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CONTENTS

EDITORIAL

DIABETES AND TENDINOPATHIES

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In patients suffering from diabetes mellitus, several rheumatologic manifestations are more pronounced (i.e., frozen shoulder, rotator cuff tears, Dupuytren's contracture, trigger finger, cheiroarthropathy in the upper limb, and Achilles tendinopathy and plantar fasciitis in the lower limb). In this review, a description of diabetes-related joint diseases, the specific pathogenetic mechanisms involved, and the role of associated comorbidities, each of which activates a complex sequence of biochemical alterations, are provided. Finally, the related therapeutic approaches are discussed.

Diabetes Mellitus (DM) has been recognized to cause a wide range of musculo-skeletal disorders, which result in significant impairment of mobility, function and quality of life (1-2). The aim of the present review is to summarize the current knowledge on the ligaments, fasciae and tendon diseases associated to DM, focusing on recent pathogenetic findings and the related therapeutic approach.

CLINICAL AND EPIDEMIOLOGICAL FEATURES

Upper limbs

Dupuytren's contracture is characterized by thickening, shortening, and fibrosis of palmar fascia (Fig. 1b). This process results in a flexion contracture of the affected fingers, which is usually painless. Trigger finger, also called flexor tenosynovitis, manifests as a locking phenomenon on finger flexion (Fig. 1d), and may occur spontaneously or be reproduced on active or passive finger flexion. Both conditions have been found in higher percentage in subjects with diabetes *vs* control population (3-6). The limited joint movement of the hand, also known as "diabetic cheiro-arthropathy", is characterized by stiff hands, with significant impairment of small joints. Also the prevalence of this condition is higher in type I and II DM, and is correlated with age, duration of DM, glycaemic control and microvascular complications (3, 5-6).

Carpal Tunnel Syndrome is due to the compression of the median nerve by the transverse carpal ligament (**7**). The symptomatology is characterized by pain and/or paresthesia over the thumb, index, middle, and lateral half of the ring fingers. The prevalence of Carpal Tunnel Syndrome in DM has been reported at 11-25% and conversely 5-8% of patients with Carpal Tunnel Syndrome may have DM (6-9). Moreover, after carpal tunnel release, the incidence of flexor tenosynovitis was found higher in subjects with DM (10).

Shoulder adhesive capsulitis ("frozen shoulder") is characterized by a limited mobility of the joint, with pain at the extremes of motion. It occurs in 10-

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20% of patients with DM and is associated with age, duration of the disease (both in type I and type II DM) and poor glycaemic control (11-13). Rotator cuff disease is very common after the age of 50 years. In the population aged 70 years or more, about 20% of people are symptomatic for shoulder problems, and MRI studies show that, after the age of 60 years, the prevalence of partial or full thickness tears (Fig. 1a) ranges from 30 to 40 % (13-14). In diabetic patients, and also in subjects with high, but yet normal, plasma glucose levels (15), the prevalence of rotator cuff tears is higher, even in absence of symptoms, and the thickness of supraspinatus and bicep tendons is significantly increased (13-14, 16-18).

This is due to the abnormal storage of collagen layers in the tendons and, therefore, is an expression of degenerative changes (11). These observations are of clinical relevance because, as shown by Yamaguchi et al. (19), in a 2.8-year follow-up study, pain and functional limitations can develop in a large percentage (50%) of people with asymptomatic tears at baseline. After surgical repair, subjects with diabetes show a restricted range of shoulder motion (20) and a higher incidence of re-tears (21). These adverse outcomes can be related to the intrinsically poor quality of the tissue that is being repaired. Indeed, experimental studies in obese and diabetic rats show that tendon repair is compromised, due to a decreased proliferation or recruitment of cells to the injury site, which ultimately contributes to defective tendon healing (22).

Lower limbs

An increased thickness of the plantar fascia and Achilles tendon (Fig. 1c) have been observed in both type I and type II DM (23-26). These changes are more severe in patients with neuropathic complications and previous foot ulcers, but can also be found in subjects without diabetic complications (27-29). At ultrasound (US) evaluation, Achilles tendon shows disorientation of collagen fibril arrangement and focal hypo-hyperechoic areas in a significantly higher percentage in comparison to healthy individuals without DM, matched for age and sex. The US abnormalities are prevalent in the body of the tendon and in the region of its attachment to the calcaneus. Evident calcifications are also found in about 30% and are exclusively localized in the Achilles enthesis. The patients with a disorganized tendon pattern are older and show a higher duration of disease in comparison to diabetic individuals without US lesions (26).

The morphologic changes are associated to biomechanical abnormalities, particularly to an increased tendon and fascia stiffness. The limited ankle movement may restrain the forward progression of the tibia on the fixed foot during the stance phase of walking. This, in turn, results in prolonged and excessive weight bearing stress under the metatarsal heads during the foot-floor interaction, which is thought to contribute to the development of foot ulcers in individuals with DM (30-32).

HISTOPATHOLOGY

The histopathological alterations were studied in experimental conditions. In rats with streptozicin-induced DM, less organized collagen fibers and an increased cellularity were found at tibial tubercle enthesis (33), and at supraspinatus tendon-bone interface (34); moreover, the AGEs deposition was increased in these entheses and the tendon-bone healing was impaired after surgical detachment. These findings were observed after few days, and therefore reflect an acute situation following an abrupt glycaemic disregulation,

On the contrary, the features of human tendons of subjects with DM are consistent with chronic degeneration. Indeed, histopathology shows that joint capsules, ligaments and tendons lose their normal glistening-white appearance. In the more affected portions, these structures become grey and amorphous, with poorly marked areas where diffuse, fusiform or nodular thickening may be observed. Electron microscopy shows that collagen fibrils appear twisted, curved, overlapping and otherwise highly disorganised. There is an increased packing density of collagen fibrils, with a decreased number of fibroblasts and tenocytes per unit of surface area. The reduction of elastic fibers is consistent. Micro-calcifications are frequent. Finally, the number of capillaries per unit of surface area is reduced (35).

This morphologic feature is in agreement with the reduced angiogenesis, observed at Doppler evaluation (36). These changes are similar to those age-related and it is current opinion that diabetes

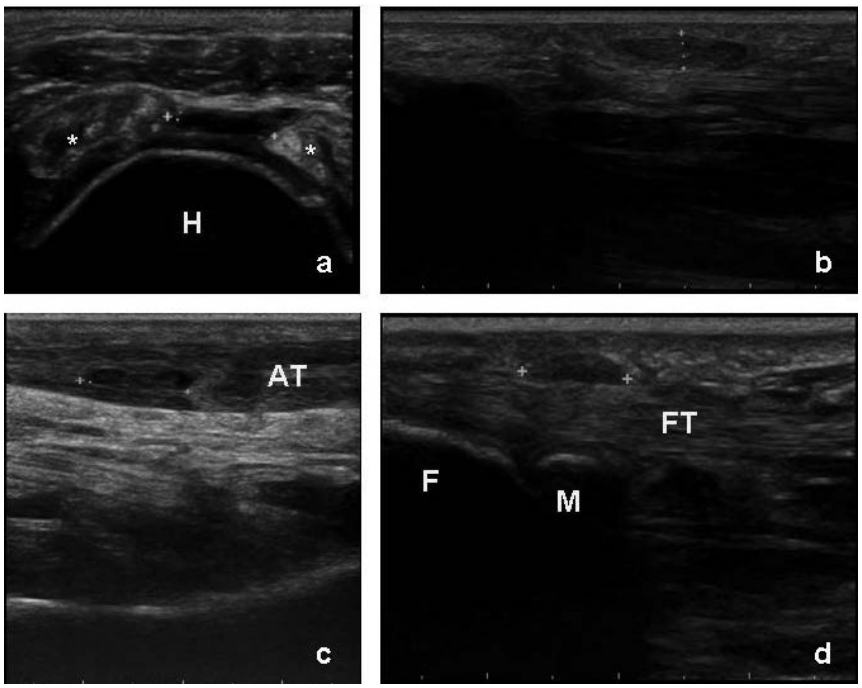

Fig. 1. *Typical ultrasound images of common tendon diseases in the upper and lower limb.* ***A)*** *Rotator cuff tear: a transverse scan of rotator cuff shows a full defect in the insertional portion of tendon, from the bursal to the articular margin, filled with anechoic fluid (calipers). H= Humeral head; *= Rotator cuff tendon ends.* ***B)*** *Dupuytren disease: an hypoechoic nodule (calipers) is depicted over the palmar fascia (longitudinal scan).* ***C)*** *Achilles tendinopathy: the longitudinally scanned Achilles tendon (AT) show a marked thickening of the midportion portion, which also appears hypoechoic (calipers).* ***D)*** *Trigger finger: the longitudinal ultrasound scan shows a thickened hypoechoic pulley (calipers) over flexor tendons (FT) in correspondence of the metacarpophalangeal joints. M= Metacarpal bone; F= phalanx.*

"accelerates" the aging process.

A specific study, performed in patients with stenosing flexor tenosynovitis, has shown that diabetic subjects are characterized by fibrocartilage metaplasia in the middle layer, associated with granulation tissue, which contains newly formed microvessels, stromal cells, a small number of inflammatory cells, and myxomatous degeneration (37). This pattern was found in 68% of the diabetic group and in 28% of the non-diabetic group, and this difference was statistically significant.

Finally, US observations on Achilles tendon confirm the experimental finding that degenerative features and calcifications are prevalent at entheseal level also in humans (38).

PATHOGENESIS

Advanced Glycation End-products

According to an accepted hypothesis, tendon damage in diabetes is caused by an excess of advanced glycation end-products (AGEs) (39).

AGEs form at a constant but slow rate and accumulate with time in the normal body. However, their formation is markedly accelerated in DM because of the increased availability of glucose. A key characteristic of reactive AGEs is their ability to form covalent cross-links within collagen fibers, altering their structure and functionality.

Essentially, collagen cross-links can generate via two different pathways: a) the enzymatically driven, hydroxylysine-derived aldehyde pathway, and b) the non-enzymatic glycation or oxidation-induced AGE cross-link (40-42). As opposed to the beneficial effects on collagen strength bestowed by enzymatic cross-links, AGE cross-linking is generally thought to deteriorate the biological and mechanical function of tendons and ligaments (43). In fact, once formed, AGEs can be degraded only when the protein they are linked to is itself degraded. Therefore, the most

extensive accumulation of AGEs will occur in tissues with low turnover, such as cartilage, bone, and tendon.

Other major features of AGEs relate to their interactions with a variety of cell-surface AGE-binding receptors (i.e. AGE-R1, AGE-R2, AGE-R3 and RAGE) (44). Ligand engagement of AGE-binding receptors activates several critical molecular pathways, and triggers a number of effects, including pro-oxidant events, via generation of reactive oxygen species, and further pro-inflammatory events via NFκβ signalling (45). This in turn accelerates AGE cross-linking in collagen fibres and leads to sustained up-regulation of pro-inflammatory mediators and to a dysfunctional cell phenotype (46-47).

Further AGE negative effects include: i) the modification of short-lived proteins, such as the Basic Fibroblast Growth Factors, which is followed by markedly decreased mitogenic activity; ii) intracellular AGE formation, which leads to the quenching of nitric oxide and impaired growth factor signalling; iii) enhanced apoptosis via oxidative stress, increased caspase activities, and/or extrinsic signalling through pro-apoptotic cytokines (48-49).

Tendon damage ensues from these complex pathways. In addition to degeneration, tendon and ligament thickness increases as expression of the abnormal storage and the architectural distortion of collagen layers (50). From the biomechanical point of view, several studies have demonstrated that collagen toughness and stiffness and the elastic modulus are strongly influenced by AGEs cross-link formation (51).

It is not surprising that these metabolic abnormalities may be present in the early clinical observation of type II DM (52). Indeed, whereas type I DM is diagnosed at an early stage because of a relatively acute clinical onset characterized by extreme elevations in glucose concentrations, type II DM is usually diagnosed later, when many patients already exhibit chronic complications. Certainly, these subjects could have glucose intolerance or mild type II DM for a significant length of time before DM is clinically diagnosed.

Other DM-related biochemical mechanisms

In addition to the AGE-mediated damage, several biochemical alterations may contribute to explain tendon degeneration. Hyperglycaemia "in se" may lead to changes in the redox environment, specifically in the polyol pathway, resulting in increased intracellular water and cellular oedema (53). It has been also shown, in porcine patellar tendons incubated with different glucose concentrations, that hyperglycaemia produces a reduction in proteoglycans levels, in an AGE-independent manner, decreasing the synthesis or sulfation of glycosaminoglycans (54). Similarly, high glucose concentration up-regulates the expression of MMP-9 and MMP-13 in tendon cells, which may account for the molecular mechanisms underlying diabetic tendinopathy (55).

Because DM is associated with an increased oxidative stress, an experimental study was performed on human cultured tenocytes to determine whether extracellular low, normal and high glucose levels alter the response to hydrogen peroxide. In low glucose, peroxide-treated cells remained fully viable and collagen synthesis was increased, suggesting an anabolic response. In high glucose, however, peroxide treatment led to increased apoptosis (56).

All the above quoted pathogenetic mechanisms are based on the idea that systemic factors related to high blood glucose levels are causally involved. Recently, a novel approach has been proposed by Lehner et al. (57), who suggest that tendon immanent cells might be directly involved in diabetic tendinopathy. These authors, by means of immune-histochemistry, laser capture microdissection, and detection of specific markers, showed that human and rat tendons harbour a population of pancreatic β-cells, both in the perivascular area and in the dense collagenous tissue. These cells express insulin and glucagon. Intraperitoneal injection of streptozotocin caused a loss of insulin and insulin mRNA in rat Achilles tendons after only 5 days, accompanied by a 40% reduction of mechanical strength. Therefore, these authors hypothesize that extrapancreatic insulin-producing cells possibly play a major role in the pathophysiology of diabetic tendinopathy.

