker was placed into the right ventricle.

Related reports

The percentage of patients with end-stage renal disease with limited central venous access requiring trans-hepatic access is unknown.

Experiences and lessons

This case represents demonstrates the challenge of establishing central venous access *via* the trans-hepatic route in a patient with no other access sites. Additionally, the authors highlight the technical challenges associated with undertaking this procedure.

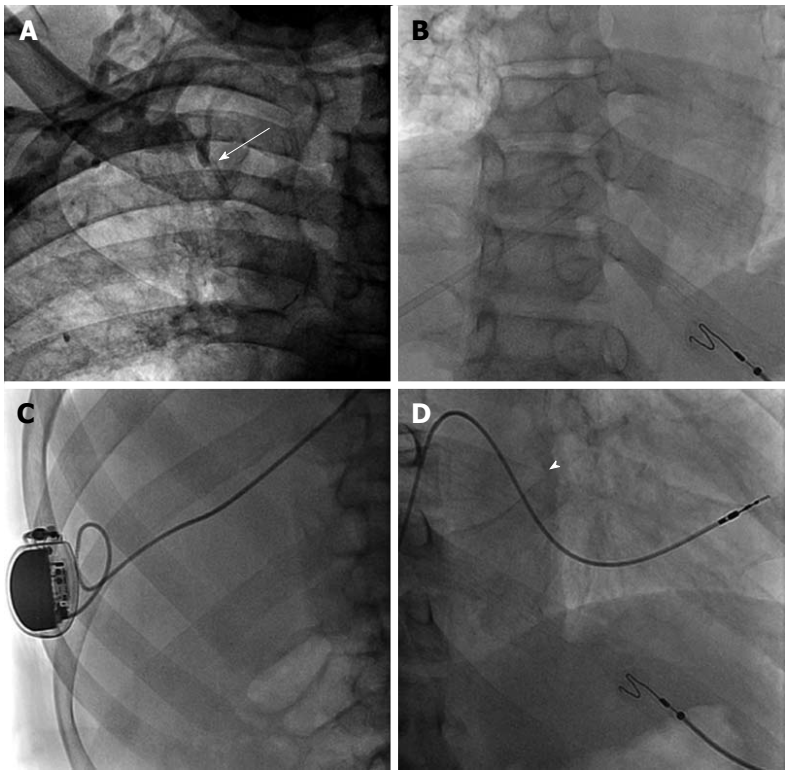

Figure 1 Transhepatic venous approach to single-lead permanent cardiac pacemaker in a 63-year-old male with bradycardia and hypotension. A: Right upper extremity venogram demonstrating total occlusion of the right innominate vein (arrow); B: Transhepatic catheter placement terminating in the right atrium; C: Placement of the pulse generator on the lateral aspect of the thoracic wall with attached right ventricular lead; D: Adequate redundancy provided to the right ventricular lead in the right atrium (arrowhead).

Peer-review

The authors have performed a good study, the manuscript is interesting.

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CASE REPORT

Horseshoe thrombus in a patient with mechanical prosthetic mitral valve: A case report and review of literature

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Author contributions: Mehra S and Marcu CB designed the research and identified the case reported; Mehra S and Espinoza C performed review of literature; Mehra S prepared manuscript; Movahed A and Marcu CB revised manuscript.

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Abstract

Patients with prosthetic cardiac valves are at high risk for thromboembolic complications and need life long anticoagulation with warfarin, which can be associated with variable dose requirements and fluctuating level of systemic anticoagulation and may predispose to thromboembolic and or hemorrhagic complications. Prosthetic cardiac valve thrombosis is associated with high morbidity and mortality. A high index of suspicion is essential for prompt diagnosis. Transthoracic echocardiography, and if required transesophageal echocardiography are the main diagnostic imaging modalities. Medically stable patients can be managed with thrombolytic therapy and anticoagulation, while some patients may require surgical thrombectomy or valve replacement. We present a case report of a patient with prosthetic mitral valve and an unusually large left atrial thrombus with both thromboembolic and hemorrhagic complications.

Key words: Thrombosis; Anticoagulation; Thrombolytic therapy; Transthoracic echocardiogram; Prosthetic valves; Transesophageal echocardiogram

Core tip: Patients with mechanical prosthetic cardiac valves require life long systemic anticoagulation. Maintaining therapeutic anticoagulation consistently is challenging, given the variable dose requirements with warfarin, especially with dietary changes and drug interactions. We present a case of a patient with fluctuating control of anticoagulation, which led to an unusually large horseshoe thrombus in her left atrium and subsequent cerebrovascular complications. We also provide a review of literature, diagnostic modalities and treatment options.

Mehra S, Movahed A, Espinoza C, Marcu CB. Horseshoe thrombus in a patient with mechanical prosthetic mitral valve: A case report and review of literature. *World J Clin Cases* 2015; 3(9): 838-842 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i9/838.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i9.838

INTRODUCTION

Patients with prosthetic cardiac valves are at high risk for thromboembolic complications and need life long anticoagulation with warfarin. Thrombosis of a mechanical prosthetic cardiac valve is a serious complication with high morbidity and mortality. Advancements in valve design and surgical techniques have improved prognosis but thrombo-embolism remains a source of complications in patients with mechanical prosthetic cardiac valves[1,2]. Warfarin is the only oral anticoagulant approved for systemic anticoagulation in patients with prosthetic cardiac valves. However, warfarin has been associated with variable dose requirements related to common genetic polymorphisms influencing their metabolism, varying amounts of dietary intake of vitamin K and interactions with other drugs being used concomitantly[3-5]. Fluctuating level of systemic anticoagulation may predispose to thromboembolic and or hemorrhagic complications. We present a case of a patient with a mechanical bi-leaflet mitral valve prosthesis who presented to our institution with an unusually shaped and large sized left atrial thrombus. The case exemplifies a recognized complication in this patient population and is followed by a review of literature including pathogenesis, clinical presentation and treatment options.

CASE REPORT

A 77-year-old female with medical history of severe rheumatic mitral stenosis treated with mitral valve replacement with 31 mm St. Jude mechanical bileaflet valve was brought to our emergency room with acute onset of right sided weakness and altered mental status by emergency medical services (EMS). Per a family member patient had complained of not feeling well and while EMS was being contacted, she became unresponsive. EMS intubated the patient for airway protection. Upon arrival to our hospital the patient was unresponsive, with heart rate of 127/min, irregular rhythm, blood pressure of 127/55 mmHg and temperature of 36.6 degrees centigrade. On chest auscultation heart sounds were soft with no clear audible valve click. Bilateral lung air entry was decreased with scattered crepitations. Neurological examination revealed pupils reactive to light but deviated to the left and right arm and leg paralysis. The patient was admitted to the medical intensive care unit. Review of her medical record showed that she had labile control of systemic anticoagulation with warfarin, with international normalized ratio (INR) fluctuating between 1.4 to 3.5 in the past six months; INR was 2.3 on admission. Initial laboratory investigations revealed leukocytosis, with white blood cells at 34.4 k/mL, hemoglobin 10.1 g/dL, platelets 845 k/mL, sodium 144 meq/L, blood urea nitrogen 23 meq/L and serum creatinine 0.7 meq/L. Electrocardiogram showed atrial fibrillation with ventricular rate of 113/min, with non-specific ST changes. Blood cultures were obtained and the patient was started on broad spectrum antibiotic coverage. Computed tomography (CT) scan of the head revealed no acute changes but old bilateral cerebellar lacunar infarcts and mild diffuse atrophy. An urgent transthoracic echocardiogram (Figure 1A) showed an oval shaped echo density (1.3 cm × 2.4 cm) attached to the atrial side of the mitral valve in a dilated left atrial cavity, likely representing a thrombus. Mean and peak pressure gradients across the mitral valve were 4 and 14 mmHg respectively (Figure 1B), which were similar to the gradients post-valve implantation. The left ventricular systolic function was mildly decreased with ejection fraction of 40%-45%.

Subsequently transesophageal echocardiogram was performed which revealed a horseshoe shaped, partially mobile, echo density, consistent with thrombus around the sewing ring of the mechanical valve on the left atrial side (Figure 2). Neurology team recommended anticoagulation with therapeutic intravenous heparin at 12 units/kg body weight per hour without initial bolus. Meanwhile patient deteriorated clinically with hypotension not responsive to fluid challenge and decreasing urine output and patient was started on pressor support. Repeat CT scan of the head showed interval development of extensive hypodensity in the left frontal, parietal, insular and temporal lobes and involving the left basal ganglia, with associated petechial hemorrhages. Heparin was subsequently discontinued due to intracerebral hemorrhage (ICH) seen on computed tomography of the head. The patient was initially anticoagulated with therapeutic intravenous heparin, which was subsequently discontinued due to ICH seen on computed tomography of the head. Thrombolytic therapy was contraindicated due to ICH. The patient passed away after withdrawal of life support per family's wishes.

DISCUSSION

According to a meta-analysis by Cannegieter *et al*[6], the incidence of valve thrombosis in prosthetic heart valve recipients was 1 per 100 patient-years in patients on warfarin therapy and around four times higher in patients on no anticoagulation therapy. The incidence of thrombosis was twice as likely for prosthetic valves in the mitral position as compared to the aortic position. Caged ball valves have a higher incidence of thrombosis compared to tilting disc and bileaflet valves[6]. Lengyel[7] reported the incidence of prosthetic cardiac valve thrombosis to be between 1%-4% despite adequate anticoagulation. Prosthetic mechanical valves in the mitral or tricuspid position, first generation Ball-cage and tilting-disc mitral valves have higher incidence of thrombosis. The pathogenesis of thrombosis is likely multifactorial, depending upon the thrombogenic effect of prosthetic material, left atrial geometry and function, presence of atrial fibrillation, inadequate anticoagulation, altered transprosthetic blood flow and any other cause for hypercoagulable state such as malignancy or

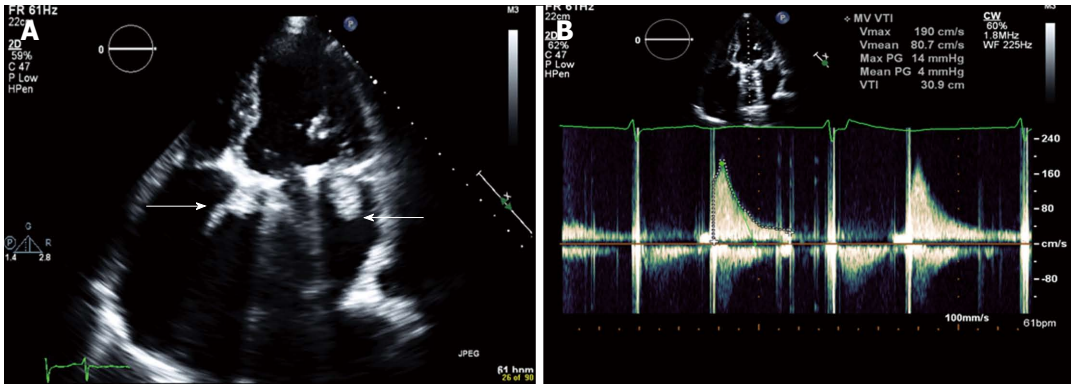

Figure 1 Prosthetic mitral valve thrombosis. A: Transthoracic two-dimensional echocardiogram in apical four-chamber view; B: Transthoracic continuous wave Doppler across mitral valve. Bi-leaflet prosthetic mitral valve seen with echo density, suggestive of thrombus, attached to the atrial side of the bi-leaflet prosthetic mitral valve. The maximum peak gradient across the prosthetic mitral valve is 14 mmHg and mean peak gradient 4 mmHg.

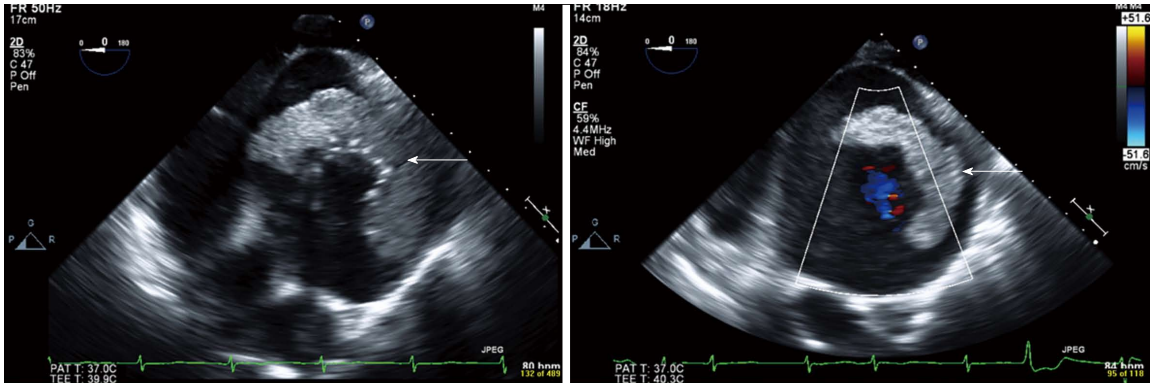

Figure 2 Transgastric views showing horseshoe shaped thrombus around the prosthetic mitral valve suture line.

drugs[8-11]. Inadequate anticoagulation could be a result of variable dose requirements of warfarin. Three new oral anticoagulants (NOACs) - dabigatran, rivaroxaban and apixaban are approved for systemic anticoagulation for patients in atrial fibrillation but not approved for patients with prosthetic cardiac valves. NOACs have several advantages over warfarin, including less dietary interference with their metabolism, fewer drug interactions and a more predictable anticoagulant effect that allows for administration of NOACs in fixed doses without the need for routine coagulation laboratory monitoring[12]. The RE-ALIGN study evaluated the use of dabigatran in patients with mechanical heart valves. The trial was terminated early because of excess of thromboembolic and bleeding events among patients in the dabigatran group[13]. Hence despite shortcomings, warfarin is the only oral anticoagulant approved for anticoagulation in patients with prosthetic cardiac valves for now.

Clinical features suggestive of cardio-embolic source include sudden onset of symptoms, loss of consciousness at onset, rapid regression of symptoms, simultaneous or sequential strokes in different arterial territories, or evidence of embolism to other organs. Presence of bi-hemispheric cerebrovascular, combined anterior and posterior circulation, or bilateral or multilevel posterior circulation infarcts on CT scan of the head are suggestive of cardioembolism[14]. In our patient's case the presentation was acute, with rapid deterioration in neurological status. Initially the head CT scan showed bilateral old infarcts, suggestive of cardioembolic source. However, the new infarcts on repeat imaging were extensive and confined to the left hemisphere.

Clinical presentation for patients with thromboembolic complications may vary from being asymptomatic to dyspnea, cardiogenic shock, cerebral or peripheral embolic episodes. Loss of prosthetic valve click on clinical examination may be suggestive of prosthetic valve thrombosis. Transthoracic echocardiogram is the initial diagnostic tool and provides information about valvular morphology and function. Transesophageal echocardiography is often required to provide more accurate images and to distinguish between pannus and thrombus. A thrombus has usually an echodensity similar to the myocardium while pannus appears more hyperechoic[15]. Fluoroscopy may also be useful for assessment of valve function by showing restriction of valve leaflet movements in case of prosthetic valve thrombosis[16].

Treatment options include thrombolytic therapy, surgical thrombectomy or surgical replacement of the valve. Depending on the clinical status of the patient,

surgical mortality can be as high as 69%. Thrombolytic therapy can also result in thromboembolic complications or hemorrhage[17]. Cáceres-Lóriga *et al*[2], have recommended that in all patients with confirmed right-sided prosthetic valve thrombosis initial treatment should be intravenous thrombolytic therapy, with serial echocardiograms. Thrombolytic therapy may be repeated if required and urgent surgical consultation for valve-replacement should be sought for failure of thrombus resolution. Medically stable patients can be managed with thrombolytic therapy and then continued anticoagulation.

Thrombosis of mechanical prosthetic cardiac valves is associated with high morbidity and mortality and treatment options available can also be associated with serious complications. This mandates the need for high clinical suspicion for thrombosis when a patient with a prosthetic cardiac valve presents with shortness of breath or with an embolic event.

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Rene Robinson-Armatta for providing assistance with acquiring echocardiographic images.

COMMENTS

Case characteristics

Acute onset of right sided weakness and altered mental status.

Clinical diagnosis

Unresponsive patient in atrial fibrillation with right arm and leg paralysis.

Differential diagnosis

Computed tomography (CT) scan of the head done to diagnose acute cerebrovascular accident and transthoracic echocardiogram done to evaluate for cardiac source of emboli.

Laboratory diagnosis

Laboratory tests showed elevated white cell count with mild anemia and subtherapeutic international normalized ratio (INR).

Imaging diagnosis

Initial CT scan of the head showed old bilateral infarcts and repeat CT head showed left frontal, parietal, insular and temporal lobes and involving the left basal ganglia, with associated petechial hemorrhages.

Treatment

Patient started on broad spectrum antibiotics for possible sepsis and initially started on intravenous heparin for subtherapeutic INR, but heparin discontinued due to subsequent intracerebral petechial hemorrhages.

Related reports

The authors have included references from the study by Cannegieter *et al* about thrombosis and bleeding complications in patients with mechanical cardiac valves. The authors have also reviewed and included in manuscript the manuscript by Barbetseas *et al* which explains how to differentiate thrombus from pannus.

Term explanation

Polymorphisms: Occurrence of more than one form; Thrombogenic: Promoting thrombosis.

Experiences and lessons

Maintaining therapeutic INR in patients with mechanical prosthetic cardiac valves is critical and high index of suspicion is required for early diagnosis of complications associated with prosthetic valves.

Peer-review

The authors present a clinical note of a 72-year-old female patient suffering from an ischemic stroke of embolic etiology secondary to thrombosis of mechanical prosthetic cardiac valve. This information is interesting from a clinical point of view.

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CASE REPORT

Do inhaled corticosteroids increase the risk of *Pneumocystis* pneumonia in people with lung cancer?

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Author contributions: All authors contributed to this work.

Institutional review board statement: The study was reviewed and approved.

Informed consent statement: The patient is dead.

Data sharing statement: No conflict interest.

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Abstract

Pneumocystis pneumonia (PCP) is a life-threatening infection in immunocompromised patients. It is relatively uncommon in patients with lung cancer. We report a case of PCP in a 59-year-old man with a past medical history of chronic obstructive pulmonary disease treated with formoterol and a moderate daily dose of inhaled budesonide. He had also advanced stage non-small lung cancer treated with concurrent chemo-radiation with a cisplatin-etoposide containing regimen. The diagnosis of PCP was suspected based on the context of rapidly increasing dyspnea, lymphopenia and the imaging findings. Polymerase chain reaction testing on an induced sputum specimen was positive for *Pneumocystis jirovecii*. The patient was treated with oral trimethoprim-sulfamethoxazole and systemic corticotherapy and had showed clinical and radiological improvement. Six months after the PCP diagnosis, he developed a malignant pleural effusion and expired on hospice care. Through this case, we remind the importance of screening for PCP in lung cancer patients under chemotherapeutic regimens and with increasing dyspnea. In addition, we alert to the fact that long-term inhaled corticosteroids may be a risk factor for PCP in patients with lung cancer. Despite intensive treatment, the mortality of PCP remains high, hence the importance of chemoprophylaxis should be considered.

Key words: *Pneumocystis jiroveci*; Lung neoplasms; Pneumonia; Inhaled corticosteroids; Prophylaxis

Core tip: *Pneumocystis* pneumonia (PCP) is relatively uncommon in patients with lung cancer. We report a case of PCP in a 59-year-old man with a past medical history of chronic obstructive pulmonary

disease treated with formoterol and a moderate daily dose of inhaled budesonide. He had also advanced stage non-small lung cancer treated with concurrent chemo-radiation. This report attempts to alert for the importance of PCP screening in lung cancer patients under chemotherapeutic regimens and with increasing dyspnea. It also alerts to the role of long-term inhaled corticosteroids as a risk factor for PCP in patients with lung cancer.

Msaad S, Yangui I, Bahloul N, Abid N, Koubaa M, Hentati Y, Ben Jemaa M, Kammoun S. Do inhaled corticosteroids increase the risk of *Pneumocystis* pneumonia in people with lung cancer? *World J Clin Cases* 2015; 3(9): 843-847 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i9/843.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i9.843

INTRODUCTION

Patients with hematological and oncological diseases are at increased risk for pneumonia caused by *Pneumocystis* pneumonia (PCP) because of their disease-related and therapy-induced immunosuppression[1]. However, PCP among lung cancer patients is probably an under-diagnosed complication whose incidence is still unknown and risk factors remain incompletely identified. We report here a case of PCP in a 59-year-old man with chronic obstructive pulmonary diseases (COPD) under long-term inhaled corticosteroid therapy and receiving a concomitant radio-chemotherapy for stage ⅢB non-small cell lung cancer.

CASE REPORT

We report the case of a 59-year-old man with a 50 pack-year smoking history. He had moderate COPD treated with formoterol and moderate doses of inhaled budesonide (800 μg/d). He was admitted to our hospital in March 2012 for hemoptysis and evaluation of a right upper lobe lung mass with invasion of the mediastinal pleura and ipsilateral mediastinal lymphadenopathy (Figure 1). Computed tomography (CT)-guided needle lung biopsies were positive for squamous cell carcinoma of the lungs. Additional work-up using abdominal and brain CT did not detect any extra-thoracic metastases. Thus, the disease was clinically classified as stage ⅢB (T3N2M0). From May to November 2012, he had undergone five cycles of etoposide 100 mg/m^2 and cisplatin 20 mg/m^2 chemotherapy given in combination with 32 rounds of radiotherapy at 74 Gray. Two months after the first chemotherapy cycle, the patient developed grade Ⅱ persistent lymphopenia (750-1000 cells/mm^3). A control CT scan performed in December 2012 showed partial remission.

Four months later, the patient came to the emergency department with a ten-day history of increasing breathlessness and fever without any other symptoms (in particular, there was neither chest pain nor hemoptysis). Upon examination, his temperature was 36.7 ℃, pulse was 120 bpm, respiratory rate was 33 breaths/min and blood pressure was 120/70 mmHg. His oxygen saturation was 99% on room air and his body mass index was 22 kg/m^2. Over the right lower lobe lung, decreased breath sounds and decreased tactile fremitus with dullness to percussion were noted. Results of the remainder of the examination were entirely normal. Chest X-ray showed a tumor in the right upper lobe. Nodular parenchymal infiltrates appeared in the left upper lobe lung. A little right pleural effusion was also noticed (Figure 2). An arterial blood gas obtained with the patient breathing room air showed pH = 7.41, pCO_2 = 40.6 mmHg, pO_2 = 68 mmHg, HCO_3^- = 27.2, and O_2Sat = 94.5%. Laboratory investigations showed a hemoglobin level of 12.7 g/dL, a leucocyte count of 8030/mm^3 with a differential of 81% polymorphonuclear leukocytes, 10% lymphocytes (lymphopenia at 803/mm^3), 7% monocytes, 1% eosinophiles and a platelet count of 329.000/mm^3. The patient's erythrocyte sedimentation rate was 86 mm/h, and C-reactive protein was 58 mg/dL. Protein level in the blood was 29 g/dL. Serum electrolytes, blood urea nitrogen, creatinine, serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase were all within normal limits. Purified protein derivative for a tuberculous skin test was non-reactive. Three acid fast bacilli smears and human immunodeficiency virus (HIV) testing were negative. The patient received 3 g of cefotaxim three times a day. On the fourth day in the hospital, his dyspnea was exacerbated and an arterial blood gas obtained on room air showed pH = 7.47, pCO_2 = 39.6 mmHg, pO_2 = 44.8 mmHg, HCO_3 = 21.9, and O_2Sat = 83.9%. A thoracic CT scan eliminated pulmonary embolism. However, it showed, in addition to the primitive tumor, widespread thin-walled cysts and nodules throughout the lungs but most prominent at the right lung. There were also a small right apical pneumothorax and a small bilateral pleural effusion (Figure 3). Pleural fluid was transudative and cultures were negative. The diagnosis of PCP was suspected based on the context of rapidly increasing dyspnea, lymphopenia following treatment with chemoradiation and the imaging findings. Bronchoscopy with bronchoalveolar lavage was indicated but could not be performed because of severe hypoxemia. Polymerase chain reaction (PCR) testing on an induced sputum specimen was positive for *Pneumocystis Jirovecii*.

Treatment for PCP was started with trimethoprim-sulfamethoxazole (TMP-SMX) given orally three times a day at a dose of 20 mg/kg per day with TMP and 100 mg/kg per day with SMX (12 tablets of co-trimoxazole daily). A short course of high-dose dexamethasone (120 mg/d) was also given intravenously for three consecutive days followed by 1 mg/kg per day of prednisone with gradual tapering.

The patient showed clinical and radiological improvement and was discharged after hospitalization for a

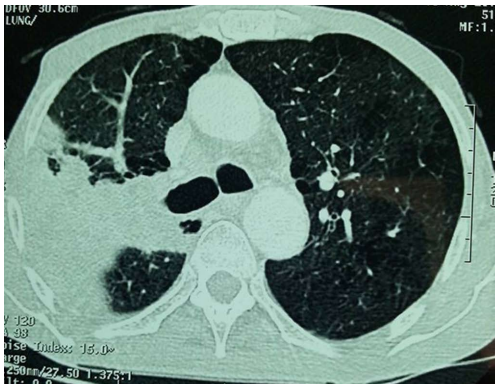

Figure 1 Thoracic computed tomography scan showed a mass in the upper lobe of the right lung with invasion of the mediastinal pleura and ipsilateral mediastinal lymphadenopathy.

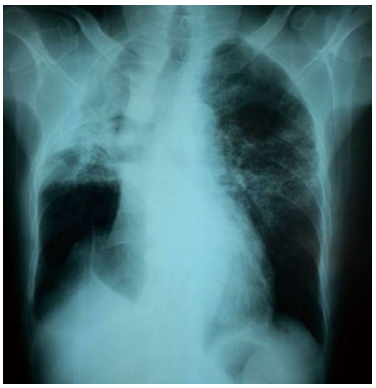

Figure 2 Chest X-ray performed at the second patient's admission showing the right upper lobe lung tumor, and nodular parenchymal infiltrates in the left upper lobe lung with a small right pleural effusion.

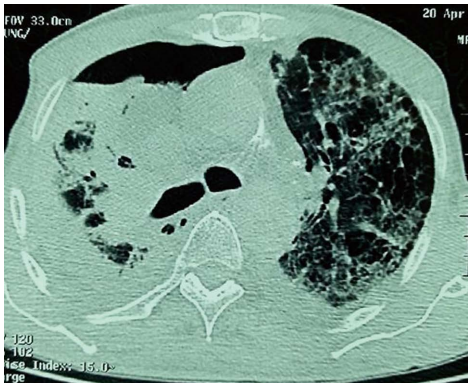

Figure 3 Thoracic computed tomography scan showing a primitive tumor with widespread thin-walled cysts and nodules throughout the lungs but most prominent at the left lung. There were also a small right apical pneumothorax and a small bilateral pleural effusion.

month with instructions to complete 21 d of treatment with TMP-SMX and one month of corticotherapy. Since then, he has been seen at our hospital once a month and he remained stable during two months. In July 2013, he was re-admitted for rapid deterioration of the general status. He reported also a shortness of breath that gradually worsened. A follow-up thoracic CT scan showed a large right pleural effusion. A needle pleural biopsy confirmed metastatic pleurisy. Therapeutic abstention was decided because of the deep deterioration of the general status, and the patient received just palliative care. He expired three months later. An autopsy was not performed.

DISCUSSION

PCP is an opportunistic life-threatening fungal infection caused by *Pneumocystis Jirovecii*. It is seen in immune-compromised individuals, primarily among HIV-infected patients[2]. However, the increasingly frequent use of immunosuppressive drugs had led to outbreak for PCP in patients not infected by HIV[1]. We report here a case of PCP in a patient with advanced stage non-small cell lung cancer treated by cisplatin/etoposide concurrent chemoradiation therapy. PCP is relatively uncommon in patients with lung cancer. Its incidence remains unknown. Recent evidence suggests that about 0.11% of the patients with solid malignancies developed PCP (30 cases of PCP among 26085 patients)[3]. In another retrospective investigation of 150 lung cancer patients receiving chemotherapy or radiotherapy during 1 year, authors found a low incidence of clinical PCP, less than 1%. However, there was a relatively higher (31%) percentage of PCP positivity in patients who developed pneumonia while being treated for lung cancer than in those being treated for other solid tumors[4]. The incidence of PCP in patients with solid tumors has recently increased, probably because of better overall survival and use of more aggressive chemotherapy[5]. The higher risk has been reported with vincristine and cyclophosphamide, but less communally with platinum-based regimens[2,5,6]. With these cytostatic agents, the risk of developing PCP depends on the severity and the duration of neutropenia[1]. Moreover, patients receiving their first cycle of chemotherapy are at a higher risk of infection than in following cycles[1]. In our case, the diagnosis of PCP was not initially suspected because of the long time from the completion of chemotherapy to the onset of pneumonitis and the absence of neutropenia. However, the patient should be regarded as a host risk for PCP due to additional factors such as the advanced stage of the underlying malignancy, associated comorbidities (COPD), the use of radiotherapy as well as inhaled corticosteroid therapy[1,7]. In fact, radiation to the thorax can increase the risk of developing PCP by producing significant lung parenchymal lesions or by causing lymphocyte-depleting as was observed in our case[7]. Paradoxically, it has been reported that PCP infiltrates may spare the area of the lung that is included in a radiation port either during the course of therapy or several months after[7,8]. In our patient, the pulmonary infiltrates were more prominent on the left lung compared to the previously irradiated right side. This presentation called "photographic negative of post-radiation pneumonia" is a distinct finding in PCP[9].

Most patients undergoing chemotherapy for solid

tumors received corticosteroids at the time of the PCP diagnosis[2,5]. In fact, it has been long known that systemic corticosteroid treatment is a major and a common factor for PCP, and accounts for 55% to 99% of published cases in non-HIV infected patients[10]. For instance, a dose of 30 mg of prednisone or the equivalent for 12 wk is considered a significant risk factor[6,8]. The mechanism could be a decrease of blood $CD4^+$ lymphocyte count[11]. Nevertheless, the impact of inhaled corticosteroid on the risk of infection is still unknown. On reviewing the medical literature, we only found one case of association between inhaled corticosteroid and PCP in a lung cancer patient with COPD[7]. Another case was also described in one asthmatic child[12]. These reports suggest that long term inhaled corticosteroid should be considered a risk factor for PCP especially in immune-compromised hosts such as COPD or lung cancer patients, even in the absence of marked leucopenia[7]. However, the magnitude of this risk, the effects of different preparations and doses, and the mechanisms of this effect remain unclear[13]. Of the inhaled corticosteroids studied, only fluticasone demonstrated a dose-related increase in risk of pneumonia in patients with COPD. This difference between fluticasone and budesonide may be explained by the longer retention of fluticasone in the airways, with potentially greater inhibition of type-1 innate immunity[14]. More studies are needed to understand the increased PCP risk with inhaled corticosteroids.

The outcome of PCP in patients without HIV infection is worse than that in HIV-positive patients[15] with a higher mortality (30%-60% *vs* 10%-20%)[16]. Despite its poor outcome, the need for primary PCP prophylaxis in patients with lung cancer is still considered less clear or even questioned by some authors. Several studies recommended that PCP prophylaxis should be performed if patients with lung cancer received prolonged systemic corticosteroids, prednisone or equivalent at least 20 mg for more than 4 wk[17]. Our patient did not receive any PCP prophylaxis because he was not under long-term steroid medication. He was just receiving long-term inhaled corticosteroids that might be, as already mentioned, a risk factor for PCP, even if it has not yet been clearly demonstrated[7]. This fact should lead to discussing prophylaxis in patients receiving inhaled corticosteroids, on a case-by-case basis, even without any specific recommendation.

Long-term inhaled corticosteroids may be linked with an increased risk of PCP in lung cancer patients with COPD, even in the absence of marked pneumonia. More studies are needed to clarify the magnitude of this risk, the effects of different preparations and doses, and the mechanisms of this effect. In lung cancer patients under long-term inhaled corticosteroids, chemoprophylaxis should be considered, although its indication and duration are still controversial.

COMMENTS

Case characteristics

A case of *Pneumocystis* pneumonia (PCP) in a 59-year-old man with chronic obstructive pulmonary diseases under long-term inhaled corticosteroid therapy and receiving a concomitant radio-chemotherapy for stage ⅢB non-small cell lung cancer.

Clinical diagnosis

Increasing breathlessness and fever.

Differential diagnosis

Bacterial pneumonia and pulmonary tuberculosis.

Laboratory diagnosis

Lymphocyte percentage was 10% (lymphopenia at 803/mm^3), erythrocyte sedimentation rate was 86 mm/h, C-reactive protein was 58 mg/dL, protein level in the blood was 29 g/dL, purified protein derivative for a tuberculosis skin test was non-reactive and three acid fast bacilli smears and human immunodeficiency virus (HIV) testing were negative.

Imaging diagnosis

Thoracic computed tomography scan showed a primitive tumor with widespread thin-walled cysts and nodules throughout the lungs, most prominent at the right lung, associated with a small right apical pneumothorax and a small bilateral pleural effusion.

Pathological diagnosis

Pneumocystis jirovecii pneumonia.

Treatment

Twenty-one days of treatment with trimethoprim-sulfamethoxazole and one month of systemic corticotherapy.

Related reports

The increasingly frequent use of corticosteroids, chemotherapy, and other immunosuppressive drugs had led to outbreak for PCP in patients not infected by HIV, in particular in oncological diseases such as lung cancer.

Term explanation

Pneumocystis jiroveci (formerly *Pneumocystis carinii*) is a fungal opportunistic pathogen found in immunocompromised patients. It has gained particular prominence since the onset of the acquired immune deficiency syndrome epidemic.

Experiences and lessons

PCP should be suspected in lung cancer patients with increasing dyspnea if they have risk factors such as chemotherapy and prolonged systemic corticosteroids or even long-term inhaled corticosteroids.

Peer-review

The subject is interesting.

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CASE REPORT

Perianal tuberculosis: A case report and review of the literature

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Abstract

Tuberculosis (TB) is still a major health problem worldwide. We present a rare case of an immuno-competent patient with perianal TB. A 38-year-old man visited a clinic with pain, swelling, and redness in the perineum. He had been persistently coughing for the past 6 mo. The abscess had formed a fistula to the perianal region, indicating perianal abscess. *Mycobacterium tuberculosis* was found in sputum and perianal abscess. Surgical drainage was performed, and oral anti-tuberculous drugs were administered for 6 mo. The patient's clinical course was favorable. On review of the literature on 58 cases of perianal TB, we found that the duration of persistent perianal lesion was much longer in patients without active pulmonary TB (APTB) than in those with APTB (66.4 mo *vs* 8.3 mo; confidence interval, 0.0760-0.9620, P = 0.0380). Thus, in cases of non-healing or recurrent perianal lesions, TB should be considered.

Key words: Tuberculosis; Abscess; Ulcer; Hemorrhoids; Fistula

Core tip: We present a case of an immuno-competent patient with perianal tuberculosis (TB) and active pulmonary TB (APTB). In our literature review of 58 cases of perianal TB, we found that the duration of persistent perianal lesions was much longer in patients without APTB than in those with APTB (66.4 mo *vs* 8.3 mo; confidence interval, 0.0760-0.9620; P = 0.0380). In cases of non-healing or recurrent perianal lesions, the diagnosis of TB should be considered and culture for *Mycobacterium tuberculosis* and histologic examination should be conducted.

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INTRODUCTION

Tuberculosis (TB) is still a major health problem worldwide and is more prevalent in Asia. In Japan, in which TB prevalence is decreasing, estimated new TB cases per 100000 population per year is 10-19, which is in the same range with some European countries[1]. Extra-pulmonary TB occupied 3%-46% of all types of TB[2]. Perianal TB is an extremely rare type of extra-pulmonary TB (0.7%)[3]; however, its prevalence might be underestimated, as it can be misidentified as Crohn's disease or other granulomatous diseases[4]. Here, we report a rare case of perianal abscess caused by *Mycobacterium tuberculosis* (MTB).

CASE REPORT

A 38-year-old Japanese man visited a clinic with chief complaints of pain in the perineum, which had developed 4 d earlier. He also had been persistently coughing for the past 6 mo. Chest radiography revealed a cavity in the right upper lung field, and acid-fast bacilli (AFB) were detected in his sputum. These findings indicated pulmonary TB, and the patient was subsequently admitted to the hospital. His past history was unremarkable: He smoked 20 pieces/d. He installed Japanese pinball machines (pachinko) for a living. He had visited North Korea 20 years ago and South Korea 3 years ago. He had no family history of TB. His blood pressure was 126/76 mmHg, pulse rate was 100 beats/min, temperature was normal at 37.3 ℃, and with clear respiratory sounds. Redness, swelling, and tenderness were noted in the right perineum (Figure 1). Thoracic computed tomography confirmed the cavity in the right upper lung field and infiltration in the right upper and left middle lung fields (Figure 2). MTB DNA was amplified using polymerase chain reaction (PCR) (Kobasu TapMan® MTB, Switzerland), and sputum culture revealed MTB. A large amount of creamy white pus was drained from the incisional perianal abscess. The abscess had formed a fistula to the perianal region, indicating a perianal abscess. AFB were also detected in the wound pus and PCR and culture analysis confirmed MTB. Further, *Escherichia coli* was concomitantly isolated from the abscess. Oral administration of isoniazid, rifampicin, ethambutol, and pyrazinamide was initiated, and the clinical course was favorable. After completing the 6-mo oral administration of anti-TB drugs, the patient was operated upon for the anal fistula. His human immunodeficiency virus (HIV) test was negative. No apparent underlying disease was detected at the 3-mo follow-up examination.

We reviewed 58 cases of perianal TB (abscess, fistula, and ulceration) reported from 1970 to 2014 worldwide (Japan, 17 cases; Taiwan, 17 cases; South Korea, 4 cases; Hong Kong, 3 cases; United States, 3 cases; United Kingdom, 2 cases; Brazil, 2 cases; Spain, 2 cases; Turkey, 2 cases; Morocco, 1 case; Iran, 1 case; India, 1 case; France, 1 case; Bulgaria, 1 case), whereas 70.7% were from Asia[5-31]. The mean age (± SD) of the patients was 45.3 (± 10.6) years, and the male-to-female ratio [52 (89.7%) men and 6 (10.3%) women] was higher than that reported previously (4:1)[4,32]. Eighteen point six percent of the patients had underlying diseases related to immunodeficiency (respiratory diseases 3, diabetes mellitus 2, hepatitis 1, hepatitis + HIV 1, chronic kidney disease 1, malignancy 1, autoimmune disease 1, cardiovascular disease 1).

We researched duration of disease, that is the time from beginning of symptoms until diagnosed with perianal TB. Among 29 patients with complete data available on the duration of perianal lesion, the mean duration of disease was 34.6 mo. Of these, 15.3% of the patients had a surgical history of perianal lesions before the diagnosis of TB.

APTB was noted in 34 (57.6%) cases; history of TB, in 12 (20.3%) cases; and no history of pulmonary TB in 12 (20.3%) cases. The existence or non-existence of pulmonary TB was not reported in 1 (1.7%) case. We compared the characteristics of patients with APTB (n = 34) with those of patients without APTB (n = 24) (Table 1). The duration of disease in patients without APTB (66.4 mo) was significantly longer than that in patients with APTB (8.3 mo), as noted in a two-sample t-test (CI: 0.0760-0.9620; $P < 0.05$).

DISCUSSION

Gastrointestinal TB accounts for less than 1% of all TB cases, and perianal disease is exceedingly rare, comprising 1% of digestive tract incidence[5].

In our literature review, only 18.6% of the patients had underlying diseases related to immunodeficiency. Therefore, not only immuno-compromised but also immuno-competent patients should be carefully evaluated, because TB can also occur in immuno-competent individuals, as noted in our case.

The mean duration of perianal TB was 34.6 mo, and 15.3% of the patients had a surgical history of perianal lesions before the diagnosis of TB. Perianal TB may have gone un-diagnosed for a long period.

APTB was noted in 34 (57.6%) cases; Since in cases of perianal TB, APTB is often concomitant with, it is important to conduct chest radiography and a detailed interview, especially to record chronic cough, fever, night sweats, or weight loss, which suggest APTB, in order to diagnose perianal TB.

We compared the characteristics of patients with APTB (n = 34) with those of patients without APTB (n = 24). We found that the occurrence of intestinal TB and TB complications (miliary TB, peritoneal TB, and

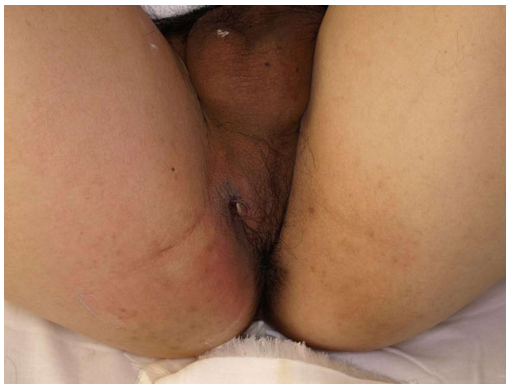

Figure 1 Redness, swelling, and tenderness is seen in the right perineum after the first drainage.

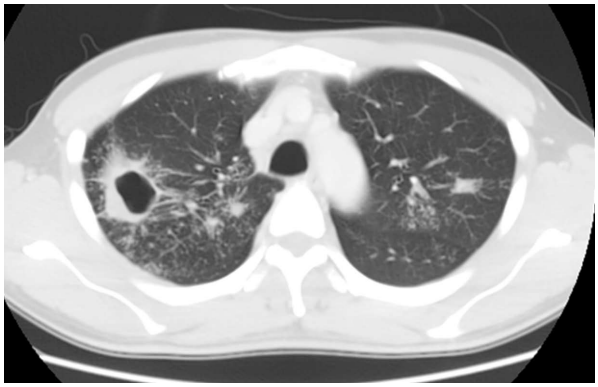

Figure 2 Thoracic computed tomography scan confirming a cavity in the right upper lung field and infiltration in the right upper and left middle lung fiel.

iliopsoas muscle abscess by TB) were similar in both patient groups. This raises a question regarding the infectious routes in patients without APTB, since TB rarely, if ever, occurs as a primary infection in a perianal region. Two possible explanations are as follows: first, hematogeneous spread after reactivation of latent lung TB could be responsible[7], and second, other foci may have not been sufficiently investigated, as seen in our cases wherein only 37 (62.7%) cases included data on intestinal examination.

The duration of disease in patients without APTB (66.4 mo) was significantly longer than that in patients with APTB (8.3 mo), as noted in a two-sample *t*-test (CI: 0.0760-0.9620; $P < 0.05$). Since perianal TB does not have any specific clinical characteristics[5,10], its occurrence without APTB may be underestimated. In cases of chronic or recurrent fistula, the diagnosis of TB should be considered, and culture for MTB and histologic examination should be performed.

The patient in our case installed Japanese pinball machines (pachinko) for a living. In Tokyo patients developed TB without any apparent contact with TB patients most frequently spent their time and maybe acquired the infection at pachinko parlors (24%) and amusement parks (24%), followed by saunas (16%)[33]. In the United States, residents and employees of congregate settings such as hospitals, correctional facilities, nursing homes, and homeless shelters are at a high risk for TB exposure[34-36]. Thus, an interview to collect information on the places that the patient stayed at or visited and employment situation often could be helpful in making a diagnosis of TB.

In conclusion, we report the case of an immunocompetent man with perianal TB and APTB. In cases of non-healing or recurrent perianal fistula, TB should be considered as a causative agent.

Table 1 Characteristics of perianal tuberculosis patients with active pulmonary tuberculosis and without active pulmonary tuberculosis *n* (%)

	Active pulmonary TB (*n* = 34)	Without APTB (*n* = 24)
Age (mean yr)	41.2	51.1
Sex (male)	30 (88.2)	22 (91.7)
Underling diseases	6 (17.6)	5 (20.8)
Previous anal surgery	4 (11.8)	5 (20.8)
TB complications	2 (5.9)	2 (8.3)
Intestinal TB	0	2 (8.3)
Anal carcinoma	2 (5.9)	1 (4.2)
Duration of anal lesion (mo)	6.8	66.5

TB complication: Miliary TB, peritoneal TB, and iliopsoas muscle abscess by TB. Underling disease: Respiratory diseases 3, diabetes mellitus 2, hepatitis 1, hepatitis + human immunodeficiency virus 1, chronic kidney disease 1, malignancy 1, autoimmune disease 1, cardiovascular disease 1. TB: Tuberculosis; APTB: Active pulmonary tuberculosis.

COMMENTS

Case characteristics

A 38-year-old male patient presented with pain in the perineum, which had developed 4 d earlier and persistently coughing for the past 6 mo.

Clinical diagnosis

The patient had redness, swelling, and tenderness in the right perineum.

Differential diagnosis

Testicular torsion, epididymitis, abscess, Fournier's gangrene, torsion of the appendix testis, trauma, testicular cancer, inguinal hernia, Henoch-Schönlein purpura (IgA vasculitis), mumps and Crohn's disease.

Laboratory diagnosis

Acid-fast bacilli were detected in the sputum and wound pus and PCR and culture analysis confirmed *Mycobacterium tuberculosis*.

Imaging diagnosis

Computed tomography scan showed a cavity in the right upper lung field.

Pathological diagnosis

Histological examination of the abscess showed epithelioid granulomas and Langhans' type multinucleated giant cells.

Treatment

Surgical drainage was performed, and oral anti-tuberculous drugs were administered for 6 mo.

Related reports

The authors reviewed 58 cases of perianal tuberculosis (TB) reported from

1970 to 2014 worldwide. And they found that the duration of persistent perianal lesion was much longer in patients without active pulmonary TB (APTB) than in those with APTB (66.4 mo *vs* 8.3 mo; CI: 0.0760-0.9620; *P* = 0.0380).

Experiences and lessons

This case report presents an immuno-competent patient with perianal TB and APTB. On literature on cases of perianal TB, the authors found that the duration of persistent perianal lesions was much longer in patients without APTB than in those with APTB. In cases of non-healing or recurrent perianal lesions, the diagnosis of TB should be considered.

Peer-review

The description of a case with perianal tuberculosis is per se of interest since this form of tuberculosis is very rare and diagnosis is delayed. The manuscript contains a complete and comprehensible description of clinical and microbiological finding.

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CASE REPORT

Two case reports of bilateral adrenal myelolipomas

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Author contributions: Yang Y, Ye LY, Yu B, and Chen Y conceived and designed the clinical studies; Yang Y, Ye LY, Yu B, Guo JX and Liu Q performed the clinical studies and contributed in management of the patients; Yang Y and Chen Y prepared and revised the paper along with managing the case; Chen Y gave final approval of manuscript.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Board of the First Affiliated Hospital of PLA General Hospital, China.

Informed consent statement: All study participants provided informed written consent.

Conflict-of-interest statement: The authors declare that they have no competing interests.

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Abstract

Primary adrenal myelolipoma is a rare, non-functioning adrenal benign tumor that is composed of mature adipose tissue and a variable amount of haemopoietic elements. Clinically, it is difficult to get diagnosed with adrenal myelolipoma because the patient usually doesn't have obvious symptoms and signs in early stage. In the present study, two cases of primary bilateral adrenal myelolipomas are reported. Clinical presentation, imaging diagnostic features, histopathological changes and surgical treatments of the two patients are discussed. Preoperative diagnostic imaging examinations (B-mode ultrasonography, computed tomography and magnetic resonance imaging sans) assisted getting a prediction diagnosis of bilateral adrenal myelolipomas. A two-stage surgery was used to successfully excise bilateral adrenal myelolipomas in the two patients. Conventional open adrenalectomy was applied to remove the adrenal myelolipomas greater than 6 cm, and laparoscopic adrenalectomy was performed to excise the adrenal tumors smaller than 6 cm. Bilateral adrenal myelolipomas of the two patients were finally confirmed by postoperative histopathological examinations. Understanding clinical, imaging diagnostic and histopathological features of bilateral adrenal myelolipomas will facilitate timely diagnosis and treatment of this condition. Surgical removal of bilateral adrenal myelolipomas is safe, curative and beneficial. The two-stage surgery appears to be the best treatment option for the patients with bilateral adrenal myelolipomas because it achieves optimal treatment effectiveness with minimized sequelae.

Key words: Bilateral adrenal myelolipomas; Magnetic resonance imaging scan; Imaging diagnosis; B-mode ultrasonography; Computed tomography scan; Two-stage surgery; Histopathological examination

Core tip: Adrenal myelolipoma is a rare adrenal benign

tumor. It is not easy to get diagnosed with the tumor due to lack of obvious clinical symptoms and signs in early stage. This report discusses clinical, imaging diagnostic and histopathological features in two patients with bilateral adrenal myelolipomas as well as a two-stage surgical strategy for removal of the adrenal tumors. Understanding clinical, imaging diagnostic and histopathological features of bilateral adrenal myelolipomas will facilitate timely diagnosis and treatment of this condition.

Yang Y, Ye LY, Yu B, Guo JX, Liu Q, Chen Y. Two case reports of bilateral adrenal myelolipomas. *World J Clin Cases* 2015; 3(9): 853-860 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i9/853.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i9.853

INTRODUCTION

Adrenal myelolipoma is a rare, benign, non-functional neoplasm of adrenal gland, which is composed of mature adipose tissue and a variable amount of haemopoietic elements. Myelolipoma is usually found to occur in unilateral adrenal gland, and rarely observed in bilateral adrenal glands[1-4]. Generally, bilateral adrenal myelolipomas are not easy to be diagnosed clinically because the patients have no obvious symptoms and don't feel pain or discomfort in the early stages of the disease. Over the last few decades, a definite diagnosis of adrenal myelolipoma could be made only with certainty at autopsy. Overall diagnosis rate of adrenal myelolipoma, which was made with autopsy, was merely 0.08%-0.2%[3,5]. In recent years, the diagnosis rate of adrenal myelolipoma has been significantly improved due to the development of modern imaging technologies [such as ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI)]. In the present study, two cases of bilateral adrenal myelolipomas that have been treated successfully in our hospital are reported. The clinical, diagnostic imaging and histopathohistological features of the two cases are retrospectively analyzed. A two-stage surgical strategy for removal of bilateral adrenal myelolipomas is discussed.

CASE REPORT

Case 1

History and physical examination: Patient A is a 40-year-old male, without family history of genetic diseases and smoking or drinking habits. His adrenal masses were found by B-mode ultrasound scanning while he had a regular health check-up in our hospital. He was admitted to the hospital after diagnosed with "left adrenal mass (tumor)". The patient had no other obvious symptoms. A thorough history and physical examination were indicated to rule out diabetes, hypertension, coronary heart disease, hepatitis and tuberculosis. Grade I hypertrophy of the prostate gland was found by prostate examination using rectal palpation technique. Patient's prostate gland was soft and spongy, but the central sulcus became shallow. Neither abnormal prostatic nodules nor rectal bleeding were found by prostate examination.

Diagnostic imaging: In order to establish a diagnosis, the patient was scanned with B-mode ultrasound, CT and MRI scans. The B-mode ultrasound scanning was done on the patient who was placed in the lateral or supine position using a Nemio 30 scanner with a 3.5- to 5-MHz curved linear transducer. A Picker PQ 6000 Spiral CT scanner with a slice thickness of 3-6 mm was used to scan the entire kidney of the patient. The patient's kidneys were also scanned using a Siemens Sensation 3.0T MRI scanner. Acquisitions included axial TSE/T2WI and FL/T1WI, and coronal TFI/T2WI sequences. The results of imaging scans (such as the density, signal strength, size, shape and location of a renal mass) were evaluated by two radiologists, respectively.

Both the left and right adrenal masses were simultaneously detected by diagnostic imaging examinations while the patient was hospitalized. Two hyper-echoic masses were observed by B-mode ultrasonography in the left and right adrenal glands. The sizes of the two hyperechoic masses were 9.7 cm × 9.5 cm in the left adrenal gland and 3.3 cm × 2.3 cm in the right adrenal gland, respectively. All masses had clear boundary and a regular shape. Color doppler flow imaging (CDFI) showed little scattered blood flow signals within the masses. CT showed a mixed density area of 9 cm × 10 cm with the absorption values ranging from 80-120 HU in the left adrenal gland, and a mixed density area of 3.5 cm × 5.5 cm with the absorption values ranging from 90-100 HU in the right adrenal gland, suggesting fat-density lesions in bilateral adrenal glands. MRI scan showed multiple round masses with unequal signals on both T1 and T2 in the left adrenal gland. The multiple round masses made the left kidney to shift downward. Among these masses, the biggest one was located in the posterior side of left adrenal gland and its size was about 8.69 cm × 7.82 cm. An enlargement of the medial limb of the right adrenal gland was also observed using MRI scan (Figure 1A-D).

After removal of the left adrenal myelolipoma, there were no obvious abnormalities found by B-mode ultrasonography in the left adrenal gland. However, B-mode ultrasonography showed a hyperechoic mass of 3.6 cm × 3.5 cm in the right adrenal gland. Both plain non-contrast and contrast-enhanced CT scans revealed that the multiple round masses had vanished from the left adrenal gland after surgery, but an irregular mass with clear boundary appeared in the right adrenal gland. The mass, which looked like a multilocular cystic lesion, had a mixed density area (mixed with fat-density and high-density shadows) with the average absorption values of 7-13 HU (Figure 1E and F).

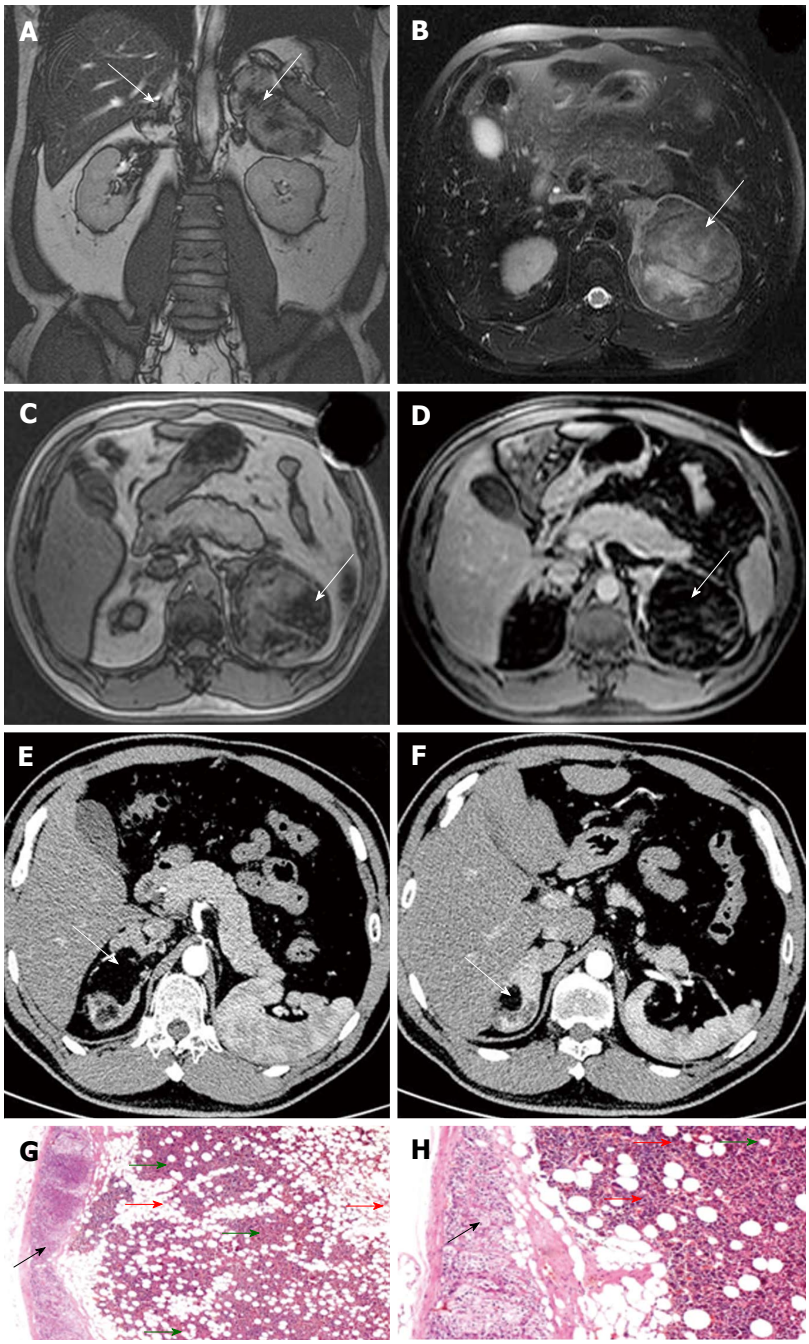

Figure 1 Diagnostic imaging and pathohistological examination results of patient A. A-D: Magnetic resonance imaging (MRI) results of bilateral adrenal myelolipomas before the first surgical procedure; A and B: 84 mm diameter circular mass (white arrow) with unequal signals on both T1 and T2 weighted MRI scans in the left adrenal gland (short T1 weighted mixed with longer T2). The mass has a clear boundary. An increased T2 signal can be seen within it; C: That an uneven T1 signal (which includes lipid signal) inside the mass reduces on anti-phase gradient echo T1WI in the left adrenal gland (white arrow). A reduced T1 signal intensity is seen on fat-suppressed image; D: There is no contrast enhancement in most part of the mass on dynamic contrast-enhanced MRI (white arrow). Separation of the lower composite signals shows the delayed linear enhancement pattern; E and F: Computed tomography scan results of the patient before the second surgical procedure. A circular mass (white arrow) with clear boundary has mixed low-density signals with the average absorption values of 7-13 Hu in the right adrenal gland. Separation of the lower composite signals shows no contrast enhancement in the mass; G and H: The adrenal myelolipomas observed on H and E stained sections. Tumor-like masses of extramedullary hematopoietic tissue is composed of adipose cells (red arrow) and hematopoietic cells (green arrow). Fat vacuoles can be seen in adipose tissue. Among adipose cells, there are medullary cells, megakaryocytes, erythrocytes and lymphoid cells with different maturity levels among fat cells. Normal adrenal gland tissue (black arrow) is surrounded by adrenal myelolipoma.

Surgery: A two-stage surgery was carried out in the patient to excise bilateral adrenal myelolipomas. Because the patient had a large myelolipoma in left adrenal gland and a small myelolipoma in right adrenal gland, a conventional open adrenalectomy was employed to remove the large myelolipoma in his left adrenal gland in first stage of surgery. During surgery, the patient was placed in supine position to make an abdominal incision after anesthesia. After excised the left adrenal myelolipoma, the resected myelolipoma

with a total volume of 11 cm × 8.5 cm × 6 cm was sent to the pathology laboratory for histopathological examination. The right adrenal myelolipoma was removed through laparoscopic adrenalectomy in second stage of surgery (10 mo later). The myelolipoma removed from right adrenal gland had an intact capsule and its total volume was 4.0 cm × 4.0 cm × 3.3 cm. A tumor tissue of 2.5 cm × 2.0 cm × 2.5 cm with the color mixing gray and red was observed inside the removed myelolipoma. Following the surgery, as a regular procedure, the indwelling urinary catheters and nasogastric tubes were kept in until the patients' urinary and gastrointestinal functions returned to normal. The patient was observed daily from the first postoperative day until discharge by the surgeon. Meanwhile, changes in the electrocardiogram (ECG), blood pressure, and blood oxygen saturation were continuously monitored. Anti-inflammatory drugs, symptomatic treatments and supportive care were provided to the patient.

Histopathology: All resection specimens of adrenal myelolipomas were examined by the pathologists using H and E staining (which was repeated twice on resection specimens). Tumor-like masses of extramedullary hematopoietic tissue were composed of adipose cells and hematopoietic cells. Fat vacuoles could be seen in adipose tissue. Among adipose cells, there were medullary cells, megakaryocytes, erythrocytes and lymphoid cells with different maturity levels among fat cells. The histopathological examination results confirmed the initial diagnosis of bilateral adrenal myelolipomas in this patient (Figure 1G and H).

Post-treatment follow-up: Follow-up data were unavailable because this patient did not schedule any post-hospital follow-up visits after he was discharged from the hospital.

Case 2

History and physical examination: Patient B is a 73-year-old male with diabetes for 14 years, but without family history of genetic diseases and smoking or drinking habits. A thorough history and physical examination were indicated to rule out hypertension, coronary heart disease, hepatitis and tuberculosis. He underwent transurethral resection of the prostate years earlier. The patient was diagnosed with bilateral renal cysts in 2009 but didn't receive further medical interventions after bilateral renal space-occupying lesions were observed during a routine physical examination. He was admitted to the hospital for further treatment after his bilateral renal space-occupying lesions were diagnosed again on B mode ultrasonography in 2011. The patients didn't have either obvious clinical symptoms or any discomfort when he was admitted to the hospital. Grade Ⅱ hypertrophy of his prostate gland was found by prostate examination using rectal palpation technique. Patient's prostate gland felt hard, but didn't have palpable nodules or shallow/disappeared central sulcus.

Diagnostic imaging: Patient B was examined with B-mode ultrasound, CT and MRI, respectively. The B-mode ultrasound scanning was done on using the Nemio 30 scanner with a 3.5- to 5-MHz curved linear transducer. The patient's kidneys were scanned by both the Picker PQ 6000 Spiral CT scanner with a slice thickness of 3-6 mm and the Siemens Sensation 3.0T MRI scanner. The medium-to-hyper echo complex masses were observed by B-mode ultrasonography in bilateral adrenal glands of the patient. The masses, which had clear boundary and regular shapes, were 8.8 cm × 7.5 cm on the left side and 9.0 cm × 8.8 cm on the right side, respectively. CDFI showed no obvious blood flow signals within the masses. CT scan found two fat-density shadows in bilateral adrenal glands, which sizes were 10.1 cm × 7.2 cm and 7.3 cm × 7.9 cm, respectively. Contrast-enhanced CT scanning made the two shadows become more inhomogeneous with a mild-to-moderate increasing density. MRI scan showed many masses in his bilateral adrenal glands. In the right adrenal gland, the largest volume of the masses was 8.7 cm × 9.97 cm × 4.92 cm and the smallest volume was 4.45 cm × 4.26 cm × 4.92 cm. In the left adrenal gland, the average volume of the masses was 7.74 cm × 7.52 cm × 4.58 cm. In MR images, the signals of these masses were non-uniform, which included a large number of signals generated by mature fat cells and intracellular lipid contents. After the right adrenal myelolipoma was removed in first stage, the MRI exam was performed again in this patient (Figure 2A-C). A round lesion with mixed signals on T1 and T2 (short T1 mixed with slightly longer T2) was found in the left adrenal gland. The lesion was characterized by decreased signal intensity in the antiphase (out-of-phase). Its size was about 7.46 cm × 7.82 cm. The upper pole region of the left kidney was found to be pressed by the lesion. The right adrenal gland still showed mixed signals on MRI (with both longer T1 and longer T2) and had unclear appearance of an anatomical structure. No lesion near the left retroperitoneal abdominal aorta was observed.

Surgery: The two-stage surgery was also carried out in patient B to excise his bilateral adrenal myelolipomas. Because the patient had large myelolipomas in both the left and right adrenal glands, a conventional open adrenalectomy was used to resect his bilateral myelolipomas. In contrast to patient A, the patients B's right adrenal myelolipoma was removed first, and his left adrenal myelolipoma was resected 3 mo later. In the first surgical procedure, an adrenal myelolipoma measuring 4.5 cm × 3.5 cm × 3.0 cm was removed from the right adrenal gland. Another adrenal myelolipoma measuring 9.0 cm × 7.0 cm × 4.5 cm was excised from the left adrenal gland in the second surgical procedure. After surgery, changes in the ECG, blood pressure, and blood oxygen saturation were continuously monitored in the patient. Anti-inflammatory drugs, symptomatic

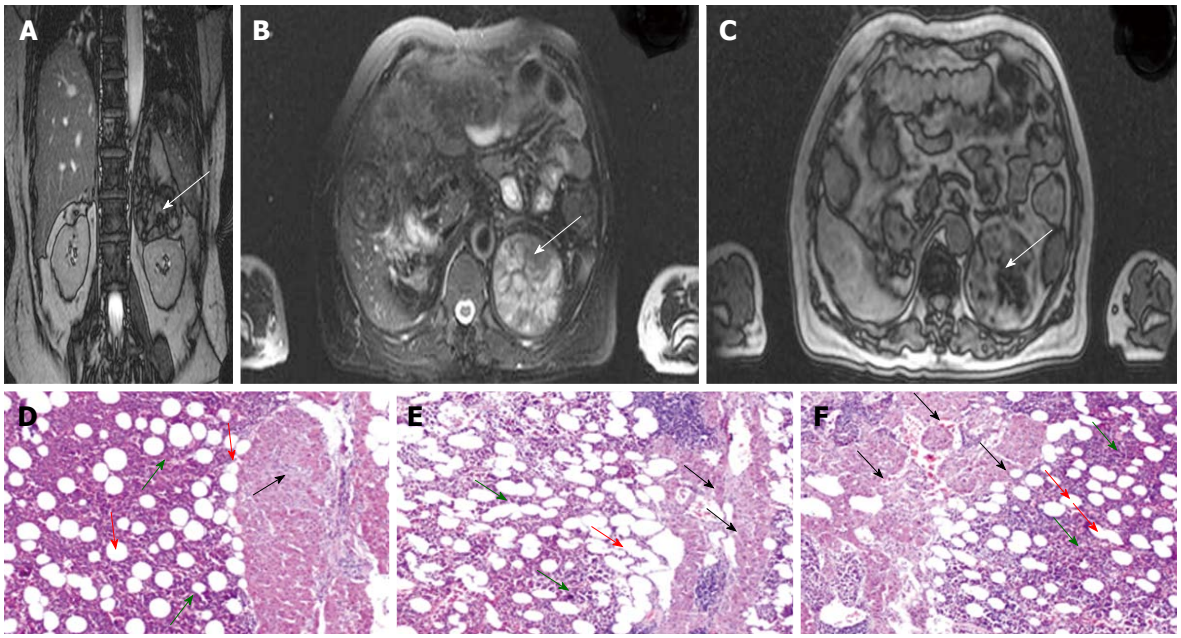

Figure 2 Diagnostic imaging and pathohistological examination results of patients B. A-C: Magnetic resonance imaging (MRI) results of adrenal myelolipomas (MRI scans were performed during the period between the first and the second surgical procedures). A 66 mm diameter circular mass (white arrow) with clear boundary shows mixed signals on T1 and T2 weighted MRI scans (short T1 mixed with slightly longer T2) in the left adrenal gland. The lesion is characterized by decreased T1WI signal intensity in the antiphase (out-of-phase); D: The histopathological features of the right adrenal myelolipoma that was excised in the first surgical procedure (stained with H and E); E and F: The histopathological features of the left adrenal myelolipoma that was removed in the second surgical procedure (stained with H and E). Tumor-like masses of extramedullary hematopoietic tissue is composed of adipose cells (red arrow) and hematopoietic cells (green arrow). Normal adrenal gland tissue (black arrow) is found to be surrounded by adrenal myelolipoma.

treatments and supportive care were also given to the patient. The patient had a good outcome after surgery.

Histopathology: All resection specimens of adrenal myelolipomas were examined by the pathologists using H and E staining (which was repeated twice on resection specimens). The histopathological examination of surgical excised specimens showed large amounts of tumor-like adipose tissue existed in bilateral adrenal medullas, in which extramedullary hematopoietic tissues were found to present. In addition, a thin layer of adrenal cortical tissues was found to surround the myelolipoma. These morphological changes corresponded to the typical histopathological features of adrenal myelolipoma, and confirmed the initial diagnosis of adrenal myelolipoma (Figure 2D-F).

Post-treatment follow-up: Follow-up data were unavailable because this patient did not schedule any post-hospital follow-up visits after he was discharged from the hospital.

DISCUSSION

Adrenal myelolipoma is a rare, benign and hormonally inactive neoplasm of adrenal gland. It occurs generally in unilateral adrenal gland, but the left and the right affected equally. As a benign tumor, myelolipoma does not spread to other body parts. Sometimes, a larger myelolipoma may cause localized tissue death and bleeding. The well-recognized complication of adrenal myelolipoma is spontaneous retroperitoneal haemorrhage[6]. Adrenal myelolipoma, which is composed of large amounts of fat and myeloid tissues, is shaped like or nearly like a round and has clear boundary. Based on its tissue components, adrenal myelolipoma can be categorized into three types: adipose tissue, myeloid tissue and a combination of both[7,8]. Primary bilateral adrenal myelolipoma is rarely observed in clinical practice[3,9,10]. In histopathology of bilateral adrenal myelolipoma, the tissue type of the left adrenal myelolipoma is usually the same as that of the right adrenal myelolipoma but it can't rule out the possibility that different tissue types of adrenal myelolipomas may exist in the left and right adrenal glands, respectively.

As early as 1905, Gierke *et al*[11] have begun to study adrenal myelolipoma. However, the etiology and pathogenesis of adrenal myelolipoma still remain unclear until now. Adrenal myelolipoma has been considered as a result of the differentiation of adrenal medullary or cortex cells into adipose tissue and extramedullary hematopoietic tissue in adrenal gland, which is caused by stimulation of some harmful factors such as stress, infection, ischemia, necrosis, *etc.*[12]. Some studies suggested that adrenal myelolipoma might result from ectopic proliferation of myeloid cells that started to occur during the embryonic period in adrenal gland[13,14]. Moreover, some researchers proposed that the tumor might be evolved from a nonfunctioning adrenal cortical adenoma[15]. As compared to unilateral adrenal myelolipoma, bilateral adrenal myelolipoma might involve more complicated pathogenic mechanism[16].

Generally, there are no specific clinical signs and symptoms observed in the patients with adrenal myelolipoma[3], and also no marked gender differences in the incidence rates of adrenal myelolipoma. It is difficult to predict the incidence of adrenal myelolipoma and to accurately identify the potential patients in the population, because so far there are not many cases to be reported worldwide. Currently, it is unavailable to obtain the authoritative statistical data about the incidence of adrenal myelolipoma. Adrenal myelolipoma is usually discovered by accident while a physical examination is performed in the patients who have suffered other types of diseases. These patients have almost no noticeable manifestations of adrenal gland disorders or endocrine disorders. A diagnosis of adrenal myelolipoma depends mainly on preoperative diagnostic imaging screening, localization and qualitative assessment[7,17,18].

B-mode ultrasonography can be employed to only screen adrenal myelolipoma, because it has limited application in the identification of the tumors that are smaller than 2 cm in diameter. CT scan, especially thin-section CT scan, could be a sensitive imaging technology to diagnose adrenal myelolipoma. However, CT signals of the adrenal masses may be doped with complicated and undesired signals from other biological components because fat tissue inside an adrenal mass is often mixed with other biological components such as calcified and hemorrhagic soft tissues. This may result in incorrect imaging information about the mass, thus leading to a wrong diagnosis.

As compared with CT scan, MRI scan is more sensitive and more accurate imaging diagnostic tool for adrenal tumors. MRI delineates soft tissue better than CT, promoting greater accuracy in preoperative assessment of adrenal myelolipoma. It can also help make a precise quantitative evaluation of adrenal masses through a comparison of unenhanced and contrast-enhanced scans. Under the unenhanced MRI scans, the masses with high proportion of fat tissue show high signal intensity on T1 and T2-weighted images. After contrast enhancement, the signal intensity of the adipose-rich masses will remain the same without any change on MRI. This will be most useful to distinguish adrenal myelolipoma from other adrenal lesions. In addition, MRI scan offers substantial advantages over CT scan for precisely locating a myelolipoma within the adrenal gland, because it uses a strong magnetic field to create high-resolution 3D images of the tumor.

Preoperative diagnostic imaging localization of an abdominal mass is most important in the differential diagnosis of adrenal myelolipoma. It will distinguish adrenal myelolipoma from renal angiomyolipoma, retroperitoneal liposarcoma and other types of abdominal masses. Therefore, MRI is very helpful to establish an accurate diagnosis of adrenal myelolipoma and to develop the surgical strategy for the treatment of this condition. Although diagnostic imaging examinations are generally effective in quantitative evaluation and diagnostic localization of adrenal myelolipoma, a final diagnosis can be made only after histopathological examinations reveal the existence of adrenal myelolipoma in the resection specimens.

Surgical removal of an adrenal myelolipoma is safe, curative and beneficial. However, surgical strategy may depend on the nature, grade, size and location of the myelolipoma[19,20]. When the patients have tumors larger than 3.5 cm in diameter or fast-growing tumors, surgical resection should be the best choice for treatment of adrenal myelolipoma. For an adrenal tumor smaller than 6 cm in diameter, laparoscopic adrenalectomy is generally recommended because this procedure provides patients with a faster recovery and less post-operative pain. However, if an adrenal tumor is greater than 6 cm in diameter or is very likely to grow quickly from its imaging characteristics, the appropriate surgical approach is a conventional open adrenalectomy.

