EFFECT OF NERVE GROWTH FACTOR ON CULTURED HUMAN CHONDROCYTES

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Nerve growth factor (NGF) is involved in several joint diseases. It participates in nociception and neurogenic inflammation and its concentrations increase in synovial fluid and tissue from arthritis. However, data about its role in articular cartilage are scant and conflicting. This study analysed effects of different NGF concentrations on cultured human chondrocytes by evaluating cell proliferation, cell phenotype, and gene expression. The MTT test excluded an influence on cell viability. Alcian blue and S100 staining demonstrated that NGF may induce de-differentiation of the chondrocyte phenotype. Real-time PCR showed that NGF did not influence gene expression of type I, II and XI collagen, TGF-β, IGF-1 and metalloproteinase (MMP)-13, while it reduced the expression of MMP-3. These findings show that NGF may have uncertain effects in human chondrocytes. Further investigations by wider gene expression and protein synthesis analyses are required to determine how chondrocytes may be influenced by NGF.

Nerve growth factor (NGF) is a dimeric protein made up of two identical, non-covalently bound polypeptide chains linked by intrachain disulfide bonds. It was the first neurotrophin (NT) to be isolated and is the best characterized. NGF interacts with two cell surface receptors, tyrosine receptor kinase A (trkA) and p75 NT receptor (p75NTR) (1). NGF is involved in nervous, endocrine and immune system function. It is also involved in some inflammatory and rheumatic diseases. It has been demonstrated to participate in pain initiation, inadequate nociception and neurogenic inflammation by stimulating neuropeptide overexpression and activating immune cells (2, 3). NGF has also been suggested to facilitate sensory nerve sensitization or growth in articular cartilage, linking osteochondral angiogenesis to arthritis pain (4). There are few *in vitro and in vivo studies* of NGF in normal and pathological articular cartilage, and their findings about its role are conflicting. NGF and trkA upregulation has been described in chondrocytes from osteoarthritis (OA) patients, suggesting a role for NGF in its pathophysiology. However, it is unclear whether it stimulates chondrocyte metabolism by promoting cartilage repair in OA or enhances the osteoarthritic process in some other way (5). The NGF-trkA complex expressed by OA chondrocytes might confer protection against OA development by slowing down chondrocyte differentiation (6).

The inconsistent literature about the role of

Key words: nerve growth factor, articular cartilage, chondrocytes, cell culture

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HYBRID COMPLEXES OF HIGH AND LOW MOLECULAR WEIGTH: EVALUATION USING AN *IN VITRO* MODEL OF OSTEOARTHRITIS

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Hyaluronan (HA) is central in joint and cartilage functions and to restore synovial fluid viscosity. In patients with osteoarthritis (OA), molecular weight (MW) and concentration of hyaluronic acid (HA) are reduced, diminishing joint lubrication. IL-1β treatment was used to mimic osteoarthritis in a chondrocytes based *in vitro* model. The aim of our research, using this model and human chondrocytes was to assess the anti-inflammatory effect of H/L-HA hybrid complexes (SINOVIAL-HL®) in comparison with HA at high (H-HA) and low molecular weight (L-HA) separately used, through the evaluation of specific biomarkers involved in cartilage degradation and correlated to osteoarthritis. Specifically, TNF-α and IL-6 mRNA were evaluated by qRT-PCR. Cytokines levels were measured using Bio-plex assays and COMP-2 through immunofluorescence staining and western blot. H/L-HA significantly reduced inflammation biomarkers respect to both L-HA or H-HA separately considered at transcriptional and protein level.

Osteoarthritis (OA), is recognized as a metabolically active, dynamic process of degradation and synthesis involving joint, cartilage, bone, synovia, ligaments and muscle (1) that has affected thousands of old and even young people worldwide each year. The main pathological characteristics of OA result from failure of the repair process of the synovial joints that is activated by different damages such as loss of chondrocytes and cartilage matrix leading to biomechanical joint failure (2). The etiology of OA is not completely understood, but considered as a long-term degenerative process and based on different factors such as inflammation, mechanical and physical injury and other metabolic causes (3). It has also been observed that OA presents an important inflammatory component, driving cytokines production into the synovial fluid (4, 5). Pro-inflammatory cytokines such as TNF-α and IL-6 are crucial factors involved in the development and progression of this degenerative pathology (6). Cartilage Oligomeric Matrix Protein (COMP) is an important degradation protein complex of articular cartilage and was investigated as marker of OA (7). As degenerative joints disease, OA progression is correlated to the production of inflammatory factor and to the enzymes responsible either for degradation or for the reduced synthesis of HA. Pharmacological treatments are based on chondroprotective agents that can stimulate the synthesis of collagen and proteoglycans by chondrocytes and production of HA by synoviocytes, also preventing fibrin formation in the subchondral and synovial vasculature. Compounds that show some of these characteristics are endogenous molecules of the articular cartilage, including HA, glucosamine, and chondroitin sulfate (8). In OA, as the cartilage wears down, the level of the HA responsible for the viscoelastic properties of synovial fluid also decrease in the joints. Actually,

Key words: Osteoarthritis, joints diseases, hyaluronan, hybrid cooperative complexes, inflammation, chondrocytes based in vitro model

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COMPARING HIBRYD HYALURONIC ACID WITH PRP IN END CAREER ATHLETES WITH DEGENERATIVE CARTILAGE LESIONS OF THE KNEE

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Cartilage lesions are very common causes of chronic knee pain in athletes. Current treatment options consist in conservative strategies, such as viscosupplementation and platelet-rich plasma injections. This randomized controlled trial aims to investigate the effect of intra-articular Hybrid Hyaluronic Acid injections compared to PRP for the treatment of cartilage lesions among athletes at the end of their career. Since March 2015, 48 professional soccer players were randomized into two groups: 24 patients received 3 injections of HHA and 23 patients received 3 intra-articular injections of PRP. All patients achieved a statistically significant clinical improvement from preoperative to postoperative time in both groups. Patients in the HHA group showed a significant superiority compared to PRP group at 3 and 6 months. Intergroup differences decrease gradually until loss of significance at 12 months follow-up. Athletes with chronic degenerative cartilage lesions of the knee responded positively both to HHA and PRP until last follow up.

Osteoarthritis (OA) is the most common joint disease and one of the most common causes of disability. Indeed, patients with OA have a low quality of life that leads to an important loss of productivity. In literature, OA is recognized as a metabolically active and dynamic process, which involves the degeneration of cartilage and all joint tissues (1). Articular cartilage of athletes is continually solicited during their physical activity; this process may lead to its premature degeneration with subsequent exposure of subchondral bone particularly rich in nociceptive receptors. Cartilage damage also leads to the release of a number of detrimental factors for the joint environment resulting in a progressive breakdown of the articular surface (2).

Metabolic and biochemical changes related to sport activity are similar to the changes described in early stages of OA and contribute to the progressive joint degeneration that occurs in end career athletes (3). The high demands on the joint, proper of impact athletes, lead to a high incidence of cartilage degeneration in asymptomatic athletes. Joint pathologies, such as meniscal tear, ACL o PCL injury or valgus-varus axis deviation, can cause symptoms and lead to a rapid progression of cartilage injury (4). Among conservative strategies, the HA viscosupplementation is currently widespread in clinical practice, with good results reported in several studies (2, 5).

HA is a highly viscous polysaccharide physiologically present in the extracellular matrix.

Key words: hybrid hyaluronic acid, platelet rich plasma, end career athletes, cartilage lesion, knee

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COMPARISON OF THREE NOVEL BIPHASIC SCAFFOLDS FOR ONE-STAGE TREATMENT OF OSTEOCHONDRAL DEFECTS IN A SHEEP MODEL

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In the last years, several tissue engineering techniques have been applied to develop different kinds of osteochondral substitutes to overcome the scarce reparative properties of this tissue. The aim of this study was to generate and compare three biphasic scaffolds in an osteochondral lesion in a large-animal model. A critical osteochondral defect was generated in the medial femoral condyle of 18 skeletally mature sheep. Three defects were left untreated, the remaining lesions were divided into three groups: 5 lesions were treated with a biphasic scaffold made of collagen type I and small cylinders of Magnesium Hydroxyapatite; 5 lesions were treated with a biphasic substituted formed by collagen type I and Wollastonite, 5 lesions were treated with a scaffold made of collagen type I and small cylinders of Wollastonite/Hydroxyapatite. Animals were sacrificed after 3 months and samples were analyzed by CT and MRI, macroscopic evaluation and histology. Our study demonstrated that one of these novel biphasic scaffolds possesses the potential for being applied for one-stage procedures for osteochondral defects.