Adipokines

Overweight and obesity (mainly visceral fat deposition) are frequently associated, and strictly intertwined, to glucose intolerance and type II DM. Therefore, pathogenetic factors linked to fat excess

must be taken into account. Prevailing hypotheses of tendon damage in obese subjects are associated with two different mechanisms: the increased yield on the load-bearing tendons and the biochemical alterations attributed to systemic dysmetabolic factors. Indeed, weight-bearing tendons are exposed to higher loads with increasing adiposity, and the higher loads lead to overuse tendinopathy. Alternatively, the systemic hypothesis is based on studies showing that the association with adiposity is equally strong for the non-load-bearing and load-bearing tendons (58).

Adipose tissue is now recognized as a major endocrine and signalling organ. In obese subjects, adipose tissue releases bioactive peptides and hormones; the adipokinome includes a full range of proteins such as chemerin, lipocalin 2, serum amyloid A3, leptin and adiponectin (59). These proteins influence several activities in various mesenchymal cell phenotypes (tenocytes, chondrocytes and osteocytes), which may directly modify tendon structure. In particular, adipokines are able to modulate cytokines, prostanoids and MMP production (60-61). The persistently raised serum levels of PGE2, TNF-a and LTB4 observed in obesity and in subjects with impaired insulin sensitivity provide supplementary evidence that a systemic state of chronic, sub-clinic, low-grade inflammation is present in these conditions and may act as a prolonged disruptor of tendon homeostasis (62-65).

Micro-angiopathy

Microvascular disease may contribute to tendon damage, leading to tissue hypoxia, overproduction of oxygen free radicals, and to a permissive apoptotic environment (66).

Microvascular disease is an ubiquitary phenomenon, which has been found also in tendons. The reduced neovascularisation inside the degenerated tendons, found by means of Power Doppler sonography (36), is consistent with several observations, which show decreased Vascular Endothelial Growth Factor levels and reduced angiogenesis in different experimental and clinical diabetic conditions (67-69). This finding enlarges our knowledge about the pathogenesis of diabetic tendinopathy. The down-regulation of this factor can limit not only vessels but also nerve ingrowth and can also affect neurogenesis, reducing neural progenitor cell recruitment, axonal outgrowth, neuronal survival and the proliferation of Schwann cells (70). The association between reduced nerve proliferation inside tendons and sensitive neuropathy reduces pain perception. Consequently, diabetic patients, who lack distress signals, may excessively exercise their tendons, making them prone to overuse damage.

Calcifications

As far as calcifications are concerned, it has been reported that calcium deposits are frequent in tendons of diabetic subjects, and mainly found in Achilles enthesis. The mechanisms of deposition of calcium salts are object of debate. In previous studies, necrosis of the tendon secondary to local ischemia, rupture of collagen fibers, hyaline degeneration have been recognized as the first step to promote calcium deposition (71). According to recent research, calcifications could be formed from the erroneous differentiation of Tendon Derived Stem Cells into chondrogenic and osteogenic cells, instead of tenocytes. Many morphogenetic proteins (osteopontin, decorin, aggrecan, byglican and fibromodulin) could be involved in the ectopic chondrogenesis and subsequent ossification (72-74). However, the mechanism by which diabetes can predispose to or promote this abnormal differentiation is unknown (75).

PREVENTION AND THERAPY

The tendon damage is strictly related to the duration of the disease, glucose levels and age of patients. Therefore, an early diagnosis and a proper control of hyperglycaemia is recommended, because it has been shown that the articular damage is more frequent and important in patients affected by DM who did not undergo a correct treatment (76).

Stretching and strengthening programmes have been widely used for a long time to prevent joint stiffness and to reduce tendon damage. However, the improvements observed are not relevant and do not result in a better overall functioning. Moreover, benefits do not last long (77).

The knowledge of basic pathogenetic mechanisms paves the way to selective therapeutic interventions with drugs which may counteract the detrimental

effects of AGEs. A plethora of different compounds are under study. They can be divided into AGE inhibitors and AGE breakers: the first ones inhibit AGE formation, working as carbonyl-trapping agents, and promoting the excretion or limiting the uptake of metal ions (iron and copper), the second ones cleave AGE cross-links in tissue proteins. These compounds, such as aminoguanidine, pyridoxamine, glucosamine, some ACE-inhibitors, aldose reductase inhibitors, genistein, and several natural derivatives (rutin, quercetin, hesperidin, polyphenols, etc), in animal models of DM have been proved effective in retarding the full range of diabetic complications, such as nephropathy, neuropathy, retinopathy and vasculopathy (52). However, only a few have entered clinical trials, but none have yet been approved for clinical use (39).

Recent studies highlight the relevance of soluble RAGE (sRAGE) isoforms in several diseases. Endogenous sRAGE isoforms have been found circulating in plasma and in tissues. Their levels are lower in vascular diseases and DM, characterized by ligand-RAGE hyperactivity, suggesting a significant inverse correlation with vascular damage (78-79).

Impressive results, obtained by administrating recombinant soluble RAGE in animal models, suggest that they may neutralize the ligand-mediated damage by acting as a decoy and blocking diabetic complications and joint inflammation in experimental models (80). It is to be hoped that in a short time new pharmacological compounds, which may counteract the negative effects of AGEs, may enter therapeutic practice and may result in a better control of AGE-related complications, including tendon and ligament damage.

Recently, a novel approach has been proposed, utilizing recombinant human adiponectin, which not only improves the metabolism of diabetic-ridden tenocytes, but also promotes progenitor cell proliferation and differentiation in tendons. Experimental studies have shown that the proliferation rate of adiponectin-treated tenocyte progenitor cells was significantly higher at 6, 8 and 10 days as compared to untreated cells. The levels of tenogenic gene expression (collagen I, III, tenomodulin and scleraxis) were also significantly up-regulated. These features supports the notion that adiponectin may be potentially beneficial in treating diabetic tendinopathy (81).

CONCLUSIONS

Frozen shoulder, rotator cuff tears, cheiro-arthropathy and Dupuytren Contracture are tendon and ligament diseases strictly related to diabetic condition. As a consequence, joint mobility is reduced with functional limitation and impairment to perform the basic and instrumental activities of daily living. Complex pathogenetic mechanisms are involved. Besides the increased AGE formation, which is considered prevalent, a plethora of other factors, acting in an AGE-independent manner, such as reduced synthesis of proteoglycans, increased production of metalloproteinases, chronic low grade inflammation and microangiopathy may play a role.

Prevention and a strict control of the metabolic disorder is mandatory because it is demonstrated that the occurrence and severity of tendinopathies are linked to the duration of disease and glycaemic levels. Several aspecific treatments are used in clinical practice, but new pharmacological compounds which may allow a better control of DM-related complications, including tendon and ligament damage, are under study.

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EDITORIAL

ENAMEL MATRIX PROTEINS AND THEIR APPLICATION IN BONE TISSUE REGENERATION. A REVIEW

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Current treatment options for skeletal repair (alloplastic materials, bone grafts, etc.) have significant limitations, especially in elderly subjects. However, bone tissue engineering seems to provide a solution for reconstructing critical size bone defects. Many of the current regenerative medicine solutions developed rely on products that combine biological agents, such as cells or biomolecules (1). In dentistry, Enamel matrix proteins (EMP) have been successfully employed to promote wound healing of severe infrabony periodontal defects with regeneration of periodontal ligament, cementum and alveolar bone (2-4). The purpose of this review is to evaluate the ability of enamel matrix proteins to promote bone tissue formation and shed light on their possible application in skeletal regenerative medicine. A systematic literature search in electronic databases (PubMed and Cochrane Library) was conducted, using the following search term combination: 'Amelogenins' or 'Enamel Matrix Proteins' or 'Enamel Matrix Derivative' and Osteoblast' or 'Bone' or 'Mineralized Tissue' or 'Tissue Regeneration'. Publications were considered for systematic review if they were published beforel January 2015 in English language and were listed as reference in selected articles. Articles were excluded if they were without histomorphometric analysis or quantitative analysis of calcium deposits *in vitro*, written in languages other than English, clinical and/or animal periodontal regeneration studies, *in vivo* and *in vitro* tooth/root developmental studies (with ameloblasts or cementoblasts or odontoblast). Assessment of the methodological quality of the studies and data extraction were carried out by three authors. A total of 405 articles were found. Only 23 publications, 15 *in vivo* and 8 *in vitro* studies, respected the inclusion criteria and were used for this review. The EMD osteoinductive property appears to be questionable and unclear if the product is used in bone tissue regeneration. In the *in vivo* reviewed articles, the best results were recorded in the presence of restraints and not in large or critical size defects, whereas EMD showed some osteopromotion in the early healing phases. Encouraging data are given on the use of Synthetic Peptide (SP) and recombinant amelogenins. Based on these data, it is necessary to carry out further investigation using amelogenin-based compounds or with their active peptides with known composition and concentration. This would help to standardize the results by increasing the effectiveness of the work in order to better clarify the role and the possible applications of amelogenins in bone tissue regeneration.

Key words: skeletal repair, bone tissue engineering, osteopromotion

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

Bone is a highly specialized tissue with support function. Any congenital or acquired defect inevitably causes a functional deficiency and reduced quality of life (5). In many cases, the amount of lost tissue exceeds the organism regenerative capacity with an insufficient migration of osteoprogenitor cells into the defect and subsequent non-healing lesions, known as Critical Size Defects (CSDs) (6-7).

In the history of bone regenerative medicine, to treat CSDs various molecules have been proposed capable of stimulating proliferation and differentiation of cells, such as platelet derived growth factor, insulin-like growth factor, trasforming growth family factor, bone morphogenic proteins (8).

In the last 20 years, several authors have studied and proposed enamel matrix proteins (EMPs) as factors that stimulate the growth of periodontal ligament and specialized connective tissue such as cement and bone (2-3, 9-11).

In general, EMPs are composed of 90% amelogenins (12-13), which are the major constituent of the organic fraction of the enamel matrix (14), and during the first phases of mineral deposition may account for 60–90% of the enamel matrix (15). The amelogenin aggregates that form the extracellular matrix of the ameloblasts have been hypothesized to create the space and environment conducive to the deposition of the mineral phase. Amelogenin may thus play a major role in the structural organization of the mineral within the developing enamel and might also regulate the nucleation and growth pattern of the enamel hydroxyapatite crystals (16). Although amelogenins are thought to be proteins of exclusively epithelial origin (17-18), recent studies demonstrated that amelogenins can be detected in other tissues such as dentin matrix (19), odontoblasts (20), remnants of Hertwig's root sheath during cementogenesis (21) and cementoblasts (22), suggesting a biological activity of amelogenins in various tissues as well as in the tooth bud. Amelogenin is also expressed in long bone marrow stromal cells, including mesenchymal stem cells (MSCs) (23). Nowadays, EMPs are produced by extraction from the tooth buds of juvenile swine (24). In periodontal surgery they have been successfully employed to promote, alone or in combination with another grafting materials (25-27), the regeneration of periodontal ligament, cementum and alveolar bone in patients with intrabony periodontal defects (3, 27-28) and recently to treat gingival recession (29). The product best known and currently used is Emdogain* (EMD).

Several studies have investigated the antimicrobial activity of Extracellular Matrix Derivatives (EMD) on the growth of some bacteria, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* (30), as well as *P. gingivalis* (31-32) on established supragingival plaque (33).

Interestingly, all authors clearly concluded that the antimicrobial effects could be attributed to the vehicle PGA. The purpose of this review is to evaluate the ability of enamel matrix proteins to promote bone tissue formation and shed light on their possible applications in skeletal regenerative medicine.

RESEARCH METHODS

A systematic literature search in electronic databases (PubMed and Cochrane Library) was conducted, using the following search term combination: 'amelogenins' or 'enamel matrix proteins' or 'enamel matrix derivative' and osteoblast' or 'bone' or 'mineralized tissue' or 'tissue regeneration'. Strategy analysis and selection phases respect the **P**referred **R**eporting **I**tems of **S**ystematic reviews and **M**eta-**A**nalyses (PRISMA) guidelines.

Titles and abstracts of the publications identified by electronic databases were screened initially by three reviewers. Publications were included for full text evaluation if the content of the abstracts met the inclusion criteria and matched to the focused question. Disagreement between the reviewers was resolved by evaluation of the full texts and discussions.

Full-text assessment was performed by the reviewers and a manual search was performed among the references of the selected publications after full text assessment.

Inclusion criteria

A literature search was performed to identify meta-analysis, and systematic reviews as well as randomized-controlled clinical trials (RCTs), case reports, or case series.

Articles considered for this sistematic review are:
- Publications published until Febrary 2015 in English language and were listed as reference in selected articles.
- *In vitro*, *in vivo* or clinical bone tissue regeneration studies with amelogenin proteins.
- All *in vivo* articles had to provide histomorphometric data concerning the question if and to what extent amelogenin affects the bone formation/regeneration.
- *In vitro* studies reporting the quantitative analysis of the calcium deposits.

The influence of the combinations with others biomaterials was additionally evaluated.

Exclusion criteria

The following were excluded:
- Articles written in languages other than English.
- *In vivo* articles without histomorphometric analysis.
- Periodontal regeneration *in vivo* or clinical studies.
- *In vivo* and *in vitro* tooth/root developmental studies (with ameloblasts or cementoblasts or odontoblast).
- *In vitro* studies without quantitative analysis of mineralized nodules or calcium deposits.
- Study with ectopic bone formation only.
- All publications with study regulation of osteogenic gene and/or cells proliferation only.
- All publications reporting the regulation of osteogenic gene alone or in combination with the cells proliferation.