Unlike that for unilateral adrenal myelolipoma, surgical strategy for bilateral adrenal myelolipoma needs to consider how to maximize both the preservation of the adrenal glands and the restoration of the adrenal function. If bilateral adrenal myelolipomas are removed at the same time, it will be inevitable that some of normal adrenal tissues are excised from both the left and right adrenal glands. This will cause bilateral adrenal insufficiency and serious postoperative complications. Therefore, the two-stage surgery should be the best treatment option for bilateral adrenal myelolipoma. Bilateral adrenal myelolipomas can be removed separately in two different stages of surgery at different times[19,21,22]. The reason for performing the two-stage surgery in the patients with bilateral adrenal myelolipoma is that the surgery can be done in a short time and causes less damage to the patients, thus reducing the risk of surgery and enhancing rapid surgical recovery. Most importantly, the two-stage surgical strategy can minimize the chance of adrenal crisis and postoperative complications.

In the present study, the two-stage surgery was successfully performed in the two patients with bilateral primary adrenal myelolipoma. Because patient A had both a large left adrenal myelolipoma and a small right adrenal myelolipoma, a conventional open adrenalectomy was employed to remove his left adrenal myelolipoma in first stage of surgery, and a laparoscopic adrenalectomy was used to excise his right adrenal myelolipoma in second stage of surgery (10 mo later). The conventional open adrenalectomy was employed to resect the patients B's myelolipomas in both left and right adrenal glands because this patient had large myelolipomas in both left and right sides. In contrast to patient A, the patients B's right adrenal myelolipoma was removed first, and his left adrenal myelolipoma was resected 3 mo later. There was no adrenal crisis or other complication to be observed in the patients after surgery. The two-stage surgical strategy has been proven to achieve optimal treatment effectiveness with minimized sequelae.

A combination of B-mode ultrasonography, CT and

MRI sans is the effective diagnostic approach to detect bilateral adrenal myelolipoma. However, before surgery, the quantitative evaluation and diagnostic localization of an adrenal myelolipoma may depend on not only the diagnostic imaging examinations, but also medical histories, clinical symptoms and signs, physical exams and laboratory tests. Surgical strategy for removal of bilateral adrenal myelolipoma should be created based on the nature, grade, size and location of the tumor. Two-stage surgical removal of bilateral adrenal myelolipoma could be the best treatment scheme to achieve optimal treatment effectiveness with minimized sequelae.

COMMENTS

Case characteristics

Rare bilateral adrenal masses were found in two patients.

Clinical diagnosis

Bilateral adrenal myelolipomas, without obvious clinical symptoms and signs.

Differential diagnosis

Retroperitoneal liposarcoma, renal angiomyolipoma, fat containing adrenocortical carcinoma and adrenal teratoma.

Laboratory diagnosis

Laboratory examinations revealed normal results.

Imaging diagnosis

B-mode ultrasonography, computed tomography and magnetic resonance imaging revealed the presence of bilateral adrenal myelolipomas.

Pathological diagnosis

Postoperative histopathological examinations confirmed finally the diagnosis of bilateral adrenal myelolipomas in the two patients.

Treatment

Bilateral adrenal myelolipomas were successfully resected using a two-stage surgical treatment scheme.

Related reports

Few cases of bilateral adrenal myelolipomas were reported with similar clinical presentation, imaging diagnostic features and surgical treatment.

Term explanation

A two-stage surgery is an operative procedure in which bilateral myelolipomas are resected separately from the left and right adrenal glands in two different stages of surgery at different times. One myelolipoma in unilateral (the left or right) adrenal gland is excised in first stage of surgery, and another myelolipoma in the contralateral adrenal gland will be removed in second stage of surgery (a couple of months later). It is a satisfying treatment scheme to achieve optimal treatment effectiveness with minimized sequelae.

Experiences and lessons

Understanding clinical, imaging diagnostic and histopathological features of bilateral adrenal myelolipomas will facilitate timely diagnosis and treatment of this condition. Two-stage surgical removal of bilateral adrenal myelolipomas appears to be the best treatment option for the patients.

Peer-review

At the outset, the authors are requested to rectify certain typographical errors which are present in the manuscript. The authors are requested to incorporate literature data concerning other reported cases of bilateral adrenal myelolipoma in respect to investigative and surgical methods. This will be helpful in determining the appropriate diagnostic and treatment modalities.

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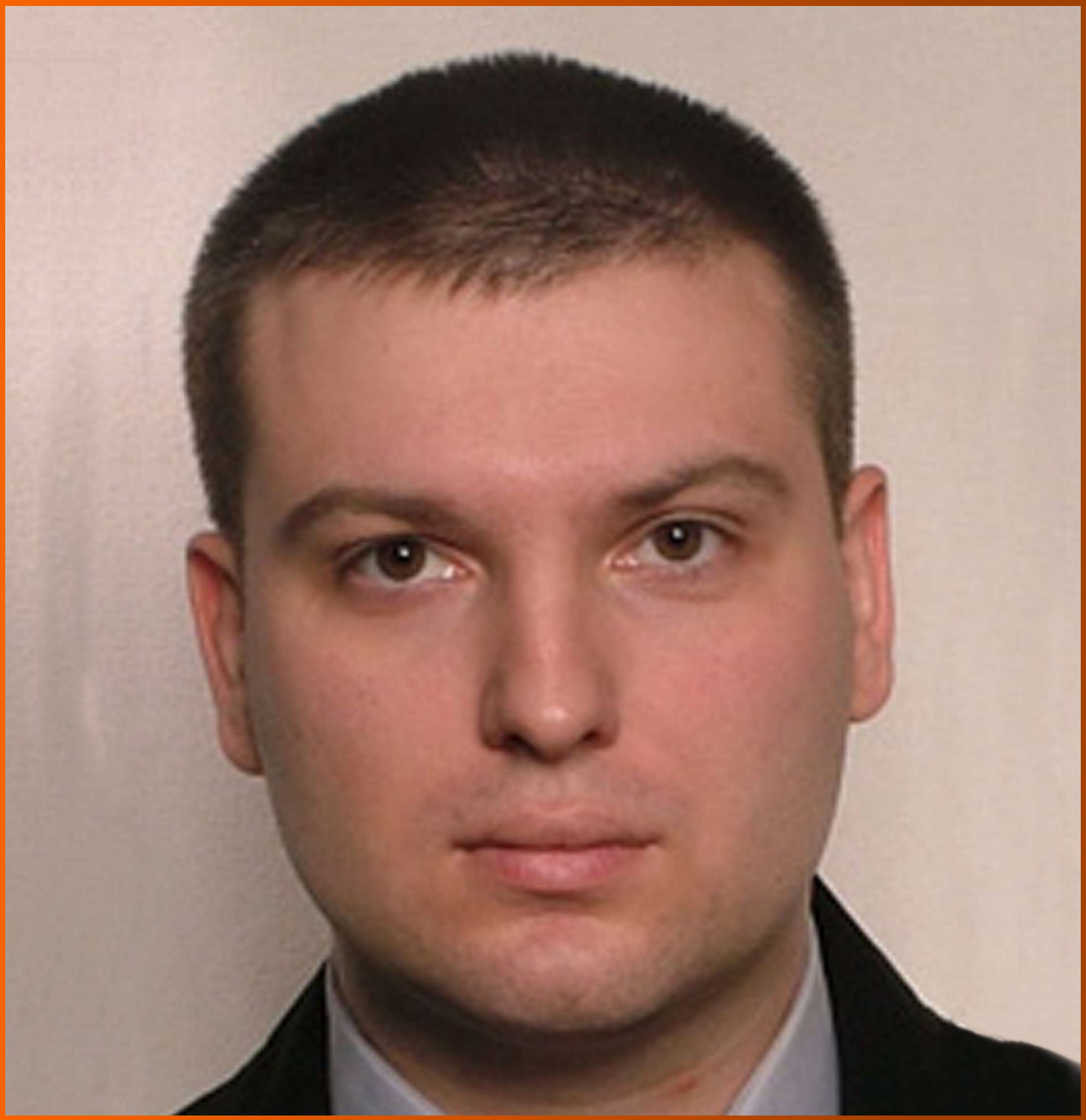

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EDITORIAL

Proliferative verrucous leukoplakia may initially mimic lichenoid reactions

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Abstract

Proliferative verrucous leukoplakia is an intriguing disease, which occurs particularly in women aged greater than 60 years, is not associated with tobacco and alcohol, and has a high risk of recurrence and malignant transformation. Although it is well known that the typical presentation is characterized by multifocal and verrucous white lesions, there is no description that its initial clinical presentation may simulate a lichenoid reaction.

Key words: Proliferative verrucous leukoplakia; Lichenoid reactions; Diagnosis

Core tip: Although uncommon, it is important for the clinician to recognize the main features of proliferative verrucous leukoplakia in order to provide the correct diagnosis and appropriate management.

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PROLIFERATIVE VERRUCOUS LEUKOPLAKIA WITH LICHENOID ASPECT

Proliferative verrucous leukoplakia (PVL) was first described in 1985 as a rare form of oral leukoplakia with a distinct clinical presentation and outcome[1]. This condition more commonly affects non-smoking and non-drinking women, aged greater than 60 years. PVL clinically begins as flat homogenous leukoplakia, becomes multifocal and tends to develop exophytic, wart-like and verrucous areas. Besides this clinical progression, PVL also has a high tendency to recur after treatment and has a high risk of malignant

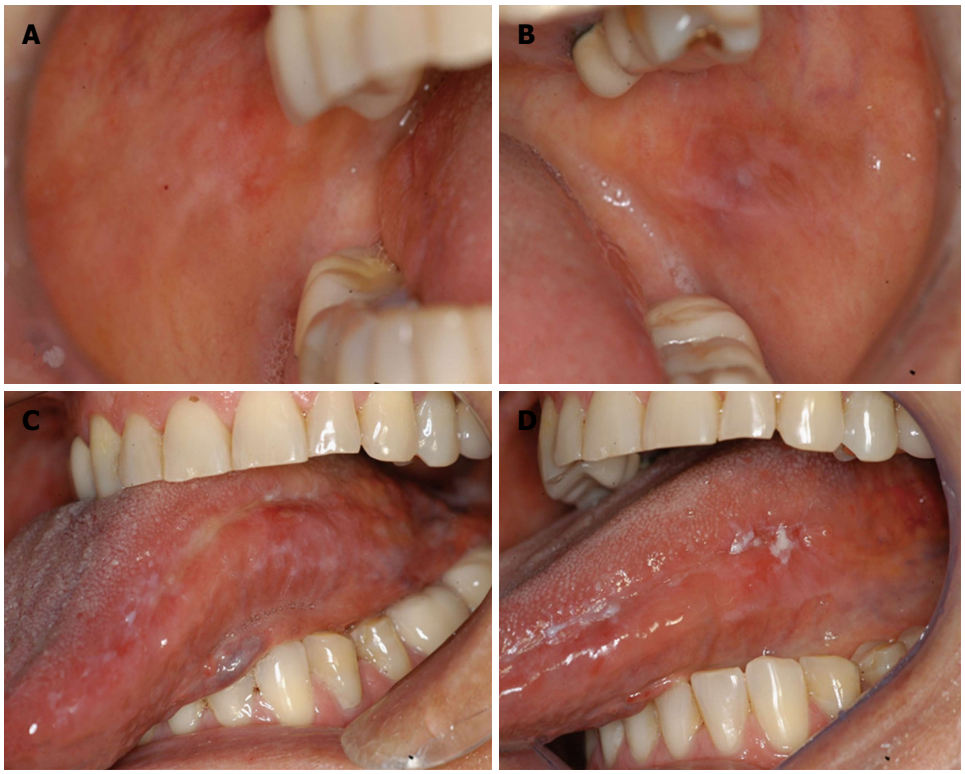

Figure 1 Lichenoid aspect on the right buccal mucosa (A), left buccal mucosa (B) and on the left lateral border of the tongue (C), leukoplakic lesions on the lateral border of the tongue (D).

transformation. According to several reports, malignant transformation rates vary between 33.3% and 100% and depend on many factors, particularly the number of patients and time of follow-up[2-4].

The characteristics reported above are well known and well accepted by the scientific community. However, there are many doubts and controversies particularly regarding etiology, diagnostic criteria and treatment[5]. The diagnosis of PVL is based on the retrospective association of clinical and histopathological features, which basically consist of observing progressive evolution of the lesions from a homogeneous and isolated area to a multifocal presentation with a verrucous appearance. As these manifestations take time, the diagnosis of PVL is often late.

In order to better recognize this condition, diagnostic criteria were recently proposed, which included 5 major and 4 minor criteria, as well as various combinations of these criteria[6]. In this proposal, one of the major criteria is "the presence of verrucous area". However, according to Aguirre-Urizar[7], the diagnosis of PVL may be delayed if verrucous appearance is considered a main diagnostic feature. In this author's opinion, the most important diagnostic criteria for this type of leukoplakia are the "proliferative" and the "multifocal" aspects. Thus, he proposed a new name for this entity: "Proliferative Multifocal Leukoplakia" with the aim of reducing under-diagnosis[7].

When attending patients with this disorder we observed that in some cases it was very clear and simple to establish the diagnosis of PVL as the patients had lesions with peculiar aspects. However, in other situations the lesions may have different clinical features such as erythroplakic changes[8], which may cause some difficulty in diagnosis. In this scenario, close follow-up is necessary and will permit observation of the development of more characteristic lesions such as in the patient presented below.

In May 2011, a non-smoking and non-drinking 64-year-old female patient was referred to our oral diagnosis service complaining of a painful area on the tongue. She reported the onset of a white lesion on the left lateral border of the tongue 3 years before attending our Clinic. At that time, she had been seen by another dental team and it was initially thought to be a fungal infection and she received treatment based on topical antifungals. As no improvement was observed, a lichenoid reaction was suspected and her dental metallic (gold) restorations were replaced and a partial fixed prosthesis was inserted. However, no improvement was observed. As the lesion persisted, an incisional biopsy was performed by an otorhinolaryngologist. Microscopically, the lesion showed moderate epithelial dysplasia and a chronic inflammatory infiltrate in the underlying connective tissue. The patient was then referred for our evaluation and the first visit to our service revealed white striations with atrophic areas on the buccal mucosa bilaterally. She also had similar alterations on the left lateral border of the tongue (Figure 1). An incisional biopsy was performed on the left lateral border of the tongue and another on the right buccal mucosa. Histopathological analysis of

both sites revealed hyperkeratosis and acanthosis with mild epithelial dysplasia. According to these clinical features and the patient's symptoms, she was treated with topical clobetasol 0.05% three times a day. After 3 wk, pain relief was observed. During the follow-up period, areas of leukoplakia were observed on the left lateral border of the tongue (Figure 1). Taking these findings into account, the diagnosis of possible PVL was suggested and the patient was advised about the need for close observation. The patient remained on regular follow-up without clinical modifications. However, after 15 mo the white lesion on the left lateral border of the tongue became more diffuse and another incisional biopsy was performed and the diagnosis of squamous cell carcinoma was established. The patient was then referred to a head and neck surgeon and a partial glossectomy was performed disclosing free surgical margins.

Recently, it was reported that the clinical presentation of oral lichen planus (OLP) has similarities to PVL based on the facts that most patients are females, without a history of tobacco or alcohol use and the presence of multifocal white lesions. In addition, as in OLP, the lesions have a predilection for the gingiva, tongue and buccal mucosa[9]. In addition to the above-mentioned similarities, we noted that some older female patients without tobacco or alcohol habits had lesions that were similar to lichenoid reactions, but the histopathological analysis proved to be hyperkeratosis and acanthosis with variable degrees of epithelial dysplasia. However, the diagnosis of lichen planus or lichenoid reaction was ruled out microscopically, and these patients later developed more leukoplakic lesions consistent with multifocal leukoplakia.

Therefore, we suggest that the initial clinical manifestation in some cases of PVL may mimic OLP or oral lichenoid reaction, and both biopsy and microscopic analysis are mandatory in order to avoid misdiagnosis, and consequently provide better patient management.

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REVIEW

Port site infection in laparoscopic surgery: A review of its management

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Abstract

Laparoscopic surgery (LS), also termed minimal access surgery, has brought a paradigm shift in the approach to modern surgical care. Early postoperative recovery, less pain, improved aesthesis and early return to work have led to its popularity both amongst surgeons and patients. Its application has progressed from cholecystectomies and appendectomies to various other fields including gastrointestinal surgery, urology, gynecology and oncosurgery. However, LS has its own package of complications. Port site infection (PSI), although infrequent, is one of the bothersome complications which undermine the benefits of minimal invasive surgery. Not only does it add to the morbidity of the patient but also spoils the reputation of the surgeon. Despite the advances in the field of antimicrobial agents, sterilization techniques, surgical techniques, operating room ventilation, PSIs still prevail. The emergence of rapid growing atypical mycobacteria with multidrug resistance, which are the causative organism in most of the cases, has further compounded the problem. PSIs are preventable if appropriate measures are taken preoperatively, intraoperatively and postoperatively. PSIs can often be treated non-surgically, with early identification and appropriate management. Macrolides, quinolones and aminoglycosides antibiotics do show promising activity against the atypical mycobacteria. This review article highlights the clinical burden, presentations and management of PSIs in LS as shared by various authors in the literature. We have given emphasis to atypical mycobacteria, which are emerging as a common etiological agent for PSIs in LS. Although the existing literature lacks consensus regarding PSI management, the complication can be best avoided by strictly abiding by the commandments of sterilization techniques of the laparoscopic instruments with appropriate sterilizing agent.

Key words: Laparoscopic surgery; Port site infection; Atypical mycobacteria; Sterilization; Surgical site infections

Core tip: Laparoscopic surgery has brought about a paradigm shift in the approach to various surgical diseases. Port site infection, although infrequent, is a complication which can undermine the benefits of the surgery. The complication is not life threatening, but definitely adds a lot to the morbidity, affects the postoperative quality of life, and spoils the aesthesis of the surgery. Leaving aside the bacterial causes, the rapidly emerging multidrug resistant atypical mycobacteria are a constant threat. By doing a thorough review of this topic, this paper aims to present the relevant literature regarding the diagnosis, currently available treatment options and commandments to prevent the occurrence of this somewhat preventable complication.

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INTRODUCTION

Rapid growths in health care technology have given the surgeon the power of not only treating diseases surgically but also limiting surgical invasiveness. The greatest example is minimal access surgery (MAS) also commonly termed laparoscopic surgery (LS) or keyhole surgery, which has caused a paradigm shift in the approach to modern surgery, by limiting the access related morbidities.

LS involves the use of reusable metallic or disposable plastic trocars inserted through small skin incisions or ports made on the skin away from the site of surgery. This ports form the portal of entry to perform the surgical procedure by means of specially devised instruments and telescope. It has gained popularity due to better aesthesis, lesser pain, early ambulation and discharge from the hospital with early return to work, minimizing the financial burden to the patient. Ever since Philips Mouret reported the first laparoscopic cholecystectomy in 1987, the approach has been adopted for many other surgical procedures including appendectomy, herniorrhaphy, colonic surgery, gastric surgery, urological and gynaecological surgery[1-5]. This is because of the combination of advancement in technology with the increasing acceptance of MAS by patients, which has led to the expansion of the horizon of LS.

LS, however, has its package of unique complications. One such complication, which is preventable although, is the port site infection (PSI). PSI soon erodes the advantages of LS, with the patient becoming worried with the indolent and nagging infection and losing confidence on the operating surgeon. There occurs a significant increase in the morbidity, hospital stay and financial loss to the patient. The whole purpose of MAS to achieve utmost cosmesis is turned into an unsightly wound, and the quality of life of patients is seriously affected.

In this article we review the current literature regarding the incidence, clinical presentation, etiopathogenesis, management and methods of prevention of PSI in LS. We emphasize on the management of PSI due to the emerging rapid growing atypical mycobacteria that do not respond to the standard anti-tubercular drugs.

Incidence of PSIs

No surgical wound is completely immune to infections. Despite the advances in the fields of antimicrobial agents, sterilization techniques, surgical techniques, and operating room ventilation, PSIs still prevail. Incidence of SSI after elective laparoscopic cholecystectomy is less than that after open elective cholecystectomy due to shorter length of incision[6]. The technique of primary port entry to the peritoneum does not show any difference in umbilical PSIs in patients undergoing laparoscopic cholecystectomy[7]. The umbilical PSI rate in LS has been reported to be 8% with 89% of the infections occurring after laparoscopic cholecystectomy, whereas 11% after laparoscopic appendectomy[8]. Francis *et al*[9] studied the factors predicting 30-d readmission after laparoscopic colorectal cancer surgery. Out of 268 patients in their study who underwent laparoscopic colorectal surgery, 48 (18%) were readmitted with surgical site infection (SSI)[9]. Several other authors have found that SSI rate is much higher in conventional surgical procedures than in MAS[10-12]. The immune functions are less affected in LS as compared to open surgery[13]. The incidences of PSI in laparoscopic cholecystectomy as per various studies[14-22] are illustrated in Table 1.

SSIs and PSIs

SSIs are infections consequent to the surgery that are present within a month of the operative procedure. Surveillance in surgeries, such as breast, cardiac, cranial, spinal and bone surgeries, with use of prosthetic material, extends to 90 d after surgery[23-25].

PSI is a type of SSI but limited to LS. The same criteria for SSIs are applicable to PSIs, but the infections are limited to superficial and deep surgical sites only as detailed below.

According to the definitions developed by the United States Centre for Disease Control (CDC), SSIs were categorized into[25]: (1) Superficial SSIs which involve skin and subcutaneous tissue; (2) Deep SSIs which involve fascia and muscle layers; and (3) Organ/Space SSIs.

Wounds are classified as (as per CDC criteria for SSI 2015)[25]: (1) Clean: A surgical wound that is neither exposed to any inflamed tissue nor has breached the gastrointestinal, respiratory, genital, or uninfected

Table 1 Studies showing frequency of port site infection following laparoscopic cholecystectomy

No.	Ref.	Year of publication	Type of study	Total number of patients	Frequency of infection
1	Karthik *et al*[14]	2013	Prospective	570	10 (1.8%)
2	Mir *et al*[15]	2013	Prospective	675	45 (6.7%)
3	Yanni *et al*[16]	2013	Prospective	100	4 (4%)
4	Taj *et al*[17]	2012	Observational	492	27 (5.48%)
5	Yi *et al*[18]	2012	NA	400	11 (2.75%)
6	Triantafyllidis *et al*[19]	2009	Retrospective	1009	14 (1.39%)
7	Chuang *et al*[20]	2004	NA	420	6 (1.4%)
8	Shindholimath *et al*[21]	2003	Prospective	113	7 (6.3%)
9	den Hoed *et al*[22]	1998	Prospective	189	10 (5.3%)

NA: Not available.

urinary tract; (2) Clean-Contaminated: Surgical wounds where there is controlled entry into the gastrointestinal, respiratory, genital, or uninfected urinary tract with minimal contamination; (3) Contaminated: Fresh wounds related to trauma, surgical wounds with major breach in sterile technique or gross contamination from the gastrointestinal tract, and incisions through nonpurulent inflammatory tissues; and (4) Dirty or Infected: Old wounds following trauma having devitalized tissue and surgical procedure performed in the presence of active infection or visceral perforation.

Most of the surgical procedures done by laparoscopy belong to Classes 1 and 2 wounds. The human body hosts a variety of microbes which can cause infections. When the host systemic immunity is suppressed due to any disease, medications or disruptions of the integrity of the skin or mucous membranes secondary to surgical insult, patients' own commensal microbial flora may cause infection. The PSIs in LS manifest in the form of seropurulent discharge from the port sites with surrounding skin inflammation or symptoms related to the organ/space infection.

The active surveillance for PSIs in LS remains a challenge, due to the early discharge and day care setting[10,12]. In the absence of post-discharge surveillance, it is estimated that a third of all SSIs will be missed[26]. The reported incidence of SSIs varies in various regions of the world. The reported incidence of SSIs in a recent article from Turkey was higher than the CDC National Healthcare Safety Network (NHSN) rates[27]. Hence, the actual incidence of the PSIs may be much higher than revealed.

There is a higher incidence of superficial incisional SSIs as compared to that of deep incisional SSIs in LS[12]. The PSI after a LS should be promptly diagnosed and treated appropriately. Although it may not be possible to achieve zero percent PSI, every attempt should be made to prevent it. Insight into the pathophysiology of incision site infections, pathogens involved and knowledge of the appropriate antibiotic is essential for successful management of PSI in LS.

Risk factors for PSIs

A number of contributing factors are somewhat responsible for the emergence of postoperative PSIs. Antibiotics always may not be the answer to this problem. Thus, using them irrationally, as is often done will only result in the emergence of multidrug resistant microbes. The majority of the reports of postoperative wound infection are of SSIs. PSIs following LS have been less reported. The risk factors for SSIs, however, may be applicable to PSIs.

Preoperative stay in hospital: Lilani *et al*[10] reported a significant increase in the incidence of SSIs with preoperative stay of more than 2 d for open surgical procedures.

Duration of operation: The study by Lilani *et al*[10] reported a nil infection rate in surgeries of less than 30 min duration. There was a significant increase in SSIs for operations of prolonged duration for two hours or more.

Other factors: Obesity, prophylactic antibiotics, and drains have no effect on the rate of SSIs following laparoscopic cholecystectomy[28]. Factors like emergency/multi-procedure surgery and surgery in acutely inflamed organs adversely affect the rate of SSIs[20,22]. The risk of SSIs increases in patients with a history of nicotine or steroid usage, diabetes, malnutrition, long preoperative hospital stay, preoperative colonization of nares with *Staphylococcus aureus*, or perioperative blood transfusion[29,30].

PSIs are more common in the umbilical port[12]; the infection rate may depend upon the port through which the specimen is extracted. The infected specimen should be removed in an endobag in order to prevent wound infection and accidental spillage of contents or occult malignant cells. An improvised endobag can be prepared from a simple surgical glove which is easy to make, cheap, readily available and disposable[17].

Microbial flora causing PSIs in LS

PSIs occur due to exposure of surgical wound to microbes which may be from an endogenous or exogenous source. The source of endogenous flora usually is from the patient's skin, mucous membranes or any of the viscera. The exogenous flora may be from any contaminated sources present in the sterile surgical

field including surgeon and team, instruments, room air, *etc*[31].

The pathogenic organisms causing SSIs differ with the surgical procedure performed. Clean surgical wounds usually harbor *Staphylococcus aureus* which may have an exogenous origin or may be from the patient's native flora. Infections in clean-contaminated, contaminated and dirty surgical wounds are polymicrobial, resembling the endogenous flora of the target organ[32].

PSIs are of two broad varieties based on the timing when they are present. The more common type manifests early, within a week of the surgical procedure. Gram positive or negative bacteria are the usual offending organisms which are contracted from the native skin or infected surgical site. They usually respond well to the commonly used antimicrobial agents. The other variety is caused by rapid growing atypical mycobacterium species, which has an incubation period of 3 to 4 wk. They show a poor response to the usual antimicrobial agents[33].

Non-mycobacterial isolates: Kownhar *et al*[34] reported superficial SSIs as the most common in both MAS and open surgical procedures, with *Staphylococcus aureus* as the most common isolate. They studied the SSIs and found various common bacteria isolated as *Staphylococcus aureus* (37%) and *Pseudomonas aeruginosa* (37%), followed by *Klebsiella pneumonia* (8%), *Acinetobacter spp*. (3.2%), *Proteus spp*. (4.8%), *Escherichia coli* (4.8%), *Citrobacter freundii* (1.6%), *Edwardsiella tarda* (1.6%) and *Enterococcus faecalis* (1.6%). *Klebsiella sp.* is the most common offending organism in deep SSIs irrespective of the surgical approach[34]. Usually hospital acquired skin flora cause superficial SSIs. Organisms causing deep SSIs usually are endogenous in origin or may be the skin commensals which reach the fascia or muscle layers through surgical incision[23]. *Bacteroides sp.* was the predominant flora (60%) causing SSIs, in a study reported by Wolcott *et al*[35]. *Bacterioides fragilis* may originate from intraoperative visceral spillage. Mir *et al*[15] in their series found pseudomonas (42.2%) as the common offending organism in PSIs following laparoscopic cholecystectomy. They found that the organisms isolated were resistant to commonly used antibiotics in their hospital[15].

Mycobacterial isolates: Several reports have established the role of rapid growing mycobacteria (RGM), particularly *M. fortuitum* and *M. chelonae* which together have been termed as *M. fortuitum-chelonae complex* that is known to cause disease in humans as well as animals[36]. The endospores of this non-tuberculous mycobacterial (NTM) complex are usually considered saprophytes which colonize in sewage, soil and even tap water. This often cause localized skin infections 3-4 wk post-surgery[37,38]. The NTM complex can cause disseminated disease in immunosuppressive diseases. These atypical mycobacteria have a predilection to involve the skin and subcutaneous tissue. *M. chelonae* and *M. abscessus* have similar characteristics, and hence together were addressed as *M. chelonae/abscessus* group. Vijayaraghavan *et al*[39] reported an outbreak of laparoscopic PSIs due to *M. chelonae* at their center. They had 145 PSIs in 35 patients in a period of 6 wk. The contaminating source was found to be the water being used for washing instruments after chemical disinfection[39]. A series of eight cases of port site tuberculosis after laparoscopy was reported by Ramesh *et al*[40] from India, caused by *M. tuberculosis*.

A case of PSI following laparoscopic cholecystectomy caused by *M. flavescens* has been reported[41]. Duarte *et al*[42] reported an epidemic (74 cases) of postsurgical infections in Brazil, due to *M. massiliense*, after video assisted surgery, which had similar characteristics to *M. abscessus*. Recently, there have been reports of rapid growing mycobacterial infection following laparoscopic gastric banding in obesity[43,44]. Atypical mycobacteria infections following surgery, although rare, are known to occur when a prosthetic material has been used[45].

Clinical presentations of PSIs

Wound discharge and erythema around the port site are the most common presentation of non-mycobacterial infection usually occurring within a week of the surgery. They are usually limited to the skin and subcutaneous tissue[12,14]. There may be surrounding tissue inflammation with pain or tenderness and low grade fever[31].

The delayed type of presentation commonly caused by mycobacteria manifests nearly a month after surgery, in the form of persistent multiple discharging sinuses or lumps/nodules, not responding to antibiotics. There may be pigmentation and induration at the port site starting in a single port and spreading to others.

There are five clinical stages of atypical mycobacterial PSI[46].

First stage: A tender nodule appears in the vicinity of the port site, and its usual timing of appearance is around four weeks following the surgery.

Second stage: Increase in the size of the nodule, and increased tenderness of the site along with other signs of inflammation with eventual formation of a discharging sinus.

Third stage: Reduced pain sensation following discharge of the purulent material and necrosis of the skin surrounding the port site.

Fourth stage: Chronic sinus discharging white or serous fluid.

Fifth stage: Hyper-pigmentation of the skin surrounding the sinus and appearance of multiple nodules at different places.

Diagnosis of the etiological agent with early management

Early PSIs: Gram stains and culture sensitivity of the pus from port site wounds are to be taken. The swabs obtained are processed aerobically and anaerobically by standard methods to find the non-mycobacterial isolates. *Staphylococcus aureus* strains are usually isolated from clean wounds. Their status of β-lactamase production and methicillin resistance needs to be assessed[10]. Daily dressing, cleaning of the wound and a course of empirical antibiotic are started. Specific antibiotics as per the culture and sensitivity report are to be given subsequently. Drainage and debridement may sometimes be required for assisting in wound healing. There are reports of port site abscess presenting as discharging sinus months after surgery due to retained stone at the port site. Wound exploration and removal of the stone is necessary for the healing of such wound[47,48]. Samel *et al*[49] reported a case of gas gangrene of the abdominal wall due to *Clostridial* agents centering around right lateral port following laparoscopic cholecystectomy. There are also reports of life threatening necrotizing fasciitis of the abdominal wall following LS. Significant erythema and wound discharge around the port site along with fever are signs of necrotizing fasciitis[50,51]. A high grade of suspicion and aggressive management are necessary to deal with these life threatening bacterial infections.

Delayed PSIs: Chaudhuri *et al*[46] have shown a raised C-reactive protein level without leukocytosis and a normal differential count in patients with atypical mycobacterial infection[46,52]. Tissue or fluid obtained by biopsy or aspiration needs to be processed for baciloscopy, culture in Lowenstein-Jensen medium and BACTEC technique (Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md). Isolation of the atypical mycobacteria by tissue culture is possible, although it takes time to grow. Moreover, maintaining the stringent environment for its culture is difficult. The most accurate method for rapid presumptive identification of *M. chelonae* is detecting resistance to polymyxin B disc (300 μg)[53]. The routine culture of pus does not grow any bacteria. The diagnosis is often based on the clinical signs and a high index of suspicion[52]. In case of growth of the organism, the isolate is to be confirmed by either biochemical reactions or the more recent nucleic acid amplification tests. Other investigations like tissue culture, real time-PCR, and serology for antitubercular antibody can support the diagnosis[53]. Even these reports are not full proof, as these tests could give a false positive result. The histopathological examination at times may reveal chronic granulomatous inflammation, comprising of epitheloid cells and lympho-plasmacytic infiltration[40].

Treatment of PSIs

Early PSIs, with bacterial isolates, are best managed with local wound care and antibiotics as per antibiogram. The study by Lilani *et al*[10] in clean and clean contaminated cases revealed *Staphylococcal sp.* as the most common isolate, which was resistant to penicillin. The isolates of *Pseudomonas aeruginosa* were totally resistant to gentamicin[10]. Mir *et al*[15] found most of the isolated strains of organisms causing SSI in elective laparoscopic cholecystectomy were resistant to antibiotics used in the hospital. They found the *Pseudomonas sp.* to be sensitive to imipenem in 89.47% of cases, but there was complete resistance to the combination of ampicillin and sulbactam and ceftrixone[15].

Management of PSIs with atypical mycobacteria lacks consensus. They respond poorly to first line anti-tubercular drug treatment. Second line anti-tubercular drugs including macrolides (clarithromycin), quinolones (ciprofloxacin), tetracyclines (doxycycline) and aminiglycosides (amikacin and tobramycin) in various combinations have been used with promising results[37,46,54]. Macrolides including clarithromycin are the only group of antimicrobials active against *M. chelonae* and *M. abscessus*[54]. *Mycobacterium fortuim-chelonae* complex has shown resistance to antibiotics because of mutation in the porin channels present in the bacterial wall, which is the site for entry of antibiotic molecules for antimicrobial activity[46,55]. Linezolid was found to be active against *M. chelonae* and has been successfully used for treatment, alone or as combination therapy[56]. The various antibiotics effectively used against the mycobacterial PSIs, as reported in various studies, are described in Table 2.

Prevention of PSIs

The million dollar question is why at all there occur PSIs in clean and clean contaminated wounds after LS. Is it because of the contamination from the endogenous source or through exogenous source? The endogenous source of infection cannot be avoided. But the incidence of PSIs after LS due to endogenous cause can be reduced by using sterile endobag for specimen retrieval.

The exogenous source of infection, however, is avoidable. Non-tuberculous mycobacteria may be present in water from various sources and soil which can contaminate hospital instruments. A breach in sterilization protocol of laparoscopic instruments is the most common cause of PSI with atypical mycobacteria[46]. The infection with atypical mycobacteria is usually limited to the laparoscopic procedure, as most of laparoscopic instruments are not autoclavable because of the heat sensitive outer insulation sheath. Moreover, as most of the laparoscopic instruments have multiple joints and crevices, where blood and tissue can collect. Frequent use of the instrument without optimal cleaning potentially results in contamination with organisms such as atypical mycobacteria. Endospores in the contaminated instrument get deposited in the subcutaneous tissue, which germinate in three to four

Table 2 Different antibiotics effectively used against *Mycobacterial sp.* in port site infections

Ref.	Type of study	Mycobacteria isolated	Treatment given
Ramesh *et al*[40]	Case series in 8 patients	*M. tuberculosis*	Standard first line antitubercular regimen Rifampicin, isoniazid, pyrazinamide and ethambutol for 2 mo followed by rifampicin and isoniazid for 9 mo
Chaudhuri *et al*[46]	Case series in 19 patients	Clinically suspected atypical mycobacterial infection. No isolates in culture	Clarithromycin and ciprofloxacin (500 mg each, twice daily) for 28 d to 3 mo For persistent local nodules, direct injection of amikacin injections into the nodules daily for 5 d (500 mg twice daily)
Verghese *et al*[37]	Case report	*M. chelonae*	Amikacin 750 mg/d and azithromycin 500 mg BD for 2 wk, followed by linezolid 500 mg BD and azithromycin 500 mg BD for 6 wk
Duarte *et al*[42]	Case series in 74 patients	*M. massiliense*	Sensitive to amikacin and clarithromycin, but resistant to ciprofloxacin, cefoxitine and doxycycline
Sethi *et al*[41]	Case report	*M. flavescens*	Ofloxacin and amikacin for 6 mo
Shah *et al*[61]	Case series in 7 patients	*M. fortuitum* *M. chelonae*	Clarithromycin and ciprofloxacin (500 mg each, twice daily) for 6-9 mo
Rajini *et al*[62]	Case report	*M. chelonae*	Clarithromycin 500 mg BD and doxycycline 100 mg OD for 4 wk

weeks to produce clinical signs and symptoms[42]. A study by Lorena *et al*[57] on *M. massiliense* BRA100 strain showed that it is resistant to even higher concentration of glutaraldehyde (GTA, 7%). Hence, they proved that GTA may not be effective for RGM. Other liquid sterilizing agents like orthophthaldehyde and per acetic acid may substitute GTA for high level disinfection with good efficacy[57].

Ten commandments for preventing PSI[58-61]: (1) Use of disposable trocars and instruments, and adequate availability of properly sterilised reusable trocars to cover all the surgical procedures in a day; (2) Use of autoclavable laparoscopic hand instruments; (3) Use of instruments with good ergonomics, limited joints and facility for proper cleaning of the debris collected in its crevices; (4) A proper cleaning of the instrument is best achieved by ultrasonic technology. Use of autoclaved water for cleaning the instruments after dismantling; (5) Proper guidelines should be followed regarding the concentration, contact time and cycles of use for instrument sterilization with liquid sterilizing agents; (6) Use of plasma sterilizer or ethylene oxide in between the consecutive surgery for instrument sterilization; (7) Avoiding inter-departmental sharing of instruments, such as using instruments used for gynecological or urological procedures; (8) Avoiding spillage of bile or gut content in the operative area or the port site; (9) Use of non-porous specimen retrieval bags for retrieving the specimen; and (10) Thorough irrigation and cleaning of the port site before wound closure.

CONCLUSION

PSI, although infrequent, can be a frustrating complication in MAS, both for the patient as well as the operating surgeon. Leaving aside the bacterial causes, the emerging rapid growing multidrug resistant non-tuberculous mycobacteria are a new threat to the surgical fraternity. Strictly abiding by the commandments of cleaning and sterilization of the laparoscopic instruments, with the appropriate sterilizing agent, the complication can be best avoided.

This review is likely to aid in understanding the relevant studies regarding the appropriate management of PSIs in LS. All the cases of PSI, especially of the atypical mycobacterium should be notified to know the exact incidence, etiology and the sensitivity pattern to various antibiotics. Macrolides, quinolones and aminoglycosides do show promising activity against the atypical mycobacterium. Further research is needed to find out appropriate guidelines for the diagnosis and treatment of this emerging problem.

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MINIREVIEWS

Arrhythmogenic epilepsy and pacing need: A matter of controversy

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Abstract

There is increasing awareness among the cardiology community regarding ictal bradyarrhythmias as a cause of loss of consciousness. A high degree of suspicion is necessary when diagnosing ictal bradyarrhythmias, and delay in diagnosing this condition may lead to morbidity associated with falls and trauma. Ictal bradyarrhythmias have also been suggested to be associated with sudden unexplained death in epilepsy, although evidence related to this association is limited. There is no guideline-directed therapy for symptomatic ictal bradyarrhythmias due to a lack of randomized, controlled trials. Cardiac pacemaker therapy is commonly used for these patients; however, currently, there is no universal agreement on the pacing indications for these patients. In this review, we focus on the pathophysiology and clinical presentation of ictal bradyarrhythmias and then discuss the pacing need based on the available literature data.

Key words: Arrhythmogenic epilepsy; Syncope; Ictal bradyarrhythmia; Pacemaker; Anticonvulsive therapy

Core tip: There is increasing awareness among the cardiology community regarding ictal bradyarrhythmias as a cause of loss of consciousness. Pacing is commonly used therapy for symptomatic ictal bradyarrhythmias. However, currently, there is no universal agreement on the pacing indications for these patients due to lack of randomized, controlled trials. In this review we will first focus on pathophysiology and clinical presentation of ictal bradyarrhythmias and then try to discuss the pacing need based on the available literature data.

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INTRODUCTION

Epileptic seizures have been associated with a variety of systemic and autonomic manifestations.

Cardiovascular autonomic manifestations include alterations in heart rate and rhythm, blood pressure and electrocardiography (ECG)[1].

Sinus tachycardia is the most frequently observed arrhythmia in patients with epilepsy, with a reported a frequency of 60%-100%[1-3]. Heart rate acceleration has been shown to precede, follow or coincide with seizure[1]. Bradyarrhythmias are rarely observed, occurring in less than 5% of all seizures[1,4]. Sinus bradycardia, AV block and prolonged asystole have been reported in a variety of case reports and case studies[1]. Most episodes have been shown to occur in the ictal state by simultaneous electroencephalography (EEG) and ECG monitoring systems. Ictal asystole (IA) has been reported to be observed in 0.27%-0.4% of patients during prolonged video EEG telemetry[5,6]. Although rare, ictal bradyarrhythmias have substantial morbidity because they are related with sudden loss of consciousness (LOC), which may lead to falls and traumatic injuries. Ictal bradycardia (IB) and IA have also been suggested to be associated with sudden unexplained death in epilepsy (SUDEP), although evidence of association is limited[7-9].

This review will focus on the pathophysiology and clinical presentation of ictal bradyarrhythmias and discuss the pacing need based on the available literature.

PATHOPHYSIOLOGY

The pathophysiology of ictal bradyarrhythmias is not entirely clear, and complex pathways have been believed to be involved in the central nervous system. Most seizure-related bradyarrhythmias have been observed in individuals with temporal lobe epilepsy and appear to be less frequent in patients with seizures originating from the frontal lobes and other brain regions[8]. It has been hypothesized that seizure-related stimulation of certain brain regions, such as insular cortex, cingulate cortex, amygdala and hypothalamus, interferes with autonomic control of the heart *via* connections with autonomic nuclei of the brain stem and spinal cord[10]. Seizure-induced stimulation of the central nervous system has been suggested to directly affect postganglionic discharges on the heart[11]. A recent comprehensive review of literature data on seizure-related cardiac arrhythmias reported that ictal bradyarrhythmias have been observed during focal dyscognitive seizures and that they were mostly commonly observed in individuals with temporal lobe epilepsy[12]. Some studies have suggested lateralization of foci related to ictal arrhythmias; *i.e.*, seizures originating from the right hemisphere have been suggested to be more frequently associated with ictal tachycardia and seizures originating from left hemisphere with ictal bradyarrhythmias[5,13,14]. However, there are inconsistent data in the literature on this lateralization hypothesis[15,16].

CLINICAL PRESENTATION

Sudden LOC is the major manifestation of prolonged IA related to complex partial seizures. Clinical presentation with sudden LOC and related falls, as well as subsequent trauma, may be similar in clinical presentation to vasovagal syncope. Schuele *et al*[17] described similar heart rate patterns during asystolic events in patients with IA and vasovagal asystole, with a tendency for tachycardia preceding the asystolic event, which then evolved into progressive bradycardia and asystole. Based on these observations, the authors suggested that both IA and vasovagal asystole might be mediated through a similar mechanism, leading to increase in vagal tone. Cerebral hypoperfusion related to prolonged asystole appears to be responsible for sudden LOC in patients with IA rather than seizure-induced activation of cortical or subcortical regions. However, absence epilepsy should also be considered in patients with sudden impairments of consciousness. Absence epilepsy is primarily observed in children and adolescent patients and is characterized by sudden cessation of movement without convulsions, impairment of consciousness, fixation of gaze and sudden termination of the epileptic episode without postictal depression[18]. Absence seizures are typically accompanied by bilateral 3-4 Hz spike-wave discharges on EEG[18].

Arrhythmogenic epilepsy should be considered in the differential diagnosis of patients with syncope[10,19]. Ictal bradyarrhythmias should particularly be suspected in patients with epilepsy and syncopal episodes[10]. IA and symptomatic IB are commonly associated with complex partial seizures. Patients commonly present with seizure-related symptoms, such as staring, unresponsiveness, epigastric aura and oroalimentary and manual automatisms, preceding the syncope[20]. Thus, patients with atypical signs and symptoms before a syncopal episode should also be evaluated for the presence of arrhythmogenic epilepsy. IA or symptomatic IB may also be the first ictal manifestation of new onset epilepsy, and a high degree of suspicion is necessary for diagnosis. Recently, Giovannini *et al*[21] published a literature review on IA cases (31 patients from 21 articles) in the context of new-onset/newly diagnosed epilepsy. They reported that symptoms suggestive of partial seizures preceding syncope were absent for most patients. Only 7 patients have been reported to display symptoms such as visual illusion, hallucinations, jamais vu, fear, psychic aura and epigastric aura prior to syncope. Four patients have been reported to display seizure-related motor activities, such as tonic-clonic contractions and automatisms. Simultaneous long-term video EEG and ECG recording appears to be the key diagnostic modality for arrhythmogenic epilepsy[21]. Long-term subcutaneous implantable loop recorders have also been useful in selected cases[22,23].

LITERATURE DISCUSSION

There is no guideline-directed therapy for IA or symptomatic IB due to the lack of randomized controlled trials[24]. Therapeutic options for symptomatic ictal

bradyarrhythmias include anticonvulsive medications, epileptic surgery and/or cardiac pacemaker implantation. Currently, there is no universal agreement on the pacing indications for these patients. Some authors have suggested that IA is a benign phenomenon, and long-term data regarding the effectiveness of pacemaker therapy for IA are missing due to low recurrence rates[17]. Schuele *et al*[25] and Moseley *et al*[26] suggested that IA promotes seizure termination by causing cerebral ischemia/anoxia. However, case studies have indicated that pacemaker implantation may reduce seizure-related falls and injuries[24,27-29]. Giovannini *et al*[21] reported that most patients (21 of 31 patients) with IA in the context of new-onset/newly diagnosed epilepsy had undergone pacemaker implantation at the time of case report publications, although outcome data for these patients are unknown. Other studies have reported some discordant outcome findings after pacemaker implantation in patients with ictal bradyarrhythmias. Ghearing *et al*[27,28] reported outcome data for 7 patients with IA who had falls and LOC prior to pacemaker implantation. Only one patient experienced seizure-related falls after pacemaker implantation at a mean follow-up duration of 27 mo. Schuele *et al*[6] performed a database search for 6825 patients undergoing long-term video EEG monitoring for episodes of IA and found that IA was recorded in 10 patients (0.27% of all patients with epilepsy). Pacemaker implantation had been performed in 6 of these patients, and none of these patients reported recurrent IA or significant bradycardia leading to pacemaker activation. However, 4 patients had been reported to have recurrent and multiple seizures after pacemaker implantation. Moseley *et al*[29] reported the outcome data of seven patients with IA who had a pacemaker implanted in their institution between 1990 and 2004. The authors stated that the mean fall rate was significantly reduced from 3.28 to 0.005 falls/month after pacemaker implantation. Seizure-related fractures and motor vehicle accidents were also reduced following pacemaker implantation.

Strzelczyk *et al*[24] reviewed 16 patients with IA or IB from 4 epilepsy centers who had been evaluated between 2002 and 2009. They reported that pacemaker implantation had been performed in 7 of these patients (43.8%). Outcome data were available for 43 patient-years. Accordingly, 5 patients (31.3%) were seizure-free in the follow-up period; 2 of these patients had experienced epilepsy surgery, 2 had received anticonvulsive therapy, and 1 had received pacemaker implantation. Nine patients (56.3%) had persisting seizures but without seizure-associated falls; 3 of these patients had received anticonvulsive therapy, and 6 had received pacemaker implantation. Two patients (12.5%) who denied epileptic surgery and did not receive pacemaker implantation had persisting seizures and continuous falls. Based on these observations, the authors proposed a clinical algorithm for treating patients with symptomatic ictal bradyarrhythmias. They recommend that cardiac pacemaker should be considered for symptomatic patients after optimizing antiepileptic therapy and discontinuing any coexisting arrhythmogenic medications. Recently, Bestawros *et al*[30] reported outcome data of 8 patients with IA who received pacemaker therapy. The authors stated that all patients remained free of syncope during a follow-up of 72 ± 95 mo.

Although most patients continued to have seizures after pacemaker implantation in the above-mentioned studies, some papers have suggested decreases in the number of seizures and in seizure intensity after pacemaker implantation[31,32]. The mechanism of this unexpected finding is unclear; however, it has been suggested to be related to the effect of cardiac pacing on cardiac vagal afferents and their connections to the brain[29]. However, in our opinion, a placebo effect of cardiac pacemaker implantation cannot be excluded, similar to the suggestion for vasovagal syncope[33].

CONCLUSION

There is increasing awareness for ictal bradyarrhythmias as a cause of LOC in the cardiology community. A high degree of suspicion is necessary for diagnosing ictal bradyarrhythmias, and a delay in diagnosing this condition may lead to substantial morbidity for the patient. Based on the available data, a cardiac pacemaker might be related to decreased morbidity associated with falls and trauma. However, literature data also suggest that optimization of anticonvulsive therapy might be effective in preventing ictal bradyarrhythmias. In our opinion, pacemaker therapy should be reserved for patients who remain symptomatic after optimizing anticonvulsive therapy. Currently, no data are available related to any effect of cardiac pacemaker implantation on preventing SUDEP. Such evidence would be very useful for a potential indication of pacemaker therapy. The results of a randomized controlled study are urgently needed to clarify the pacemaker need in patients with ictal bradyarrhythmias.

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Disease that should be remembered: Sacrococcygeal pilonidal sinus disease and short history

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Abstract

Pilonidal sinus disease has led to heated debates since it was first described in the medical literature. Although a consensus has been built on its etiology and pathogenesis, the same course has not progressed for treatment modality. This review is a short article about the process of pilonidal sinus disease from past to present. Some important points were mentioned between the years 1833, which is accepted as the milestone for the awareness of the disease, in which it was first reported until the year of 1880, in which it was given its name. Although its name has been the same for about two centuries, some other names such as "Jeep Disease" have also been used depending on the population affected by the disease. At present, it is indisputable that the disease is acquired. Large series were presented about the treatment in the last two decades. Some surgical methods were even named after the ones who first described them and they have many supporters. However, since the treatment modalities have some advantages and disadvantages and they do not have marked superiority over others, debates still continue. We hope that pilonidal sinus disease will not lose its significance and be underrated in parallel with the developments in technology and specialization in medicine.

Key words: Pilonidal sinus; History; Anorectal disease

Core tip: Pilonidal sinus disease has been a debate for about 2 centuries, about which many articles and reviews have been written until now. In this paper, some points that can be accepted as milestones were chronologically presented from the date in which it was first described until today. Since the debates still continue and there is no consensus on the treatment, we suggest that the debates will continue. For this reason and since this article shortly and clearly explains pilonidal sinus disease milestones, we think that it will contribute to the surgeons dealing with the issue.

Kanat BH, Sözen S. Disease that should be remembered: Sacrococcygeal pilonidal sinus disease and short history. *World J Clin Cases* 2015; 3(10): 876-879 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i10/876.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i10.876

INTRODUCTION

Science, medicine in particular, cannot be evaluated without writing. Unwritten things are unreadable. Therefore, unread science and interventions cannot be learned and cannot be known. The basic rules of knowledge of medical experiences and interventions are written. It should be published for people to reach, after it is written; so the information can be transferred to the new generations.

HISTORICAL OVERVIEW

Therefore, the beginning dates of many diseases as old as the history of humans is the date that they were written for the first time. This date is 1833 for pilonidal sinus disease. Herbert Mayo, British Physiologist, Anatomist and Surgeon (3rd April 1796-28th June 1852), described it as a sinus containing hair follicles located in the sacrococcygeal region in a woman, in 1833[1,2].

Afterwards, an article named "Hair Extracted from an Ulcer" published by Anderson[3] in "Boston Medical Surgical Journal" in 1847 was found. He reported a case of a 21-year-old male with a Scrophuloderma on his back, in his article written as a letter to the editor. He reported that he drained the cavity after 3 wk and a structure looking like a mesh made of multiple hairs of 2 inches long and after complete drainage and cleaning of the hair in the cavity, the wound healed quickly[3,4]. Seven years later, in 1854 Warren reported 3 similar cases and this study is the first case series known in the history of pilonidal sinus disease[5].

The disease was given many names until 1880. Widely used ones are; sacral, coccygeal or sacrococcygeal infundibulum, dermoid and dermoid fistula, congenital dermal sinus and sacrococcygeal ectodermal sinus[6].

Eventually, in 1880, Hodges[7] named the disease with the statement of "I venture to give the name of pilo-nidal (pilus, a hair, nidus, a nest) sinus to this rather singular lesion." He produced the word "pilonidal" by conjoining the word "pilus" which means hair in Latin and "nidus" which means nest[8].

ETIOLOGY AND PATHOGENESIS

Discussions about pilonidal sinus disease are still hot even though it was described 200 years ago. In the previous years, there were many fevered arguments and many theories to describe whether the disease is congenital or acquired.

80 years ago, Gage[9] reported that pilonidal cyst and sinuses are congenital and he was supported. According to the congenital disease theory, it might have originated from caudal remnants of the neural tube, dermal inclusions produced by sequestrated epithelial structures or dermal tractions that are produced during the involution of the tail during embryonic development[9-11].

The disease was a commonly seen problem among soldiers in World War Ⅱ, during which important explorations and developments were seen in medicine. It was detected to be particularly common among jeep drivers. It was emphasized that compression and irritation reaching the coccyx is important in the etiology and Buie[12] stated that the disease is acquired in his article named "The Jeep Disease".

In 1946, after the war, Patey and Scarff[13] demonstrated that it might be seen in other regions of the body. He wrote that a granulomatous reaction takes place following hair penetration of sub dermal tissue. In addition, he claimed that it is acquired, as it is also seen in the hands of barbers. Afterwards the idea of the disease being acquired became stronger with articles written by King[14,15] in 1947 and 1950.

Two important names that shook the last 20 years of modern surgery in pilonidal disease supported and explained acquired disease theory as the discussions go on. Bascom[16-19] says: "Only the bones get up when people stand up. Sacrum has to stick on to and pull up skin, fat and muscles to move the buttocks. This pulling process produces a vacuum effect all over the gluteal region. Hair enters the pit in case of a minor folliculitis as a result of the vacuum produced by the movement of the gluteal region".

Karydakis[20], who published the largest pilonidal sinus case series in 1992, developed the most logical theory about the etiology and etiopathogenesis of the disease. He reported as a result of his 35 years of work on pilonidal sinus that the etiology is acquired. Especially minor local trauma is the most important predisposing factor of the disease. Hair penetration process is the basis of pilonidal sinus according to Karydakis[21]. Three main factors play a role in embedding of hair: Invaders formed by free hair (H-hair), the force that provides hair embedding the (F-force), and the vulnerability of the skin that lets the embedding of the hair deeper in the gluteal region (V-vulnerability). Pilonidal sinus disease develops in cases in which these three factors are present together and the disease development possibility could be calculated with HxFxV formula[20,21]. As a result, recently most of surgeons are in the opinion that the disease is acquired.

TREATMENT

What about the treatment besides the discussions about the name and etiology? No consensus is obtained about the treatment even though tens of treatment options are written and discussed.

One might think who cares about the treatment of a pilonidal sinus as there are many life threatening diseases in the field of general surgery. However tens of surgical and non-surgical treatment options are described. Discussions continue as the treatment options have advantages and disadvantages, and no option is preferable to the other ones significantly. Different surgical procedure descriptions and modification of surgeons' different procedures, lead to increase the numbers of surgical techniques[22].

The ideal treatment for pilonidal disease should be simple, with short hospitalization, less pain, local anesthesia if possible, low cost, the patient should go back to daily activities in a short time and recurrence rates should be low after treatment. Combination of all these measures is not possible for all treatment options. Therefore, treatment procedures must be planned according to the patient.

Conservation or a surgical method should be chosen when the treatment is planned according to the patient. Unnecessary surgical operations should be avoided for patients that could be treated conservatively and also time and workforce waste should be avoided for a patient that requires surgical treatment by trying a conservative treatment.

Many surgical techniques are present from simple surgical treatment methods such as incision, drainage, unroofing, curettage, and secondary healing, to the described and modified techniques such as excision flap, Karydakis, Bascom, MacFee[16-23]. In addition, conservative methods such as phenol solution, crystalized phenol technique, cauterization, and alcohol injection have also been used[24-27]. No consensus was obtained as all authors advocate their own method. Treatment has to be planned according to the disease and the patient. Natural evaluation, recurrence reasons of the disease must be known very well and the state of the sacrococcygeal region should be evaluated carefully.

Pilonidal sinus caused interest in many aspects. Many materials such as the effect on quality of life and relationship with hormones were investigated and found place in the literature[25,28]. Besides all these processes there is consensus about the symptoms and clinical presentation of the disease. Patients present with 4 different forms as symptomatic, acute pilonidal abscess, chronic fistulizing form or complex pilonidal sinus disease. Chronic fistulizant form is the most common clinical presentation[26].

Where and how does the pilonidal sinus disease stand in general surgery? General surgeons used to take care of orthopedic emergencies, plastic, cardiovascular and thoracic surgery in 1950s. However, increased number of specializations emerged with the development of technology. Today, especially after the millennium a big portion of general surgeons in academic field are interested in specific fields of general surgery. Pilonidal sinus became a part of colorectal surgery as many diseases are addressed to specific fields. For example surgeons and centres interested in hepatobiliary surgery, peripheric vascular surgery or transplantation surgery are distant to the subject.

I hope, surgeons working outside of big centres with specialization in specific surgical fields and colorectal surgeons will continue to pay adequate attention and each of us will take his/her part.

In surgery, there is no such thing as major or minor. Therefore, pilonidal sinus disease should not be underestimated. Sometimes treatment might disappoint both the surgeon and the patient. At a point that you think everything is going very well, you are face to face with repeating surgeries, insecurity and dissatisfaction of the patient, and fear of surgical failure.

With the hope that pilonidal sinus is never underestimated nor forgotten…

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ORIGINAL ARTICLE

Retrospective Study

Improved bowel preparation increases polyp detection and unmasks significant polyp miss rate

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Author contributions: Papanikolaou IS, Polymeros D, Dimitriadis GD and Triantafyllou K conceived the idea and designed the study; Papanikolaou IS and Magdalinos N performed the research; Beintaris I, Lazaridis LD and Malli C collected the data; Triantafyllou K analyzed the data; Papanikolaou IS, Sioulas AD and Triantafyllou K drafted the manuscript; all authors read and approved the final manuscript.

Institutional review board statement: IRBS was considered to be non-obligatory given that patients received the standard-of-care treatment in both endoscopic departments. Additionally, our retrospective research project involves use of existing information collected from human participants, but there are not any identifiers linking individuals to the data.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: None to declare.

Data sharing statement: There is no additional data available.

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Abstract

AIM: To retrospectively compare previous-day *vs* split-dose preparation in terms of bowel cleanliness and polyp detection in patients referred for polypectomy.

METHODS: Fifty patients underwent two colonoscopies: one diagnostic in a private clinic and a second for polypectomy in a University Hospital. The latter procedures were performed within 12 wk of the index ones. Examinations were accomplished by two experienced endoscopists, different in each facility. Twenty-seven patients underwent screening/surveillance colonoscopy, while the rest were symptomatic. Previous day bowel preparation was utilized initially and split-dose for polypectomy. Colon cleansing was evaluated using the Aronchick scale. We measured the number of detected polyps, and the polyp miss rates per-polyp.

RESULTS: Excellent/good preparation was reported in 38 cases with previous-day preparation (76%) *vs* 46 with split-dose (92%), respectively (P = 0.03). One

hundred and twenty-six polyps were detected initially and 169 subsequently ($P < 0.0001$); 88 *vs* 126 polyps were diminutive ($P < 0.0001$), 25 *vs* 29 small ($P = 0.048$) and 13 *vs* 14 equal or larger than 10 mm. The miss rates for total, diminutive, small and large polyps were 25.4%, 30.1%, 13.7% and 6.6%, respectively. Multivariate analysis revealed that split-dose preparation was significantly associated (OR, *P*) with increased number of polyps detected overall (0.869, $P < 0.001$), in the right (0.418, $P = 0.008$) and in the left colon (0.452, $P = 0.02$).

CONCLUSION: Split-dose preparation improved colon cleansing, enhanced polyp detection and unmasked significant polyp miss rates.

Key words: Colonoscopy; Bowel preparation; Polyp miss rate; Polyp detection; Colorectal cancer

Core tip: Colonoscopy and polypectomy are currently considered as the gold standard to prevent colorectal cancer. However, a significant proportion of precancerous lesions are missed during the procedure, limiting its efficacy and giving rise to interval cancers. Adequate bowel cleanliness represents a major factor with regards to colonoscopy quality. This study demonstrates that split-dose bowel preparation results to significantly better mucosal cleansing compared to previous-day preparation. Moreover, we showed that preparation with the split-dose regimen significantly enhanced polyp detection, especially of the diminutive ones. Finally, better inspection of the colonic epithelium unmasked a notable polyp miss rate.

Papanikolaou IS, Sioulas AD, Magdalinos N, Beintaris I, Lazaridis LD, Polymeros D, Malli C, Dimitriadis GD, Triantafyllou K. Improved bowel preparation increases polyp detection and unmasks significant polyp miss rate. *World J Clin Cases* 2015; 3(10): 880-886 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i10/880.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i10.880

INTRODUCTION

Colonoscopy is currently regarded as the modality of choice, in order to reduce the incidence of colorectal cancer (CRC) and its associated mortality[1]. The rationale behind this is its ability to detect and remove polyps that represent precancerous lesions[2,3]. However, interval CRC, namely cases that are diagnosed between screening and post-screening surveillance examinations, do exist[4,5]. The majority of them are thought to originate from missed polyps during colonoscopy. Polyp and adenoma miss rates reach 28% and 24%, respectively, in several studies reducing colonoscopy preventive efficacy against CRC[6,7].

Numerous technical-, patient- and endoscopist-related factors influence the detection of polyps during colonoscopy[8-11]. In this setting, international associations of endoscopy include adenoma detection rate (ADR) among principal colonoscopy quality indicators[12,13]. Poor bowel preparation is regarded as an impediment to the detection of both small and large polyps[14]. Therefore, multiple interventions have been proposed to improve bowel cleansing and thus increase the quality of colonoscopy[15-17].

Using a tandem colonoscopic evaluation we investigated the impact of different timing of purgative administration in colon cleansing and polyp detection. Polyp miss rates, as well as variables affecting polyp detection were also assessed.

MATERIALS AND METHODS

Study population

This retrospective study was performed on a consecutive series of patients from January to December 2012. All patients were diagnosed with colon polyps during colonoscopy in a small private clinic on an island near Athens and were referred for polypectomy in the Endoscopy Unit of "Attikon" University General Hospital. Exclusion criteria included: (1) age less than 18 or more than 80 years; (2) history of bowel resection; (3) history of inflammatory bowel diseases; (4) suspicion of polyposis syndrome; (5) incomplete colonoscopy (in one of the two examinations); (6) poor bowel preparation as assessed with the Aronchick scale; and (7) ongoing anticoagulation treatment.

Bowel preparation

Prior to the index colonoscopies, patients received the full dose of a 4-L polyethylene glycol (PEG) regimen (Fortrans, Ipsen, Athens, Greece) in the previous day. On the other hand, split dosing (3 L on the previous and 1 L on the same day) was preferred for the subsequent colonoscopies. In all cases patients were advised to maintain a low-fiber diet during the day preceding the examinations.

The quality of bowel cleansing was evaluated by the performing endoscopists using the Aronchick scale. This assesses the preparation quality of the entire colon as excellent (a small volume of clear liquid or greater than 95% of the surface seen), good (a large volume of clear liquid covering 5% to 25% of the surface but greater than 90% of the surface was seen), fair (some semisolid stool that could be suctioned or washed away, but greater than 90% of the surface was seen), poor (semisolid stool that could not be suctioned or washed away and less than 90% of the surface was seen), or inadequate (repeat preparation and colonoscopy was needed)[18]. The evaluations of bowel cleanliness were further summarized as adequate (excellent/good) and inadequate (fair/poor).

Colonoscopy procedure

Two equally experienced endoscopists with experience of more than 5000 colonoscopies each did all the examinations. Specifically, one endoscopist conducted the diagnostic examinations using uniquely previous-day preparation and the other performed the second series with split-dose preparation. The endoscopist who performed the polypectomies was not aware of the number, size and location of polyps detected during the first colonoscopies and had no data regarding the quality of bowel preparation during index colonoscopies. Procedures were done using olympus CF-Q145L standard-definition white-light colonoscopes (Olympus Corporation, Tokyo, Japan). Polypectomies were accomplished by means of forceps, snares or endoscopic mucosal resection, as needed.

All patients signed a standard informed consent form prior to the exam. Institutional ethics committee approval for our study was not needed, since all patients received the standard-of-care without reference to any study.

During the examinations, pulse rate, arterial blood pressure, oxygen saturation and consciousness level were monitored. Supplemental oxygen was routinely delivered *via* nasal catheters at 2 L/min. Intravenous conscious sedation and analgesia including midazolam (Dormicum, Roche Hellas, Athens, Greece) and pethidine hydrochloride (Petidina cloridrato, Molteni Farmaceutici Cilteni, Scandicci, Firenze, Italy) was administered depending on patient's willingness along with comorbidities and baseline vital signs assessment. Reversal agents including flumazenil (Anexate, Roche Hellas, Athens, Greece) and naloxone (Naloxon, B. Brown Melsungen AG, Melsungen, Germany) were available in case of sedation-related complications. No antispasmodics were administered.

In the first colonoscopies, the colonoscopes were advanced to the cecum and polyps were identified during both insertion and withdrawal, counted, but not removed. In the second examinations, all detected polyps were resected and sent for histologic evaluation. Adenomas larger than 1 cm and/or with high-grade dysplasia or a villous component more than 25% were defined as advanced adenomas. To note, numerous tiny hyperplastic polyps in the rectosigmoid area were not subject to assessment.

For each procedure eligible for analysis, the following data were collected: (1) patients' characteristics (age, gender, American Society of Anesthesiologists-ASA grade); (2) indication for colonoscopy; (3) sedation and oxygen administration; (4) bowel preparation quality; (5) polyp features (size, location, shape); and (6) other findings. According to their size, polyps were categorized as diminutive ($\leqslant$ 5 mm), small (6-9 mm) and large ($\geqslant$ 10 mm). Polyp size was determined by comparison with opened biopsy forceps. All colonoscopies were performed between 8:00 a.m. and 2:00 p.m.

Statistical analysis

Polyps per patient were calculated as number of detected polyps/number of patients. Polyp miss rates were calculated as: number of missed polyps/total number of missed polyps + total number of polyps on initial examination and presented as percentages. Both parameters were calculated overall and within strata of polyp size and location. Ideally, a third gold-standard preparation methodology against which comparisons regarding polyp miss rates were applied should be available. Since that was not the case in our retrospective trial we decided to use as reference the type of bowel preparation that showed better results regarding colon cleanliness. Therefore, no OR (95%CI) were calculated in the univariate analysis.

Continuous variables were presented as means or medians and standard deviations, while categorical ones were expressed as absolute values and percentages. Differences in the number of detected colon polyps (overall, right- and left-sided) between the two endoscopic procedures were examined using non-parametric related samples (Wilcoxon Signed Rank Test) tests.

A multivariate linear regression analysis model was constructed to examine variables associated with the number of polyps (overall, right- and left-sided) detected at colonoscopies (dependent variable). Independent variables include: patients' age; sex (male *vs* female), ASA grade (1 *vs* 2), indication for colonoscopy (screening/surveillance *vs* symptoms evaluation) and the quality of bowel preparation (adequate *vs* inadequate). The OR (95%CI) and the level of significance were calculated. A P value of less than 0.05 indicated statistical significance.

Statistical analysis of data was carried out by international business machines corporation (IBM) SPSS Statistics Client for Trial 32. bit 22.0 Microsoft Windows Multilingual (IBM, New York, USA).

RESULTS

Clinical characteristics

A total of 50 patients (28 male) completed both examinations; 4 patients were excluded. Reasons for exclusion were poor bowel preparation (n = 3) and failure to complete the second colonoscopy secondary to sedation-related hypoxemia (n = 1). Mean age was 58.4 ± 11.1 years. Indication for the index colonoscopies were: screening (n = 22), blood in stool (n = 7), abdominal pain (n = 12), family history of CRC (n = 2), altered bowel habits (n = 4) and post-polypectomy surveillance (n = 3). Median interval period between the two exams was 6 wk (range: 1-12).

Bowel preparation quality

Bowel preparation according to the Aronchick scale in the 2 series of colonoscopies was described as excellent in 17 (34%) *vs* 24 (48%) patients, good in 21 (42%)

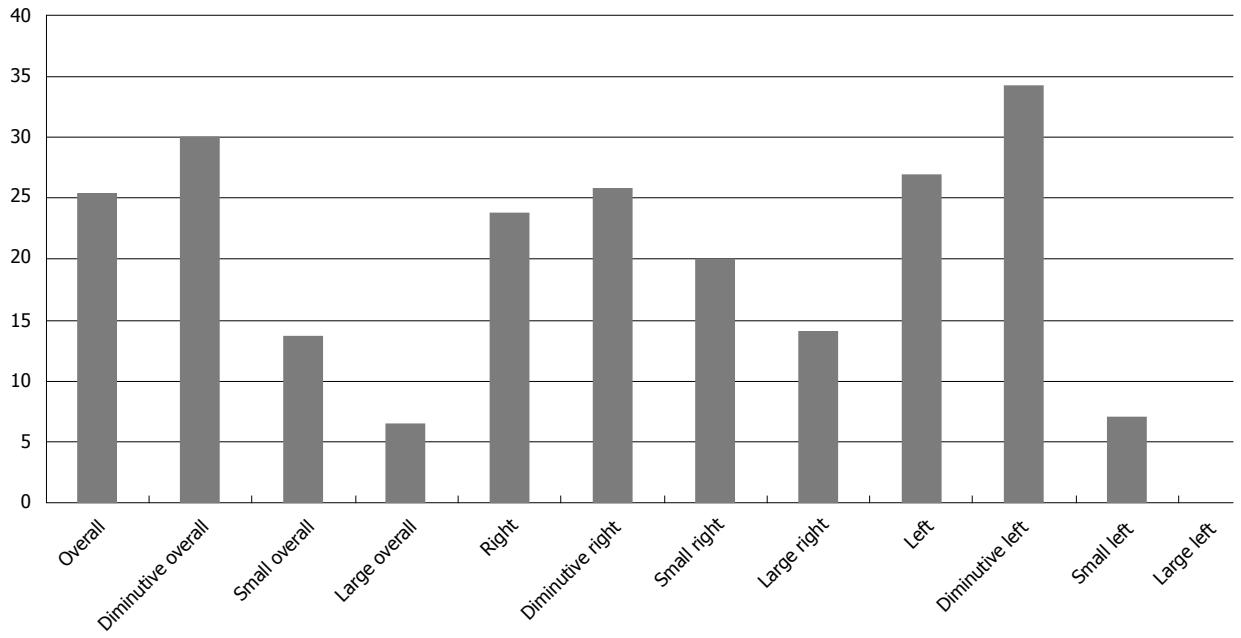

Figure 1 Polyp miss rates (as % percentages).

Table 1 Differences in number of detected polyps between the 2 colonoscopies

	Previous day	Split-dose	*P* value
Overall	126	169	< 0.001
Diminutive overall	88	126	< 0.001
Small overall	25	29	0.046
Large overall	13	14	0.317
Right	64	84	< 0.001
Diminutive right	46	62	< 0.001
Small right	12	15	0.083
Large right	6	7	0.317
Left	62	85	< 0.001
Diminutive left	42	64	< 0.001
Small left	13	14	0.317
Large left	7	7	1.000

vs 22 (44%) patients and fair in 12 (24%) *vs* 4 (8%) patients, respectively. When the evaluations of bowel cleanliness were classified as adequate (excellent/good) and inadequate (fair/poor), the second group of colonoscopies showed a significant increased rate of adequate preparations (92% *vs* 76%, $P = 0.03$).