In the synovial joint, articular cartilage and subchondral bone form a load-bearing system that provides a large range of joint motion with excellent lubrication, stability and uniform distribution of high acting loads (1). Articular cartilage and subchondral bone frequently undergoes degeneration as a result of traumas or disease (2). The osteochondral tissue has a poor healing potential; thus great debate persists about the best available treatment for osteochondral defects. Current surgical procedures (i.e. microfractures or mosaicplasty) lead to the formation of a fibrocartilaginous tissue, which does not exhibit the wear characteristics of native hyaline cartilage (3). Recently, the application of the tissue engineering approaches for the repair of osteochondral defects has received an increasing

Key words: cartilage, osteochondral lesion, scaffold, tissue engineering, animal models

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TRANEXAMIC ACID EFFECTS ON CARTILAGE AND SYNOVIAL TISSUE: AN *IN VITRO* STUDY FOR A POSSIBLE SAFE INTRA-ARTICULAR USE

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The possible toxic effects of intra-articular tranexamic acid (TA) are still debated. The aim of this study was to evaluate TA effects on human cartilage fragments and synovial biopsies. Explant culture of minced articular cartilage underwent prolonged TA exposure. Histological analysis, immunofluorescence and colorimetric assay for quantification of s-GAG and DNA were performed at the end term. Synoviocytes were cultured for 48h in presence of TA. Light microscopy and flow cytometry analysis were performed at the end of the exposure to TA and one week after the treatment. TA exposure did not influence i) the chondrocyte outgrowth and migration, ii) the expression of chondrogenic and proliferative markers and iii) the s-GAG/DNA ratio. TA treatment did not affect synoviocytes' morphology and treated cells were phenotypically similar to control cells. This study demonstrated that TA does not negatively affect chondrocytes and synoviocytes cultured in vitro. Thus, our findings may be clinically relevant in order to validate the intra-articular TA administration during orthopedic procedures.

Blood loss is a common event in orthopedic surgery and it is usually treated with blood transfusions. Tranexamic acid (TA) is an anti-fibrinolytic drug, which has been introduced to prevent excessive bleeding (1-3). The direct application of TA in the joint fluid has been described since the '70s and it was initially proposed for the treatment of joint bleedings in hemophiliac patients during intra-articular operations (4). Currently, TA is routinely used in joint replacements (5) and more recently, in ACL reconstruction (6).

TA is a synthetic analog of the amino acid lysine. TA prevents the formation of plasmin through the inhibition of the proteolytic activity of plasminogen activators. Furthermore, at high concentrations, it is also a noncompetitive inhibitor of plasmin. Thus, TA inhibits tissue fibrinolysis, it prolongs thrombin effects and it ultimately enhances and stabilizes the clot formation.

Intra-articular injection of TA could have different advantages: it would allow for an optimal concentration at the site of bleeding and it would reduce systemic absorption, thus, it would reduce any possible systemic side effects, such as vascular occlusive events (7). Several clinical studies have proven the safety of topical TA administration during joint replacement surgery, however, the effects of this drug on synovium and cartilage are still under

Key words: tranexamic acid, chondrocytes, synoviocytes, in vitro culture

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INTRAOPERATIVE APPLICATION PLATELET RICH FIBRIN, POSTOPERATIVE INJECTIONS OF PRP OR MICROFRACTURE ONLY FOR OSTEOCHONDRAL LESIONS OF THE KNEE: A FIVE-YEAR RETROSPECTIVE EVALUATION

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Cartilage lesions are the most common cause of chronic knee pain. Micro-fracturing is reliable, effective, easy to perform and inexpensive. We propose a novel approach to cartilage lesions where microfractures are performed contextually to intra-operative or post-operative administration of platelet concentrates. We retrospectively evaluate 48 patients divided in 3 groups. Group 1: 15 patients underwent microfractures and intraoperative administration of PRF (PRF group); group 2: 16 microfractures and postoperative injections of PRP (PRP group); group 3: 17 patients with isolated microfractures (Microfractures group). Clinical scores (IKDC, VAS pain) were administered at 2 and 5 years postoperative and MRI was performed to evaluate the lesions of patients according to the MOCART criteria (2006). Patients treated with platelet concentrates achieved better clinical results compared to patients treated with microfracture only. The PRF group showed better results than the PRP group at 2 years, with loss of significance at 5 years. At MOCART score, PRF group obtained better results earlier than the other two groups.

Cartilage lesions of the knee occur in 60% of patients complaining of knee pain. (1, 2). They affect athletes as a consequence of traumatic injuries and sedentary patients, as part of the spectrum of degenerative joint disease and are very painful (3).

Cartilage can tolerate a great amount of stress but avascularity and its low mitotic activity make it less suitable for intrinsic healing or repair process. In fact, normal hyaline cartilage has little or no potential to heal spontaneously when injured (4, 5).

As primary end-points, the short-term aim is symptom relief and resuming daily activities and sports, while prevention of degenerative changes is wished over time. In the management of grade II-III cartilage lesions, restorative procedures seem to be encouraging. Micro-fracturing is reliable, effective, easy to perform and inexpensive, however, the repaired tissue is fibro-cartilage, which is less resilient and stiff than hyaline cartilage (6), Autologous Chondrocytes (ACI) have been successfully implanted in young physically active patients (7) although this procedure is relatively invasive, two stage, and expensive.

A new approach is to administer platelet

Key words: platelet rich plasma, cartilage lesions, microfractures, osteoarthritis, platelet rich fibrin

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PAIN REDUCTION AND IMPROVEMENT OF FUNCTION FOLLOWING ULTRASOUND-GUIDED INTRA-ARTICULAR INJECTIONS OF TRIAMCINOLONE HEXACETONIDE AND HYALURONIC ACID IN HIP OSTEOARTHRITIS

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The scientific literature has shown positive results regarding intra-articular injections of hyaluronic acid in osteoarthritic joints. When injecting in the hip joint, the guidance of ultrasound can provide higher injection accuracy and repeatability. However, due to the methodological limitations in the current available literature, its recommendation in the current practice is still controversial. This study shows that ultrasound-guided intra-articular injections of triamcinolone hexacetonide and hyaluronic acid can improve pain, function and quality of life in patients with symptomatic and radiographic hip osteoarthritis. In addition, the administration of triamcinolone hexacetonide and hyaluronic acid to the hip joint in these patients can delay the need for interventional surgery.

Osteoarthritis (OA) is one of the most common causes of functional disability and loss of autonomy in the elderly population worldwide, causing a massive socio-economic burden. Hip OA prevalence estimates ranges from 3.9% to 6.1% in persons aged between 40 and 75 years, being the second most frequent form of OA, slightly lower than knee OA (1).

The etiology of OA has known to be multifactorial and currently is not completely understood (2), and includes non-genetic (age, gender, body mass index, joint mechanical stress, sedentary lifestyle, joint trauma, participation in sports or work-related activities) and genetic (modified gene expression patterns of the cartilage and subchondral bone) risk factors (3). This systemic and chronic joint disorder involves a slow and progressive deterioration of the articular cartilage, including changes in the underlying subchondral bone and in the lubricating properties of the synovial fluid, leading to a gradual increase on pain and loss of function (3-5). In this sense, several conservative therapeutic approaches have been developed, aiming for the reduction of pain and inflammation, improvement of joint function and reducing the progression of OA (3,6,7).

Key words: hyaluronic acid, viscosupplementation, osteoarthritis, hip, ultrasound-guided

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

HOMOLOGOUS PLATELET-RICH PLASMA FOR THE TREATMENT OF KNEE INVOLVEMENT IN PRIMARY SJÖGREN'S SYNDROME

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Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease characterized by dry eyes, dry mouth, and other clinical manifestations. The most common extraglandular manifestation of pSS is articular involvement and to date their management is unclear. The aims of the current pilot study were to assess the safety and the outcomes of homologous platelet-rich plasma (HPRP) injections in pSS cohort affected by knee arthralgia/arthritis at short-term follow up. This pilot study provides the first evidence that HPRP injections are a safe treatment and induce a short-term clinical improvement. Although the lack of a control group, randomization and long-term follow up prevents the assessment of the real effectiveness of this treatment, further studies are needed to confirm these findings and to determine the mechanism of action, biological changes and disease-modifying properties of PRP.

Sjögren's syndrome (SS) is a chronic inflammatory autoimmune disease, characterized by a focal lymphocytic infiltration of exocrine glands, with a higher incidence in female patients (9:1) and a prevalence of 0.5% in the general population. It is usually diagnosed between the ages of 40 and 60, but it also affects children and the elderly (1). SS is best defined as the triad of dry eyes, dry mouth, and evidence of an autoimmune process mediating these and other clinical manifestations. The most widely accepted classification criteria are those proposed in 2002 by the American-European Consensus Group (AECG) (2). SS is termed "secondary"when it occurs with another systemic rheumatic disease and "primary (pSS)" when such a second disease is absent..

The spectrum of pSS extra-glandular manifestations is broad and includes fatigue, vasculitis, peripheral neuropathy, kidney involvement, interstitial lung disease, lymphoproliferative disease, immunological abnormalities and joint involvement (3). Articular manifestations seem to affect both genders similarly, and are some of the most common extra-glandular pSS manifestations, with a frequency range of 15-90% (4). Joint symptoms can precede the onset of sicca symptoms (17%), occur simultaneously (52%) or also develop after pSS diagnosis with a mean period of 51 months (31%) (5). The most common symptoms reported are arthralgia involving equally small and large joints (6) and intermittent symmetrical non-erosive poliarthritis characterized by tenderness, swelling or effusion affecting mainly the small joints (7).