We divided the works into two groups: *in vitro* studies and *in vivo* studies.

In the *in vitro* study group, we evaluated: cell type, type of amelogenins, other amelogenin protein-associated materials, time of culture, qualitative and quantitative calcified nodule evaluation methods and their results.

In the *in vivo* study group, we evaluated: animal used, type of amelogenins, other amelogenin protein-associated materials, investigation timing and histomorphometic analysis results.

RESULTS

A total of 577 records in the two electronic databases were identified. Of these, 171 were duplicates and 406 records were subjected to the first phase of title and abstract screening. For eligibility assessment of full-text, only 53 articles were selected and 353 were excluded.

After a first text evaluation, based on inclusion and exclusion criteria previously reported, we excluded a further 28 articles (Table I). At the second stage of text evaluation another two *in vitro* studies (37, 39) were excluded due to method of investigation of calcium deposits. These authors used only the Von Kossa method which is not specific only for calcium based compounds. Finally, we selected 23 papers meeting the inclusion/exclusion criteria (34-36, 38, 40-58).

Of the included articles, 15 are *in vivo* (44-58) and 8 (34-36, 38) are *in vitro* studies (Table IIa; IIb and IIIa; IIIb). No studies on human subjects were found.

Table I. *Articles excluded after text evaluation and reasons.*

AUTHORS	EXCLUSION REASONS
Boyan et al. 2000; Donos et al. 2006; Kim et al. 2005; Koike et al. 2005; Wang et al. 2011;	***Ectopic bone formation investigated***
Cangini et al. 2005; Donos et al. 2004; Izumikawa et al. 2012; Min et al. 2012; Miron, Caluseru et al. 2014; Miron, Bosshardt et al. 2014, Miron et al. 2013; Wu et al. 2014; Yoneda et al. 2003;	***No Histomorphometric analysis***
Fawzy El_Sayed et al. 2014; Hattar et al. 2005; Jeong et al. 2014; Jiang et al. 2001; Kakegawa et al. 2010; Schwarz et al. 2004; Palioto et al. 2011; Neeley et al. 2010; Pischon et al. 2006; Miron et al. 2012; Wang et al. 2014; Weishaupt et al. 2008; Yang et al. 2014; Zhe Qu et al. 2011;	***No bone formation investigated***
Keila et al. 2004; Miron et al. 2011;	***Staining with Von Kossa method***

In vitro study group

Articles on *in vitro* investigation were published between 2004 and 2014 with 50% of them after 2010. Six articles were conducted using human cells. In particular, five were conducted using adult stem cells and one with embrionic stem cells. From the 8 selected items, three authors tested enamel matrix derivative (EMD) alone, one compared and combined EMD with other synthetic proteins, one compared EMD with recombinant amelogenin, and three authors tested synthetic amelogenin proteins alone. Seven works reported the calcium concentration, of these, one did not run the staining with Alizarin red. Only one author had calculated the size of calcified nodules using a computer morphometric analysis (size in pixels). The maximum cell culture incubation period was not more than 15 days in two studies, no more than 21 days in 4 studies and more than 30 days in a single study (Table IIa and IIb).

Three authors (34, 40, 42) had investigated the effects of different EMD concentrations, different density of PGA veicle and their use as coated or not to the well plates, respectively.

Galli (34) used as cell model human jaw osteoblasts to evaluate calcific nodule formation. These cells were cultured in the absence (G1, control group) or in the presence of EMD at 20 (G2), 50 (G3), and 100 (G4) μg/mL in Dulbecco's modified Eagle's medium (DMEM) containing fetal bovine serum (FBS) 10%, ascorbate, dexamethasone and β-glycerophosphate. At 21 days, cells were fixed and stained with a Alizarin red and authors showed that calcific nodule formation in the presence of EMD appeared more irregular and less round-shaped in the control group (G1). Number and size of mineralized nodules were statically higher ($P<0.001$) in groups 3 and 4 compared to the other two groups. The best studies on the size and the number of nodules were reported for the groups G3 and G4, respectively. The Authors had not calculated the calcium concentration in histological preparations.

Van den Dolder (42) and Nagano (40) used a similar culture medium for rat bone marrow cells (rBMCs) and human periodontal ligament (hPDL) cells respectively, incubated in alpha modification of Eagle's medium containing 10% FBS and antibiotics.

Van den Dolder mesured calcium concentration in rat embrionic stem cells (mES) cultured with EMD (100 μg/mL) coated well plates or EMD not coated (in solution). The author carried out the experiment in two phases. In the first phase, calcium measurements showed that the EMD-coated well group had a significantly enhanced calcium content compared with the uncoated EMD cells on day 32 ($p < 0.01$). In the second phase, this difference was not observed by the author.

Nagano (40) compared effects between Propylene glycol alginate (PGA) alone (G1), Emd-Gel (G2) and EMD + PGA (G3) in human Periodontal ligament cell (hPDL) cultures. The histological samples obtained were stained with Alizarin red S at the end of the experimental period (day 15) and the calcium content was measured by a Calcium C-test kit and protocol after the dissolution of the compartments of cells by hydrochloric acid. The results obtained by the authors showed that the calcium content stimulated by the Emd-Gel was almost 1.7-fold higher than that resulting from the addition of EMD + PGA (0.037 and 0.025 mg/cm^2, respectively). Significant differences were shown between G1, G2 ($p < 0.01$), and G3 ($p < 0.05$) on calcium content.

The effect on different type and EMD composition on hPDL cells isolated from non-impacted premolars extracted for orthodontic reasons were investigated (38). Cells were maintained in differentiation medium (DMEM with 2% Fetal calf serum, 50 μg/mL L-ascorbate 2-phosphate) alone (G1 control) or with home made Recombinant poly(His) tagged mouse 180 amino acid amelogenin (rp(H)M180) at 5 μg/mL (G2), EMD at 50 μg/mL (G3), rhBMP 2/7 at 100 ng/mL (G6), desametasone (Dex) (G7) at 10 nM and following combinations rp(H)M180) + BMP 2/7 (G5) and EMD + rhNoggin protein (rhNG) (G4) at 500ng/mL. Calcium deposits were stained with Alizarin red, and calcium concentration was determined by measuring absorbance at 562 nm with a spectrophotometer. After 21 days of incubation, the authors showed that Alizarin red staining was significantly greater with EMD or rp(H)M180 + rhBMP2/7 stimulation (4.2- and 3.8-fold increase, respectively) compared to dexamethasone, rhBMP2/7, or rp(H)M180 (3.1-, 2.7- and 1.6-fold increase, respectively). The addition of rhNg to the medium also partially inhibited the EMD induced matrix mineralization in hPDL cell cultures, confirming the involvement of BMPs in these responses ($p = 0.004$).

At day 21, calcium deposits by hPDL cells stimulated with rp(H)M180 were moderate, showing greater values when stimulated with Dex, rhBMP2/7 alone, and the greatest deposit when treated with EMD or rp(H)M180 + rhBMP2/7. Incubation of hPDL cells with rhNoggin (Ng) incompletely inhibited the EMD induced mineralization ($p < 0.05$).

One author (41) tested the response of human mesenchimal stem cells derived from bone marrow stimulated with two different culture mediums (osteoinductive and normal growth medium) with EMD at 100 ng/mL, or recombinant human full-length amelogenin (rh174) at 10 and 100 ng/mL. Calcium concentration was determined using a Calcium C-Test and measuring the absorbance using the Bio-Rad Model 550 microplate reader (Bio-Rad) at 570 nm. The Authors concluded that calcium concentration in the EMD treated group, was significantly higher than that in the control group from day 18 ($p < 0.05$) to day 26 ($p < 0.01$), and the staining level with Alizarin red S became stronger by the addition of rh174 as well as of EMD in a dose-dependent manner.

The role of a small portion of the amelogenin protein, corresponding to seven amino acid sequence (WYQNMIR) codified by the amelogenin gene (XX) exon 5, was synthesized (SP) and tested in two independent papers (35-36).

A greater number of calcified nodules deposed by human PDLSCs treated with normal culture medium with the synthetic oligopeptide (SP) derived from enamel matrix derivative was observed by Kato (36), after samples staining treated cells with Alizarin Red S solution (1%) and measuring the calcium deposition by Calcium E-test protocol. He reports that calcium deposition is increased from day 14 to day 21, after the inclusion of SP. However, mineralization was higher in the presence of SP (100 ng/mL) compared with its absence at both 14 ($p = 0.012$) and 21 ($p = 0.00003$) days.

Using the same SP investigated by Kato, Katayama (35) conducted an *in vitro* experiment investigating, for the first time, the role of this SP on the production of Procollagen Type 1 C-Peptide (PIP) and calcified nodules of treated human mesenchymal stem cells (MSCs). In this exsperimental study, SP was used at the concentrations of 0, 1, 10, 100 and 1000 ng/ml in MSC culture medium.At concentration of 10 ng/ml, the authors observed that SP had the strongest effect of human MSCs. After 7 and 14 days the authors reported that SP promotes the number of mineralized nodules and PIP production (day 7 and 14, $p < 0.05$, *vs* SP 0 ng/ml, respectively). Moreover, their results suggest that SP promotes cell proliferation, osteoblast differentiation and mineralization in human MSCs through the ERK 1/2 pathway and that SP is advantageous because it can be artificially synthesized and is not likely to induce antibodies in the host.

In another work (43), authors tested amelogenin-null (KO) and wild tipe (RW4) embrionic stem cells (ESCs) in two different type of culture medium. The quantification of calcium accumulation in the matrix was achieved using the Quanti Chroma calcium assay kit to measure the amount of free calcium. The visual record from Alizarin red staining showed that calcium concentration in the control group was significantly higher than the basal group. The calcium accumulation in the Leucin rich amelogenin protein (LRAP)-treated group was significantly higher than the control group in both RW4 and KO ES cells. Comparing RW4 and KO ES cells, a significant decreased level of calcium accumulation was observed in the basal, control, and LRAP treated groups. Noticeably, LRAP could partially rescue the reduced level of calcium deposited in the matrix created by the KO ES cells.

In vivo study group

In vivo articles were published between 2001 and 2014, and 33% of them after 2010 (see Table III a and b). Two articles were conducted using dogs, four articles using rabbits, nine with rats and one with minipigs. Unlike *in vitro* studies previously treated, no author has tested peptides or recombinant amelogenins enriched with leucinein but they used enamel matrix derivative (EMD) only. In particular, in four studies (44, 46, 50, 55) EMD alone were used, and the other 11 authors (45, 47-49, 51-54, 56-58) used EMD in combination with other bone substitute materials.

Four papers (44-45, 57-58) evaluated the effect of EMD around titanium implant.

In the paper from Birang (44) , the authors created bone defects in the tibia of rabbits. The defect on the right leg was filled with EMD, and the

Table II. a, **b**) *In vitro selected studies*.

a.

Author	Cells	Enamel Protein (EP)	Groups	Investigated	Culture Timing	Nodule Size	Ca Concentration	Significance Level
Galli et al. 2006	Human jaw osteobals	EMD	G1: no EMD (control)	Staining with Alizarin Red	21 days	G1: 240 pixel	/	p < 0,001
			G2: 20 micro gr/mL			G2: 260 pixel		
			G3: 50 micro gr/mL	Computer morphometric analysis (Nodules Size in pixel)		G3: 450 pixel		
			G4: 100 micro gr/mL			G4: 400 pixel		
Katayama et al. 2014	Human Cartilage MSC	SP	G1: no SP (control)	Alizarin Red staining	7(*) and 14(+) days	/	G1: 4,9* ; 17+ mg/dL	P < 0.05
			G2: 1 nano gr/mL				G2: 5,4* ; 18+ mg/dL	
			G3: 10 nano gr/mL	Ca concentration			G3: 5,7* ; 19+ mg/dL	
			G4: 100 nano gr/mL				G4: 5,6* ; 19+ mg/dl	
			G5: 1000 nano gr/mL				G5: 5,5* ; 18+ mg/dL	
Kato et al. 2013	hPDLSCs	SP	G1: no SP (control)	Alizarin Red staining	14(*) and 21(+) days	/	G1: 8 * ; 20+ mg/dL	at 14 days P= 0.012; 21 days P = 0.00003
			G2: 100 nano gr/mL	Calcium concentration			G2: 16* ; 26+ mg/dL	
Kémoun et al. 2011	hPDLSCs	rp(H)M180 (G2) and EMD (G3)	G1: Control	Alizarin Red staining	21 days	/	G1: 160 micro moli x well	p < 0.01 compared to control
			G2: 5 micro gr/mL (rp(H)M180)				G2: 250 micro moli x wel	
			G3: 50 micro grammi/ mL (EMD)	Ca concentration with spectrophotometer			G3: 660 micro moli x wel	
			G4: 50 micro + 500 nano /mL (EMD + RhN)				G4: 300 micro moli x wel	
			G5: 100 nano + 5 micro gr/mL (BMP2/7 + rp(h) M180)				/	
			G6: 100 nano gr/mL BMP 2/7				/	
			G7: desametasone				G5: 620 micro moli x wel	
Nagano et al. 2004	hPDL	EMD Sol and Gel	G1: Control with PGA only	Alizarin Red staining	15 days	/	G1: 0,018 mg/cmq	p < 0,01 compared to control
			G2: 1 mg/mL GEL				G2: 0,037 mg/cmq	
			G3: 1 mg/mL EMD	Calcium concentration			G3: 0,025 mg/cmq	p < 0,05 compared to control

defect on the opposite leg was left unfilled as control. In the left and in the right tibia, authors placed the implants with and without EMD, respectively. In this study, the postoperative protocol consisted of the administration of antibiotics and intensive care until the animals were sacrified.The dogs were sacrified 2, 4 and 6 weeks after implantation. Histomorphometric evaluation was performed via measurement of the percentage of the woven, lamellar, and total generated bone. Authors reported that the percentage of total generated bone in the test group was higher in respect to the control group, although not statistically significant (P = 0.917).