Polyp detection and polyp miss rates

One hundred and twenty-six polyps were detected during the first examinations. Of those, 88 were diminutive, 25 small and 13 large; 43 additional polyps were identified during the tandem colonoscopies divided in 38 diminutive, 4 small and 1 large. Importantly, better colonic cleansing with the split-dose preparation contributed to significantly increased numbers of identified overall, right- and left-sided polyps ($P < 0.0001$). Significantly more diminutive polyps were detected throughout the colon ($P < 0.001$), while a marginal increase in the number of small polyps was also revealed (Table 1).

The calculated miss rates regarding overall, diminutive, small and large polyps were 25.4%, 30.1%, 13.7% and 6.6%, respectively. The overall miss rates for polyps located in the right colon (cecum, ascending and transverse colon) was 23.8% compared with 27% in the left colon (distal to splenic flexure). Based on size, the miss rates for right-sided diminutive, small and large polyps were 25.8%, 20% and 14.2%, respectively, in comparison to 34.3%, 7.1% and 0%, respectively, for left-sided ones (Figure 1).

Linear regression analysis revealed that increased patients' age and split-dose bowel preparation were the only variables associated with the number of polyps detected overall. Split-dose bowel preparation entered the model first [OR = 0.869 (95%CI: 0.456-1.283); $P < 0.001$], followed by increased age [OR = 0.054 (95%CI: 0.017-0.092); $P = 0.005$]. The same variables were also associated with the number of polyps detected in the right colon. Split-dose bowel preparation entered the model first [OR = 0.418 (95%CI: 0.111-0.724); $P = 0.008$], followed by increased age [OR = 0.032 (95%CI: 0.004-0.060); $P = 0.024$]. Split-dose bowel preparation was the only variable associated with the number of polyps detected in the left colon [OR = 0.452 (95%CI: 0.076-0.828); $P = 0.02$].

Polyp histology

A total of 169 polyps were found and resected in 50 patients during the second series of colonoscopies. Histologic examination of resected polyps revealed tubular adenomas ($n = 110$), advanced adenomas ($n = 18$), serrated lesions ($n = 7$), hyperplastic polyps ($n = 51$) and adenocarcinoma ($n = 1$). Of note, 9 advanced adenomas and 4 serrated lesions were detected in the

right colon, while 9 and 3 respectively, similar lesions, were located in the left colon.

DISCUSSION

This study demonstrates that split-dose bowel preparation results to significantly better mucosal cleansing compared to previous-day preparation. Moreover, we showed that better preparation with the split-dose regimen significantly enhanced overall, right- and left-sided polyp detection, especially referring to diminutive ones. Furthermore, improved view of the colonic epithelium unmasked a noteworthy polyp miss rate, inversely linked to their size.

Colonoscopy is currently considered as the gold standard for the detection of colonic neoplasia. However, emerging data demonstrate that a significant proportion of precancerous lesions are missed during the procedure, limiting its efficacy and leading to interval cancers[19].

It is established that variations in colonoscopy quality reflect differences in numerous patient-, procedure- and endoscopist-related parameters. Taking that into consideration, a great body of interventions has been conducted aiming to decrease colonoscopy's native imperfections, including internal audits and feedback to individual endoscopists, education in quality indicators, implementation of mandatory withdrawal times, bowel preparation modifications, discussion with poor-performers, introduction to emerging technologies, routine sedation administration, repeat attempts for cecal intubation and report card utilization[20-22].

In terms of pre-colonoscopy bowel preparation, numerous interventions have been suggested. These include dietary modifications and various purgatives alone or combined with adjunctive agents (*e.g.*, prokinetics, enemas, simethicone). Timing of bowel preparation administration has been tested in several randomized controlled trials focusing on bowel cleanliness and lesion detection. Recently, the European Society of Gastrointestinal Endoscopy adopted the results of a meta-analysis recommending split dose preparation for morning colonoscopies[10,23,24]. In line with this, our study highlights the significantly better colon cleansing achieved with split-dose preparation, as well as its contribution to increase polyp detection. However, our splitting of PEG dose was 3:1, in contrast to the recommended 2:2. Additionally, we did not collected data with respect to patients' satisfaction, impact on daily activities and willingness to repeat the same bowel preparation in the future, if indicated.

Our data supports the importance of better bowel preparation in the detection of additional polyps. This finding is in line with the results of Gurudu *et al*[21] demonstrating improved polyp detection rates (PDR) and ADR with split-dosing. Unfortunately, we cannot provide information for possible differences in adenoma detection in the present study, as the index series of colonoscopies was diagnostic. However, PDR and ADR seem to correlate well, at least in segments proximal to the splenic flexure[25].

Miss rates for total, diminutive, small and large polyps were 25.4%, 30.1%, 13.7% and 6.6% respectively. These results indicate that the smaller the polyp size, the higher the polyp miss rate, which is in accordance to findings of previous studies[6,26]. Location did not affect the polyp miss rates similarly to a recent study conducted by Ahn *et al*[27]. Interestingly, other data suggests that the risk of missing a polyp is related to left colon location[28]. However, it should be clearly stated that no gold-standard bowel preparation method against which our studied alternatives (*i.e.*, previous-day *vs* split-dose preparation) were compared in terms of polyp miss rates was available. Therefore, we favored split-dose preparation's findings to serve as comparator given that it yielded significantly better results as regards colon cleanliness. This reflects the current knowledge that the risk of missing polyps and adenomas during colonoscopy is affected by bowel preparation quality[29]. Nevertheless, our assumption encompasses a disadvantage of this study and weakens its conclusions.

As obvious, this study bears several limitations. First, we enrolled a small number of patients, which limits the power of our results. Second, we used as as reference methodology the results of the split-dose examination to calculate miss rates, as presented above. Third, we did not assess the inter-observer agreement considering bowel preparation status evaluation. Our results could have been affected by a possible significant discrepancy between the two examiners in rating preparation quality. Fourth, we could not provide data regarding histological features of polyps identified in the first series of colonoscopies (as they were not removed). Fifth, no reports of patients' preference in terms of timing of purgatives administration and comfort during the examinations were collected (the majority of patients had received sedation). Sixth, we did not captured data regarding withdrawal times which seem to influence ADRs. Additionally, we did not utilize validated scales such as Boston or Ottawa scales to assess the quality of cleansing in each bowel segment, as the Aronchick scale is closer to what an endoscopy unit uses in its "normal" -outside a study-practice, which was what we actually wanted to assess. Finally, we are not aware of the true polyp miss rate, since we considered the second colonoscopy as the gold standard.

In conclusion, our results support that split-dose bowel preparation improves the quality of colonoscopy in terms of mucosal cleanliness and polyp detection. However, future efforts to identify barriers and develop interventions aiming to further enhance colonoscopy effectiveness in the prevention of CRC are also necessary, as there are many factors that contribute to a high-quality examination.

COMMENTS

Background

Several factors influence colonoscopy quality and affect its potential to decrease

colorectal cancer incidence. Quality of bowel preparation represents one of the most studied ones. In this setting, numerous regimens, combinations and administration timings have been tested. Apart from rating bowel cleanliness achieved, polyp and adenoma detection seems to improve in parallel to the quality of preparation. This retrospective study assesses two different schedules of preparation regimen administration in terms of bowel cleansing and polyp detection.

Research frontiers

In this study it is suggested that splitting preparation regimen results in better quality of colon cleanliness than that achieved by previous-day dosing and leads to improved polyp detection.

Innovations and breakthroughs

The authors' 3:1 splitting of polyethylene glycol (PEG) regimen is shown to significantly improve the adequacy of bowel preparation and increase the number of detected polyps in both entire and colon segments. A remarkable polyp miss rate is substantially unmasked.

Applications

The results of this study serve as additional evidence aiming to improve colon cleanliness and polyp detection rates in every day clinical practice.

Terminology

Polyps per patient: number of detected polyps/number of patients. Polyp miss rates: number of missed polyps/total number of missed polyps + total number of polyps on initial examination. PEG is an osmotic laxative containing PEG, water and added electrolytes that is used in bowel preparation prior to colonoscopy and surgery.

Peer-review

The manuscript "Improved bowel preparation increases polyp detection and unmasks significant polyp miss rate" is clear and well-written. The manuscript reports on the comparison of two methodologies, full dose *vs* spilt dose, in colonoscopy and concludes with the report, that split-dose regimen enhanced polyp detection and reduced polyp miss rate.

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ORIGINAL ARTICLE

Prospective Study

New tapered metallic stent for unresectable malignant hilar bile duct obstruction

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Institutional review board statement: This study was conducted under approval of our ethical committee, and was registered as prospective clinical trial.

Clinical trial registration statement: UMIN Clinical Trial Registry (UMIN000004758).

Informed consent statement: All the treatment procedures were performed after obtaining the informed consent in writing from the patients.

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Data sharing statement: I share data in the group of us.

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Abstract

AIM: To examine the usefulness of a new tapered metallic stent (MS) in patients with unresectable malignant hilar bile duct obstruction.

METHODS: This new tapered MS was placed in 11 patients with Bismuth Ⅱ or severer unresectable malignant hilar bile duct obstruction, as a prospective study. The subjects were six patients with bile duct carcinoma, three with gallbladder cancer, and two with metastatic bile duct obstruction. Stenosis morphology was Bismuth Ⅱ: 7, Ⅲa: 3, and Ⅳ: 1. UMIN Clinical Trial Registry (UMIN000004758).

RESULTS: MS placement was 100% (11/11) successful. There were no procedural accidents. The mean patency period was 208.401 d, the median survival period was 142.000 d, and the mean survival period was 193.273 d. Occlusion rate was 36.4% (4/11); the causes of occlusion were ingrowth and overgrowth in 2 patients each, 18.2%, respectively. Patients with occlusion underwent endoscopic treatment one more time and all

were treatable.

CONCLUSION: The tapered MS proved useful in patients with unresectable malignant hilar bile duct obstruction because it provided a long patency period, enabled re-treatment by re-intervention, and no procedural accidents occurred.

Key words: Malignant hilar bile duct obstruction; Metallic stent; Tapered metallic stent

Core tip: Placement of a tapered metallic stent in patients with unresectable malignant hilar bile duct obstruction proved useful because it allowed a longer patency period without procedural accidents.

Sakai Y, Tsuyuguchi T, Nishikawa T, Sugiyama H, Sasaki R, Sakamoto D, Watanabe Y, Nakamura M, Yasui S, Mikata R, Yokosuka O. New tapered metallic stent for unresectable malignant hilar bile duct obstruction. *World J Clin Cases* 2015; 3(10): 887-893 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i10/887.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i10.887

INTRODUCTION

The guidelines for biliary cancer diagnosis recommend drainage as frequent as possible in patients with unresectable malignant hilar bile duct obstruction for improvement of patient's quality of life or when performing chemotherapy[1]. As an approach route for hilar bile duct occlusion, there are surgical, percutaneous, and transpapillary routes; and the region where drainage can be carried out differs depending on the location of the tumor, thus it is very difficult to establish treatment strategies. An endoscopic approach is recommended as the drainage route because of the low invasiveness and high success rate of internal drainage[1]. Metallic stents (MSs) are considered useful for internal drainage in terms of patency period[1]. Even past randomized controlled trials reported that MSs are associated with a longer patency period and lower occlusion rate than plastic stents (PSs)[2,3]. Under such considerations, it may be necessary to set the strategies to use MSs in patients with unresectable malignant hilar bile duct obstruction. In this study we examine the usefulness of a new tapered MS developed for exclusive use in patients with unresectable malignant hilar bile duct obstruction.

MATERIALS AND METHODS

The patients with unresectable malignant hilar bile duct obstruction and showed a remarkable increase of hepatobiliary enzymes, had Bismuth Ⅱ or higher degree stenosis according to Bismuth classification[4] and had been treated between July 2011 to December 2012 were included in this study (Table 1). There were 11 patients (7 men and 4 women) aged 72.273 ± 10.771 (59-85) years. The diagnosis was established based on a combination of images plus pathological findings. The cause of obstruction was bile duct carcinoma in 6, gallbladder cancer in 3, and metastatic bile duct obstruction in 2 patients. We evaluated the intrahepatic bile duct with a little contrast media. Stenosis morphology was Bismuth Ⅱ in 7, Ⅲa in 3, and Ⅳ in 1 patient. The stenosis was 22.727 ± 8.545 (10-35) mm long. Remarkable increase of hepatobiliary enzymes was defined as a value double or more the normal value of ALT (IU/L), ALP (IU/L), or T-Bil (mg/dL), or a combination of them. ALT (IU/L) was 114.055 ± 96.915, ALP (IU/L) was 1157.09 ± 420.250, and T-Bil (mg/dL) was 5.427 ± 4.4365 prior to drainage. Inclusion criteria were: (1) Patients with unresectable malignant hilar bile duct obstruction; (2) No criteria on underlying disease, age or sex; and (3) Patients who give gave their informed consent. Exclusion criteria were: (1) Patients in whom the endoscopic approach was difficult; (2) Patients with a bleeding tendency; (3) Patients who had suffered serious procedural accidents; (4) Patients who did not provide their informed consent; and (5) Patients who were determined not to be appropriate by the physician in charge. The MS was placed in all the patients *via* the endoscopic retrograde cholangiopancreatography (ERCP) route. Magnetic resonance cholangiopancreatography was performed in all of them before drainage. There was no case of cholangitis. Chemotherapy was performed in 6 patients and 5 received the best supportive care. The patients were followed up from MS placement to their death, and if patients were alive by March 2014 they were evaluated. Before ERCP, all patients were given the standard premedication consisting of intravenous administration of midazolam (3 to 10 mg), and the dose depended on age and tolerance. Scopolamine butylbromide or glucagon was used for duodenal relaxation. During ERCP, arterial oxygen saturation was continuously monitored using a pulse oximeter. Patients were kept fasting after the procedure for at least 24 h with drip infusion of 2000 mL and stayed in the hospital for at least 72 h. They received 8-h infusion of a protease inhibitor (nafamostat mesilate, 20 mg/d) and were prescribed antibiotics (SBT/CPZ, 2 g/d) for 2 d. For cannulation, catheters PR-104Q, R110Q-1 and PR233Q were used. Wire-guided cannulation was not performed. A 0.025-inch or 0.035-inch guidewire (Jagwire: Microvasive, Boston Scientific Corp., Natick, MA, Revo Wave: PIOLAX, or VisiGlide: Olympus Corp., Tokyo, Japan) was used. The endoscopes used were JF240, JF260V, TJF260V (Olympus Corp.), backward side-viewing endoscopes. After cholangiography, a guidewire was placed in the bile duct to conduct endoscopic sphincterotomy (EST). Clever-Cut3V

Table 1 Patient background

Case	Sex	Age	Disease	Stent no.	Stenosis morphology	Stenosis length (mm)	Treatment
1	Male	60	Intrahepatic bile duct carcinoma	2	Bismuth Ⅱ	25	BSC
2	Male	59	Colon cancer	1	Bismuth Ⅲa	33	Chemotherapy
3	Female	85	Intrahepatic bile duct carcinoma	1	Bismuth Ⅲa	28	Chemotherapy
4	Female	85	Bile duct carcinoma	2	Bismuth Ⅳ	35	BSC
5	Male	67	Intrahepatic bile duct carcinoma	2	Bismuth Ⅱ	18	Chemotherapy
6	Female	61	Gallbladder cancer	2	Bismuth Ⅱ	17	BSC
7	Male	65	Gallbladder cancer	1	Bismuth Ⅱ	18	Chemotherapy
8	Female	85	Gallbladder cancer	2	Bismuth Ⅱ	22	BSC
9	Male	81	Colon cancer	1	Bismuth Ⅱ	32	BSC
10	Male	79	Bile duct carcinoma	1	Bismuth Ⅲa	10	Chemotherapy
11	Male	68	Bile duct carcinoma	1	Bismuth Ⅱ	12	Chemotherapy

BSC: Best supportive care.

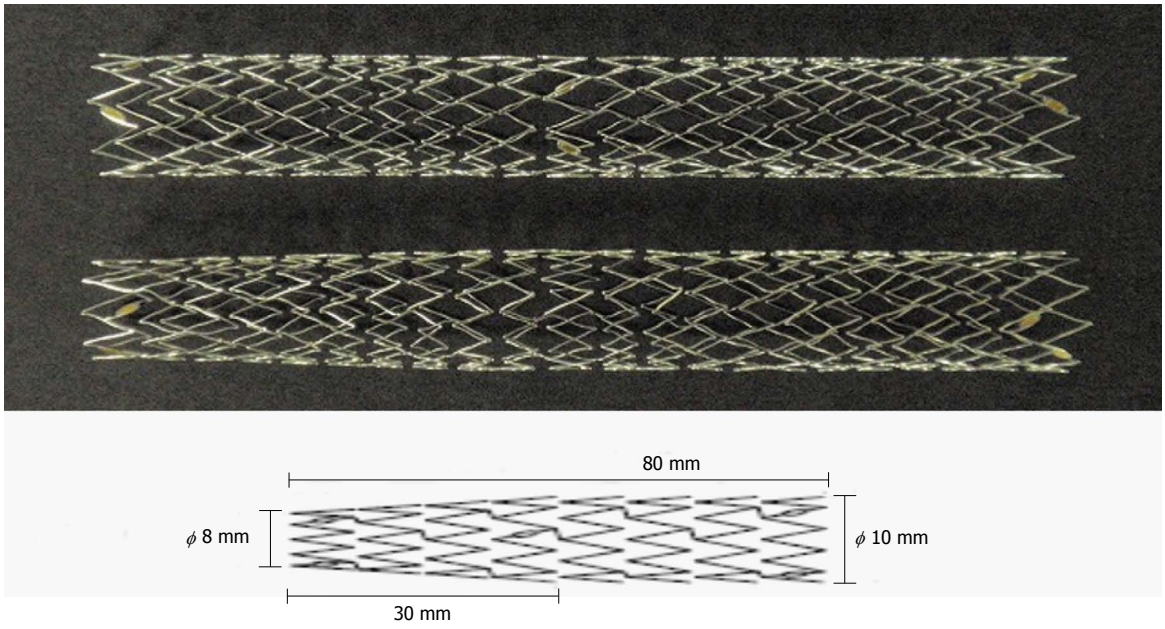

Figure 1 The metallic stent at the top is the ordinary laser-cut uncovered metallic stent. The one at the bottom is the laser-cut uncovered metallic stent (PIOLAX: Japan) created for exclusive use in the liver. This metallic stent is 8 cm in full size with a 3-cm tapered tip and a mesh space of 6-8 mm in the center of the stent; its internal diameter is 10 mm in the papillary side and 8 mm in the hepatic side.

(Olympus Corp.) was used as the knife for EST. EST was conducted using a single electrosurgical current generator (PSD-20, Olympus Corp.) at a power of 25 watts. EST was carried out in all the patients. The effect of drainage was determined by placing an endoscopic nasobiliary drainage (ENBD), or a PS in either the right or left bile duct. The effect of drainage was evaluated 7 d after drainage placement, and it was determined effective if the T-Bil was normal or 2/3 or less; then a tapered MS was placed. In patients without effective drainage, ENBD or PS was placed in the bile duct in the side where the drainage is not placed. An ENBD tube of 7 Fr. was used (FLEXIMA: Boston Scientific Corp., Natick, MA, or SD9: SILUX Straight type). Tube stents of 7 Fr., 8.5 Fr. and 10 Fr. were used (FLEXIMA: Boston Scientific Corp., or SD9: SILUX Straight type). And if the drainage was effective, the new tapered MS was placed in the region. As for tapered MSs, the delivery system is a laser-cut MS created for use exclusively in the liver. These MSs are 7 Fr in size, with a full length of 8 cm and a 3-cm tapered tip. The mesh space is 6-8 mm at the center of the stent, and its internal diameter in the papillary side is 10 mm, while in the hepatic side it is 8 mm (PIOLAX: Japan) (Figure 1). In patients for whom two tapered MSs were required, stenting was performed in the partial stent-in-stent manner[5-7]. The axial force and radial force of this MS were evaluated as follows. Axial force is the unbending force of the MS from the curved part. To measure the axial force, a portion of the stent was pushed perpendicularly by a force gauge (model DPX-5TR, Imada, Tokyo) until the angle became 60 degrees, and the force necessary to keep it in place was recorded. The measurement was made in an oven at 37 ℃ for 3 points distant 20, 40, and 60 mm from the bending point. Radial force is the dilating force of the MS. Radial force was measured using a radial force

Table 2 Comparison of alanine transaminase values before drainage and after metallic stent insertion

Case	ALT before drainage (IU/L)	ALT after MS insertion (IU/L)	*P*-value
1	63	54	
2	165	26	
3	182	32	
4	263	53	
5	75	14	
6	326	52	
7	69	25	
8	37	25	
9	115	25	
10	39	22	
11	212	35	
Average	114.055 ± 96.915	33.00 ± 13.892	$P < 0.05$

MS: Metallic stent; ALT: Alanine transaminase.

Table 3 Comparison of alkaline phosphatase values before drainage and after metallic stent insertion

Case	ALP before drainage (IU/L)	ALP after MS insertion (IU/L)	*P*-value
1	1524	1288	
2	1113	490	
3	1200	386	
4	1726	775	
5	605	354	
6	1289	254	
7	1524	956	
8	638	256	
9	1611	956	
10	610	238	
11	888	283	
Average	1157.09 ± 420.250	566.91 ± 365.157	$P < 0.05$

MS: Metallic stent; ALP: Alkaline phosphatase.

measurement machine (RX 500, Machine Solutions, Flagstaff, Ariz) in an oven at 37 ℃. An MS sample in a fully expanded state was placed in the cylindric space of the machine, and the cylinder was contracted to shrink the MS to its minimum size of 2 mm. Then the force on the cylinder was reserved by an expansion force of the MS until it achieved its fully expanded state of 10 mm in diameter. The placement success rate, patency period, occlusion rate, and success rate of re-intervention of this MS were examined. Procedural accidents during ERCP-related procedures were evaluated according to Cotton's classification[8]. When the jaundice level was T-Bil 3 mg/dL less, we started chemotherapy. This study was conducted under approval of our ethical committee, and was registered as prospective clinical trial. UMIN Clinical Trial Registry (UMIN000004758).

Statistical analysis

Fisher's exact probability test, student's *t*-test, and the Mann-Whitney *U*-test were used for statistical analyses to compare the blood test findings prior to drainage insertion and post MS insertion. A *P* value < 0.05 was regarded as significant. Cumulative stent patency and survival were estimated using the Kaplan-Meier estimator. Data were analyzed using SPSS software version 17 (SPSS, Chicago, IL).

Table 4 Comparison of T-Bil values before drainage and after metallic stent insertion

Case	T-Bil before drainage (mg/dL)	T-Bil after MS insertion (mg/dL)	*P*-value
1	13.9	3.8	
2	3	1	
3	12	1	
4	1.4	0.7	
5	3.1	0.9	
6	10.2	2.1	
7	2.3	1.3	
8	3	1	
9	1.8	1.3	
10	3.8	1	
11	5.2	1	
Average	5.427 ± 4.4365	1.373 ± 0.8833	$P < 0.05$

MS: Metallic stent.

RESULTS

Initial drainage was successful in 6 (54.5%) of the eleven patients, and the remaining 5 (45.5%) had poor drainage thus drainage in the right and left bile ducts was performed. In the end, drainage was successful in all the patients. Since drainage was effective, MS was placed in all of them. In the six patients who underwent unilateral bile duct drainage one MS was placed, while in the five patients who underwent right and left bile duct drainage, two MS were placed; stenting was successful in all the patients. The mean number of MSs used was 1.545 ± 0.522 (1-2). All the parameters assessed at one week after stenting showed significant improvement compared with those before drainage insertion: ALT 33.00 ± 13.892 (IU/L), ALP 566.91 ± 365.157 (IU/L), and T-Bil 1.373 ± 0.8833 (mg/dL) (Tables 2-4). There were no procedural accidents due to stenting. The axial force of this MS was 0.156 ± 0.017 N when evaluated at bending point 20 mm, and radial force was 4.76 ± 0.18 N when evaluated at a dilated diameter of 4 mm. The patency of MS is shown in Table 5. The mean patency period was 208.401 d, the median survival period was 142.000 d (mean 193.273 d). The occlusion rate was 36.4% (4/11), and the occlusion causes were ingrowth in 2 (18.2%) patients and overgrowth in another 2 (18.2%). Patients with occlusion underwent endoscopic treatment one more time and in all of them it was 100% (4/4) successful. In patients who developed overgrowth in the contralateral hepatic side, an MS was placed in the partial stent-in-stent manner. In patients with an MS in each bile duct who developed overgrowth, an MS was additionally placed. In two patients with two MSs in the right and

Table 5 Achievements of metallic stent placement

Case	Survival period (d)	Alive or dead	Patency period (d)	Absence or presence of occlusion	Occlusion cause	Re-intervention
1	142	Dead	126	+	Ingrowth	PS
2	122	Dead	86	+	Ingrowth	PS
3	213	Dead	213	-	-	-
4	93	Dead	93	-	-	-
5	245	Dead	245	-	-	-
6	78	Dead	78	-	-	-
7	533	Alive	130	+	Overgrowth	MS
8	123	Dead	123	-	-	-
9	145	Dead	75	+	Overgrowth	MS
10	127	Dead	127	-	-	-
11	305	Dead	305	-	-	-
Mean	142		208.401	-	-	-
Median	193.273		-	-	-	-

MS: Metallic stent; PS: Plastic stent.

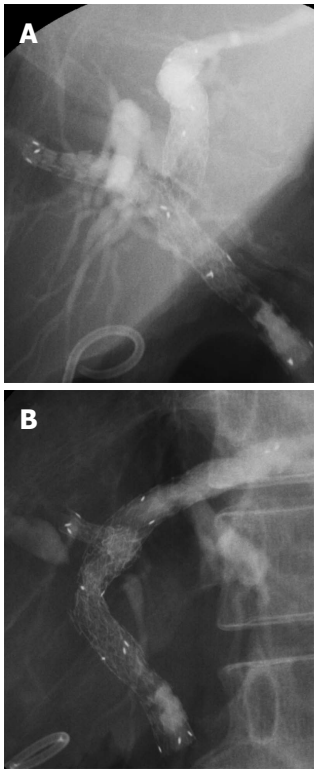

Figure 2 New tapered metallic stent (fluoroscopic image). A: Tapered laser-cut metallic stent placed in the liver. Stenting along the shape of the bile duct was possible (fluoroscopic image: front view); B: Fluoroscopic image: oblique view.

left bile duct who developed ingrowth, two PSs were placed in the right and left bile duct in the stent-in-stent manner. Re-treatment was successful in all the occlusion patients. The accidental occurrence symptom about the ERCP related procedures did not accept it.

DISCUSSION

In this study we evaluated a new tapered MS for unresectable malignant hilar bile duct obstruction. The MS used in this study was the laser-cut MS that enables precise stenting because shortening is structurally less[5]. Although evaluation may be partially difficult due to the small sample size, we experienced no procedural accidents during insertion, the patency period was long, and re-intervention was successful in all the patients; thus we consider this is a useful stent. This MS has moderate radial force at low axial force[9]. With regard to procedural accidents, this MS has low axial force, which enables stenting along the bile duct and may prevent kinking. When an MS is placed, usually procedural accidents such as acute pancreatitis or acute cholecystitis do not occur, however, abdominal pain may occur[10]. There may be various causes for this, including stress on the bile duct due to high axial force or strong radial force of the MS, or to a mismatch of the bile duct and MS regarding diameter, especially if the MS is of a diameter larger than that of the hepatic bile duct. The MS used in this study has a low axial force and a moderate radial force as shown in past reports; thus it is useful to treat stenosis and carry out stenting while applying low pressure on the bile duct. Furthermore, the tip is tapered, enabling good positioning of the stent (Figure 2). This may reduce the risk of abdominal pain due to stenting and of procedural complications such as hepatic abscess because the Glisson's sheath is not compressed. In this study no procedural accidents occurred; still if pancreatography is performed frequently during the procedure or it is difficult to catheterize the bile duct, pancreatitis might occur after ERCP[11,12].

As for the patency period, the sample size was small and thus evaluation is difficult. However, the patency period was in the same range as that found in a previous report of similar sample size, and which was considered as satisfactory[13]. The nature of the tumor, effect of chemotherapy, and characteristics of the MS itself may influence the patency period; yet, these should be evaluated in a study involving a large number of patients in the future.

Re-treatment was 100% (4/4) successful. Recent advancement of endoscopes and medical devices has enabled re-treatment in a comparatively easy

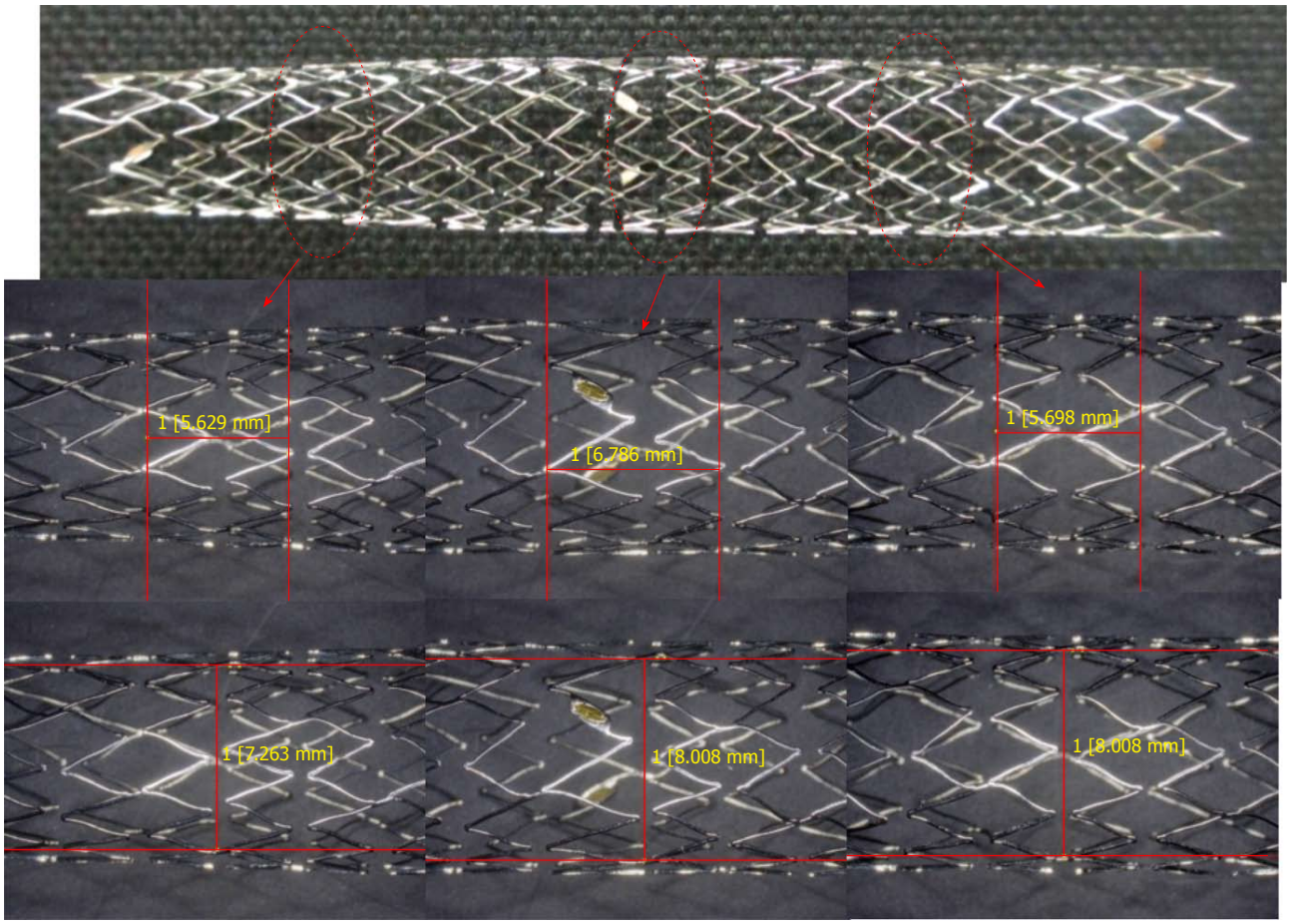

Figure 3 Laser-cut metallic stent with a comparatively large mesh space of about 6.8 mm × 8.0 mm in the center.

way. This MS has a large mesh space that facilitates re-intervention. Indeed, Mukai *et al*[14] reported that in the liver MSs with a large mesh space were an excellent choice because it was easier to re-intervene. Furthermore, other authors have also reported on the usefulness of MSs with a large mesh space that were created for exclusive use in the liver in patients with unresectable malignant hilar bile duct obstruction[13,15]. The MSs used in these reports were of the braided type with a mesh space of 7 mm; that is, a space similar to that of the mesh space of the laser-cut tapered MS used in this study (Figure 3). The laser-cut tapered MS used in this study has a large mesh space, which facilitates manipulation through the mesh and re-intervention. From such results and reports, it is currently considered that MSs with a large mesh space may be an excellent choice for use in the liver. Compared with the braided MS, the laser-cut MS used in this study hardly suffered shortening and enabled precise placement. However, in the future it may be necessary a randomized clinical trial to assess which one is best regarding placement success rate and patency period.

Our results suggested that the new tapered MS was useful for patients with unresectable malignant hilar bile duct obstruction because the patency period was long, re-treatment was possible, and there were no procedural accidents during their insertion.

COMMENTS

Background

Even past randomized controlled trials reported that metallic stents (MSs) are associated with a longer patency period and lower occlusion rate than plastic stents. Under such considerations, it may be necessary to set the strategies to use MSs in patients with unresectable malignant hilar bile duct obstruction.

Research frontiers

In this study, the authors examine the usefulness of a new tapered MS developed for exclusive use in patients with unresectable malignant hilar bile duct obstruction.

Innovations and breakthroughs

It may be necessary to set the strategies to use MSs in patients with unresectable malignant hilar bile duct obstruction.

Applications

A new tapered MS developed for exclusive use in patients with unresectable malignant hilar bile duct obstruction.

Terminology

The results suggested that the new tapered MS was useful for patients with unresectable malignant hilar bile duct obstruction because the patency period was long, re-treatment was possible, and there were no procedural accidents during their insertion.

Peer-review

This study prospectively estimated the efficacy of an uncovered metal stent with slightly tapered shape in its distal end for the patients with malignant biliary obstruction at the liver hilum.

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CASE REPORT

Littoral cell angioma: A case report

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Author contributions: Bailey A wrote the case report and compiled the table; Vos J contributed the pathology analysis and provided the collection of pathological images; Cardinal J critically revised the intellectual content and contributed to the design of the table.

Institutional review board statement: This study has been approved by West Virginia University.

Conflict-of-interest statement: There are no conflicts of interest to be declared by the authors of this paper.

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Abstract

Primary splenic lesions are rare entities among which littoral cell angioma (LCA) is a recently described, uncommon vascular lesion that is unique to the spleen. It has heretofore been described primarily in pathologic series and has been found mostly to behave as a benign entity. A few reports of malignant variants have been reported. We present a case report of a solitary LCA discovered after splenectomy for an incidentally discovered splenic lesion, along with a literature review.

Key words: Littoral cell angioma; Splenic tumor

Core tip: Littoral cell angioma (LCA) is a rare benign vascular lesion of the spleen. LCA can range from no symptoms to a vague set of symptoms such as: abdominal pain, splenomegaly, thrombocytopenia, anemia, fever, chills, weakness and fatigue. Diagnosis is made by histopathology after splenectomy.

Bailey A, Vos J, Cardinal J. Littoral cell angioma: A case report. *World J Clin Cases* 2015; 3(10): 894-899 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i10/894.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i10.894

INTRODUCTION

Primary splenic tumors are uncommon and are classified as lymphoid tumors, non-lymphoid tumors, and tumor like lesions[1-12] (Table 1). Among non-lymphoid tumors, vascular neoplasms are the most common and arise from the vascular elements that compose the splenic red pulp. Conversely, the lymphatic tissue containing splenic white pulp is from where lymphoid neoplasms arise. In regards to vascular tumors of the spleen, the biologic behavior can be both benign and malignant.

Littoral cell angioma (LCA) of the spleen is a rare vascular tumor that was first described in 1991 by Bhatt *et al*[13]. Initially thought to be benign, the biologic

Table 1 Classification of splenic tumors with associated clinical, pathological and radiological factors[1-12]

Category	Type	Clinical	Pathological	Radiological
Lymphoid	Non Hodgkin lymphoma	Fevers, sweats, change in weight are common symptoms	Derived from B or T cells, lympho-proliferative	CT: Hypodense nodules, diffuse or military distribution MRI: Isotense on precontrast images, hypotense on postcontrast images
	Hodgkins lymphoma	Spleen is a rare primary site	Nodular sclerosis subtype, Reed-Sternberg cells	CT: Hypodense nodules with nodular sclerosis
	Inflammatory pseudotumor	Secondary to inflammatory response to infection or injury Benign	Spindle cells, lymphocytes in fibroblastic stroma	CT: Well circumscribed +/- calcifications, hypoattenuating MRI: Hypo- or isointense on T1 images. Variable signaling on T2 images
	Plasmacytoma	Rare diagnosis	Diffuse infiltration of plasma cells	Not well categorized findings
	Histocytic lymphoma	Non specific symptoms, elevated ESR	Nodules with central necrosis	US: Cystic appearance CT: Sharply demarcated with central necrosis
	Hemangioma	Benign, slow growth, asymptomatic	Sinusoidal epithelium, proliferation of vascular channels	Solid to cystic components US: Echogenic solid to complex mass CT: Iso- to hypoattenuation associated with calcification MRI: Hypo- to isointense on T1 images, hyperintense on T2 images
	Hamartoma	Benign, asymptomatic. Associated with tuberous sclerosis and Wiskott Aldrich	Solid nodules, well circumscribed, well defined gross appearance. Unorganized vascular channels with fibrotic cords	US: More sensitive than CT, solid mass +/- calcification CT: Isoattenuating MRI: Isointense on T1 images, hyperintense
	Lymphangioma	Asymptomatic, benign, mostly in children	Multiple solitary nodules, Flattened endothelium with proteinaceous material in a capillary, cavernous or cystic presentation	US: Splenic cysts hypoechoic septations CT: Thin walled low attenuation masses, subcapsular location MRI: Hypointense on T1 images, hyperintense on T2 images
Vascular	Littoral cell angioma	Asymptomatic, benign with malignant potential	Well delineated nodules of anastomosing vascular channels with endothelial cells	US: Hypoechoic to hyperechoic CT: Iso to hypoattenuating with contrast enhancement MRI: Low intensity lesions
	Angiosarcoma	Older patients, malignant, nonspecific symptoms	Diffuse involvement of spleen arises from sinus endothelial cells, high mitotic rate	US: Complex mass, heterogenous, necrotic degeneration CT: Ill-defined mass with heterogenous enhancement, punctate calcification MRI: Mixed signal intensity on T1 and T2
	Hemangioendothelioma	Nonspecific symptoms, young adults	Variable morphologic appearance	US: Hypoechoic mass CT: Low attenuated mass with enhancement of solid portions MRI: Heterogenous solid mass. Hypointense on T1 and T2 images
	Fibrosarcoma	Asymptomatic	Well differentiated, spindle shaped, fibroblasts, collagen is commonly present	Non specific imaging findings
Non-lymphoid	Lipoma	Asymptomatic	Adipose tissue, no atypia, cytoplasmic vacuoles	CT: Well defined fat density mass
	Kaposi sarcoma	Associated with HIV/AIDS +/- skin lesions	Spindle cell proliferation, spongelike vascular channels	CT: Ill-defined nodules, homogeneous US: Hyperechoic nodules
	Peliosis	Associated with anabolic steroid, TB, AIDS, cancer. Asymptomatic	Cyst like blood filled cavities within splenic parenchyma	US: Echogenic mass CT: Hypoattenuating, multiloculated with septa
Tumor like	Nonparasitic cysts	Congenital or neoplastic in origin. Benign.	Varies according to type of cyst including dermoid cyst	US: Cystic lesions with solid components CT: Hypoattenuating lesions, well defined
	Granulomas	Associated with chronic granulomatous disease and sarcoidosis	Granulomas non-necrotizing or necrotizing	CT: Hypodense nodules MRI: Hypointense T1 and T2

ESR: Erythrocyte sedimentation rate; CT: Computed tomography; MRI: Magnetic resonance imaging; US: Ultrasound; HIV: Human immunodeficiency virus; AIDS: Acquired immunodeficiency syndrome; TB: Tuberculosis.

behavior of LCA has not been firmly established, as there have been several reports of LCA with malignant features[14,15]. LCA may occur at any age and has no gender predilection. To date, a total of 110 cases have been reported in the literature with 4 published pathologic series and 3 published case series[13,16-32].

LCA is discovered as a splenic lesion in patients who are undergoing a workup for laboratory evidence

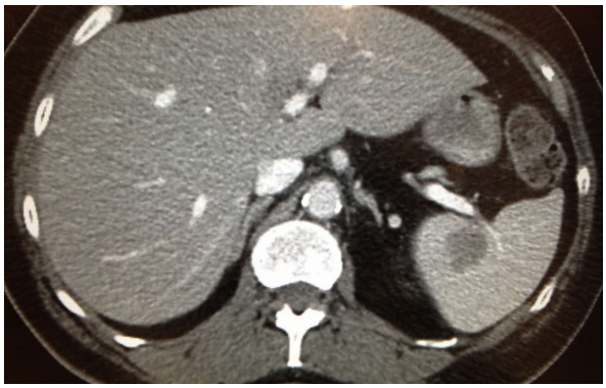

Figure 1 Computed tomography abdomen and pelvis, axial view of hypodense splenic lesion.

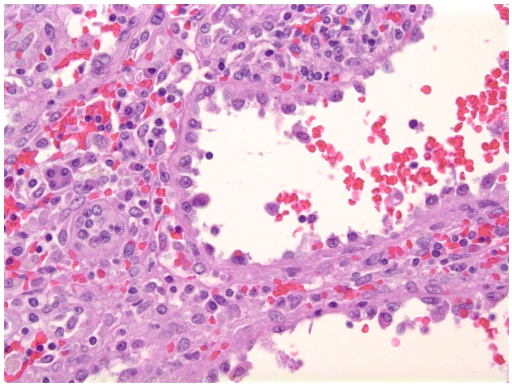

Figure 2 High power view of the tumor demonstrates tall columnar endothelial cells that line the cyst-like spaces. These cells show no cytologic, nuclear atypia or mitotic figures (H and E stain, × 400).

of anemia or thrombocytopenia[33-36]. Imaging findings of LCA are nonspecific and splenomegaly, to a varying degree, is a common finding. Due to the nonspecific findings that often result from the diagnostic workup, splenectomy is often performed for both diagnostic and therapeutic purposes. In the present report, a case of an incidentally discovered LCA is described.

CASE REPORT

A 65-year-old female presented to the outpatient oncology surgery clinic for surgical evaluation of a 2.2 cm splenic lesion. The lesion was discovered incidentally on a computed tomography (CT) abdomen/pelvis study to evaluate recurrent urinary tract infections (Figure 1). Also, the CT scan revealed a second incidental finding of a 1.1 cm right adrenal nodule. The patient was asymptomatic without abdominal pain, persistent fever, chills, weight loss, or other constitutional symptoms. Her past medical history included hypertension, diabetes mellitus, gout and peripheral neuropathy. Physical examination was unremarkable except for abdominal wall scars from prior open hysterectomy, cholecystectomy and left nephrectomy, the latter of which which was performed at a young age for a nonfunctioning

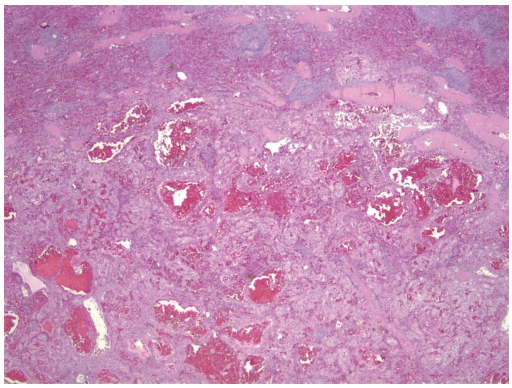

Figure 3 Low power view of the well-demarcated tumor with uninvolved spleen. The tumor has anastomosing vascular channels and cyst-like hemorrhagic spaces.

left kidney secondary to congenital ureteropelvic junction obstruction. A biochemical workup to exclude a functioning adrenal tumor was performed and included serum renin and aldosterone levels as well as 24 h urinary fractionated metanephrine and cortisol levels, all of which were within the limits of normal. Of note, she was not leukopenic, anemic or thrombocytopenic.

Given the size of her incidentally discovered splenic lesion, she was offered operative resection for diagnostic purposes. Based on her extensive prior surgical history, an open approach to the splenectomy was planned. The patient received preoperative pneumococcal, meningococcal and haemophilus B vaccinations. The operation and recovery were uneventful and the patient was discharged to home on postoperative day four.

Grossly, the spleen weighed 270 g and measured 23.3 cm × 18.1 cm × 7.2 cm. The splenic lesion measured 2 cm × 2 cm × 2 cm. Histopathologically, the tumor was found to have anastomosing vascular channels with large cyst formations which were lined predominately by tall, histiocytoid cells which projected into the vascular spaces along with interspersed flat endothelial cells (Figures 2 and 3). Immunohistochemically, the cells compromising the tumor stained positive for CD68 and lysozyme (Figures 4A and B). The specimen also showed variable expression of S100. CD34 and CD31 stains were positive on the endothelial cells, however negative on the histiocytoid cells (Figures 4C and D). Final pathologic diagnosis was littoral cell angioma.

DISCUSSION

LCA is a rare vascular neoplasm of the spleen. It has been found to affect both men and women in an equal distribution. Given the relative lack of symptom specificity, LCA is most often found incidentally as a splenic mass on abdominal imaging; however, two cases of LCA presenting with splenic rupture and hemoperitoneum have been reported[37,38]. The sonographic appearance of LCA is variable, and ranges from a hypoechoic to a hyperechoic mass with a mottled texture[14]. On contrast

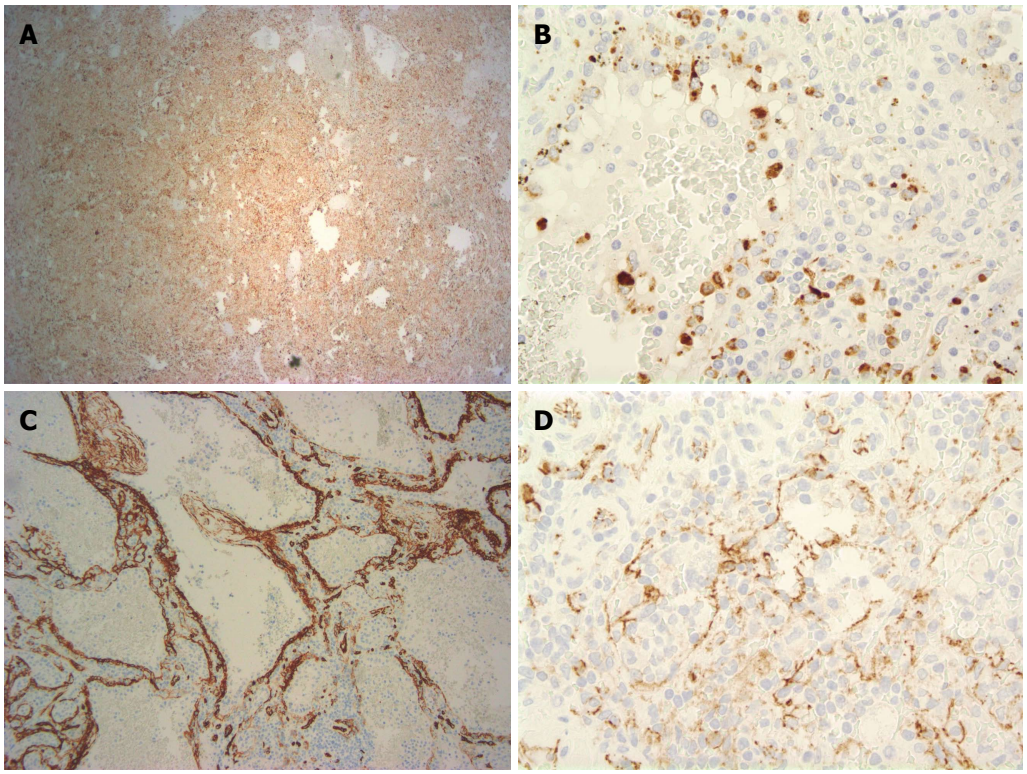

Figure 4 Endothelial cells lining the cyst-like spaces are immunoreactive. A: CD68 (CD68 stain, × 100); B: Histiocytic marker lysozyme (lysozyme stain, × 400); C: Endothelial marker CD34 and the histocytoid cells are negative for CD34 (CD34 stain, × 400); D: Endothelial marker CD31 (CD31 stain, × 400).

enhanced CT, LCA is isodense to slightly hypodense as related to the surrounding splenic parenchyma in both the arterial and early portal venous phase[39,40]. Magnetic resonance imaging characteristically shows a T1 and T2 hypointense mass. LCA is often multifocal and lesions can be variable in size[14]. The differential diagnosis of lesions that can mimic LCA on imaging includes lymphangioma, hamartoma, lymphoma, Kaposi' s sarcoma, and hemangioma. Therefore, a definitive diagnosis can only be obtained pathologically[41].

Pathologically, LCA is a vascular tumor of the spleen that represents a tumoral counterpart of the normally present littoral cells that line the splenic sinus channels of the red pulp[30]. First described by Falk *et al*[33] in 1991 in a pathologic series of 17 cases, this new entity was described histologically as consisting of anastomosing vascular channels with cyst like spaces and papillary projections. The endothelial cells lining the channels are tall and plump compared to the flat endothelial cells lining the channels in a normal spleen. Immunohistochemically, LCA is characteristically CD 34 negative, CD 68 positive, CD 21 positive and CD 8 negative[22]. Additionally, the epithelial cells in LCA do occasionally express S-100 protein[16]. High expression of formin homology domain protein 1 (FHOD1) distinguished littoral cells from LCA. FHOD1 protein is expressed by normal littoral cells, not by LCA[42]. Further research has been done evaluating molecular markers and LCA to help aide in the accurate diagnosis of LCA tumors. O'Malley *et al*[43], looked at splenic lesions and the activity of the Ets Related Gene (*ERG*) and the Wilms Tumor-1 gene (*WT-1*). They found that LCA splenic lesions had a pattern of ERG positive and WT-1 negative[43]. Of the other types of splenic lesions evaluated cavernous hemangiomas were found to have the same pattern, therefore these markers are not specific enough alone to make the diagnosis of LCA.

LCA has most commonly been described as a benign process. However, observations of malignant behavior have been described[41]. In one case, metastatic lesions were found in the liver and retroperitoneum four years after splenectomy for LCA[44]. This case initially had symptoms of ureteral obstruction and renal failure. In comparison, our patient did not have ureteral obstruction however did have recurrent UTI's and a history of congenital ureteropelvic junction obstruction. Kranzfelder *et al*[45], showed a case of familial individuals with LCA and primary splenic angiosarcoma, raising the question of possible malignant transformation. There were no similar signs and symptoms between their case and the presented case. Harmon *et al*[17], published a case report of a patient with transitional cell carcinoma of the bladder with suspected splenic metastasis. The pathology revealed LCA and not splenic metastasis. Ben-Izhak *et al*[14], showed a case of malignant littoral cell tumor naming it littoral cell hemangioendothelioma. This case report featured a symptomatic patient with liver metastasis eight years after splenectomy. In reviewing all of these cases, the immunohistochemical pattern was similar giving the diagnosis of LCA.

LCA has been shown to be rarely associated with visceral malignancies including colorectal adenocarcinoma, pancreatic cystadenocarcinoma, pancreatic neuroendocrine tumor, renal cell cancer, hepatocellular carcinoma, non-small cell lung cancer, seminoma, ovarian cystadenocarcinoma, papillary thyroid cancer and transitional cell carcinoma of the bladder[17]. Furthermore, there have been a few reports describing an association of LCA with immunological disorders, such as, ankylosing spondylitis, myelodysplastic syndrome, non-Hodgkin lymphoma, Crohn's disease, Wiskott Aldrich syndrome, chronic glomerulonephritis, aplastic anemia and Gaucher's disease[17,22,46]. Given the association of LCA with other malignancies as well as the few reported cases of malignant behavior, patients should undergo close follow up after splenectomy; however, no established postoperative surveillance guidelines exist.

Littoral cell angioma is a rare vascular tumor of the splenic red pulp, and is typically an incidental finding on abdominal imaging. The splenic lesion can only truly be differentiated from other splenic masses by histologic examination. Splenectomy is the appropriate treatment, as LCA has a variable behavior pattern of which malignant tendencies are worrisome. Furthermore, longitudinal surveillance in the postoperative phase is recommended.

COMMENTS

Case characteristics

Littoral cell angioma (LCA) can range from no symptoms to a vague set of symptoms such as: abdominal pain, splenomegaly, thrombocytopenia, anemia, fever, chills, weakness and fatigue.

Clinical diagnosis

The main clinical finding is a splenic lesion.

Differential diagnosis

The differential diagnosis of a splenic lesion is lymphoid, vascular, non lymphoid and tumor like which can be distinguished by pathology.

Imaging diagnosis

Ultrasound, computed tomography and magnetic resonance imaging are all acceptable modalities for imaging and diagnosing a splenic tumor; all of which are non-specific for LCA.

Pathological diagnosis

The splenic specimen is analyzed for abnormal littoral cells along with immunohistochemical stains to provide definitive diagnosis of LCA.

Treatment

Treatment is surgical resection with close surveillance as a malignant variant is possible.

Related reports

Over a hundred cases of LCA have been reported since 1991, research continues into the realm of pathological markers and surveillance is new territory with cases of malignant variants being reported.

Term explanation

Hemangioendothelioma is a term to describe a vascular neoplasm that may be considered benign as well as malignant.

Experiences and lessons

This case teaches that there is malignant potential for LCA lesions of the spleen.

Peer-review

This is the well-written case report of LCA.

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CASE REPORT

Acute hepatitis after amiodarone infusion

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Author contributions: Fonseca P, Dias A and Gonçalves H wrote the case report; Albuquerque A and Gama V revised the manuscript.

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Informed consent statement: The patient gave his informed consent to the publication of this clinical case.

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Abstract

Acute hepatitis is a very rare, but potentially fatal, adverse effect of intravenous amiodarone. We present a case of an 88-year-old man with history of ischemic dilated cardiomyopathy and severely depressed left ventricular function that was admitted to our coronary care unit with diagnosis of decompensated heart failure and non-sustained ventricular tachycardia. A few hours after the beginning of intravenous amiodarone he developed an acute hepatitis. There was a completely recovery within the next days after amiodarone withdrawn and other causes of acute hepatitis have been ruled out. This case highlights the need for close monitoring of hepatic function during amiodarone infusion in order to identify any potential hepatotoxicity and prevent a fatal outcome. Oral amiodarone is, apparently, a safe option in these patients.

Key words: Polysorbate 80; Hepatitis; Hepatotoxicity; Idiosyncratic reactions; Amiodarone

Core tip: We report a rare case of acute hepatitis induced by intravenous amiodarone in a patient with non-sustained ventricular tachycardia. The physiopathology of this adverse effect is still unclear. Close monitoring of hepatic function during amiodarone infusion is essential to avoid any potential hepatotoxicity.

Fonseca P, Dias A, Gonçalves H, Albuquerque A, Gama V. Acute hepatitis after amiodarone infusion. *World J Clin Cases* 2015; 3(10): 900-903 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i10/900.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i10.900

INTRODUCTION

Long-term oral amiodarone therapy is associated with many extracardiac adverse effects, such as thyroid dysfunction, photosensitivity, corneal microdeposits and pulmonary and hepatic toxicities. The frequency

of most adverse effects is related to the total drug exposure. Hepatic toxicity in these patients ranges from an asymptomatic elevation of serum aminotransferases (in approximately 25%) that is usually transient and resolves after dose reduction or withdrawal, to severe liver disease (1%-3%)[1]. Acute hepatic toxicity during intravenous amiodarone has been rarely described[2-6].

CASE REPORT

An 88-year-old man with history of ischemic dilated cardiomyopathy and severely depressed left ventricular function presented at Emergency Department due to progressive worsening of dyspnea, orthopnea and peripheral edema during the previous week. He was on long-term treatment with aspirin, ramipril, furosemide, transdermal nitroglycerin, sinvastatine and pantoprazole. He had no history of alcohol abuse or chronic acetaminophen intake.

On physical examination, blood pressure was 110/60 mmHg and percutaneous peripheral oxygen saturation was 87% on air. He had bilateral basilar rales on pulmonary auscultation and moderate lower leg edema. Arterial gasometry confirmed type 1 respiratory failure and blood tests were unremarkable, including liver function. Electrocardiogram monitoring revealed periods of non-sustained ventricular tachycardia (NSVT).

He was admitted to our coronary care unit with the diagnosis of acute decompensated heart failure and NSVT. He was started on intravenous amiodarone with a bolus dose of 300 mg followed by a continuous infusion of 900 mg over 24 h. Control blood tests performed 18 h after starting amiodarone showed an abrupt elevation of aminotransferases (aspartate aminotransferase 3398 U/L, alanine aminotransferase 1964 U/L), lactate dehydrogenase (2127 U/L), direct bilirubin (2.47 mg/dL) and international normalized ratio (2.79). Gamma-GT and alkaline phosphatase were normal. Despite hemodynamic and ventricular electric stability, he evolved with worsening of hepatic function associated with thrombocytopenia, metabolic acidosis and acute kidney injury. Abdominal ultrasonography showed a liver with normal appearance and excluded hepatic artery and vein thrombosis and any bile duct abnormalities. Viral hepatitis serologies (hepatitis B and C, cytomegalovirus, Epstein-Barr and herpes zoster viruses) and autoimmune markers (antinuclear antibody, anti-smooth muscle antibody, anti-liver/kidney microsomal antibody type 1) were negative.

Drug-induced liver injury secondary to amiodarone was the main diagnostic hypothesis and amiodarone was withdrawn about 40 h after its beginning (total dose of 1800 mg). Since then, he improved gradually with progressive normalization of renal and hepatic function (Figures 1 and 2). At day 4, he restarted amiodarone in oral form, at loading doses of 200 mg three times daily, without any additional liver injury. There was no recurrence of VT and he was discharged on day 12 with nearly normal hepatic tests.

DISCUSSION

This report describes a severe acute hepatitis induced by intravenous amiodarone. Among the few cases reported in literature of idiosyncratic reactions to intravenous amiodarone, some had a fatal outcome[4-6].

American College of Gastroenterology guidelines recommends that the causality assessment in patients with drug-induced hepatic injury should rely primarily on consensus expert opinion following a thorough evaluation for competing etiologies[7]. The causal relationship between intravenous amiodarone exposure and acute hepatitis has been established based on the following principles: (1) Sudden hepatic tests abnormalities within 24 h after starting amiodarone administration; (2) Presence of a pattern of hepatocellular injury with peak aminotransferases levels of more than 50 times the upper limit of normal; (3) Rapid improvement after amiodarone withdrawal; and (4) Exclusion of other causes.

It's often difficult to distinguish between this entity and acute hepatic ischemia since many of these patients on intravenous amiodarone present hemodynamic instability. In this case, the patient was under invasive monitoring and maintained mean arterial pressure superior to 75 mmHg, which makes the diagnosis of ischemic hepatitis unlikely. He also didn't have a severe congestive heart failure that could possibly explain a congestive hepatopathy. Thrombocytopenia and acute kidney injury were assumed to be secondary to acute hepatic injury. Because of his favorable evolution, we did not perform a hepatic biopsy.

According to the Council for International Organizations of Medical Sciences/Roussel Uclaf Causality Assessment Method scale[8] this case fulfilled the criteria of a highly probable amiodarone adverse effect.

The physiopathology of this adverse effect is still unclear. Different potential mechanisms have been proposed, including an immunologically mediated mechanism[9,10], a free radical mechanism, in which formation of free radicals leads to peroxidative injury of membrane lipids and necrosis[11,12] and a mechanism based in increased expression of the *PPAR-*α gene secondary to disrupted hepatic lipid homeostasis[13]. The mechanism of oral amiodarone induced hepatotoxicity seems to be different from that induced by intravenous amiodarone. Some reports, including our own, showed that introduction of oral amiodarone in these patients did not result in any additional liver injury. Based on this observation, Rhodes *et al*[14] proposed that polysorbate 80, the solvent of intravenous formulation of amiodarone, could be involved in this adverse effect since it is present in the intravenous but not in the oral form of amiodarone. Polysorbate 80 has been implicated in the E-ferol syndrome, which has been described in infants after intravenous administration of E vitamin with this component[15]. The E-ferol syndrome shows significant similarities to the cases of liver toxicity due to amiodarone[14]. In addition, polysorbate 80 has a short

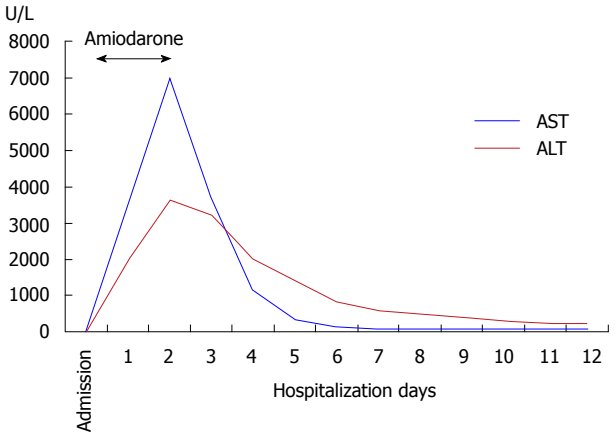

Figure 1 Evolution of aminotransferases levels during hospitalization. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

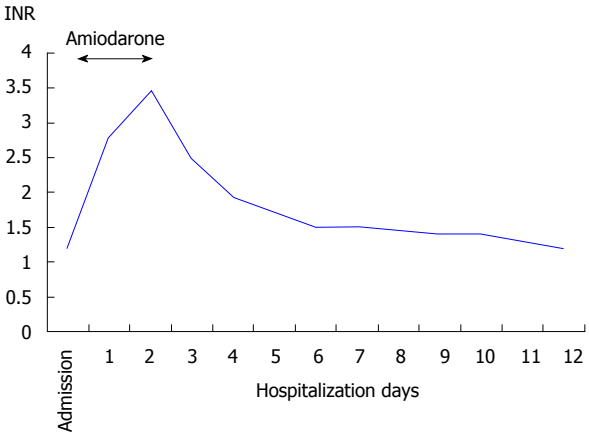

Figure 2 Evolution of international normalized ratio levels during hospitalization. INR: International normalized ratio.

plasma half life, which could justify the rapid recover of hepatic failure after discontinuation of intravenous amiodarone. In 2008 Food and Drug Administration approved a polysorbate-free formulation of amiodarone (Nexterone, Baxter Healthcare Corporation, Deerfield, IL), however it's still not available in several hospitals.

In conclusion, acute hepatotoxicity is a rare, but potentially fatal, adverse effect of intravenous amiodarone. This case highlights the need for close monitoring of hepatic function during amiodarone infusion in order to identify any potential hepatotoxicity and prevent a fatal outcome. If available, it should be considered the use of polysorbate-free formulation of intravenous amiodarone. Oral amiodarone is, apparently, a safe option in these patients.

COMMENTS

Case characteristics

An 88-year-old man admitted with acute decompensated heart failure and non-sustained ventricular tachycardia underwent intravenous amiodarone.

Clinical diagnosis

The patient developed severe acute hepatitis induced by intravenous amiodarone.

Differential diagnosis

Acute viral hepatitis, autoimmune hepatitis, ischemic liver injury, congestive hepatopathy.

Laboratory diagnosis

Aspartate aminotransferase 3398 U/L, alanine aminotransferase 1964 U/L, lactate dehydrogenase 2127 U/L, direct bilirubin 2.47 mg/dL and international normalized ratio 2.79; viral hepatitis serologies and autoimmune markers were negative.

Imaging diagnosis

Abdominal ultrasonography showed a liver with normal appearance and excluded hepatic artery and vein thrombosis and any bile duct abnormalities.

Pathological diagnosis

Hepatic biopsy was not performed due to favorable evolution after amiodarone withdrawal.

Treatment

The treatment was mainly supportive after amiodarone withdrawal.

Related reports

Few cases of acute hepatic injury after intravenous amiodarone have been reported in literature. Some of them had a fatal outcome.

Term explanation

Idiosyncratic reactions are unpredictable adverse drug reactions that do not occur in most patients, but can be life-threatening.

Experiences and lessons

This case highlights the need for close monitoring of hepatic function during amiodarone infusion in order to identify any potential hepatotoxicity and prevent a fatal outcome. Oral amiodarone is, apparently, a safe option in these patients.

Peer-review

This is a well written case report on a serious complication following *iv* amiodarone administration.

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CASE REPORT

Novel variant syndrome associated with congenital hepatic fibrosis

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Abstract

Congenital hepatic fibrosis is part of many different malformation syndromes, of which oculo-encephalo-hepato-renal syndrome is the most common. These syndromes largely overlap, and so accurate classification of individual patients may be difficult. We present herein three syndromic siblings who were products of a consanguineous marriage. We investigated in detail at least six organ systems in these patients, namely the liver, brain, eye, kidneys, skeleton, and gonads. The common features observed in these three cases were congenital hepatic fibrosis, retinitis pigmentosa, truncal obesity, rotatory nystagmus, mental retardation, advanced myopia, and high-arched palate. The clinical dysmorphology in these patients was distinct and lacked the major features of the known syndromes associated with congenital hepatic fibrosis. Although some features of these presented cases are similar to those found in Bardet-Biedl syndrome (BBS), the absence of some major criteria of BBS (polydactyly, renal abnormality, and hypogonadism) suggests that this may be a new syndrome. All three patients remain under follow-up in the departments of Gastroenterology, Ophthalmology, and Neurology at Hacettepe University.

Key words: Congenital hepatic fibrosis; Nystagmus; Mental retardation; Retinitis pigmentosa; High-arched palate

Core tip: Congenital hepatic fibrosis is an inherited disorder that may also accompany other congenital syndromes. Here, we present three siblings with a new variant syndrome characterized by congenital hepatic fibrosis, retinitis pigmentosa, mental retardation, nystagmus, high-arched palate, truncal obesity, and advanced myopia.

Bayraktar Y, Yonem O, Varlı K, Taylan H, Shorbagi A, Sokmensuer C. Novel variant syndrome associated with congenital hepatic fibrosis. *World J Clin Cases* 2015; 3(10): 904-910 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i10/904.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i10.904

INTRODUCTION

Congenital hepatic fibrosis (CHF) is an autosomal recessive inherited malformation defined pathologically by a variable degree of periportal fibrosis and irregularly-shaped proliferating bile ducts[1,2]. The exact incidence and prevalence of CHF are not known, but it is a rare disease. By 1981, only 200 patients with CHF had been reported in the literature[3]. The first manifestations of the disease in most patients are signs or symptoms related to portal hypertension, especially splenomegaly and varices, often with gastrointestinal bleeding[4]. The clinical manifestations of CHF are non-specific, making the diagnosis of this disorder difficult. Although the onset of symptoms and signs is highly variable (ranging from early childhood to the 6th decade of life), CHF is most frequently diagnosed during adolescence or young adulthood[4]. The late appearance of symptoms and their clinical evolution suggest that CHF is a dynamic and progressive condition.

CHF occurs in association with a range of both inherited and non-inherited disorders. Described herein are three siblings from consanguineous parents, all of whom had CHF in conjunction with retinitis pigmentosa, truncal obesity, rotatory nystagmus, mental retardation, advanced myopia, and high-arched palate. The aim of this report was to evaluate the clinical findings of these three cases and to compare these findings with relevant syndromes; Joubert, Bardet-Biedl, cerebellar vermis hypoplasia, oligophrenia, ataxia, coloboma, hepatic fibrosis (COACH), Arima, and Meckel, among others.

CASE REPORT

Patient 1

The eldest child of the family is a 34-year-old female patient who presented with nystagmus, truncal obesity [body mass index (BMI): 29, waist circumference: 97 cm], and blurred vision. The family history was unremarkable, with the exception that her parents were first cousins. She reported that her liver disease and splenomegaly were discovered when she presented to the hospital for pneumonia at the age of seven, whereupon she underwent splenectomy and then cholecystectomy.

Laboratory studies revealed the following: hemoglobin 13.4 g/dL, white blood cell count 9200/mm^3, platelet count 246000/mm^3, international normalized ratio 1.09, partial thromboplastin time 28.3 s, aspartate aminotransferase (AST) 27 U/L, alanine aminotransferase (ALT) 25 U/L, gamma glutamyl transpeptidase (GGT) 32 U/L, total bilirubin 0.59 mg/dL, and albumin 3.4 g/dL. Serum electrolytes, renal function, and urinary examination were normal. The real time and Doppler ultrasonographic examination revealed portal vein cavernous transformation, a heterogeneous liver, and normal kidneys. A needle biopsy of the liver showed an increased number of irregularly-shaped bile ducts, with nodularity of the liver parenchyma accentuated by fibrous septa typical of CHF (Figure 1).

Her neurological examination demonstrated mild mental retardation, normal motor examination aside from hypoactive deep tendon reflexes (+1), and normal cerebellar tests. She had a high-arched palate, dystonia in her hands, and pes planus. Her vibration and position senses were decreased. Brain magnetic resonance imaging (MRI) revealed bilateral substance deposition in the globus pallidus, suggesting the presence of chronic liver disease.

She had exhibited signs of blurred vision in infancy/childhood, but her family was not concerned until she attended primary school. She had significant myopia and rotatory nystagmus with normal facial expression (Figures 2 and 3). Fundus examination revealed a pale optic disc, abundant bone spicules involving even the macula, and advanced arterial sclerosis. She also had myopia and rotatory nystagmus. Her bilateral visual acuity was restricted to hand movements only. Electroretinography yielded findings of retinitis pigmentosa (Figure 4). Examination of other cranial nerves yielded normal findings. No respiratory or cardiac abnormalities were found, and she had normal secondary sex characteristics.

Patient 2

The second patient, a 31-year-old female and the sister of the first patient, also presented with blurred vision, nystagmus, and truncal obesity (BMI: 33, waist circumference: 107 cm).

Laboratory tests revealed the following: hemoglobin 13.4 g/dL, white blood cell count 6600/mm^3, platelet count 223000/mm^3, ALT 19 U/L, AST 23 U/L, GGT 64 U/L, alkaline phosphatase 70 U/L, total bilirubin 0.72 mg/dL, blood urea nitrogen (BUN) 8 mg/dL, creatinine 0.69 mg/dL, and albumin 4.2 g/dL. Urinary examination was normal. Findings of a liver biopsy of this patient were also consistent with CHF (Figure 5).

Her neurological examination showed mental retardation, normal motor examination aside from hypoactive deep tendon reflexes, normal cerebellar tests, and a

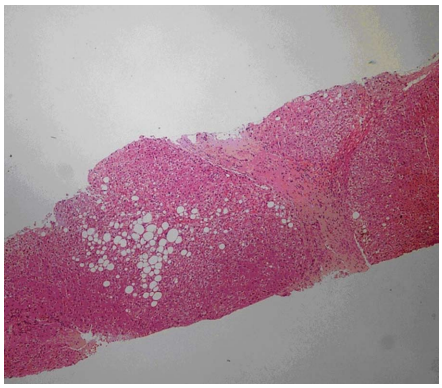

Figure 1 Liver biopsy showing an increased number of abnormal bile ducts, with nodularity of liver parenchyma accentuated by fibrous septa.

negative Romberg test. Her sensation of vibration and position was decreased. Her brain MRI yielded normal findings. She had a high-arched palate.

The patient's blurred vision was noticed while in primary school. Although decreased, her visual acuity was better than her elder sister; 2/20 with significant myopia bilaterally. Fundus examination revealed a pale optic disc, abundant bone spicules involving even the macula, and advanced arterial sclerosis. She also had rotatory nystagmus. Electroretinography yielded findings of retinitis pigmentosa. Examination of other cranial nerves yielded normal findings. No respiratory, cardiac, or renal abnormalities were found, and she had normal secondary sex characteristics with regular menses.

Patient 3

The third patient, a 30-year-old male and the brother of the first two patients, also presented with blurred vision, nystagmus, and truncal obesity (BMI: 28, waist circumference: 97 cm). Laboratory tests revealed the following: hemoglobin 15.3 g/dL, white blood cell count 5900/mm^3, platelet count 92000/mm^3, ALT 67 U/L, AST 46 U/L, GGT 122 U/L, alkaline phosphatase 107 U/L, total bilirubin 1 mg/dL, BUN 14 mg/dL, creatinine 0.85 mg/dL, and albumin 4.6 g/dL. Urinary examination was normal. Liver biopsy of this patient was also consistent with CHF (Figure 6).