Recurrent monoarthritis or oligoarthritis are also reported in 10-20% of cases (8). Despite the fact that joints involvement has been reported in the last 30 years, their treatments have been paradoxically poorly studied. Joint symptoms are not adequately controlled by the wide spectrum of pharmacologic options used in clinical practice and chronic therapy

Key words: primary Sjogren's syndrome, homologous platelet, knee involvement, synovial membrane

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BONE MARROW CONCENTRATED CELLS AND STROMAL VASCULAR FRACTION CELLS INJECTIONS FOR OSTEOARTHRITIS TREATMENT: A SYSTEMATIC REVIEW

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The aim of this systematic review is to examine current clinical evidences supporting the intra-articular injection of bone marrow concentrate cells (BMC) and adipose-derived stromal vascular fraction cells (SVF) for the treatment of osteoarthritis (OA). The research was performed on PubMed (Medline), EMBASE and Cochrane Library considering the English literature. Only clinical trials have been included. The systematic research identified twelve clinical trials. Articles included in the study, were one of level II, four of level III, six of level IV and one level V. Among clinical trials, none were randomized, four were comparative, seven were case series, and one was a case report. Seven studies were focused on the use of SVF (1332 patients) and five on the use of BMC (963 patients), with preliminary interesting findings in the OA treatment. Despite the growing interest in this biological approach for OA, knowledge on this topic is still preliminary. Randomized controlled trials are needed to support the potential of BMC and SVF injections and to evaluate advantages and disadvantages with respect to the available treatments.

Osteoarthritis (OA) is the most common joint disease, affecting about 10 to 25% of the population. It is characterized by an increasing incidence in western world because of the longer life expectancy and obesity (1). OA is characterized by functional impairment and clinical symptoms such as pain, stiffness, tenderness and joint swelling. The main pathologic feature of OA is degradation of articular cartilage, but other joint tissues, such as synovial fluid and membrane and subchondral bone are involved (2).

Therapeutic strategies for OA include systemic administration of non-steroideal inflammatory drugs (NSAIDs), glucosamine and/or chondroitin-sulfate, intrarticular injections of hyaluronic acid or growth factors as platelet rich plasma (PRP) (3). To date, all the described therapies have demonstrated to partially relieve symptoms, minimize disability and temporary increase joint function. None of them are able to regenerate joint tissue, with a real impact on the progression of the condition. A new possible regenerative approach has been hypothesized since the identification of stem/progenitor cells. Mesenchymal stem cells (MSCs) have been isolated in several tissues such as bone marrow, adipose tissue, synovia, skeletal muscle, placenta, amniotic fluid (4).

In the last few decades, MSCs have gained interest as a possible strategy for OA treatment, in particular in early-moderate stages (5). *In vitro* and preclinical studies demonstrated that the use of MSCs shows benefits both in terms of cartilage

Key words: osteoarthritis, stem cells, bone marrow concentrate, stromal vascular fraction

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THE CYTOKINOME IN OSTEOARTHRITIS, A NEW PARADIGM IN DIAGNOSIS AND PROGNOSIS OF CARTILAGE DISEASE

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At present, diagnosis and progression monitoring of osteoarthritis (OA) is made through radiological and clinical assessment. Several studies investigated the role of synovial fluid analysis, to find out whether joint disease could be characterized by the pattern of cytokines, which acts during the pathogenic process or in specific stages of it. Online PubMed-Medline search was performed in order to retrieve evidence concerning synovial fluid analysis of cytokines involved in OA degenerative process. Concerning pro-inflammatory cytokines, it has been shown that interleukin (IL)-6, TNF-α and IL-17 are mainly over-expressed in the synovial fluid of OA joints, as well as anti-inflammatory cytokine IL-10. Variations of cytokines levels occur with radiological and clinical progression. It was also reported that metalloproteinases are involved. Synovial fluid analysis may be helpful in defining stage and type of OA, but more research is needed, especially focusing on the variation of sets of cytokines during OA stages and correlating these patterns with clinical features.

The term "cytokinome" was first used in 2010 to define the array of immunological molecules which play a role in an inflammatory process of a given tissue. Costantini et al. (1) utilized this term in the perspective of "omics" definition system, to refer to the whole of molecules, their action and their complex interaction network, which act in an organism.

Actually, the term can be applied to every tissue where an inflammatory pathogenesis occurs and cytokines are mainly involved. The exact pattern of molecules implied in a given process is useful in defining the process itself and to determine it as unique. This statement derives from the observation that cytokines always act in concert with other ones, in order to create a defined system of cell-cell signalling (2). Furthermore, given the molecular changes which occur during the disease progression, multiple stages can also be found which analyse the alternation of molecular patterns. Osteoarthritis (OA) is a degenerative disease of cartilage which affects joints of the human body, yielding also an inflammatory process which involves cartilage and synovia (3, 4). It has been well documented that cytokines, including interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), cover a main role in degenerative process, along with metalloproteinases (MMP), in degradation of matrix which the chondrocytes are surrounded by (5).

It has been well established that chondrocytes are the main target of cytokines during arthritic process, although exact timing and action of these molecules is far from being clarified. Furthermore, the source of production of cytokines in the joint is not well known (6). Synovia, the tissue which surrounds cartilage and other articular structures and which provide them with nutrition, is commonly involved in OA inflammatory process. In fact, synovitis can be retrieved in many OA models in human and animals. It is commonly accepted that fibroblast-like and macrophage-like cells of the synovia are

Key-words: osteoarthritis, cytokines, Synovial fluid, cytokinome, molecular signalling, cartilage disease

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

THE MENISCUS VASCULARIZATION: THE DIRECT CORRELATION WITH TISSUE COMPOSITION FOR TISSUE ENGINEERING PURPOSES

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Meniscal lesions still represent an unsolved problem in clinical practice. Like the articular cartilage, meniscus has a scarce healing potential. Thus, when this tissue is damaged, the joint biomechanics is completely altered, leading to the development and progression of premature osteoarthritis. Therefore, in the last years, several tissue-engineering strategies have been developed to regenerate the meniscus with debated results. The comprehension of complex processes underlying meniscus maturation and structure is essential for a correct approach for the generation of a biomimetic meniscal substitute. In this chapter, we will first review the morphology of the meniscus during growth, focusing on the unique pattern of vascularization, and then we will discuss the most common tissue engineering strategies for meniscus repair.

The development of an engineered meniscus represents one of the most promising methods to regenerate a tissue, which, in the common clinical practice is rarely able to spontaneously repair or regenerate, with the consequent loss of functionality of the entire knee joint. The production of meniscal substitute requires a profound knowledge of the native meniscus morphology, of its biochemical composition and of cell phenotype in its different regions. Current information on meniscus is the result of several studies in human but also in animal models; for example, the swine meniscus is very similar to the human one and it is therefore useful both for the characterization of the native meniscus and for the validation of an engineered scaffold (1). Although several studies have been performed to clarify the cellular and structural heterogeneity of the meniscus, there are still some open questions regarding its structure and its insufficient repairing and regenerating capability. In particular, as the reduction of the vascularization is strongly connected to meniscus maturation (2), it is important to determine a correlation between vascular changes and the modulation of cell phenotype during the course of meniscus development. These two processes could be regulated by common molecules that are already known as vascular modulators but are not yet associated in the signalling involved for the regulation of cell phenotype. New insights about the pathways involved in vascular and phenotypical changes will guide the generation of better meniscal substitute and will enlighten the physiological development of the meniscus.

Meniscus Morphology

The meniscus is a fibrocartilaginous tissue, which is essential for proper knee function, playing an active role in the biomechanics of the knee joint.

Key words: meniscus, vascularization, tissue engineering, animal models

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ARTHROSCOPIC ALL-INSIDE TREATMENT OF POPLITEOMENISCAL FASCICLES TEARS: SURGICAL TECHNIQUE AND RESULTS FROM THE FIRST 6 CONSECUTIVE PATIENTS

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Athletes whose knees are subjected to sudden changes of direction and high jumps such as martial arts athletes, dancers, wrestlers and football players are at higher risk of injuring popliteomeniscal fascicles. Painful squatting and mechanical symptoms such as locking sensation are common. Current available treatments includes open or arthroscopic in repair. Arthroscopic repair with all-inside device can relieve symptoms and restore knee function. Six patients from two surgical centers with isolated popliteomeniscal fascicles tears were treated with arthroscopic all-inside repair. The surgical technique is thoroughly described. All patients showed consistent symptoms and MRI findings, as well as meniscal hypermobility during arthroscopic probing. Moreover, four out of six showed a chondral lesion of the lateral femoral condyle. All of them had their lateral meniscus sutured with one or more sutures. Symptoms were relieved and all but one were able to return to play at the pre-injury level. No postoperative complications were encountered. The diagnosis of the disruption of popliteomeniscal fascicles is challenging and often seen in athletes that play sports which involve repetitive twisting. However, patients' complaints are consistent. Arthroscopic repair with an all-inside device showed to be a reliable and easy technique for addressing the condition, although some issues still need to be investigated, such as how much constraint the repair should provide. Arthroscopic all-inside repair of popliteomeniscal tears prove to be safe and effective in the short-term follow-up, allowing for sport activity resumption.

The popliteomeniscal fascicle is a thin synovial mesh connecting the lateral meniscus at the popliteal hiatus (1).