Casati (45) created buccal dehiscence defects (3.5 x 5.0 mm) in dogs before and 2 months after implant placement. The buccal dehiscence defects were treated with a resorbable membrane (GBR) or EMD alone or a combination of both. The percentage of bone to implant contact (BIC) and new bone area (NBA) of each implant was determined. After 3 months, no statistically significant differences were observed between the groups in terms of BIC.

b.

Author	Cells	Enamel Protein (EP)	Groups	Investigated	Culture Timing	Nodule Size	Ca Concentration	Significance Level
Tanimoto et al. 2012	hMSCs	rh174	G1: ODM with out rh174	Alizarin Red staining by absorbance 405 nanometri	14, 26 days	/	G1: 50 ppm at 18 days	Staining: G1 vs G3 $p < 0.01$
			G2: ODM with rh174 10 nanogr/mL				G1: 185 ppm at 26 days	G2 vs G3 $p < 0,05$; G1 vs G6 $p < 0.05$
			G3: ODM with rh174 100 nanogr/mL				G3: 75 ppm at 18 days	G1,G2,G3 vs G4 $p < 0,01$
		EMD	G4: GM with rh174 100 nanogr/mL	Calcium concentration			G3: 210 ppm at 26 days	Calcium: Control Vs Experim. $P < 0,01$
			G5: GM with EMD 100 nanogr/mL				/	
			G6: ODM with EMD 100 nanogr/mL				/	
Van den Dolder et al. 2006	Rat BMCs	EMD	G1: EMD coated 100 micro gr/mL	Calcium content	8, 16, 24 and 32 days	/	G1: 470 micro gr/mL	$p < 0,01$
			G2: EMD no coated 100 micro gr/mL				G2: 375 micro gr/mL	
Warotayanont et al. 2008	Mouse ESCs	LRAP	G1: Basal media	Alizarin Red staining	20 days	/	G1: 0,7	$P < 0.05$
			G2: Control media				G2: 2,3	
			G3: 10 nano gr/mL	Calcium concentration			G3: 6,3	
			G4: 100 nano gr/mL				G4: 5,7	

G: Group; EMD: Enamel matrix derivative; Emdogain, Straumann; SP: Syntetic oligopeptide; hPDLSCs: Human periodontal ligament stem cells; hPDL: human periodontal ligament cells; MSCs: Mesenchimal stem cells; BMCs: Bone mesenchimal stem cells; ESCs: Embryonic stem cells; rp(H)M18: Recombinant poly(His) tagged mouse 180 amino acid amelogenin. RhN: RhNoggin. rhBMP 2/7: Recombinant human bone morphogenetic protein heterodimer 2/7; rh174: Recombinant human full-length amelogenin; LRAP: Leucine-rich amelogenin peptide.

However, the EMD + GBR group presented a greater ($p < 0.05$) area of new bone compared to the control group. The groups treated by EMD or GBR alone showed no statistically significant differences in NBA compared to controls or to the EMD + GBR group.

In his work, Shimizu (57) inserted cylinder-shaped mini titanium implants (1.6 x 3.5 mm) and filled the medullary cavities with either EMD or its carrier (PGA) alone. The author did not declare the number of rats used. Mean percentage of newly formed trabecular bone per medullary cavity was assessed. In morphometric analysis, the newly formed trabecular bone area within medullary cavities was significantly ($P < 0.05$) greater in EMD-treated femurs than in PGA-treated femurs at 30th day post-implantation.

Stenport (58) placed one implant in each femur and two in each tibia of rabbits, after EMD or PGA injection into the surgically sites. After 6 weeks, histomorphometrical quantifications were made on ground sections by measurements of the percentage of bone to metal contact, bone area inside the threads as well as outside the threads. Authors have reported that EMD-treated implants had a higher mean value than the implants treated with the vehicle gel only, however, these differences were not statistically significant.

Two authors (47, 52) tested bone formation ability of EMD in rat jaw and rabbit calvaria using teflon and titanium caps with or without bone substitutes.

Donos (47) randomly divided 20 Wistar rats into four groups and surgically inserted on external mandibular ramus surface PTFE capsules with an

internal diameter of 5 mm and a wall thickness of 0.5 mm, empty, with EMD, with deproteinized bovine bone mineral (DBBM) or EMD + DBBM. The animals were sacrificed at 60 and 120 days after the surgical procedure. After histological preparation and planimetric measurements, the percentage of newly formed bone into the capsules were determined. Results showed a statistically significant difference between G1c and G2c ($P = 0.034$), G1a and G2b ($P = 0.027$) and G1c and G2d ($P = 0.021$). The best results were obtained in the samples with the empty capsules at 60 and 120 days (35.8 and 39.7% of capsule volume, respectively). The authors concluded that the use of EMD in the capsule did not offer any added benefit to the use of the capsule alone in terms of new bone formation and that neither the application of EMD nor the use of DBBM or the combination of EMD and DBBM results in enhanced amounts of bone formation in comparison with the GBR procedure alone.

Murai and colleagues (52) evaluated the effects of EMD and β-TCP on bone augmentation within a hemispherical titanium cap in calvaria of 14 white rabbits, which were sacrificed at 30th and 90th days post surgery. After 1 and 3 months of healing, authors did not find statistically significant differences in the percentage of mineralized bone in the newly generated tissue in control sites, when compared with test sites ($P = 0.075$ and 0.0917, respectively). The present findings indicate that the combined application of EMD and β-TCP did not increase bone formation compared with use of β-TCP alone.

Three authors (46, 50-51) created femoral and tibial defects in their animals to test the percentage of new bone formed with and without EMD application. In the Cornelini (46) paper, the authors created an 8 mm-defect under sterile conditions, 1 per tibia, for a total of 2 defects per rabbit. The defects on the right legs were filled with derived enamel matrix until the material was almost extruding from the defects. The left leg defects were left unfilled (control). After 4 and 8 weeks the differences in the percentage of bone regeneration (new bone) between test and control sites were evaluated. The authors reported a higher percentage of new bone in the EMD group throughout the experimental period. However, only at eight weeks a statistically significant difference was recorded betweenthe control and test grous.

Kawana (50) perforated the femurs of 4 Wistar rats with a sterile cylindrical bar (1.0 mm in diameter), and injured medullary cavities were immediately filled with EMD. Because EMD contains propylene glycol alginate (PGA) as suitable vehicle for local application (3), this carrier, PGA, was used for experimental controls. On 4, 7, 14, and 28 days post-operation, the rats were sacrificed. After dissection of the perforated areas of the femurs, the three-dimensional (3D) architecture of bone samples by micro-computed tomography analysis and quantitative analysis of Ca and P weight % and Ca/P ratio of new bone were examined. Data from the quantitative analysis indicate that the newly-formed trabecular bone volume fraction in EMD-applied femurs was significantly greater ($P < 0.05$) than in PGA-applied controls at 7 days post operation, but there was no significant difference in bone volume fraction between the two experimental groups at 14 and 28 days post operation.

Miron (51) treated twenty seven rats with either natural bone mineral (NBM) or NBM + EMD and carried out histological analysis at 2, 4, and 8 weeks after the surgical procedure. The three groups were randomly divided into three clusters of six defects. Each animal received two types of treatment such as control defects and NBM alone, or control defects and NBM + EMD, or NBM and NBM + EMD at each time point. Defect morphology and mineralized bone were assessed by μCT and conventional histological approach was utilized to quantify new bone formation using morphohistometric analysis. Significantly much more newly formed bone was observed around both NBM + EMD and NBM alone when compared to the drilled control group at all time points ($P < 0.05$). Statistical analysis revealed new bone formation was significantly higher in the NBM + EMD group at 4 weeks when compared to NBM alone ($P < 0.05$). At 8 weeks, no significant difference between NBM + EMD and NBM could be observed although the results still demonstrated increased bone formation in the defects treated with NBM + EMD.

In his work, Intini (48) created 8 mm calvaria critical size defect (CSD) in a rat model. He used five groups of five rats each. The two test groups were DFDBA- and EMD-treated. A negative control consisted of a defect without any biomaterial

implanted, a positive control consisted of a defect filled with collagen carrying rhBMP-2, and a non-surgical control consisted of the intact rat calvaria. Eight weeks after implantation of the biomaterials, the animals were sacrificed and histologic analysis was carried out for qualitative assessments and microcomputed tomography was used for the quantitative assessments of bone formation. The quantitative analysis reported that volume of bone formed within the 8-mm bone defects of DFDBA and EMD groups was not statistically different from the volume of bone formed in the negative control defects ($P = 0.674$ and $P = 0.847$, respectively). Compared to the positive control (rhBMP-2) and the non-surgical control groups, the surface of bone formed by DFDBA and EMD was statistically different ($P < 0.001$) and equal to approximately one-fifth of the bone present in the 8-mm diameter area of normal calvaria (non-surgical control). The Author showed that neither DFDBA nor EMD was able to regenerate a critical size bone defect. Histologically, only DFDBA showed signs of bone repair at the center of the defect. EMD did not show any sign of bone repair in the center of the defect, and the deposition of newly formed osteoid was only seen at the margins of the defect.

In Plachokova's (53) research, we considered only the orthotopic study. Poly(D,L-lactic-coglycolic acid)/calcium phosphate implants, unloaded or loaded with different concentrations (0.25, 0.50 or 0.80 mg per implant) of EMD, were inserted into the cranial defects of 24 rats. Their evaluation consisted of descriptive histology and histomorphometry assessments 4 weeks after sample implantation. The results showed that bone formation was most abundant for unloaded implants and lowest for the 0.25 mg EMD ($P < 0.05$). Statistical analyses identified no significant differences ($P > 0.05$) in the amount of the newly-formed bone among the EMD groups.

Potijanyakul (54) created bone defects (2 mm in diameter) in the left and right rat parietal bone using a trephine bur (5 mm diameter) in a slow speed micromotor under copious saline irrigation with depth equal to the full thickness of the calvarial bone. The bone defects were filled with bioactive glass (BAG) alone, with EMD alone, with BAG + EMD or unfilled. New bone formation was evaluated by radiomorphometry and histomorphometry assessment after a healing period comprised between 2 and 8 weeks. The author found no significant difference in the mean optical density between bioactive glass with EMD and bioactive glass alone; with no defect completely regenerated with bone. The histologic analysis revealed that defects filled with bioactive glass plus EMD in all groups contained slightly more percentage of new bone than those filled with bioactive glass alone; however, the difference was not statistically significant ($P > 0.05$). The highest percentage of new bone formation was present at 8 weeks in the bioactive glass plus EMD group.

Sawae et al. (55) perforated the parietal bones of Wistar rats with a sterile round bur (0.8 mm diameter). The injured bone areas were immediately filled with EMD (test) or its PGA carrier (control) and allowed to heal for 4, 7, 14, 30, and 60 days. The results were expressed as the mean percentages of newly formed bone areas per perforated space. Morphometric analysis showed that only at 60 days post surgery, new bone formation in the EMD-treated parietal bones was significantly greater ($P < 0.05$) in respect to PGA-treated controls.

Shahriari (56) conducted an experimental randomized single blind study with white rabbits. Four equal cranial bone critical size defects ($3 \times 6 \times 0.5$ mm) were created in frontal and parietal bone and randomly grafted with deproteinized bovine bone materials (Bio Oss, Group 1), EMD (Group 2), EMD + Bio-Oss (Group 3), and one of them was left unfilled to serve as a control group (Group 4). After 2, 4, 8, and 12 weeks the defects were evaluated and histological and histomorphometric analyses showed that the amount of the regenerated bone was significantly higher in the EMD + Bio Oss group after 8 (87%) and 12 weeks (96.6%). Bone regeneration of the EMD + Bio-Oss group in 8 and 12 weeks was significantly higher in respect to the other remaining groups ($P = 0.000$).

Jensen (49) created six non-critical size defects in mandibles of 18 minipigs and filled them with G1: autogenous bone chips, G2: biphasic calcium phosphate (BCP), G3: Polyethylene glycol based hydrogel (PEG) + BCP, G4: EMD + PEG + BCP, G5: Parathyroid hormone (PTH) + PEG + BCP, or G6: PTH + amino acid sequence Arg-Gly-Asp (RGD) +

PEG + BCP. The 18 animals were divided into three groups of six animals and were allowed to heal for 2 weeks, 4 weeks, or 8 weeks, respectively. There were no signs of any qualitative differences of the tissues invading the defects or the cells populating the BCP particles when EMD, PTH, or PTH-RGD was added to the BCP-PEG matrix.

DISCUSSION

Tissue engineering is a rapidly growing area of research that aims to create various tissues for the replacement of damaged tissue either through disease or trauma. This is a relatively new and

Table III. a,b) *In vivo selected studies.*

a.