Patient 3 also had mental retardation, although less pronounced than his sisters. He did not have any motor deficit, aside from hypoactive deep tendon reflexes (+2). His cerebellar tests were normal and Romberg test was negative. His brain MRI revealed normal findings.

The patient's blurred vision and night blindness were noticed while in primary school. He managed to finish primary school in a special facility for mentally retarded children. His visual acuity was 1/20 bilaterally, with significant myopia. Fundus examination revealed a pale optic disc, abundant bone spicules involving even the macula, and advanced arterial sclerosis. He also had rotatory nystagmus. Electroretinography yielded findings of retinitis pigmentosa. Examination of other cranial nerves yielded normal findings. No respiratory, cardiac, or renal abnormalities were found. He had normal secondary sex characteristics, was married, and had one child.

DISCUSSION

CHF has been described frequently in combination with other abnormalities, such as renal disease, cerebellar malformations, and mental retardation[5,6]. The term oculo-encephalo-hepato-renal syndrome is currently employed to report this association. This syndrome is not a single entity, but rather a group of disorders including COACH[7], Meckel[5], Joubert[8], and Arima syndromes[9] (Table 1). These syndromes largely overlap, and so accurate classification of individual patients may be difficult. It has been suggested that the basic defect in COACH, Joubert syndromes, and other similar conditions might be a disturbance in normal epithelial-mesenchymal interactions due to different genetic mutations[8].

A special subgroup of CHF is COACH syndrome, which is characterized by hypoplasia of the cerebellar vermis, oligophrenia, congenital ataxia, coloboma, and hepatic fibrosis[7]. The abnormalities observed in this syndrome appear to be variable. Numerous congenital anomalies were reported to accompany this syndrome, including slender long bones, postaxial polydactyly, pulmonary stenosis, and atrial septal defect. We found no anomalies involving the kidneys, lungs, or heart in any of our patients, and they did not have ataxia. Furthermore, the absence of the primary features of COACH syndrome (*i.e.*, oligophrenia and ocular coloboma, polydactyly, ataxia, and cerebellar vermis hypoplasia) in our patients excludes that diagnosis.

Another member of this group of disorders, Arima syndrome, is characterized by cerebellar vermis hypoplasia, psychomotor retardation, ocular abnormalities including nystagmus, and polycystic kidneys. Of those, only mental retardation and nystagmus was evident in our patients. Moreover, death in infancy is common in this syndrome, usually due to respiratory failure, and survivors usually have severe mental retardation[10].

Joubert syndrome is an autosomal recessive condition distinguished by hypoplasia of the cerebellar vermis, hypotonia, retinal dystrophy characterized by abnormal eye movements, and impaired psychomotor development together with an abnormal respiratory pattern[11]. This syndrome is genetically heterogeneous with mutations in two genes (*AHI-1* and *CEP290*) identified to date[12]. Although not a constant feature, CHF has also been reported to co-exist with Joubert syndrome. Molar tooth sign (MRI appearance of hypoplasia of the cerebellar vermis and accompanying brainstem abnormalities in an axial plane through the junction of the midbrain and pons) is nearly a pathognomonic finding for this syndrome. In our previous study, we reported two sisters with Joubert syndrome and CHF

Table 1 Comparison of our cases with other related syndromes

	Joubert syndrome	Bardet-Biedl syndrome	COACH syndrome	Arima syndrome	Meckel syndrome	Our cases
Cerebellar vermis hypoplasia	+[1]		+[1]	+[1]		
Ataxia	+[1]		+[1]			
Abnormal breathing pattern	+[1]					
Abnormal eye movements	+[1]					
Hypotonia	+[1]					
Retinitis pigmentosa	+[3]	+[1]				+
Polydactyly		+[1]	+[3]		+[1]	
Truncal obesity		+[1]				+
Mental retardation		+[1]				+
Psychomotor retardation				+[1]		
Hypogonadism/genital abnormalities		+[1]				
Renal abnormalities		+[1]		+[1]	+[1]	
Speech disorder/delay		+[2]				
Strabismus/cataract/astigmatism		+[2]				
Brachydactyly/syndactyly		+[2]				
Mild hypertonia		+[2]				
Dental abnormalities		+[2]				
High-arched palate		+[2]				+
Cardiovascular abnormalities		+[2]	+[3]			
Encephalocele					+[1]	
Diabetes mellitus		+[2]				
Oligophrenia			+[1]			
Ocular coloboma			+[1]			
Nystagmus	+[3]			+[1]		+
Hepatic fibrosis	+[3]	+[3]	+[1]	+[1]	+[3]	+
Advanced myopia						+

[1]One of the primary features of this syndrome; [2]One of the secondary features of this syndrome; [3]Not a constant feature but has been reported in the literature.

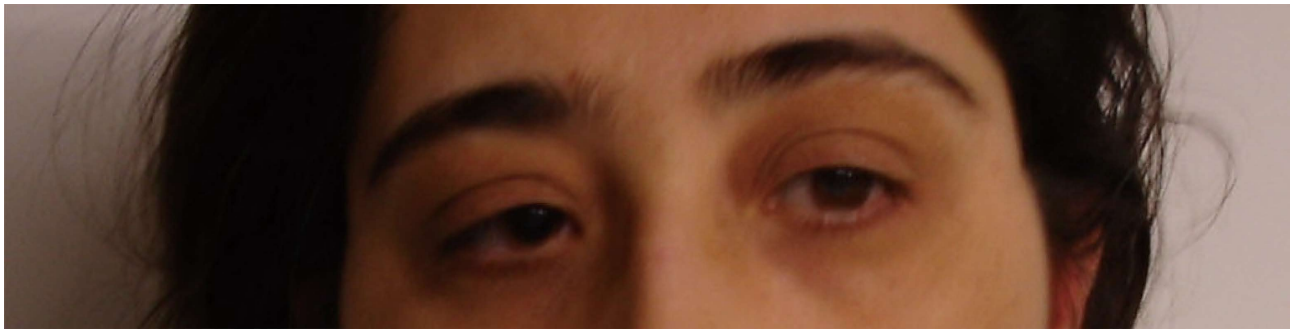

Figure 2 Eyes of the first patient.

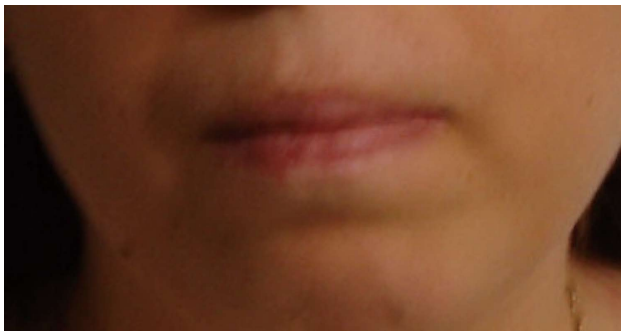

Figure 3 Mouth of the first patient. This appearance helps us to make a differential diagnosis of Cohen's syndrome, in which a distinct cheerful facial expression is noted.

who presented with abnormal eye movements, speech disorder, and mental motor retardation. Their MRIs were suggestive of Joubert syndrome[6]. However, none of the current three patients had molar tooth sign on MRI, essentially excluding Joubert syndrome. The overlapping features of our patients with Joubert syndrome included poor vision, nystagmus, retinitis pigmentosa, and CHF. These are not constant findings for Joubert syndrome, but have been reported in the literature as co-existent.

Cohen's syndrome is one of the rare autosomal recessive disorders that are over-represented in the Finnish population[13]. The phenotype in Finnish patients is highly homogenous, consisting of non-progressive mild to severe psychomotor retardation, motor clumsiness, microcephaly, characteristic facial features, childhood hypotonia and joint laxity, progressive retinochoroidal dystrophy, myopia, intermittent isolated neutropenia,

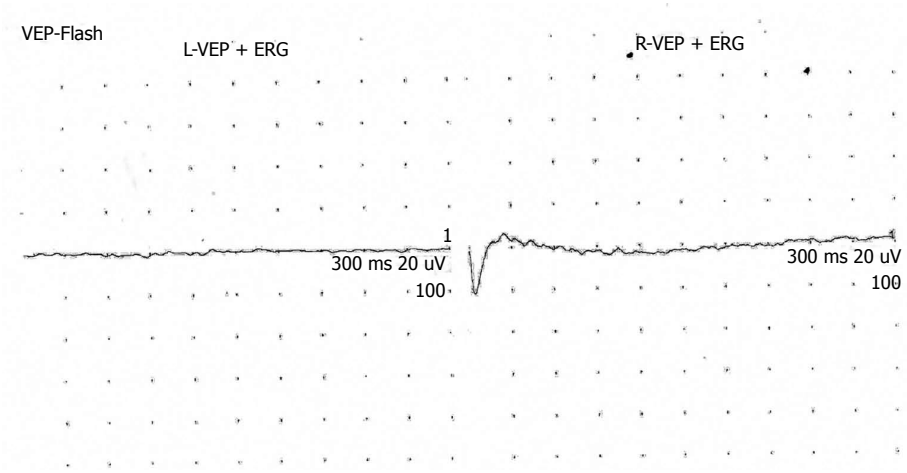

Figure 4 Electroretinography of case 3 showing no response in eyes bilaterally. VEP: Visual evoked potantial; ERG: Electroretinography.

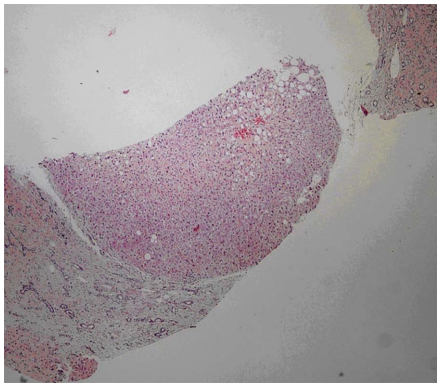

Figure 5 Liver showing nodular appearance due to fibrous bands in which elongated and angulated bile ducts are seen.

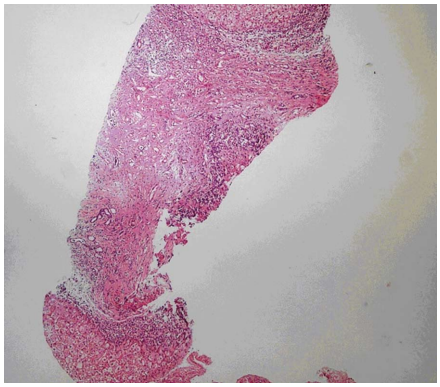

Figure 6 Liver showing nodular appearance due to fibrous bands in which bile ducts are elongated and periportal ductular proliferation is seen.

and a cheerful disposition. The characteristic facial features include high-arched or wave-shaped eyelids, a short philtrum, thick hair, and low hairline. Truncal obesity appearing during or after mid-childhood can be seen in a minority of individuals with the syndrome[14]. Our cases share some features of Cohen's syndrome, such as retinitis pigmentosa, myopia, mental retardation, and obesity, but facial dysmorphism and the other aforementioned features which are highly typical for Cohen's syndrome were absent in our cases.

Retinitis pigmentosa is the term given to a set of hereditary retinal diseases that feature degeneration of rod and cone photoreceptors[15]. A major form of syndromic retinitis pigmentosa, Bardet-Biedl syndrome (BBS), is variably associated with obesity, cognitive impairment, polydactyly, hypogenitalism, and renal disease[16]. BBS has also been found to be associated with CHF. Three families with BBS mapped to the BBS2, BBS3, and BBS4 loci of 2q31 were recruited in one study for a comprehensive eye exam and, in selected cases, electroretinography testing. The results of that study suggested that BBS3 and BBS4 mutations may play a role in the development of myopia[17]. Our patients share primary (truncal obesity and retinitis pigmentosa) and secondary (high-arched palate and CHF) features of BBS. Truncal obesity was defined in our patients according to the International Diabetes Federation 2005 criteria[18]. However, the primary features of BBS, such as postaxial polydactyly, hypogonadism, and renal abnormalities, were absent in the presented cases. Different mutations in unknown genes could possibly be responsible for the advanced myopia in our patients, similar to the situation in BBS. Although myopia has been rarely reported in BBS, advanced myopia was noted in our cases.

In forming the diagnostic criteria for each syndrome, it is important to consider anatomical malformations in conjunction with the clinical signs and symptoms. We investigated six organs in detail, namely the liver, brain, eye, kidneys, skeleton, and gonads. There are major anatomical malformations of the kidney, hands (polydactyly), and gonads in BBS, and of the brain in Joubert, Arima, COACH, and Meckel syndromes. As shown in Table 1, our cases, who presented with CHF, advanced myopia, rotatory nystagmus, truncal obesity, retinitis pigmentosa, mental retardation, and high-arched palate, did not have all or even at least three major components of any listed syndrome. The authors

entertain the possibility that our cases may represent a new syndrome. As shown in Table 1, the presented cases most closely resemble BBS. Beales *et al*[16] reviewed the diagnostic criteria of BBS after evaluating 112 cases based on their clinical findings and they added new criteria; however, liver fibrosis was not included as part of BBS. The predominance of liver fibrosis and the absence of polydactyly, renal abnormality, and hypogonadism in our cases distinguish them from BBS. These clinical findings suggest that our patients might represent a new syndrome.

Our report may contribute to a better delineation of the variable clinical expression of cases within the spectrum of oculo-encephalo-hepato-renal syndromes.

COMMENTS

Case characteristics

Three patients presented with blurred vision and truncal obesity.

Clinical diagnosis

Abnormal signs on physical examination were mental retardation, high-arched palate, pes planus, myopia, rotatory nystagmus, and retinitis pigmentosa on electroretinography.

Differential diagnosis

Cerebellar vermis hypoplasia, oligophrenia, ataxia, coloboma, hepatic fibrosis (COACH), Meckel, Joubert, Arima, Cohen, Bardet-Biedl syndromes.

Laboratory diagnosis

Laboratory test results were essentially within normal limits, with the exception of mildly-elevated alanine aminotransferase in two patients and mild thrombocytopenia in one patient.

Imaging diagnosis

Doppler examination in one patient revealed portal cavernous transformation, while magnetic resonance imaging of the brain was within normal limits in all patients.

Pathological diagnosis

Liver biopsy revealed an increased number of irregularly-shaped bile ducts, with nodularity of the liver parenchyma accentuated by fibrous septa typical of congenital hepatic fibrosis (CHF) in all patients.

Treatment

The patients did not require immediate treatment, but were treated for their ophthalmological disturbances.

Related reports

The co-existence of CHF, retinitis pigmentosa, mental retardation, nystagmus, high-arched palate, truncal obesity, and advanced myopia in the patients may indicate a new variant syndrome different from the known oculo-encephalo-hepato-renal syndromes (*i.e.*, COACH, Meckel and Joubert).

Term explanation

CHF has been described frequently in combination with other abnormalities, such as renal diseases, cerebellar malformations, and mental retardation. The term oculo-encephalo-hepato-renal syndrome is currently employed to report this association.

Experiences and lessons

CHF may present as part of a syndrome affecting the central nervous system and eyes.

Peer-review

The authors have described three cases of CHF associated with ophthalmological and neurological findings which could represent a new syndrome.

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CASE REPORT

Acute dapsone poisoning in a 3-year-old child: Case report with review of literature

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Author contributions: Sunilkumar MN collected the clinical data and prepared the case report; Ajith TA had edited and critically revised the intellectual content; Parvathy VK approved the final version of the manuscript to be published.

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Informed consent statement: The subject had given verbal consent for publishing this case report.

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Abstract

Dapsone (DDS-diamino diphenyl sulphone) is a sulfone antibiotic being used for a variety of clinical conditions. Poisoning in children by DDS is rarely reported. Poisoning in acute cases will be frequently unrecognized due to relative lack of severe signs and symptoms. Methemoglobinemia is the major life-threatening situation associated with poisoning of DDS. Hence, any delay for medical attention can lead to increased rate of mortality. In this case, we describe acute DDS poisoning in a 3-year-old child and the successful management using intravenous methylene blue.

Key words: Dapsone; Methemoglobinemia; Ascorbic acid; Methylene blue; Hemolysis

Core tip: Dapsone (DDS-diamino diphenyl sulphone), a sulfone antibiotic poisoning in children is rarely reported. Methemoglobinemia is the major life-threatening situation associated with DDS poisoning. Delay in seeking medical attention can lead to increased rate of mortality. Methylene blue 0.1% (2 mg/kg) as slow *iv* is the first line therapy. Furthermore, therapies like exchange transfusions and hyperbaric oxygen therapy are options especially in cases where contraindicated in glucose-6-phosphate dehydrogenase deficiency or if methylene blue therapy is ineffective.

Sunilkumar MN, Ajith TA, Parvathy VK. Acute dapsone

poisoning in a 3-year-old child: Case report with review of literature. *World J Clin Cases* 2015; 3(10): 911-914 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i10/911.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i10.911

INTRODUCTION

Dapsone (DDS-diamino diphenyl sulphone), a sulfone antibiotic being used for the prophylactic therapy of various infections in an immunocompromised individual[1]. DDS poisoning in children are rarely reported. In initial stages of acute poisoning, there will not be any major manifestations and hence there may be delay in seeking medical attention. The life threatening events occur as a result of DDS-induced methemoglobinemia which will eventually affect the oxygen delivery to cells[2]. Hence, it will be worthwhile to discuss the manifestations and managements of DDS poisoning in order to prevent its adverse effects. In this case study, we present a 3-year-old child with accidental ingestion of DDS.

CASE REPORT

A 3-year-old boy brought to the hospital with complaints of accidental ingestion of DDS. At the onset of admission, the symptoms were lethargy, vomiting and unsteadiness. The child had persisted vomiting and later developed lethargy. He was conscious with blood pressure 106/68 mmHg, respiration rate 68/min, temperature 98.6 °F and pulse rate 150 beats/min. Oxygen saturation (SpO_2) was 91%. He had mild peripheral cyanosis, ataxia and nystagmus. Pupils were equally reacting to light; reflexes were brisk with plantar withdrawal with tone increased in all limbs. Later, the child becomes agitated and stuporous. Arterial blood gas (ABG) analysis showed pO_2 84 mmHg with hematocrit 29% and SpO_2 91.6% (Table 1). Evidence for hemolysis characterized by progressive drop in haemoglobin levels and hematocrit values. Packed red cell transfusion was given on 2nd and 3rd day as there was ongoing hemolysis. The initial methemoglobin (MHbA) level was 19.4%. Acute DDS-induced methemoglobinemia and CNS involvement was confirmed. O_2 inhalation and ascorbic acid (CELIN-1000 mg) was administered *via* nasogastric tube along with ranitidine and ondansetron as *iv* Methylene blue 0.1% (2 mg/kg) as slow *iv* was given. SpO_2 was increased to 96%, pO_2 88 mmHg with hematocrit 29%. Liver function test showed abnormal rise in enzymes till 5th day (Table 1). The renal function test and urinalysis were normal. On 5th day, the MHbA level was found to be 10.2%. On the day 7, another dose of methylene blue was given as he became lethargic with SpO_2 of 82%. The child was improved and discharged on day 14th after admission. During the follow-up, he had no neurological deficits and haemoglobin level was 11.8 g/dL.

DISCUSSION

DDS is an antimicrobial used to treat leprosy, dermatoses, malaria, *etc*[1]. The most frequent reaction that occur with higher doses of DDS toxicity is hemolytic anemia and methemoglobinemia[3]. Landers *et al*[4] reported decrease in hemoglobin (1-2 g/dL) and reticulocyte count (2%-12%) levels in patient with DDS toxicity. Therefore, when the methemoglobinemia causes symptomatic hemodynamic instability, discontinuation of DDS therapy is recommended.

The possibilities for DDS ingestion and poisoning in children are high. The blood MHbA level determines the clinical severity of the symptoms and signs. Most of the patients are found to be asymptomatic until approximately 30% of hemoglobin is presented as MHbA[1]. However, levels especially greater than 15% may be associated with cyanosis. In this child, the initial MHbA was 19.4%. Headache, lethargy, tachycardia and dizziness may be presented at levels between 20%-45%, whereas dyspnea, acidosis, seizures, cardiac dysrhythmias, heart failure and coma may occur at level above 45%. Furthermore, high mortality rate is associated with levels above 70%[5]. The patient in this case study had metabolic acidosis as evidenced from the lowered blood pH and bicarbonate level one day after the admission.

Acquired methemoglobinemia can be caused by nitrites and nitrates, nitric oxide, sulphones (*e.g.*, dapsone), local anesthetics (*e.g.*, benzocaine), aniline dyes, chlorates, pyridium, phenacetin, sulphonamides, *etc*[6]. Use of topical DDS as treatment for acne vulgaris has also been associated with MHbA levels as high as 20%[7]. In oxygenated and deoxygenated hemoglobin, iron remains in the ferrous (Fe^{2+}) form which is essential for the oxygen transportation. Oxidation of Fe^{2+} to ferric form yields MHbA, which does not bind to oxygen. Followed by the MHbA formation, the oxygen affinity of any remaining Fe^{2+}-hemes in the hemoglobin tetramer is increased and the oxygen dissociation curve is "left-shifted". Therefore, the circulating MHbA as well as the remaining oxyhemoglobin which has increased oxygen affinity can cause impaired oxygen delivery to the tissues. The net effect is that patients with acutely increased concentrations of MHbA have a functional anemia (*i.e.*, the amount of functional hemoglobin is less than the measured level of total hemoglobin). The existence of underlying diseases of lung, heart or blood may exacerbate the toxicity of MHbA. About 3% of the Fe^{2+} of deoxy Hb is slowly oxidized to MHbA per day. The intra-erythrocytic MHbA reducing enzyme systems such as NADH-dependant cytochrome b5 reductase, mainly and NADPH–MHbA reductase and NADPH-glutathione reductase, to a lesser extent help to keep its level below 1%. Level of MHbA above 2% is abnormal.

Management of DDS includes oral administration of activated charcoal and intravenous treatment with methylene blue. In this patient, we could not administer activated charcoal due to persistent vomiting. The

Table 1 Laboratory investigations

Investigations	1 d after admission	5 d after admission
Hemoglobin (g/dL)	10.5	9.7
Platelet count/μL	250000	210000
Total WBC count/μL	10760	14094
Differential leucocyte count (%): Neutrophils; Lymphocytes; Eosinophils; Monocytes; Basophils	65; 26; 3.3; 2.5; 2	48; 43; 5; 2; 2
Serum Na^+ (mmol/L); K^+ (mmol/L)	137; 4.3	138; 4.1
Bicarbonate (mmol/L)	17	23
Serum glutamic-pyruvic transaminase (U/L)	150	162
Blood gas analysis pH; pCO_2 (mmHg); pO_2 (mmHg); hematocrit (%) and SpO_2 (%)	7.37; 16; 84; 29; 91.6	7.41; 22; 88; 29; 96.6
Methaemoglobin (%)	19.4	10.2

plasma elimination half-life of DDS was found to be dose dependent which varies from 10 to 80 h. The renal excretion of unchanged DDS is limited to approximately 20% of the administered dose. DDS is metabolised in the liver for its elimination resulted a moderate elevation of SGPT (150-162 U/L) during the initial few days but normalised on 14th day (45 U/L). After the initial dosage of methylene blue, an additional dose may be repeated if there is an insufficient response. In this case, an additional dose of methylene blue was given since the MHbA level was high on 5th day. This may be due to the enterohepatic circulation of DDS which resulted in a rebound methemoglobinemia as high as 60% up to 18 h of methylene blue injection.

Treatment with methylene blue can be complicated by the presence of underlying glucose-6-phosphate dehydrogenase deficiency. Therefore, alternative therapies like exchange transfusions and hyperbaric oxygen therapy are the remaining options in patients with glucose-6-phosphate dehydrogenase deficiency or if methylene blue therapy is ineffective[8]. But the efficacy of these therapies not yet been elucidated. Ascorbic acid rarely reduces the cyanosis associated with chronic methemoglobinemia but has no role in treatment of acute acquired methemoglobinemia. Furthermore, Cimetidine, used as a selective inhibitor of N-hydroxylation, may be effective in increasing patient tolerance to dapsone, chronically lowering the MHbA level by more than 25%. Since it works slowly, cimetidine is not helpful for the management of acute symptomatic methemoglobinemia arising from the use of DDS.

Methylene blue is a phenothiazine-related heterocyclic aromatic molecule most commonly used as a reducing agent in the treatment of methemoglobinemia and for the treatment of cyanide and carbon monoxide poisoning[3,9]. It has dose-dependent effect on cardiac index and pulmonary artery occlusion pressure as well as oxygen delivery and lactate concentrations. The dosing of methylene blue is not entirely clear, but 1-2 mg/kg is used for the treatment of methemoglobinemia. However, methylene blue above 7 mg/kg is associated with adverse effects such as paradoxical induction of MHbA, hemolytic anemia and detrimental effects on pulmonary function[10,11]. Therefore, methylene blue should not be recommended in patients with pulmonary hypertension, underlying glucose-6-phosphate dehydrogenase deficiency and acute lung injury[11]. Clinicians should also be aware of potential adverse effects and drug interactions with serotonergic agents when considering therapy with methylene blue[12].

According to Wright *et al*[13], the diagnosis may be complicated by the effect of MHbA on arterial blood gas and pulse oximeter oxygen saturation results. In the presence of the increased MHbA fraction, pulse oximeter values will trend toward 85% underestimating the actual oxygen saturation. Guay[14] demonstrated the discrepancy between the pulse oximeter saturation ($\leq$ 90%) and the arterial oxygen partial pressure ($\leq$ 70 mmHg) in subjects with MHbA. Therefore, the routine pulse oximetry is generally inaccurate for monitoring oxygen saturation in the presence of methemoglobinemia. Acute hemolytic anemia in DDS can be explained with the DDS-induced continued oxidative stress or may also be due to the doses of methylene blue[15]. Charcoal hemoperfusion has also been reported for the rapid clearing of dapsone[16].

This case report concluded that patient with dapsone poisoning should be evaluated for serial measurements of methemoglobin levels following treatment with methylene blue in order to evaluate for the subsequent worsening and the need for additional treatment.

COMMENTS

Case characteristics

A 3-year-old boy presented with persisted vomiting and lethargy.

Clinical diagnosis

The patient had mild peripheral cyanosis, ataxia and nystagmus.

Differential diagnosis

Other causes for the drug induced acquired methemoglobinemia.

Laboratory diagnosis

Methemoglobinemia greater than 2% and lowered haematocrit value.

Treatment

Methylene blue 0.1% (2 mg/kg) as *iv*.

Related reports

Accidental acute dapsone poisoning in children are rarely reported. Management includes charcoal hemoperfusion, exchange transfusions and hyperbaric oxygen therapy.

Term explanation

Dapsone, a sulfone antibiotic being used for the prophylactic therapy of various infections in an immunocompromised individual, induces methemoglobinemia at higher doses. The level of methemoglobin in the blood determines the clinical severity of the symptoms and signs.

Experiences and lessons

Patient with dapsone-induced methemoglobinemia required serial measurements of methemoglobin levels following treatment with methylene blue in order to evaluate the subsequent worsening and the need for additional treatment. Routine pulse oximetry is generally inaccurate for monitoring oxygen saturation in the presence of methemoglobinemia.

Peer-review

This is a useful review of dapsone poisioning and its treatment.

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CASE REPORT

Mediastinal small cell carcinoma with liver and bone marrow metastasis, mimicking lymphoma

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Abstract

Primary mediastinal neuroendocrine tumors are a rare malignancy that accounts for < 10% of all mediastinal tumors. The case presented here involves a 52-year-old man who had been suffering for 3 mo from chronic cough, anorexia and substantial weight loss, as well as 2 wk of jaundice prior to his admission. A computed tomography scan showed a 4.3 cm × 6.6 cm mediastinal mass with multiple liver nodules scattered along both hepatic lobes. Endoscopic ultrasound showed a large heterogeneous hypoechoic mass at the mediastinum with multiple target-like nodules in the liver. Fine-needle aspiration specimens revealed numerous, small, round cells with hyperchromatic nuclei, scarce cytoplasm, and frequent mitotic features. Immunohistochemical study revealed positive results for AE1/AE3, CD56 and chromogranin A, with negative findings for synaptophysin, CK20, vimentin, CK8/18 and CD45. The patient was subsequently diagnosed with a poorly differentiated neuroendocrine carcinoma, small cell type. A bone marrow biopsy also revealed extensive involvement by the carcinoma.

Key words: Bone marrow metastasis; Liver metastasis; Lymphoma; Mediastinal mass; Neuroendocrine tumor

Core tip: Neuroendocrine tumors are rare tumors that arise from the gastrointestinal tract and bronchopulmonary system. Primary mediastinal neuroendocrine

tumors are exceptionally rare malignancies, accounting for < 10% of all mediastinal tumors. The common clinical manifestation of this rare tumor is a mediastinal mass, but the condition can mimic lymphoma in advanced cases. The liver is the most common site of metastasis.

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INTRODUCTION

The most common malignancy of the mediastinum is Hodgkin's lymphoma, which usually involves the anterior mediastinum and typically presents as adjacent organ invasion, superior vena cava obstruction, pleural effusion, or erosion of the sternum. The most common site of metastasis for Hodgkin's disease is the liver[1]. In contrast, primary mediastinal neuroendocrine tumors are a rare malignancy that account for < 10% of all mediastinal tumors, with a reported incidence of 0.2-2.0 per 100000 people. These tumors are typically detected as incidental findings from chest radiography, as 40%-70% of advanced cases present with chronic cough, chest pain, dyspnea, or superior vena cava obstruction syndrome[2].

According to Travis[3], pulmonary neuroendocrine tumors are classified as: (1) typical carcinoid tumors; (2) atypical carcinoid tumors; (3) large cell neuroendocrine carcinomas; and (4) small cell neuroendocrine carcinomas. The prognoses of these mediastinal neuroendocrine tumors differ, with typical carcinoids associated with the best prognosis due to their slow growth and late metastasis, and the worst prognosis found with small cell neuroendocrine tumors[4]. Only a few cases of these tumors have been reported, with the liver as the most common site of metastasis[5,6]. This report describes a rare case involving a mediastinal mass with clinical manifestations mimicking lymphoma. To our knowledge, this is the first case report of primary neuroendocrine carcinoma with bone marrow and liver metastases.

CASE REPORT

A 52-year-old man presented to our hospital with a chronic cough, anorexia and substantial weight loss that had occurred over the previous 3 mo. He also presented with painless jaundice that had appeared 2 wk prior to his admission. He reported that he was a heavy smoker and had not experienced fever or shivering. His physical exam showed marked pallor and moderate jaundice, with a normal chest examination. The abdominal examination revealed hepatomegaly without splenomegaly and a negative finding for peripheral lymphadenopathy. Laboratory blood tests showed marked anemia with 6.8 g/dL hemoglobin (reference range: 12.0-18.0 g/dL), and normal white blood cell (7.7×10^3 cells/μL; reference range: 4-11 × 10^3 cells/μL) and reduced platelet (139×10^3 cells/μL; reference range: 150-440 × 10^3 cells/μL) counts. Liver chemistry results were as follows: total bilirubin, 4.2 mg/dL (reference range: 0-1.2 mg/dL); direct bilirubin, 3.7 mg/dL (reference range: 0-0.3 mg/dL); aspartate transaminase, 138 U/L (reference range: 0-32 U/L); alanine transaminase, 141 U/L (reference range: 0-32 U/L); and alkaline phosphatase, 403 U/L (reference range: 35-105 U/L). A markedly elevated lactate dehydrogenase level was noted at 3971 U/L (reference range: 240-480 U/L). Carcinoembryonic antigen measured 3.1 ng/mL (reference range: 0-5.0 ng/mL), with carbohydrate antigen 19-9 at 60.6 U/mL (reference range: 0-37.0 U/mL) and alpha-fetoprotein at 3.02 ng/mL (reference range: 1.09-8.04 ng/mL). The anti-HIV test was negative.

A computed tomography (CT) scan of the chest and upper abdomen showed a 4.3 cm × 6.6 cm mediastinal mass (Figure 1) with multiple liver nodules scattered along both hepatic lobes without any noteworthy pulmonary lesions. The provisional diagnosis was lymphoma, and the patient was therefore scheduled for endoscopic ultrasound and tissue sample collection. After deep sedation was induced using intravenous propofol with full anesthetic monitoring, a curvilinear endoscopic ultrasound scope (EG530UT2; Fujifilm, Minato-ku, Tokyo, Japan) was used for scanning. The echoview showed a large heterogeneous hypoechoic mass > 6 cm in diameter at the mediastinum, with multiple target-like nodules in the liver (Figure 2). Next, fine-needle aspiration of the mediastinal mass was performed with four passes using a 22-gauge needle (EchoTip Procore; Cook Group Inc., Bloomington, IN, United States) (Figure 3). A diagnosis of primary mediastinal lymphoma with liver metastasis was strongly suspected.

The patient's clinical status worsened despite administration of intravenous corticosteroids. The histopathologic results finally revealed numerous small, individual, round cells with hyperchromatic nuclei, scarce cytoplasm, and frequent mitotic features. An immunohistochemical study was positive for AE1/AE3, CD56 and chromogranin A (Figure 4), but negative for synaptophysin, CK20, vimentin, CK8/18 and CD45. Based on these findings, a diagnosis of poorly differentiated neuroendocrine carcinoma, small cell type was made.

A bone marrow biopsy was also performed, which showed extensive involvement by the carcinoma (Figure 5). However, intravenous chemotherapy was not administered due to the poor performance status at this point. The patient subsequently died a few weeks later, after developing progressive liver failure, edema in both legs, and a sudden onset of dyspnea and cyanosis, due

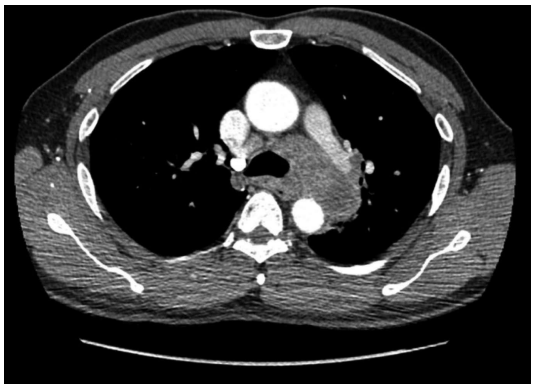

Figure 1 Computed tomography findings. A 6.6 cm × 4.3 cm mediastinal mass was observed.

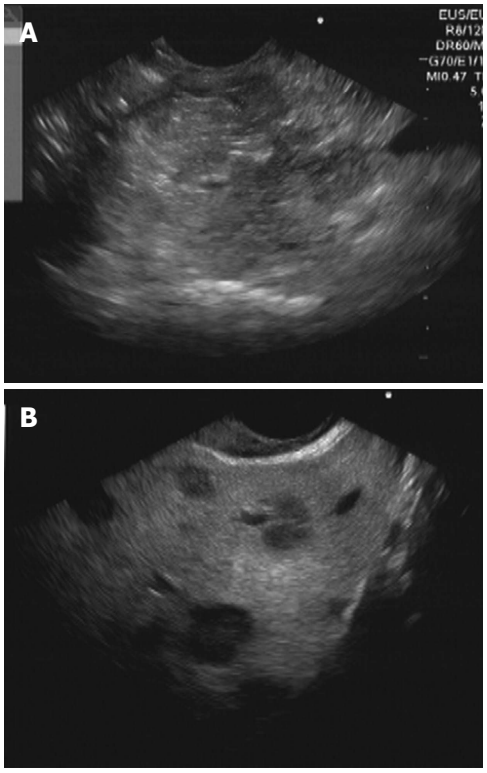

Figure 2 Endoscopic ultrasound findings. A: Echoview showed a large mediastinal mass; B: Liver nodules with target-like appearance were also observed.

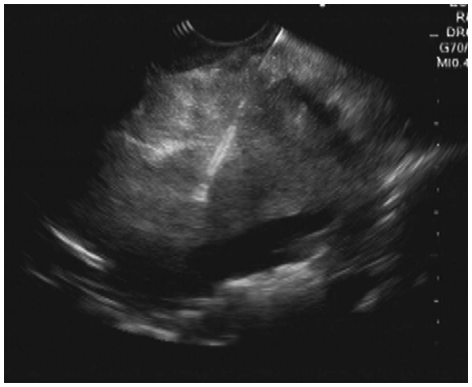

Figure 3 Fine-needle aspiration cytology was performed.

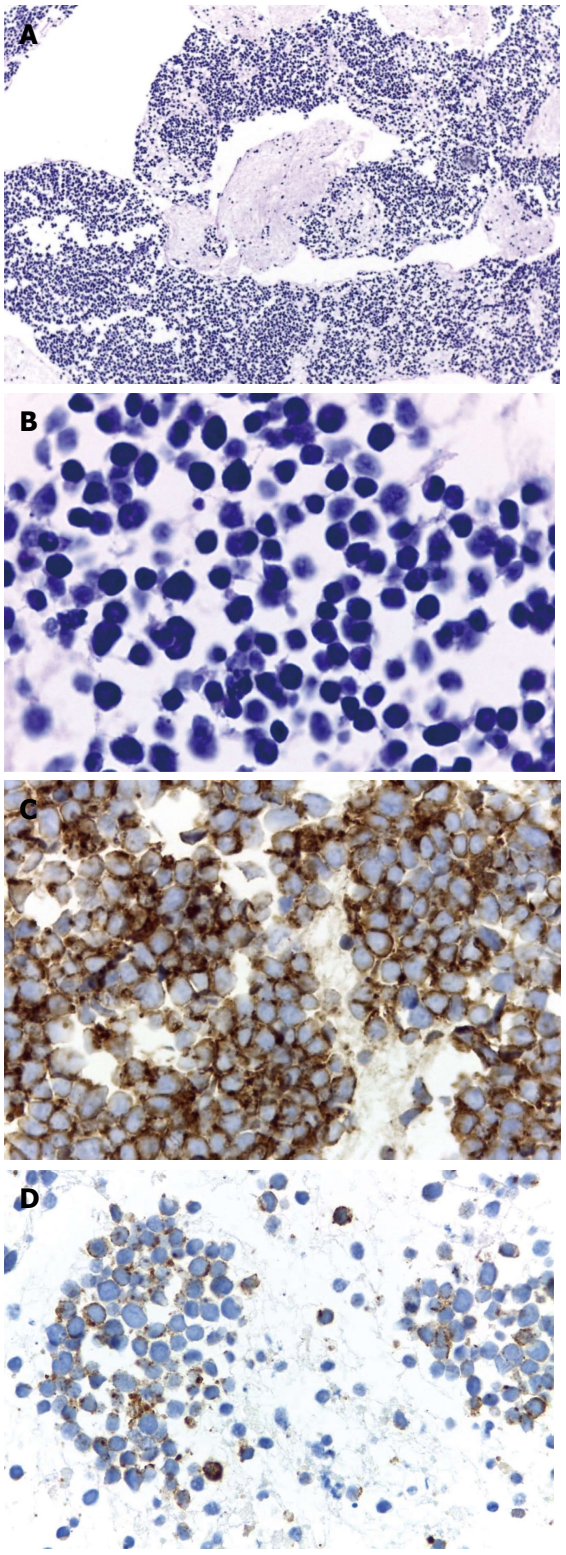

Figure 4 Histopathologic findings. A and B: Cell-block preparation from fine-needle aspiration of the mediastinal mass showed numerous small, individual, round cells with hyperchromatic nuclei, scarce cytoplasm, and frequent mitotic features. Rare instances of nuclear molding were also observed (hematoxylin-eosin staining, magnification × 10 and × 40, respectively); C and D: Immunohistochemical study revealed that the tumor cells were positive for AE1/AE3 (C) and chromogranin A (D; magnifications × 40).

to a suspected acute pulmonary embolism. No autopsy was performed in this case.

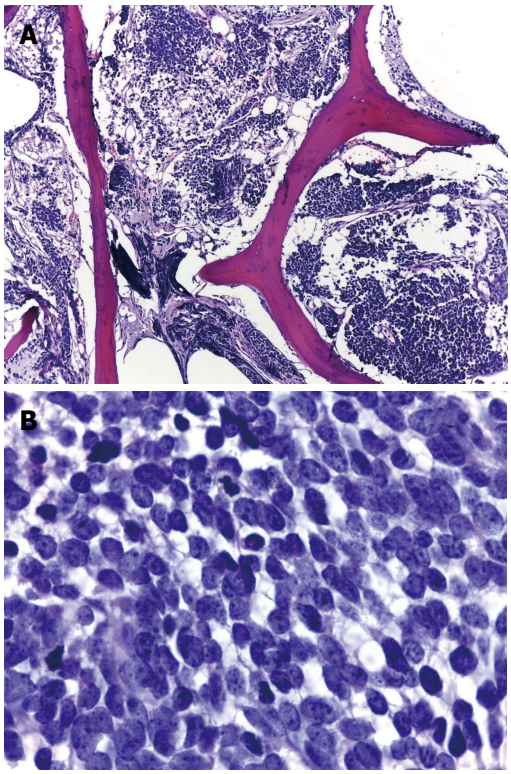

Figure 5 Bone marrow biopsy showed extensive infiltration by cohesive sheets of small, round cells with fine granular chromatin and scarce cytoplasm.

DISCUSSION

Neuroendocrine tumors are rare tumors that typically involve the gastrointestinal tract and the broncho-pulmonary system. Primary mediastinal neuroendocrine tumors are extremely rare[7-13], and can present as lymphoma, particularly Hodgkin's type. Indeed, the case described here initially presented with similar clinical symptoms, such as chronic cough, anorexia, weight loss, and a bulky mediastinal mass with liver metastasis. However, a final diagnosis of a poorly differentiated neuroendocrine tumor, small cell type was made after histopathologic and immunohistochemical study. The multiple liver nodules were strong indicators of liver metastasis, and this is the first reported case of mediastinal neuroendocrine tumor with liver and bone marrow metastases.

Li *et al*[14] reported a case series of six patients with primary small cell neuroendocrine carcinoma and found that most of the cases were in advanced stages, with tumors > 6 cm. Moreover, more than two-thirds of those cases involved the anterior-middle mediastinum with 44% scattered punctate calcification on CT. However, the immunohistochemical studies were positive for different markers, indicating the tumors had differentiated from a carcinoid tumor, mediastinal lymphoma, germ cell tumor of mediastinum, and thymoma. The poor prognosis for primary small cell neuroendocrine carcinoma was demonstrated by only half of the cases responding to chemotherapy, and the 2-year mortality rate of 50%[14].

The patient described in this case report had an advanced stage neuroendocrine tumor with fatal outcome. Definitive treatment for this small cell carcinoma should be guided by the definite histopathological and immunohistochemistry results.

COMMENTS

Case characteristics

A 52-year-old man presented with a chronic cough, weight loss, anemia and progressive jaundice.

Clinical diagnosis

Metastatic small cell neuroendocrine tumor with liver and bone marrow involvement.

Differential diagnosis

Lymphoma; Carcinoid tumor; Mediastinal lymphoma; Germ cell tumor of mediastinum; Thymoma.

Laboratory diagnosis

Hemoglobin, 6.8 g/dL; White blood cell count, 7.7 × 10^3 cells/μL; Platelet count, 139 × 10^3 cells/μL; Total bilirubin, 4.2 mg/dL; Direct bilirubin, 3.7 mg/dL; Aspartate transaminase, 138 U/L; Alanine transaminase, 141 U/L; Alkaline phosphatase, 403 U/L; Lactate dehydrogenase, 3971 U/L; Carcinoembryonic antigen, 3.11 ng/mL; Carbohydrate antigen 19-9, 60.62 U/mL; Alpha-fetoprotein, 3.02 ng/mL.

Imaging diagnosis

Computed tomography scan of the chest and upper abdomen showed a 4.3 cm × 6.6 cm mediastinal mass with multiple liver nodules scattered along both hepatic lobes without any marked pulmonary lesions.

Pathological diagnosis

Poorly differentiated neuroendocrine carcinoma, small cell type.

Treatment

Palliative treatment.

Related reports

Neuroendocrine tumors are a very rare malignancy of the mediastinum, and there are very few reports in the literature.

Experiences and lessons

Mediastinal neuroendocrine tumors may have a clinical presentation similar to lymphoma of the mediastinum. Histologic diagnosis and immunohistochemical study are crucial for definite diagnosis.

Peer-review

The authors describe a case of primary mediastinal neuroendocrine tumor with liver and bone marrow metastases, which mimicked lymphoma. This is a rare tumor with malignant lymphoma as the primary differential diagnosis.

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CASE REPORT

Repeated pancreatitis-induced splenic vein thrombosis leads to intractable gastric variceal bleeding: A case report and review

Shan-Hong Tang, Wei-Zheng Zeng, Qian-Wen He, Jian-Ping Qin, Xiao-Ling Wu, Tao Wang, Zhao Wang, Xuan He, Xiao-Lei Zhou, Quan-Shui Fan, Ming-De Jiang

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Abstract

Gastric varices (GV) are one of the most common complications for patients with portal hypertension. Currently, histoacryl injection is recommended as the initial treatment for bleeding of GV, and this injection has been confirmed to be highly effective for most patients in many studies. However, this treatment might be ineffective for some types of GV, such as splenic vein thrombosis-related localized portal hypertension (also called left-sided, sinistral, or regional portal hypertension). Herein, we report a case of repeated pancreatitis-induced complete splenic vein thrombosis that led to intractable gastric variceal bleeding, which was treated by splenectomy. We present detailed radiological and pathological data and blood rheology analysis (the splenic artery - after a short gastric vein or stomach vein - gastric coronary vein - portal vein). The pathophysiology can be explained by the abnormal direction of blood flow in this patient. To our knowledge, this is the first reported case for which detailed pathology and blood rheology data are available.

Key words: Splenic vein thrombosis; Intractable gastric variceal bleeding; Recurrent pancreatitis; Review

Core tip: Here, we report a case in which chronic

pancreatitis-induced complete splenic vein thrombosis led to intractable gastric variceal bleeding, which is effectly treated by splenectomy. We have provided details regarding the imaging and pathology data, and we describe the hemodynamic characteristics. Then, we reviewed the disease onset and treatment methods, which may provide a reference for the clinical diagnosis and treatment of similar patients.

Tang SH, Zeng WZ, He QW, Qin JP, Wu XL, Wang T, Wang Z, He X, Zhou XL, Fan QS, Jiang MD. Repeated pancreatitis-induced splenic vein thrombosis leads to intractable gastric variceal bleeding: A case report and review. *World J Clin Cases* 2015; 3(10): 920-925 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i10/920.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i10.920

INTRODUCTION

Gastric varices (GV) are among the most common complications affecting patients with portal hypertension, which has a mortality rate that can reach as high as 20% within 6 wk[1]. Currently, histoacryl injection is recommended as the initial treatment for bleeding GV, and this approach has been confirmed to be highly effective for most patients in many studies[2-5]. However, this treatment might be ineffective for some types of GV, such as splenic vein thrombosis-related localized portal hypertension (also called left-sided sinistral or regional portal hypertension). Herein, we report a case of recurrent pancreatitis-induced complete splenic vein thrombosis that led to intractable gastric variceal bleeding, which was treated by splenectomy. We present detailed radiological and pathological data and blood rheology analysis results (splenic artery - after a short gastric vein or stomach vein - gastric coronary vein - portal vein). The pathophysiology can be explained by the abnormal direction of blood flow in this patient. To our knowledge, this is the first reported case for which detailed pathology and blood rheology data are available.

CASE REPORT

A 58-year-old man was admitted to our hospital due to recurrent melena lasting for over a month and vomiting lasting for two hours. His past history revealed a history of heavy drinking of at least 200 g daily that exceeded 30 years; however, approximately 7 years before, his alcohol consumption had decreased. Over the past 7 years, he had experienced recurrent pancreatitis five times, and all incidences resolved. Approximately one month prior to admission, this patient began to experience melena with no obvious cause. Endoscopy showed that the gastric mucosa was elevated with fundal varices without active bleeding. After conservative treatment, the melena became intermittent. Then, another endoscopic examination revealed severe GV, and the patient received five histoacryl injections. Subsequently, he experienced intermittent melena and vomited approximately 200 mL of blood. Physical examination showed anemia, splenomegaly spanning three ribs across the liver, and active bowel sounds (7/min). Blood examinations revealed the following: Red blood cell, 2.98×10^{12}/L; hemoglobin concentration, 67 g/L; and platelet count, 90×10^{9}/L. Both liver and kidney functions were normal. Abdominal enhanced computed tomography (CT) showed cirrhosis and an enlarged portal vein.

The patient was diagnosed with alcoholic cirrhosis, portal hypertension, splenomegaly and GV. Then, emergency endoscopy revealed bleeding GV, and a second histoacryl injection treatment was performed. However, this patient was also experiencing intermittent vomiting, which had become more frequent because the histoacryl injection did not effectively stop the bleeding from fundus varices. Emergency transjugular intrahepatic portosystemic shunt placement was performed as a hemostatic treatment. Portal vein puncture was successful, and portal vein radiography showed an enlarged portal vein; however, the splenic vein and gastric coronary vein were not imaged (Figure 1). Then, another abdominal enhanced CT and portal systemic vascular reconstruction were performed. The enhanced CT scan revealed an enlarged portal vein from the origin of the gastric coronary vein and an enlarged and circuitous gastric coronary vein (Figure 2A). The splenic vein did not show any flow signals in the portal venous phase (Figure 2B). The portal systemic vascular reconstruction image did not show the splenic vein or spleen signals. These data indicated that the intractable gastric variceal bleeding was not induced by alcoholic cirrhosis or portal hypertension but rather by regional portal hypertension promoted by complete splenic vein thrombosis after recurrent pancreatitis.

Taking into account the poor general condition of the patient, splenic artery embolization could have led to serious complications. Therefore, laparotomy was performed for splenectomy. After opening the abdomen, normal liver size, color and texture were observed. During surgery, we found adhesions of the spleen to organs and tissues, such as the stomach, transverse colon and kidney. Approximately two hours were spent separating the extensive adhesions.

After separation of the surrounding tissues and ligation of the splenic artery and short gastric vessels, we successfully removed the spleen and found that the pancreas was very hard to the touch. After anatomical resection of the spleen, we found that the splenic vein was completely blocked by thrombosis (Figure 3A), and the pathology results further confirmed splenic vein thrombosis (Figure 3B). One month after splenectomy, endoscopic examination revealed that the fundal varices had markedly reduced, and ultrasound examination revealed a normal-sized portal vein.

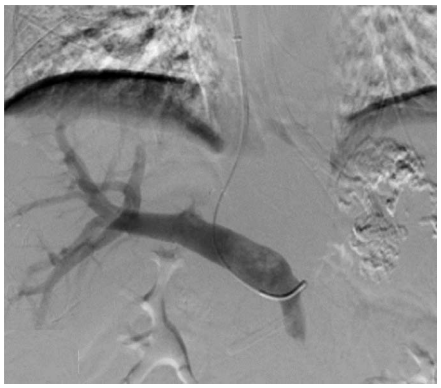

Figure 1 Direct portal vein radiography shows an enlarged portal vein; however, the splenic vein and gastric coronary vein could not be imaged.

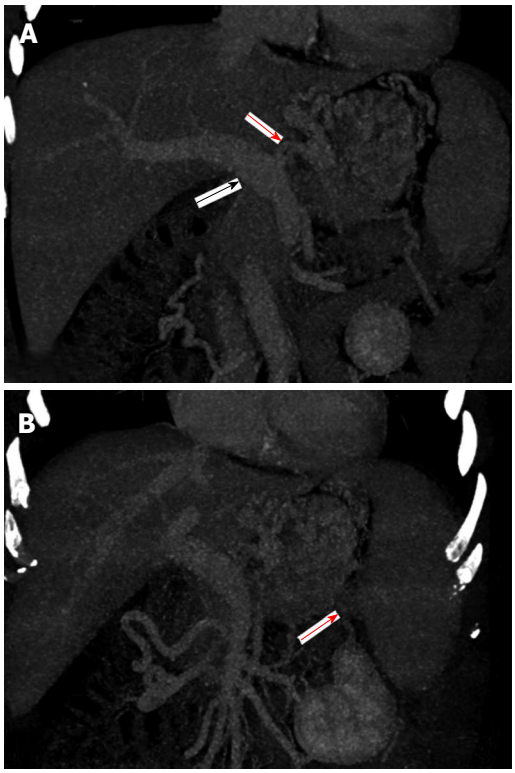

Figure 2 Enhanced computed tomography scan. A: An enlarged portal vein from the origin of the gastric coronary vein and an enlarged, circuitous gastric coronary vein; B: Splenic vein flow signals in the portal venous phase are absent.

DISCUSSION

Gastric variceal bleeding due to regional portal hypertension with splenic vein thrombosis is a severe, life-threatening condition, which is very difficult to control[6]. Patients with splenic vein thrombosis-induced GV, who usually have normal hepatic function, are unlike those with generalized portal hypertension[7], and their mortality risk is higher than that of patients with variceal hemorrhage due to other causes[6,8]. A previous study has shown that as many as 37 different specific etiologies lead to splenic vein thrombosis[9], the most common of which is pancreatitis[10]. The rate of splenic vein thrombosis is 7% to 20% in patients who have previously suffered from pancreatitis[11]. Splenic vein thrombosis induced by pancreatitis was first reported by Hirschfeldt[12]. Other causes of this disease include myeloproliferative neoplasm[13,14], gastrointestinal, pancreatic and hepatobiliary cancers, liver cirrhosis[15], abdominal compression and vibration[16], pancreatic exocrine cancer[17], factors secondary to splenic metastatic cancer[18], minimally invasive distal pancreatectomy[19], and splenic laceration[20].

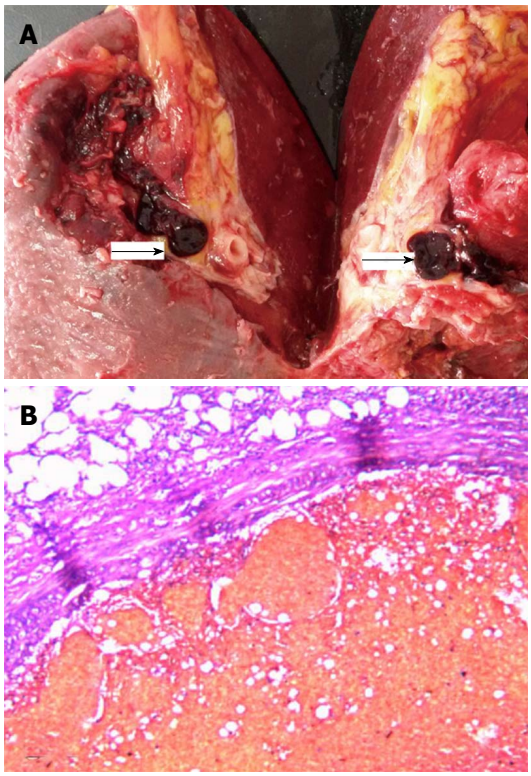

Figure 3 Anatomical resection of the spleen and hematoxylin-eosin staining staining. A: The splenic vein is completely blocked by thrombosis; B: The splenic vein is completely filled by thrombosis.

The splenic vein originates in a large and non-tortuous vessel from the spleen, lies inferior to the splenic artery, and runs behind the pancreatic body and tail. Therefore, the splenic vein endothelium can be damaged by inflammation in the nearby pancreatitis, which can induce splenic vein thrombosis and obstruction. Since the first report of splenic vein thrombosis induced by pancreatitis in 1920[12], five types of pancreatitis have been identified, including chronic, acute, familial, traumatic and autoimmune pancreatitis (Table 1), the most common of which is chronic pancreatitis[11,21-24]. Recently, we have reported a patient with chronic pancreatitis-associated splenic vein thrombosis caused by regional portal hypertension who was treated by partial splenic artery embolization[25]. Acute pancreatitis has been reported to be another common cause of splenic vein thrombosis[23,26,27]. In addition, familial[28], traumatic[29] and autoimmune[30] pancreatitis-induced splenic vein thrombosis and GV have been reported.

Table 1 Etiologies of pancreatitis-induced splenic vein thrombosis

Chronic pancreatitis
Longstreth *et al*[21], 1971
Little *et al*[22], 1981
Moossa *et al*[23], 1985
Bernades *et al*[24], 1992
Heider *et al*[11], 2004
Tang *et al*[25], 2015
Acute pancreatitis
Moossa *et al*[23], 1985
Madsen *et al*[26], 1986
Rogers *et al*[27], 1989
Familial pancreatitis
McElroy *et al*[28], 1972
Traumatic pancreatitis
Salam *et al*[29], 1973
Autoimmune pancreatitis
Ishikawa *et al*[30], 2012

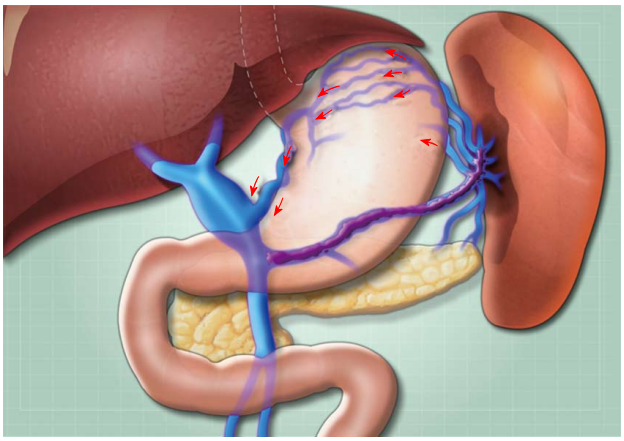

Figure 4 A schematic diagram of the pathophysiological and blood flow changes in this patient.

Herein, we report a case of pancreatitis-induced complete splenic vein thrombosis that led to intractable gastric variceal bleeding. This patient was first misdiagnosed with alcoholic cirrhosis-induced portal hypertension. After direct portal venography and portal vein reconstruction, the patient was finally diagnosed with regional portal hypertension induced by complete splenic vein thrombosis after pancreatitis. Normally, blood flows through the splenic artery and short gastric vein from the fundus back to the portal vein. After passing through the spleen, blood flows through the splenic vein[31]. However, when the splenic vein is completely blocked, splenic artery blood cannot flow back through the splenic vein, which causes the spleen to become congested and enlarged. Blood must reflux to the gastric fundus vein through the short gastric vein, which results in a significant increase in gastric fundus pressure, varices, and reflux to the vena cava through the stomach, the renal vein shunt and other branches. When the pressure of the gastric fundus vein is higher than that of the portal vein, the gastric coronary vein will become enlarged, and blood will reflux to the portal vein through the gastric coronary vein, inducing portal vein enlargement (Figure 4). Therefore, these blood rheology findings explain all of the symptoms, signs, laboratory test results and imaging data of the patient.

Antithrombotic therapy has been recommended for venous thromboembolic disease[32-34]. An institutional (Mayo clinic) database search has revealed that a total of 2454 patients were diagnosed with acute pancreatitis from January 1996 to December 2006, with splenic vein thrombosis noted in 45 (1.8%) patients, and the use of oral anticoagulation was considered to be reasonably safe in these patients[35]. However, for chronic pancreatitis, the incidence of splenic vein thrombosis can reach 20% to 40%[36-38]. For complete splenic vein thrombosis patients, antithrombotic therapy may aggravate the risk of bleeding due to fundal varices. Therefore, splenic artery embolization is one of the best treatments for bleeding GV induced by splenic vein thrombosis[14,39-42]. However, "post-embolization syndrome" is a common side effect experienced after splenic artery embolization and includes abdominal pain, fever, vomiting, and purulent infection depending on the arterial embolism size and the patient's condition. Another study has suggested that transjugular endovascular recanalization of the splenic vein is a safe and effective therapeutic option in patients with regional portal hypertension and is not associated with an increased risk of procedure-related complications[43]. As the condition of the patient in the present report was poor due to massive blood loss, we chose splenectomy *via* laparotomy, which was successful.

This paper describes a case of chronic pancreatitis-induced complete splenic vein thrombosis, which led to intractable gastric variceal bleeding. We have provided details regarding the imaging and pathology data and have described the hemodynamic characteristics. In addition, we have reviewed the disease onset and treatment methods, which may provide a reference for the clinical diagnosis and treatment of similar patients.

COMMENTS

Case characteristics

A 58-year-old man with recurrent melena lasting for over a month and vomiting lasting for 2 h.

Clinical diagnosis

Chronic pancreatitis-induced complete splenic vein thrombosis led to intractable gastric variceal bleeding.

Laboratory diagnosis

Red blood cell, 2.98 × 10^{12}/L; hemoglobin concentration, 67 g/L; and platelet count, 90 × 10^{9} /L.

Imaging diagnosis

Enhanced computed tomography scan revealed an enlarged portal vein from the origin of the gastric coronary vein and an enlarged and circuitous gastric coronary vein. The splenic vein did not show any flow signals in the portal venous phase.

Treatment
Laparotomy was performed for splenectomy.

Experiences and lessons
This is the first reported case for which detailed pathology and blood rheology data are available.

Peer-review
A very interesting paper.

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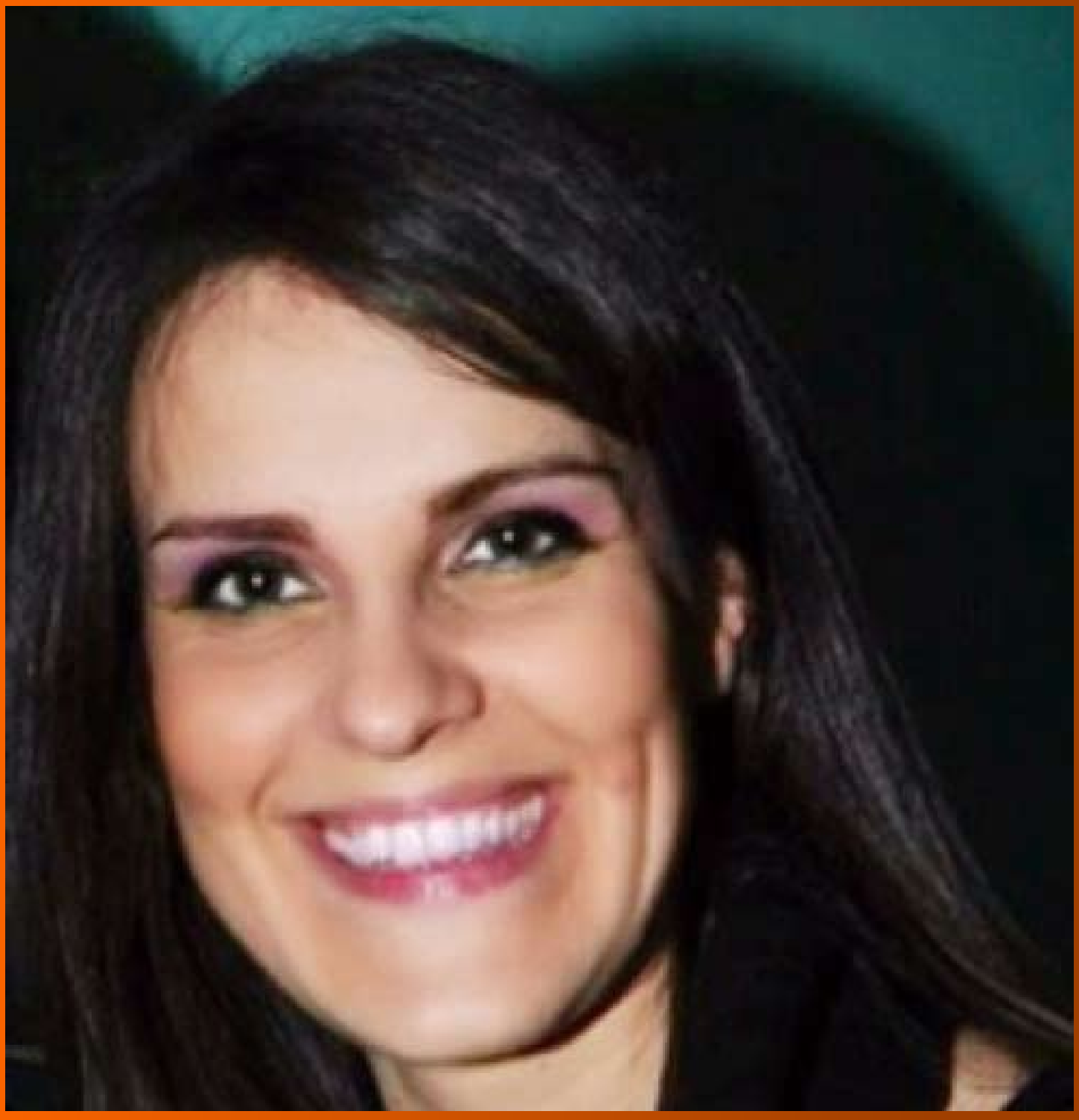

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EDITORIAL

Ultrasound: A promising tool for contemporary airway management

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Abstract

Airway evaluation and its management remains an ever emerging clinical science. Present airway management tools are static and do not provide dynamic airway management option. Visualized procedures like ultrasound (US) provide point of care real time dynamic views of the airway in perioperative, emergency and critical care settings. US can provide dynamic anatomical assessment which is not possible by clinical examination alone. US aids in detecting gastric contents and the nature of gastric contents (clear fluid, thick turbid or solid) as well. US can help in predicting endotracheal tube size by measuring subglottic diameter and diameter of left main stem bronchus. US was found to be a sensitive in detecting rotational malposition of LMA in children. Also, US is the fastest and highly sensitive tool to rule out a suspected intraoperative pneumothorax. In intensive care units, US helps torule out causes of inadequate ventilation, determine the tracheal width and distance from the skin to predict tracheotomy tube size and shape and assist with percutaneous dilatational tracheostomy. US can help in confirming the correct tracheal tube placement by dynamic visualisation of the endotracheal tube insertion, widening of vocal cords (children), and bilateral lung-sliding and diaphragmatic movement. Thus, ultrasonography has brought a paradigm shift in the practise of airway management. With increasing awareness, portability, accessibility and further sophistication in technology, it is likely to find a place in routine airway management. We are not far from the time when all of us will be carrying a pocket US machine like stethoscopes to corroborate our clinical findings at point of care.

Key words: Airway; Ultrasound; Evaluation; Difficult; Management

Core tip: Airway evaluation and its' management is conventionally based on clinical examination and

radiological imaging. They remain static and do not provide dynamic airway management option. Visualized procedures like ultrasound (US) provide point of care real time dynamic views of the airway in perioperative, emergency and critical care settings. US also aids in detecting gastric contents and the nature of gastric contents (clear fluid, thick turbid or solid). This detection is important for preventing complication of aspiration during airway management. The ultrasonography has brought a paradigm shift in the practise of airway management. With increasing awareness, portability, accessibility and further sophistication in technology, it is likely to find a place in routine airway management. We are not far from the time when all of us will be carrying a pocket US machine like stethoscopes to corroborate our clinical findings at point of care.

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Contemporary anaesthesia practise is richly blessed with technology based solutions. Technology has served to reduce human error in enumerable ways. Ultrasonography is one such extremely useful tool which is finding increasing applications in anaesthesia. It is already being considered as "gold standard" for central venous cannulations and peripheral nerve blockade. Visualized procedures improve safety and outcomes as compared to conventional techniques. In recent past, accumulated evidence is favouring its utility for various aspects of airway management for preoperative airway assessment, intraoperative management, predicting weaning from ventilation and successful extubation[1-3]. Various closed claim database and national level audits continue to implicate failure in airway management as a major contributor to perioperative morbidity and mortality[4-6]. Hence, constant efforts have been directed towards finding a "fail-safe" device for assisting us with airway management. Ultrasound (US) is turning out to be one such promising tool.

US provides point of care real time dynamic views of the airway inperioperative, emergency and critical care settings. It is free of ionizing radiation, painless, portable, convenient, reproducible, accurate and easily mastered skill and anaesthesiologist need not be dependent on their radiology colleagues. Because of superficial location of larynx, US can provide images of even higher resolution than advanced imaging modalities like computed tomography (CT) or magnetic resonance imaging (MRI)[6].

Conventional airway assessment fails predict difficult intubation in all patients. US can provide dynamic anatomicalassessment which is not possible by clinical examination alone. Various studies have suggested that US can help in predicting difficult airway by measuring the soft tissue thickness measured on anterior aspect of trachea along with neck circumference[1], hyomental distance ratio[7], width of tongue base and lateral pharyngeal wall thickness[8]. Intraoral sublingual approach to US is being investigated as a useful approach to predict difficult airway[9]. If difficult airway is suspected US can assist in preparing the airway (superior laryngeal and recurrent laryngeal) for awake intubation[10] and identify the cricothyroid membrane so that transtracheal cricothyrotomy cannula can be placed in a "cannot ventilate cannot intubate" (CVCI) scenario[11,12]. Though fasting guidelines are well known, however, gastric emptying is quite variable. US aids in detecting gastric contents and thenature of gastric contents (clear fluid, thick turbid or solid) as well[13].

US can help in predicting endotracheal tube size by measuring subglottic diameter and diameter of left main stem bronchus (for placement of double lumen tube) and help in deciding the appropriate size of the endotracheal tube (ETT)[14,15]. US can also be used to confirm correct laryngeal mask airway (LMA) placement[16]. Its use instead of fiberoptic confirmation averts the hypercapnia associated with the later[17]. US was found to be a sensitive in detecting rotational malposition of LMA in children[18]. Also, US is the fastest and highly sensitive tool to rule out a suspected intraoperative pneumothorax[2].

In intensive care units, US helps to rule out causes of inadequate ventilation, determine the tracheal width and distance from the skin to predict tracheotomy tube size and shape and assist with percutaneous dilatational tracheostomy (PDT)[19,20]. US guided PDT provides real time visualisation of the needle path and guide wire placement using linear array probe. It permits visualisation of pretracheal blood vessels, selection of puncture site, decreases posterior tracheal wall puncture, decreases injury to thyroid isthmus and increases the overall success[21-23]. US has been found to be a better alternative to FOB guided PDT and may replace it in coming years.

US scan help in confirming the correct tracheal ETT placement by dynamic visualisation of the ETT tube insertion, widening of vocal cords (children), and bilateral lung-sliding and diaphragmatic movement[23-25]. Additional advantage of US guided ETT placement is that esophageal intubation can be diagnosed prior to initiation of mechanical ventilation, thus reducing gastric insufflations and its consequences. Recent studies have suggested that bedside US is feasible and faster substitute to conventional techniques (auscultation and waveform capnography) and may replace them in future[24].

Expanding literature in recent years is indicating the utility of US in diagnosing various pathologies that can have implication in clinical decision making, *e.g.*, vocal cord malfunction[3], swallowing abnormalities[25], sialolithiasis[26], supraglottic hemangiomas[27], respi-

ratory papillomatosis[28], laryngeal stenosis[29], Zenker's diverticulum[30-34], *etc*.

Recent advances in airway US include transesophageal US which can provide distal airway images from mid-trachea to bronchi[33]. Additionally, endoscopic high frequency US of larynx has been described where a thin catheter high frequency probe with rotating mirror can produce 360° image of larynx[34]. With advent of multiplanar 3D US in airway imaging, spectrum of its application has further widened as spatial information obtained is more detailed and measurements obtained are more precise[35]. A recent report describing the use of 3D US concluded that airway anatomy, anteroposterior diameter of subglottic area and transverse diameter of upper trachea can be accurately measured and correlated with MRI findings[35]. Pocket sized smartphone based system can increase its applicability even in remote areas[36].

US has steep learning curve as depicted by many studies[37]. Inexpensive training models like gel phantom model can help improve US assessment and interventional skills and safety[38]. However, like and any other skill based technique, a degree of manual dexterity and knowledge is required to be proficient in its use. Hence, its accuracy remains operator dependent.

To conclude, ultrasonography has brought a paradigm shift in the practise of airway management. With increasing awareness, portability, accessibility and further sophistication in technology, it is likely to find a place in routine airway management. We are not far from the time when all of us will be carrying a pocket US machine like stethoscopes to corroborate our clinical findings at point of care.

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MINIREVIEWS

Physician disruptive behaviors: Five year progress report

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Abstract

Disruptive behaviors in health care can have a significant adverse effect on staff interactions that can negatively impact staff satisfaction, staff performance, and patient outcomes of care. As referenced in a previously published article, the Obstetrics and Gynecology specialty is one of the service areas where these behaviors occur more frequently. Despite growing evidence of the ill effects of these types of behaviors many organizations are still having a difficult time in addressing these issues in an effective manner. Gaining a better understanding of the nature, causes, and impact of these behaviors is crucial to finding the right remedies for solution. Nobody intentionally starts the day planning to be disruptive, it's just that things get in the way. A combination of deep seated factors related to age and gender preferences, culture and ethnicity, life experiences, and other events that help shape values, attitudes and personalities, and more external factors related to training, environmental pressures, stress and burnout, and other personal issues all contribute to the mix. Given the complexities of today's health care environment, each person needs to recognize the importance of being held accountable for appropriate actions and behaviors that affect work relationships and care coordination that impact patient care. Early recognition, early intervention, and taking a pro-active supportive approach to improve individual behaviors will result in better relationships, less disruption, more satisfaction, and better outcomes of care.