Athletes whose knees are subjected to sudden changes of direction and high jumps such as martial arts athletes, dancers, wrestlers and football players are at higher risk of injuring popliteomeniscal fascicles (2-5). Current literature also reports how the tear of polpiteomeniscal fascicle often occurs in association with acute anterior cruciate ligament injuries in over 25% of cases (6). This leads to most of the cases being ignored because the ACL rupture draws all the post-injury diagnostic and therapeutic care. Isolated lesions of the popliteomeniscal fascicle are not always symptomatic, presenting pain over the posterolateral region and locking of the knee joint (6, 7).

Clinical (8) and radiological (9) diagnosis of this lesion can be very difficult. Despite a careful physical examination of the knee and a scrupulous visualization of MRI images, sometimes only the arthroscopic visualization of the structures and the surrounding area, is able to lead to the correct identification of the lesion (2). Currently, few data

Key words: popliteomeniscal fascicles, lateral meniscus, menisco-capsular lesion, all-inside repair

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TEARS OF POPLITEOMENISCAL FASCICLES, DIAGNOSTIC AND CLINICAL IMPLICATIONS. A REVIEW OF THE EVIDENCE

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Postero-lateral corner of the knee is composed of several structures including the popliteo-meniscal fascicles (PMFs). These fibrous structures form a stable ligamentous complex around the popliteus tendon, which stabilize the lateral meniscus, increasing the strength of postero-lateral corner. Studies were retrieved through an electronic search of CINAHL, EMBASE, and Pub-Med, until May 2016. Studies in English, Italian, French, and Spanish were considered for inclusion. Randomized controlled trials, prospective and retrospective comparative studies, case series, and case reports were included. Studies eligible for inclusion concerned PMFs anatomy, biomechanics, diagnostic assessment of PMFs tears and clinical options for tears management. Thirteen studies were included in this review. There were: 7 case series, 4 case reports and 3 anatomical studies. Through anatomic dissection, two or three PMFs (antero-inferior fascicle, aiPMF; postero-superior fascicle, psPMF; postero-inferior fascicle, piPMF) can be indentified and isolated. Evaluation through MRI can be a useful diagnostic tool in detecting PMFs tears, especially using proton density (PD) sequences. The biomechanical analysis assessed that lateral meniscus (LM) motion is directly related with PMFs integrity and increased with section of one or both the fascicles. The clinical studies clearly state that a snapping syndrome, associated with lateral knee pain, can develop when one or both PMFs are torn. The three PMFs described are considered as relevant components of the popliteal hiatus, in the posterolateral aspect of the knee. MRI evaluation can detect these fibrous fascicles with good sensitivity. More studies with larger samples would be needed for a clear comprehension of PMFs function and clinical management of PMFs tears, especially with large case series and modern biomechanical testing.

The anatomy of the lateral meniscus and more broadly the postero-lateral corner (PLC) of the knee is a relevant topic with still unclear issues (1). It is known that PLC is composed of several structures, including the lateral meniscal wall, the popliteus muscle tendon and the arcuate popliteal ligament, all of them reinforced by the deep lateral collateral ligament (2). Its function has been clearly defined as the stabilization of the knee during tibial internal rotation and change of direction (3).

Several anatomical studies, described accurately the anatomy of the PLC, focusing on some small, though relevant, structures called popliteo-meniscal fasciculi (PMFs) (4). These fibrous structures form a stable ligamentous complex around the popliteus tendon, which stabilize the lateral meniscus,

Key words: menisci, knee, biomechanics, knee arthroscopy, sports medicine

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TGF-β1 DIFFERENTIALLY MODULATES THE COLLAGEN VI α5 AND α6 CHAINS IN HUMAN TENDON CULTURES

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Collagen VI is a microfibrillar collagen with a potential regulatory role in tendon repair mechanism. We studied the expression of collagen VI α5 and α6 chains in normal human tendon fibroblast cultures, both under basal condition and in response to TGF-β1, a potent regulator of tendon healing. Under basal condition, we found that the α5 chain was expressed, although to a lesser extent with respect to the α3 chain; in contrast, the α6 chain was absent. The treatment with TGFβ1 induced an opposite effect on the expression of the α5 and α6 chains; in fact, while the α5 chain was dramatically reduced, the α6 chain was induced and released in the culture medium. These data indicate that collagen VI α5 and α6 chains are differentially involved in tendon matrix homeostasis. The α6 chain may represent a new potential biomarker for monitoring TGFβ1-related events in tendon, as healing and fibrotic scar formation.

Tendon is a dense connective tissue composed of a highly ordered extracellular matrix (ECM), mainly constituted by collagen fibrils which are hierarchically organized to withstand tensile forces transmitted from muscle to bone axis. Fibrils contain mostly type I collagen and other ECM components which contribute to tendon collagen fibrillogenesis (1).

Collagen VI, a microfibrillar collagen widely expressed in most tissues, has been identified in the matrix of both developing (2) and mature (3) tendon. It has been detected as a network of beaded filaments anchored to the cell surface of tenocytes (3). Recently, we found that collagen VI localizes in the pericellular matrix of tendon fibroblasts, by interacting with the NG2 proteoglycan (4).

In humans, five distinct collagen VI α chains have been identified (5). The best characterized and widely expressed form of collagen VI is the α1α2α3 chain containing heterotrimer that assembles intracellularly into dimers and tetramers. After secretion, tetramers associate end to end constituting typical 100 nm-spaced beaded microfilaments, which may form fibrils, by parallel alignment, or web-like structures, depending on the association with cell receptors and ECM-binding proteins (6). In humans, two novel collagen VI chains, the α5 and α6 chains, have been recently identified, which structurally resemble the α3 chain but display a more restricted and often alternative tissue specific pattern (5, 7, 8). With respect to the collagen VI α3 chain which is ubiquitous, the α5 chain is selectively detected in areas subjected to tensile stress, such as the dermo-epidermal junction (7) and the myotendinous junction (8). However,

Key words: collagen VI, transforming growth factor β1, tendon, myotendinous junctions

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HEAT SHOCK INDUCES THE EXPRESSION OF PRO-INFLAMMATORY CYTOKINES IN HUMAN ACHILLES TENDON TENOCYTES

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The aim of our study is to investigate the behaviour of healthy and tendinopathic human tenocytes after a heat shock. After we harvested tendinopathic and healthy human tendon samples, we split tenocytes into 4 groups: 3 groups were submitted to heat shock, followed by different periods of post-heating (2, 4 and 20 h). The other group represents our negative control. The target genes were analysed using Real Time PCR. IL-1β and IL-6 expression were significantly increased in tendinopathic samples after heat shock. COL1 and COL3 expression were increased in non-stimulated tendinopathic tenocytes, but their levels significantly decreased after heat shock ($p<0.01$). COL3 levels increase in healthy samples after 20 h post-heating ($p<0.01$). COL1 and COL3 decreased after heat shock as a sign of the failure of repair mechanisms in tendinopathic tendons. Heat shock in *in vitro* models was insufficient to trigger pro-inflammatory cytokines in healthy human tenocytes.

Tendinopathy is the best generic descriptive term for the clinical conditions in and around tendons arising from overuse (1, 2). The term "tendinopathy" defines the clinical syndrome characterised by a combination of pain, swelling and impaired performance (3). Tendon injuries produce considerable morbidity, and the disability that they cause may last for several months despite what appropriate management. The aetiology of tendinopathy remains unclear and many causes have been hypothesised (4). Hypoxia, ischaemic damage, oxidative stress, hyperthermia, impaired apoptosis, inflammatory mediators, fluoroquinolones, and matrix metalloproteinase imbalance have all been implicated as mechanism of tendinopathies (4). Biomechanical factors, functional alterations, repetitive mechanical loading, aging and metabolic disorders may predispose to tendinopathy, with high risk of re-injury (1, 2, 5).

The process of tendinopathy involves both the collagen matrix and the tenocytes (6, 7, 8). Overloading of tendons after intensive exercise and training induces micro-rupture of tendon (9), with damage expression associated molecular patterns (DAMPs) in an attempt to produce tissue healing (10). Several models have recently implicated pro-inflammatory cytokines to initiate the catabolic process, such as Tumor Necrosis Factor-α (TNF-α),

Key words: Stress-related changes, pro-inflammatory cytokines, heat shock, in vitro, human tenocytes

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RISK FACTORS FOR POST-OPERATIVE SHOULDER STIFFNESS: ARE THERE NEW CANDIDATES?

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D. Cucchi and A. Menon contributed equally to this work.

The aim of this study was to document the incidence of postoperative shoulder stiffness (SS) after arthroscopic rotator cuff repair and evaluate the role of risk factors for its development. Seventy-five consecutive patients that underwent arthroscopic rotator cuff repair were included. The incidence of post-operative SS was prospectively investigated and the presence of 20 potential risk factors was documented retrospectively. The incidence of post-operative SS was 10.4%. All patients were women, and sex was significantly associated to pathology development (p=0.0067). The presence of gastroesophageal diseases was found to be significantly associated with post-operative SS development (p=0.0046). A significant association between the occurrence of post-operative SS and the presence of gastroesophageal diseases was identified. This finding, not yet reported in literature, deserves further investigation. The incidence of post-operative SS fell among previously reported ranges, with females significantly more affected than men.