Author	Aim	Animals	EP	Groups	Bone Investigation Methods	Timing	New bone formation	Significance Level
							Mean volume % of new bone	
Birang et al. 2012	Bone formation around implant	Dogs	EMD	G1: Left tibia with EMD	light microscopy	14*, 28# and 42§ days	G1: 50.83 ± 29.25	0,6* - 0,6# - 0,917§
				G2: Right tibia without EMD	% of new bone (fotomicrography)		G2: 42.15 ± 21.87	
Casati et al. 2002	GBR around implant dehiscence	Dogs	EMD	G1: Control	% of new bone formation with fotomicrography	30 and 90 days	G1: 34.07 ± 22.08 / 36.95 ± 25.10	T1 0.055; T2 0.100
				G2: EMD			G2: 41.82 ± 22.03 / 55.55 ± 11.81	
	Dehiscence T1 and T2 (after 2 months)			G3: GBR	Computer/softwerw analysis		G3: 42.89 ± 18.08 / 53.89 ± 16.35	T1 0.700; T2 < 0.05
				G4: EMD + GBR			G4: 34.43 ± 15.75 / 62.15 ± 18.47	
Cornelini et al. 2004	Bone regeneration in tibia defects	Rabbits	EMD	G1: Emdogain in right tibia	light microscopy staining with toluidine blu	30 and 60 days	G1: 30.2 ± 2.28 / 39.8 ± 6.31	T1: .860
				G2: no EMD in left tibia	% of new bone formation		G2: 30.4 ± 4.98 / 41.8 ± 1.31	T2: .507
Donos et al. 2005	GBR in mandible ramus with EMD and/or DBBM	Rats	EMD	G1a: ePTFE capsule only	Section stained with toluidine blue and red fuchsin	G1a: 60	G1a: 35.8	G1a vs G2b 0.027
				G1b: Capsule + EMD		G1b: 60	G1b: 15,2	
				G1c: PTFE capsule only		G1c: 120	G1c: 39,7	
				G1d: Capsule + EMD		G1d: 120	G1d: 17,5	G1c vs G2c 0.034
				G2a: GBR + DBBM	Planimetric measurements	G2a: 60	G2a: 19	
				G2b: GBR + DBBM + EMD		G2b: 60	G2b: 9,5	
				G2c: GBR + DBBM		G2c: 120	G2c: 15,1	G1c vs G2d 0.021
				G2d: GBR + DBBM + EMD		G2d: 120	G2d: 12	
Intini et al. 2008	Bone format. in calvaria critical size defects	Rats	EMD	G1: control	Histologic qualitative evaluation	60 days	G1: 2 mm cubic (Volume)	0.001
				G2: DFDBA			G2: 2 mm cibic (Volume)	
				G3: EMD	microcomputed tomography quantitative eval.		G3: 1 mm cubic (Volume)	
				G3: rhBMP2			G3: 16 mm cubic (Volume)	
Jensen et al. 2011	Bone formation in standard mandible defects	Minipigs	EMD	G1: BCP	% of new bone formation	14, 30, 60 days	G1: 32.27	0.001
				G2: PEG + BCP			G2: 36.57	
				G3: EMD + BCP + PEG			G3: 38.49	
Kawana et al. 2001	Bone regeneration in femur defects	Rats	EMD	G1: EMD	Quantitative MCT analysis of bone volume	7, 14 and 28 days	G1: 2.79 ± 3.64	at 7 days p < 0.05
				G2: PGA (control)	Ca/P ratio of new bone		G2: 2.52 ± 2.65	not significant at 14 and 28 days

b.

Author	Aim	Animals	EP	Groups	Bone Investigation Methods	Timing	New bone formation	Significance Level
Miron et al. 2014	Bone regeneration in femur defects	Rats	EMD	G1: Control		14, 28 and 60 days	G1: 0.1 ; 3.8 ; 5.2	< 0.01
				G2: NBM	micro TC		G2: 8.5 ; 12 ; 14	
				G3: NBM + EMD			G3: 9.5 ; 15.5 ; 16	
Murai et al. 2005	Calvaria GBR with caps	14 Rabbits	EMD	G1: EMD + β-TCP	Histologic evaluation were recorded using a computerizd image analysis	30 and 90 days	G1: 42.2 ± 13.1 ; 43.3 ± 3.3	0.075 at 1 month
				G2: β-TCP alone			G2: 36.8 ± 10.3 ; 41.2 ± 10.6	0.917 at three months
Plachokova et al. 2008	Bone regenerative in cranial defects	24 Rats	EMD	G1: PLGA + CPC	Histomorphometry	28 days	G1: 54 ± 15	no significant differences
				G2: PLGA + CPC + 0.25 ηg			G2: 19 ± 22.5	P > 0.05 tra G1 e G2
				G3: PLGA + CPC + 0.5 ηg			G3: 40 ± 23.6	
				G4: PLGA + CPC + 0.8 ηg			G4: 26 ± 17.6	
Potijanyakul et al. 2010	Bone formation in calvarium defects	20 Rats	Emdogain	G1: BAG + EMD	Radiomorphometry	G1 and G2 : 14, 28 and 56 days	G1: 5.2 - 7 - 18	> 0.05
				G2: BAG alone			G2: 3.5 - 6.8 - 5.9	
				G3: EMD only	Histomorphometry	G3 and G4: 56 days	G3: 12	
				G4: Empty			G4: 7.5	
Sawae et al. 2002	Bone tissue format. in rat parietal defects	30 Rats	EMD	G1: EMD 3 mg + PGA 0.1 ml	Scannin elect. Miscroscopy. MCT analysis	4, 7, 14, 30 and 60 days	G1: 0.37 ± 0.14%; 0.61 ± 0.18%; 0.72 ± 0.08%; 0.97 ± 0.04%	< 0.05 at 60 days
				G2: PGA 0.1 ml (control group)			G2: 0.33 ± 0.05%; 0.61 ± 0.08%; 0.73 ± 0.03%; 0.74 ± 0.17%	
Shahriari et al. 2012	Bone formation in calvarial defects	20 Rabbits	EMD	G1: Bio-oss	Histomorphometry	14, 28, 56 and 98 days	G1: 9.8 ± 0.7; 25.08 ± 1.1; 40.12 ± 0.7; 70 ± 0.8	Best performance G3 < 0.000
				G2: EMD			G2: 8.9 ± 0.8; 40.14 ± 0.9; 78.1 ± 1.0; 83.3 ± 0.9	
				G3: EMD + Bio-oss	Histologic evaluation		G3: 8.9 ± 0.8; 40.14 ± 0.9; 78.1 ± 1.0; 83.3 ± 0.9	
				G4: Empty (control group)			G4: 14.1 ± 1; 49.48 ± 1.2; 60.74 ± 0.7; 74.4 ± 0.5	
Shimizu-Ishiura et al. 2001	Bone induction after bioinert titanium implantation in femur	undeclared n° of Rats	EMD	G1: EMD	Back scattered electron (BSE) microscopy	4, 7, 14 and 30 days	Day 14 - G1: 16.23 ± 1.40	Day 14: p < 0.05
					Energy-dispersive X-Ray (EDX) microanalysis		Day 14 - G2: 12.67 ± 2.06	
				G2: PLGA alone (controls)	LM and TEM observations		Day 30 - G1: 12.82 ± 1.42	Day 30: p< 0.05
					Immunocytochemistry		Day 30 - G2: 9.88 ± 2.31	
Stenport et al. 2003	Bone formation and osteointegration of Ti implants in femur/tibia	6 Rabbits	EMD	G1: 0.5ml EMD	Histomorphometry	6 weeks	G1 EMD: 53 ± 16% NBA	0.51; 0.5
							G2 PGA: 58 ± 11% NBA	
				G2: PGA (controls)			G1 EMD: 4.5 ± 2% BL	0.007
							G2 PGA: 6.0 ± 1.8% BL	

G: Group; EMD:Enamel matrix derivative; Emdogain, Straumann; GBR: Guited bone regeneration; ePTFE: expanded polytetrafluoroethylene; DBBM: deproteinized bovine bone mineral; DFDBA: Human demineralized freeze-dried bone allograft; rhBMP2: Recombinant human bone morphogenetic protein. BCP: biphasic calcium phosphate bone substitute; PEG: Polyethylene glycol–based hydrogel; PGA: propylene glycol alginate.

multidisciplinary science. In recent years, bone tissue engineering has emerged as one of the main research areas in the field of regenerative biomedicine. Despite the exceptional regenerative capacity and/or reparative potential of bone tissue, when creating defects of critical size, the amount of newly formed tissue may prove insufficient, necessitating surgery. Bone tissue engineering (BTE) may represent an alternative approach to conventional bone grafts. BTE is a complex and dynamic process that begins with the migration and recruitment of osteoprogenitor cells followed by their proliferation, differentiation and subsequent formation of the matrix with bone remodeling. BTE has made tremendous progress over the years thanks to the use of increasingly efficient scaffold to which are associated growth factors, drugs and gene deliveries. Between different bioactive molecules proposed in the literature for BTE, in this review we focused on the ability of EMPs to promote bone tissue formation. EMPs are mainly composed of amelogenin which is a peptide expressed by tooth germs during development as an adhesion molecule and possesses growth stimulating effects (59). In fact, it has been demonstrated that EMD has a significant influence on cell adhesion, cell proliferation and cell differentiation of many cell types by mediating cell attachment, spreading, proliferation and survival as well as expression of transcription factors, growth factors, cytokines, extracellular matrix constituents and other molecules involved in the regulation of bone remodelling (60-63).

In the present study, twenty-three papers were selected, 15 *in vivo* and 8 *in vitro*. The clinical studies were related to the use of EMD in periodontal defects, and for this reason were excluded. This choice was made because in the regenerative periodontal surgery procedures amelogenins interact with a large number of cell types from different teeth supporting tissues, taken together with other numerous variables (defect morphology, blood clot stability, etc.) make it difficult to understand the osteoinductive potential of EMPs.

The best-known commercially available product containing EMD is EMDOGAIN. The exact composition of Emdogain is not known. However, Gestrelius (9) et al. used different immunoassays to examine whether certain factors were present. Granulocyte macrophage colony stimulating factor (GM-CSF), calbindin D, epithelial growth factor (EGF), fibronectin, basic fibroblast growth factor (bFGF), interferon a, interleukin-1a, -2, -3 and -6, insulin growth factor-1 (IGF-1) and -2, neurotrophic growth factor (NGF), platelet-derived growth factor (PDGF), tumour necrosis factor (TNF) and TGF-a were examined and none was detected.

In this review, 86.96% of the studies, 5 *in vitro* and all *in vivo*, tested Emdogain. In *in vitro* papers, only one study (42) did not report positive results regarding the ability of EMD in calcium phosphate accumulation and cell proliferation. Of the 15 *in vivo* articles, seven (46.67%) reported no benefits in terms of bone formation. Of these, one author (47) specifies that in all groups there were cases in which the capsule had moved from the original position. Overall 44.4% of empty and 48.15% with EMD and/or total DBBM capsules had a slight displacement. This complication makes the results unreliable because there was no stabilization of the blood clot (64).

Of the remaining articles, four (26.67%) reported limited ability but were not statistically significantly and, on the contrary, four (26.67%) showed positive effects in terms of newly formed bone.

Three studies (50, 55-56) noted a significantly higher bone fraction volume of newly formed bone trabeculae 7 days after injury in the EMD-treated group. The results reported by Miron (51) suggest that additional benefits may exist for NBM particles precoated with EMD as this combination improved new bone formation both 4 and 8 weeks post implantation. These results support the clinical evidence that EMD may not only be confined to cementum and PDL regeneration but used also for bone. This evidence also seems to hint that EMD target cells early in their differentiation process and offers rationale for the variegated results when EMD is used to treat osseous defects. Additionally, the activity of EMD can differ depending on lot number (35). Therefore, to best use the clinical data obtained regarding the regeneration effects and postoperative stability of EMD, a completely synthetic peptide containing the active component of EMD is required (35).

These results are in agreement with those reported by Cornelini (46) on the duration of the activity

of EMD (Emdogain is completely resorbed after 4 weeks). Also EMD-based preparations do not favor the stability of the blod clot and space mainteinance (64). In fact, EMD failed to stimulate significant new bone formation in critical and large size defects (47-48, 54).

New stimulating studies have been carried out that have used biomolecules different from EMD. A synthetic oligopeptide (SP), a seven amino acid sequence (WYQNMIR) in amelogenin that corresponds to a portion of the amelogenin gene exon 5 (65), has been produced. The results of the Katayama (35) study suggest that SP promotes both the number of mineralized nodules stained with Alizarin red, as well as the extracellular calcium deposition in treated MSCs. The authors hypothesized that cell proliferation and osteoblastic differentiation of human MSCs are regulated by the ERK 1/2 signaling pathway as well as EMD. Therefore, using the ERK 1/2 inhibitor, PD98059, they examined the molecular mechanism by which SP affects cell proliferation and osteoblastic differentiation in human MSCs. In fact, the inhibitor significantly suppressed cell proliferation and osteoblastic differentiation induced by SP. Therefore, they propose that SP promotes cell proliferation and osteoblastic differentiation thorough the ERK 1/2 signaling pathway. Nevertheless, the detailed mechanism was not completely revealed and it is necessary to examine the mechanism by which SP induces cell proliferation and osteoblastic differentiation. Kato (36) came to the same conclusions using cells of the periodontal ligament. Amelogenin protein, such as rp(H)M180, has recently been shown to enhance the growth rate for human PDL cells and BMSCs (66-68).

The effect of rh174 on the osteogenic differentiation of MSCs, in the Tanimoto (41) research, is consistent with the effect of EMD demonstrated in previous studies (69-70). EMD enhanced mineralization of human bone marrow MSCs from cancellous bone in monolayer culture (70) and immortalized human bone marrow MSCs in 3-dimensional culture with coralline hydroxyapatite scaffold (69). From these results, they suggested that full length amelogenin enhances the mineralization of human bone marrow MSCs. Another peptide investigated was the Leucine-rich Amelogenin Peptide (LRAP), a 59 aa residue protein translated from exon 2, 3, 5, 6 and 7 of amelogenin mRNA (Fincham 1983). It was found to induce osteogenesis in various cell types, including rat muscle fibroblasts (71), mouse cementoblasts (72), and mouse oral mucosal cells (73). Warotayanont (43) confirmed that LRAP induced cell differentiation along osteogenic lineage and mineral deposition.