Key words: Disruptive behaviors; Patient safety; Patient outcomes; Staff relationships; Communication; Teamwork

Core tip: Disruptive behaviors in health care can have a significant adverse effect on staff interactions that can negatively impact staff satisfaction, staff performance, and patient outcomes of care. Disruptive incidents are more likely to occur in high risk settings such as the Obstetrical arena. Despite growing evidence of the ill effects of these types of behaviors many organizations are still having a difficult time in addressing these issues in an effective manner. Gaining a better understanding of the nature, causes, and impact of these behaviors and providing appropriate early and supportive interventions is crucial to finding the right remedies for solution.

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INTRODUCTION

It's been several years since I published a paper on the impact of disruptive behaviors in the Obstetrical setting highlighting its negative impact on staff relationships, care coordination, and patient outcomes of care[1]. Follow up reactions to the article have been very positive, but due to the nature of problem, and issues around reporting, internal organizational dynamics, and confidentiality, it's difficult to assess valid statistics as to how this has impacted the frequency of occurrence or consequences of these episodes. There have been a number of recent reports suggesting that the problem continues despite recurring evidence linking disruptive behaviors to patient harm[2-4]. Further evidence of its continued recurrence comes from research we conducted for an upcoming article in a law journal where we found a large number of cases reaching the appeal courts for incidents related to physician disruptive behaviors. The question is, why does this continue to be an ongoing problem?

HISTORY

We first reported on the impact of physician disruptive behaviors in 2002 highlighting the types of disruptive behaviors, the frequency, the specialties most involved, and its impact on nurse satisfaction and retention[5,6]. Phase two of our research extended the scope of analysis to include the incidence of disruptive behaviors in nursing and other disciplines and its impact on behaviors affecting communication and task performance leading to medical errors and other adverse events negatively impacting patient care. Our article in *The Joint Commission Journal of Quality and Patient Safety* was timed with the release of the Joint Commission Sentinel Event Alert #40 and the initiation of the new Joint Commission accreditation standard requiring hospitals to have a disruptive policy in place and to provide resources for its support as one of the leadership standards for accreditation[7,8]. During our research we noted that disruptive behaviors had the greatest likelihood of occurrence in high risk settings such as Obstetrics, Surgery, and the Emergency Department, and we reported on special studies conducted specifically in these areas[1,9,10]. In actuality, disruptive behaviors can occur anytime and anywhere across the full spectrum of care with similar detrimental effects on organizational culture, patient and staff satisfaction, morale, work relationships, task accountability, care efficiency, patient safety, and quality of patient care.

Table 1 Barriers

Organizational responsiveness (code of silence)
Reluctance to act (financial/hierarchy)
Structure and process (policy/reporting)
Process review (bias/conflicts of interest)
Intervention (skill sets)
Recommended action
Physician liabilities (personality)

PROGRESS?

We have definitely made some progress in this area. Many organizations have initiated a culture of zero tolerance for disruptive behaviors supported by setting appropriate behavioral standards described in either a code of conduct or disruptive behavior policy holding individuals accountable for their actions with set ramifications for non-compliance. Some organizations have taken a more pro-active approach in trying to reduce the incidence of disruptive behaviors by providing specific training programs in diversity management, cultural competency, emotional intelligence, conflict management and/or additional training to improve communication and team collaboration skills[11]. Programs focusing on skills taught in the airline industry (crew resource management), NASCAR (pit crew mechanics), and the nuclear power industry have shown significant benefit for team based care in Obstetrics, Surgery and Critical Care[12]. But problems still persist.

BARRIERS

Table 1 lists a number of different barriers that influence organizational effectiveness in addressing disruptive behaviors.

One of the first barriers is the issue of organizational responsiveness. This starts with organizational awareness. Many events go unnoticed or are not reported due to a hidden code of silence, an inconsistent reporting system, or fears of repercussion or retaliation for making a report. Ways to enhance organizational awareness include distributing a confidential internal survey assessment and making it safe for individuals to speak up. The second part is responsiveness. The underlying organizational culture and leadership need to develop and support a zero tolerance policy for disruptive behaviors and be willing to take the necessary steps to intervene when they occur.

A second more disturbing barrier is that of tolerance and acceptance. Many of these behaviors occur in physicians who are high revenue producers and the organization may be reluctant to confront the physician in fear of an antagonistic response and threats to bring his or her patients elsewhere. There may also be issues related to hierarchy, boundaries, or "sacred saints" leading to an unwillingness to

Table 2 Risks of non-action

Organizational morale
Recruitment and retention
Staff/patient satisfaction (HCAHPS)
Community reputation
Patient complaints/malpractice
Care efficiency (process flow/delays/utilization/productivity)
Poor compliance (documentation/metric based performance)
Communication gaps/medical errors/adverse events

HCAHPS: Hospital Consumer Assessment of Healthcare Providers and Systems.

intervene.

A third issue is that of structure and process. Do you have the right policies and procedures in place? Do you have a consistent reporting process? Do you have a standardized intervention plan where evaluation, assessment, and recommendations can be made in professional non- biased manner?

One of the key liabilities of any disruptive behavior policy is the process for event review, assessment, and follows up intervention. Some organizations may turn the issues over to the Chief Medical Officer, a Department Chair, or another delegated individual or task force, but do they have the right skills necessary to adequately assess the full situation, avoid preconceived biases or conflicts of interest, diffuse anger, resolve conflict, maintain focus on the key issues, offer support, and provide appropriate recommendations for next steps? In many cases the success of the intervention is more dependent on the effectiveness of the individual doing the intervention than the scope of the disruptive behavior described.

Probably the biggest challenge has to do in dealing with the underlying personality traits of the physician involved. Physicians are by nature very competitive, task driven, perfectionists, with very strong egocentric personalities. Medical training further accentuates the problem with its focus on gaining scientific knowledge (at the expense of developing interpersonal skills) which breeds a sense of autonomy, dominance, and need to control (at the expense of emotional sensitivity). All these factors can lead to a challenging personality who may at time be difficult to deal with.

As far as the question as to whether or not disruptive behavior will go away, recent changes in the health care environment may actually make the situation worse. Issues around Health Care Reform, changing models, metrics, and financial incentives for care, and greater accountability for performance outcomes have dramatically increased physician frustration, dissatisfaction, and levels of stress and burnout which can lead to both physical and emotional states that adversely affect attitudes and behaviors[13,14]. Recognizing these underlying issues are critically important when it comes to making appropriate recommendations for improvement.

Table 3 Recommendations

Awareness and responsiveness
Address organizational culture
Solicit project champions
Develop policies and procedures
Implement a consistent reporting and review process
Follow established process
Document all interactions
Intervention with trained personnel
Prevention
Provide physician/staff education (recognition/accountability)
Provide physician training (diversity/conflict management/communication skills)
Offer physician assistance and support (coaching/counseling/behavioral intervention)
Enhance physician engagement (input/motivation/alignment/satisfaction)
Recognize efforts

RISKS OF NON-ACTION

Sometimes we have to deliver a wake-up call for the organization to take appropriate action. Budget issues, resource issues, and the naïve sense of "no harm done" may override thoughts and willingness for organizational time and investment. Actually, it's quite the opposite[15]. Table 2 lists a number of different "costs" that may result from inaction.

One of the most obvious impacts is on employee morale. Perceptions of working in a "toxic" non-caring work environment leads to problems with staff retention and turnover and problems in recruiting new staff. The average cost to recruit a new nurse is over $60000 and at least twice as much to recruit a new physician. Anger and frustration lead to not only staff dissatisfaction, but also filters through to patient dissatisfaction which for Medicare is a key metric affecting hospital reimbursement[16]. With the growing public focus on the effects of workplace bullying, a further consequence is a tarnished community reputation which may impact market share and contract negotiations. More extreme situations may lead to patient complaints and a higher risk of costly malpractice suits[17].

Care efficiency can also suffer. Failure to follow best practice guidelines, failure to comply with hospital policies and procedures, failure to return calls, failure to collaborate, and failure to document can lead to wasted dollars related to inappropriate utilization, waste, duplication, process delays, mistakes, and reduced reimbursement.

The most serious effects occur when these behaviors disrupt care leading to costly medical errors and adverse events[15].

RECOMMENDATIONS

The discussion above highlights opportunities for improvement which are summarized in Table 3.

The first and most obvious need is organizational awareness of what is happening and the willingness to respond. The case for inaction is inexcusable.

At the core of reaction is organizational willingness to endorse and support a culture that values staff contributions and work ethic and reinforce the importance of a positive work environment which will not tolerate inappropriate behaviors. There are many articles emphasizing the importance of organizational culture and its relationship to staff satisfaction and patient outcomes of care[18]. Soliciting the help of project champions (both clinical and non- clinical) provide an excellent opportunity to further advance organizational initiatives.

Policies and procedures need to be developed to define appropriate standards of behavior and establish a consistent process for review. The organization then needs to follow due process in how it moves forward with the intervention. Not following due process and/or lack of documentation are two key issues to be considered if subsequent legal action is initiated.

The actual intervention process is probably the most critical part of the entire process and should be conducted by individuals trained in facilitation and conflict resolution techniques. The degree of intervention will depend upon the circumstances. Many disruptive behaviors occur unknowingly by the physician. In these cases just raising awareness and discussing alternative reactions will often help them self-correct. These types of informal interventions are often described as "coffee time" discussions. For more serious and repetitive disruptive behaviors the organization needs to take a more formal approach concluding with specific recommendations of what the physician needs to do to avoid these types of behaviors in the future. Depending on the circumstances additional training in diversity management, anger management, stress management, or conflict management may be appropriate. More severe cases may require individualized coaching or counseling services. These interventions can either be conducted internally or through a variety of outside programs offered by organizations that specialize in dealing with disruptive individuals. In some cases more intense behavioral modification therapy is needed which may includes assessment of possible substance abuse. In cases where the physician is resistant to change, sanctions, suspension, or termination of privileges may be the only alternative.

The best overall strategy is prevention. Most physicians don't plan to be disruptive, it's just that things may get in the way. Training in emotional intelligence, communication, and team collaboration skills will help provide essential tools to improve staff relationships and lower the incidence of disruptive events. If stress and burnout is an issue providing support services through either human resources, Physician Wellness Committees, a Physician EAP (Employee Assistance Program), or through the use of outside agencies to help the physician better adjust to the pressures of today's health care environment will ease some of their emotional liabilities.

Even better, take a proactive stance in trying to increase overall physician engagement. Take time to educate them about Health Care Reform and other current issues impacting their medical practice. Provide opportunities for discussion, listen to them, and respond to some of their needs and concerns. This can be done through discussion forums or town hall meetings, agenda items at Department meetings, or though one on one discussions[19]. Allowing physician input and participation around health care matters will increase physician alignment, engagement, satisfaction, and compliance, all of which will reduce the likelihood of a disruptive event. Be responsive to their needs and when possible offer appropriate administrative, operational, or clinical support to help ease the burden of running a demanding clinical practice.

In the end, physicians, and all staff, should be regarded as a precious resource. Show them respect, recognize and thank them for what they do, and work with them to re-invigorate their passion for providing medical care.

CONCLUSION

Disruptive behaviors can have a significant impact on patient care. Most physicians are just trying to do their job and in many cases don't even recognize the downstream effects of inappropriate behaviors. Many of these problems occur with strong personality traits further perpetuated by medical training that results in dominant, authoritative, egocentric, demanding behaviors with little emotional intelligence about the world around them. The current changes in today's medical environment are putting even more pressures on physicians which are increasing levels of stress and burnout that can change attitudes, perspectives, and behaviors that impact patient care. Physicians often don't recognize that they're under stress or what it does, and even if they do, feel like they can handle it themselves. Egos and concerns about competency and confidentiality with further limit their willingness to seek outside help. All of these issues can lead to disruptive behaviors. Yes organizations need to have policies and procedures in place to address the issue and definitely need to intervene when staff relationships and patient care may be compromised. Unfortunately, that's the punitive approach. Better yet would be for the organizations to take a different direction by taking a more pro-active approach to gaining insight into physician concerns, providing education, training, guidance, and behavioral support, and providing additional resources to help ease the burden of medical care. We can't leave it up to physicians to take care of themselves. Compassion and early intervention will do the job.

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Cost-effectiveness in *Clostridium difficile* treatment decision-making

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Abstract

AIM: To develop a framework for the clinical and health economic assessment for management of *Clostridium difficile* infection (CDI).

METHODS: CDI has vast economic consequences emphasizing the need for innovative and cost effective solutions, which were aim of this study. A guidance model was developed for coverage decisions and guideline development in CDI. The model included pharmacotherapy with oral metronidazole or oral vancomycin, which is the mainstay for pharmacological treatment of CDI and is recommended by most treatment guidelines.

RESULTS: A design for a patient-based cost-effectiveness model was developed, which can be used to estimate the cost-effectiveness of current and future treatment strategies in CDI. Patient-based outcomes were extrapolated to the population by including factors like, *e.g.*, person-to-person transmission, isolation precautions and closing and cleaning wards of hospitals.

CONCLUSION: The proposed framework for a population-based CDI model may be used for clinical and health economic assessments of CDI guidelines and coverage decisions for emerging treatments for CDI.

Key words: *Clostridium difficile* infection; Guidance; Cost-effectiveness; Model; Standardisation; Decision making

Core tip: Current clinical guidelines seldom include cost-effectiveness evaluations. Conclusions are typically based on clinical data only and sometimes referral is made to prices of therapies for justification of the treatment sequence advised. However, the price of a therapy as such is just a single criterion and does not reflect the balance between effectiveness and costs associated with the application of that therapy. This results often in a restricted position of new therapies in the treatment algorithm. Integration of cost-effectiveness using the population-based variant of cost-effectiveness evaluations as an instrument in guidelines for *Clostridium difficile* infection may be provide better decision making framework.

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INTRODUCTION

Escalating costs resulting from ageing of the population and an increase of innovative, expensive, medical technologies have become a major concern for health care professionals, decision-makers and the public. In addition to considering clinical benefits and the price of the new treatment, decision makers have taken a broader perspective by including cost-effectiveness evaluations, which also include related costs in the health care system.

Clostridium difficile infection (CDI) is considered a hospital-acquired infection. Diarrhea due to pathogenic *Clostridium difficile* (*C. difficile*) can occur if the bowel microbiota (bacterial content of the bowel) of a patient is disturbed, which is usually the result of antibiotic use prior to the CDI. With the increasing use of broad-spectrum antibiotics over the past two decades, the incidence of CDI has risen[1,2] and CDI is responsible for 15%-25% of cases of antibiotic associated diarrhea (AAD)[1]. CDI is usually self-limiting, but severe disease leading to colectomy and intensive care admission may occur. Mortality rates of 2%-7% have been reported[2,3], and seem even higher with the hypervirulent strain polymerase chain reaction (PCR) ribotype 027[4-6]. The chance of contracting CDI increases with a longer hospital stay[7] and both spread of *C. difficile* between patients as well as auto-re-infection, by this spore forming bacterium, have been demonstrated. In 55% of hospitalized patients with CDI, hospital stay was prolonged to more than 4 wk[8].

Pharmacotherapy of an initial episode of CDI with oral metronidazole or oral vancomycin is the recommended treatment in most guidelines[9-11]. However, following antibiotic treatment of CDI, recurrence and re-infection within 30 d occurs in approximately 15%-35% of patients, while 33%-65% of patients with > 2 previous CDI episodes will recur[12]. Recurrence of CDI is a serious and difficult-to-treat problem, impacting on the length and overall cost of hospitalisation[13]. The guidelines of the European *Society of Clinical Microbiology and Infectious Diseases* (ESCMID) have identified recurrence as being the most important challenge in the treatment of CDI[14].

Recently published guidelines[11-13] have incorporated relatively new treatments strategies with the antibiotic fidaxomicin and fecal microbiota transplantation (FMT or donor feces infusion), although their role is restricted because of the high price of fidaxomicin and the complexity (unconventional and unstandardized nature) of FMT. The latest Netherlands guideline suggests weighing fidaxomycin's high price versus the advantage of fewer recurrences[10,12,15]. English guidelines also recommends oral metronidazole for initial treatment in non-severe CDI, because it is cheaper than oral vancomycin, and because of concern about the selection of vancomycin resistant enterococci[13]. The median cost to treat a patient with CDI was €33840, showing an almost five fold higher and significant difference compared with the non-infected matched controls[16]. The estimated cost of CDI within the European Union (EU) is about €3 billion per year[17], and may further increase with aging. In most studies, hospitalisation is the main cost driver in patients with CDI[18]. Patients with CDI spend on average an extra 7-21 d in hospital, compared with non-infected controls[16,19,20]. The high rates of treatment failure and high rates of currently recommended antibiotics (metronidazole and vancomycin)[15,21,22] significantly affects costs[23]. The influence on clinical outcome and costs of this limited treatment efficacy is particularly apparent for patient groups with multiple comorbidities and a high risk of recurrence. In addition, minimizing the risk of person-to-person transmission of *C. difficile* in hospital wards seems of utmost importance. Taken together it is evident there is a large socio-economic and clinical unmet need to evaluate all these different factors in a single decision support model[1].

MATERIALS AND METHODS

Classic patient-based cost effectiveness model for infectious diseases tend to ignore the supra-patient social-economic consequences such as, for example, person-to-person transmission and closing of hospital wards due to infectious outbreaks. Preparing for an

Table 1 Summary of the most relevant therapeutic issues in *Clostridium difficile* infection

Level	Issues
Patient level	Recurrence of CDI is a serious and difficult-to-treat problem[26]
	Patient groups at high risk of recurrence or those for whom the impact of recurrence would be most dramatic include those with multiple comorbidities, who are immunocompromised, who are receiving certain concomitant antibiotics[26], who have had CDI previously, who are renally impaired, who are aged 65 yr or over, patients awaiting further treatment (for example chemotherapy) or rehabilitation (for example after cerebrovascular event)
Population level	The rate of person-to-person transmission of *C. difficile* is a complicating problem
	The risk for development of vancomycin-resistant enterococci or other antibiotic induced resistant bacteria, although it is not a major issue in daily practice

CDI: *Clostridium difficile* infection.

Table 2 Summary of the most relevant economic issues in *Clostridium difficile* infection

Level	Issues
Patient level	The cost of recurrence of CDI is high
	CDI leads to additional costs: extra diagnostic tests, extra antibiotics and other medication, time spent by nurse and physician on the ward
	The additional circumstances of these seriously ill patients (*e.g.*, not completing primary therapy, thereby complicating cure or improvement of their disease state) due to CDI should be reflected in the CEA
Population level	The rate of person-to-person transmission of *C. difficile* is a complicating problem with high costs
	The increased length and overall cost of hospitalization with CDI, including the costs of measures to isolate the patient and other clinical measures to prevent person-to-person transmission, as well as the costs of closing and cleaning wards
	The consequences of developing vancomycin-resistant enterococci or other antibiotic induced resistant enterococci are not integrated in standard cost-effectiveness evaluations

CDI: *Clostridium difficile* infection; CEA: Carcinoembryonic antigen.

all-inclusive model an expert procedure was convened considering the clinical and economic issues supposed to have an influence on cost-effectiveness evaluation of preventive and therapeutic measures for CDI. Step one was an extensive literature search on all different aspects of such a model. Subsequently, a base model was constructed and presented to experts. Next, the therapeutic and economic issues that were mentioned by the individual experts were incorporated in the model. This model was the input for a plenary discussion. The most relevant therapeutic and economic issues were defined and discussed. Based on scientific sources (literature and professional guidelines) as well as practice based sources, the issues were validated and a framework for the integration of these relevant issues into cost-effectiveness modeling was finalized. The outcomes of the expert procedure are described below.

Clinical and economic relevant issues

Clinical and economic relevant issues are shown in Tables 1 and 2.

The clinical and economic consequences of CDI in terms of morbidity, survival and costs underline the therapeutic need for innovative cost-effective solutions. Payers require cost-effectiveness analyses when deciding whether or not to reimburse new therapies/approaches for CDI. In such an analysis the first step is typically to develop a patient-based cost-effectiveness model. Such a model for CDI is shown in Figure 1.

Instructions are defined possible different stages for a CDI patient (health states).

Treatment stages hospital setting: (1) In the hospital, discontinuation of the antibacterial therapy that may have precipitated CDI is often not possible; (2) Patients with CDI are usually treated with antibiotics (metronidazole); (3) Patients may die or stay alive and surviving patients may or may not respond to metronidazole; (4) Patients who respond to metronidazole may be cured or experience a recurrence (or re-infection), which may occur during the hospitalization period or after discharge. In both cases the initial treatment with metronidazole is restarted, or vancomycin or fidaxomicin is prescribed instead; (5) If no response to metronidazole is seen, patients may be switched to vancomycin or fidaxomicin; (6) Then again patients may die or stay alive and surviving patients may respond or not respond to vancomycin or fidaxomicin; (7) Patients who respond to vancomycin or fidaxomicin may be cured or experience a recurrence (or re-infection), which may occur during hospitalization or after discharge. In both cases the initial treatment with vancomycin or fidaxomicin is restarted; and (8) Patients not responding will be switched to third-line treatment. For patients failing on third-line treatment, not many treatment options are left. If third line treatment is FMT, this can be repeated several times. Otherwise, patients may have to use vancomycin more or less continuously.

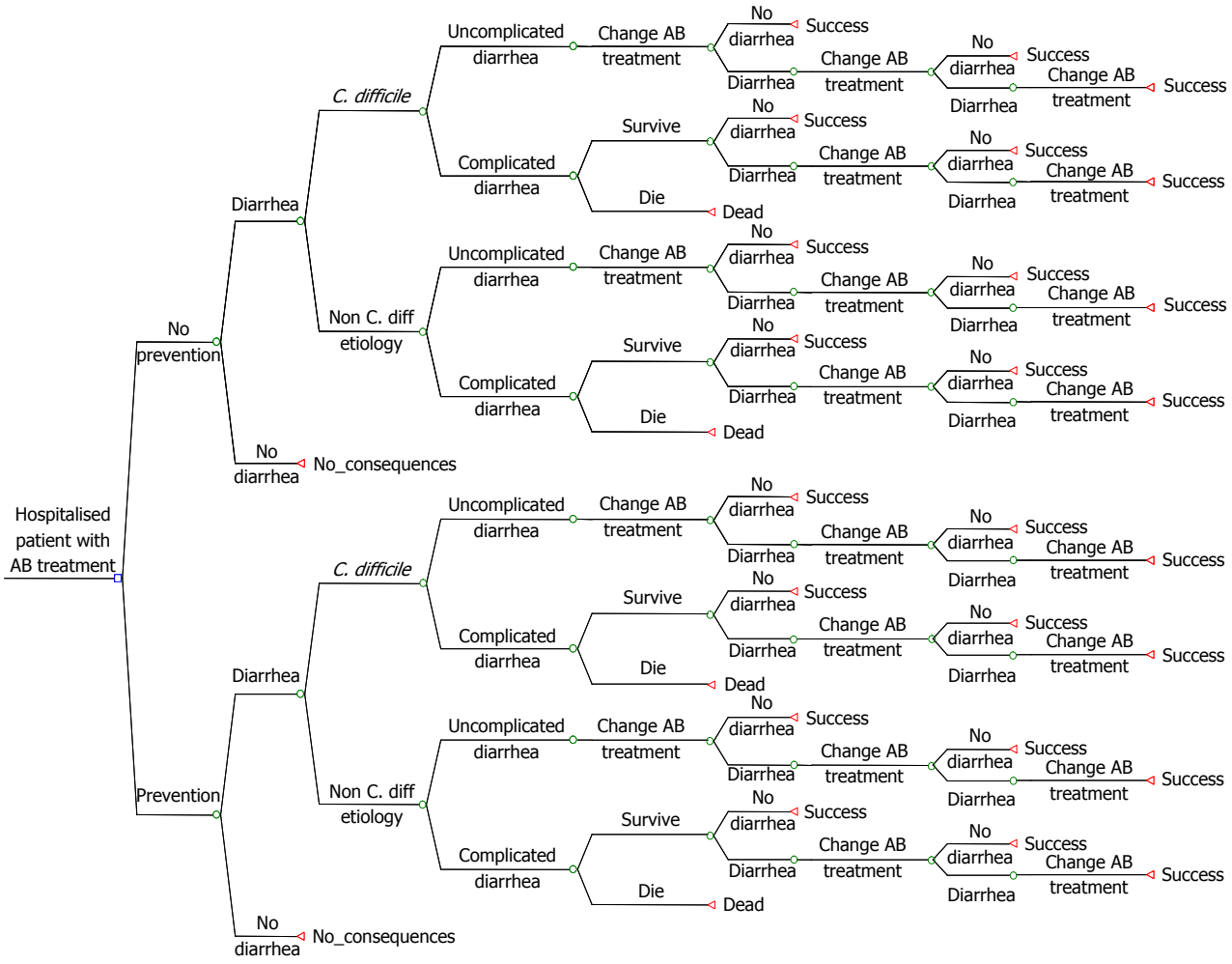

Figure 1 Flow diagram for cost-effectiveness modeling for Clostridium difficile infection.

Treatment stages community setting: The treatment stages are similar, as described above, but the difference is that patients may or may not be hospitalized, whereas in the previous section patients are already hospitalized.

The incremental cost per QALY gained is seen as the preferred cost-effectiveness outcome, but is often of limited value in a health economic analysis, which only covers the hospitalization period. The QALY gain is calculated by combining the utility gain (quality of life gain) with the number of life years gained. QALY gained may therefore be the result of longer life expectancy, utility gain, or both. If the cost per QALY gained is not viewed as the most appropriate outcome (for example when transmission and/or ward contamination are a problem), other cost-effectiveness outcomes may be considered, for example, the cost per recurrence avoided, which reflects the additional costs for the prevention of one recurrence. As recurrence is not only a clinical issue, but also may have major economic consequences, this outcome might be relevant for decision makers.

Population-based model for cost-effectiveness analysis

The cost-effectiveness outcome based on the patient-based model only provides a limited health economic outcome in terms of time horizon and perspective and more importantly, disregards the impact on other patients. The relevant economic issues, such as resistance, person-to-person transmission, isolation measures and closing of wards of hospitals, are all supra-patient effects. These consequences of CDI on hospitals, payers and society, that go beyond the individual scope of the patient, are not integrated in standard CEA's. Therefore, the outcomes of a patient-based cost-effectiveness model should be considered cautiously because they present only a conservative and limited outcome. For all-encompassing cost-effectiveness evaluations of CDI therapies, these supra-patient economic aspects cannot be disregarded. Therefore, we propose performing cost-effectiveness analyses for CDI using a population-based model, which incorporates all of the clinically important elements of the patient-based model as well as the supra-patient therapeutic and economic issues (Table 3).

RESULTS

The flow diagram (Figure 1) does not contain a particular choice for a specific therapy but serves as

Table 3 Similarities and differences between a patient-based and population based cost-effectiveness model

Patient-based cost-effectiveness model	Population-based cost-effectiveness model
Similarities	
Patient-related therapeutic and economic measures for clinical and economic evaluations	
Differences	
	The relevant economic issues, as indicated for CDI like: increasing incidence of CDI, person-to person transmission of CDI, development of vancomycin-resistant enterococci (VRE), or other antibiotic induced resistant bacteria, impact for department of microbiology diagnostic testing isolation measures and closing of wards of hospitals other supra-patient effects
Limited health economic outcome in terms of time horizon and perspective	
The patient-based cost-effectiveness model only captures the short-term time horizon of the CDI episode within the hospital setting at a patient level	

CDI: *Clostridium difficile* infection.

a blueprint for cost-effectiveness modeling. Based on the developments in CDI treatment, we suggest applying different treatment sequences for testing the effects on cost-effectiveness outcomes. Other suggestions for application are stratification of the patient population according to potential co-variables, such as risk factors for recurrence (for example, prolonged hospital stay or ICU admission) or underlying diseases (for example, patients after surgery, patients with a malignancy receiving chemotherapy, and renally impaired patients).

Three types of recent therapies could be candidates for comparison using a population-based model: the antibiotic fidaxomicin, fecal microbiota transplantation (FMT), and preventive use of probiotics.

Fidaxomycin is a novel antibiotic with targeted activity against *C. Difficile* with a similar safety profile as vancomycin. After treatment of an initial episode of CDI, the cure rate after 30 d was increased after fidaxomycin (82%) compared to vancomycin (70%)[16,17,24]. FMT helps restore the normal colonic micro flora in patients with refractory and recurrent CDI[25,26]. The procedure involves single or multiple infusions (*e.g.,* by enema) of a feces based solution from a healthy donor. A recently published randomized trial confirmed the efficacy of FMT in patients with recurrent CDI. For assessment of preventive treatments, the framework (Figure 1) can be used to estimate the costs and benefits of co-prescription of probiotics with antibiotics to prevent CDI. Recently, a patient-based cost-effectiveness evaluation for probiotics showed probiotics "could lead to substantial cost savings"[27]. To further investigate the economic consequences of the use of probiotics to prevent CDI, a population-based model could be applied and although the expected clinical benefit may be limited, total cost savings compared to no preventive treatment, and a predicted (Cochrane) drop in therapy induced side effects, may still be relevant.

DISCUSSION

Current clinical guidelines seldom include cost-effectiveness evaluations. Conclusions are typically based on clinical data only and sometimes referral is made to prices of therapies for justification of the treatment sequence advised. However, the price of a therapy as such is just a single criterion and does not reflect the balance between effectiveness and costs associated with the application of that therapy. This results often in a restricted position of new therapies in the treatment algorithm.

Among health authorities, it is common to include evidence of cost-effectiveness in decision-making about coverage under the health insurance package. Even though the cost per QALY outcome might fall below the threshold of a country, health authorities might decide to reject coverage based on the high weight they place on the budget impact[28]. This may be considered a paradox, because the cost-effectiveness guidelines were written by the same authorities and payers.

Estimates of the cost-effectiveness of a medicine may only have a limited impact on the use of that medicine within a hospital, as a result of a "silo mentality" found within the hospital as well as within the budget management structure existing at the payer, local and national levels. In that case, a treatment (medication or medical therapy) that is more expensive than existing treatments may exceed the amount of money reserved within the hospital budget or the pharmacy budget.

Another paradox, since exceeding this "local" budget might generate a multiplier and create substantial savings in the total system/hospital.

Achieving changes in the "silo structure" within hospitals as well as the budget management structure by payers depends on the generation of basic information on these cost-effective aspects. We propose that usage of the current flow diagram will

generate facts and figures, as well as enable motivated implementation of these facts into guidance documents from professional societies to policy makers and payers (locally or regionally as well as nationally).

Integration of cost-effectiveness using the population-based variant of cost-effectiveness evaluations as an instrument in guidelines for CDI should be considered.

This may help healthcare professionals, patients, hospitals, payers and society to make better decisions about the optimal way to reduce the health and economic impact of CDI.

COMMENTS

Background

Current clinical guidelines seldom include cost-effectiveness evaluations. Conclusions are typically based on clinical data only and sometimes referral is made to prices of therapies for justification of the treatment sequence advised. However, the price of a therapy as such is just a single criterion and does not reflect the balance between effectiveness and costs associated with the application of that therapy. This results often in a restricted position of new therapies in the treatment algorithm.

Research frontiers

Recent high rates of treatment failure and recurrent infection have vast economic consequences emphasizing the need for innovative and cost effective solutions in *Clostridium difficile* infections (CDI). The price of new therapies and approaches cannot always compete with the relatively low, generic prices of current standard therapies with metronidazole and vancomycin. The question is then, how should professional societies integrate new and more effective, but also more expensive, remedies into their guidelines and how health authorities make reimbursement decisions.

Innovations and breakthroughs

The cost-effectiveness outcome based on the patient-based model only provides a limited health economic outcome in terms of time horizon and perspective and more importantly, disregards the impact on other patients. The relevant economic issues, such as resistance, person-to-person transmission, isolation measures and closing of wards of hospitals, are all supra-patient effects. These consequences of CDI on hospitals, payers and society, that go beyond the individual scope of the patient, are not integrated in standard cost-effectiveness analyses. Therefore, we developed a guidance model for coverage decisions and guideline development in CDI based on a population-based cost-effectiveness model.

Applications

The authors propose performing cost-effectiveness analyses for CDI using a population-based model, which incorporates all of the clinically important elements of the patient-based model as well as the supra-patient therapeutic and economic issues.

Terminology

CDI is responsible for 15%-25% of cases of antibiotic associated diarrhea (AAD) and is typically seen in elderly hospitalised patients, resulting in significant morbidity and mortality. Pharmacotherapy of an initial episode of CDI with oral metronidazole or oral vancomycin is the mainstay for pharmacological treatment of CDI and is recommended by most treatment guidelines.

Peer-review

This guideline article is interesting and has a high scientific value.

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CASE REPORT

Contrast induced neurotoxicity following coronary angiogram with Iohexol in an end stage renal disease patient

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Author contributions: All authors contributed to the acquisition of information, writing and revision of this case report.

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Abstract

Neurotoxicity is an infrequent adverse reaction to iodinated contrast agents. Contrast induced neurotoxicity following coronary angiogram is very rare. Renal disease is a risk factor for contrast induced neurotoxicity. We report a case of contrast induced neurotoxicity following coronary angiogram and intervention using Iohexol (Omnipaque 350) in an end stage renal disease patient on peritoneal dialysis who had prior exposure to iodinated contrast without any adverse reaction. Hemodialysis had to be initiated for rapid removal of the contrast agent with subsequent complete resolution of neurological deficits. This case highlights the need for interventionalists to be aware of an important adverse reaction to iodinated contrast agents, especially in individuals with renal dysfunction, and that neurotoxicity is a possibility even with prior uneventful exposures. The role and timing of hemodialysis in contrast induced neurotoxicity in patients with chronic kidney disease and in those without chronic kidney disease needs further deliberation.

Key words: Coronary angiogram; End stage renal disease; Hemodialysis; Iodinated contrast agent; Neurotoxicity

Core tip: Contrast induced neurotoxicity following coronary angiogram is very rare. Interventionalists should be aware of this rare complication especially in patients with end stage renal disease (ESRD). Iodinated contrast media can be effectively removed from the blood by dialysis. Hemodialysis is a better modality for rapid removal of contrast agent compared to peritoneal

dialysis. Hemodialysis should be considered in life-threatening adverse reactions when supportive care alone is not sufficient. More studies are needed to further delineate the role and timing of hemodialysis following coronary angiogram and the optimal dosage of contrast media in ESRD patients to prevent this infrequent but potentially life threatening adverse reaction.

Gollol Raju NS, Joshi D, Daggubati R, Movahed A. Contrast induced neurotoxicity following coronary angiogram with Iohexol in an end stage renal disease patient. *World J Clin Cases* 2015; 3(11): 942-945 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i11/942.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i11.942

INTRODUCTION

Iodinated contrast agents are an important tool in medical practice. It is estimated that nearly 75 million doses are administered worldwide every year[1]. Modern iodinated contrast agents are mostly nonionic and low osmolar (2-3 times the osmolality of serum) or iso-osmolar (same osmolality of serum). They are safe and adverse reactions, when occur, are mild and self-limiting but serious and life threatening reactions can occur occasionally[2]. Here we describe a case of contrast induced neurotoxicity (CIN) following coronary angiogram in a patient with end stage renal disease (ESRD).

CASE REPORT

A 44-year-old African American female with coronary artery disease, hypertension, severe functional mitral regurgitation, ESRD on peritoneal dialysis, status post failed renal transplant on slow taper of immunosuppressants and diabetes mellitus type 2, was admitted for unstable angina. A month prior to this presentation patient had undergone a diagnostic right and left heart catheterization revealing 70% stenosis of first obtuse marginal branch and 80% stenosis of mid right coronary artery. Seventy ml of Iohexol (Omnipaque 350), a low-osmolar nonionic contrast media, had been used during the procedure. A coronary angiogram with percutaneous coronary intervention was planned. Three days prior to the cardiac intervention, a computerized tomography (CT) of abdomen and pelvis with and without contrast was performed for evaluation of hematuria. Seventy milliliter of Iodixanol (Visipaque 320), an iso-osmolar nonionic contrast media, was used. Patient tolerated this without any untoward events. Three days later patient underwent coronary angiogram and percutaneous intervention with drug eluting stent to first obtuse marginal branch of left circumflex and right coronary artery. Around 190 mL of Iohexol (Omnipaque 350) was used during the procedure. Patient tolerated the procedure without any immediate complications but within few hours developed headache with left sided weakness. A non-contrast head CT, four hours after the coronary intervention, showed extensive intravascular contrast with cortical staining, primarily over the right cerebral hemisphere and left cerebral hemisphere watershed territories (Figure 1). No other acute abnormalities were noted. Subarachnoid hemorrhage was considered unlikely and the clinical picture was considered likely secondary to contrast induced neurotoxicity. Supportive care and manual peritoneal dialysis exchanges were initiated. During this process, patient became more encephalopathic with subsequent seizure like activity. Antiepileptic medications, lorazepam and levetiracetam, were given to control seizures. Patient was transitioned to hemodialysis for rapid removal of contrast agent. A magnetic resonance imaging (MRI) of the brain taken 24 h after the coronary intervention showed hyperintense cortical signal on T2, fluid attenuated inversion recovery (FLAIR) and diffusion weighted images throughout the right greater than left cerebral hemisphere (Figure 2). No definite restricted diffusion was observed. There was mild mass effect without hemorrhage or herniation. An electro encephalogram was negative for seizure

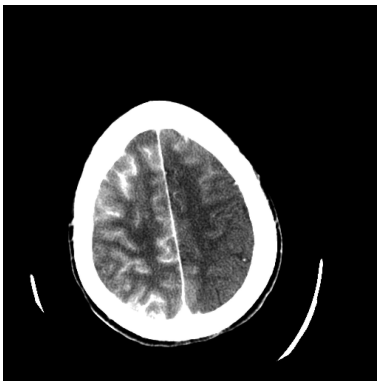

Figure 1 Non-contrast head computerized tomography showing extensive intravascular contrast with cortical staining, primarily over the right cerebral hemisphere.

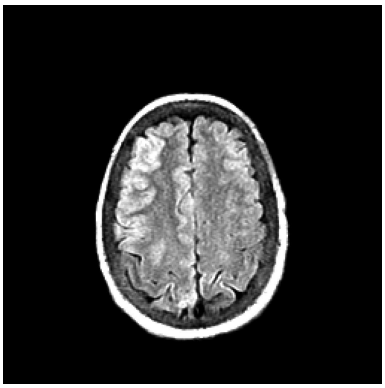

Figure 2 Magnetic resonance imaging fluid attenuated inversion recovery image showing hyperintense cortical signal of the cerebral hemispheres.

activity. These findings were considered consistent with contrast induced neurotoxicity (CIN). Supportive care and hemodialysis were continued for the next three days with gradual improvement and complete resolution of neurological abnormalities. Patient was transitioned back to peritoneal dialysis and discharged from the hospital in stable condition. Repeat imaging was not performed prior to discharge.

DISCUSSION

CIN is an infrequent adverse reaction to iodinated contrast agents. Intraarterial and neurointerventional procedures are more commonly associated with CIN[3]. CIN following coronary angiogram is very rare and the reported incidence is 0.06%[4]. This adverse reaction following coronary angiogram using Iohexol has been noted before[5]. All types of iodinated contrast agents irrespective of their ionic state or osmolality can cause CIN[3]. Neurological deficits are either focal or global. The exact etiology of CIN is unclear. Prior and subsequent exposure may not cause the same complication[4]. It is considered to be an idiosyncratic reaction to the contrast agent. An intact blood brain barrier is impermeable to contrast agents under normal conditions. Direct chemotoxic effects and hyperosmolality result in increased permeability of blood brain barrier and resultant cerebral edema, and also the possibility of increased hydrostatic pressure transmitted during neurointerventional procedures and subsequent changes in cerebral autoregulation predisposing to contrast extravasation has also been postulated[4,6]. Symptoms range from headache to seizures, hemiparesis, ophthalmoplegia, transient global amnesia, and transient cortical blindness. Neurological deficits are mostly transient but could also be persistent, especially with ophthalmic involvement[3,4]. Symptoms appear within 2 to 12 h of contrast injection and usually resolve in 24 to 72 h[7]. Imaging studies are recommended to rule out thromboembolic or hemorrhagic complications. Unenhanced CT images may be normal or show combination of poorly localized cortical and or subcortical enhancement, cerebral edema, and hyperdensity in the subarachnoid space similar to subarachnoid hemorrhage. MRI imaging may demonstrate hyperintense areas on T2, FLAIR, and diffusion weighted images[5]. Risk factors for CIN include renal disease, hypertension, and route, number of administration, and duration of exposure[3,7]. Larger dose of contrast is considered a risk factor but CIN has been reported even with very small doses[4]. Kocabay *et al*[3] recommend a maximum of 170 mL contrast agent for coronary angiograms to prevent CIN but none of the patients in their case series had ESRD. Our patient had ESRD and had prior exposure to iodinated contrast agents including Omnipaque 350 and to Visipaque 320 without any adverse events supporting the concept of CIN being an idiosyncratic reaction. In our case, for the culprit coronary angioplasty procedure, 190 mL of Omnipaque 350 had been utilized. General consensuses for preventive measures include adequate hydration prior to contrast exposure and using lowest possible contrast dose. Treatment is mostly supportive and hydration. Contrast media can be effectively removed from the blood by dialysis[8].

Contrast induced neurotoxicity following coronary angiogram is very rare. Interventionalists should be aware of this rare complication especially in patients with ESRD. Iodinated contrast media can be effectively removed by dialysis. Hemodialysis is a better modality for rapid removal of contrast agent compared to peritoneal dialysis. Hemodialysis should be considered in life-threatening adverse reactions when supportive care alone is not sufficient. More studies are needed to further delineate the role and timing of hemodialysis following coronary angiogram, and the optimal dosage of contrast media in ESRD patients to prevent this infrequent but potentially life threatening adverse reaction to iodinated contrast agents.

COMMENTS

Case characteristics

A 44-year-old female with coronary artery disease and end-stage renal disease on peritoneal dialysis underwent coronary angiogram and intervention for unstable angina.

Clinical diagnosis

Status post coronary angiogram and intervention, patient developed acute encephalopathy with no focal neurological deficits.

Differential diagnosis

Cerebrovascular accident, contrast neurotoxicity.

Imaging diagnosis

A non-contrast computerized tomography of the head showed extensive intravascular contrast with cortical staining, primarily over the right cerebral hemisphere. A magnetic resonance imaging showed hyperintense cortical signal of the cerebral hemispheres.

Treatment

Hemodialysis and supportive care.

Related reports

Contrast induced neurotoxicity is very rare following coronary angiogram. Correct diagnosis will avoid erroneous treatment for cerebrovascular accident.

Term explanation

Contrast induced neurotoxicity (CIN) is an infrequent adverse reaction to iodinated contrast agents. End stage renal disease (ESRD) is a risk factor for the development of CIN.

Experiences and lessons

Contrast induced neurotoxicity is very rare following coronary angiogram. Prior exposures to iodinated contrast agents may not have resulted in any adverse reaction supporting CIN to be an idiosyncratic reaction. Hemodialysis is an effective tool in contrast removal and management of CIN, especially in ESRD patients.

Peer-review

Very good case report about contrast induced neurotoxicity. It is very well written, summarized but completed.

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CASE REPORT

First description of cervical intradural thymoma metastasis

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Author contributions: All the authors contributed to this manuscript.

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Informed consent statement: The study participant provided informed verbal consent prior to study enrollment.

Conflict-of-interest statement: The authors declare no conflicts of interest.

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Abstract

Thymoma and thymic carcinoma are rare epithelial tumors, which originate from the thymus gland. According to the World Health Organization there are "organotypic" (types A, AB, B1, B2, and B3) and "non-organotypic" (thymic carcinomas) thymomas. Type B3 thymomas are aggressive tumors, which can metastasize. Due to the rarity of these lesions, only 7 cases of extradural metastasis are described in the literature. We report the first and unique case of a man with cervical intradural B3 thymoma metastasis. A 46-year-old man underwent thymoma surgical removal. The year after the procedure he was treated for a parietal pleura metastasis. In 2006 he underwent cervical-dorsal extradural metastasis removal and C5-Th1 stabilization. Seven years after he came to our observation complaining left cervicobrachialgia and a reduction of strength of the left arm. He underwent a cervical spine magnetic resonance imaging, which showed a new lesion at the C5-C7 level. The patient underwent a surgery for the intradural B3 thymoma metastasis. Neurological symptoms improved although the removal was subtotal. He went through postoperative radiation therapy with further mass reduction. Spinal metastases are extremely rare. To date, only 7 cases of spinal extradural metastasis have been described in the literature. This is the first case of spinal intradural metastasis. Early individuation of these tumors and surgical treatment improve neurological outcome in patients with spinal cord compression. A multimodal treatment including neoadjuvant chemotherapy, surgery and postoperative radiation therapy seems to improve survival in patients with metastatic thymoma.

Key words: Thymoma; Metastasis; Intradural lesion; Spinal tumor; Spinal surgery

Core tip: To date, only 7 cases of spinal extradural thymoma metastasis have been described in the literature. We report the first case of spinal intradural thymoma metastasis. Early individuation of these tumors and surgical treatment improve neurological outcome in patients with spinal cord compression. A multimodal treatment including neoadjuvant chemotherapy, surgery and postoperative radiation therapy seems to improve survival in patients with metastatic thymoma.

Marotta N, Mancarella C, Colistra D, Landi A, Dugoni DE, Delfini R. First description of cervical intradural thymoma metastasis. *World J Clin Cases* 2015; 3(11): 946-950 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i11/946.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i11.946

INTRODUCTION

Thymoma and thymic carcinoma are uncommon epithelial lesions, which originate from the thymus gland. To date, the true incidence is not known, but it is estimated to be 0-15 cases/100000 people. Between the fifth and sixth decades of life, this pathology represents 0.2%-1.5% of all malignancies[1]. According to the World Health Organization there are "organotypic" (types A, AB, B1, B2, and B3) and "non-organotypic" (thymic carcinomas) thymomas. Types A, AB, B1 and B2 thymomas are benign tumors. Type B3 thymomas are aggressive tumors of intermediate malignancy[2]. Spinal metastases are very uncommon. In the literature, only 7 patients with spinal extradural metastasis have been described. We report the first and unique case of a man with cervical intradural B3 thymoma metastasis.

CASE REPORT

A thymoma was resected in a 46-year-old male in 1989. He did not submit himself to adjuvant therapy. A year after the procedure he underwent parietal pleura metastasis resection. The patient remained disease-free until 2006, when he complained a reduction of strength of the left arm, for the presence of a cervico-dorsal extradural metastasis. He underwent resection of the lesion and C5-Th1 stabilization (Figure 1). The lesion was totally removed (R0) and the dura mater appeared intact.

In 2013, the patient came to our observation complaining left cervicobrachialgia and a reduction of strength of the left arm. A cervical spine magnetic resonance imaging (MRI) showed a new lesion at the C5-C7 level (Figure 2). The lesion showed a homogeneous enhancement after gadolinium administration in the T1-weighted sequences, and enclosed the spinal cord, especially on the left side. From the imaging, it was not clear if the lesion was extradural or intradural. Intraoperatively, there was not pathological tissue in the epidural space; it appeared only after opening the dura mater. The patient underwent a sub-total resection, in order to preserve the spinal cord from the surgical manipulation. The lesion was intradural-extramedullary. It was adherent to the spinal cord surface in its lateral and anterior portions.

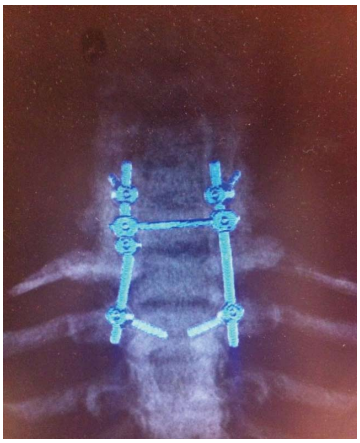

Figure 1 Maximum intensity projection reconstruction from computed tomography images shows C5-Th1 stabilization after the first intervention.

A spinal metastasis of the type B3 thymoma according to the World Health Organization 2004 was diagnosed (well-differentiated thymic carcinoma according to Marino and Muller-Hermelick)[3]. The immunohistochemical examination demonstrated positivity of neoplastic cells for CK19 and p63 and CD1a positive T-cells (Figure 3). Even if the resection was not total, no postoperative neurological deficits were observed. After 3 mo, the cervico-thoracic spine MRI showed a small residual tumor, especially anteriorly to the cervical spinal cord (Figure 4). Subsequently, focal radiotherapy (25.5 grays) was given. Cervical MRI after radiation treatment showed almost total disappearance of the mass (Figure 5). The patient is free of symptoms.

A search was carried out about spinal thymoma metastasis in the English language literature. We excluded one work because it is available only in Japanese language[4] and another because it did not report sufficient data to be included in our series. We used the following key words: "thymoma metastasis", "thymic carcinoma", "spinal cord compression", and "spinal metastasis". The data were extracted according to these parameters: (1) author and year of publication; (2) age; (3) sex; (4) time to spinal metastasis; (5) site of spinal metastasis; (6) sign and symptoms; (7) surgery; (8) follow-up; (9) radiotherapy; and (10) classification of histological type of the thymoma[5-9].

DISCUSSION

We found 6 papers, in which 7 cases were described.

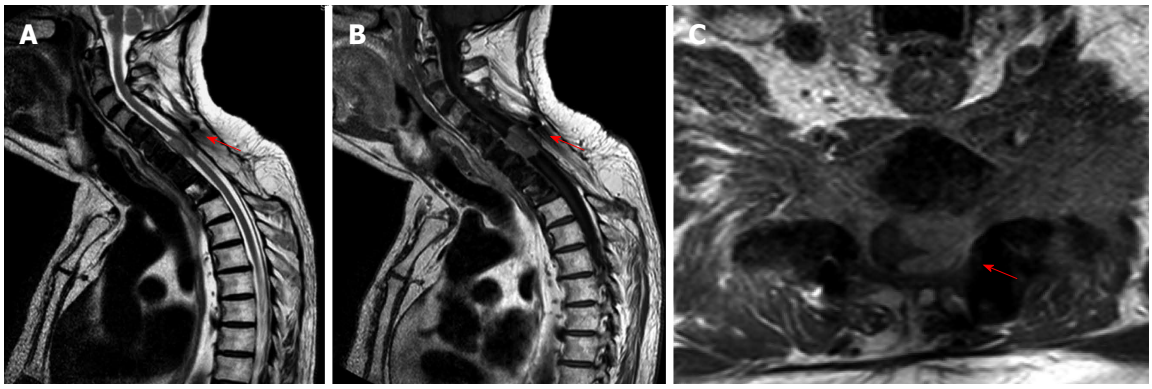

Figure 2 Cervical spine magnetic resonance imaging. A: A C5-C7 lesion with homogeneous enhancement after gadolinium administration in the T1-weighted sequences; B: T2 weighted sequences showing the enclosed spinal cord; C: Especially on the left side (red arrow).

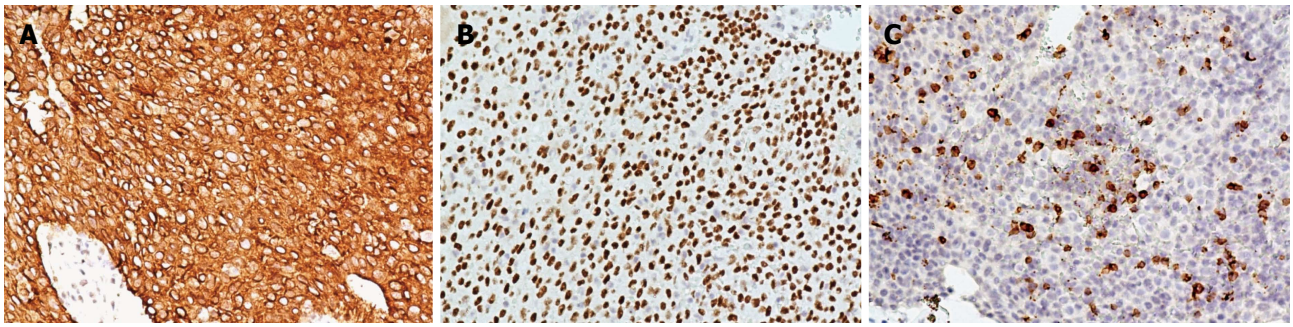

Figure 3 The immunohistochemical examination demonstrated positivity of neoplastic cells for (A) CK19, (B) p63 and (C) CD1a positive T-cells.

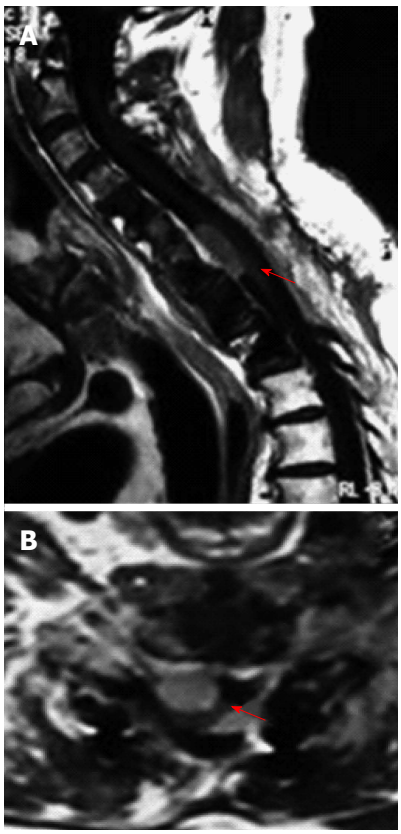

Figure 4 Magnetic resonance imaging of the cervico-thoracic spine at 3-mo follow-up showing (A) a small residual tumor, especially anteriorly to the cervical spinal cord and (B) axial view (red arrow).

We included our case in the review, for a total number of 8 cases of spinal thymoma metastasis. Four patients were male and 3 were female. About the age, the average was 51.4 years old (range, 29-70 years). The median time from thymoma diagnosis to spinal metastasis was 7.9 years (range, 1-17 years); in one case the spinal metastasis was diagnosed before primary tumor; the case with longer time to spinal metastasis was our case. The spinal metastases were localized at the thoracic level in 4 cases, at the cervico-thoracic site in 1 case, cervical level in 2 cases, and lumbar level in 1 case. Patients presented symptoms related to the thymoma in 2 cases (myasthenia gravis); in all cases they had neurological symptoms related to the spinal metastasis (motor deficit, sensitive deficit or pain). All patients underwent surgical treatment. During the follow-up, 3 patients died after 3 mo, 5 mo and 2 years, respectively; 4 patients survived with a time of follow-up from 9 mo to 8 years (our case). Five patients did not receive radiation therapy, and 2 underwent RT. Histological diagnosis was not based on the actual WHO classification in 2 cases; the histological subtype was B2 in 1 case, B3 in 2 cases, and C in 2 cases.

Thymoma and thymic carcinoma are uncommon epithelial lesions, which originate from the thymus gland. Thymoma is a rare tumor, commonly associated with the myasthenia gravis (15%); local dissemination occurs quickly, but distant spinal metastasis may occur up to 16 years after the diagnosis of thymomas. They

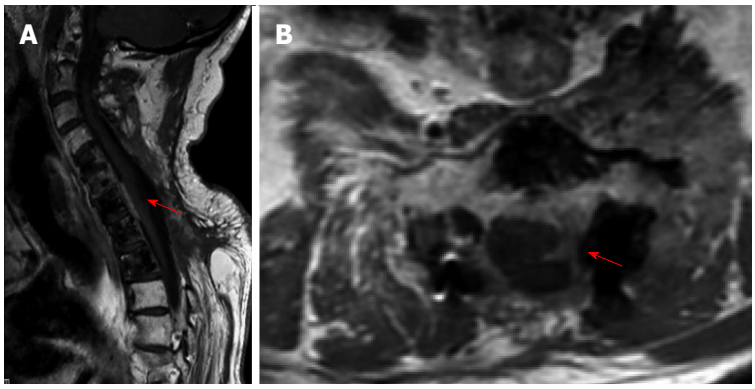

Figure 5 Cervical magnetic resonance imaging after radiation treatment showing (A) almost total disappearance of the mass and (B) axial view (red arrow).

can metastasize to the pleura regional nodes, liver and lung. Spinal metastases are very uncommon. In the literature, only 7 patients with spinal extradural metastasis have been described. We report a unique case of spinal intradural metastasis. On MRI, the vertebral metastases appear hypointense on T1-weighted images and hyperintense on T2-weighted images. Infiltration of the paravertebral muscles and vertebral elements, evident on axial views, is possible. Imaging after gadolinium administration shows an important enhancement. A computed tomography scan shows infiltration of the vertebral body, with both osteolytic and osteoblastic lesions. The spinal metastasis can cause, in fact, vertebral collapse, spinal instability and neurological deterioration. Early diagnosis and surgery have the goal to control pain and reduce motor deficits. It is fundamental to take in account that an invasive thymoma can metastasize both extradurally and intradurally, because patient's survival may be extended by an early diagnosis followed by an appropriate treatment.

We report the first case of spinal intradural metastasis. It is not yet known the gold standard of treatment, but a multimodal treatment which includes neoadjuvant chemotherapy, surgery and postoperative radiotherapy seems to improve survival in patients with metastatic thymoma.

COMMENTS

Case characteristics

Left cervicobrachialgia and a reduction of strength of the left arm.

Differential diagnosis

It is fundamental to take in account that an invasive thymoma can metastasize both extradurally and intradurally.

Imaging diagnosis

The lesion showed a homogeneous enhancement after gadolinium administration in the T1-weighted sequences, and enclosed the spinal cord, especially on the left side.

Pathological diagnosis

A spinal metastasis of the type B3 thymoma according to the World Health Organization 2004 was diagnosed (well-differentiated thymic carcinoma according to Marino and Muller-Hermelick).The immunohistochemical examination demonstrated positivity of neoplastic cells for CK19 and p63 and CD1a positive T-cells.

Treatment

Surgical sub-total resection and focal radiotherapy.

Related reports

The spinal metastasis can cause, in fact, vertebral collapse, spinal instability and neurological deterioration. Early diagnosis and surgery have the goal to control pain and reduce motor deficits.

Term explanation

Extradural, outside the dura mater.

Experiences and lessons

Multimodal treatment which includes neoadjuvant chemotherapy, surgery and post-operatory radiotherapy seems to improve survival in patients with metastatic thymoma.

Peer-review

The present case report is worth publishing, as it documents an extremely rare case of an intradural thymoma metastasis.

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CASE REPORT

Laparoscopic splenectomy for a littoral cell angioma of the spleen: Case report

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Author contributions: All the authors equally contributed to this work.

Institutional review board statement: According to Italian regulation, ethical committee approval is not requested for case reports analyzing anonymized routinely collected data.

Informed consent statement: The patient gave her informed verbal consent prior to study enrollment.

Conflict-of-interest statement: The authors declare that they have no competing interests.

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Abstract

A littoral cell angioma (LCA) is a primary vascular tumor of the spleen, that can have malignant potential and may present association with other malignancies. This is a case of LCA that was discovered incidentally in a 79-year-old woman who presented with a polycythemia at the time of consultation. The neoplasm was evaluated by ultrasound and computed tomography. The patient underwent a splenectomy that revealed LCA by pathological evaluation. The post-operative outcome was favorable with no complications or recurrent disease. This case presentation, clinical, radiographic, and pathological features of an uncommon splenic tumor can be studied in order to advance our knowledge in our understanding of LCA.

Key words: Laparoscopy; Splenectomy; Splenomegaly; Angioma; Littoral cell

Core tip: We invite readers to read "laparoscopic splenectomy for littoral cell angioma of the spleen: Case report", because we understand how pathological evaluation, after splenectomy, allows the definite diagnosis of this rare vascular neoplasm and given its

potential malignancy and its association with other cancer types, splenectomy should be always performed.

Marzetti A, Messina F, Prando D, Verza LA, Vacca U, Azabdaftari A, Rubinato L, Reale D, Favat M, Barbujani M, Agresta F. Laparoscopic splenectomy for a littoral cell angioma of the spleen: Case report. *World J Clin Cases* 2015; 3(11): 951-955 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i11/951.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i11.951

INTRODUCTION

LCA was first reported by Falk *et al*[1] in 1991. They reviewed 200 surgical specimens of benign vascular tumors of the spleen and described 17 similar tumors correlated to the cells lining the red pulp splenic sinuses[1]. The littoral cell, the original one, presents both epithelial and histiocytic features[1].

Both sexes at any age are affected by this tumor. Usually patients are asymptomatic and the dignosis leads to an incidental founding. When patient presents symptoms they arise later and are splenomegaly, thrombocytopenia and anemia.

The neoplasm take its origin in the sinus of the red pulp of the spleen. These endothelial cells show the same immunoreactivity for markers CD31 and factor Ⅷ, as showed by hemangiomas situated in different places.

Pathogenetic mechanism of this tumor is still uncertain, but has been thought as a possible one an immune system dysfunction because it has been observed an important association with autoimmune disorders like Crohn's disease and metabolic diseases like Gaucher's one[2,3].

CASE REPORT

Our patient, a 79-year-old woman, entered to the hospital for a polycythemia and during medical examinations it was discovered an hypointense lesion of the spleen and splenomegaly without hypersplenism (Figure 1). The patient did not present abdominal pain, nausea, episodes of vomite and changes in bowel habits. Her BMI was 35.8. The evaluation of EPO (5 IU/L) and genetic mutation of Jak2 (30%) revealed a chronic myeloproliferative syndrome. Ultrasound and computed tomography (CT) scan were performed. The ultrasound showed a round, solid, hypoechogenic mass of 45 mm of size at the superior pole of the spleen (Figure 2). A large, solitary, low-density lesion appeared in the early arterious phase of the contrast-enhanced CT (Figure 1). Laboratory tests showed normal liver enzymes and renal function, they only revealed an elevated count of platelets (587000/mcL) and a polycythemia (RBC = 5920000/mm, Hb = 17 g/dL) that was the primary reason why the patient entered to the Hospital.

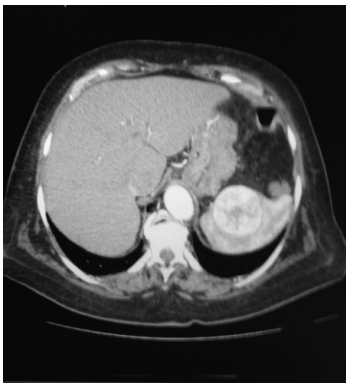

Figure 1 Computed tomography scan.

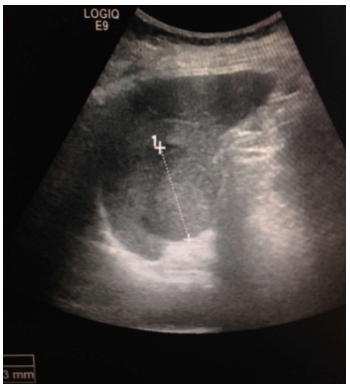

Figure 2 Ultrsound.

Imaging

Our patient underwent CT scan, that showed a hypoattenuating nodule of 4 cm × 5 cm of size with contrast enhancement on the arterious phase (Figure 1) and a hypointense lesion at Ultrasonography (Figure 2). Several studies have described the contrast-enhanced sonographic findings of various splenic lesions[4-6]. Characteristically benign vascular tumors show isoenhancement or a little hyperenhancement during the arterial phase and isoenhancement or hypoenhancement during the venous phase.

Rarely imaging can dignose the benign vascular neoplasm because many other splenic tumors mimic LCA. It is important a differential diagnosis with other splenic tumors that have a similar appearance with LCA like lymphangiomatosis, hamartoma, hemangiomatosis, hemangioendothelioma, hemangiopericytoma, and angiosarcoma; it has to be included in the differential diagnosis also lymphoma, metastases, Kaposi sarcoma and infectious diseases like Pneumocystis and Mycobacterium.

Histopathologic features

The neoplasm arises from the littoral cells in the splenic red pulp sinuses. Hemangiomas of other sites share

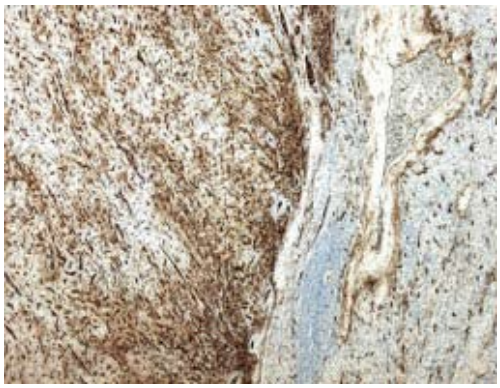

Figure 3 Positivity of vascular proliferation for immunohistochemical marker CD34; on right side there is normal splenic parenchyma (CD3450X).

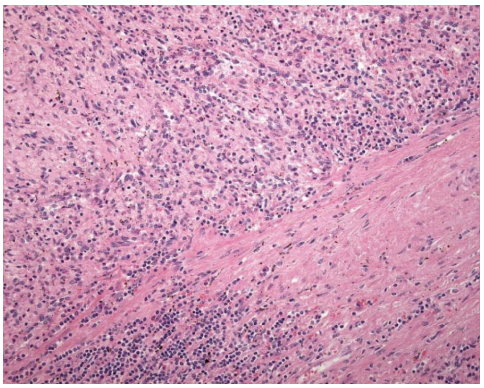

Figure 4 Vascular structures anastomosed (E-E 200 ×).

the same immunoreactivity for vascular endothelial markers CD31 and factor Ⅷ. It is interesting to observe that the tumor cells express histiocytic marker CD68 that explain why this neoplasm may begin in the splenic sinus lining cells or littoral cells.

DISCUSSION

Pathological evaluation, after laparoscopic splenectomy, allowed the definite diagnosis of this rare vascular neoplasm.

The surgical outcome was favorable without post-operative complications or recurrence.

Given certain diagnosis by pathological evaluation, associated to clinical features and imaging of an uncommon splenic tumor can be studied in order to advance our knowledge in our understanding of LCA.

The pathogenesis of LCA is nowadays still indefinite. We speculate as a possible pathogenic mechanism an immune system dysfunction, as demonstrates the association with autoimmune disorders like Gaucher's disease and Crohn's disease.

Pathological evaluation, after splenectomy, allows the definite diagnosis of this rare vascular neoplasm. Given its potential malignancy, splenectomy is usually performed.

The lining of vascular splenic sinuses, made by endothelial cells, presents both phagocytic and hematopoietic characteristics, that is the reason why they are thought to be unique since the 1930s[2].

The littoral cell of the spleen, the cell of origin, presents both epithelial and histiocytic features[7,8]. Overall the incidence of hemangioma of the spleen changes from 0.003% to 14% in autopsy reports[9], however the real incidence of LCA is unrecognized.

From a clinical point of view, the majority of patients with LCA are asymptomatic, while usually symptoms are anemia, thrombocytopenia both can be hypersplenism associated, splenomegaly, or unrecognized fever[10].

Abdominal pain can be also the main symptom at the time of consultation, otherwise the neoplasm is an incidental finding[11].

Our patient infact, entered to the hospital for a polycythemia and during medical examinations it was discovered an hypointense lesion of the spleen and splenomegaly without hypersplenism. She did not describe episodes of abdominal pain.

Normally, splenectomy performed for other reasons leads to diagnosis of LCA.

Only a minority of patients presents with splenomegaly, fever and features of hypersplenism like anemia and thrombocytopenia[12].

The vascular neoplasm can look like a single or multiple lesions in the spleen, while a massive splenomegaly can mimic a pancreatic tumor.

Recently LCA has been associated with tumors of the colon, kidney, pancreas, lung and ovary[13]. Have been described also associations with leiomyosarcoma, melanoma and lymphoma. Considering this malignancy association in patients with LCA, it should be always excluded visceral tumor.

The laparoscopic approach permitted to operate an old age, obese and cardiopathic patient and to dismiss her in 6 d without any postoperative complication.

Reports[10] comparing laparoscopic splenectomy to open splenectomy for diagnosed diseases like idiopathic thrombocytopenic purpura, reveal superiority of miniinvasive procedure because of its several benefits of lower post-operative pain and quicker post-operative recovery. Kercher *et al*[11] found that a laparoscopic approach was beneficial for massive splenomegaly (defined as craniocaudal dimension $\geqslant$ 17 cm and weight $\geqslant$ 600 g), which is also supported by our experience with this patient. Brodsky *et al*[12] also support laparoscopic splenectomy for several splenic diseases, also in case of splenomegaly. Rosen *et al*[13] found laparoscopic splenectomy to be safe for benign and malignant hematologic conditions, including Idiopathic Thrombocytopenic Purpura and a case of LCA, with a conversion rate of 5%.

Our patient was discharged by postoperative day 2 and did not present any complication, so

that splenectomy allowed the definite dignosis of LCA, without compromise the outcome in an old, cardiopathic, obese patient.

Pathological evaluation, after splenectomy, allows the definite diagnosis of this rare vascular neoplasm and given its potential malignancy, even though most of LCAs are benign, and its association with other cancer types, splenectomy should be always performed. So their differential diagnosis must observe primary and secondary malignancy.

This rare case shows the interest of studying vascular tumors of the spleen even most of all are incidental foundings.

Considering these concepts, gold standard management appears to be splenectomy (in our experience and in literature, laparoscopic approach reveals a better surgical outcome) and a strict follow-up to recognize synchronous tumors or metastatic lesions.

COMMENTS

Case characteristics

The authors' patient, a 79-year-old woman, entered to the hospital for a polycythemia and during medical examinations it was discovered an hypointense lesion of the spleen and splenomegaly without hypersplenism (Figure 1). The patient did not present abdominal pain, nausea, episodes of vomite and changes in bowel habits.

Clinical diagnosis

Splenomegaly, polycythemia, chronic myeloproliferative syndrome, elevated counts of platelets.

Differential diagnosis

Lymphangiomatosis, hamartoma, hemangiomatosis, hemangioendothelioma, hemangiopericytoma and angiosarcoma; lymphoma, metastases, Kaposi sarcoma and infectious diseases like Pneumocystis and Mycobacterium.

Laboratory diagnosis

Laboratory tests showed normal liver enzymes and renal function, they only revealed an elevated count of platelets (587000/mcL) and a polycythemia (RBC = 5920000/mm, Hb = 17 g/dL) that was the primary reason why the patient entered to the Hospital.

Imaging diagnosis

CT scan showed a hypoattenuating nodule of 4 cm × 5 cm of size with contrast enhancement on the arterious phase, and US revealed an hypointense splenic lesion.

Pathological diagnosis

Splenectomy revealed at the pathological examination vascular structures anastomosed and positivity of vascular proliferation for immunohistochemical marker CD34 , diagnosing LCA (Figures 3 and 4).

Treatment

Laparoscopic splenectomy.

Related reports

Recently LCA has been associated with neoplasms of the colon, kidney, pancreas, lung and ovary. Have been described also associations with leiomyosarcoma, melanoma and lymphoma. Considering this malignancy association in patients with LCA, it should be always excluded visceral tumor.

Term explanation

LCA is primarily a benign tumor, however may be associated with others visceral neoplasms and may have malign potential.

Experiences and lessons

Pathological evaluation, after splenectomy, allows the definite diagnosis of this rare vascular neoplasm because radiological and clinical foundings do not lead to a certain diagnosis. So, given its potential malignancy, even though the vast majority of LCAs are benign, and its association with other cancer types, splenectomy remains the golden standard in the managment of this disease.

Peer-review

Best management of this disease remains splenectomy (in our experience and in literature, laparoscopic approach reveals a better surgical outcome) and a strict follow-up to recognize synchronous tumors or metastatic lesions.

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CASE REPORT

Differential diagnosis of a vanishing brain space occupying lesion in a child

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Abstract

We describe clinical, diagnostic features and follow up of a patient with a vanishing brain lesion. A 14-year-old child admitted to the department of Neurology at September 2009 with a history of subacute onset of fever, anorexia, vomiting, blurring of vision and right hemiparesis since one month. Magnetic resonance imaging (MRI) of the brain revealed presence of intra-axial large mass (25 mm × 19 mm) in the left temporal lobe and the brainstem which showed hypointense signal in T1W and hyperintense signals in T2W and fluid attenuated inversion recovery (FLAIR) images and homogenously enhanced with gadolinium (Gd). It was surrounded by vasogenic edema with mass effect. Intravenous antibiotics, mannitol (2 g/12 h per 2 d) and dexamethasone (8 mg/12 h) were given to relief manifestations of increased intracranial pressure. Whole craniospinal radiotherapy (brain = 4000 CGy/20 settings per 4 wk; Spinal = 2600/13 settings per 3 wk) was given based on the high suspicion of neoplastic lesion (lymphoma or glioma). Marked clinical improvement (up to complete recovery) occurred within 15 d. Tapering of the steroid dose was done over the next 4 mo. Follow up with MRI after 3 mo showed small lesion in the left antero-medial temporal region with hypointense signal in T1W and hyperintense signals in T2W and FLAIR images but did not enhance with Gd. At August 2012, the patient developed recurrent generalized epilepsy. His electroencephalography showed the presence of left temporal focus of epileptic activity. MRI showed the same lesion as described in the follow up. The diffusion weighted images were normal. The seizures frequency was decreased with carbamazepine therapy (300 mg/12 h). At October 2014, single voxel proton (1H) MR spectroscopy (MRS) showed

reduced N-acetyl-aspartate (NAA)/creatine (Cr), choline (Cho)/Cr, NAA/Cho ratios consistent with absence of a neoplasm and highly suggested presence of gliosis. A solitary brain mass in a child poses a considerable diagnostic difficulty. MRS provided valuable diagnostic differentiation between tumor and pseudotumor lesions.