Rotator cuff (RC) tears are a common contributing factor to shoulder pain and occupational disability, and RC surgery evolved rapidly from a minor niche to a fully recognized subspecialty in orthopaedic surgery. Nowadays, arthroscopic RC repair is accepted as gold standard in surgical treatment of RC tears. This technique has proven to be effective, safe and has high clinical success rate that is durable in time (1). There are however some possible complications described in literature. Restriction in glenohumeral joint range of motion (ROM) has been reported with variable incidence in literature and has been defined as post-operative shoulder stiffness (SS) (2, 3). The term SS has been recommended to replace the sometimes misleading previously used "frozen shoulder" and "adhesive capsulitis" (2).

SS may have various aetiologies, including immobilization, trauma or surgical interventions, and numerous risk factors for primary and post-operative SS have been described. This article evaluates the role of pre-operative conditions as risk factors for SS in the development of a specific type of post-operative condition, namely post-operative SS after arthroscopic RC repair.

MATERIALS AND METHODS

After institutional review board approval (55/INT/2016),

Key words: shoulder stiffness, rotator cuff repair, post-operative complication

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SURGICAL TREATMENT OF INSERTIONAL ACHILLES TENDINOPATHY: A SYSTEMATIC REVIEW

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Insertional Achilles tendinopathy is a frequent cause of pain and performance impairment of the ankle. It is more common in runners, but may also affect general population. Conservative treatment is the gold standard in the early phases but 10% to 30% of patients require surgery. The aim of this study is to review the current literature in order to evaluate current surgical strategies for Insertional Achilles tendinopathy and to analyze the effectiveness of the available techniques. We performed a systematic review of the literature, to identify studies reporting clinical outcome after surgical treatment for Insertional Achilles tendinopathy in any population group with at least 6 months follow-up. The quality of the articles included was evaluated by the Coleman Methodology Score and correlated with the reported outcome and year of publication. We identified 16 studies reporting on 465 surgically treated Insertional Achilles tendinopathy with a mean follow-up of 29.8 months. Average age at the time of surgery was 53 years. Two different categories of surgical treatment were distinct: debridement alone or debridement with augmentation in case of excessive tendon loss. Results were excellent or good in 89.6% of cases and fair or poor in 10.4%. Average complications rate was 18.3%, with 15.7% of minor and 2.6% of major complications with no difference in the two groups. Negative correlation was found between Coleman Methodology Score and the reported outcome and positive correlation was found between Coleman Methodology Score and year of publication. Good or excellent outcome can be expected after surgical treatment for Insertional Achilles tendinopathy whatever the adopted procedure, but there is no specific evidence regarding which surgical technique provides a better outcome or a lower rate of complications. Research with higher levels of evidence and methodology that is more rigorous are needed in order to evaluate the optimal surgical strategy for patients with IAT.

Achilles tendon problems are very common in active people but they can also affect general population. According to anatomical location two different pathologic entities can be identified, the non-insertional or midportion Achilles tendinopathy and the less common insertional tendinopathy (1). Achilles tendon problems are reported to be non-insertional in 66% of the cases and insertional in 23%, rarer are the mixed forms.

Insertional Achilles tendinopathy (IAT) is more common in runners, especially if occasional athletes with an incidence of 9%, nevertheless up to 5% of professional athletes during their careers suffer for IAT (2). Non-athletes, typically older than 40 years, are the second most common category in whom symptoms appear. Older people are more commonly

Key words: Achilles tendon, tendinopathy, tendonitis, tendinosis, tendinitis, tendon transfer

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EXTRACORPOREAL SHOCK WAVES INDUCE OSTEOGENIC DIFFERENTIATION OF HUMAN BONE-MARROW STROMAL CELLS

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The effects of treatment with shock waves (SW) on osteoblastic cells have already been described. Furthermore, the effects of treatment with SW are also determined by the contextual stimulation of other cell lines, in particular of mesenchymal cells. This is the first experimental study of stimulation of a human mesenchymal stem cell line, taken from bone marrow, using SW (electromagnetic device), with two energy levels. The results showed a significant increase in expression of the main osteoblastic differentiation genes: BMP2, alkaline phosphatase, osteocalcin, COL1A1, RUNX2. The monitoring within 96 hours demonstrated a progressive increase of cell adhesion and an intense cell proliferation at 48 h. The differentiation response and proliferation of stem cells after treatment with SW shows that this therapy is an effective method of regenerative medicine.

In the last 10 years, Extracorporeal Shockwave Therapy (ESWT) has been used in the treatment of pseudoarthrosis and non-union, with a success rate of 70% (1). The Shock Waves (SW) is an acoustic wave characterized by a rapid increase in blood pressure and a following decrease that guarantees the focalization in tissue depth. The clinical effects are justified by the neo-angiogenesis actions, proliferation of osteoblasts and differentiation of stem cells into osteoblastic. This latter effect of SW has been reported only in animal models (2, 3).

The bone healing of a fracture follows the sequence of osteogenesis and bone remodelling that ensures the skeletal reconstruction integrity (4, 5). In the healing of a fracture, the mesenchymal cells are attracted and induced to differentiate into osteoblasts in order to activate bone tissue regeneration (6). When there is non-union, this means there has been a failure of the fracture healing process. In this case, the role of existing osteoblasts may have been inefficient. Moreover, the role of the differentiation of mesenchymal cells into osteoblasts is also very important (7-9). The important role assumed by stem cells in the consolidation of a fracture is already recognized and adopted in the therapeutic field. Recently, innovation in biotechnology has allowed the use, in fractures, of different substances, such as growth factors and bone morphogenetic proteins (BMP), which are able to stimulate the differentiation of progenitor cells present in the site (10). With these biological mediators, it is possible to speed up and optimize the response of fracture healing (11).

The aim of our study was to verify the effect of the shock waves on human pre-osteoblast mesenchymal cells, particularly as regards the expression of osteoblast differentiation genes and the adherence and proliferation of cells.

Key words: *shock waves, stem cell, differentiation, bone*

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ASSOCIATION BETWEEN MARKERS OF BONE LOSS AND URINARY LITHOGENIC RISK FACTORS IN OSTEOPENIC POSTMENOPAUSAL WOMEN

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In this study, we explored if urinary lithogenic risk parameters could have some application for monitoring bone health status. We recruited 20 women with postmenopausal osteopenia and a negative medical history for nephrolithiasis. Markers of lithogenic risk were evaluated on 24-h urine and fasting-morning urine. Serum levels of bone turnover markers (BTM) were measured in fasting-blood samples. We found that cross-linked telopeptide of type I collagen (CTX) was significantly correlated with 24-h calcium excretion. N-terminal propeptide of type I procollagen (PINP) correlated with 24-h excretion of potassium, calcium and citrate. CTX had considerably increased in patients with pH <5.5. Low citrate levels (<3.3 mmol/24 h) were associated with lower levels of CTX and PINP. Our findings suggest that a low-grade acidosis and some lithogenic risk factors are detectable in a proportion of patients with postmenopausal osteopenia. Further studies are necessary to confirm that this evaluation could be clinically relevant.

The microarchitectural deterioration of bone tissue is a current picture in postmenopausal women, with consequent lowering of bone mass and increased susceptibility to fracture (1). The bone loss starts early and continues for many years, depending on many causes, leading to a unique epiphenomenon that is the imbalance between the bone resorption and formation (2). The pathophysiology of postmenopausal bone weakening may also be influenced by adverse metabolic conditions. Bone tissue is directly involved in essential functions, such as the regulation of acid-base balance. In order to buffer the systemic acidosis, the skeleton acts as an ion exchange column modifying the composition of the mineral portion (3). There is a linear correlation between elimination of calcium and acidosis: the higher is the acidosis, the higher will be the loss of calcium from bones. Estrogen deficiency (1), a diet high in meat protein (4) and the decreased renal function due to ageing (5) are factors favouring a high acid loading that may exceed the neutralization capacity, leading to a latent or low-grade acidosis (3). The relationship among renal function, risk of osteoporosis and hip fracture has been investigated and many studies show that bone mineral density (BMD) decreases not only in end-stage renal disease (5), but also in patients with nephrolithiasis (6). In fact, the urinary stone formation is frequently associated with calcium metabolism alterations and high bone turnover and is favoured by the increased acid production occurring in subclinical acidosis (7). Recent findings demonstrated that lithogenic risk

Key words: osteopenia, acidosis, bone turnover markers, urolithiasis

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PREGNANCY-ASSOCIATED OSTEOPOROSIS (PAO) WITH MULTIPLE VERTEBRAL FRAGILITY FRACTURES: DIAGNOSIS AND TREATMENT IN A YOUNG PRIMIGRAVID WOMAN

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PAO is an uncommon condition affecting pregnant women during last trimester or early post-delivery period; it is often asymptomatic or presents with pain related to some acute fragility fractures. The diagnosis is often delayed or missed, the etiology remains unknown and no guidelines about treatment have been published. We present one case of PAO in a 33-year-old primigravid woman presenting acute worsening back pain. Our patient was treated with a TLSO brace, oral 25 (OH)-vitamin D supplementation and Teriparatide for 6 months. A short review of the literature has been included and useful advice about how to suspect and diagnose this uncommon disease were given in order to recognize and treat such a debilitating and severe condition for young mothers as best as possible, based on the available scientific

Pregnancy- and lactation-associated osteoporosis (PAO) was first described by Nordin and Roper in 1955 as an idiopathic form of acute osteoporosis occurring in late pregnancy or early post-partum period (1). Literature on PAO is scarce and mostly focused on description of isolated cases. Prevalence in general population of PAO is unknown and aetiology of this condition remains unclear. It has been suggested that genetic predisposition or hormone factors may play a role in development of PAO (2), but it is also possible that mechanical factors associated with pregnancy, (e.g. decreased level of activity, increased weight) may be involved. The most common clinical presentation of PAO is in a young, primigravid woman, with severe low back pain occurring in the last trimester or in the early post-delivery period (3, 4). Thoracic and lumbar vertebral compression fractures are the most frequently occurring fractures, although hip, ribs, and pubic rami fractures can also occur.