In conclusion, on the basis of what is reported in literature, the EMDOGAIN osteoinductive property appears to be questionable and unclear if the product is used in bone tissue regeneration. *In vitro* results seem to be more encouraging than those *in vivo*. However, there are discrepancies. In addition to cell type, most studies differ in cell source. Some researchers used calvarial cells to examine the osteogenic capacity, while others used bone marrow cells. Moreover, different types of rats were used for cell retrieval. All these discrepancies in primary conditions can have severe implications for the final results.

In the reviewed *in vivo* articles, the best results were recorded in the presence of restraints and not in large or critical size defects. Whereas EMD showed some osteopromotion in the early healing phases. It is important to emphasize that the observation periods of maximum 4 weeks are very short compared to the time of complete bone repair, about 36 months (74).

Encouraging data are given on the use of SP and recombinant amelogenins, that allow the standardization of the procedure and could minimize the variability due to the preparation of the commercially available EMDOGAIN. However, studies in the literature are few and there are no *in vivo* studies. Based on these data it is necessary to perform further research using compounds based on amelogenins or their active peptides with known composition and concentration. This would help to standardize the results by increasing the effectiveness of the work in order to better clarify the role and the possible application of amelogenins in bone tissue regeneration.

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CASE SERIES

MID-VASTUS FOR TOTAL KNEE REPLACEMENT: IS IT A SAFE APPROACH? AN ULTRASONOGRAPHIC STUDY

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The Mid-vastus surgical approach for total knee replacement has been introduced as a possible alternative to open standard median parapatellar approach to preserve the extensor mechanism avoiding patellar alterations and pain during early postoperative recovery. The goal of this study is to perform a qualitative ultrasonographic evaluation of the vastus medialis oblique muscle (VMO) in order to identify possible ultrasound-visible impairments resulting from surgical stress.

For many years Total Knee Arthroplasty (TKA) approach has become a controversial decision in achieving a good result of the implantation. Although the standard median parapatellar approach is the most used for comfort and better surgical view of the joint, minimally-invasive surgery (MIS) approaches, such as Mid-vastus or Sub-vastus, offer a lesser insult for the extensor knee mechanism as well as a faster and better recovery after surgery (1). This technique in recent years had the support of the development of instruments, allowing small skin incision and bone-cut, less tissue trauma and a good reproducibility (2). In literature we find there are very few studies regarding the instrumental evaluation of the extensor mechanism after total knee replacement using ultrasonography or electromyography rather than comparison of different approaches based on Gait Analysis or functional scales such as KOOS, Oxford Knee Score etc. (3-4).

The aim of this study was to evaluate ultrasonographic qualitative alterations in the vastus medialis oblique (VMO) after mid-vastus approach in patients undergoing TKA.

MATERIALS AND METHODS

After approval of our Institutional Review Board, we enrolled in our study all patients undergoing surgery for primary TKA and available for at least a 2-year follow-up. We excluded all patients undergoing surgery for revision TKA or affected by psychological or neurological situations that could compromise follow-up. Ten patients (7F, 3M) were included consequently in the first data analysis that we used for this pilot study. All patients signed informed consent before enrollment in the study.

Ultrasonography was carried out with an Esaote Mylab 70XVG with multi-frequency linear transducer 7.5 to 13 MhZ one day before surgery, at 20 days and 6 months after surgery. The operator was a rheumatologist, expert in musculoskeletal ultrasound. The examination was made with the patient in a supine position with the knee

Key words: midvastus, TKA, approach, mini-invasive surgery

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extended. Firstly, the VMO muscle at the insertion on the patella (Fig.1) was found, then a qualitative evaluation was made of the muscle in the mid-vastus approach zone, identifying alterations such as fibrotic scar tissue, sero-hematic effusion, muscle ectopic ossifications and other anomalies. All data were blind collected and registered, the examiner was aware whether the ultrasound evaluation was pre- or post-surgery.

All the surgical operations were carried out by the same surgeon, expert in prosthetic joint replacement, who had been performing Mid-Vastus approach in TKR since 2010. The surgical technique was the same described by Laskin (5) and Haas (6) (Fig.2). The surgical technique is initiated with a midline skin incision and deep dissection exposing the VMO. With the knee flexed, the direction of the muscle fibers of the VMO is identified and, following this course, an incision approximately 2-4 cm is made along the VMO fascia and parenchyma. The arthrotomy proceeds distally, in the usual way, through cutting of the medial patellofemoral ligament and capsule to reach the tibial tuberosity. Most of the VMO, including the entire portion attached to the quadriceps tendon, is preserved. The implant used for all patients was the cemented Biomet Vanguard PS Knee replacement system. All the operations were made without tourniquet and no patella was replaced.

On the first day after surgery patients started physical rehabilitation which is the standard protocol of our Department, and consists of a Continuous passive motion (CPM) machine for the recovery period, isometric quadriceps exercises, hamstring stretching. On discharge from the hospital (mean length of stay: 5.5 days) patients had to be able to walk with 2 crutches on the level and going up and down stairs, stand-up from sitting-position or from bed. All patients were recommended to continue the rehabilitation program after recovery for at least 3 months.

RESULTS

Ten patients (7F, 3M; mean age 68.3 yrs) were included in the study. Of these, there were 5 with varus and 3 with valgus knee deformity, and 2 with normal knee mechanical axis before surgery. Two

Table I. *Study population.*

10 patients	7 females, 3 males, mean age 68.3 yrs
Knee Mechanical Axis	5 varus, 3 valgus , 2 normal axis
Ahlback OA Classification	Three grade 5, Five grade 4, Two grade 3

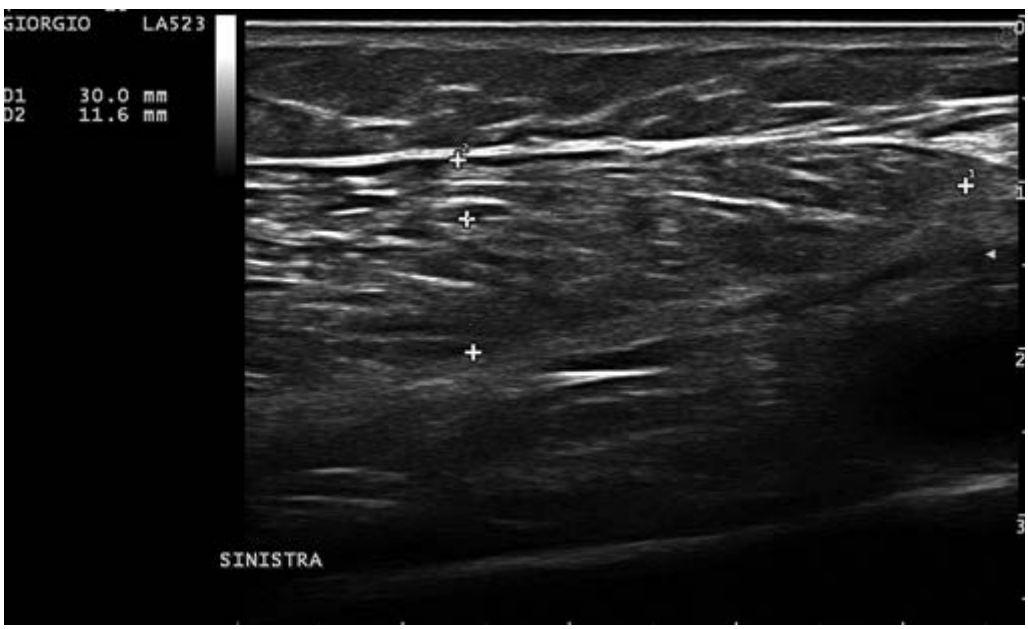

Fig. 1. *Ultrasonography of VMO.*

Fig. 2. *Vastus medialis oblique split.*

patients had a contralateral TKA. According to Ahlback classification system of osteoarthritis (OA) (7) three patients had grade 5, five had grade 4 and two had grade 3 (Table I).

The ultrasonographic evaluation before surgery found no alterations of VMO; in 7 patients there was a large articular effusion. The first control was made 20 days after surgery. We detected in all ten patients absorbable suture knots of VMO, and in just one patient a small seroma in the contest of the VMO belly was found. No other types of alterations were noted. Presence of moderate articular effusion was found in all patients.

At 6 months there was no hyperechoic sign of fibrotic scar in the VMO zone of approach, no absorbable suture knots and no presence of calcifications. The one seroma found at 20 days had disappeared.

DISCUSSION

The result of this pilot study answer in an excellent way our question about the reliability in extensor mechanism sparing of the mid-vastus approach for TKA. At the beginning of the study we expected some fibrotic scar tissue or problems with wound-healing in the zone of VMO. At 6 months ultrasonography of the mid-vastus approach zone was comparable to the pre-operation stage, suggesting that there was no surgical insult. A post-traumatic ectopic calcification inside the VMO was another possible alteration expected as a consequence of the surgical stress (8-9), however, the examiner did not report ultrasonographic signs of myositis ossificans at follow-up. In literature Lin et al. (10) made an ultrasound evaluation for the subvastus approach regarding the transverse cross-section of vastus intermedius, lateralis and medialis. He found significant thickness differences between the operated and healthy knees at 2 months, as well as a greater effusion in the operated knees. However, no-significant difference was seen at 6 months after surgery. It is not specified at what height and zone of the muscles the ultrasound test was made. Dalury et al. (10) had a similar doubt about the midvastus approach regarding possible damage of the VMO. He evaluated 20 patients undergoing bilateral TKA, comparing the standard median parapatellar in one knee and midvastus approach in the other. He decided to evaluate and compare the two approaches by radiographs, electromyography, nerve conduction studies, range of motion tests and knee society function tests. Regarding the instrumental tests at 6 weeks there was an abnormal result of nerve conduction in the mid-vastus group; since this EMG abnormality was transient and the authors did not report a VMO recruitment deficit at 12 months, further studies are needed to clarify this problem. Several studies, such as Nestor et al. (11) or Karachalios et al. (12), reveal that the gain for the patient undergoing TKA with a mid-vastus rather than a standard approach is only for the first month after surgery, and at 12 months after surgery there are no-significant differences between the two approaches. Bonutti et al. (13) compared the sub-vastus to the mid-vastus approach in 51 patients with bilateral TKA. No-significant differences comparing the two MIS techniques suggest that both are excellent as alternatives to the standard one.

One possible limit of this pilot study is the

short follow-up (6 months). Other possible limits are that we did not evaluate patients' outcomes with functional scores or gait analyses, and we did not evaluate the thickness of VMO comparing with the contralateral healthy knee. Despite these limitations we have to underline that the absence of VMO damage, as assessed by the ultrasonography, contributes to the most important goals of the surgical approach to the knee: extensor mechanism sparing, patellofemoral stability, faster recovery of quadriceps muscle strength and reduction of the need to perform lateral release.

Six months after TKA using the mid-vastus approach, there is no qualitative difference in VMO compared to the pre-operative test and with the healthy contralateral knee, which suggests that this approach is a safe alternative for knee arthroplasty without damage or wound complications of vastus medialis oblique.

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THERAPEUTIC STUDY

A CLINICAL CASE OF CHARCOT NEUROARTHROPATHY TREATED WITH A WEDGE SHORTENING MIDFOOT OSTEOTOMY: SURGICAL TECHNIQUE AND GAIT ANALISYS AFTER TREATMENT

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Charcot neuropathic osteoarthropathy (CN) is a chronic arthropathy with progressive evolution and with multifactorial pathogenesis. Reported here is a case of a 50-year-old woman with Type X diabetes of X years duration with a Type II-III Charcot foot and a plantar ulceration treated with a wedge shortening midfoot osteotomy. Gait analysis at 18 months postoperative confirmed a good result, proving to be a valuable tool for understanding the validity of the surgical reconstruction performed. This personalized approach, a multidisciplinary team and patient education may be the best solution to treatment of the complicated Charcot foot.

Charcot neuropathic osteoarthropathy (CN) is a chronic arthropathy with progressive evolution, characterized by the lesions of bones and joints associated with a peripheral and somatic neuropathy. The pathogenesis is multifactorial. Many theories have been developed: repeated microtrauma, mechanical stress and peripheral vascular disease caused by the neuropathy of the autonomous nervous system may change the bones and joints of the foot (1).

More recent studies suggest the possibility of changes of bone metabolism: the most probable seems to be an uncontrolled inflammation process: changes in the so-called RANK-L complex could interfere with the function of osteoclasts (2).

Clinically the diagnosis of acute Charcot foot must be considered for any patient with diabetes, unilateral swelling, pain and heat of the foot. Weight-bearing radiographs, MRI or CT, are recommended for all patients to aid in determination of the extent of bone and joint degeneration. MRI is the gold standard in early phases of the disease to evaluate the subtle changes of a complicated foot in diabetic patients and has a high sensitivity and specificity for osteomyelitis (3).

The most used classifications are: the Eichenholtz classification that divides 3 stages of the disease; and Sanders and Fryberg RG classification in relation to the clinical presentation of osteo-articular destruction. The Authors stated 5 groups: Type 1 forefoot; Type 2 Lisfranc joints; Type 3 Chopart joints and naviculo-cuneiforms joints, Type 4 ankle and subtalar joints, and a Type 5 calcaneus (4-5).

The most important aspect in the medical

Key words: Charcot's foot deformity, diabetic neuroarthropathy, shortening osteotomy

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treatment of CN is to offload the foot and prevent foot and ankle fractures and additional deformities. Offloading with a total contact cast is the treatment of choice. Therapy with bisphosphonates and bone stimulation with growth factors should be considered (3).