Key words: Vanishing brain mass; Gliosis; Unconfirmed diagnosis; Lymphoma; Granuloma

Core tip: A vanishing brain space occupying lesion is defined as reduction or disappearance of a brain lesion spontaneously or after steroid treatment to ⩽ 70% of its size before establishing its definitive diagnosis. A vanishing solitary neoplastic/non-neoplastic (pseudotumor) (*e.g.*, infection/abscess, granuloma, radiation necrosis, multiple sclerosis) brain mass in a child poses a considerable diagnostic difficulty particularly deeply seated lesions in which tissue diagnosis is difficult to be done. In clinical practice, neuroimaging has to be done every 6-12 mo for at least 3-5 years to follow up after complete remission of the patient. Magnetic resonance spectroscopy (MRS) has been proved to be valuable for diagnostic differentiation between tumor and pseudotumor lesions. MRS provides information related to the metabolic activity in the culprit lesion (*e.g.*, neoplastic processes, demyelination, cell necrosis or gliotic changes).

Hamed SA, Mekkawy MA, Abozaid H. Differential diagnosis of a vanishing brain space occupying lesion in a child. *World J Clin Cases* 2015; 3(11): 956-964 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i11/956.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i11.956

INTRODUCTION

Cases with solitary brain space occupying lesions (*e.g.,* tumor, intracranial infection/abscess, granuloma, neurocystocercosis, tuberculoma, multiple sclerosis) may pose a diagnostic difficulty particularly when brain biopsy can't be done due to deep location of the lesion. For such cases, clinicians often start corticosteroids to reduce manifestations of increased intracranial pressure (ICP) (caused by the intracranial mass and the surrounding vasogenic edema) and to give brain radiation if there is a high suspension for the presence of malignant lesion[1,2]. Also such patients should be routinely followed by magnetic resonance imaging (MRI) even after the disappearance of the enhancing lesion for at least 3-5 years[3]. The disappearance or decrease of the initial brain space occupying lesion (SOL) volume to ⩽ 70% either spontaneously or after steroid treatment before establishing the definitive diagnosis, has been referred as a vanishing brain lesion[4].

In the last decade, magnetic resonance spectroscopy (MRS) has been considered as a diagnostic test which helped to distinguish normal from abnormal brain tissue. Proton (1H) MRS measures some unique tissue metabolites which provide valuable information regarding the severity of the brain lesion, pathogenesis, prognosis and response to therapy. Briefly, in MRS, peaks which are proportional to the concentration of the given metabolite, are arranged along a flat baseline according to their radiofrequency (measured in units called parts per million or ppm)[5]. The most commonly defined brain metabolites (^{1}H-MRS spectrum) in which their patterns can be observed and correlate with different types of lesions (from right to left) include: free lipids (Lip), lactate (Lac), N-acetyl-aspartate (NAA), glutamate/glutamine (Glx), creatine (Cr)/phosphocreatine (the Cr peak), choline (Cho; the Cho peak), and myo-inositol (mI). In spectra obtained at long echo time (⩽ 135 ms), the peaks for NAA, Cr, Cho, and lactic acid are prominent and sharp. They are also detected and quantified at short echo time (⩾ 30 ms) MRS. In contrast, Lip, Glx and mI signals are detected only in short echo time MRS. In normal brain, NAA is synthesized in neurons, diffuses along axons and broken down in oligodendrocytes. In MRS, NAA is a marker of intact number of neurons in gray matter and the density of intact axons in white matter. The most prominent peak of NAA in MRS is the resonance at 2.0 ppm and it has concentration of 7.9-16.6 mmol/kg. NAA is a non-specific marker as its value is reduced in any disease associated with neuronal or axonal loss[6]. Cr is present at higher concentrations in the glia and it is a marker of brain energy. The most prominent peak of Cr in MRS is the resonance at 3.0 ppm and concentration of 5.1-10.6 mmol/kg[7]. Cho is a marker of brain injury of non-specific type. Cho level reflects the brain membrane metabolism with cellular turnover. The most prominent peak of Cho in MRS is the resonance at 3.2 ppm and concentration of 0.9-2.5 mmol/kg[6]. Lipids comprise about 20% of brain weight. Lipids are normally absent from 1H spectrum, and its appearance at 0.9-1.4 ppm resonances indicates presence of necrotic tissue (*i.e.*, breakdown of cell membrane and release of fatty acids)[8]. The mI is a marker of glial proliferation. It resonates at 3.6 ppm and concentration of 3.8-8.1 mmol/kg[9]. The glutamate/glutamine peak represents a mixture of excitatory and inhibitory brain neurotransmitters. Glutamate is mainly stored in neurons whereas glutamine concentration is higher in astrocytes. Both, Glx have two groups of resonances, the first group resonances at 3.6-3.9 ppm whereas the second group resonances at 2.0-2.6 ppm and concentration of 6.0-12.5 mmol/kg for glutamate and 3.0-5.8 mmol/kg for glutamine. Excess glutamate in active lesions could contribute to axonal damage, brain atrophy and neurological disability[10].

Malignant brain tumors are differentiated from other focal lesions, including multiple sclerosis (MS), radiation necrosis and infections/abscess by absent

NAA, excess Cho and lip. Spectra with elevated Cho, a Cho/Cr index greater than 1.3 and diminished NAA levels are associated with aggressive neoplasms (*e.g.,* malignant lymphoma and glioma). Tumor recurrence is characterized by high Cho/Cr and Cho/NAA ratios. A multi-voxel Cho/Cr ratio of > 1.54 and Cho/NAA ratio of > 1.05 was found to have 93.1% and 89.7% accuracies for diagnosis of tumor recurrence, respectively[11,12]. In MS, the abnormal increases in total Cr, total Cho, mI, Lac, lipids and macromolecules are markers for acute demyelination in MS. NAA is reduced in acute MS lesions and in normal appearing white matter, even distant to acute and chronic-lesions. Increased NAA to subnormal values occurs during remyelination. mI is increased in chronic MS (a marker for astrocytic gliosis). Reduced NAA peak represents neuronal/axonal dysfunction or loss. Elevated Cho peak represents enhanced cell-membrane turnover and is seen in demyelination, remyelination, inflammation, or gliosis[13]. In radiation necrosis (defined as a death of normal brain tissue caused by radiation therapy), in which the pathological features include progressive cellular necrosis (coagulative necrosis), inflammatory changes and reactive glial cell proliferation and gliosis, MRS shows elevated Cho, mI, lactate and lipid peaks[14].

This paper described a child with a symptomatic parenchymal brain mass but showed marked clinical recovery and disappearance of the original brain mass shortly after starting treatment. In this case report, the phenomenon of a tumor/or pseudo-tumor remission and the problems related to its differential diagnosis and treatments were discussed. The significance of using MRS to distinguish neoplastic from non-neoplastic nature of the intra-axial lesion in our patient was also discussed.

CASE REPORT

At September 2009, a 14-year-old child presented with a history of fever, anorexia, generalized body ache, loss of weight and headache since two months, which progressed to repeated vomiting, nausea, lethargy and blurring of vision in the last month. Prior to neurologic consultation, the patient was admitted to a fever hospital for one week because of the unexplained high grade fever. One year ago, the patient had past history of body aches and recurrent arthritis which was attributed to recurrent tonsillitis and based on the advice of the ear, nose and throat (ENT) physician, the patient did tonsillectomy. The mother said that although it was expected that the patient will be better after tonsillectomy but unfortunately, he had recurrent fever and generalized body aches till the time of presentation. On neurological examination, the child was feverish and looked toxic. He was alert, oriented and his higher mental functions testing were normal. He had right hemiparesis and right upper motor neuron facial paralysis. His fundus examination was normal. MRI-brain [1.5-Tesla, standard (T1W) pre- and post-contrast, T2W, fluid attenuated inversion recovery (FLAIR) brain imaging] revealed presence of a large intra-axial mass (25 mm × 19 mm) in the left temporal lobe with extension to the adjacent brainstem. It showed hypointense signal in T1W, hyperintense signals in T2W and FLAIR images and homogenous enhancement with gadolinium (Gd). It was surrounded by moderate perifocal vasogenic edema with mass effect in the form of compression of the third ventricle with midline shift (Figure 1). The clinical and radiological findings were highly suggestive of a neoplastic lesion (malignant lymphoma or glioma). The patient was examined for lymphadenopathy and organomegaly. He underwent laboratory workup [for complete blood count, erythrocyte sedimentation rate (ESR), glucose, electrolytes, lactic dehydrogenase (LDH), liver and renal functions], abdominal ultra-sonography and chest radiographs to rule out the presence of systemic lymphoma but no bone marrow evaluation was done. Blood tests revealed leukocytosis (16000 cells/μL) and elevated ESR (30/52). In view of the presence of fever, manifestations of increased intracranial pressure (ICP) and the prominent cerebral edema associated with the intracranial lesion; intravenous antibiotics (cefotax 1 g/12 h per 7 d), mannitol (2 g/12 h per 48 h) and dexamethasone (8 mg/12 h) were initiated. The oncologist recommended whole craniospinal irradiation (brain = 4000 CGy/20 settings per 4 wk; spine = 2600CGy/13 settings per 3 wk) which was started 10 d after presentation. Within 15 d and even before the start of radiotherapy, the patient exhibited marked clinical recovery (up to complete improvement) but he developed subjective cognitive deterioration as a side effect of radiotherapy which was recovered after its discontinuation. Tapering of steroids was done over the next 4 mo. Follow up of the patient was done every 3 mo. The follow up MRI after 3 mo from onset showed disappearance of the original mass but presence of small lesion with hypointense signal in T1W and hyperintense in T2W and FLAIR signals in the antero-medial part of the left temporal lobe but did not show enhancement with Gd. At further follow up (September 2010) the patient condition was unremarkable and his MRI had the same small non-enhanced lesion (Figure 2). At August 2012, the patient developed recurrent generalization tonic-clonic convulsions. His EEG showed left temporal focus of epileptic activity. His MRI had the same small non-enhanced lesion (as that of 2010) with no restricted diffusion in diffusion weighted images (DWI) (Figure 3). The seizures frequency was reduced with carbamazepine therapy (300 mg/12 h). At October 2014, his follow up MRI had the same small non-enhanced lesion (as that of 2010 and 2012) with no restricted diffusion in DWI. Single voxel proton (1H) spectroscopy at long and short echo times showed reduced values of choline to creatine (Cho/Cr: long ET = 0.05; short ET = 0.907), N-acetyl-aspartate to

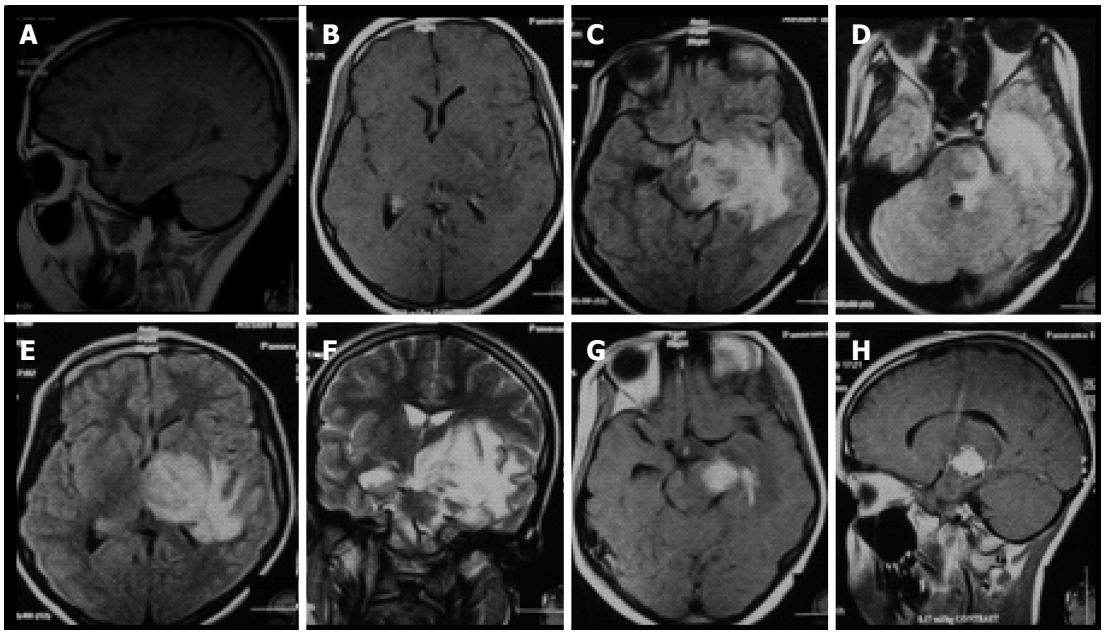

Figure 1 Cranial magnetic resonance imaging brain (on admission at September 2009) showing (A, B) sagittal and axial T1-weighted views with a solitary hypointense lesion in the left temporal lobe; (C-E) axial fluid attenuated inversion recovery and T2-weighted (F) images showing hyperintense lesion in the left temporal lobe encroaching on the adjacent brainstem with perifocal edema and mass effect; (G, H) axial and sagittal T1-weighted views showing homogenous solitary enhanced lesion in the left temporal lobe encroaching on the adjacent brainstem and surrounded by a moderate hypointensity.

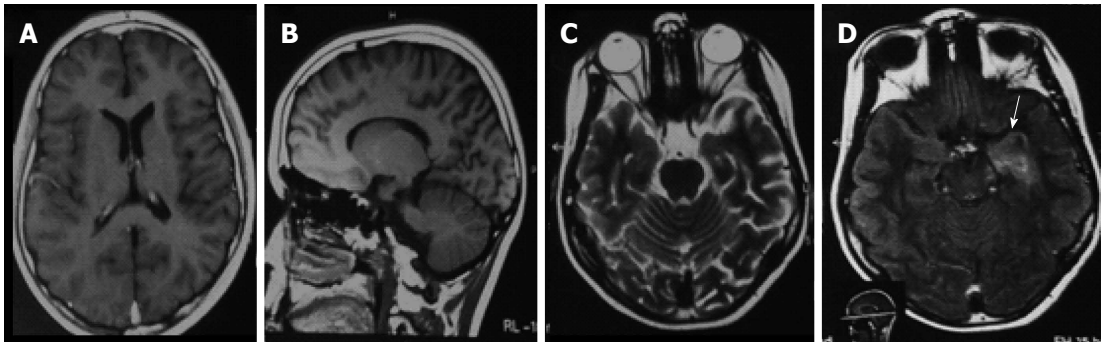

Figure 2 Cranial magnetic resonance imaging brain (September 2010) showing (A, B) normal axial and sagittal TIW and (C) axial T2W images but (D) hyperintense lesion in the antero-medial region of the left temporal lobe (white arrow) in fluid attenuated inversion recovery image.

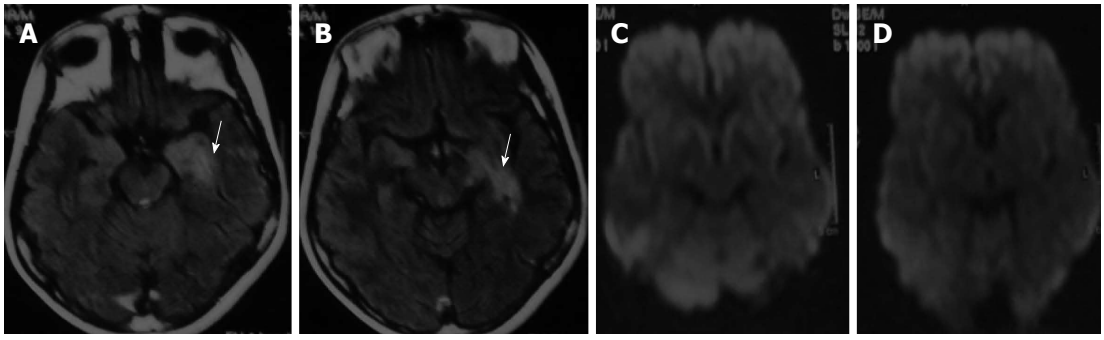

Figure 3 Cranial magnetic resonance imaging brain (August 2012) showing (A, B) hyperintense lesion in the antero-medial region of the left temporal lobe (white arrow) in axial fluid attenuated inversion recovery images (white arrow) and (C, D) normal diffusion weighted axial images.

creatine (NAA/Cr: long ET = 1.31; short ET = 1.107) and N-acetyl-aspartate to choline (NAA/Cho: long ET = 0.037; short ET = 0.38) ratios which confirmed the absence of neoplastic activity but highly suggestive of gliotic lesion (Figure 4).

This study was conducted according to the principles established in Helsinki and approved by Assiut University Hospital ethics committee. Informed written

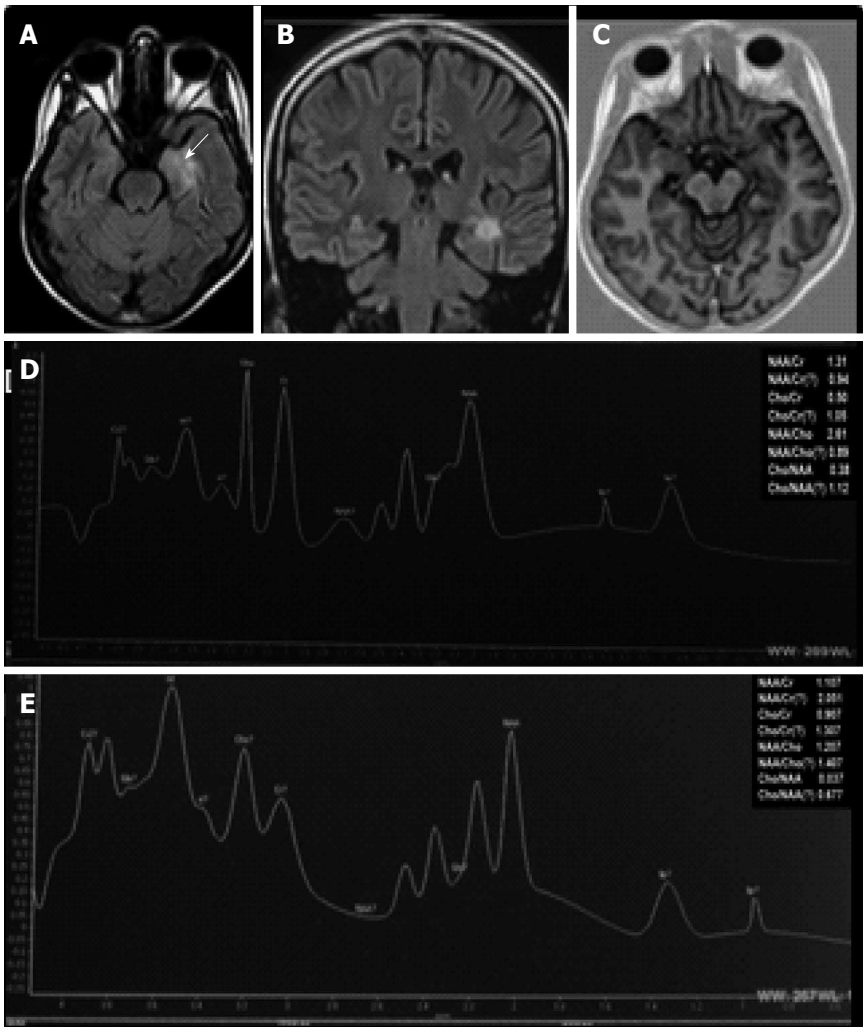

Figure 4 Cranial magnetic resonance imaging and magnetic resonance spectroscopy (MRS) brain (October 2014) showing (A, B) hyperintense lesion in the antero-medial region of the left temporal lobe in axial and coronal fluid attenuated inversion recovery images (white arrow); and (C) no restricted diffusion in axial diffusion weighted axial images; (D, E) long and short time echo MRS showing reduced values of Cho/Cr (long ET = 0.05; short ET = 0.907), NAA/Cr (long ET = 1.31; short ET = 1.107) and NAA/Cho (long ET = 0.037; short ET = 0.38) ratios. NAA: N-acetyl-aspartate; Cr: Creatine; Cho: Choline.

consent was obtained from the patient and his parents to publish the details of his clinical history, laboratory and imaging data.

DISCUSSION

This case is significant as the parenchymal brain mass could not be distinguished from a neoplastic space occupying lesion (*e.g.,* glioma or lymphoma) at presentation. Brain biopsy was not done due to deep location of the lesion. Complete clinical improvement was observed within 15 d on medical treatment including steroids and even before the start of radiotherapy. Corticosteroids were used empirically to reduce the manifestations of increased ICP and improve the surrounding vasogenic edema caused by the intracranial mass. The decision to give whole craniospinal irradiation was based on the high suspicion of a neoplastic lesion (*e.g.,* lymphoma or glioma). Marked reduction of the intracranial mass with disappearance of enhancement was observed in MRI within 3 mo of the onset. Follow up of the patients up to 5 years showed absence of recurrence of the original lesion.

Based on the above findings, the suggestive differential diagnosis of the vanishing space occupying lesion in this child at presentation include: (1) tumor [*e.g.,* primary central nervous system lymphoma (PCNSL) or glioma]; (2) tumor like demyelinating lesion or tumefactive multiple sclerosis (TMS); in clinical practice, most vanishing brain masses are frequently diagnosed as malignant tumors or multiple sclerosis (MS); and (3) intracranial infection/abscess/granulomas or tuberculoma.

For our patient, earlier at presentation, PCNSL was suggested. In patients presented with unclear intra-axial brain masses which regress with steroids, the diagnosis of PCNSL has to be considered[15]. PCNSL is a rare extranodal non-Hodgkin's lymphoma[16]. PCNSL represents approximately 3%-4% of newly diagnosed

central nervous system (CNS) tumors[17]. The typical MRI features of PCNSL include the presence of intra-axial single or multiple masses adjacent to cerebrospinal fluid space (CSF) with intermediate- to low-signal-intensity in T1W images and hypointense signal relative to the gray matter on T2W images, surrounding vasogenic edema, mass effect, restricted diffusion in DWI and intense homogenous enhancement with Gd[18,19]. Although, PCNSL is extremely rare in children and immunocompetent individuals[20,21], we suggested the diagnosis of PCNSL based on the MRI appearance of large solitary deep hemispheric infiltrative lesion[22] and rapid remission with steroid even before the start of radiotherapy. However, PCNSL is a malignant neoplasm and never considered as a self-limiting and recurrence is common within 18 mo. No cases have been reported yet for malignant brain tumors that recurred more than 5 years after spontaneous regression[23]. For our patient, the lacks of recurrence on follow ups for more than 5 years making such diagnosis less likely. This was also confirmed by the findings of MRS which will be discussed in the following section.

Also for our patient, the presence of fever prior to presentation and rapid remission with Ⅳ antibiotics and steroid suggest the diagnosis of TMS[24] or abscess/granuloma[25] but not the diagnosis of tuberculoma[26]. TMS is defined as a solitary large intracranial lesion larger than 2.0 cm in diameter associated with peri-lesional edema and mass effect[24]. TMS represents 1-2/1000 of cases of MS. TMS has been reported to be extremely rare in children in comparison to tumors and abscesses[27]. Diagnosis of MS depends on combination of clinical, neurophysiological, elevation of CSF immunoglobulin G (IgG) index and oligoclonal bands, and MRI of the brain and spine. Immunosuppressants (including steroids) and immunomodulators are the main therapies of TMS[28]. TMS lesion usually appears as open-ring (directed toward the cortical surface or to the basal ganglia) or closed ring or has diffuse, homogeneous, punctate, or concentric enhancement with Gd[29]. Although, CSF examination and gadolinium-enhanced MRI scan should differentiate between the MS and PCNSL, however, CSF may also be normal in fulminant conditions and short duration of the disease[30].

Furthermore, for our patient inflammatory pseudotumors or non-neoplastic lesions (*e.g.,* abscess/granulomas) of unknown etiology and respone to steroids was also suggested[25]. Patients with intracranial infection/abscess/granulomas commonly have history of risk factors (*e.g.,* immunocompromised state, dental, pulmonary or ear abscesses and intravenous drug use), fever, abnormal labs (as high erythrocytic sedemintation rate or C-reactive protein) and abnormal CSF suggesting CNS infection. Presentation is usually of acute onset with manifestations of increased ICP, seizures and focal neurological deficits. MRI-brain of brain abscess usually shows ring enhancement which is often complete with regular margin[31].

Magnetic resonance spectroscopy (MRS) was not done to the patient at presentation (2009) to distinguish neoplastic from non-neoplastic nature of the mass due to lack of availability. However and fortunately, it was available later and was done to the patient when he developed epilepsy (2012). For our patient, the focal lesion in the left antero-medial region of the temporal lobe found in the MRI (2010-2014) is the cause of the patients' left temporal lobe epilepsy with secondary generalization. The suggested differential diagnosis of the lesion according to the conventional MRI include: (1) tumor recurrence; (2) radiation necrosis; (3) multiple sclerosis; and (4) post-infective/inflammatory gliosis. MRS helped to distinguish tissue changes due to different brain lesions as discussed below.

For our patient, the reduced Cho/Cr (short ET = 0.907; long ET = 0.05), NAA/Cr (short ET = 1.107; long ET = 1.31) and NAA/Cho (short ET = 0.38; long ET = 0.037) ratios confirm the absence of tumor recurrence. Furthermore, the lack of reduced diffusion in DWI also confirms the absence of tumor recurrence[32].

For our patient, the diagnosis of radiation necrosis was suggested based on the facts that children are more susceptible to radiation necrosis than adults[33] and it usually occurs approximately 2-32 mo after radiotherapy, with 85% of cases occurring within 2 years. Delayed radiation-induced brain injury is a relatively common complication of radiation therapy representing 3%-24%[34]. Radiation necrosis is usually presented as a solitary periventricular white matter lesion, because of excess oligodendrocytes in these areas and a poor blood supply that produces ischemia[35]. The typical MR appearance of radiation necrosis is a soap bubble or Swiss cheese-like enhancing periventricular mass[36-38] and elevated Cho, mI, lactate and lipid peaks in MRS[39,40]. Radiation necrosis is related to both the volume of irradiated brain and the total administered radiation dose[41]. It has been reported radiation necrosis is extremely rare (5%) at doses < 45 Gy given over 25 fractions, or when the fractional dose is < 2 Gy/d but often occurs with total doses of > 60-70 Gy[34] or when the fractional dose is ⩾ 2 Gy/d. Our patient was given whole brain radiotherapy in a dose of 4000 CGy/20 settings per 4 wk (*i.e.,* < 2 Gy/d) making the diagnosis of radiation necrosis less likely. Also the absence of enhancement of the new lesion further confirms that the lesion in our patient is not a radiation necrosis.

Although, the results of MRS of our patients may suggest remyelination and gliosis following TMS, however, the presentation with seizures and lack of relapse with enhanced lesions after 5 years of follow up makes the diagnosis of MS less likely[13].

For our patient, the reduced values of Cho/Cr, NAA/Cr and NAA/Cho and lack of enhancement of the lesion are consistent with the diagnosis of gliosis. Gliosis is the process of scarring in the central nervous

system[42]. It results from the proliferation of glial cells or in a damaged brain tissue. It represents a healing process of brain injury whatever its nature. When neurons are injured, astrocytes proliferate in the region and manufacture glial-fibrillary acidic protein. This compound causes the astroglia to form a dense and fibrous tissue: The glial scar. Gliosis can take from a few days to many months to reach its final form. Gliosis is diagnosed by immunohistochemistry or MRI[43]. Gliosis occurred as a result of an acquired brain injury (most likely abscess, granuloma, inflammation) and it is the cause of temporal lobe epilepsy. Cr and Cho are glial markers. Gliosis typically presents with reduced levels of Cho, NAA, and Cr and observed lip peaks. Moderate levels of Cho and/or a Cho/Cr index < 1.3 are frequent with gliosis. This is supported by the followings: (1) the development of epilepsy in our patient occurred as a result of a focal lesion in the antero-medial region of the left temporal lobe which is suggestive of gliosis with no evidence of neoplastic activity as confirmed by MRS; and (2) mesial temporal lobe epilepsy (due to hippocampal sclerosis) is characterized by hippocampal atrophy, decreased NAA, and a low NAA/Cr ratio which are attributed to neuron loss and gliosis[39].

ACKNOWLEDGMENTS

I would like to thank the patient's parents for their cooperation and providing approval to publish the clinical, laboratory and imaging results of this case presentation.

COMMENTS

Case characteristics

A 14-year-old child with history of acute manifestations of increased intracranial pressure and right sided hemiparesis which improved completely within two weeks and followed after 3 years by epilepsy.

Clinical diagnosis

The patient was having right sided hemiparesis due to brain space occupying lesion which was complicated by recurrent generalized epilepsy.

Differential diagnosis

Brain tumor (*e.g.,* primary central nervous system lymphoma or glioma); tumor like demyelinating lesion or tumefactive multiple sclerosis and intracranial infection or abscess/granulomas.

Laboratory diagnosis

Blood tests revealed mild leukocytosis and elevated erythrocyte sedimentation rate (ESR).

Imaging diagnosis

Initially at presentation, magnetic resonance imaging-brain showed a large intra-axial mass (25 mm x 19 mm) in the left temporal lobe with hypointense signal in T1W, hyperintense signals in T2W and fluid attenuated inversion recovery (FLAIR) images and homogenous enhancement with Gd suggesting neoplastic lesion (malignant lymphoma or glioma) while follow up after 5 years, MRI showed small non-enhanced lesion in the antero-medial part of the left temporal lobe with hypointense signal in T1W and hyperintense in T2W and FLAIR signal and reduced choline (Cho)/creatine (Cr), N-acetyl-aspartate (NAA)/Cr and NAA/Cho ratios in magnetic resonance spectroscopy (MRS) which confirmed absence of neoplastic activity but suggestive of gliosis.

Pathological diagnosis

Inflammatory brain space occupying lesion complicated by gliotic lesion in the antero-medial part of the left temporal lobe.

Treatment

Brain dehydrating measures, antibiotics, corticosteroids and a course of craniospinal irradiation.

Related reports

In clinical practice; a vanishing brain space occupying lesion is commonly diagnosed as a neoplasm (*e.g.,* lymphoma) or multiple sclerosis.

Term explanation

A vanishing brain space occupying lesion is defined as reduction or disappearance of a brain lesion spontaneously or after steroid treatment to ⩽ 70% of its size before establishing its definitive diagnosis.

Experiences and lessons

In clinical practice, neuroimaging [including MRS or magnetic resonance imaging (MRI)] has to be done every 6-12 mo for at least 3-5 years to follow up after complete remission of the patient with a vanishing brain lesion.

Peer-review

The case report presents a vanishing brain space occupying lesion in a child over 5 years course of recovery and MRI follow-up. The prognosis was fortunately better. Text is well wrote and easily comprehensible with clear figures. The authors discussed the potential differential diagnosis, and recommended that MRS may be helpful to identify a potential vanishing brain space occupying benign lesion from tumor lesion in clinic.

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CASE REPORT

Case of Fitz-Hugh-Curtis syndrome in male without presentation of sexually transmitted disease

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Author contributions: Yi H and Kim GW designed research; Yi H, Kim GW, Kim JS and Choi IZ were attending doctors for the patients; Shim CS organized and revised the report; and Yi H wrote the paper.

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Informed consent statement: Patient provided informed written consent for this publication.

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Abstract

Fitz-Hugh-Curtis syndrome is a type of perihepatitis that causes liver capsular infection without infecting the hepatic parenchyma or pelvis. Fitz-Hugh-Curtis syndrome is known to occur commonly in women of childbearing age who do not use oral contraceptives and have sexual partners older than 25 years of age. However, the syndrome has been reported to occur rarely in males. The clinical symptoms are right upper quadrant pain and tenderness, and pleuritic right sided chest pain. The clinical presentation is similar in male and female. We experienced a case of Fitz-Hugh-Curtis syndrome in a 60-year-old man with the chief complaint of right upper quadrant abdominal pain. Despite a previous history of gonorrhea, we have also described our experiences of improved symptoms and recovery with allopathic medicines and have thereby reported the present case with a literature review.

Key words: Male; Right upper quadrant pain; Fitz-Hugh-Curtis syndrome; Perihepatitis; Sexually transmitted disease; Liver capsular infection

Core tip: Fitz-Hugh-Curtis syndrome is known to occur commonly in sexually active women and very rarely in males. We experienced a case of Fitz-Hugh-Curtis syndrome in a 60-year-old man with the chief complaint of right upper quadrant abdominal pain on inspiration. Despite of negative laboratory result, we diagnosed as Fitz-Hugh-Curtis syndrome by symptom and liver computed tomography scan. We have also described our experiences of improved symptoms and recovery with allopathic medicines.

Yi H, Shim CS, Kim GW, Kim JS, Choi IZ. Case of Fitz-Hugh-Curtis syndrome in male without presentation of sexually transmitted disease. *World J Clin Cases* 2015; 3(11): 965-969 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i11/965.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i11.965

INTRODUCTION

Fitz-Hugh-Curtis syndrome is a type of perihepatitis that causes liver capsular infection without infecting the hepatic parenchyma or pelvis. In 1920, Carlos Stajano first described the surgical confirmation of an adhesion that connected the anterior peritoneum and hepatic parenchyma in a gonorrheal patient who had complained of right upper quadrant abdominal pain. In 1930, Curtis[1] reported an adhesion of the peripheral hepatic parenchyma in a patient with salpingitis. In 1934, Fitz-Hugh[2] identified *Neisseria gonorrhoeae* (*N. gonorrhoeae*) on a peripheral liver adhesion in a patient complaining of right upper quadrant abdominal pain and reported that these findings were associated with the violin string-shaped adhesions around the liver observed in female patients with pelvic infection and salpingitis, which was at that time suggested as a new syndrome in terms of the pathophysiology of venereal diseases. Initially, only *N. gonorrhoeae* was considered a causative bacterium; however, in recent years, additional causative bacteria such as *Chlamydia trachomatosis* (*C. trachomatosis*) have been reported, and this condition was also found to be caused by other bacterial sexually transmitted diseases in addition to gonorrhea[3-5].

Traditionally, Fitz-Hugh-Curtis syndrome is known to occur commonly in women of childbearing age who do not use oral contraceptives and have sexual partners older than 25 years of age; because of unclear statistical results, it is uncertain whether this condition is accompanied by pelvic inflammation. However, since its detection in men after the 1970s[6], more extensive studies of this disease have been performed.

Based on the diagnostic criteria of a disease history and clinical patterns, cases in which a violin string-shaped abdominal adhesion is confirmed through laparoscopy or laparotomy to exclude other diagnoses and in which causative bacteria are identified in the liver capsule exudate are generally diagnosed as Fitz-Hugh-Curtis syndrome. In 2003, Nishie *et al*[7] and colleagues observed more definite hepatic capsule enhancement in the arterial phase relative to other phases during a computed tomography (CT) scan. Using abdominal dynamic CT scans, Joo *et al*[8] detected Fitz-Hugh-Curtis syndrome with a sensitivity of 88% and specificity of 95%, and Woo *et al*[9] diagnosed Fitz-Hugh-Curtis syndrome with 95.5%. In recent years and in consideration of surgical complications, non-invasive diagnoses not requiring surgery have increased for cases with mild symptoms by integrating the outcomes of clinical patterns, culture tests, and CT scan results.

In South Korea, Fitz-Hugh-Curtis syndrome was previously reported in women; however, in 2010, *Mycoplasma genitalium* was first identified *via* blood testing, an abdominal dynamic CT scan, and urine culture testing in a 35-year-old male patient complaining of right upper quadrant abdominal pain[10]. In addition to a literature review, the authors herein report a case of non-invasively diagnosed Fitz-Hugh-Curtis syndrome in a sexually inactive, hepatitis B virus (HBV)-positive elderly patient who presented with right upper quadrant abdominal pain; this diagnosis was achieved *via* blood testing and an abdominal dynamic CT scan, and excluded other diseases despite the inability to identify the causative bacteria.

CASE REPORT

A 60-year-old male patient was admitted to the emergency room with right upper quadrant abdominal pain that had gradually increased in severity beginning three days earlier. This pain was not affected by meals, and became sharp and severe upon inhalation. The patient had a disease history of gonorrhea while in his 20s, although this had completely recovered, and had no family medical history. He was divorced, a non-smoker, drank 1 bottle of soju 3-4 times a week, and had no external injuries. At the time of admission, his vital signs were as follows: Blood pressure, 140/70 mmHg; pulse rate, 80 times/min; respiration, 20 times/min; and body temperature, 36.5 ℃. He presented with acute symptoms, no specific sphygmoscopic findings, a soft abdomen, normal bowel sounds, oppressive pain in the right upper abdominal quadrant, no rebound tenderness or abdominal distension, and Murphy's sign negativity. He did not present with shifting dullness or fluid waves indicative of ascites, enlarged organs or masses, or bilateral costovertebral pain.

Peripheral blood tests revealed the following (Table 1). The following serum biochemical test results (Table 1) were observed: Increased C-reactive protein, aspartate aminotransferase, and alanine aminotransferase. Urine tests revealed no specific findings, and the results of simple chest and abdominal radiography were normal.

An abdominal pelvic CT scan was performed, and linear capsular enhancement of the inferior segment of the liver was observed in the arterial phase (Figure 1A). No specific findings were observed in other abdominal and pelvic organs, including the hepatic parenchyma, gallbladder, biliary tract, and pancreas. Hepatitis virus tests were planned to evaluate the increased hepatosomatic index, and a fluid treatment involving daily intravenous levofloxacin administration (500 mg) as well as experimental antibiotics were planned to treat suspected Fitz-Hugh-Curtis syndrome. Based on the findings observed in known cases of this disease, examinations of previous urinary tract

Table 1 Clinical value on initial admission

Peripheral blood test	
Leukocyte	3610/mm^3
Neutrophils	50.90%
Lymphocytes	35.50%
Hemoglobin	146 g/L
Platelet	164000/mm^3
Erythrocyte sedimentation rate	25 mm/h
Serum biochemical test	
C-reactive protein	15.6 mg/L
Total protein	67 mg/L
Albumin	37 mg/L
Total bilirubin	5.8 mg/L
Aspartate aminotransferase	52 IU/L
Alanine aminotransferase	47 IU/L
Alkaline phosphatase	93 IU/L
Blood urea nitrogen	148 mg/L
Creatinine	6.1 mg/L
Na	134 mmol/L
K	4.01 mmol/L
Cl	98.3 mmol/L
Creatine kinase-myocardial band	2.27 ng/mL
Troponin-T	0.003 ng/mL
Amylase	61 U/L

Table 2 Tumor marker test

Carbohydrate antigen 19-9	0.6 U/mL
Alpha-fetoprotein	19.28 ng/mL
Carcinoembryonic antigen	4.69 ng/mL
Protein induced by vitamin K absence or antagonist Ⅱ	17 nAU/mL

infections or venereal diseases, blood tests, and urine culture tests were performed. In a subsequent examination, the patient reported having 1 sexual relationship 20 d earlier; all results for the following tests were negative: Human immunodeficiency virus (HIV) Ag/Ab combi test, urine culture test, and PCR for *N. gonorrhoeae*, *C. trachomatis*, Ureaplasma urealyticum, M. genitalium, M. hominis, Trichomonis vaginalis, Treponema palladium, *Candida albicans*, Herpes simplex Ⅵ and Ⅶ, Haemophilus ducreyi, and Condyloma 6, 11, which was performed after prostate massage. The hepatitis virus test indicated the following: Hepatitis A virus IgM, HBV surface antibody (enzyme-linked immunoassay; EIA), HBV extracellular antigen (HBeAg), and hepatitis C virus Ab negativity; HBV surface antigen (EIA) and HBV extracellular antibody (HBeAb) positivity; and an HBV DNA copy number of 1.59×10^7/mL. A tumor marker test measured to exclude cancer and showed no abnormalities (Table 2). Gastroscopy and colonoscopy were performed to exclude cancer, and no specific findings were observed besides chronic superficial gastritis and a gastric ulcer. No findings indicative of liver cancer besides the previously observed capsular enhancement of the inferior segment of liver were observed on a liver abdominal dynamic CT scan.

On the second day of hospitalization, the right upper quadrant abdominal symptoms improved and on the third day of hospitalization, the patient's pain upon inhalation had decreased. The patient requested outpatient clinic care and he was discharged from the hospital. Two weeks after the initial medical treatment, the right upper quadrant abdominal pain disappeared during outpatient clinic care, and pain during inhalation decreased to 1-2 times per day. After four weeks of antibiotic treatment, the right upper quadrant abdominal pain improved, and the liver capsule enhancement completely disappeared on a liver abdominal dynamic CT scan performed three months after treatment (Figure 1B).

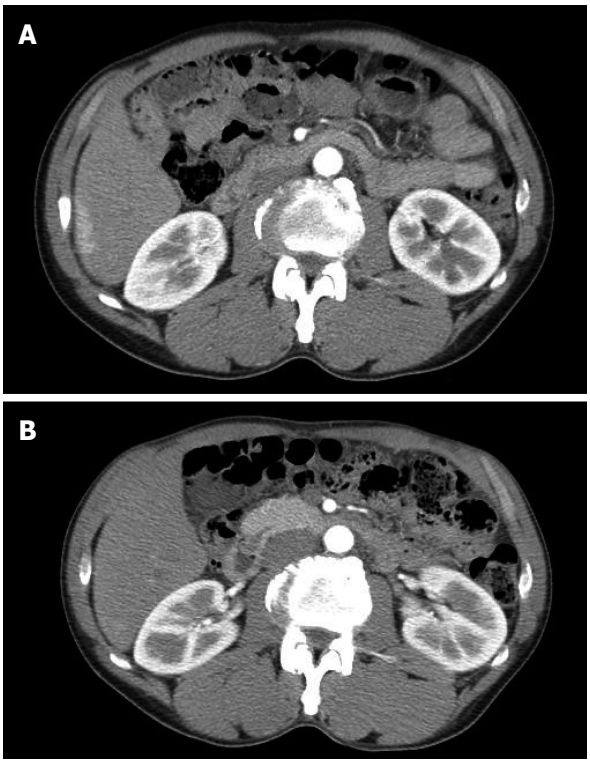

Figure 1 Abdominal computed tomography findings. A: Linear capsular enhancement of the inferior segment of the right lobe is visible on an arterial phase image; B: On an arterial phase image obtained 3 mo after treatment, the liver capsule enhancement over the right lobe has disappeared completely.

DISCUSSION

Fitz-Hugh-Curtis syndrome refers to perihepatitis accompanied by pelvic inflammation in 5%-15% of cases; young women of childbearing age are mainly affected by this disease. The symptoms can be divided into acute and chronic phases. Patients in the acute phase have characteristic pathologic findings of exudative hepatic capsule inflammation with inflammatory reactions and bleeding of the inferior liver and vessels in the abdominal wall adjacent to the liver[11]. Accordingly, in the acute phase, the right upper quadrant abdominal pain is sharp and pleuritic and occasionally radiates to the right shoulder or inside of the arm. In the chronic phase, exudative inflammation causes a violin string-like adhesion between the

hepatic capsule and inferior abdominal wall or between the hepatic capsule and diaphragm. Although this adhesion rarely causes clinically significant symptoms, laparoscopic synecotomy can be performed in cases involving persistent right upper quadrant abdominal pain that are refractory to antibiotic therapy[12].

Previously, *N. gonorrhoeae* was identified as the causative bacterium of Fitz-Hugh-Curtis syndrome, but in recent years *C. trachomatis* has also been identified as a major causative bacterium. In the present case, the patient had a previous history of gonorrhea and was an HBV carrier; however, there are no previous reports of secondary Fitz-Hugh-Curtis syndrome onset resulting from a previous gonorrheal infection and HBV positivity.

Regarding the infection route, traditionally direct infection has been dominant as determined *via* culture tests of the uterine tubes and hepatic lesions. However, blood-mediated infection has been reported in other cases[13] *via* blood culture tests, supporting the use of antibiotic treatment. However, most case reports remain controversial because of a lack of positive culture test results. Infection *via* lymphatic vessels could explain the cases of perihepatitis in both men and women without gonohemia, although as yet there is no clear evidence to support this. Money *et al*[14] suggested an immune reaction-based pathophysiology following a comparison of IgG values in the context of chlamydia infection, but currently this hypothesis remains unconfirmed.

Long-term complications of Fitz-Hugh-Curtis syndrome are rare and include pelvic inflammatory complications, chronic pain, small intestinal obstruction due to adhesion, and infertility.

A suspicion of Fitz-Hugh-Curtis syndrome is most important when diagnosing and detecting pleural or right upper quadrant abdominal pain in young, sexually active women in the absence of clear evidence for other diseases such as acute cholecystitis. As mentioned earlier, invasive surgical procedures such as laparoscopic surgery or laparotomy are required to confirm a diagnosis of Fitz-Hugh-Curtis syndrome, but these are not desirable or practically feasible in many cases. Therefore, in actual clinical settings, it is common to diagnose and treat this syndrome under only a presumptive diagnosis and the identification of characteristic strains after excluding other diagnoses. Blood tests mostly reveal a normal or elevated leukocyte count and erythrocyte sedimentation rate and a normal or slightly increased hepatosomatic index, which assist with the diagnosis. CT scans reveal hepatic capsule contrast enhancement, a characteristic finding of perihepatitis, in the arterial phase and are thereby used as a non-invasive method for diagnosing Fitz-Hugh-Curtis syndrome. Although cervical exudate is mainly used for stain identification, vaginal, anal, urethral, and pharyngeal exudates may also be used. Generally, culture tests are most widely used although genetic tests such as PCR or gene amplification provide better sensitivity and specificity. According to recent studies, however, increasing numbers of cases have been observed with *C. trachomatosis* negative PCR results but positive antigen-antibody reactions, and it is therefore recommended that antibody tests for causative bacteria identification should be performed concurrently[15]. In the present case, both culture tests and PCR were conducted to identify sexually transmitted microbes; however, an antigen-antibody test was not performed and the causative bacteria could not be identified.

Fitz-Hugh-Curtis syndrome can be treated experimentally with antibiotics, according to the principle of using antibiotics suitable for each identified causative bacterium. However, because of the rejection of some patients with venereal diseases, some cases have reportedly been treated with experimental antibiotics in the absence of causative bacteria identification or even attempted identification. Experimental antibiotics use is based on pelvic inflammatory treatment, and antibiotics such as cefotetan, doxycycline, clindamycin, gentamicin, and ofloxacin have been used[16]. Cefotetan and doxycycline are mainly administered intravenously, and levofloxacin and metronidazole can also be used. Intravenous antibiotics are continued for 48 h after the improvement of clinical symptoms, and metronidazole or levofloxacin are used orally for 2 wk. If pain persists even with proper treatment, a peripheral liver adhesion should be confirmed *via* laparoscopy[17].

Fitz-Hugh-Curtis syndrome is known to be extremely rare in men. In 1970, Kimball and Knee first reported a case of Fitz-Hugh-Curtis syndrome caused by *N. gonorrhoeae* in a 22-year-old man[6]. In 1982, Davidson and Hawkins[18] reported the development of this syndrome from gonorrheal sepsis concomitant with pustular bacterid in a 35-year-old bisexual man with an identified *N. gonorrhoeae* infection. In 1985, Winkler *et al*[19] reported a case of Fitz-Hugh-Curtis syndrome and discussed the possibility that *N. gonorrhoeae* entered *via* damaged rectal mucous membranes and directly spread through the abdominal cavity in a 35-year-old homosexual man with a history of acquired immune deficiency syndrome.

In the present case, we have reported a diagnosis of Fitz-Hugh-Curtis syndrome *via* blood tests and abdominal dynamic CT in a 60-year-old man with the chief complaint of right upper quadrant abdominal pain. Blood culture tests, urine culture tests, and PCR of sexually transmitted disease-causing microbes were performed to identify the causative agent, although *N. gonorrhoeae* was not proven to be the causative bacterium, despite a previous history of gonorrhea. We have also described our experiences of improved symptoms and recovery with allopathic medicines and have thereby reported the present case with a literature review.

COMMENTS

Case characteristics

A 60-year-old male patient was admitted to the emergency room with right upper quadrant abdominal pain that had gradually increased in severity beginning 3 d earlier.

Clinical diagnosis

Fitz-Hugh-Curtis syndrome.

Differential diagnosis

Hepatocellular carcinoma (right upper quadrant abdominal pain) -gastroscopy, colonoscopy, abdominal computed tomography (CT), and tumor marker test.

Laboratory diagnosis

Aspartate aminotransferase was 52 IU/L and Alanine aminotransferase was 47 IU/L.

Imaging diagnosis

Abdominal CT showed linear capsular enhancement of the inferior segment of the right lobe is visible on an arterial phase.

Pathological diagnosis

Blood culture tests, urine culture tests, and PCR of sexually transmitted disease-causing microbes were negative.

Treatment

Empirical intravenous antibiotics were administered, maintained oral medication.

Related reports

Fitz-Hugh-Curtis syndrome occurs rarely in male. Cases reported in English and Korean literature were reviewed.

Term explanation

Fitz-Hugh-Curtis syndrome is a type of perihepatitis that causes liver capsular infection without infecting the hepatic parenchyma or pelvis is known to be locally aggressive and requires extensive surgical resection.

Experiences and lessons

Fitz-Hugh-Curtis syndrome was considered as sexually transmitted disease. Although causative pathogen was not proven, we diagnosed and treated patient.

Peer-review

This article can nicely contribute to increasing the awareness of Fitz-Hugh-Curtis syndrome.

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CASE REPORT

Lower gastrointestinal tract bleeding caused by dieulafoy-like lesion synchronous meckel diverticulum: A rare case report

Song-Hu Li, Guang-Yao Wu, Xiao-Dong Lin, Zong-Quan Wen, Mei-Ting Huang, Shao-Ping Yu, Hao Zhang

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Author contributions: Li SH, Wu GY contributed the main work of this article, they are both co-first authors; the study was guided by Yu SP; the endoscopy operation was performed by Wen ZQ, assisted by Lin XD; Zhang H performed the colectomy and exploring laparotomy; data were obtained by Li SH, Wu GY, Lin XD and Huang MT; data were analyzed by Wu GY; the report was written by Li SH and Wu GY; all authors approved the final version.

Institutional review board statement: This case report was exempt from the Ethics Committee of Dongguan Kanghua Hospital.

Informed consent statement: The patient involved in this study gave his written informed consent authorizing use and disclosure of his protected health information.

Conflict-of-interest statement: No conflicts of interest exist.

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Abstract

Meckel diverticulum is an embryonic remnant of the Gastrointestinal duct which causes symptoms < 5% in the 2% population. Painless bleeding and abdominal pain are the most often reported symptoms. Dieulafoy lesion/dieulafoy-like lesion often cause upper gastrointestinal (GI) tract bleeding, but massive lower gastrointestinal bleeding is rare. We reported a 19-year-old male presented massive lower GI tract bleeding caused by Meckel diverticulum synchronous dieulafoy-like lesion.

Key words: Lower gastrointestinal tract; Bleeding; Dieulafoy-like lesion; Meckel diverticulum; Endoscopy

Core tip: Dieulafoy-like lesion often causes upper gastrointestinal (GI) tract bleeding, and meckel diverticulum is another common cause of GI tract bleeding. The two of them happened at the same person is rare. We observed a 19-year-old man complained of upper stomachache was admitted to hospital. He underwent left hemi-colectomy on day 5 after admitted. Pathology confirmed the diagnosis of dieulafoy-like lension of descending colon. The bleeding ceased for 2 d. But another attack came on day 3 after surgery. He underwent a second laparotomy which united endoscopy, a 2 cm × 1.5 cm meckel diverticulum

in terminal ileum was detected. Resection was performed. Pathology revealed meckel diverticulum. He was fully recovered with no sign of bleeding in the next year's following up.

Li SH, Wu GY, Lin XD, Wen ZQ, Huang MT, Yu SP, Zhang H. Lower gastrointestinal tract bleeding caused by dieulafoy-like lesion synchronous meckel diverticulum: A rare case report. *World J Clin Cases* 2015; 3(11): 970-972 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i11/970.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i11.970

INTRODUCTION

A 19-year-old man was admitted to hospital due to upper stomachache for 6 d, hematochezia for 3 d and syncope twice. He had recurrent massive hematochezia and conservative therapy was inefficacy. He had left hemi-colectomy on day 5 after admitted. Pathology confirmed the diagnosis of dieulafoy-like lesion of descending colon. The bleeding ceased for 2 d. He had another attack of hematochezia on day 3 after surgery (day 7 after admitted). He underwent a second laparotomy which united colonoscopy, a 2 cm × 1.5 cm meckel diverticulum in terminal ileum was detected. Resection was performed. Pathology revealed meckel diverticulum, atopia gastric mucosa polyp accompany with chronic ulcer. He recovered well and was discharged 20 d later. The next year's following up shows no sign of bleeding.

CASE REPORT

A 19-year-old man was admitted to hospital due to upper stomachache for 6 d, hematochezia for 3 d and syncope twice. He looked pale and weak when admitted. Blood count reveals RBC 2.44 × 10^{12}/L, Hb 71 g/L, HCT 0.20. There is no sign of abnormal of his colon except retention of fresh blood in the following day's colonoscopy. Tc-99m pertechnetate scan in the third day revealed no positive sign neither. The patient had a third time of hematochezia for about 300 mL. Bleeding from the left hemicolon was highly suspected during the second colonoscopy, but exact bleeding point was not observed. He was treated with hemostasia to stop the bleeding and fluid infusion therapy. A fourth time hematochezia occured in day 5. Digital subtraction angiography supported bleeding from left hemi-colon (Figure 1). He was sent to OR for left hemi-colectomy. Pathology confirmed the diagnosis of dieulafoy-like lension of descending colon (Figure 2). The bleeding ceased for 2 d. On day 3 post colectomy (day 7 after admitted), he had the fifth hematochezia, discharged about 1000 mL of blood. Blood transfusion and blood coagulant were used to stop bleeding. Those treatments did not stop the bleeding. He underwent a second laparotomy united colonoscopy. During the surgery laparotomy united colonoscopy, a 2 cm × 1.5 cm meckel diverticulum in terminal ileum was detected (Figure 3). A wide base, smooth, about 0.6 cm × 0.6 cm hemispherical polyp beside it was observed, resection was performed meanwhile. Pathology revealed meckel diverticulum, atopic gastric mucosa polyp accompany with chronic ulcer. The patient recovered in 20 d with neither bleeding nor other complications and was discharged. There was no more hematochezia in the next year's follow up.

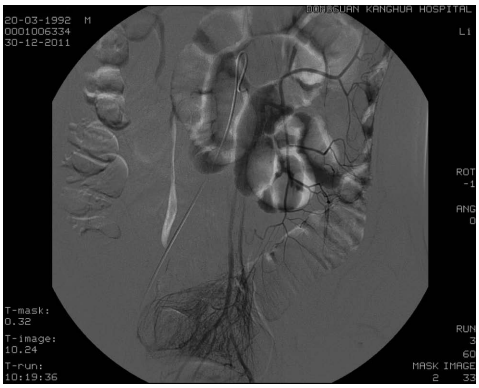

Figure 1 Digital subtraction angiography supported bleeding from left hemi-colon.

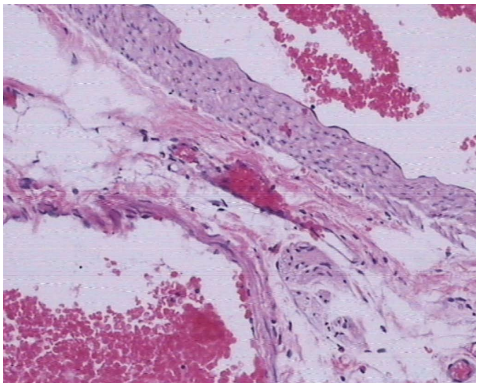

Figure 2 Pathology after left hemi-colectomy confirmed the diagnosis of dieulafoy-like lension of descending colon.

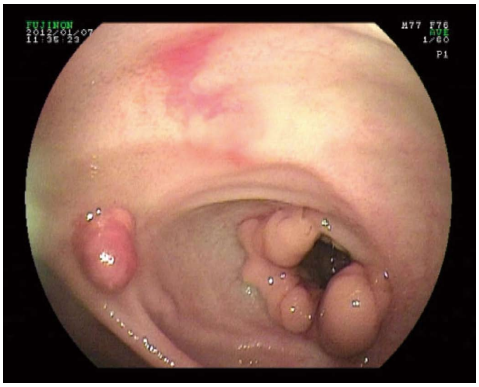

Figure 3 A 2 cm × 1.5 cm meckel diverticulum in terminal ileum was detected by colonoscopy during the second laparotomy which united endoscopy.

DISCUSSION

Dieulafoy lesion/dieulafoy-like lesion is a cause of GI tract bleeding which cannot be ignored, most commonly results the proximal stomach bleeding, but very rare entity that can cause massive lower gastrointestinal track bleeding[1]. Meckel diverticulum is an embryonic remnant of the gastrointestinal duct which is presented in approximately 2% of the population and is estimated to cause symptoms < 5% of the time. It generally results in painless bleeding or abdominal pain[2]. The technetium 99m pertechnetate scan is the best of choice for detecting Meckel diverticulum, with a reported sensitivity of 85% to 90% in the pediatric population[3]. In adults, however, the sensitivity falls on to only 62%[4]. It is an ideal noninvasive and sensitive way to detective meckel diverticulum. We performed surgery though this patient's technetium 99m pertechnetate scan is negative and a meckel diverticulum was found. This case presented of massive lower gastrointestinal bleeding caused by descend colon dieulafoy-like lesion and small bowl meckel diverticulum at same time is a rare condition.

COMMENTS

Case characteristics

A 19-year-old man complained of upper stomachache was admitted to hospital.

Clinical diagnosis

Massive lower gastrointestinal bleeding caused by descend colon dieulafoy-like lesion and small bowl meckel diverticulum at same time.

Differential diagnosis

Tumor of the colon, ischemic colitis, Crohn's disease, Ulcerative colonitis.

Laboratory diagnosis

Blood count reveals RBC 2.44 × 10^{12}/L, Hb 71 g/L, HCT 0.20.

Imaging diagnosis

Tc-99m pertechnetate scan in the third day revealed no positive sign. Digital subtraction angiography supported bleeding from left hemi-colon.

Pathological diagnosis

Pathology confirmed the diagnosis of dieulafoy-like lension of descending colon at the first surgery. Pathology reveals meckel diverticulum, atopic gastric mucosa polyp accompany with chronic ulcer after the second surgery.

Treatment

Left hemi-colectomy and resection of the bowl with meckel diverticulum.

Related reports

Dieulafoy lesion of the colon is reported to be bleeding cause of lower GI tract bleeding, and so does meckel diverticulum.

Experiences and lessons

Massive lower gastrointestinal bleeding caused by descend colon dieulafoy-like lesion and small bowl meckel diverticulum at same time is a rare condition. Both of them may be missed at the first endoscopy examination. Repeated endoscopy examination may be needed when bleeding occurs over and over again.

Peer-review

This case is interesting.

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EDITORIAL

Facial nerve paralysis in children

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Abstract

Facial nerve palsy is a condition with several implications, particularly when occurring in childhood. It represents a serious clinical problem as it causes significant concerns in doctors because of its etiology, its treatment options and its outcome, as well as in little patients and their parents, because of functional and aesthetic outcomes. There are several described causes of facial nerve paralysis in children, as it can be congenital (due to delivery traumas and genetic or malformative diseases) or acquired (due to infective, inflammatory, neoplastic, traumatic or iatrogenic causes). Nonetheless, in approximately 40%-75% of the cases, the cause of unilateral facial paralysis still remains idiopathic. A careful diagnostic workout and differential diagnosis are particularly recommended in case of pediatric facial nerve palsy, in order to establish the most appropriate treatment, as the therapeutic approach differs in relation to the etiology.

Key words: Facial paralysis; Seventh cranial nerve; Children; Bell's palsy; Therapy

Core tip: Pediatric facial nerve palsy can be congenital or acquired and its etiology can remain unknown. Bell's palsy is the most frequent form of facial paralysis also in children; about 70% of these cases has a favorable prognosis with spontaneous resolution. An accurate differential diagnosis is necessary to assess the prognosis and the therapeutic options. In Bell's palsy, the use of oral corticosteroids is recommended also in children, preferably within 3 d from onset. In children presenting a permanent congenital or acquired facial palsy, the therapeutic strategy consists in surgical techniques associated to rehabilitative approaches.

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INTRODUCTION

Pediatric facial nerve palsy can be congenital or acquired. Despite efforts to define its etiology, the cause of paralysis can often remain unknown. Idiopathic facial paralysis, even in childhood, is commonly known as Bell's palsy,

Table 1 Possible causes of facial nerve palsy in children

Causes of facial nerve palsy in childhood	
Idiopathic	Bell's palsy
Congenital	Delivery traumas:
	Primiparity
	Birth weight > 3500 g
	Forceps
	Cesarean section
	Prematurity
	Syndromic malformative:
	Möbius syndrome
	Goldenhar syndrome
	Syringobulbia
	Arnold-Chiari syndrome
	Genetic:
	Hereditary myopathies
	3q21-22 and *10q21.3-22.1* mutations
Acquired	Infectious:
	Ramsay Hunt syndrome
	Epstein-Barr virus
	Haemophilus influenzae
	Tubercolosis
	Lyme disease
	Cytomegalovirus
	Adenovirus
	Rubella
	Mumps
	Mycoplasma pneumoniae
	Human immunodeficiency virus
	Acute otitis media
	Chronic otitis media/cholesteatoma
	Inflammatory:
	Henoch-Schönlein porpora
	Kawasaki syndrome
	Neoplastic:
	Schwannomas of the Ⅶ c.n.
	Hemangiomas
	Rhabdomyosarcoma
	Temporal bone histiocytosis
	Leukemia
	Parotid gland tumors
	Traumatic:
	Temporal bone fractures
	Iatrogenic

named after the Scottish surgeon Sir Charles Bell, who, in 1821, firstly described a "weakness" of the facial nerve[1].

Aim of this paper is to describe the most common causes of facial nerve palsy in children and therefore the most appropriate available treatments.

STUDY METHODS

Narrative review. PubMed database was searched up to April 2015, for meta-analysis, systematic reviews, and controlled trials, going back for 10 years. The search was conducted independently and was restricted to children. Full text articles were required when the title, abstract or keywords indicated that the study could be suitable for this review. Additional papers were also identified from the references in the chosen literature.

The medical subject heading used included "facial paralysis"; "Bell's palsy"; "children"; "seventh nerve"; "therapy".

EPIDEMIOLOGY AND ETIOPATHOGENESIS

There are many possible causes of facial nerve paralysis in children. These can be classified as congenital (traumatic, syndromic and non-syndromic malformations, genetic) or acquired (infectious, inflammatory, neoplastic, traumatic)[2,3] (Table 1).

Unfortunately, in about 50% of the cases, the etiology remains unknown: these forms are classified as Bell's palsy. In children, Bell's palsy has an estimated incidence of about 6.1 cases per year per 100000 in those aged between 1 and 15 years[2,3]. It is believed that it can be caused by viruses such as Herpes simplex 1. About 70% of Bell's palsy has a favorable prognosis with spontaneous resolution within 3 mo, without sequelae. The paralysis severity at onset can influence the degree of recovery: a severe paralysis hardly obtains a complete recovery of nerve function[4-7].

Congenital facial paralysis can result from developmental defects or delivery traumas. Perinatal traumas are the most frequent causes of congenital paralysis. The main reported risk factors associated to traumatic facial paralysis are: mother's first child, birth weight greater than 3500 g, use of forceps, cesarean birth and prematurity. These cases have usually a favorable prognosis, with infants recovering the full functionality of the seventh cranial nerve within few months without sequelae[8,9].

A congenital facial nerve paralysis, although other cranial nerves such as the Ⅲ, Ⅳ, Ⅴ, Ⅷ can be involved, is presented within the Möbius syndrome. The reported prevalence of this syndrome is about 1/150000 live births[9-12]. It is reported to be due to hypoplasia of the motor nuclei of the cranial nerves within the brainstem, probably due to a hypoxic-ischemic encephalopathy[10]. Those affected by Goldenhar syndrome (hemifacial microsomia, with a spectrum of congenital malformations involving the structures derived from the first and second branchial arch) can also present a congenital facial paralysis[11]. Congenital pseudobulbar palsy (Syringobulbia) is a condition that clinically manifests with facial paralysis, dysphagia and speech difficulties, while in the Arnold-Chiari syndrome, congenital facial paralysis is usually associated to other cranial nerves paralysis (especially the Ⅵ one) due to malformations of the posterior fossa that allow herniation of brain structures through the foramen magnum[12].

Genetic causes of facial nerve paralysis includes hereditary myopathies, such as myotonic dystrophy and myasthenia. Also two loci responsible for isolated hereditary forms of facial paralysis (chromosome 3q21-22 and 10q21.3-22.1) have been identified[9,10,13].

Acquired facial paralysis can frequently be due to viral infections. The reactivation of Herpes Varicella-Zoster

may be responsible, even in children, of Ramsay Hunt syndrome (zoster oticus); in this case, facial palsy can be associated to the presence of vesicular lesions of the external auditory canal and/or of the auricular concha. The incidence of this syndrome under 10 years of age is reported to be 2.7/100000[9,11,14]. Not frequently, a bilateral facial nerve palsy may be the onset of a Epstein-Barr virus, Haemophilus influenza, tuberculosis or Borrelia burgdorferi infection. Lyme disease has become the most common cause of acute facial paralysis in children in those areas where Borrelia Burgdorferi infection is endemic[9,15]. Other agents that may cause facial nerve palsy in children are cytomegalovirus, adenovirus, rubella, mumps, Mycoplasma pneumoniae and HIV[3,13].

Facial nerve palsy may also be present as a complication of several diseases such as acute and chronic otitis media, cholesteatoma, mastoiditis and meningitis[16,17].

Other inflammatory diseases such as vasculitis and Henoch-Schönlein porpora or Kawasaki syndrome can also occur with facial nerve palsy[9].

Rarely, in children, facial nerve paralysis can be due to tumors such as schwannomas or hemangiomas of the seventh nerve or bone tumors such as rhabdomyosarcoma and histiocytosis.

Pediatric facial nerve paralysis has been also described associated to leukemia (in many cases bilateral) or to parotid gland tumors[9,18].

Finally, traumas such as temporal bone fractures (longitudinal, transverse and oblique) can cause facial nerve palsy in children[19], while iatrogenic paralysis can occur after surgery of the parotid gland, middle ear or mastoid[8,9].

CLINICAL FEATURES

The peripheral paralysis of the seventh cranial nerve is characterized by motorial, sensorial and visceral deficits of the hemi-face involved. There is a facial asymmetry at the examination of the face: the facial creases and the nasolabial fold disappear; the affected side also presents a dropping mouth rim (with possible saliva leakage), eyelid widening and lagophthalmos (static signs). Dynamic signs are represented by the inability to whistle, puffing cheeks, frown, close the eyelid. Signs of Bell and Nigro can be present. Hyperacusis, due to paralysis of the stapedius muscle, can be present, too[1,5,15].

The little patient may also report paresthesias or pain of the pinna or of the concha. Lacrimal and salivary production can be reduced (visceral deficit). Lagophthalmos can promote corneal irritation; furthermore the child may complain of a metallic taste in the mouth due to the taste alteration of the anterior 2/3 of the tongue[1,5,10,20].

In a very young children and in newborns, the unilateral facial paralysis can be suspected when, in absence of front and nasolabial groove motility, there is also asymmetry of the face with buccal deviation when crying. In cases of severe paralysis, the child cannot close the eye due to a complete absence of movement on the affected side and there is an asymmetry of the face at rest. In newborns, this condition can also hamper breastfeeding[10,21].

In all the cases, the occurrence of facial nerve palsy in children represents a serious clinical problem also due to the functional and aesthetic outcomes affecting the quality of life; this feature is cause of significant concern in the little patients and their parents as well as in doctors.

DIAGNOSIS

A comprehensive history evaluation is always important for the correct diagnosis. It is necessary to investigate about the onset and the time course of the paralysis and its eventual progression (*e.g.,* a gradual onset, > 3 wk, may suggest a neoplastic etiology). All the associated symptoms should be identified, as well as any other comorbidities affecting the child[14,15,18-21].

During the ENT examination, particular attention should be given to the inspection of the external auditory canal, the eardrum and the mastoid region. The facial nerve evaluation, in terms of facial movements and spontaneous expressions, should be classified according to House-Brackmann grading system, whenever the child is cooperative. Both the eye and palpebral region as well as the lower face should be careful observed at rest and at movement, eventually documenting the asymmetry using a camera or a video-camera. Computer systems can also provide tools for measuring the facial asymmetry[22].

The audiological evaluation is important in order to assess the presence of stapedial reflexes (topo-diagnosis) and eventually to evidence the presence of hearing loss[15,21].

Blood pressure and blood count should be verified in all cases of pediatric paralysis. Particularly, in children it has been described that high blood pressure levels can be associated to recurrent facial palsy[14,21]. Furthermore, a moderate increase of monocytes and lymphocytes is compatible with Bell's palsy, as far as this analysis does not place definitive diagnosis nor exclude an inflammatory process. The lumbar puncture is performed only when suspecting a meningitis (severe headache, fever, papilledema, neck stiffness) or a Guillain-Barré syndrome: in this last case, the analysis of the cerebrospinal fluid shows a characteristic increase in protein not accompanied by a consensual cells increasing (albumin-cytological dissociation)[1,5,9,13,21].

Specific laboratory and imaging tests are not routinely indicated, but are recommended for patients with recurrent paralysis or when there has been no improvement after 3 wk of therapy. With the purpose of diagnose the Ramsey Hunt syndrome in children, an ELISA serum searching for IgM and IgG antibody titer against Herpes Varicella-Zoster is recommended[14]. Serologic tests for Lyme disease should be carried

out when the history of the patient suggests a possible exposure, while in case of clinical suspicion of a neoplastic etiology, the computed tomography of petrous bone and the brainstem magnetic resonance imaging must be performed. Radiological images are required even when the child shows other neurological manifestations or in suspected chronic otitis media, acute mastoiditis or temporal bone fracture[14,15,18-21].

Electrophysiological studies can be useful to identify the cause of the paralysis, to define the prognosis and follow-up of functional recovery, but they are still not considered necessary in all pediatric patients[14,15,18-21].

PROGNOSIS

To assess the prognosis of facial paralysis can be difficult, especially in children, even if the possibility of a complete functional recovery is greater in pediatric cases than in adult ones.

The degree of paralysis represents a prognostic element: patients with partial paralysis have a better prognosis. Actually, the Ⅱ degree according to House-Brackmann scale has a good outcome, while the Ⅲ and the Ⅳ degrees are associated to moderate residual dysfunctions. The Ⅴ and the Ⅵ degrees, instead, have poor possibility of recovery[1,23].

Perinatal traumatic forms usually have a good prognosis, with a possible spontaneous resolution within 4 mo of life[9,13,21]. Bell's palsy has a generally optimal functional recovery in a short period of time; a favorable prognostic indicator is represented by a clinical improvement within 3 wk by the onset[1,13,23]. Ramsey Hunt syndrome has a worse prognosis compared to Bell's palsy: only 10% of severe paralysis due to reactivation of Herpes Varicella-Zoster have a full recovery[14].

The prognosis of facial paralysis caused by tumors is of course related to the type and stage of the tumor and the treatment performed[8,9,13,15,21].

It has been reported that in about 5% of cases, the affected side may develop residual sequelae like contractures, spasms, synkinesis[1,15]. The latter, in particular, affect the symmetry and facial expressiveness and usually recognize three possible pathogenetic mechanisms: an aberrant axonal regeneration, an aberrant nerve impulse transmission and a hyperexcitability of the nucleus of the facial nerve. The most common synkinesis affects the eye and mouth muscles: during a voluntary movement of the mouth, for example a smile, there could be an involuntary eye closure and *vice versa*. Less frequently, involuntary movements of the chin can be seen during voluntary movements of the mouth or the voluntary eye closure[24]. A similar phenomenon can occur with the autonomic fibers: for example, when eating, the activation of salivation causes also lachrymation (phenomenon known as "crocodile tears")[1,15,25].