Due to the non-specific clinical presentation of PAO, the correct diagnosis is often missed or delayed. Chronic low back pain is a common occurrence in pregnant women. Furthermore, concerns over the use of conventional radiological investigations (e.g. x-ray or bone densitometry) during pregnancy may delay diagnosis. Therefore, clinical suspicion should be formulated when a young primigravid woman complains of severe back pain during pregnancy or lactation, without any remission of symptoms with common analgesia. When symptoms become continuous and quality of life gets worse (immobilization, inability to take care of the newborn, etc.) a gynecologist or a pediatrician should be consulted in order to decide when to safely perform X-ray examination and bone densitometry scan (DEXA). Furthermore, serum levels of calcium, phosphorus and alkaline phosphatase, thyroid and

Key words: pregnancy associated osteoporosis, vertebral fragility fractures, teriparatide

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TREATMENT OPTIONS OF SIMPLE BONE CYSTS: THE ROLE OF BONE SUBSTITUTES, GROWTH FACTORS AND LITERATURE REVIEW

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The solitary bone cyst is a typical tumor-like lesion of the immature skeleton, whose etio-pathogenesis is still unclear. The purpose of this work is to perform a review of the literature about the different surgical approaches focusing on the role of bone substitutes and growth factors. Literature analysis shows injection techniques of substances such as methylprednisolone, autologous bone marrow, demineralized bone matrix, calcium sulphate and surgical techniques that involve the resection and curettage associated with bone graft and/or intramedullary nailing. Although there are good results currently associated to these techniques and the different ways of treatment, the only evidence-based treatment is given by injections of steroids. However, given the high rate of failure, autologous bone marrow and platelet gel represent a viable therapeutic option.

The solitary bone cyst (SBC) is a typical tumor-like lesion of the immature skeleton (between 3 and 14 years), characterized by the presence of an intramedullary cavity full of liquid frequently localized in the proximal metaphyseal and diaphyseal area of the humerus and femur, that has a tendency to grow, weakening the bone (1).

Multiple etio-pathogenethyc theories have been proposed, ranging from the presence of dysplastic area in the lesions that develop in response to trauma, to venous occlusion in medullary spaces, inflammation and congenital remains of intraosseous synovial tissue, while the origin in correspondence to the growth plate, supports the hypothesis that it represents more of a growth disorder rather than a neoplastic process (2).

Most lesions are asymptomatic and the diagnosis is often incidental or associated to simple or moderately displaced pathological fractures after a low-energy trauma (1).

The localization of the SBC near or across the physis in young children is indicative of an active cyst, while the localization in diaphyseal region and an older age is indicative of an inactive cyst. It tends to regress or heal spontaneously once they reach skeletal maturity in the majority of cases but not always (1, 2).

The aim of SBC treatment is to prevent pathological fractures, reduce pain and prolonged physical restraint, but despite the high frequency, the treatment lacks guidelines and consensus regarding timing and the type of surgical technique. It is possible that the treatment of an active cyst may not be successful while the treatment of an inactive cyst could be, but at the same time may not be necessary. However, although there are no guidelines, it is

Key words: simple bone cyst, unicameral bone cysts, tumor-like lesions, bone tumor

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AUTOLOGOUS BONE MARROW CONCENTRATE COMBINED WITH PLATELET-RICH PLASMA ENHANCE BONE ALLOGRAFT POTENTIAL TO INDUCE SPINAL FUSION

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Bone marrow cells concentrate (BMCs) is a source of osteoprogenitor cells and platelet-rich plasma (PRP) is a source of growth factors. The objective of the study was to determine whether BMC and PRP could increase the potential of bone allograft to induce posterolateral-lumbar spinal fusion compared to the bone allograft alone. A prospective nonrandomized radiographic study has been conduced on 10 patients with posterolateral instrumented fusion for degenerative lumbar disease with 1-year follow-up using CT scan. A fresh frozen bone allograft alone and bone allograft with a mixture of autologous BMC and PRP blended with thrombin were apposed in the right and left posterolateral side, respectively. CT showed good right fusion masses (allograft alone) in 4 patients and poor in 6; good left masses (BMC and PRP plus allograft) in 9 patients and poor in 1. The differences detected between right-side and left-side masses show an advantage in adding BMC and PRP to the bone allograft to increase spinal fusion rate.

Lumbar spinal fusion with pedicle screw fixation is a widely used surgical procedure and the most common technique performed to obtain fusion in the lumbar spine. In the United States, the number of lumbar fusions doubled between 1998 and 2008 (1). It gives important advantages in patients affected by degenerative disc disease, and it is based on intertransverse or interarticular bone formation being protected by fixation (2). However, reported failure rates still range from 5% to 45% (3). Therefore, procedures that may enhance bone repair and fusion still arise great interest in the scientific community.

Autologous iliac crest bone graft (ICBG) has been considered the gold standard in lumbar spinal fusion because of its osteoconductive and osteoinductive potential (1). However, its harvesting is associated with morbidity including hematoma, fracture, impaired wound healing, infection and most of all donor site pain (4). Local bone harvested from the laminae and spinous processes during the decompressive maneuvers of a lumbar surgery is also widely used as a suitable autologous graft to avoid donor site morbidity (1).

Allograft may represent an alternative source of bone graft and a valid substitute to the autologous bone graft. However, it lacks osteoinductive potential and osteogenic cells because of the processing that it undergoes to decrease its antigenicity (5). Therefore, the bone formation by the use of allograft may be improved by adding both osteoprogenitor cells, such as bone marrow cells, and growth factors to the fusion site.

Bone marrow cells concentrate (BMC) is an option to promote bone formation in spinal fusion (6). BMC contains mesenchymal stromal/stem cells (MSCs), which have been demonstrated to be able

Key words: Bone marrow, Stem cell, platelet rich plasma, spine fusion, bone marrow concentrate, allograft

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MESENCHYMAL STEM CELLS FOR INTERVERTEBRAL DISC REGENERATION

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Low back pain (LBP) is one of the most common disabling symptoms affecting the adult population throughout the industrialized world. The main cause underlying this condition is intervertebral disc degeneration (IDD), which is characterized by progressive decrease of the proteoglycan content within the nucleus pulposus (NP), leading to disc dehydration and loss of its morpho-functional and biomechanical properties. To date, LBP treatment is based upon conservative and invasive procedures which are not capable of restoring the degenerative alterations of the disc, as they only help relieve the symptoms and/or slow down disc degeneration and are, nonetheless, characterized by significant comorbidities, costs and secondary risks. The potential use of different mesenchymal stem/stromal cells (MSCs) for treating IDD has been promisingly tested *in vitro* and *in vivo*. The combination of different cell types, preconditioning culture conditions, engineered scaffolds and delivery systems have yielded proof of disc matrix reconstitution, increased cell viability and tissue regeneration in several experimental settings. This article reviews the current literature on stem cell-based therapy for IDD and the outcomes that diverse approaches have achieved.

Low back pain (LBP) is a musculoskeletal symptom affecting more than 80% of the general population throughout their life, leading to high morbidity with great psychological, social and economic burdens. LBP attests itself as the first cause of disability in people under 45 years of age, especially among female individuals, hence resulting in huge national economic losses in developed countries (1).

LBP is mainly caused by degeneration of the intervertebral disc (IVD). The IVD consists of three highly specialized tissues: the inner nucleus pulposus (NP), the outer annulus fibrosus (AF) and the cartilaginous end-plate, which connects the disc with the adjacent vertebrae. The AF is a fibrocartilaginous ring, composed of concentric, dense lamellae of highly orientated type I collagen fibers which constitute an organized matrix hosting fibroblast-like cells. The NP is an amorphous, gelatinous matrix rich in proteoglycans (mainly aggrecan) and type II collagen. Aggrecan is a large-aggregating proteoglycan which is capable of interacting with hyaluronan and is provided with several negatively charged sulfated glycosaminoglycans (namely chondroitin sulfate and keratan sulfate) that are able to bind water. The high level of hydration produces a swelling pressure which is responsible for maintaining the disc height and contributes to provide the disc with mechanical resistance to twisting, bending and compression. Small chondrocyte-like cells can be found within the NP and are responsible for synthesizing the proteoglycans thus maintaining the matrix environment (2).