For the surgical treatment of the instability in the CN, literature reports the correction and stabilization of the deformity with osteosynthesis with plates and locking screws, external fixators, the forefoot and midfoot arthrodesis with locking screws and ankle arthrodesis (6).

C*ase report*

A 50-year-old woman with Type II diabetes, under insulin treatment for over 10 years, developed a Charcot foot of type II-III, resulting in valgus abduction of the forefoot in relation to the midfoot. Over a 12 month period, a wide plantar-medial neuropathic ulceration (grade A II Texas wound Classification) measuring 6x5cm developed secondary to the midfoot pronation and due to the use of unprotected shoes (7). The ulceration was resistant to medical therapy consisting of non-weight-bearing casts and orthosis (Fig. 1 A-B).

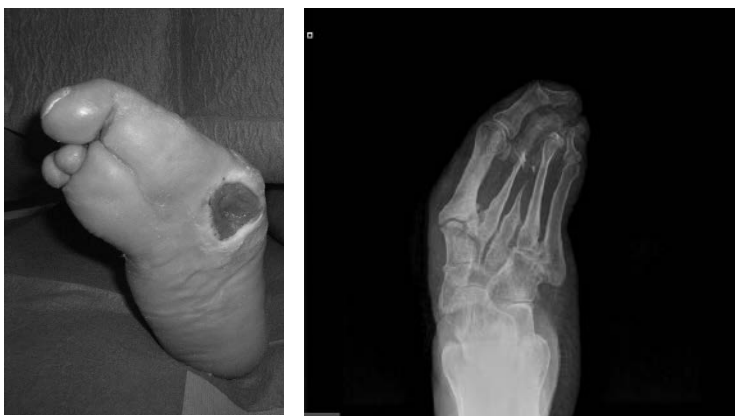

Fig. 1. ***A-B****) Charcot foot Type II-III with a wide plantar ulcer and AP x-ray view. The deformities and remodelling bone of the foot are obvious.*

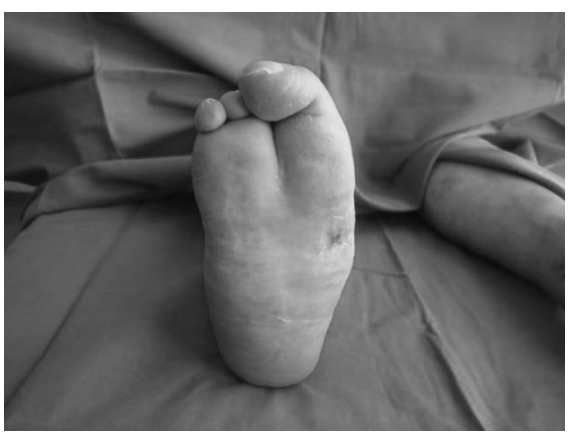

Fig. 2. *Resolution of ulcer and return to a plantigrad foot without recurrence 18 months after surgery.*

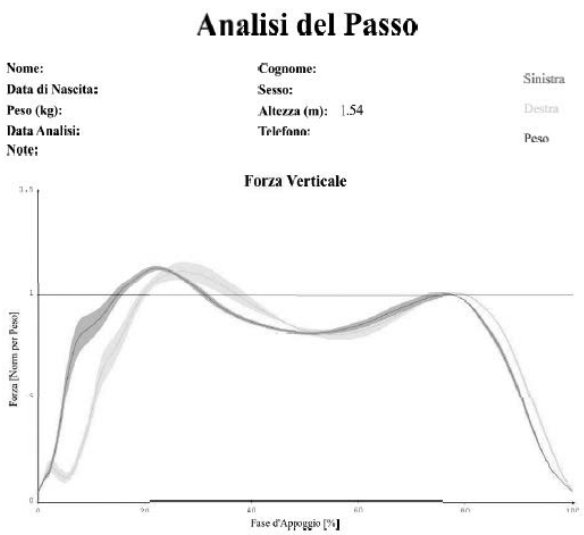

Fig. 3. *Gait analysis exam with shoes. All phases of the step are present.*

There were no clinical signs of inflammation or infection. Peripheral pulses were appreciable. Active infection was excluded through clinical examination, laboratory tests (ESR, CRP) and a microbiological exam of plantar ulcer (negative for bacteria and fungi). Echo-Doppler was performed which excluded any component of ischemia. On the radiographs we observed collapse of the midfoot and deformities at the Lisfranc joint with forefoot abduction, there it was decided not to carry out further investigations and, in agreement with the patient, to operate.

The patient was positioned supine on the operating table with an ankle tourniquet at 280mmHg maintained for 60'. Through a dorsal longitudinal incision centered over the first inter-metatarsal space measuring approximately 10 cm, the neurovascular bundle was identified and protected with lateral retraction. The cuneiform bones were then identified with direct fluoroscopic imaging and a wedge shortening trans-cuneiform osteotomy with the base medially and the apex laterally was performed. Under fluoroscopic guidance, the valgus deformity and pronation of the forefoot was noted to be reduced. The osteotomy was fixated with two 2 mm Kirschner wires.

Restoration of the longitudinal axis of the foot reduced tension on the plantar skin enabling primary closure to be performed. The closure was dressed in a soft, compressive dressing. The patient was kept non-weight bearing for 8 weeks. The Kirschner wires were removed at 6 weeks postoperative and a control X-ray was carried out. This technique allowed to obtain healing of the neuropathic ulcer, a good correction of the deformity and a rapid and stable consolidation.

At two months postoperative, the patient was allowed partial weight-bearing in an aircast tutor for two months. At four months postoperative, the patient was allowed to be full weight bearing in a a hard-soled shoe with gradual return to normal activities. The patient had no recurrent wound at 18 months postoperative (Fig. 2).

A gait analysis was performed at 18 months postoperative with the patient barefoot and then in her normal shoes. Gait analysis revealed alteration of the stance phase (midstance period) still present on the operated foot when the patient was barefoot. Conversely, with shoes, the step cycle was significantly improved, allowing to clearly distinguish all stages of the step and demonstrating good correction of the longitudinal axis of the foot (Fig. 3).

In presence of a peripheral neuropathy that changed pain perception, the wide plantar ulcer and the considerable foot deformity, it was not possible to use any validated clinical score (e.g., SF-36, AOFAS score, Foot Function Index). The patient was asked to rate her satisfaction after surgery with her ability to walk with shoes and perform normal daily activities, declaring the result as excellent, good, fair, or poor. The satisfaction was rated excellent.

DISCUSSION

The treatment of complicated Charcot foot is very complex. A surgical treatment pathway is not well-defined. Each patient represents a significant challenge, even for an experienced foot surgeon. The case reported here shows a very good result with a patient-specific technique. Most papers are case reports and case series with low numbers and the results vary widely from case to case. Failures are even reported in experienced hands (8). Grant et al. published a retrospective analysis of 44 patients (50 feet) treated with a surgical approach divided into four phases: Achilles tendon lengthening, arthrodesis, bone stimulation by autologous growth factors and final stabilization with external fixators, reporting 36 stable syntheses. Reported complications were: 13 cases of infection of the pin, 9 of wound dehiscence, 8 osteomyelitis and 2 cases of amputation (9). A paper by El-gafary et al. on 20 patients treated with external fixators, showed 75% of infections of the pin (10). Assal et al. evaluated 15 patients treated with arthrodesis of the medial column and reported one case of amputation and 4 of nonunion (11). Sammarco reported on 22 patients treated with midfoot arthrodesis, 6 cases of breakage and 6 of migration of the screws plus eight aspecific complications (12). Lowery et al., in a systematic review of 95 articles, emphasized that all these were level IV and V of evidence (8). Varma et al. recommended the need of an individualized approach for each patient and a multidisciplinary team to treat the patient in the best way (3).

In our case, gait analysis 18 months after

treatment, confirmed the good results of our approach, with restoring a proper gait cycle and a correct weight distribution due to ulcer healing, to the restoration of a plantigrade foot and to the use of specific footwear.

For the success of the treatment, the accurate planning of each case, a multidisciplinary team and the patient compliance are absolutely necessary factors. The presence of these factors, combined with an individualized preoperative planning, may provide the best solution for the treatment of Charcot foot.

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LETTER TO THE EDITOR

THE RISK FACTORS AND SURGICAL TECHNIQUE ANALYSIS OF ROTATOR CUFF RETEAR AFTER ARTHROSCOPIC REPAIR

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Rotator cuff tears (RCTs) are an ubiquitous cause of shoulder pain and disability which seriously influence the quality of life of patients. Nowadays, arthroscopic repair has become the mainstay in the treatment of significant RCTs which have failed conservative therapy. However, despite small- and medium-sized RCTs being successfully repaired in the vast majority of cases, high rates of failure of arthroscopic rotator cuff repairs (ARCR) have been reported for large and massive tears. The failures are attributed to multiple factors, including age, size of the tear, and surgical technique. The purpose of this paper was to review the risk factors for retear of the ARCR.

Rotator cuff tears (RCTs) are a common source of shoulder pain and occupational disability, the prevalence of which is rising, particularly among the elderly. Reported incidences of RCTs range from 13% in subjects aged between 50 and 59 years old, 20% to 30% in individuals aged between 60 and 80 years old and up to 50% in individuals older than 80 years (1-2). Arthroscopic rotator cuff repair (ARCR) has now become the mainstay in the treatment of significant RCTs which have failed conservative therapy. Compared with the traditional open technique, ARCR offers patients less soft-tissue trauma and improved postoperative rehabilitation (3-4). However, despite small- and medium-sized RCTs being successfully repaired in the vast majority of cases, concern remains regarding healing failures for large and massive tears. Many studies have documented structural failure up to 90% of large and massive tears during postoperative 1-2 year follow-up (5-6). These high failure rates of are attributed to multiple factors, including age, smoking, size of the tear, poor tendon quality, suture device failure (suture breakage, knot slippage, suture anchor pull-out), trauma, and surgical technique. The purpose of this paper was to review the risk factors for retear of the ARCR.

GENERAL FACTORS

Age

Age is an important prognostic factor for the healing of RCR. Lichtenberg et al. (7) found that the failure rate of isolated supraspinatus repair rose from 9% among patients under 55 years of age to 53% among patients over 65 years. Cho et al. (8) assessed the tendon healing of the ARCR using MRI. After a minimum of six months follow-up, the results showed that complete healing was observed in 43 (87.8%) out of 49 shoulders ≤50 years, in 54 (79.4%) out of 68 shoulders 51≤ and ≤60years, and in 34 (65.4%) out of 52 shoulders ≥61 years. The incidence of rotator cuff recurrent tears tended to

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increase with age at the time of surgery.

Smoking

Smoking is an important risk factor for the development of RCTs (9). Lundgreen et al. (10) assessed the effect of smoking on supraspinatus tendon degeneration. They found the supraspinatus tendons from smokers presented more pronounced degeneration with associated reduced cellularity, increased proliferation, and increased apoptosis when compared with non-smokers (10). Carbone et al. (11) found there was an increasing daily average number of cigarettes and a total number of cigarettes smoked in life across patients with increasing severity of tears. Smoking has also been associated with poorer outcomes in patients with rotator cuff repair (RCR). Mallon et al. (12) examined the outcomes of patients who underwent surgical treatment for RCTs. There were 95 smokers and 129 non-smokers. After 1 year postoperative follow-up, they found that the non-smokers had a significantly greater increase in total University of California, Los Angeles (UCLA) shoulder rating scale scores and a significantly higher improvement in pain scores than smokers. Santiago-Torres et al. (13) reviewed the effect of smoking on rotator cuff and glenoid labrum surgery. They found that smoking had a negative influence on RCR clinical outcomes and was associated with decreased healing of small-medium RCTs after repair (13). It was reported that nicotine delayed tendon-bone healing after RCR perhaps due to increasing persistence of inflammatory markers, contracting vessel and reducing the delivery of oxygen to tissues (14).

Diabetes

It is well known that diabetes is a strong risk factor for rotator cuff pathologies (15-16). Abate et al. (17) evaluated the prevalence of shoulder lesions in 80 asymptomatic elderly subjects (48 subjects with diabetes and 32 controls) using ultrasound examination. The authors demonstrated that the degenerative features and tears in the supraspinatus tendon were more frequently observed in diabetics. Diabetes may also affect tendon-bone healing after RCR (18). Cho et al. (19) compared the clinical and structural outcomes between diabetic and non-diabetic patients after ARCR using MRI. After a minimum of six months (range, 6-12 months) follow-up, the retear rate in diabetic patients was significantly higher than that in non-diabetic patients (35.9% *vs* 14.4%). According to the severity of sustained hyperglycemia in the diabetic group, the retear rate in controlled diabetic patients was significantly lower than that in uncontrolled diabetic patients with poor glycemic control (25.9% *vs* 43.2%). Based on this study, the risk of structural failure after RCR in diabetic patients with poor glycemic control may be higher than that in euglycemic patients. In addition, after a surgical repair, diabetic patients may have worse results than non-diabetic patients (20). Dhar et al. (21) compared the results of 56 patients with diabetes and 67 patients without diabetes, all of whom underwent ARCR with 1 year of follow-up. They found that patients with diabetes had a lower improvement in range of motion (forward flexion, abduction, and external rotation), American Shoulder and Elbow Surgeons (ASES) score, and Penn Shoulder Score (PSS) although there were no significant differences in recurrent tears between them.