THERAPY

The treatment of facial palsy is related to the etiology and the severity of the palsy itself. When a specific cause is identified, treatment is aimed to resolve the underlying cause. The therapeutic approach in children often involves a multidisciplinary team, comprehending otolaryngologists, pediatricians, neurologists, ophthalmologists, maxillofacial surgeons, plastic surgeons, physiotherapists (Table 2).

Drug therapy

In the idiopathic cases of facial palsy, the main limitations regarding drug therapy in children concern the lack of controlled clinical trials on children with Bell's palsy and its favorable natural history[9,13]. Since most of these forms in childhood recover spontaneously, aim of the drug therapy is to minimize the possibility of incomplete resolutions and reduce the risk of sequelae, such synkinesis, autonomic dysfunctions (*e.g.,* crocodile tears), facial spasms[26]. When Bell's palsy occurs in adults, it is well known that glucocorticoids in combination with antiviral therapy (acyclovir or valacyclovir) are recommended[27-30]. In children, the use of oral corticosteroids is recommended preferably within 3 d from onset of symptoms (the suggested treatment regimen is prednisone 1-2 mg/kg per day for 10 d, gradually decreasing the dose)[13,31] as the majority of patients improves in the first three weeks[32], although several studies did not find significant differences between the outcomes of children treated with corticosteroids and not[20,33-35]. The Ramsay Hunt syndrome, instead, should be treated as soon as possible with intravenous steroid associated with antivirals in children older than 2 years (*e.g.*, acyclovir 80 mg/kg per day every 6 h for 5 d or, in children older than 12 years, valacyclovir 20 mg/kg three times per day, up to a maximum of 1000 mg three times daily), in order to obtain a full recovery in 75% of cases if treated within the first three days from onset[5,13,36].

The majority of children has a spontaneous recovery, but for both congenital and acquired forms, particular attention should be paid to the corneal protection, resorting to the use of protective devices and lubrication with artificial tears to prevent irreversible corneal lesions. Rarely, persistent paralysis with an important lagophthalmos may require a tarsorrhaphy or the implantation of a temporary weight in the upper eyelid. Moreover, in infants with difficulty in suction due to mouth muscles involvement, it is essential to provide an alternative nutritional support[1,9,13,15,21,37].

Children with persistent severe paralysis require a long follow-up. The absence of signs of functional recovery after six weeks requires a comprehensive reassessment of the diagnostic-therapeutic approach[13].

Infants with congenital paralysis for perinatal trauma, usually have a good prognosis even without treatment. For those presenting a neural damage, there are surgical solutions in combination with steroid therapy, depending on the severity of the case[9]. The direct neurorrhaphy has an excellent prognosis, due to the large neuronal plasticity and the excellent regenerative capacity in the childhood. Alternatively the use of a nerve graft is

Table 2 Therapeutic approaches to facial nerve palsy in childhood

Therapy of facial nerve palsy		Outcome[1]
Drugs	Bell's palsy	
	Oral steroids within 3 d of onset	70% recovery after 3 wk[32]
	Ramsay Hunt syndrome	
	Intravenous steroids as soon as possible	75% recovery at 6 mo if treated within 3 d from onset; 30% recovery at 6 mo if treated after 7 d from onset[36,44]
	Antiviral agents	
	Other conditions	
	Targeted therapies for specific diseases	N/A
Protective measures	Eye protection	N/A
	Artificial tears	N/A
	Tarsorrhaphy	N/A
	Eyelid weight implant	N/A
	Nutritional support	N/A
Surgery	Traumatic palsy	
	Neurorrhaphy within 72 h	N/A
	Nerve grafting within 72 h	N/A
	Other conditions	
	Dynamic facial reanimation	
	Temporalis elongation mioplasty	80% recovery within 1 mo[38]
	Gracilis muscle microvascular free flap	89% recovery within 4-6 mo[45]
	Sural nerve grafting	N/A
	Cross-facial nerve grafting	83% recovery within 1 yr[46]
Rehabilitation approaches	Botulinum toxin	100% recovery (temporary)[24]
	Physiotherapy	N/A
	Biofeedback therapy	N/A
Regenerative therapy	Bioelectrical interface/electrode	N/A
	Stem cells and bio-scaffolds	N/A

[1]When available.

also described, with discrete functional and aesthetic results[3,9]. In both cases, the repair of the nerve should be completed within 72 h from the trauma onset[3,13].

Surgical therapy

In the pediatric population, the surgical decompression of the facial nerve in its labyrinthine segment is not recommended[3,9], primarily due to the lack of systematic clinical studies demonstrating its real effectiveness and secondly due to the risk of sensorineural hearing loss occurrence. In children presenting a permanent congenital or acquired facial palsy, surgical techniques of dynamic facial reanimation can be considered in order to tentatively restore a static and dynamic facial symmetry. Among these, the most performed are locoregional muscles transfers and muscle and nerve grafts[10,13]. In particular, a frequently performed intervention is the temporalis elongation myoplasty: the tendon of the temporal muscle is moved from the mandibular coronoid process to the lips, with 80% of children regaining a sufficient symmetry within a month[38]. A similar intervention is the bilateral anterior third of the masseter muscle transfer above the corners of the mouth. Also the employment of microvascular free flaps of gracilis muscle has been proposed[10,13]. Another microsurgical technique consists of nerve grafts (usually sural nerve) between the branches of the facial nerve of the healthy side of the face and those of the injured side (cross facial nerve grafting). This practice allows the healthy facial nerve to send a symmetrical and synchronous pulse to the paralyzed side[3,10,24,37,39,40]. Children have the best chances of success with this type of surgery[10,15]. When it is not possible to perform a cruciate graft, a neural transposition from a donor site of the same side of the facial paralysis can be proposed (*e.g.*, the hypoglossal nerve or the trigeminal motor branch): the nerve is partly or completely dissected and anastomosed to the distal part of the paralyzed facial nerve[10,15].

Rehabilitation approaches

Among the proposed treatments for synkinesis and emifacial spasms, the botulinum toxin has been proposed also in childhood[15,24]. Unfortunately, the toxin has a temporary effect, making necessary to repeat the injections. Moreover, the periods of relief from synkinesis become more and more short. Better results have been reported with the use of botulinum toxin after a cross facial nerve grafting[15,24]. Although with less evidences, other rehabilitative approaches, such as physical therapy, biofeedback therapy, relaxation exercises with massages therapy, coordination and facial expression exercises, can reduce muscle stiffness, facilitating facial movements. Relatively to acupuncture and electrical nerve stimulation (in order to accelerate healing by stimulating muscle), there are still not enough data in the literature in order to

certify the real efficacy[15,24-27].

Regenerative therapy

In the recent years, innovative technologies are improving the possibilities for facial reanimation with bioelectrical interfaces by using tissue-engineered constructs. An emerging strategy in order to restore a symmetrical smile is a direct neural interface: Inputs of the interfaced nerves induce stimuluses in the injured facial nerve. Regenerative electrodes are used in case of traumatic injuries of the nerve: These could be implanted at the end of the facial nerve and could allow its regrowth through the construct[41].

Among regenerative therapy, peripheral nerve regenerative strategies are clinically not available yet. Experimental procedures described in the literature have shown different achievements and consist of a combination of stem cells and bio-scaffolds.

Different types of stem cells have been proposed in order restore neuronal integrity; among these, embryonic stem cells, nerve and mesenchymal stem cells, adipose and bone marrow derived stem cells and also other types have been proposed[42].

Bio-scaffolds aim to maintain cell feasibility, but should also sustain proliferation and allow intercellular communication and cellular growth. Carbon nanotubes, hyaluronic acid-based scaffolds, polymeric scaffolds and other similar solutions have been proposed with the aim of piloting the neuronal/assonal regrowth[43].

Nonetheless, this therapeutic strategy is indeed complex; if it will become available, hopefully, it could offer new potential approaches for future treatments.

CONCLUSION

Pediatric facial nerve palsy is a condition with several implications, particularly when occurring in childhood. It causes significant concerns in doctors and in parents as well, mainly due to the functional and aesthetic outcomes.

The causes of paralysis in children are many, however idiopathic facial paralysis, or Bell's palsy, is the most frequent form of facial paralysis in children too. A careful diagnostic workout and differential diagnosis are always recommended, in order to establish the most appropriate treatment. Hopefully, in the future, regenerative medicine could offer new options for the treatment of this condition.

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MINIREVIEWS

Myasthenia gravis and thymic neoplasms: A brief review

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Abstract

Thymoma is the most common mediastinal tumor. They have varied presentation ranging from asymptomatic incidental mediastinal masses to locally extensive tumor with compressive symptoms and distant metastases. They have frequent association with various paraneoplastic syndromes (PNS). The most common PNS associated with thymoma is myasthenia gravis (MG). Patients of thymoma with MG have a favourable outcome due to early disclosure of the disease. Histologically they are classified into five subtypes and Masaoka-Koga staging system is used for staging. Surgery, chemotherapy and radiotherapy play an important role along with anti-myasthenia drugs. This review would like to highlight the association of thymoma with MG and associated clinical and therapeutic issues.

Key words: Thymoma; Myasthenia gravis; Surgery; Radiotherapy; Chemotherapy

Core tip: This article is a comprehensive review of literature of a rare neoplasm. This article outlines the various newer details of diagnosis, staging and treatment aspects of thymoma and myasthenia gravis (MG). The importance of association between thymoma and MG is reviewed in detail.

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INTRODUCTION

Thymoma is a neoplasm of the thymus gland originating from its epithelial tissue. Thymomas are the most common neoplasm of the anterior mediastinum in adults and the most common tumor of the thymus[1]. They account for approximately fifty percent of the anterior mediastinal masses[1]. But, thymomas are rare human neoplasms accounting for less than 0.5% of all cancers. The incidence of thymoma is 0.15 per 100000 person years[2]. The peak incidence in seen in the fourth and fifth decades of life with 52 years as the mean age at presentation[2]. No sexual predilection exists. They have frequent association with various paraneoplastic syndromes (PNS)[1]. The most common PNS associated

Table 1 World Health Organization histopathological classification

Type	Histologic description
A	Medullary thymoma
AB	Mixed thymoma
B1	Predominantly cortical thymoma
B2	Cortical thymoma
B3	Well-differentiated thymic carcinoma
C	Thymic carcinoma

with thymoma is myasthenia gravis (MG). This review would like to highlight the association of thymoma with MG and associated clinical and therapeutic issues.

CLINICAL ASSOCIATION

Thymomas are associated with PNS in 50% to 70% of cases[3]. The common PNS associated are MG (30% to 50%), Cushing syndrome, hypogammaglobulinemia, pure red blood cell aplasia, rheumatoid arthritis and limbic encephalitis. Thymoma is detected early in patients with MG as compared to patients without PNS due to regular clinical evaluation for treatment of MG[4]. The most common symptom is cough. They usually have a slow growth and spreads by local extension. Metastasic spread is to the pleura, pericardium, or diaphragm, while spread to extrathoracic sites are uncommon[2].

MG is a disease affecting the neuromuscular junction and manifests as muscular weakness and fatigability due to acetyl-choline receptor (AChR) antibodies in 85% of the cases[5]. Thymoma MG is seen in approximately fifteen percent of all MG cases[6]. Basic pathogenesis is caused by humoral immune response to an epitope on the thymoma cells which is similar to the epitope on the neuromuscular junction components[7]. The neoplastic thymoma cells encircled by the T cells expresses epitopes that cross-react with AChR. All patients with thymoma MG have detectable AChR antibodies in serum. The AChR antibody attacks the neuromuscular junction, specifically aimed at the nicotinic AChR at the endplate region of the postsynaptic membrane, resulting in muscle weakness[8]. Other non-AChR autoantibodies are also seen in 95% of thymoma MG cases and in 50% patients with late-onset MG (after 50 years of age) which cross-reacts with striated muscle titin and RyR antigens[9].

PATHOLOGY

Thymomas are classified into 5 World Health Organization (WHO) histopathological subtypes (Table 1)[10]. Thymoma arises from thymic epithelial cells and is associated with a variable degree of T lymphocyte proliferation. These T lymphocytes are generated *de novo* within the thymoma from the bone marrow progenitor cells under the influence of the cortical epithelial cell-like function of the thymoma's transformed epithelial cells. The WHO classification is based on the morphology of these epithelial cells and the amount of associated T lymphocyte, which is an indicator of the biologic function of the thymoma cells. While thymomas of WHO types A, AB, B1, B2, and B3 all show a certain amount of immature T lymphocytes, thymic carcinomas do not have a measurable number of immature T lymphocytes and are thus undifferentiated. Cortical thymoma (Type B2) is associated with MG in 50% of cases while medullary thymoma (Type A) is seldom associated with MG.

STAGING

Different staging systems are used but the most widely used system is Masaoka-Koga staging system, based on the per-operative and histopathological findings (Table 2)[11,12]. TNM staging of thymoma is not widely accepted, because it is not more useful than the Masaoka system[13].

INVESTIGATIONS

Histopathological diagnosis is the gold standard. Contrast enhanced computed tomography (CECT) thorax shows the local and regional extent of the disease. Magnetic resonance imaging aids in better soft tissue delineation and surgical planning. Routine hemogram and blood biochemistry is needed for assessing the patient's status. Metastatic workup requires further ultrasound of the abdomen or CECT abdomen. Serum titers of antiacetylcholine receptor antibody are done to assess the myasthenia status.

TREATMENT

Surgery

Surgery is the mainstay modality for the management of thymomas. Surgery helps for exact histopathological evaluation and staging, and is the first-line treatment modality in most of the cases[14]. Immediate and complete surgical resection is advised for resectable tumors. Surgery can be approached transternally or by video assisted thoracoscopic surgery, both having similar clinical outcome[15]. Radical removal of thymoma is curative for thymic tumors in most of the cases, but patients do suffer from MG after surgery. Thus, pharmacological treatment for MG and continuous followup is necessary even after surgery. In locally advanced cases when the tumour invades pleura or pericardium, complete radical surgery is not possible and adjuvant treatment in form of radiotherapy (RT) and chemotherapy (CT) is required. Presurgical plasmapheresis or immunoglobulin (IgG) intravenous infusion helps in removal of circulating pathogenic antibodies to a significant level[16].

RT

Postoperative adjuvant RT is should be given in patients with incompletely resected tumors[17]. Stage Ⅱ and

Table 2 Masaoka-Koga staging system

Stage	Definition
I	Encapsulated tumor with no gross or microscopic invasion
II	Macroscopic invasion into the mediastinal fat or pleura or microscopic invasion into the capsule
III	Invasion of the pericardium, great vessels, or lung
IVA	Pleural or pericardial dissemination
IVB	Lymphogenous or hematogenous metastasis

Ⅲ patients after complete resection also benefit from adjuvant RT in reducing the local recurrence rates. RT doses ranging from 40 to 60 Gy is advised which includes a radiation boost to the tumor bed in incompletely resected or nonresected lesions, with a fractionation scheme of 1.8 to 2 Gy daily over a period of 4-6 wk[18]. Patients with poor performance status and advanced diseases with compressive symptoms are given palliative RT in doses of 30 Gy in 10 fractions or 20 Gy in five fractions.

CT

CT in thymomas is preferred in locally advanced, unresectable and metastatic disease[18]. The common chemotherapeutic drugs used in thymoma are cisplatin, adriamycin, etoposide, cyclophosphamide and ifosfamide. Various standard CT regimens include the following: cyclophosphamide, adriamycin, cisplatin (CAP)[19]; cisplatin and etoposide (PE)[20]; adriamycin, cisplatin, vincistine, cyclophosphamide (ADOC)[21]; and etoposide, ifosfamide, cisplatin (VIP)[22]. Response to CT is ranges between 32% and 92% and around 10%-43% of patients have complete responses[23]. Adjuvant CT has a favorable influence on survival in stage Ⅲ and Ⅳ A resected thymomas. CT can be used as the initial modality in stage Ⅲ and Ⅳ A unresectable thymomas[24]. For stage Ⅳ B thymomas with disseminated disease, CT is preferred with local RT for palliation of local symptoms[18].

Treatment of MG crisis in thymoma

In MG crisis, the standard management is plasmapheresis and immunoglobulin treatments[25]. Intense pharmacological treatment should be used along with the immunoglobulin and plasmapheresis treatment in these patients.

Pharmacological treatment of thymoma MG

The drug of choice is acetylcholinesterase inhibitors[26]. Immunosuppressive drugs are the second choice when additional drug treatment is required. Several immunosuppressive drugs are used, namely steroids, azathioprine, cyclosporine, tacrolimus, cyclophosphamide, methotrexate, mycophenolate mofetil and rituximab.

PROGNOSIS

Prognostic factors predicting recurrence was evaluated by Detterbeck *et al*[27] in a systemic review. The significant factors were Masaoka Stage and completeness of resection. Other factors such as age, sex, size of tumor and MG were not statistically significant in multivariate analysis.

CONCLUSION

The management of thymoma involves a multimodality approach and needs cooperation between surgeons, radiologists, pathologists and oncologists from establishing diagnosis, deciding the therapeutic strategy and evaluating the prognosis. With advances in medical science in new techniques and drugs, there is a remarkable improvement in the management of thymoma.

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CASE REPORT

Simultaneous Erb's and Klumpke's palsy: Case report

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Abstract

Mapping nerve deficits during a physical exam after trauma to the upper extremity can help determine not only if the brachial plexus was injured but also which nerve roots were involved. A 28-year-old male presented with simultaneous signs and symptoms of Erb's (C5) and Klumpke's (C8, T1) palsy, with sparing of the C6 and C7 roots. The patient presented several months ago to his local emergency room with shortness of breath, which was determined to be caused by left diaphragmatic paralysis through clinical and radiographical evidence. However, the etiology of the current nerve dysfunction in the upper extremity remained unknown. With persistent questioning and establishing the patient's trust in the caregivers, it was revealed that the patient had attempted suicidal hanging. We describe the clinical features and the likely mechanism of injury leading to this previously unreported combination of brachial plexus injuries. The unique injuries to this patient's brachial plexus can be explained by the sequence of events during the attempted suicidal hanging. The upper brachial plexus was injured during the initial moments where the neck was excessively stretched and the lower brachial plexus was injured due to the patient reaching up and holding himself by his arm for an extended period of time.

Key words: Erb's palsy; Klumpke's palsy; Hanging injury

Core tip: This report describes a unique case in which only the upper and lower roots of the left brachial plexus were injured, sparing the C6 and C7 roots. Careful questioning revealed that the injury was caused during an attempted suicidal hanging. The damage to C5 occurred due to stretching of the neck and the damage to the C7 and C8 roots occurred after the patient reached up to free himself and hung by his arm for a period of

time.

Dawson A, Vasquez E, Garrett Jr D, Harris FS, El Nihum IM, Dayawansa S, Huang JH, Singel S. Simultaneous Erb's and Klumpke's palsy: Case report. *World J Clin Cases* 2015; 3(12): 984-987 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i12/984.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i12.984

INTRODUCTION

The brachial plexus is a group of nerves from C5-T1 that provide motor and sensory innervation to the upper extremity. Injury to the brachial plexus usually results in a pattern of functional losses that indicate which region of the brachial plexus was injured. Traumatic brachial plexus injuries are the most common type of brachial plexus injury[1]. Total lesioning of the brachial plexus (C5-T1) occurs in 75% of traumatic brachial plexus injuries. Traumatic brachial plexus injuries are typically associated with motor vehicle collisions. The typical presentation of a total brachial plexus injury is the rupture of the C5 and C6 roots (distal to the dorsal root ganglia) and avulsion of C7-T1 roots (proximal to the dorsal root ganglia)[2]. It can be extrapolated from this that the lower brachial plexus is susceptible to greater injury in traumatic brachial plexus injuries.

Erb's palsy or Erb-Duchenne palsy is an upper brachial plexus injury involving nerve roots C5 and C6. Klumpke's palsy or Dejerine-Klumpke palsy describes injury to the lower roots (C8, T1). These are usually brought about by a specific injury mechanism resulting in stretch of either the upper plexus (due to widening of the angle between the shoulder and head) or the lower plexus (seen in hyperabduction of the arm), in a mutually exclusive manner[3].

We report a case of an initially puzzling neurological deficit consisting of both upper and lower brachial plexus injuries. We assume this is the first reported case of such a combination injury.

CASE REPORT

A young male patient was referred to the clinic with a history of weakness and numbness in his left arm. Complaints of shortness of breath when he presented to his local emergency room several months prior had prompted radiographic workup revealing slight elevation of the left hemi diaphragm. Detailed neuroimaging with MRI and MRA were normal.

On motor exam at the clinic, there was difficulty raising the left arm above the horizontal plane against resistance. Slight atrophy of both the deltoid and supraspinatus muscles along with atrophy and weakness of the hypothenar and intrinsic hand muscles of the left side were noted. Full strength and normal muscle bulk were demonstrated in the left biceps and triceps. On sensory exam, reduced sensation to light touch and pinprick were found along the upper lateral shoulder supporting left C5 nerve root involvement. In addition, sensory deficits of the ulnar aspect of the forearm, hypothenar and the fifth digit were consistent with left C8 and T1 nerve root involvement. Denervation and early regeneration potentials were also confirmed for the left C5, C8 and T1 roots at the trunk level of the brachial plexus.

The patient was initially evasive regarding the events leading up to his neurological defects. He had repeatedly denied any trauma. Serological exam did not reveal any inflammatory process. With persistent inquiry, the patient ultimately disclosed that several months ago he had attempted suicide by hanging, using a rope and jumping from height. He reported that just when the rope tightened, his neck was stretched upward. He then reached up and grabbed the rope. He remained hanging on his outstretched arm before he was able to free himself.

Conservative treatment including physical therapy was initiated, with gradual improvement of the deficits over several months. Psychological counseling and psychiatric care were established.

DISCUSSION

There are only a few case reports describing complications of hangings despite its high prevalence. One reason is the high rate of completion of this method of suicide[4]. Additionally, unless the clinician is able to develop a rapport with the patient, patients are less likely to reveal the true scenario which led to the presentation; another potential reason for under reporting.

Based on the radiographic findings and history, it was determined that partial diaphragmatic paralysis was responsible for the shortness of breath the patient experienced. Hanging has previously been shown to cause temporary bilateral diaphragmatic paralysis[5]. In addition, several other mechanisms that can give rise to shortness of breath have been reported in the literature and were considered in the differential diagnosis. Post-obstructive pulmonary edema (POPE) is a common finding in attempted hangings. Young men, such as this patient, are especially susceptible to POPE because of the greater negative intrapleural pressure that is generated in this patient group[4,6]. Lack of pulmonary opacities on radiography made this unlikely.

It is believed that the primary fatal component of suicidal hanging is not from a cervical fracture, airway compression, or sympathetic stimulation, but rather internal jugular vein and carotid artery compression. Venous and arterial compressions are then followed by airway compression that ultimately leads to global hypoxia[4,7,8]. This theory is further reinforced by cases of patients with tracheotomies completing suicide with ligatures above the tracheotomy. Perfusion with hypoxic blood has been shown to be sufficient to produce respiratory distress in animal models, which reinforces this hypothesis[8]. Deaths in

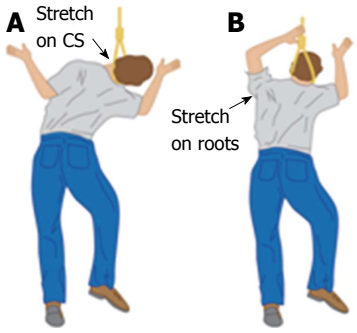

Figure 1 The likely injury mechanism can lead to a similar clinical presentation. A: Artist's rendering of the upper brachial plexus stretch injury; B: Artist's rendering of the lower brachial plexus stretch injury.

attempted hangings are typically associated with respiratory failure or bronchopneumonia[4,8].

In attempted suicidal hangings that did not result in death, nerve lesions have been reported and are described as upper motor neuron lesions caused by damage to the basal ganglia due to cerebral anoxia. Patients present with muscle spasticity and hyperreflexia[9]. Nerve damage due to anoxia in this patient was ruled out during the physical exam, which showed atrophy and decreased strength in the involved muscles, indicating that the deficits were lower motor neuron type lesions. Lower motor neuron lesions without ongoing or past inflammatory episodes in a healthy individual suggests trauma. Although the patient denied any trauma during the initial encounters, it was still high on the differential diagnosis list.

Physical examination of the patient revealed left partial diaphragmatic paralysis with atrophy of the intrinsic muscles of the hand as well as the hypothenar, supraspinatus and deltoid muscles on the left side. The nerves involved in innervating these muscles were studied to determine if there was a pattern in the distribution of nerve loss that could be traced back to the brachial plexus. The diaphragm is innervated by the phrenic nerve from C3-C5. The hypothenar and intrinsic muscles of the hand are innervated by the ulnar nerve from C8-T1. The supraspinatus muscle is innervated by the suprascapular nerve from C5-C6. The deltoid muscle is innervated by the axillary nerve from C5-C6[10]. The pattern of these involved nerves indicate injuries in the distribution of the left C5, C8 and T1 nerve roots. This combination of deficits and electrophysiological findings point to a stretch injury to both the upper and lower plexus. It is noteworthy that the C7 and possibly C6 nerve roots were spared in the case described here. Therefore, it is likely the damages occurred due to successive movements during the course of the hanging attempt that stretched first the upper then the lower regions of the brachial plexus.

Figure 1 shows the likely injury mechanism that can lead to a similar clinical presentation. Tension on the upper plexus likely occurred when the neck was abruptly pulled upward by the tightening rope (Figure 1A). The survival instinct led the patient to reach up and transfer his weight to his outstretched arm, causing stretch of the lower trunk (Figure 1B).

Traction injuries to the brachial plexus make up the majority of brachial plexus lesions. It is advised that the patient should be observed for up to 3-5 mo following the injury as edema should decrease and minor injuries resolve[11]. Natural recovery usually occurs within 6 mo, but recoveries without operative intervention are poorly documented[1]. As expected, this patient was also less symptomatic during subsequent follow-up appointments. Unfortunately we were unable to follow him long-term and address the physical and psychological issues completely, due to the poor compliance of the patient.

This patient's injuries are consistent with a sequence of events resulting in first a lateral stretch of the neck followed by a stretch of the lower brachial plexus. The movements behind these injuries can be explained by the patient's history of an attempted suicide followed by a survival response and extended period of time where the patient's weight was suspended by his arm above his head.

ACKNOWLEDGMENTS

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COMMENTS

Case characteristics

A young male with numbness and weakness in his left arm and previous difficulty breathing.

Clinical diagnosis

Left diaphragmatic paralysis with atrophy and weakness of the deltoid, supraspinatus, hypothenar and intrinsic hand muscles on the left side with reduced sensation to light touch and pinprick along the upper lateral shoulder, ulnar aspect of the forearm, hypothenar and the fifth digit.

Differential diagnosis

Brachial plexus trauma, nerve impingement.

Laboratory diagnosis

Serological studies did not reveal inflammatory processes.

Imaging diagnosis

Radiographic workup showed slight elevation of the left hemi diaphragm and magnetic resonance imaging/magnetic resonance angiography neuroimaging showed no abnormalities.

Treatment

Conservative treatment including physical therapy was initiated and psychological counseling and psychiatric care were established.

Related reports

To our knowledge, this is the first case report to describe both Erb's and Klumpke's palsy resulting from a suicidal hanging attempt.

Term explanation

Erb-Duchenne palsy refers to damage of the C5 and C6 nerve roots resulting in sensory and motor deficits in the distribution of commonly the suprascapular, musculocutaneous and axillary nerves. Dejerine-Klumpke palsy refers to

damage of the C8 and T1 nerve roots resulting in sensory and motor deficits in the distribution of the ulnar nerve. Post-obstructive pulmonary edema is negative pressure, non-cardiogenic pulmonary edema that can occur during (type Ⅰ) or after relief of (type Ⅱ) a pulmonary obstruction.

Experiences and lessons

This case presents a unique distribution of nerve damage after attempted suicidal hanging that we do not believe has been previously described.

Peer-review

This is an interesting case of a combined upper and lower brachial plexus injury associated with an attempted suicide.

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CASE REPORT

Sarcoma-associated sarcoid reaction: Report of cutaneous sarcoid reaction in a patient with liposarcoma

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Abstract

Sarcoidosis is a systemic inflammatory condition in which noncaseating epithelioid cell granulomas appear within one or several body sites. Sarcoid reaction (also referred to as sarcoidal or sarcoid-like reaction) occurs in patients who do not fulfill the diagnostic criteria for systemic sarcoidosis but present with similar clinical and histological features. As sarcoma-associated sarcoid reactions are rare, we describe the features of sarcoid reaction that developed in a man with liposarcoma and summarize reports of other oncology patients with sarcoma-associated sarcoid reactions. A 68-year-old man with retroperitoneal liposarcoma presented for evaluation of erythematous dermal plaques on his left leg. Microscopic examination of a tissue specimen revealed multiple epithelioid granulomas in the superficial and mid-reticular dermis. Correlation of the clinical presentation and histopathologic findings established a diagnosis of liposarcoma-associated cutaneous sarcoid reaction. Sarcoid reactions have been described in only seven individuals with sarcoma, including two patients with leiomyosarcoma and one patient with either carcinosarcoma, Kaposi sarcoma, liposarcoma, malignant peripheral nerve sheath tumor, rhabdosarcoma, or synovial sarcoma. Sarcoidal granulomas most commonly develop within the locoregional draining lymph nodes. Sarcoid reactions may also affect other organs, such as the lungs, skin, and spleen.

Key words: Liposarcoma; Malignancy; Sarcoid; Sarcoid reaction; Sarcoidosis; Sarcoma

Core tip: Sarcoid reaction is an inflammatory condition in which noncaseating epithelioid cell granulomas develop within one or several body sites. Several malignancies,

including lymphomas and carcinomas, have been linked to sarcoid reaction. We describe the first case of a patient presenting with liposarcoma-associated sarcoid reaction and summarize the literature on rare patients with sarcoma-associated sarcoid reactions. It is imperative that clinicians consider sarcoid reaction in the evaluation of oncology patients prior to initiating treatment.

Beutler BD, Cohen PR. Sarcoma-associated sarcoid reaction: Report of cutaneous sarcoid reaction in a patient with liposarcoma. *World J Clin Cases* 2015; 3(12): 988-992 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i12/988.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i12.988

INTRODUCTION

Sarcoidosis is a multisystem inflammatory condition characterized by the appearance of noncaseating epithelioid cell granulomas within one or several body sites. Sarcoid reaction-also called sarcoidal or sarcoid-like reaction-refers to the presence of noncaseating epithelioid cell granulomas in patients who do not fulfill the diagnostic criteria for systemic sarcoidosis. Many malignancies-including cancers of the thyroid, breast, and kidney-have been associated with sarcoid reaction. However, sarcoma-associated sarcoid reactions are rare[1].

Sarcoid reactions most commonly affect the lungs, intrathoracic lymph nodes, and skin. In addition, oncology patients often develop sarcoidal granulomas within the locoregional lymph nodes that drain the cancer. Diagnosis is typically established through imaging and/or biopsy. Similar to sarcoidosis, sarcoid reaction is typically asymptomatic and self-limiting; therefore, treatment is seldom required[2]. Immunohistochemical analyses have revealed that granulomas found in sarcoid reactions are B cell-positive while those found in sarcoidosis are B cell-negative.

We describe a man with liposarcoma who presented with cutaneous sarcoid reaction and summarize the characteristics of other sarcoma patients with sarcoid reaction.

CASE REPORT

In July 2014, a 68-year-old man with liposarcoma, which was diagnosed in 2008, presented for evaluation of a red rash on his leg that had been present for 30 mo. The tumor was 20 cm × 15 cm and located in the retroperitoneal space. The liposarcoma was inoperable and therefore treatment with oral pazopanib hydrochloride (200 mg taken four times per day) had been initiated. However, metastasis to the lymph nodes was subsequently detected.

Cutaneous examination revealed multiple smooth-surfaced erythematous dermal plaques affecting his left pretibial area (Figure 1). Pathologic examination of a punch biopsy showed multiple epithelioid granulomas in the superficial and mid-reticular dermis. Histiocytes could also be seen within the interstitium. There was mild lymphocytic and neutrophilic inflammation surrounding the sarcoidal granulomas (Figures 2 and 3). Bacterial, fungal, and mycobacterial cultures of biopsy-obtained skin specimens were negative for organisms.

Laboratory studies revealed an elevated erythrocyte sedimentation rate of 68 mm/h (reference range: 0-20 mm/h). With the exception of his serum albumin level being low at 2.92 g/dL (reference range: 3.5-5.5 g/dL), his serum chemistry levels were normal. Notably, his alpha-2-macroglobulin was elevated at 1.17 g/dL (reference range: 0.6-1.1 g/dL); this finding was consistent with subacute tumor-associated inflammation. Laboratory results for the following studies were negative or normal: anti-nuclear antibody, angiotensin converting enzyme, anti-dsDNA, anti-La (Sjogren's syndrome B), anti-Ro (Sjogren's syndrome A), anti-streptolysin O titer, glycohemoglobin, hepatitis antibodies, lipid profile, rheumatoid factor, Smith antibody, syphilis enzyme immunoassay, thyroxine 4, thyroid stimulating hormone, and vitamin D1,25-dihydroxy.

Based on correlation of the clinical presentation, histopathologic findings, and laboratory studies, a diagnosis of liposarcoma-associated cutaneous sarcoid reaction was established. The patient did not fulfill the criteria for systemic sarcoidosis. His skin condition was asymptomatic; therefore, no treatment was initiated. He died of malignancy-associated kidney failure shortly after the diagnosis of sarcoid reaction was established.

DISCUSSION

The first account of sarcoid reaction can be traced back to 1869, when the British physician Jonathan Hutchinson described a patient with "peculiar patches of dark purplish color on his extremities"[3]. The condition was further characterized throughout the late-19th and early-20th centuries. In 1899, Boeck[4] described 24 patients with "benign miliary lupoids". Twelve years later, Wolbach[5] detailed the histologic features of sarcoidal granulomas in a report of five patients with "widely distributed miliary sized lesions of granulomatous character". However, the association between sarcoid reaction and malignancy was not identified until 1917, when Herxheimer[6] observed sarcoidal granulomas affecting patients with breast, rectal, and cystic duct carcinomas.

Investigators continued to study sarcoid reaction throughout the first decades of the 1900s. By 1937, it was clear that sarcoid reaction could be definitively distinguished from systemic sarcoidosis. In a report of six patients with sarcoidal granulomas, Nickerson explained that sarcoid reaction refers to the localized development of noncaseating epithelioid cell granulomas; sarcoidosis, in contrast, is a multisystem inflammatory disease characterized not only by the development of noncaseating epithelioid cell granulomas, but also by various systemic symptoms and serum abnormalities[7]. Interestingly,

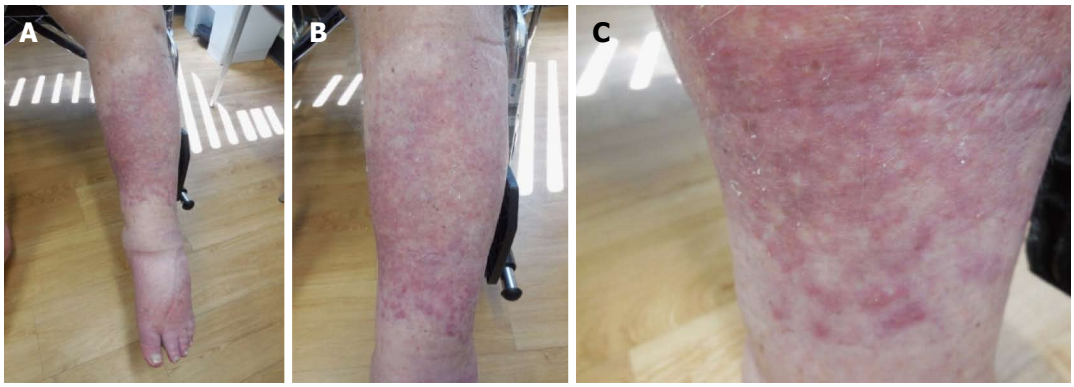

Figure 1 Distant (A), intermediate (B), and close (C) views of erythematous dermal plaques on the left leg of a 68-year-old man. The lesions were later diagnosed as cutaneous sarcoid reaction.

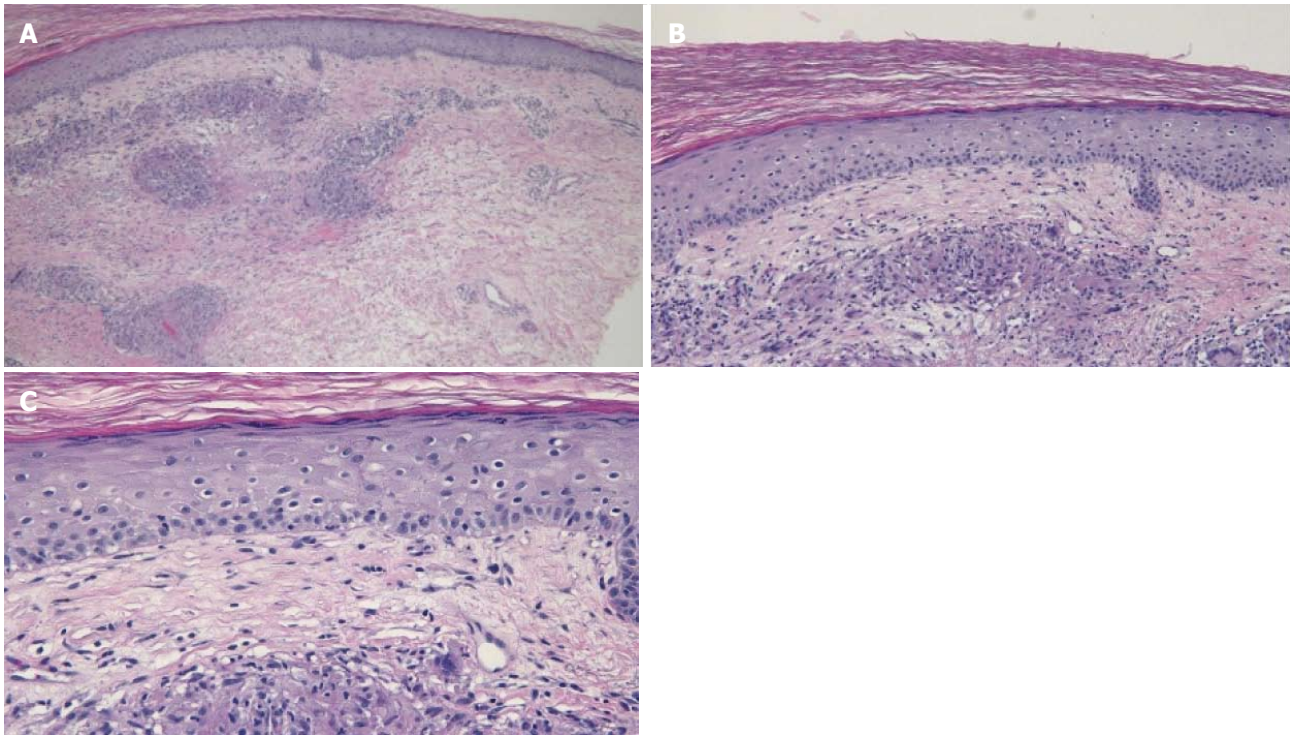

Figure 2 Low (A), intermediate (B), and high (C) magnification views of a sample of a lesion taken from the left leg of a 68-year-old man. Multiple epithelioid granulomas can be observed in the superficial and mid-reticular dermis. Interstitial histiocytes can also be seen within the interstitium (hematoxylin and eosin: A: × 10; B: × 20; C: × 40).

although sarcoidal granulomas are histologically identical in both conditions, immunohistochemical analyses have revealed that granulomas found in sarcoid reactions are B cell-positive while those found in sarcoidosis are B cell-negative[8].

Sarcoid reactions have been described in association with numerous hematologic malignancies and solid tumors[9]. However, sarcoma-associated sarcoid reactions are rare. Indeed, to the best of our knowledge, only eight patients (including our patient) with sarcoma-associated sarcoid reactions have been described in the English literature (Table 1)[10-16].

Leiomyosarcoma-either of the rectum or stomach-was associated with sarcoid reaction in 25% of individuals (two of eight). The other patients had either carcinosarcoma, Kaposi sarcoma, liposarcoma, malignant peripheral nerve sheath tumor, rhabdosarcoma, or synovial sarcoma. However, the number of patients is too small to observe any definitive relationship between specific sarcoma type and the development of sarcoid reaction.

Sarcoma-associated sarcoid reaction was observed in five men and three women. The age at sarcoma diagnosis ranged from 22 to 74 years; the median age was 57.5 years. The women were significantly younger than the men; they ranged in age from 22 to 58 years, with a median age of 48 years. In contrast, men ranged in age from 30 to 74 years; their median age was 60 years.

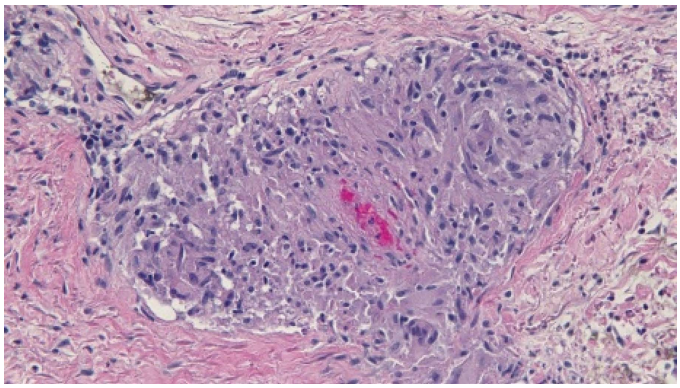

Figure 3 High magnification view of a sarcoidal granuloma from a sample of a lesion taken from the left leg of a 68-year-old man. There is mild lymphocytic and neutrophilic inflammation surrounding the granuloma (hematoxylin and eosin: × 40).

Table 1 Characteristics of patients with sarcoma and sarcoid reaction

C	Dx: SM	Dx: SR	G	Sarcoma	Diagnostic tests	Sarcoid reaction	Ref.
1	22	24	F	Synovial sarcoma of the left thigh	CT; biopsy	Left lower lobe subpleural nodules	[10]
2	30	30	M	Malignant peripheral nerve sheath tumor[1]	FDG PET/CT; biopsy	Hilar and mediastinal lymphadenopathy	[11]
3	48	50	F	Leiomyosarcoma of the stomach	Chest radiograph; biopsy	Bilateral hilar and paratracheal lymphadenopathy	[12]
4	57	57	M	Leiomyosarcoma of the rectum	Biopsy	Granulomas within the tumor tissue	[13]
5	58	59	F	Uterine carcinosarcoma	FDG PET; CT; biopsy	Hilar, pretracheal, and mediastinal lymphadenopathy	[14]
6	60	60	M	Rhabdomyosarcoma of the esophagus	Biopsy	Granulomas within the lymph nodes draining the neoplasm and in the spleen	[15]
7	62	68	M	Retroperitoneal liposarcoma	Biopsy	Red dermal plaques of granulomas	Current report
8	74	74	M	Kaposi sarcoma of the foot	Biopsy	Red-purple cutaneous patches; granulomas within the tumor tissue and in the stroma surrounding the tumor	[16]

[1]Malignant peripheral nerve sheath tumor is also referred to as neurofibrosarcoma, neurosarcoma, and malignant schwannoma. C: Case; CT: Computed tomography; Dx: SM: Age at sarcoma diagnosis (years); Dx: SR: Age at sarcoid reaction diagnosis (years); F: Female; G: Gender; M: Male; FDG PET/CT scan: Integrated 2-[18F]-fluoro-2-deoxy-D-glucose positron emission tomography and computed tomography scan.

The primary site of origin was most commonly the gastrointestinal tract; three of eight cases presented with neoplasms affecting this region. These included tumors from the esophagus, stomach, and rectum. Other sites of origin, each present in one individual, included the genitourinary tract (uterus), pelvic cavity, retroperitoneum, skin (Kaposi sarcoma), and synovium.

Five of eight cases were diagnosed with sarcoma and subsequently developed sarcoid reaction. The interval between the diagnosis of sarcoma and sarcoid reaction ranged from three months to six years, with a mean interval of 2.3 years. The two conditions were diagnosed concurrently in the remaining three patients (cases 4, 6, and 8).

The sites of sarcoma-associated sarcoid reaction in 4 of 8 cases were the lungs and mediastinal lymph nodes. Cutaneous involvement was observed in two patients, including our own. In addition, two individuals-either with Kaposi sarcoma or leiomyosarcoma of the rectum-developed nodules within the tumor tissue. Other sites of sarcoma-associated sarcoid reaction included the locoregional draining lymph nodes, the spleen, and the stroma surrounding the neoplasm.

The diagnosis of sarcoma-associated sarcoid reaction was established primarily by imaging studies and histologic examination of tissue samples. Imaging studies were performed on five patients-either chest radiography (one patient), computed tomography scan (two patients), or combination positron emission tomography and computed tomography scan (two patients). In addition, all eight patients underwent biopsy in order to identify noncaseating epithelioid cell granulomas and exclude disease metastasis. In most patients, sarcoid reactions resolve spontaneously; therefore, treatment is rarely required.

The pathogenesis of sarcoma-associated sarcoid reaction is unknown. It has been postulated that the development of sarcoidal granulomas in oncology patients represents a host immune defense mechanism. Indeed, the occurrence of sarcoid reaction within tumor tissue is associated with a better prognosis, a reduced risk of metastasis or recurrence, or both; T lymphocytes and dendritic cells, which are typically found within granulomas, are thought to play a central role in this

response[17,18]. Sarcoid reaction is characterized by the development of noncaseating epithelioid cell granulomas in patients who do not fulfill the diagnostic criteria for sarcoidosis or sarcomas.

COMMENTS

Case characteristics

A 68-year-old man with a six-year history of retroperitoneal liposarcoma presented for evaluation of a red rash on his leg that had been present for 30 mo.

Clinical diagnosis

Multiple smooth-surfaced erythematous dermal plaques affecting the left pretibial area.

Differential diagnosis

Discoid lupus erythematous, granuloma annulare, lichen planus, lymphocytoma cutis, plaque psoriasis.

Laboratory diagnosis

Elevated erythrocyte sedimentation rate of 68 mm/h (reference range: 0-20 mm/h) and elevated alpha-2-macroglobulin at 1.17 g/dL (reference range: 0.6-1.1 g/dL).

Pathological diagnosis

Multiple epithelioid granulomas in the superficial and mid-reticular dermis.

Treatment

The cutaneous condition was asymptomatic and therefore no treatment was administered.

Related reports

Sarcoid reactions have been described in only eight individuals, including the authors' patient, with various sarcomas; these include two patients with leiomyosarcoma and one patient with either carcinosarcoma, Kaposi sarcoma, liposarcoma, malignant peripheral nerve sheath tumor, rhabdosarcoma, or synovial sarcoma.

Term explanation

Sarcoidosis is a systemic inflammatory disease in which noncaseating epithelioid cell granulomas develop in multiple organ systems. In contrast, sarcoid reaction - also called sarcoidal or sarcoid-like reaction - refers to the presence of noncaseating epithelioid cell granulomas in patients who do not fulfill the diagnostic criteria for systemic sarcoidosis.

Experiences and lessons

Sarcoid reaction may occasionally mimic metastases in patients with solid tumors, including sarcomas, and should therefore be considered in the evaluation of oncology patients in order to prevent misdiagnosis and unnecessary treatment.

Peer-review

This is a straightforward clinical case study reporting on a liposarcoma patient that presents with a cutaneous sarcoid reaction. At the same time the authors review the literature listing all cases of soft tissues sarcoma that are linked to a sarcoid reaction.

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CASE REPORT

Histopathological confirmation of similar intramucosal distribution of fluorescein in both intravenous administration and local mucosal application for probe-based confocal laser endomicroscopy of the normal stomach

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Author contributions: Nonaka K and Ohata K designed the report; Ban S, Ichihara S and Takasugi R performed the pathological analyses; Minato Y, Tashima T, Matsuyama Y, Takita M and Matsuhashi N performed the examination; Nonaka K, Ban S and Neumann H analyzed the data and wrote the paper.

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Abstract

Probe-based confocal laser endomicroscopy (pCLE) is capable of acquiring *in vivo* magnified cross-section images of the gastric mucosa. Intravenous injection of fluorescein sodium is used for confocal imaging. However, it is still under debate if local administration of the dye to the mucosa is also effective for confocal imaging as it is not yet clear if topical application also reveals the intramucosal distribution of fluorescein. The objective of this study was to evaluate the intramucosal distribution of fluorescein sodium after topical application and to compare the distribution to the conventional intravenous injection used for confocal imaging. pCLE of the stomach uninfected with *Helicobacter pylori* was performed in a healthy male employing intravenous administration and local

mucosal application of fluorescein. The mucosa of the lower gastric body was biopsied 1 min and 5 min after intravenous administration or local mucosal application of fluorescein, and the distribution of fluorescein in the biopsy samples was examined histologically. Green fluorescence was already observed in the cytoplasm of fundic glandular cells in the biopsied deep mucosa 1 min after local mucosal application of fluorescein. It was also observed in the foveolar lumen and inter-foveolar lamina propria, although it was noted at only a few sites. In the tissue biopsied 5 min after the local mucosal application of fluorescein, green fluorescence was more frequently noted in the cytoplasm of fundic glandular cells than in that 1 min after the local mucosal application of fluorescein, although obvious green fluorescence was not identified in the foveolar lumen or inter-foveolar lamina propria. The distribution of intravenously administered fluorescein in the cytoplasm of fundic glandular cells was also clearly observed similarly to that after local mucosal application of fluorescein. Green fluorescence in more cells was observed in many cells 5 min after intravenous administration compared with that after 1 min. The presence of fluorescein in the mucosa was observed within a short time after local mucosal application of fluorescein, suggesting that pCLE images similarly to those after intravenous fluorescein administration can be acquired by local mucosal application of fluorescein.

Key words: Confocal laser endomicroscopy; Fluorescein; Local application; Intravenous; Distribution

Core tip: In this study, we demonstrated the presence of fluorescein administered by local mucosal application in the lamina propria. We consider this study valuable because it demonstrated that confocal laser endomicroscopic images can be acquired by local mucosal application of fluorescein. In addition, the fluorescein distributions after intravenous administration and local mucosal application were the same, which is also of interest.

Nonaka K, Ohata K, Ban S, Ichihara S, Takasugi R, Minato Y, Tashima T, Matsuyama Y, Takita M, Matsuhashi N, Neumann H. Histopathological confirmation of similar intramucosal distribution of fluorescein in both intravenous administration and local mucosal application for probe-based confocal laser endomicroscopy of the normal stomach. *World J Clin Cases* 2015; 3(12): 993-999 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i12/993.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i12.993

INTRODUCTION

Probe-based confocal laser endomicroscopy (pCLE) is a novel endoscopic procedure capable of observing in real time *in vivo* horizontal cross-section images of a fixed depth of the gastrointestinal mucosa with a magnifying power of 1000 times using a probe-type endoscope (Cellvizio; Mauna Kea Technology, Paris, France)[1,2]. To visualize gastrointestinal mucosal tissue by pCLE, staining with a fluorescent dye is necessary, and generally, images are acquired by detecting fluorescence of intravenously administered fluorescein[1-3]. It has been assumed that intravenously administered fluorescein visualizes the capillary vascular network in the superficial layer of the mucosa immediately after administration and leaks gradually into the lamina propria, resulting in visualization of the contour of the mucosal gland structure[3,4]. However, its dynamics has not been investigated in detail.

We have reported that pCLE images equivalent to those acquired employing intravenous fluorescein administration can be acquired by local mucosal application of fluorescein[5-7]. However, it has not been investigated whether or not pCLE images were acquired through the distribution of locally-applied fluorescein in the tissue similarly to that after intravenous administration.

In this study we examined whether the fluorescein locally applied on the gastric mucosal surface penetrates the mucosa tissue by histologically investigating the intramucosal distribution of fluorescein of the biopsy. In addition, it was compared with the distribution of intravenously administered fluorescein in the gastric mucosa to verify the validity of pCLE performed using local mucosal application of fluorescein.

CASE REPORT

Subject

The subject was a 39-year-old healthy male (author, Kouichi Nonaka). Before examination, written informed consent regarding gastrointestinal local mucosal application and intravenous administration of fluorescein was obtained. With respect to examination using pCLE and fluorescein administration, approval was obtained from the Ethics Review Board of our hospital.

pCLE and biopsy

Firstly, pCLE was performed without fluorescein administration in the subject, and one site of the probe-contacted mucosa in the lower gastric body was biopsied as a negative control.

For the local mucosal application of fluorescein, 0.1 mL of 10% fluorescein solution was prepared in advance and applied to the dye-spraying tube, so that the dye alternated with air (Figure 1A). Using the prepared dye-spraying tube, 2 drops of fluorescein were topically applied on the mucosa of the greater curvature of the lower gastric body of the subject, followed by pCLE (Figure 1B). One biopsy specimen of the applied mucosa was obtained after 1 min, and another specimen was biopsied from the same region 5 min after local mucosal application.

pCLE employing intravenous administration of 2.5 mL of 10% fluorescein solution was performed in the same healthy subject after 4 wk. One biopsy specimen was obtained from the mucosa of the greater curvature of the lower gastric body, which was the pCLE observation

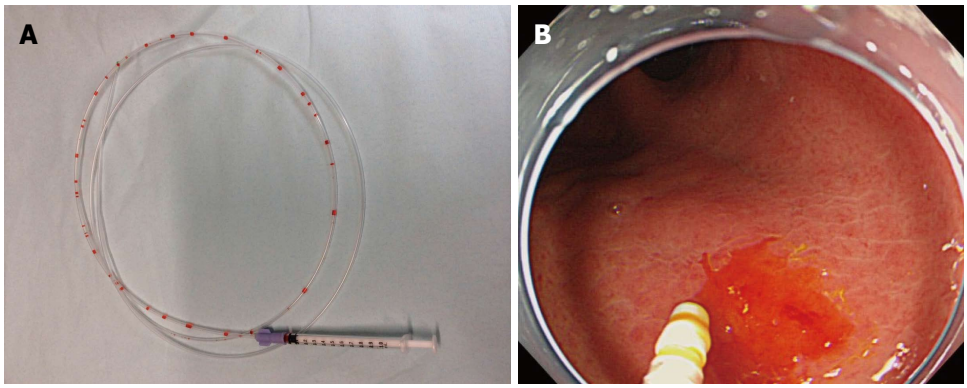

Figure 1 Local mucosal application of fluorescein for probe-based confocal laser endomicroscopy. A: Preparation for local mucosal application of fluorescein. 0.1 mL of 10% fluorescein solution is applied to the dye-spraying tube so that the dye alternates with air; B: Endoscopy findings immediately after local mucosal application of fluorescein onto the gastric body mucosa.

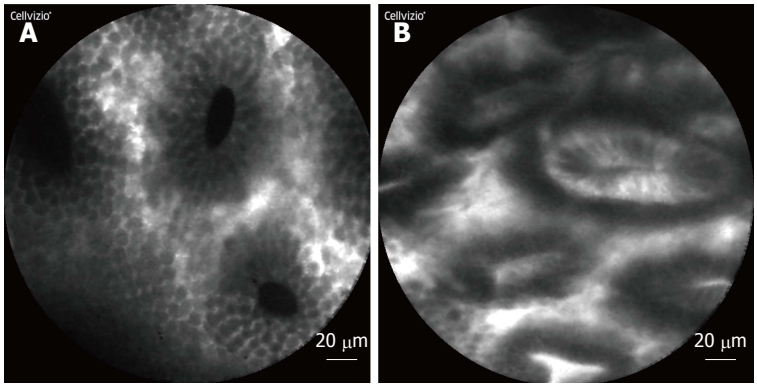

Figure 2 Probe-based confocal laser endomicroscopy images of normal fundic glands mucosa of the stomach. A: Image employing intravenous fluorescein administration. Regular round or oval foveolar lumina with homogeneous epithelial cells are visualized, showing dark images; B: Image employing the local mucosal application of fluorescein. Regular round or oval foveolar lumina with homogeneous epithelial cells are visualized, showing dark images of the foveolar epithelial cells and bright images of the foveolar lumina.

region, one minute after fluorescein administration. One biopsy specimen was similarly obtained from the mucosa of the same region 5 min after administration.

The 5 biopsy specimens of the gastric mucosa were histologically investigated.

Preparation of frozen sections and observation of fluorescence

Each biopsied specimen was embedded in a cryoembedding medium (Tissue-Tek Optical Cutting Temperature Compound; Sakura Finetek Japan, Tokyo, Japan) immediately after biopsy and fresh-frozen in liquid nitrogen. Using a cryomicrotome (CM1860UV; Leica Microsystems, Wetzlar, Germany), frozen sections with a thickness of approximately 5 μm were prepared.

The prepared frozen sections were dried with cold air and rapidly dipped in pure water and 100% ethanol to remove the embedding medium. The sections were dried again with cold air, covered with a cover glass after adding 5 μL of 4'-6-diamidine-2'-phenylindole dihydrochloride (DAPI) of the HER2 FISH KIT (J17539, JOKOH, Tokyo, Japan), and immediately observed under a fluorescence microscope (BIOREVO BZ9000; KEYENCE, Osaka, Japan) at 20 times objective lens magnification (200 times). Images of green fluorescence were acquired at an excitation wavelength of 470 nm, which were merged with images of nuclei derived from DAPI fluorescence.

Probe-based confocal laser endomicroscopy

Regular round/oval foveolar with homogeneous epithelial cells were visualized on pCLE by both intravenous fluorescein administration and local mucosal application of fluorescein (Figure 2). Foveolar epithelial cells were dark on pCLE of both methods. The foveolar lumen was dark in the intravenous administration whereas it was bright in local mucosal application of fluorescein. Although more clear images were obtained in the intravenous administration, images in the local mucosal application of fluorescein were also of sufficient quality for evaluation. No difference of pCLE images was noted between 1 min and 5 min after fluorescein administration both in the intravenous administration and in the local mucosal application.

Histological assessment

In the negative control, no green fluorescence regarded as derived from the administered fluorescein was observed.

In the mucosal tissue obtained one minute after local mucosal application of fluorescein (Figure 3), green fluorescence was observed in the cytoplasm of fundic glandular cells. It was also observed in the foveolar lumen and inter-foveolar lamina propria, although it was noted at only a few sites. Green fluorescence was more frequently noted in the cytoplasm of fundic glandular cells of the biopsy tissue obtained 5 min after local mucosal application of fluorescein compared with that obtained one minute after local mucosal application of fluorescein, although no fluorescence was observed in the foveolar lumen and inter-foveolar lamina propria (Figure 4).

Intravenously administered fluorescein was also

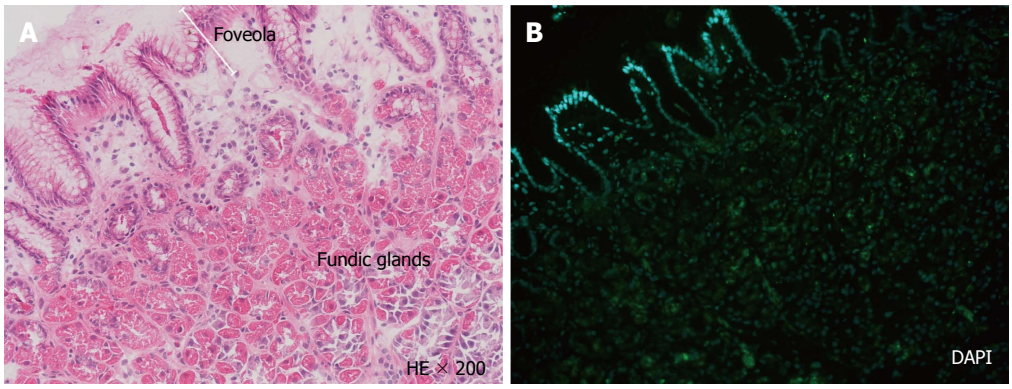

Figure 3 Histology one minute after local mucosal application of fluorescein. A: HE staining of biopsied tissue (original magnification × 200); B: Green fluorescence was observed in the cytoplasm of fundic glandular cells in the deep mucosa (non-HE staining; original magnification × 200). Although it was noted at only a few sites, green fluorescence was also observed in the glandular crypt lumens and lamina propria.

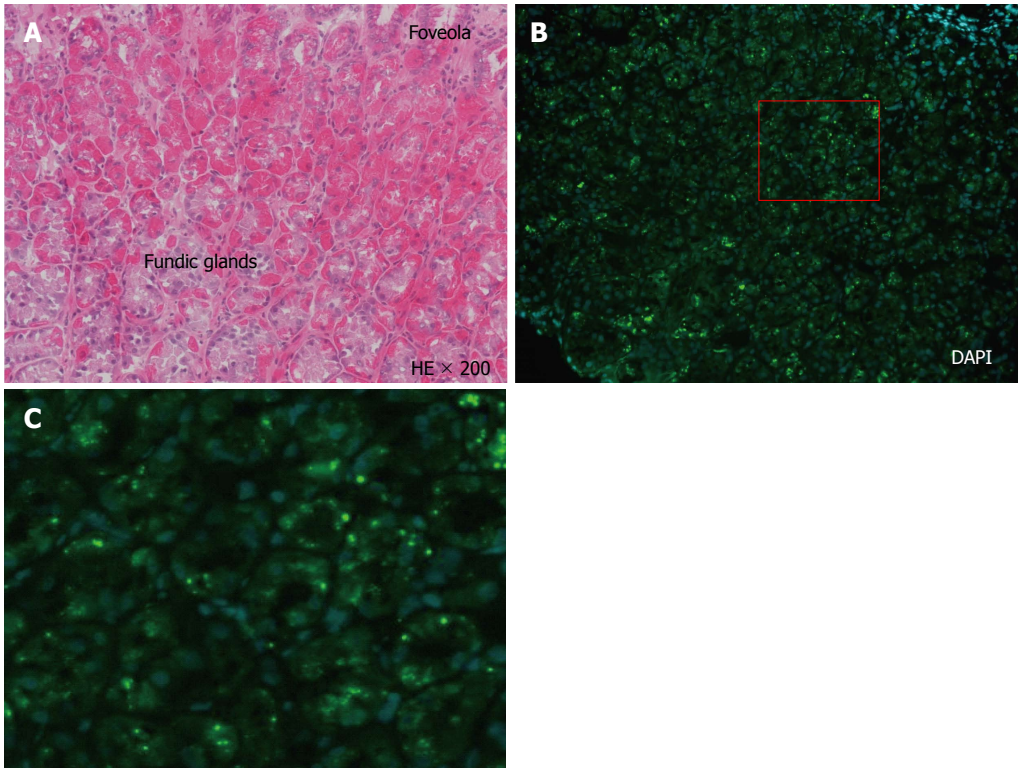

Figure 4 Histology 5 min after local mucosal application of fluorescein. A: HE staining of biopsied tissue (original magnification × 200); B: Fluorescence observed in the serial section of Figure 4A. Green fluorescence is observed in more fundic glandular cells compared with that at one minute (non-HE staining; original magnification × 200); C: The region in the red square is magnified. Green fluorescence is noted around the nuclei (light blue) of fundic glandular cells.

localized in the cytoplasm of fundic glandular cells, and fluorescence was more frequently noted in the biopsy tissue obtained 5 min after administration (Figure 5) than that obtained 1 min after administration (Figure 6), similar to the distribution after local mucosal application of fluorescein.

DISCUSSION

Confocal laser endomicroscopy has been spreading mainly in Europe, and its usefulness to differentiate cancer and non-cancer in the digestive tract and detect dysplasia, cancer in Barrett's esophagus, and inflammatory bowel disease has been reported over the last several years[8-16]. However, only a few studies on the distribution of fluorescein in the mucosa after intravenous administration[4,17], which is essential to acquire black-and-white images of confocal laser endomicroscopy, have been reported. Moreover, the penetration of fluorescein locally applied on the mucosa from the superficial layer has not previously been elucidated, which we investigated in this study.

In this study, in both fluorescein local mucosal application and intravenous administration, the fluorescence was observed in fundic glandular cells showing similar distribution, which suggests that the fluorescein locally

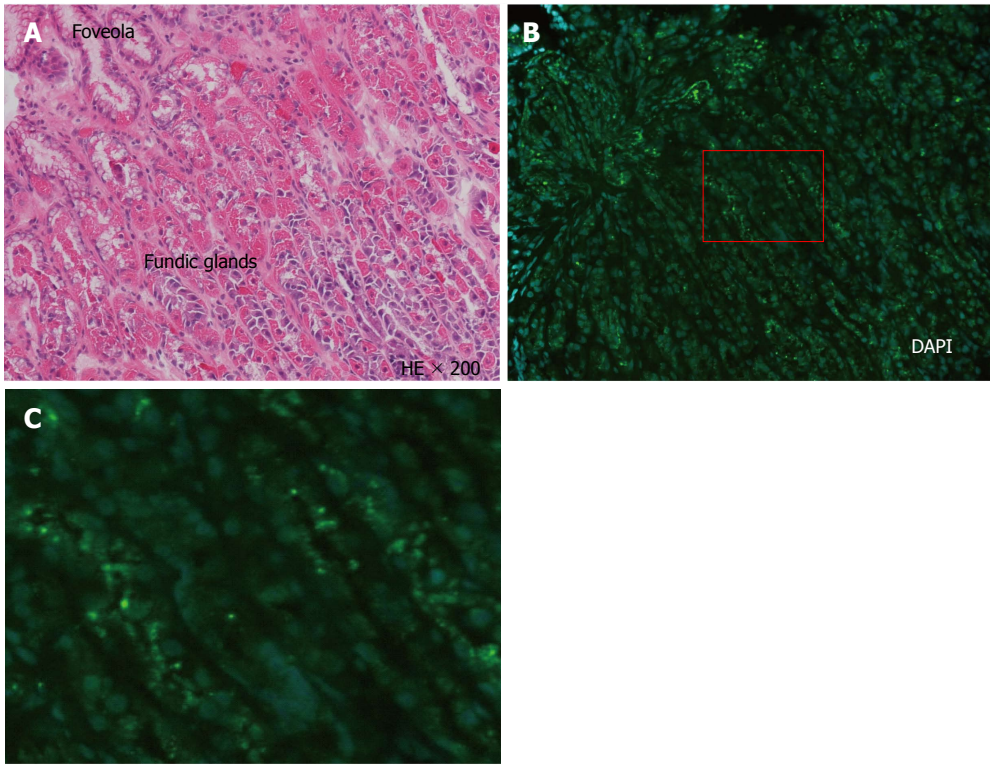

Figure 5 Histology 5 min after intravenous fluorescein administration. A: HE staining of biopsied tissue (original magnification × 200); B: Fluorescence observed in the serial section of Figure 5A. Green fluorescence in the cytoplasm of fundic glandular cells is more clearly observed compared with that at one minute (non-HE staining; original magnification × 200); C: The region in the red square is magnified. Green fluorescence is noted around the nuclei (light blue) of fundic glandular cells, suggesting that fluorescein was incorporated into the cytoplasm of the cells.

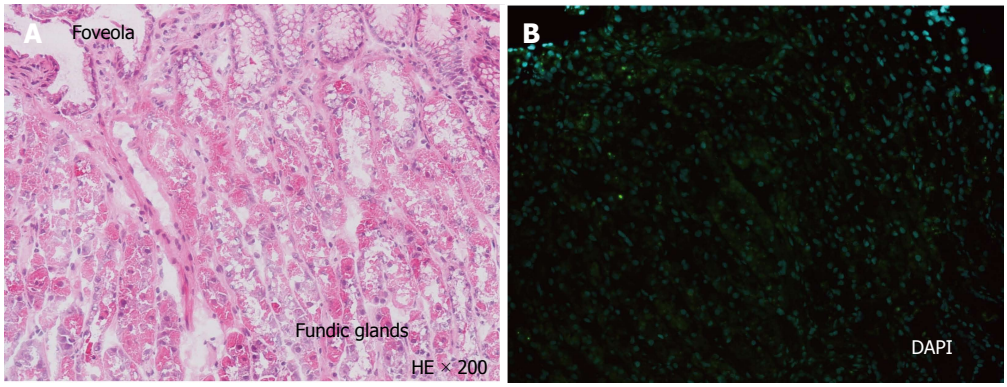

Figure 6 Histology one minute after intravenous fluorescein administration. A: HE staining of biopsied tissue (original magnification × 200); B: Fluorescence observed in the serial section of Figure 6A. Green fluorescence is scattered in the cytoplasm of fundic glandular cells (non-HE staining; original magnification × 200).

applied on the mucosal surface can be incorporated into the mucosal tissue within a short time as with the case for intravenous administration, supporting the validity to pCLE images using local mucosal application of fluorescein.

However, the region observed on pCLE is the foveolar area within the depth of 100 μm from the surface of the mucosa. In this region, just a small amount of fluorescence was observed in the foveolar lumen and inter-foveolar lamina propria of the biopsy sample obtained one minute after local mucosal application of fluorescein. When the air-dried frozen sections were observed without removing the cryoembedding medium in our preliminary experiment, intense fluorescence was noted around the tissue, which made it difficult to identify fluorescence in the tissue (Figure 7). Considering that fluorescein might readily dissolve in the cryoembedding medium (aqueous solution containing polyethylene glycol), the procedure was modified: the sections were dried with cold air and then rapidly dipped in pure water and 100% ethanol to remove the embedding medium surrounding the tissue. Although trying to remove the medium as rapidly as possible, it was plausible that fluorescein was eluted from the tissue while being passed through the solvents (pure water and alcohol).

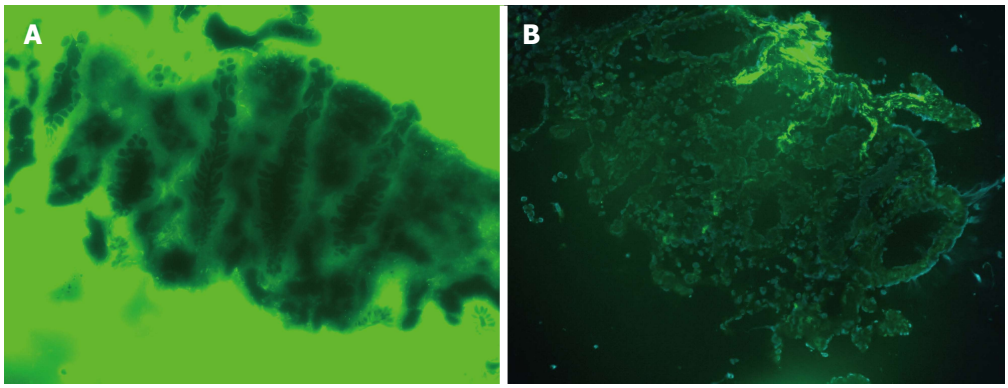

Figure 7 Fluorescence observed in tissue sections with or without removal of the cryoembedding medium. A: Lower gastric body mucosa tissue obtained 5 min after local mucosal application of fluorescein. When fluorescence is observed without removing the embedding medium, intense green fluorescence is noted around the tissue, and it is difficult to observe fluorescence in the tissue; B: Fluorescence in the tissue can be observed clearly after removing the embedding medium.

In addition, the procedure was performed in a room under fluorescent light, which possibly gave rise to additional attenuation of the fluorescence with time. The larger amount of fluorescein might have been present in the tissue before the procedure, and the detection of fluorescence at a low level in the foveolar area may be due to this condition.

Although the level was low, some fluorescence was observed in the foveolar lumen and inter-foveolar lamina propria, which corresponded to bright images of the foveolar lumens on pCLE after local mucosal application of fluorescein and suggested that fluorescein locally applied on the mucosal surface was incorporated into the lamina propria. In contrast, no fluorescence indicating the incorporation of fluorescein into the foveolar epithelium was confirmed, and this may correspond to dark images of foveolar epithelium on pCLE. Ji *et al*[17] reported that gastric epithelium with intestinal metaplasia was positive for fluorescein immunostaining, suggesting that fluorescein is incorporated into the epithelium, but goblet cells were negative. Fluorescein may not be readily incorporated into the foveolar epithelial cells, which have abundant intracytoplasmic mucus similar to goblet cells.

According to the study by Ji *et al*[17], the intercellular permeability of intact foveolar epithelia was low. However, if fluorescein is unlikely to be incorporated into the cytoplasm of foveolar epithelial cells, the locally applied fluorescein is may diffuse into the lamina propria through intercellular spaces of the foveolar epithelia, but it is also possible that it diffuses through the cell membrane and cytoplasm, and reaches the lamina propria without accumulating in the foveolar epithelia. Fluorescein for medical use is water-soluble sodium salt with a molecular weight of 376.27 and a negative charge. The cell membrane is mostly composed of a phospholipid bilayer containing various proteins and glycolipids. Since fluorescein sodium salt is negatively charged as are phospholipids of the cell membrane, repellence of the charge prevents the fluorescein from entering the cell membrane. However, fluorescein has both a hydrophilic region (negatively charged region) and a hydrophobic region corresponding to the lipid fraction, as the lipid bilayer of the cell membrane. This hydrophilic-hydrophobic balance is similar to that of the cell membrane lipid bilayer, which may enable fluorescein to pass through the cell membrane. On the other hand, regarding fundic glandular cells, it was suggested that the fluorescein does not simply pass through the cells, but also is actively incorporated into the cells from the lamina propria or gland lumen side and accumulates in the cells.