Intervertebral disc degeneration (IDD) in an age- and disease-related chronic process which is characterized by a progressive decrease of the proteoglycan (and consequently water) content in the NP, with the subsequent loss of the disc ability to resist compressive forces, leading to instability (3).

IDD may evolve to several spinal disorders such

Key words: stem cells, intervertebral disc degeneration, mesenchymal stromal cells, disc regeneration, spine surgery

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SIMULTANEOUS DOUBLE ROD AND EN-BLOC DIRECT VERTEBRAL ROTATION TECHNIQUE FOR CORRECTION OF MAIN THORACIC ADOLESCENT IDIOPATHIC SCOLIOSIS: RETROSPECTIVE ANALYSIS OF 14 CASES

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Adolescent idiopathic scoliosis (AIS) is a triplanar deformity associated with rib hump, especially when a principle thoracic curve is present. The aim of this study is to evaluate the results of AIS correction retrospectively, using simultaneous double rod derotation manoeuvre technique followed by en-bloc direct vertebral rotation (DVR). Fourteen patients were included in this study. Coronal and sagittal thoracic Cobb angle, global coronal balance, sagittal balance, rib hump prominence, Scoliosis Research Society outcome instrument score (SRS-22) and Walter Reed visual assessment scale (WR-VAS) values were recorded pre- and postoperatively and evaluated. Results were evaluated at a mean follow-up of 2 years. Good to excellent radiographic and clinical results were obtained in all patients. No major perioperative complications occurred. This technique has proved to be effective for surgical correction of the deformity in Lenke type 1 AIS with good clinical and radiological results and low rate of complications.

Adolescent idiopathic scoliosis (AIS) is a triplanar deformity on coronal, sagittal and axial plane. In the majority of cases this leads to a hypokyphosis associated with a rib hump especially for severe Lenke type 1 curve or main thoracic form (1, 2).

Harrington first introduced deformity correction in 1962 using only concave distraction and convex compression forces. Although this technique achieved considerable coronal correction, it did not affect neither the sagittal deformity nor the rotational components (3). Subsequently, in order to obtain a better deformity control, Cotrel et al. developed the Cotrel-Dubousset (CD) multihook segmental instrumentation which allowed a superior coronal plane Cobb angle correction but also a partial sagittal curvature improvement (4).

Today pedicle screw constructs are considered the mainstay for scoliosis correction surgery because of their three-dimensional deformity correction capacity (5, 6). Pedicle screws are the only available instrumentation able to transmit forces onto the entire vertebra so as to allow direct vertebral rotation (6).

Based on implant density, pedicle screw constructs are considered segmental if more than 60% of the available anchor sites are fixated. Non-segmental pedicle screw fixation showed to be equivalent to segmental fixation in case of mild and flexible curves but for rigid and severe curve, segmental fixation is preferred to avoid excessive concentration of mechanical forces around the screws (1, 6, 7).

The direct vertebral rotation technique (DVR) was first introduced by Lee in 2004 aiming to restore the axial plane deformity by applying direct rotational forces on apical vertebrae (AV) (1). Both segmental and en-bloc DVR have been described based on

Key words: stem cells, intervertebral disc degeneration, mesenchymal stromal cells, disc regeneration, spine surgery

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IS A MINIMALLY INVASIVE ANTERIOR APPROACH EFFECTIVE IN OLD PATIENTS? A PILOT STUDY

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Minimally invasive approach to the hip is a blood preserving surgery, with rapid rehabilitation, and low dislocation rate. Intuitively, these characteristics render this approach extremely suitable in the elderly patient. The aim of this study was to analyze the early clinical and radiographic results in the first 30 consecutive patients above 70 years of age undergoing THR through a minimally invasive anterior approach. Clinical evaluations showed an improvement of the Harris Hip Score and WOMAC score after surgery. Radiographic assessment showed cup orientation averaging 47° (range 40°–59°) and no valgus stem aligned. Allogeneic blood transfusion was required in only 6 patients (19.8%). One patient experienced an intraoperative fracture of the greater trochanter. No early implant dislocation was observed in the study population. In conclusion we advise a minimally invasive anterior approach for THR in older patients when a careful patient selection has been done.

Minimally invasive procedures for total hip replacement (THR) gained increasing popularity over recent years in an effort to reduce soft tissue damage and improve patients' recovery. Focusing predominantly on early rehabilitation and quick recovery following THR, the majority of these techniques have been proposed for young and active patients (1-4). The direct anterior approach to the hip is a tissue sparing procedure that allows the surgeon to reach the joint using an intermuscular and internervous plane and reducing soft tissue traumatization (5).

However, minimally invasive approach to the hip is also characterized as a blood preserving surgery and is related to an early rehabilitation and recovery of ambulation. Intuitively, these characteristics render this approach extremely suitable for the fragile elderly patient, in which multiple comorbidities are associated to intra and postoperative complications. Elderly patients in fact are more likely to suffer cardiac complications due to postoperative anemia and neurological or vascular complications for delayed weight bearing. Furthermore, implant dislocation rate is higher in elderly patients especially using a traditional postero-lateral approach (6). The theoretical advantages of minimally invasive anterior approaches are the reduced postoperative blood loss, the early weight bearing on first postoperative day and the very low dislocation rate (7, 8).

The higher complication rate and the longer learning curve compared to traditional approaches to the hip have been shown to counterbalance the advantages of the rapid rehabilitation using this approach (9, 10). In this context, because of the aging of the population, it can be hypothesized that an increasing number of elderly patients should be scheduled for THR in the future and take advantage of a minimally invasive approach to the hip (11, 12-14) but at present very little has been published on the results of minimally invasive procedure in this

Key words: total hip replacement, minimally invasive anterior approach, old age, complications

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ISOLATE ACETABULAR CUP REVISION THROUGH THE DIRECT ANTERIOR HIP APPROACH: SURGICAL TECHNIQUE, EARLY EXPERIENCE AND REVIEW OF THE LITERATURE

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Direct anterior approach to the hip allows perfect exposure of the acetabulum and an easy proximal and medial extension that makes it eligible for isolate acetabular cup revision although it is seldom used and there are only few published studies. On 23 consecutive acetabular revision (16 cases Paprosky grade 1 or 2, 5 cases 3A, 1 case 3B and 1 case 4) at an average 28-month follow up, we did not record failures or major complications. Early complications included prolonged wound healing in 4 cases and transient femoral cutaneous nerve palsy in 2 cases, the mean postoperative Harris Hip Score was 82.2 with 82.5% of excellent and good results. Our results are consistent with those reported in the literature with similar techniques. The direct anterior approach has shown excellent results for isolated cup revision, though is probably better suited for surgeons that have some experience with the same approach for primary cases.

The direct anterior (DA) approach through the Smith-Petersen or Heuter interval has been gaining popularity for primary hip arthroplasty and while some limitations are reported for femoral exposure, none are reported for the acetabulum (1). The DA allows a perfect exposure of the acetabulum and an easy proximal or medial extension that could be very useful in case of acetabular protrusio to isolate and protect the iliac vascular bundle (Fig. 1). Furthermore, the DA approach has the potential to decrease the postoperative dislocation rate since the dislocation rate with the same approach for primary hips is known to be low (2, 3). However, few papers have described acetabular revision through this approach. Thus, the aim of this paper is to describe our midterm experience of acetabular revisions through the DA approach.

MATERIALS AND METHODS

Twenty-three consecutive acetabular revisions operated in our Department between 2012 and 2015 were performed through a DA approach. Sixteen cases were rated grade 1 or 2 according to Paprosky, 5 cases 3A, 1 case 3B and 1 case 4. All cases underwent a preoperative protocol with bone scan, RCP and biopsy to exclude infection. Clinical and radiological 28-month follow up was performed in each case except one.

Surgical technique

As DA is usually performed on a patient that has undergone primary surgery through another approach, a long incision for a good exposure is not usually required. Since the femoral component is not subject to revision, special leg positioners or dedicated devices are not required. In case of cup revisions with an acetabular Paprosky 1 and 2 bone loss, we usually perform a simple tissue sparing Heuter approach as for primary surgery (Fig. 2).

After the administration of general or spinal anaesthesia and third generation cephalosporins prophylactic, the patient is placed supine on a standard table with the

Key words: hip, revision, anterior approach, acetabulum, failure

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

RESTORING THE FEMORAL OFFSET PREVENT EARLY MIGRATION OF THE STEM IN TOTAL HIP ARTHROPLASTY: AN EBRA-FCA STUDY

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The use of modular stems is still debated and controversial. Some authors have highlighted a number of disadvantages of modular prostheses including high costs, the tendency to fracture, the fretting and corrosion and the increased production of debris. Other authors have emphasized several advantages to adapt the prosthesis to the morphometric differences of patients, to allow better accuracy in restoring the anatomy and biomechanics of hip joint. The advantages of the modular devices appear to be more evident in patients with developmental dysplasia of the hip (DDH). In our study we compared 96 patients, operated for arthritis of the hip with 55 modular neck prostheses (PROFEMUR®, Wright® Arlington, Tennesse, USA) and 41 standard femoral stems (SYMAX®, Striker® Kalamazoo, Michigan, USA). The precision of restoring the natural offset during surgery was correlated with the clinical outcome and the radiological early migration of each stem measured using the computer-assisted EBRA-FCA method. The average preoperative HHS (Harris Hip Score) was 44 (23-66); the postoperative 86.56 in the 55 patients operated with modular prostheses and 81.70 in the 41 patients with monoblock stem. The worst HH Scores were seen in patients in whom the offset was not restored properly. On the contrary, the best scores have been reached in patients in which that value is closer to the "target" value (offset value of the contralateral hip). Restoring the proper offset seems to determine an appropriate tension of the abductor muscles of the hip and implies a better functioning of the joint and a better primary stability of the implant, with less early migration. This has to be a primary objective of THA surgery.