Size of the tear

Whereas small- and medium-sized RCTs are successfully repaired in the vast majority of cases, the re-rupture rates of large and massive RCTs remain very high. Galatz et al. (5) evaluated the anatomic results of eighteen patients who had complete arthroscopic repair of a tear measuring >2 cm in the transverse dimension (fifteen of them had tears of >3 cm) using ultrasound examination. After a minimum of 2 year postoperative follow-up, they found that the retear rate reached 94% (17 of 18). Furthermore, Bishop et al. (6) reported the overall retear rate, after ARCR, was 47% (19/40). According to the size of tears, the retear rate was 16% in less than 3 cm tears, 76% in greater than 3 cm tears, and 88% in greater than 5 cm tears. According to the measurements performed during surgery, Cho et al. (8) classified the RCTs into small-sized, medium-sized, large-sized and massive. After a minimum of six month follow-up, complete healing was observed in 29 (96.7%) out of 30 small tears, in 62 (87.3%) out of 71 medium tears, and in 40 (58.5%) out of 68 large and massive tears. It appears that the bigger the intraoperative tear size, the lower the rate

of complete healing.

Rotator cuff muscle atrophy and fatty infiltration

Rotator cuff muscle atrophy and fatty infiltration can affect the tendon-bone healing after RCR (22-23), which leads to subsequent failure of ARCR (24-25). Cho et al. (8) evaluated the fatty degeneration for each muscle of rotator cuff tears using the five stage grading system developed by Goutallier et al. (24). The global fatty degeneration index (GFDI) was calculated for each shoulder and the postoperative MRI was performed at a minimum of six months after surgery. Complete healing was found in 92.5% of shoulders with GFDI ≤0.25, in 88.3% of shoulders with an index of 0.25≤ and ≤1, in 52.9% of shoulders with an index 1≤ and ≤1.5, and in 38.5% of shoulders with an index 1.5≤ and ≤2. The recurrence of tears was observed in all nine shoulders with an index ≥2. The findings suggested that as the severity of the preoperative fatty degeneration of the cuff muscles was higher, the rate of complete healing was lower and the retear rate was greater. Therefore, the presence of fatty degeneration is a major prognostic factor for the outcome of RCR.

Cortical thickness and bone density

Roth et al. (26) found that the ultimate pull-out strength and fatigue life of suture anchors depend directly on the cortical thickness. Decortication of the rotator cuff footprint decreases the biomechanical stability of anchors in the proximal humerus. As a result, loss of anchor fixation strength leads to failure of the RCR. Therefore, the footprint of the rotator cuff should not be completely decorticated in order to preserve the biomechanical stability of the anchors. Furthermore, the bone density where the anchors are implanted may also affect the biomechanical stability of anchors at this site (27-28). Tingart et al. (27) evaluated the bone density of greater tuberosity in a biomechanical cadaveric model using quantitative CT scans. The authors demonstrated that the anchors' pull-out strength was significantly higher in regions of the proximal humerus with higher bone density than in lower ones (29). Pietschmann et al. (28) similarly evaluated the suture anchor fixation strength in osteopenic *vs* non-osteopenic bone for RCR. They found that under cyclic loading, there was a positive correlation between higher trabecular bone density and pull-out strength for the anchors. Chung et al. (30) used computed tomography arthrography (CTA) or ultrasonography to verify the postoperative cuff integrity of 272 patients. The result showed that the overall failure rate of rotator cuff healing was 22.8% (62 of 272). After the multivariate analysis, they found that bone mineral density (BMD), as well as fatty infiltration of the infraspinatus and amount of retraction, was an independent determining factor affecting postoperative rotator cuff healing.

Suture anchor pull-out

The suture anchors pull-out during the early postoperative period may also play a role in the RCR failure (29, 31). Benson et al. (32) reviewed 269 patients (550 metallic suture anchors) who underwent ARCR and found that early anchor pull-out occurred in 6 patients (9 anchors). The overall incidence of early metallic suture anchor pull-out in this cohort was 2.4%. The incidence in RCTs less than or equal to 3 cm was 0.5%, and the incidence in tears greater than 3 cm was 11%. It showed that the risk of suture anchor pull-out increased with larger tear sizes. Furthermore, larger diameter exhibited significantly greater pull-out strength relative to smaller diameter for screw-type anchors (33).

Suture abrasion

Suture abrasion can occur against the anchor eyelet, which leads to subsequent suture failure and pull-out. The orientation of the suture to the eyelet may have an effect on suture abrasion. Bardana et al. (34) found that under cyclic loading, sutures oriented at 45° to the anchor are significantly more prone to abrasion and breakage. Meyer et al. (35) also confirmed the increased suture abrasion and decreased suture failure load at 45°. Besides the orientation of the suture, anchor composition and eyelet design may also influence the suture abrasion. Because of their sharper and rougher edges, metallic anchors have much higher rates of suture rupture following eyelet suture abrasion from cyclic loading when compared with their bio-absorbable counterparts (36-37). In addition, the suture anchor depth also affected the mechanical properties and mode of failure of suture anchor constructs (38). More specifically, deeper anchor placement led to suture degradation.

Others

Trauma can also cause rotator cuff retear. It may occur in the early postoperative period (first 3 months) or in the late period (after rotator cuff healing). A single traumatic event, such as a fall on the outstretched hand, or patient non-compliance to correct physical therapy limitations (such as overly aggressive postoperative rehabilitation) can result in early failure. Similar to primary RCTs, late traumatic failure can result from acute injuries or repetitive trauma. Furthermore, infection may affect the tendon-bone healing after RCR, which leads to subsequent RCR failure.

TECHNICAL CONSIDERATIONS

Suture tension and number

Because of advances in suture anchor design and knot-tying techniques, the most common mode of failure is at the suture-tendon interface, especially the loaded suture pull-out from rotator cuff tendon (31, 36). A clinical study by Cummins et al. (31) examined the mode of failure at the time of rotator cuff revision surgery. They found that 19 of the 22 shoulders requiring revision surgery showed failure at the tendon-suture interface, with the sutures cutting through the tendon. So, how to decrease the load of the suture-tendon interface has become a research hotspot. Denard et al. (39) considered that increasing the number of points of fixation was one of the simplest ways to improve construct strength. With multiple fixation points, the load per fixation point is decreased, and thus the load that sutures cut out from tissue is similarly decreased. Cummins et al. (40) used an ovine model to study the best combination of anchors and suture techniques for RCR. They found that increasing the number of anchors and sutures per anchor significantly increased the load to failure. Because the number of suture anchors which can be placed in rotator cuff footprint is limited by the size of the bone bed, the best way to increase the number of fixation points is to increase the number of sutures by using double or triple-loaded suture anchors. Jost et al. (41) performed single-row repair with two, four, or six mattress sutures in an ovine infraspinatus tendon repair model and found that cyclic gap formation in the two-suture group was greater than that in the four and six-suture groups after 200 cycles. The average loads to failure of the two, four, and six-suture single-row groups were 274, 362, and 572 N, which indicates that increasing the number of sutures decreased cyclic gap formation and increased load to failure.

Orientation of anchors

In order to increase the suture anchor pull-out strength, many orthopaedic surgeons insert suture anchors at what is termed the pull-out angle (i.e. deadman's angle) ≤45° to the bone surface (42). However, more recent studies have revisited the ideal pull-out angle for RCR. In a cadaveric RCR model, Liporace et al. (43) inserted suture anchors at angles of 90°, 75°, 45°, and 30° relative to the cortical border at the junction of the greater tuberosity and the articular surface, and loaded each specimen to failure. They found that anchors inserted at 75° showed the highest load to failure (219 N) and anchors inserted at 45° showed the lowest load to failure (169 N). Based on these findings, the authors concluded that the recommended suture anchor insertion angle of ≤45° should be reconsidered. Strauss et al. (44) also used a cadaveric shoulder model to investigate the mechanical effects of suture anchor insertion angle for RCR. They found that repairs made with the anchors inserted at 90° to the superior junction of the greater tuberosity and the humeral head articular surface provided better soft tissue fixation stability than repairs made with the anchors inserted at the deadman's angle of 45°. The authors considered that the applied force vector on the repaired tendon occurring with the anchors inserted at the deadman's angle of 45° might have a greater component of shear force than that seen on the repair with the anchors inserted at 90°, leading to early fixation failure. Clevenger et al. (45) also found anchors placed at more acute angles, that is, anchors placed closer to the so-called deadman's angle, failed at lower loads and provided less construct stiffness than anchors placed at angles greater than 90°. Green et al. (46) assessed the effect of the insertion angle and angle of applied load on the pullout strength of screw-in suture anchors. They found that anchors inserted at 90° and applied load of 90° showed the highest load to failure (306 N), while a simulated deadman's angle with a 45° insertion angle and 150° applied load failed at 127

N. Their results showed significant differences with combined insertion angles and angles of applied load and highlighted that the deadman simulation produced one of the weakest constructs tested. Given the fact that repair failure often occurs at the suture-tendon interface, Strauss et al. (44) suggested that suture anchors should be placed in an orientation that optimizes forces across the suture-tendon interface, rather than the anchor-bone interface.

Single-row and double-row repair technique

The most common approach to ARCR involves the use of suture anchors in either a single-row or a double-row configuration. However, which method is better still remains a controversy. Biomechanical studies suggested that double-row repairs have improved the tendon-bone contact area, better initial fixation strength and stiffness, decreased gap formation, and increased load to failure when compared to single row repairs (47-55). Kim et al. (48) evaluated the differences between single-row and double-row repairs on cyclic loading, failure loads and gap formation. They found that double-row repairs had 42% less gap formation, 46% more stiffness and 48% more ultimate load-to-failure when compared with single-row repairs. However, there has been no clinical evidence that the double-row repairs provide an improved functional outcome compared with single-row repairs (56-65). In a multicenter, randomized, double-blind controlled study, Lapner et al. (64) compared the functional outcomes and healing rates of ninety patients who received either a single-row or a double-row repair of the rotator cuff. The anatomical outcomes assessed with MRI or ultrasonography demonstrated that a smaller initial tear size and double-row fixation were associated with higher healing rates. However, the Western Ontario Rotator Cuff Index (WORC) score, Constant score, ASES score, and strength did not differ significantly between groups at any time point (0, 3, 6, 12, and 24 months). Ma et al. (65) also compared the clinical and imaging outcomes of single-row and double-row suture anchor fixation in ARCR. The clinical results showed that the UCLA score, ASES score, and muscle strength in abduction and external rotation had no significant differences between the 2 groups with a minimum 2-year follow-up. Moreover, the imaging results also showed no significant difference in postoperative cuff integrity in both groups at 6-month and minimum 2-year follow-up. Only one study by Park et al. (66) found that tears greater than 3 cm had significantly better outcomes for double-row repair than single-row repair. Koh et al. (62) reported a similar opinion that double-row repair was not required for small RCTs but it might be ideal for large RCTs.

Suture bridge and margin convergence technique

According to Burkhart (67), RCTs can be classified as (1) crescent-shaped, (2) U-shaped, (3) L-shaped, and (4) massive, contracted, immobile tears. Small- and medium-sized tears can be easily repaired with minimal tension. Massive tears generally have greater tendon retraction, usually extending to the glenoid, or even medial to the glenoid. This would cause relatively high tensile stress when single-row or double-row ARCR is performed, which cause failure or retear of the repair. Suture bridge technique has been recognized to provide better resistance to shear and rotational forces over earlier-generation constructs (68). Cho et al. (69) evaluated the clinical results and repair integrity of 123 shoulders (120 patients) that underwent arthroscopic suture bridge repair for full-thickness RCTs. The results showed that suture bridge repair still had a relatively high rate of recurrent defects (33.3%), despite excellent pain relief and improvement in the ability to perform the activities of daily living. Burkhart et al. (67, 70-71) suggested that margin convergence repair should be performed to reduce tension on the repaired tendon edges and enhance the security of fixation at this time. Chen et al (72) used a kangaroo RCR model to evaluate the results of margin convergence versus suture anchors. They found that RCR with margin convergence +/- suture anchor were far stronger than suture anchor alone, both in gap formation and ultimate failure load. However, they also found that progressive gap formation after RCR, regardless of techniques, is present after a number of loading cycles, suggesting that gap formation would inevitably occur after repair of RCTs (72). Burkhart et al. (73-74) estimated that if a gap of 5 mm or greater forms between the tendon edge and bony footprint during the early postoperative period, the likelihood of failure will increase. Repetitive, low-level loading

of the RCR with routine muscle contraction before healing is thought to be the likely mechanism of this gap formation, which causes acute rupture of the repair (73-74). If the gap formation is minimized during early healing period, the rate of tendon healing may be improved. This presents the surgeon with the dilemma of whether to protect a repair by immobilization or to preserve function by early mobilization to prevent stiffness and weakness. The question therefore is: what postoperative treatment regime will protect the repair and still allow enough movement to improve range of movement.

With advances in techniques and instruments, ARCR has become increasingly popular. However, despite good clinical results having been acquired, recent studies have reported a high rate of structural failure after ARCR, especially for large and massive tears. Randelli et al. (75) found that the re-rupture was the most frequently encountered complication associated with ARCR. Galatz et al. (5) reported that the recurrence rate reached 94% following arthroscopic repair of massive RCTs. The ARCR has a number of significant prognostic factors for failure of the repaired cuff. Besides age, smoking, size of the tear, fatty infiltration of rotator cuff muscle belly, suture anchor pull-out and suture abrasion, the surgical technique has also been demonstrated to play an important role in the RCR failure. Although many patients of rotator cuff retears after rotator cuff surgery are not clinically symptomatic (76-77), the high rate of structural failure creates a concern that these tears may eventually become larger and symptomatic with time. And according to prior studies, better clinical results tend to occur in completely healed tendons after surgery (78-80). Therefore, how to promote the tendon healing after ARCR, decrease the retear rate, and improve the clinical outcomes have become urgent and important issues which need further study and discussion.

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