This study was performed with only normal gastric mucosa in one subject, so further investigation is necessary. However, it was confirmed that fluorescein locally applied on the gastric mucosal surface was distributed in the mucosa within a short time, validating that pCLE images can be acquired by local mucosal application of fluorescein.

COMMENTS

Case characteristics

A 39-year-old healthy male with no symptoms.

Laboratory diagnosis

All labs were within normal limits.

Imaging diagnosis

Regular round/oval foveolar with homogeneous epithelial cells were visualized on probe-based confocal laser endomicroscopy (pCLE) by both intravenous fluorescein administration and local mucosal application of fluorescein.

Pathological diagnosis

In the mucosal tissue obtained after local mucosal application of fluorescein, green fluorescence was observed in the cytoplasm of fundic glandular cells.

Related reports

Previously it was considered that CLE imaging requires intravenous injection of fluorescein. However, the acquisition of images just by applying a small volume of fluorescein onto the region of interest has been reported recently.

Term explanation

pCLE is a novel endoscopic procedure capable of observing gastrointestinal mucosa at 1000 times magnification in real time.

Experiences and lessons

It was confirmed that fluorescein locally applied on the gastric mucosal surface was distributed in the mucosa within a short time.

Peer-review

The presence of fluorescein in the mucosa was observed after local mucosal application of fluorescein, suggesting that pCLE images similarly to those after intravenous fluorescein administration can be acquired by local mucosal application of fluorescein.

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CASE REPORT

Colovesical fistula caused by glucocorticoid therapy for IgG4-related intrapelvic mass

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Author contributions: Yabuuchi Y and Matsubayashi H participated in writing the case report and revising the draft; Matsubayashi H, Matsuzaki M, Shiomi A, Moriguchi M and Kawamura I were engaged in his treatment; Ito I helped in the pathological diagnosis; Ono H helped to supervise and approve the final manuscript.

Institutional review board statement: Institutional Review Board of Shizuoka Cancer Center ethically approved current case report (Institutional code number: 25-J123-25-1-3).

Informed consent statement: The patient provided informed written consent prior to this study.

Conflict-of-interest statement: All authors disclose no conflict.

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Abstract

IgG4-related disease (IgG4-RD) is an immune-mediated fibroinflammatory disorder that can occur in almost all systemic organs and generally responds to corticosteroid treatment. We report a rare case of an IgG4-related intrapelvic mass lesion that responded to steroid therapy but caused a fistula between the sigmoid colon and bladder. A 71-year-old man was followed after treatment for hepatocellular carcinoma. Follow-up computed tomography (CT) incidentally depicted left hydronephrosis with an ill-demarcated intrapelvic mass lesion. This lesion was histologically diagnosed as IgG4-RD by open biopsy, and peroral steroid therapy was initiated. One month after starting steroids, a colovesical fistula was detected by follow-up CT. A colostomy and urethral catheterization were emergently performed. The patient recovered and the mass lesion was drastically minimized by the initiation of glucocorticoids; however, he still needs urethral catheterization. IgG4-RD develops in various systemic organs and generally responds well to steroids. Clinicians must be watchful for the complications of responses to corticosteroids, such as fistulization, when the mass lesion of IgG4-RD is adjacent to multiple luminal organs.

Key words: IgG4-related disease; Intrapelvic mass; Steroid therapy; Colovesical fistula; Colostomy; Urethral catheterization

Core tip: IgG4-related disease (IgG4-RD) is an immune-mediated fibroinflammatory condition that is effectively treated with glucocorticoids. Progression of this disease may cause serious complications or several symptoms; hence, glucocorticoid therapy is often inevitable. To date, critical adverse events caused by steroid treatment for IgG4-RD have seldom been reported. The current case report describes a rare, but severe, adverse event of colovesical fistula following a good response to glucocorticoids.

Yabuuchi Y, Matsubayashi H, Matsuzaki M, Shiomi A, Moriguchi M, Kawamura I, Ito I, Ono H. Colovesical fistula caused by glucocorticoid therapy for IgG4-related intrapelvic mass. *World J Clin Cases* 2015; 3(12): 1000-1004 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i12/1000.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i12.1000

INTRODUCTION

IgG4-related disease (IgG4-RD) is recognized as an autoimmune disorder that can affect multiple systemic organs through the formation of fibroproliferative and/or tumefactive lesions. Today, the disease concept and diagnostic criteria of IgG4-RD are globally widespread, and opportunities to diagnose and treat this disease are increasing. Patients with active IgG4-RD tend to demonstrate multiple systemic lesions[1,2], and therefore, often meet the indications for glucocorticoid therapy. Glucocorticoid is effective for most IgG4-related lesions; however, the period before a response to steroid is observed may vary depending on the affected organ or the degree of fibrosis[3]. A response is usually recognized in two weeks to a month following the initiation of steroids[1,4].

In general, corticosteroids improve the lesions or symptoms associated with IgG4-RD, while concerns regarding adverse effects of glucocorticoid therapy are well known and limited. We herewith report a patient who developed a colovesical fistula caused by glucocorticoid therapy to treat an IgG4-related intrapelvic mass and subsequently required surgical treatment.

CASE REPORT

The patient, a 71-year-old man with a history of hepatitis C and hepatocellular carcinoma, had been treated with interferon, percutaneous ethanol injection, transcatheter arterial chemoembolization, and radio frequent ablation, and had been continuously followed up with clinical images. Follow-up computed tomography (CT) incidentally revealed left hydronephrosis and an ill-demarcated mass lesion extending to the left lower ureter, left margin of the bladder, and sigmoid colon (Figure 1). ^{18}F-fluorodeoxyglucose positron emission tomography demonstrated strong uptake around the left lower ureter (SUV max: 19.0) (Figure 2). Colonoscopy showed a severe extrinsic compression at the sigmoid colon (Figure 3). Cystoscopy demonstrated whitish edematous protrusions at the upper left area of the bladder but the left ureteral orifice was intact. Under the suspected diagnosis of left-lower ureter cancer invading the adjacent organs, transurethral resection was performed for a histological diagnosis. Histology of the TUR specimens revealed intraepithelial papillary urothelial carcinoma (G1, low grade, pTa), which was not invasive, contradicting our previous diagnosis. An open biopsy was done for a conclusive diagnosis of the intrapelvic mass lesion. Histology of the biopsied material did not show cancerous tissue, but dense fibrous tissue with abundant lymphoplasmacyte infiltration. Immunohistochemistry by using mouse anti-human IgG4 monoclonal antibody in enzyme immunoassay revealed $\geqslant$ 10 IgG4 positive cells per high-power field (Figure 4), although the ratio

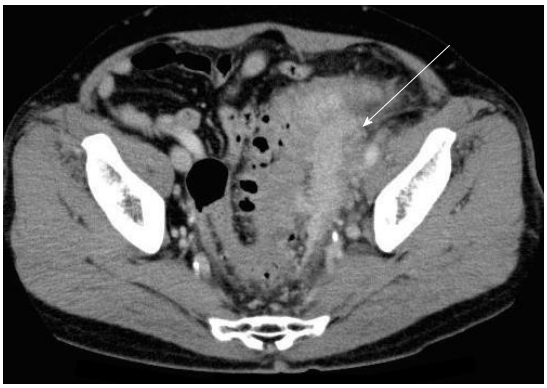

Figure 1 Follow-up computed tomography. Computed tomography shows an ill-demarcated intrapelvic mass lesion extending to the left lower ureter, left margin of the bladder, and sigmoid colon, as denoted by arrow.

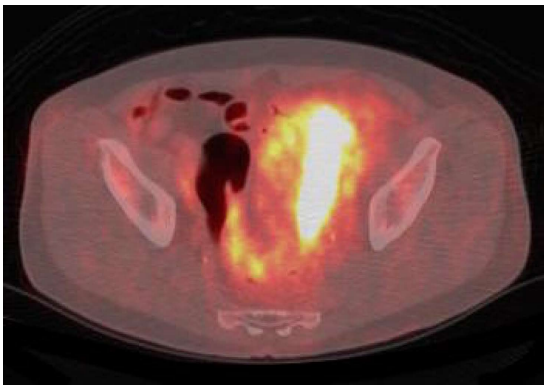

Figure 2 ^{18}F-fluorodeoxyglucose positron emission tomography for screening. ^{18}F-fluorodeoxyglucose positron emission tomography shows strong uptake around the left lower ureter.

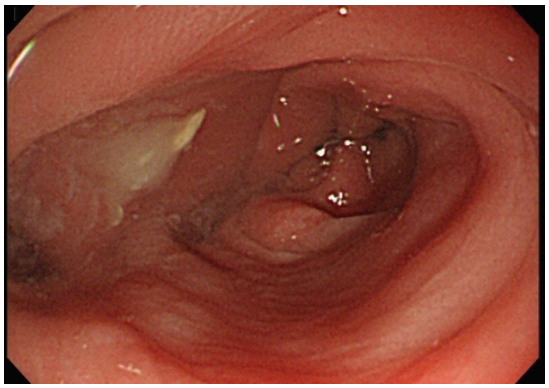

Figure 3 Colonoscopy for screening. Colonoscopy shows a severe extrinsic compression.

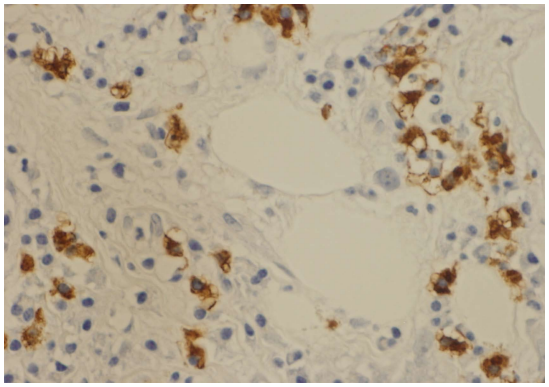

Figure 4 Immunohistochemical analysis of resected specimen. More than 10 IgG4 positive cells are distributed per high-power field.

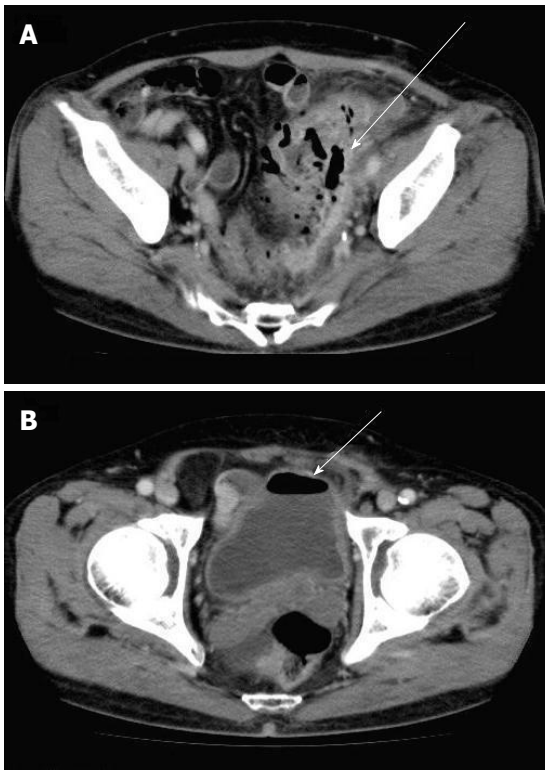

Figure 5 Computed tomography one month after the initiation of glucocorticoid therapy. Computed tomography shows shrinkage of the mass and extraluminal air in the mass (A) and in the bladder (B), as denoted by arrow.

of IgG4/IgG positive cells was < 40%. Serum IgG4 was then examined and found elevated (190 mg/dL, normal range: 4.8-105 mg/dL). With these results, we diagnosed "Possible IgG4-RD", based on the comprehensive diagnosis criteria[5]. Glucocorticoid therapy was initiated with 40 mg/d of prednisolone and tapered in the standard pitch[6] to treat the hydronephrosis due to the IgG4-related mass lesion and subsequently recover renal function.

One month later, the patient visited the hospital for evaluation of his steroid response, but he had a continued moderate fever, diarrhea, and urine with a feces odor for a few days. CT demonstrated shrinkage of the intrapelvic mass lesion, as well as extraluminal air in the mass lesion and bladder (Figure 5). The laboratory findings showed leukocytosis (white blood cell: 21480/μL), thrombocytopenia (platelet: 3.4 × 10^4/μL), elevated C-reactive protein (14.87 mg/dL), and renal dysfunction (creatinine: 1.64 mg/dL). Large quantities of bacteria (*Escherichia coli* and *Enterococcus faecalis*, *etc.*) were detected in urine cultures, and fistulization between the urinary bladder and sigmoid colon with enteric bacterial infection of the mass lesion was confirmed. A percutaneous drainage was placed into the infected mass lesion, and colostomy was performed with urethral catheterization and antibiotic administration as emergent management. The patient gradually recovered and was discharged in two weeks. He has been in good health subsequently, and the intrapelvic mass has remained minimized with a maintenance dose of prednisolone (Figure 6). However, he has sometimes experienced urinary incontinence from the anus when the urethral catheter is occluded.

Eight months after steroid initiation with 5 mg/d of prednisolone, the screening cystoscopy revealed a polypoid lesion near the fistula. The recurrence of superficial bladder cancer was ruled out by a repeat TUR. This histology and response to glucocorticoid provided diagnostic confirmation[3,5,7]. Today, the remaining fistula is still visible with a cystoscope, so that the urethral catheter cannot be withdrawn and the artificial anus has been unclosed for nearly two years.

DISCUSSION

Standard therapy for IgG4-RD is peroral steroid therapy in the form of prednisolone initiated at a dose of 0.6 mg/kg with subsequent tapering[3,6,7]. Glucocorticoids are highly effective in treating IgG4-RD and a response to steroids can be seen in most systemic lesions within 2-4 wk as determined by clinical images[1,6]. The current case also responded well to standard glucocorticoid therapy; however, this response caused a fistula between the urinary bladder and sigmoid colon.

To date, severe complications have been described in

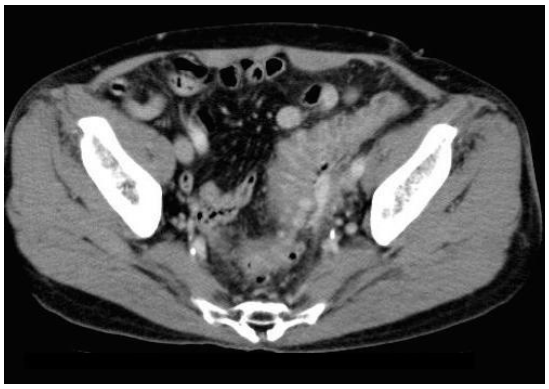

Figure 6 Computed tomography 6 mo after the initiation of glucocorticoid therapy. Computed tomography shows that the mass remains minimized.

many cases with IgG4-RD; however, these are related to the occurrence or progression of the disease, rather than adverse steroid responses. For example, complications of IgG4-RD have included clinical emergencies such as leptomeningitis with rapidly progressive cognitive decline, gastric-pericardial fistula, aortoduodenal fistula, stenosis of the coronary arteries, stenosis of the colon, and splenic haemorrhage[8-13]. A case of IgG4-related inflammatory pseudotumor of the urinary bladder causing a colovesical fistula, similar to the present case, has also been reported[14]. However, these complications are all caused by the progression of the disease and are not results of glucocorticoid therapy. To the best of our knowledge, based on a PubMed keyword survey, this is the first report of a severe complication due to a good response to corticosteroid therapy in a case of IgG4-RD.

A colovesical fistula can occur in various diseases. Common causes are colonic diverticula, inflammatory bowel disease, and carcinomas of the colon, urological, and gynecological organs. A gastrointestinal foreign body, trauma, and iatrogenic injuries are rare causes[15]. A colovesical fistula usually develops as a consequence of the progression of these diseases; however, fistulization rarely occurs in cases of malignancy treated with chemotherapy or radiation therapy[16]. Fistulas are thought to arise simply due to more rapid shrinkage and necrosis of the tumor compared with slow repair of the interstitial tissue. A similar explanation is speculated for the fistula formation observed in the current case.

Predicting the risk of the fistulization is difficult in cases with IgG4-RD. In the present case, the mass lesion was adjacent to multiple luminal organs. This condition might be one of the risk factors that led to fistulization. A widespread area affected by the stromal tissue, the level of inflammatory cell infiltration, and colonic diverticula might be also the risk factors. Earlier detection could increase the chance of a cure or prevention of severe complications.

After the initiation of steroids, we examined abdominal ultrasonography at two weeks and CT at one month, as the response to glucocorticoid can be usually obtained within this timeframe in cases with IgG4-RD[1,6]. Although long-term risk of complications by the accumulated radiation dose is a concern, CT is a suitable modality for the evaluation of the steroid response, colovesical fistula, and adjacent anatomical structures[17]. During the early period after the initiation of glucocorticoids, the IgG4-related mass lesions located at rare sites must be followed using a suitable image modality.

In conclusion, fistulization between the bladder and sigmoid colon occurred after glucocorticoid therapy in a case with IgG4-RD. An intrapelvic mass adjacent to multiple luminal organs, a wide range of spread of the mass lesion and the good response to glucocorticoids may have caused this severe complication. Early follow-up imaging as well as blood examination is necessary for the evaluation of the steroid response as well as the detection of complications arising in response to glucocorticoid treatment.

COMMENTS

Case characteristics

One month after the initiation of glucocorticoid therapy for IgG4-related disease, a 71-year-old man had a continued moderate fever, diarrhea, and urine with a feces odor for a few days.

Clinical diagnosis

The patient slightly had lower abdominal pain.

Differential diagnosis

Fistulization between the bladder and colon due to colonic diverticulitis, inflammatory bowel disease and carcinomas of the colon or urological organs.

Laboratory diagnosis

The laboratory findings showed leukocytosis, thrombocytopenia, elevated C-reactive protein and renal dysfunction, with large quantities of bacteria in urine cultures.

Imaging diagnosis

Computed tomography demonstrated shrinkage of the intrapelvic mass lesion, as well as extraluminal air in the mass lesion and bladder.

Treatment

A percutaneous drainage was placed into the infected mass lesion, and colostomy was performed with urethral catheterization and antibiotic administration.

Related reports

Fistulization have been described in some cases with IgG4-related disease, such as gastric-pericardial fistula and aortoduodenal fistula. However these are related to the progression of the disease.

Experiences and lessons

This case was a rare complication of IgG4-related disease due to the good response to glucocorticoid therapy. Early follow-up imaging is necessary for the evaluation of the steroid response as well as the detection of complications arising in response to glucocorticoid treatment.

Peer-review

This case report is interesting and well documented.

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CASE REPORT

Probable case of drug reaction with eosinophilia and systemic symptom syndrome due to combination therapy with daclatasvir and asunaprevir

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Author contributions: Suga T collected, analyzed and interpreted the data; Sato K drafted the article and analyzed and interpreted the data; Yamazaki Y, Ohyama T, Horiguchi N, Kakizaki S and Kusano M analyzed the data; Yamada M approved the final version of the manuscript.

Institutional review board statement: Institutional review board in our institute does not require more than obtaining written informed consent regarding "Case report".

Informed consent statement: We obtained written informed consent.

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Abstract

A 66-year-old, interferon-ineligible, treatment-naive man who was diagnosed with chronic hepatitis C due to hepatitis C virus genotype 1b began combination therapy with daclatasvir and asunaprevir. On day 14 of treatment, hepatic reserve and renal function deterioration was observed, while his transaminase levels were normal. Both daclatasvir and asunaprevir were discontinued on day 18 of treatment, because the patient complained of dark urine and a rash on his trunk and four limbs. After discontinuing antiviral therapy, the abnormal laboratory finding and clinical manifestations gradually improved, without recurrence. Our case fulfilled the diagnostic criteria of probable drug reaction with eosinophilia and systemic symptom (DRESS) syndrome. Despite the 18-d treatment, sustained virological response 12 was achieved. Based on the clinical course, we concluded that there was a clear cause-and-effect relationship between the treatment and adverse events. To our knowledge, this patient represents the first case of probable DRESS syndrome that includes concomitant deterioration of hepatic reserve and renal function due to combination therapy with daclatasvir and asunaprevir, regardless of normalization of transaminase levels. Our case suggests that we should pay attention not only to the transaminase levels but also to allergic symptoms associated with organ involvement during combination therapy with daclatasvir

and asunaprevir.

Key words: Hepatitis C; Daclatasvir; Renal dysfunction; Asunaprevir; Hepatic reserve deterioration

Core tip: Oral combination therapy with daclatasvir and asunaprevir for chronic hepatitis C demonstrates a relatively favorable safety profile. Although the incidence of hyperbilirubinemia, hypoalbuminemia, and a decreased prothrombin activity have been reported, as well as renal damage, concomitant deterioration of hepatic reserve and renal function without transaminase elevations have not been reported. We observed probable drug reaction with eosinophilia and systemic symptom syndrome, including concomitant deterioration of hepatic reserve and renal function due to the combination therapy, regardless of normalized transaminase levels. Thus, our case highlights the importance of paying attention not only to transaminase levels but also to the allergic symptoms associated with organ involvement during combination therapy.

Suga T, Sato K, Yamazaki Y, Ohyama T, Horiguchi N, Kakizaki S, Kusano M, Yamada M. Probable case of drug reaction with eosinophilia and systemic symptom syndrome due to combination therapy with daclatasvir and asunaprevir. *World J Clin Cases* 2015; 3(12): 1005-1010 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i12/1005.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i12.1005

INTRODUCTION

Combination therapy with daclatasvir and asunaprevir has been approved as oral antiviral therapy for chronic hepatitis C due to hepatitis C virus (HCV) genotype 1b. This new type of therapy resulted in a sustained virological response (SVR) rate of over 80% after 24 wk of therapy for chronic hepatitis C patients with genotype 1b[1,2]. In Japan, combination therapy with daclatasvir and asunaprevir has been recommended for interferon-ineligible patients based on the Japan Society of Hepatology guidelines for managing HCV infection[3].

One of the most frequent adverse events during combination therapy with daclatasvir and asunaprevir is elevated alanine aminotransferase (ALT) levels. In an open-label trial of oral antiviral therapy conducted in Japan[1,4,5], ALT elevations occurred in 17.6% of the patients treated with a combination of daclatasvir and asunaprevir. The incidence of hyperbilirubinemia, hypoalbuminemia or prothrombin activity decrease, which reflect deteriorated hepatic reserve[6-8], were 3.9%, 1.2% or 0.8%, respectively[1,4,5]. The incidence of renal damage was 0.4%[1,4,5]. However, concomitant deterioration of hepatic reserve and renal function without transaminase elevations has not been reported. Here, we report a case of chronic hepatitis C with concomitant deterioration of hepatic reserve and renal function that fulfilled the diagnostic criteria of probable drug reaction with eosinophilia and systemic symptom (DRESS) syndrome[9] due to combination therapy with daclatasvir and asunaprevir. We got written informed consent from the patient in advance.

CASE REPORT

A 66-year-old man, diagnosed with chronic hepatitis C (genotype 1b) at the age of 50, was started on a scheduled 24-wk course of daclatasvir and asunaprevir for elevated transaminase levels. The patient suffered from a duodenal ulcer, bronchial asthma, hypertension and cerebral hemorrhage. He had undergone antihypertensive therapy with nifedipine and olmesartan for 5 years. These drugs were maintained throughout the antiviral treatment. No relevant family history was noted. The patient did not have a past history or predisposing factors for liver disease, except for chronic hepatitis C. He had no history of drug allergies, although he did have bronchial asthma. Combination therapy with daclatasvir (60 mg once daily) and asunaprevir (100 mg twice daily) was selected because the patient was both interferon-ineligible and interferon-naïve due to his past history of hypertension and cerebral hemorrhage. A V170I mutation in the NS3 lesion was detected by direct sequencing prior to the therapy. Before beginning therapy, the following measurements were noted: serum ALT, 57 IU/L; total bilirubin, 0.5 mg/dL; albumin, 4.1 g/dL; creatinine, 0.7 mg/dL; prothrombin activity, 102%; total white cell count, 5000/μL (eosinophils 150/μL); platelet count, 11.1 × 10^4/μL; C-reactive protein, < 0.1 mg/dL, and HCV RNA, 5.9 Log IU/mL.

On day 14 of treatment, the patient's laboratory findings showed concomitant deterioration of hepatic reserve and renal function, without any subjective symptoms. Although the ALT level had decreased to 22 IU/mL, serum total bilirubin had worsened to 1.9 mg/dL, albumin to 3.3 g/dL, creatinine to 1.4 mg/dL, prothrombin activity to 66.4%, total white cell count to 12130/μL (eosinophils 121/μL), platelet count to 7.9 × 10^4/μL and C-reactive protein to 3.5 mg/dL (Table 1). Abdominal ultrasonography revealed splenomegaly, which was not present before the therapy. There was no evidence of biliary obstruction. Daclatasvir and asunaprevir were continued. The patient complained of headache, dark urine and a partly fused erythematous rash on his trunk and four limbs on day 18 of treatment. In view of the skin lesions, a dermatologist diagnosed the skin rash as a drug eruption or untypical urticarial lesion (Figure 1), although the patient's vital signs were normal: temperature 36.5 ℃, blood pressure 121/58 mmHg and heart rate 72/min. Both daclatasvir and asunaprevir were discontinued based on the dermatologist's advice and the patient's request, and an antihistamine was administered for 2 wk. When observed on day 21 of the initiation of treatment,

Table 1 Laboratory findings before antiviral therapy and at the onset of adverse events

On day 14 of antiviral therapy				Virological analysis before antiviral therapy	
Hematology		Virus markers		Virus marker	
WBC (μL)	12130	IgM-HAV Ab	(-)	HCV RNA (Log IU/mL)	5.9
Neutrophils (%)	82	HBs Ag	(-)		
Lymphocytes (%)	10	IgM-HBc Ab	(-)	Drug resistant variants	
Monocytes (%)	6	HBV DNA	(-)	NS3/V36A	(-)
Eosinophils (%)	1	IgA-HEV Ab	(-)	NS3/T54A	(-)
RBC (μL)	4000000	IgM-HSV Ab	(-)	NS3/T54S	(-)
Hb (g/dL)	13.2	IgG-HSV Ab	(+)	NS3/Q80L	(-)
Plt (μL)	79000	IgM-HHV6 Ab	(-)	NS3/Q80R	(-)
		IgG-HHV6 Ab	(+)	NS3/R155K	(-)
Blood chemistry		IgM-CMV Ab	(-)	NS3/R155Q	(-)
TP (g/dL)	7.4	IgG-CMV Ab	(+)	NS3/R155T	(-)
Albumin (g/dL)	3.3	IgM-EBV VCA Ab	(-)	NS3/A156S	(-)
T-Bil (mg/dL)	1.9	IgG-EBV VCA Ab	(+)	NS3/A156T	(-)
D-Bil (mg/dL)	0.7	EBNA Ab	(+)	NS3/A156V	(-)
AST (IU/L)	15			NS3/D168A	(-)
ALT (IU/L)	22	Endocrine		NS3/D168E	(±)[2]
ALP (IU/L)	284	TSH (μIU/mL)	1.7	NS3/D168H	(-)
γ-GTP (IU/L)	33	fT3 (pg/mL)	2.81	NS3/D168T	(-)
LDH (IU/L)	147	fT4 (ng/dL)	1.1	NS3/D168V	(-)
ChE (IU/L)	257			NS3/V170I	(+)[2]
T. Chol (mg/dL)	126	Serology		NS5A/L31F	(-)
BUN (mg/dL)	28	IgA (mg/dL)	130	NS5A/L31M	(-)
Cr (mg/dL)	1.4	IgE (mg/dL)	526	NS5A/L31V	(-)
UA (mg/dL)	5.4	IgG (mg/dL)	2225	NS5A/Y93H	(±)[2]
CPK (IU/L)	60	IgM (mg/dL)	104		
CRP (mg/dL)	3.5	RF	(-)		
BNP (pg/mL)	20.1	ANA	× 40 (+)		
Fe (μg/dL)	123	AMA	(-)		
Ferritin (ng/mL)	115	ASMA	(-)		
Hyaluronic acid[1] (ng/mL)	179				
Collagen-4[1] (ng/mL)	298	Tumor markers			
		PIVKA-2 (mAU/mL)	19		
Blood coagulation		AFP (ng/mL)	6.6		
PT (%)	66.4				
PT-INR	1.22	DLST			
		Daclatasvir	(-)		
		Asunaprevir	(-)		

[1]Hyaluronic acid and collagen-4 were measured before antiviral therapy. Drug resistant variants in the NS3 and NS5A regions were measured by invader method; [2]D168E and Y93H mutations were not detected by direct sequencing. Only V170I was detected by direct sequencing. γ-GTP: γ-glutamyl transpeptidase; Ab: Antibody; AFP: α-fetoprotein; Ag: Antigen; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AMA: Antimitochondrial antibody; ANA: Antinuclear antibody; ASMA: Antismooth muscle antibody; AST: Aspartate aminotransferase; BNP: Brain natriuretic polypeptide; BUN: Blood urea nitrogen; ChE: Cholinesterase; CMV: Cytomegalovirus; CPK: Creatine phospho kinase; CRP: C-reactive protein; D-Bil: Direct bilirubin; DLST: Drug-induced lymphocyte stimulation test; EBNA: Epstein–Barr virus nuclear antigen; EBV: Epstein-Barr virus; fT3: Free triiodothyronine; fT4: Free thyroxine; HAV: Hepatitis A virus; Hb: Hemoglobin; HBc: Hepatitis B virus core; HBs: Hepatitis B virus surface; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HEV: Hepatitis E virus; HHV: Human herpes virus; HSV: Herpes simplex virus; Ig: Immunoglobulin; INR: International normalized ratio; LDH: Lactic dehydrogenase; PIVKA: Protein induced by vitamin K absence or antagonist; Plt: Platelets; PT: Prothrombin time; RBC: Red blood cell; RF: Rheumatoid factor; T-Bil: Total bilirubin; T.Chol: Total cholesterol; TP: Total protein; TSH: Thyroid stimulating hormone; UA: Uric acid; WBC: White blood cell.

the clinical manifestations had disappeared, and the laboratory findings had returned to normal ranges, except for the serum albumin (3.4 g/dL), prothrombin activity (74.5%) and eosinophilia (2673/μL) levels. These measurements also returned to normal ranges, until day 35 of the initiation of treatment. On day 42 of the initiation of treatment, molecular biological analysis detected Epstein-Barr virus (EBV) DNA but not human herpesvirus (HHV)-6 and HHV-7 DNA in the serum. Although daclatasvir and asunaprevir were discontinued on day 18 of treatment, SVR 12 was achieved (serum HCV RNA was negative after 12 wk of treatment). The clinical course of our case and the time course for the critical laboratory values are shown in Figure 2 and Table 2, respectively.

DISCUSSION

Here, we described a case of deteriorated hepatic reserve and renal function during combination therapy with daclatasvir and asunaprevir, regardless of normalized transaminase levels at the development of the symptoms.

Table 2 Time course of critical laboratory values

	Before antiviral therapy	Day 14 of treatment	Day 21 of the initiation of treatment (day 3 after discontinuation of the treatment)	Day 35 of the initiation of treatment (day 17 after discontinuation of the treatment)
Hematology				
WBC (μL)	5000	12130	7800	5950
Neutrophils (%)		82	35	47
Lymphocytes (%)		10	26	43
Monocytes (%)		6	6	7
Eosinophils (%)		1	32	3
Plt (μL)	111000	79000	130000	115000
Blood chemistry				
Albumin (g/dL)	4.1	3.3	3.4	4.2
T-Bil (mg/dL)	0.5	1.9	0.4	0.5
D-Bil (mg/dL)	0	0.7	0.1	0.1
AST (IU/L)	57	15	17	28
ALT (IU/L)	57	22	18	23
ChE (IU/L)	311	257	204	276
T. Chol (mg/dL)	170	126	140	179
BUN (mg/dL)	13	28	6	16
Cre (mg/dL)	0.7	1.4	0.6	0.8
CRP (mg/dL)	0.1	3.5	0.6	0
Blood coagulation				
PT (%)	102	66.4	74.5	95.5
PT-INR	1.01	1.22	1.16	1.04

WBC: White blood cell; Plt: Platelets; PT: Prothrombin time; T-Bil: Total bilirubin; T. Chol: Total cholesterol; CRP: C-reactive protein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ChE: Cholinesterase; BUN: Blood urea nitrogen; D-Bil: Direct bilirubin; INR: International normalized ratio.

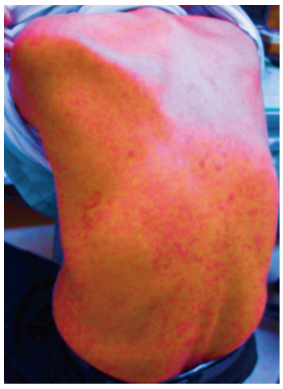

Figure 1 Skin rash on the patient's trunk on day 18 of the treatment with daclatasvir and asunaprevir.

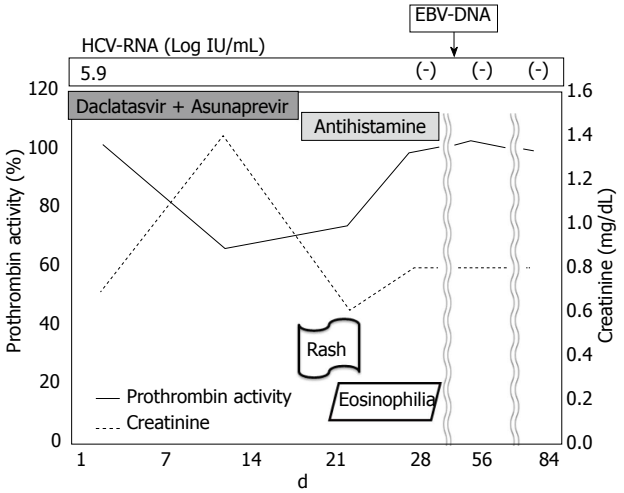

Figure 2 Clinical course of a patient with concomitant deterioration of hepatic reserve and renal function due to combination treatment with daclatasvir and asunaprevir. HCV: Hepatitis C virus; EBV: Epstein-Barr virus.

The decreased cholinesterase and total cholesterol values were also consistent with deteriorated hepatic reserve[10,11]. Drug-induced liver injury generally results in abnormal biochemical liver tests, including transaminase or alkaline phosphatase[12]. In our case, the normal values of transaminase and alkaline phosphatase did not fulfill the diagnostic criteria of drug-induced liver injury. However, the clinical course was similar to that of drug-induced liver injury, key elements of which are drug exposure preceding the onset of liver injury, the exclusion of underlying liver disease, including obstructive jaundice, and improvement after discontinuing the drug[12,13]. Because there have been case reports showing severe liver injury or hepatic decompensation during the therapy of direct-acting antivirals, including telaprevir[14] or simeprevir[15], daclatasvir or asunaprevir may also induce liver injury, with deteriorated hepatic reserve.

The present case also demonstrated renal damage and rash on the trunk and four limbs, which were accompanied by inflammatory reaction and eosinophilia after starting therapy, thereby indicating the possibility of drug-induced hypersensitivity syndrome (DIHS). The patient's atopic background, including bronchial asthma, was also a risk factor of drug allergy[16]. In fact, a case associated with DIHS during combination therapy with daclatasvir and asunaprevir has been reported[17]. The

renal damage was not due to dehydration or urinary tract infection; thus, organ involvement associated with a series of symptoms was considered. Rapidly enlarging rash and deteriorating hepatic reserve, as well as eosinophilia and EBV reactivation[18], are the typical hallmark features of DIHS, which might be responsible for the pathophysiology in our case. However, the lack of fever, lymphadenopathy, and HHV-6 reactivation were inconsistent with DIHS. Thus, our case did not fulfil the diagnostic criteria of even atypical DIHS, according to the diagnostic criteria of DIHS[19]. During this therapy, our case was fundamentally different from the case associated with DIHS[17] because our patient did not have lymphadenopathy, fever, ALT elevation but had other organ involvement, such as renal damage.

However, as a severe, adverse, drug-induced reaction, DRESS syndrome is similar to DIHS. DRESS syndrome is characterized by a delayed onset, usually 2-6 wk after the initiation of drug therapy, as well as the possible persistence or aggravation of symptoms, despite the discontinuation of the causative drug; severe skin eruption, fever, hematologic abnormalities (eosinophilia or atypical lymphocytes) and internal organ involvement are also associated with DRESS syndrome. In this case, the patient showed eosinophilia (more than 1500/μL, with a DRESS score of 2), and skin rash (more than 50%), which were indicative of DRESS; in addition, there was liver and kidney involvement and the absence of other potential causes (each with a DRESS score of 1). There were no enlarged lymph nodes or atypical lymphocytes, and the skin biopsy was not performed, thus each received a DRESS score of 0, and high fever was not observed (less than 38.5 ℃, with a DRESS score of -1). However, the resolution within 15 d is uncertain. If we estimate this evaluation item at a score of -1, the score of our case was at least 5; therefore, our case was considered to be a probable case, according to the RegiSCAR scoring system[9]. The possible mechanisms of the DRESS syndrome are detoxification defects, which can lead to reactive metabolite formation and subsequent immunological reactions, slow acetylation, and reactivation of human herpesvirus, including EBV, HHV-6 and -7[9].

Both daclatasvir and asunaprevir are metabolized by CYP3A, and asunaprevir is a substrate of OATP1B1[20]. CYP3A activity and OATP1B1 activity are known to be decreased in advanced liver disease and cirrhosis[21,22]. Prior to antiviral therapy, the platelet count, hyaluronic acid and type Ⅳ collagen levels suggested hepatic fibrosis in our case[23,24]. Moreover, nifedipine (one of the concomitant drugs) is metabolized by CYP3A and, thus, may compete against daclatasvir and asunaprevir as the substrate. Another concomitant drug, olmesartan, is a substrate of OATP1B1 and, thus, may compete against daclatasvir and asunaprevir as the substrate. Drug interactions may also increase the blood concentration of daclatasvir and asunaprevir. Therefore, higher blood levels of daclatasvir or asunaprevir (based on low hepatic metabolic ability) might cause unexpected adverse events. Indeed, in our case, the achievement of SVR12 supported this hypothesis. As a combination therapy, daclatasvir and asunaprevir are also approved in compensated cirrhosis patients (Child-Pugh A), and careful observation is particularly needed for these patients.

Based on the decreased prothrombin activity and platelet count, disseminated intravascular coagulopathy (DIC) was a differential diagnosis in our case. Unfortunately, we did not measure the fibrin degradation products, D-dimer, and fibrinogen levels at the onset of the deteriorated laboratory data. According to the ISTH Diagnostic Scoring System for DIC, the score in our case was considered to be 5, at most. Thus, we think that overt DIC was a possible but not the probable diagnosis.

In summary, we experienced an unusual case of probable DRESS syndrome due to combination therapy with daclatasvir and asunaprevir. We should pay attention not only to the transaminase levels but also to the allergic symptoms associated with organ involvement during combination therapy with daclatasvir and asunaprevir.

COMMENTS

Case characteristics

Dark urine and a rash on his trunk and four limbs.

Clinical diagnosis

Drug reaction with eosinophilia and systemic symptom (DRESS) syndrome due to combination therapy with daclatasvir and asunaprevir.

Differential diagnosis

The ISTH Diagnostic Scoring System for disseminated intravascular coagulopathy and diagnostic criteria of drug-induced hypersensitivity syndrome.

Laboratory diagnosis

Blood drawing and urine collection showed that hepatic reserve and renal function deterioration, eosinophilia and dark urine, respectively.

Imaging diagnosis

Inspection of skin and photographic evidence of a skin rash.

Treatment

Both daclatasvir and asunaprevir were discontinued and an antihistamine was administered for 2 wk.

Related reports

The reference #1 for the understanding of combination therapy with daclatasvir and asunaprevir, and #9 for the understanding of The DRESS syndrome.

Term explanation

DRESS syndrome: DRESS syndrome is characterized by a delayed onset, usually 2-6 wk after the initiation of drug therapy, as well as the possible persistence or aggravation of symptoms, despite the discontinuation of the causative drug; severe skin eruption, fever, hematologic abnormalities (eosinophilia or atypical lymphocytes) and internal organ involvement are also associated with DRESS syndrome.

Experiences and lessons

We should pay attention not only to the transaminase levels but also to the

allergic symptoms associated with organ involvement during combination therapy with daclatasvir and asunaprevir.

Peer-review

It is a very interesting and well explained case worth sharing with the scientific community.

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CASE REPORT

Burkitt's lymphoma of maxillary gingiva: A case report

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Author contributions: All the authors contributed to the acquisition of data, writing and revising the manuscript; Kane S helped with the immunohistochemical staining and diagnosis.

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Abstract

Burkitt's lymphoma (BL) is an aggressive form of non-Hodgkin's B-cell lymphoma with three variants namely endemic, sporadic, and immunodeficiency-associated types. It is endemic in Africa and sporadic in other parts of the world. While the endemic form is widely reported to occur in early childhood and commonly involves the jaw bones, the sporadic form typically presents as an abdominal mass. This presentation reports a rare case of sporadic form of BL clinically manifesting as a generalized gingival enlargement in an immunocompetent adult male which demonstrated an aggressive behavior. The patient reported with a prominent anterior gingival swelling of 6 mo duration which slowly enlarged in size and associated with multiple lymph node involvement. Microscopic examination of the lesion using H, E and immunohistochemical diagnosis confirmed the diagnosis as BL. The patient succumbed to the disease before any therapy could be instituted. Since a wide array of causes can be attributed to gingival enlargements, it is necessary to consider malignancies as one of the important differential diagnosis so as to facilitate the need for appropriate diagnosis and prompt treatment.

Key words: Lymphoma; Diagnosis; Differential; Non-Hodgkin's; Gingival overgrowth; Prognosis; Pathology; Oral; Immunohistochemistry

Core tip: Burkitt's lymphoma (BL) is an aggressive form of non-Hodgkin's B-cell lymphoma with three variants namely endemic, sporadic, and immunodeficiency-associated types. It is endemic in Africa and sporadic in other parts of the world. We report a rare case of sporadic BL presenting as generalized gingival enlargement. The purpose of this case report is to illustrate the fact that gingival enlargements may be caused by any benign non-neoplastic lesions or aggressive malignancies like BL and bespeaks the need for prompt recognition and life-saving referral by the dental practitioner.

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INTRODUCTION

Lymphomas are malignant neoplasms of lymphocyte cell lines and ranks second to squamous cell carcinoma in frequency of occurrence in head and neck. They are of two types: Hodgkin's (HL) and Non-Hodgkin's (NHL). HL often presents as a nodal disease with predilection for head and mediastinal nodes. NHL are heterogenous group of neoplasms arising primarily within the lymph nodes but up to 24%-40% cases occur in extra nodal sites such as gastrointestinal tract, skin, bone and Waldeyer's ring[1].

Burkitt's lymphoma (BL) is an aggressive form of non-Hodgkin's B-cell lymphoma that is endemic in Africa and sporadic in other parts of the world. It is usually diagnosed in children and young adults but rarely in middle-aged adults. The endemic form typically involves the mandible, maxilla, and abdomen. BL of orofacial region typically occurs in the jaw bones associated with tooth mobility, dental pain and jaw expansion. Sudden and unexpected development of life threatening complications such as airway and abdominal obstruction and acute renal failure has been reported with BL[2]. In contrast, the sporadic form commonly presents as an abdominal mass involving the mesenteric lymph nodes or ileocecal region. Jaw involvement occurs in only a small number of sporadic cases[3].

A wide range of local and systemic lesions may manifest in the form of gingival enlargements. The most common cause occurs secondary to prolonged exposure to dental plaque resulting as a consequence of poor oral hygiene[4]. Other causes include drug induced enlargement; conditioned enlargements secondary to pregnancy, puberty, vitamin C deficiency; idiopathic enlargements and those associated with chronic microbial infections. While majority of the causes are benign and non-neoplastic in nature, malignant neoplasms also at time manifest in the form of gingival enlargements. Gingival enlargement is an important manifestation of malignancies like leukemia and lymphoma encountered by a dental specialist[5]. In the oral cavity lymphomas of the NHL type occurs as a primary disease or in conjunction with disseminated disease. All the variants of NHL occur in older patients except BL which usually occurs in children. The patient may present with nonspecific pain parasthesia, bony swelling with eventual perforation and soft tissue enlargement[6].

The present article reports a sporadic form of BL in an imuunocompetent adult male manifesting with atypical clinical presentation primarily involving the oral soft tissue.

CASE REPORT

A 38-year-old male patient reported to Oral Pathology clinic at our center for evaluation of an oral mass. Patient's chief complaint was painful swelling in the anterior gingiva with difficulty in eating. He noticed the swelling 6 mo back in the upper anterior gingiva which progressively increased in size and gradually involved the entire gingiva of both the arches. Patient visited a local dentist and oral prophylaxis was performed. No clinical changes were evident post prophylaxis and the lesion attained the present size within seven days. The patient's medical and dental history was insignificant. Personal history included regular tobacco chewing along with lime 5-6 times a day for the past 7-8 years accompanied with occasional alcohol consumption.

On extra-oral examination mild facial asymmetry with elevation of upper lip was noted. No abnormalities were detected on TMJ examination. Multiple, ipsilateral, nontender, matted, submandibular lymph nodes of approximate size 2.5 cm^2 along with superficial cervical lymph nodes of approximate 1.5-2 cm^2 were palpable on right side. Intra oral examination revealed generalized diffuse enlargement of both maxillary and mandibular gingiva. The swelling was particularly prominent in the anterior maxillary region involving both the palatal and labial gingiva extending from right maxillary lateral incisor to left maxillary lateral incisor, of size 3 cm × 3 cm, covering 2/3 of the crown portion of the incisors (Figures 1 and 2). The maxillary central incisors were displaced. The anterior lesion possessed smooth, shiny, non-stippled surface and was red in color. The other areas were red, edematous, stippled with non ulcerated surface. On palpation the texture was soft with bleeding in few areas and fibrous in others. There was no evidence of pulsation or thrill. Generalized Grade 1 mobility of the teeth was present. Oral hygiene status of the patient was poor. A provisional diagnosis of human immunodeficiency virus (HIV) associated gingival enlargement or neoplastic gingival enlargement was considered.

Orthopantamographic examination revealed a generalized horizontal alveolar bone loss. The mass was primarily located in the soft tissues. No other bony changes related to the swelling were observed.

Routine blood investigation was performed and all parameters were within normal limits. ELISA and Western blot were negative for HIV. Peripheral blood smear stained with Leishman-Romanowsky stain showed atypical lymphocytes with pale cytoplasm and nuclei at the periphery with coarse chromatin and multiple nucleoli (Table 1).

The light microscopic examination of the H and E stained tissue section under low power magnification showed a dense infiltrate of monotonous appearing darkly stained round cells, which appeared to be of lymphoid origin, with minimal stroma in the submucosal region. On a higher magnification the tumor cells were homogenous in size and shape with round to oval intensely basophilic nuclei and minimal cytoplasm. They were separated by thin fibrous septae with scattered large pale staining macrophages resembling starry sky pattern. The neoplastic cells showed nuclei with coarse chromatin and several nucleoli indicating a high mitotic index. Microscopically the morphological picture was

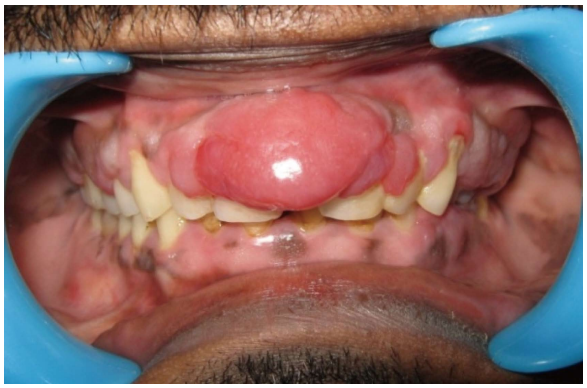

Figure 1 Generalized gingival enlargement with prominent swelling in the anterior maxilla.

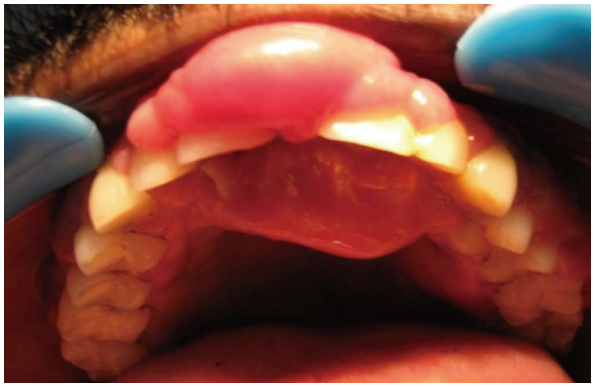

Figure 2 Swelling in the hard palate covering one third of the palate.

Table 1 Details of lab investigations

Test	Values
HB	14.1 g/dL
RBC	5.1 million/mm^3
Neutrophils	26
Lymphocytes	41
Eosinophils	4
Monocytes	3
Abnormal cells	26
Platelet count	23000
HIV	Negative
HBs Ag	Negative

RBC: Red blood cell; HIV: Human immunodeficiency virus.

Table 2 Immunohistochemical expression of various markers in the present case

Marker	Expression	Interpretation
LCA	Diffusely positive	Suggestive of lymphoma
Cytokeratin	Negative	Excludes undifferentiated carcinoma
MPO	Negative	Ruled out granulocytic origin
Tdt	Negative	Rules out blast cell origin
CD3	Positive in scattered T cells	Excludes T-lymphocyte predominance
CD20	Focally positive	Indicates mature cell origin
CD10	Positive	Indicative of B-cell origin
CD138	Negative	Excludes plasma cell origin
MIB 1	Positive (almost 100%)	Indicative of aggressiveness

consistent with NHL. Based on the histopathological findings a diagnosis of NHL probably BL was made (Figure 3).

Immunohistochemical (IHC) analysis was performed with a panel of antibodies to confirm the diagnosis (Table 2). A negative cytokeratin staining along with diffuse positivity of LCA confirmed the lymphoid origin. Granulocytic origin was ruled out by MPO negativity. CD3 positivity in scattered cells revealed that the cells were not of T-cell lineage. The origin of B cells was confirmed with CD10 positivity. Tdt was negative which indicated the presence of mature cells and not blast cells. CD 20 was focally positive which confirmed the presence of mature B cells. MIB 1 was > 95% positive which confirmed increased DNA synthesis and increased mitotic activity. Plasma cell neoplasms were ruled out on CD 138 negativity.

The histopathological picture along with IHC findings confirmed the diagnosis of BL.

The patient's condition deteriorated with the platelet count falling below 10000 within few days and he succumbed to the disease.

DISCUSSION

A wide range of etiological factors are responsible for gingival enlargements. They may result from acute or chronic inflammatory changes, systemic diseases such as Wegener's granulomatosis[7] tuberculosis and sarcoidosis or neoplastic enlargements which may clinically mimic inflammatory enlargements[8]. Malignant neoplasms of epithelial origin are more common in gingivobuccal area[9] and are to be primarily considered followed by leukemia[6]. The present case manifested in the form of gingival enlargement with aggressive behavior and poor clinical outcome.

Lymphoma is a malignant neoplasm of lymphocytic cell lines and includes two clinical types namely HL and NHL lymphoma. HL often presents as a nodal disease with predilection for head, neck and mediastinal lymph nodes. NHL is a heterogenous group of neoplasms arising primarily within the lymph nodes but up to 24%-40% cases occur in extra nodal sites[1]. Oral lesions are often a component of disseminated disease process that may involve regional lymph nodes or may at times represent the primary extra nodal form of the disease[10]. Isolated oral lymphoma is extremely rare and oral NHL in Indian sub population is more aggressive compared with western population[11].

BL is an aggressive childhood to early adulthood variant of NHL which is endemic in Africa and is often associated with Epstein-Barr virus (EBV). Majority of the endemic form occurs in children 5 years or lesser[12],

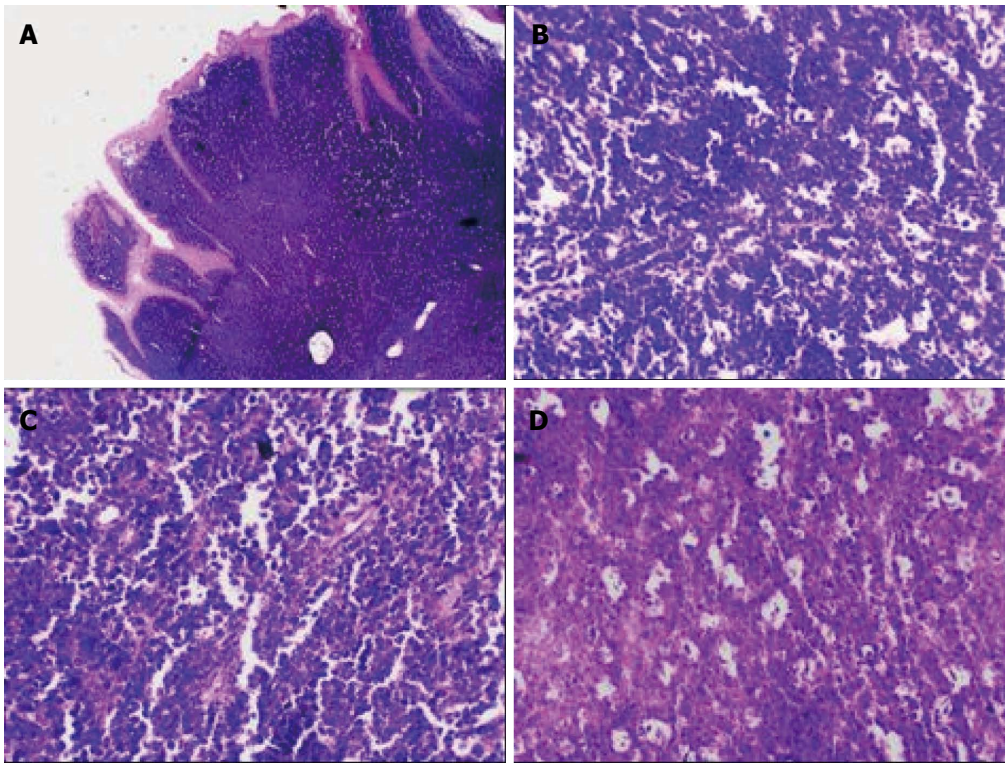

Figure 3 Microscopically the morphological picture. A: Monotonous population of basophilic round cells with minimal stroma beneath the surface epithelium (4 ×); B: Homogenous tumor cells with basophilic nuclei and minimal cytoplasm separated by thin fibrous septa (10 ×); C: Tumor cells showing coarse chromatin and multiple nucleoli indicating high mitotic index (10 ×); D: Macrophages with foamy cytoplasm are seen scattered amongst tumor cells (10 ×).

suggesting a stimulatory effect from the growth factors associated with jaw or tooth development[13]. The sporadic form has no geographic predilection and most commonly develops in adults as an abdominal mass[14]. BL of orofacial region typically occurs in the jaw bones associated with tooth mobility, dental pain and jaw expansion. Sudden and unexpected development of life threatening complications such as airway and abdominal obstruction and acute renal failure has been reported with BL[2]. BL affecting the gingival soft tissue only, has been occasionally reported in the literature while the present article present an unique case with diffuse enlargement involving both maxillary and mandibular gingiva.

BL is classified as a highly aggressive peripheral B cell tumor and demonstrating the highest proliferation rate of any neoplasms in humans, with a potential doubling time of 24 h and a growth fraction of nearly 100%[15]. It is further classified morphologically by WHO into classical BL and two variants namely BL with plasmacytoid differentiation and atypical Burkitt's like lymphoma[16]. All cases of BL show characteristic translocation between IgH locus on either chromosome 14/2/22 and the *c-MYC* gene on chromosome 8. An increase in c-Myc expression results from the translocation. This c-MYC dysregulation seems to be the defining abnormality that eventuates into BL[17].

The microscopic features of typical BL shows tumor cells that are monotonous, intermediate sized with round nuclei containing coarse chromatin, multiple small nucleoli admixed with tingible body macrophages creating starry-sky pattern visible at low power. Mitotic and apoptotic activity are typically a prominent feature. Expression of pan B-cell markers as well as Bcl-6 and CD-10 suggests a germinal centre origin for the tumor cells[16]. Histologically oral BL has to be differentiated from diffuse large B-cell lymphoma (DLBCL) and plasmablastic lymphomas (PBL). DLBCL is predominant variant in oral cavity and this is explained by its proclivity to present at single extra nodal site[18]. The characteristic microscopic feature of PBL is a diffuse sub mucosal proliferation of monomorphic large sized tumor cells with deep ulceration of the overlying mucosa. PBL is characterized by a diagnostic triad of predilection for gingivobuccal complex mucosa, classical plasmablastic morphology with lack of neoplastic plasma cells and a limited histochemical panel including a high Mib-1 index[19].

This paper presents a unique sporadic case of BL in a HIV adult male with the oral lesion limited to the gingival soft tissue only without involvement of the jaw bones. Diffuse involvement of gingiva on both the arches along with rapid growth showed the aggressiveness of the lesion. This lesion has clinical characteristics that mimicked a variety of other aggressive orofacial pathologies including malignant neoplasms and illustrates the inherent difficulty in diagnosis based on the lesion's uncharacteristic clinical appearance. Biopsy and histopathological examination of the tissue with immunohistochemistry was mandatory to arrive at a diagnosis.

Gingival enlargement is a common pathology in

the general population caused by a variety of local and systemic factors. While plaque induced inflammation is the most important cause, enlargement can also be induced by non-neoplastic and neoplastic factors of systemic origin. The clinician can often diagnose the cause of enlargement by careful history, by location and by clinical presentation. It is imperative that the clinician maintain a high degree of suspicion and act promptly in lesions with unusual appearance and behavior. A detailed investigation including biopsy is mandatory to correctly diagnose and treat such lesions. Our case reported some unusual presentation of gingival involvement secondary to malignancy and thus highlights the importance of considering such lesions in the differential diagnosis of gingival enlargements.

COMMENTS

Case characteristics

A 38-year-old male patient reported with a chief complaint of painful swelling in the upper anterior gingiva with difficulty in eating since 6 mo.

Clinical diagnosis

Human immunodeficiency virus associated gingival enlargement was considered as the provisional diagnosis.

Differential diagnosis

Neoplastic gingival enlargement, chronic granulomatous lesion and oral lesions secondary to systemic diseases like leukemia.

Laboratory diagnosis

All lab findings were within normal limits.

Imaging diagnosis

Orthopantamographic examination revealed a generalized horizontal alveolar bone loss without any other relevant changes.

Pathological diagnosis

Burkitt's lymphoma (BL).

Treatment

Patient succumbed to the disease before institution of therapy.

Related reports

BL in an adult immunocompetent individual is less reported in literature and needs to be treated early owing to its aggressive nature.

Term explanation

BL is a malignant neoplasm belonging to group of non-Hodgkin'ss lymphoma with aggressive behavior and poor prognosis.

Experiences and lessons

Oral manifestations of malignant neoplasms such as leukemia and lymphoma should be considered in the differential diagnosis of rapid gingival enlargements and clinical practitioners should be aware of its consequences.

Peer-review

It's a simple case report about oral BL concurrency associated with gingival enlargement. This is a well written case report with a very rare pathology.

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CASE REPORT

Unilateral neuropathic arthropathy of the shoulder secondary to syringomyelia: Diagnostic challenges

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Author contributions: Chakraborty PP was the clinician responsible for care of the patients; Datta S and Ray S drafted the manuscript; Bhattacharjee R revised the manuscript; Ray S reviewed the literature; Chowdhury S provided expert opinion; all the authors contributed to the intellectual content and approved the final version.

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Informed consent statement: The patient involved in this study gave her written informed consent authorizing use and disclosure of her health information.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

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Abstract

Neuropathic arthropathy of the shoulder is a rare disorder characterized by joint degeneration, and is associated with loss of sensory innervation. Syringomyelia is a disease in which fluid-containing cavities (syrinxes) form within the spinal cord. Here, we report a case of neuropathic arthropathy of the shoulder secondary to syringomyelia in a 40-year-old woman. X-rays of the left shoulder revealed damage to bone and joint architecture. Blood tests indicated vitamin D deficiency and secondary hyperparathyroidism. Magnetic resonance imaging of the cervical spine showed a large syrinx from the second cervical spine to the second dorsal spine. Although neuropathic arthropathy is uncommon, it should be considered in cases of unexplained pain, discomfort, or limited range of motion of the affected joint. Symptoms related to the affected joint may precede or overshadow neurological deficits. Appropriate radiological examinations and diagnoses are imperative to prevent misdiagnosis or undetected bone and joint disorders.

Key words: Neuropathic arthropathy; Charcot shoulder; Syringomyelia; Magnetic resonance imaging; Vitamin D deficiency

Core tip: Neuropathic arthropathy, also called Charcot shoulder, is a chronic, degenerative condition associated with decreased sensory innervation. Syringomyelia patients typically suffer with shoulder and elbow involvement. Since joint symptoms often appear before other signs,

neurological deficits are often overshadowed, and the condition is misdiagnosed. In cases of unexplained joint pain, discomfort, and limited range of motion, syringomyelia should always be considered even in the absence of detectable neurological features. To prevent misdiagnoses, clinicians should be aware of the clinical and radiological signs of this rare condition. Timely diagnoses are important to avoid unwanted operative procedures that could lead to unsatisfactory outcomes.

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INTRODUCTION

Neuropathic arthropathy of the shoulder, also called Charcot shoulder, is a chronic, degenerative condition associated with decreased sensory innervation. Patients with diabetes mellitus, syphilis, or syringomyelia are at most risk for this disease[1]. Diabetic patients are most commonly afflicted with foot and ankle disease, while the knee is typically involved in syphilis patients. Syringomyelia patients usually present with pain in the upper limbs, shoulder joint, and elbow[2]. Approximately 6% of patients with neuropathic arthropathy have shoulder joint involvement[3]. Here, we report a case of neuropathic arthropathy of the shoulder secondary to syringomyelia. The case was of particular interest, as it was initially misdiagnosed as a pseudofracture secondary to osteomalacia. Accurate, timely diagnoses are important to avoid operative procedures.

CASE REPORT

A 40-year-old woman visited her family physician with a three-month history of gradually increasing discomfort and restricted movement of the left shoulder. It was not preceded by any trauma. She was found to be non-diabetic, but her serum 25-hydroxy vitamin D level was low (12 ng/mL). She was initially thought to have frozen shoulder and was advised to undergo physiotherapy. However, upon examination of X-ray results and the presence low vitamin D levels, she was then diagnosed with osteomalacia with pseudofracture and was advised to take oral cholecalciferol and undergo physiotherapy. She had no other symptoms related to osteomalacia, such as bone pain, muscle weakness, or difficulty walking.

Due to lack of improvement, the patient was subsequently referred to our facility. Physical examination showed asymmetry of the shoulder joints and drooping of the left shoulder. The shoulder joint was not palpable and the patient had painless restriction of joint movement in all axes. She denied any history of long-term steroid use or other drug use. She had normal menstrual cycles without any history of low trauma fracture elsewhere. She had normal sensations and preserved powers in all the four limbs with normal deep tendon reflexes and downgoing plantars. No muscle wasting, or peripheral nerves thickening were evident. Despite getting a normal neurological examination, we suspected a neuropathic joint.

A thorough look at the radiographs of the left shoulder revealed resorption of the left humeral head and parts of the glenoid cavity, multiple loose bodies, and dislocation of the left shoulder joint (Figure 1). We also obtained blood counts, measured her erythrocyte sedimentation rate, tested for venereal diseases, and measured serum vitamin B12 and serum intact parathormone (iPTH) levels. A skeletal survey and magnetic resonance imaging (MRI) of cervical and thoracic spine were also performed. Besides an elevated iPTH level (120 pg/mL), the rest of the blood and serum results were normal. Skeletal radiographs did not indicate the presence of looser zones, which are common in patients with osteomalacia. However, the MRI of the cervicodorsal spine revealed a hyperintense intramedullary signal change extending from C2 to T2, determined to be a syrinx. The craniovertebral junction appeared normal (Figure 2). Based on the patient's symptoms, the clinical examination, X-ray and MRI findings, we re-diagnosed the patient with neuropathic arthropathy of the shoulder. She was transferred to the physical therapy and rehabilitation department for conservative treatment.

DISCUSSION

The relationship between syringomyelia and neuropathic arthropathy is well described. Syringomyelia is a chronic disease anatomically characterized by the development of a tubular cavitation within the spinal cord. It can be congenital or can be caused by trauma, tumors, degenerative diseases, or infection[4]. The usual manifestations of syringomyelia include dissociated anesthesia in a "cape" distribution, areflexia, weakness, and of upper limb along with neuropathic joints. Less commonly, syringomyelia presents with atypical symptoms (*e.g.*, limb hypertrophy, joint pain, swelling) with or without detectable neurological symptoms[5]. Neuropathic arthropathy is a form of chronic destructive arthropathy that occurs secondary to sensory loss of the involved joint. Twenty-five percent of patients with syringomyelia develop neuropathic arthropathies, 80% of which involve an upper limb[6].

Neuropathic arthropathy of the shoulder is a rare disorder, with very few cases reported in the English literature. A review by Hatzis *et al*[3] found 31 documented cases of neuropathic arthropathy of the shoulder. Although neuropathic arthropathy of the shoulder had varied clinical presentations, shoulder pain and swelling were the most common symptoms. Stiffness and decreased range of movement were the next most frequent symptoms. These more common symptoms often

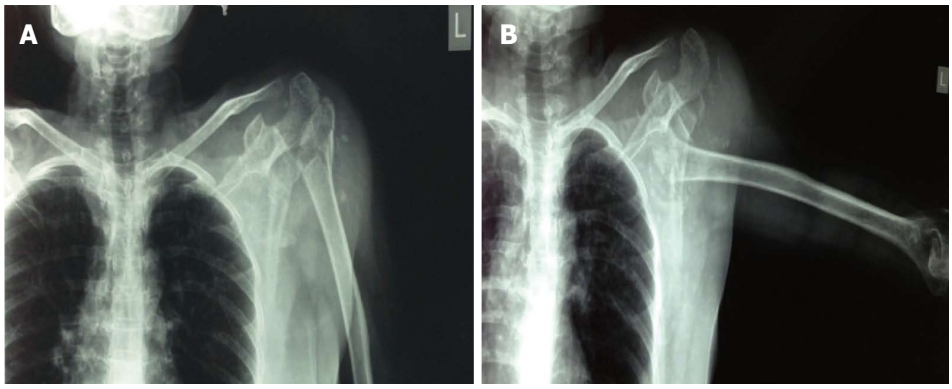

Figure 1 Neuropathic arthropathy of the shoulder in a 40-year-old woman with a syrinx. A: Antero-posterior X-ray of the left shoulder showing concentric bone atrophy of the upper end of the humerus with dislocation of shoulder joint; B: Scapula and clavicle appear to be intact.

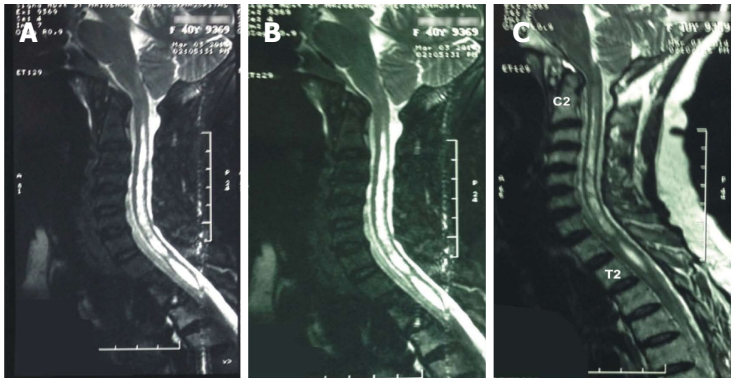

Figure 2 Magnetic resonance imaging images. Sagittal T2 fat suppressed (A and B) and T2-weighted (C) and magnetic resonance imaging images showing a long segment syringohydromyelia through the cervical cord, extending into the upper thoracic region.

precede and overshadow neurological deficits. The patient first visited her family physician with discomfort and restricted movement of shoulder joint, which was suggestive of frozen shoulder.

Neuropathic arthropathy of the shoulder generally progresses slowly, but rapid progression may happen over months or even weeks. The symptoms of neuropathic shoulder may mask the symptoms of syringomyelia, which is often characterized by shoulder instability. Complete dislocation of the neuropathic shoulder may even occur. Since neurological symptoms occur much later than pain or discomfort, patients typically first visit a primary care physician or an orthopedic surgeon[3]. If neuropathic arthropathy of the shoulder is suspected, X-rays and an MRI of the cervical cord should be taken to look for syringomyelia since it is the most common underlying disease.

Low dietary intake of calcium often leads to vitamin D deficiency in the Indian population. In reproductive *vs* post-menopausal age groups, 25-hydroxy vitamin D tests show a deficiency (< 20 ng/mL) in 76% *vs* 16.5% of those measured, or an insufficiency (20-30 ng/mL) in 7.5% *vs* 70%[7]. Vitamin D deficiency can lead to low bone mass, muscle weakness, and an increased risk of osteoporotic fractures. In our patient, the vitamin D deficiency was detected by her primary care physician. She was referred to us with a suspicion that her shoulder pathology was secondary to osteomalacia. Secondary hyperparathyroidism was detected upon further investigation at our clinic based on elevated iPTH levels. Although the presence of low 25-hydroxy vitamin D levels and high iPTH levels made osteomalacia highly likely, a low serum 25-hydroxy vitamin D is a poor indicator of osteomalacia. We therefore performed skeletal radiographs to look for pseudofractures or looser zones, which are common in patients with osteomalacia. These are lucent lines seen perpendicular to cortex which spans the cortex incompletely looser zones occur most commonly in ribs, outer borders of scapulae, and the pubic rami. However, the shoulder radiograph in this case strongly suggested a neuropathic joint. Neuropathic arthropathy of the shoulder leads to rapid, extensive degradation of the proximal humerus and glenoid cavity, and is sometimes destroyed in less than 6 wk[8]. Radiographs of neuropathic arthropathy often show osseous fragmentation and debris. Fractures are lesser-known manifestation of the disorder. The treatment strategy for neuropathic arthropathy is conservative. The prevention of trauma to the joint with proper splinting is the key to treatment. Aspiration of large effusions and splinting prevents further ligamentous laxity[9,10]. Our patient showed satisfactory improvement upon conservative treatment.

The case presented with shoulder-related signs and symptoms. Since a vitamin D deficiency was detected, the patient's physician suspected osteomalacia rather than a neuropathic disorder. Neuropathic arthropathy, although rare, should be considered as the possible

source of unexplained joint pain, discomfort, and limited range of motion. Neuropathic arthropathy is often misdiagnosed, especially that occurring at non-weight-bearing joints. It is commonly misattributed, even with radiographs, to other etiologies such as osteoarthritis, infection, or tumors. Even in the absence of prominent neurological features, syringomyelia should always be considered as the underlying cause of neuropathic arthropathy of the shoulder.

COMMENTS

Case characteristics

A 40-year-old woman presented with stiffness and restricted movement of the left shoulder.

Clinical diagnosis

Neuropathic arthropathy of the shoulder secondary to syringomyelia.

Differential diagnosis

Neuropathic joint, osteonecrosis, and pathological fracture from neoplasms.

Laboratory diagnosis

Blood and serum levels were normal, with the exception of elevated intact parathormone levels (120 pg/mL) and low serum 25-hydroxy vitamin D levels (12 ng/mL).

Imaging diagnosis

X-rays of left shoulder revealed resorption of the left humeral head and parts of the glenoid cavity, multiple loose bodies, and dislocation of the left shoulder joint. Magnetic resonance imaging of the cervicodorsal spine showed a hyperintense intramedullary signal change extending from C2 to T2, indicating the presence of a syrinx.

Treatment

Patient received physical therapy.

Related reports

Neuropathic arthropathy involving the shoulder joint is an uncommon disorder, with less than 70 cases reported in the English literature.

Term explanation

Neuropathic arthropathy represents a spectrum of bone and joint destructive processes associated with neurosensory deficit. Loss of proprioception has been implicated as a causative factor in pathogenesis of the disease.

Experiences and lessons

Even in the absence of prominent neurological features, syringomyelia should always be considered as a differential diagnosis of upper limb neuropathic joints.

Peer-review

This is a good article.

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