The hip is one of the most replaced joints in the world. Total Hip arthroplasty (THA) is a worldwide recognized treatment in patients suffering from arthritic disease of the hip, reducing pain and improving function (1).

The first problem of a total hip arthroplasty (THA) is the longevity of the implant. Infection, polyethylene wear, aseptic loosening, design faults, material mismatch and poor surgical technique cause failures. Being able to control the orientation of the prosthetic components is of critical importance in normalising the biomechanics of the hip (2-4). It is important to achieve a stable joint with the ideal range of motion for patients, in order to fulfil their daily activities (5).

The primary stability and early migration of the prosthesis components can be measured on plain radiographs, with Roentgen stereophotogrammetry (RSA) or Ein-Bild-Roentgen-femoral component analysis (EBRA-FCA) (6, 7). RSA is the most precise option with an accuracy of 0.1-0.4 mm but unfortunately needs tantalum markers and special instruments (8). The accuracy of EBRA-FCA to detect implant migration has been reported to be ±1

Key words: total hip arthroplasty, femoral offset, primary stability, longevity of the implant, early migration, EBRA-FCA

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THE ADULT ZEBRAFISH AS POLYHEDRIC MODEL FOR SKELETAL STUDIES

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In the last decade, several examples have been produced by scientific literature about zebrafish as a model to study human bone diseases. In fish, bone turnover, reparation and remodeling of the adult bone tissue cannot be studied in embryonic or juvenile stages. In addition, fins and scales represent unique anatomical features useful to study adult bone metabolism and diseases. For these reasons, the adult zebrafish represents an innovative and readily available resource for studying the bone metabolism at cellular and molecular level. Although the adult fish is less used than the embryo, several applications have been found in the last years with the production of innovative pathological models in adult zebrafish, helpful to understand the mechanisms of bone physiopathology. The use of mutants, regenerating organs, transgenic fish and scales have increased the power of this model in the last years.

The bone tissue from Danio rerio to Homo sapiens

Danio rerio (zebrafish) is an elective model organism for the study of vertebrate development. This is due to the unique characteristics of the embryo such as large clutches (up to 250 embryos/ week), small size, rapid external development and transparency of the larval body. Such advantages encourage the use of live imaging and powerful genetic tools based on mutagenesis. Moreover, automated systems have been coupled with zebrafish embryo to create one of the most important *in vivo* methods for drug screening, drug discovery and toxicity testing. The combination of these characteristics makes zebrafish an excellent animal model for basic biomedical research, drug development and translational medicine studies (1). Bone is a heterogeneous tissue composed by a mineral phase, hydroxyapatite, organic phase (type I collagen, other structural proteins, lipids) and water. Bone tissue is not a simply protective and static scaffold for the body but a dynamic organ that is constantly remodeled. Moreover, bone stores crucial nutrients, proteins, minerals, and lipids. In addition, in recent years has emerged the endocrine role of the skeletal tissue because of its implication in the hormonal network, energy metabolism and the physiological regulation of the several organs such as kidney, bone marrow, muscles etc. (2).

All these functions are common in the vertebrates, from human to fish. The similarity of the skeletal structure between *Danio rerio* and *Homo sapiens* has hired zebrafish as animal model to study different aspects of skeletal physiology: osteogenesis, bone metabolism, tissue turnover, resorbing activity etc. (3). In the last decade, several examples have been produced by the scientific literature about the introduction of zebrafish as model to study human bone diseases. Different and useful approaches can be used in zebrafish field, from embryo to adult.

Key words: zebrafish, animal model, bone, cartilage, regeneration

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TENDINOPATHIC SUPRASPINATUS TENOCYTES MAY HAVE A NEUROENDOCRINE-LIKE FUNCTION, SECRETING CGRP, SP AND VEGF: A PILOT IMMUNOHISTOCHEMISTRY STUDY

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We wanted to observe and compare the appearance of neurovascular tissue from tendon *ex vivo*, in patients with and without painful rotator cuff tendinopathy. Supraspinatus tendons were biopsied from 5 participants with painful tendinopathy and normal tendon from a young male. Slides were stained with haematoxylin & eosin and toluidine blue for histological assessment. Immunohistochemical markers for general nerves (protein gene-product 9.5 and synaptophysin), sensory nerves (calcitonin gene-related peptide; substance-P) and vascularisation (vascular endothelial growth factor) were used. PGP9.5 and CGRP-immunoreactive fibres were associated with vessels in cases and control. Synaptophysin-labelled fibres were observed in close relation to vessels in tendinopathy. PGP9.5, CGRP, SP and VEGF-immunoreaction also labelled tenocyte-like cells in degenerative areas and fibres in regions of fat and collagen. Sensory innervation and vascularity are increased in tendinopathy. The evidence for innervation and vascularity of symptomatic rotator cuff tendon may aid the development of novel investigations and therapies in the management of patients with this ailment.

Rotator cuff tendinopathy is a common cause of pain and disability (1). Its pathogenesis of rotator cuff tendinopathy is unclear and the response to intervention is frequently slow and often incomplete (2), the result of a combination of extrinsic or intrinsic factors.

Symptomatic and pain-free Achilles tendons, patellar tendons and the common extensor tendons of the wrist are supplied by general and sensory innervation, intimately associated with blood vessels (3). Using immunohistochemical techniques (3), general nerves are revealed by staining tissue with the neuronal marker, protein gene-product 9.5 (PGP9.5), and sensory fibres labelled with neuropeptides calcitonin gene-related peptide (CGRP) and substance-P (SP). Pathological tendons demonstrated an increased immunoreactivity for sensory neuropeptides which can cause pain (3). The innervation patterns in the supraspinatus tendon are unclear.

Additionally, neovascularisation has been associated with Achilles tendinopathy (4). Vascularised tendons may maintain an inflammatory state by supplying cytokines to the injured area (5). Angiogenesis is also associated with proliferation of sympathetic nerve fibres, promoting neoinnervation (6). The role of neovascularisation remains unclear. Some studies (7) reported hypovascularity in torn tendons, while others evidenced florid neovascularity (8). At ultrasound, Lewis et al (9) demonstrated a greater degree of neovascularity in rotator cuff tendinopathy compared with asymptomatic tendons.

Key words: tendon, shoulder, rotator cuff, supraspinatus, immunostaining

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QUANTITATIVE STUDY ON VANCOMYCIN RELEASE FROM CEMENT IN 3 DIFFERENT FORMULATIONS: PRELIMINARY RESULTS AND ANTIMICROBIAL ACTIVITY

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The purpose of this study is to investigate the best preparation method of the cement powder mixture, solvent and antibiotic in order to obtain the greatest amount of antibiotic in the joint for the longest time as possible. At time T0 the three samples, packed in a sterile environment in different formulations, were placed in sterile tubes, adding to each one 5 ml of saline phosphate buffer solution (PBS) and put in a stove at 37°C for 24 h. A sample of PBS without cement (T control) was also created. Qualitative and quantitative assessment of the incubated liquid with cement was performed along with biochemical analysis with High Performance Liquid Chromatography (HPLC). The analysis of the raw data demonstrated that at T1 there was a prevalence of antibiotic release from sample , compared to sample 2 and 3. This difference was maintained until the T20; from T21 the antibiotic release gradually leveled in 3 samples. The elution of the antibiotic remained detectable up to T60. Our work shows that the sample preparation is decisive on the quantity of released antibiotic. These results are confirmed by microbiological tests. It is useful to know the actual kinetics of antibiotics in articulation. Further studies are necessary to determine the effectiveness of antibiotic against micro-organisms and how long it acts.

Periprosthetic joint Infections (PJI) are one of the most dangerous complications in orthopedics. Although over the years surgeons have tried to prevent them, these occurrences are still common. Nowadays the gold standard treatment is the "two-stage surgery" using an antibiotic-loaded acrylic bone cement (ALABC) spacer.

The function is double: keeping the length of the limb (while waiting for the definitive prosthesis) and resolving the infection, acting directly on the septic focus, keeping high local concentration of antibiotic and minimizing systemic side effects. Recently, attention was focused on the elution of the antibiotic by polymethylmethacrylate (PMMA), in particular on the amount of released antibiotic, on how long it is released and on its efficacy. However, there are no studies in literature to describe how different preparations of the mixture ALABC could modify the elution of the antibiotic.

The aim of this study is to verify *in vitro* the different elution of antibiotic from three different preparations of the mixture (ALABC).

MATERIALS AND METHODS

The antibiotic used was Vancomycin (Hospira,

Key words: cement, prosthetic joint infections, antibiotic elution, PMMA

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