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Abstract	For the past 20 years, autologous Platelet-Rich Plasma (PRP) has been safely employed and its use has been documented in many areas, including orthopedics, sports medicine, dentistry, neurosurgery, ophthalmology, urology, wound healing, cosmetics, cardiothoracic, otorhinolaryngology and maxillofacial surgery.

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Chapter 2
Platelet-Rich Plasma in Pain Medicine

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Summary

For the past 20 years, autologous Platelet-Rich Plasma (PRP) has been safely employed and its use has been documented in many areas, including orthopedics, sports medicine, dentistry,

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neurosurgery, ophthalmology, urology, wound healing, cosmetics, cardiothoracic, otorhinolaryngology and maxillofacial surgery.

In the past few years, scientific research and technology have afforded fresh perspectives for understanding the wound healing process. In the beginning, platelet use was instituted solely to assist in clotting. However, we have learnt that platelets are also responsible for releasing many bioactive proteins and growth factors responsible for recruitment of macrophages, mesenchymal stem cells, and osteoblasts, which not only promotes necrotic tissue removal, but also improves the quality of tissue regeneration and the healing process.

Based on this principle, platelets are now introduced for the purpose of simulating the supraphysiological release of cells responsible for healing, so as to increase healing potential, mediate inflammatory processes and reduce pain through the release of substances such as serotonin, histamine and dopamine. Because of this, PRP has become an important prophylactic alternative in pain medicine and in treatment of chronic injury.

Introduction

Platelet-rich plasma (PRP) is an autologous biomaterial that can be obtained by centrifuging whole blood. PRP may be defined as a fraction of autologous plasma with platelet concentration above baseline level. Studies have shown that ideal concentration is at least a fourfold increase in initial concentration, or around 1,000,000 mm^3 [1].

Platelets are responsible for hemostasis promotion, new connective tissue formation and revascularization. A sample of whole blood usually contains 93 % red blood cells, 6 % platelets and 1 % white cells (leukocytes).

Justification for the benefits of PRP rests on inverting the proportion of these cells in the blood, reducing the red layer to 5 %, since red blood cells are less useful in the healing

process, and increasing platelets and leukocytes to 94 % to stimulate tissue regeneration [1].

Other benefits that justify PRP use are the diverse growth factors contained in the concentrate, such as transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), insulin-like growth factor-l (IGF-l), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) [2–4]. There have been *in vitro* and *in vivo* reports of the effects of PRP and growth factors on the stimulation of fibroblast proliferation by IGF-l, bFGF, and PDGF and of collagen synthesis and extracellular matrix synthesis by TGF-β in tendons and ligaments [5–8], resulting in enhanced regeneration and increased tissue strength and consistency.

PRP Processing

PRP processing involves the separation and concentration of platelets, leukocytes, and growth factors, considered initiators in any repair process. However, there is no standard technique for obtaining PRP and different preparations are described in the literature. There is wide variability in the ability to concentrate these cells and, for the most part, this variability relies on equipment, manufacturer and materials utilized.

Several authors have presented their proposals for classifying and suggestions for standardizing the infinite number of terms observed in current studies. These classifications are directly related to the cell composition of each concentrate.

Mishra [9] classifies PRP on the basis of the presence or absence of leukocytes, on the utilization of activating agents and on final platelet concentration, enabling a framework wherein products are divided into four types: type 1, high leukocyte concentration, non-activated; type 2, high leukocyte concentration, activated; type 3, non-activated, no leukocytes or low leukocyte concentration; and type 4, activated, no leukocytes or low leukocyte concentration. All these prod-

uct types may be further classified as A, corresponding to platelet concentration equal to or five times higher than the baseline level, or B, platelet concentration five times lower than baseline concentration.

Everts et al. (2008) [10] based their terms on two underlying principles: (1) the impossibility of obtaining a leukocyte-free product and (2) the need for platelet activation which generates a gelled product, internal or externally. Thence they proposed the term "platelet and leukocyte gel" (PLG) to denominate any and all platelet-derived products, assuming the veracity of premises 1 and 2.

Anitua et al. (2009) [11] advocate production of a leukocyte-free platelet concentrate which, according to the authors, can promote proinflammatory effects because of the proteases and acid hydrolases contained within. The authors suggest use of the term PRGF (plasma rich in growth factors), hinged upon the principle that activation of any platelet concentrate will result in the release of growth factors, essential agents in the healing cascade.

Ehrenfest et al. (2010) [12] uphold that it is impossible to obtain a leukocyte-free platelet concentrate, and suggest classification based on the presence or absence of leukocytes and on fibrin network architecture. This classification allowed for putting the different products into four categories: pure platelet-enriched plasma (P-PRP), which includes PRGF proposed by Anitua et al. (1999) [13] and leukocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), leukocyte- and platelet-rich fibrin (L-PRF).

Another much-debated and researched point is the use of the leukocyte layer in association with the platelet concentrate. Some studies have shown that white blood cells contained in PRP naturally safeguard this substance from infectious and allergic processes [14, 15].

However, there is still much debate surrounding leukocyte use, and studies suggest that when leukocytes are present, neutrophils are able to release metalloproteinases which cause cell matrix degradation as well as release free radicals [16]. This could lead to a delayed healing response from muscles [17].

According to Sundman et al. (2006), growth factor concentrations and catabolic cytokines are influenced by PRP cell composition. Platelets augment anabolic signaling whereas leukocytes increase catabolic signaling molecules, thereby suggesting reduction or non-utilization of the leukocytes contained in platelet concentrates [18].

Ehrenfest et al. (2012) also studied platelet concentrates, linking fibrin network and leukocyte content to capacity and speed of release of some growth factors. The leukocyte-dense concentrate was responsible for the slower and more intense release of growth factors, mainly, TGFβ1. The researchers concluded that polymerization and final architecture of the fibrin network strongly influence intensity and growth factor release speed, mainly TGFβ1, and that leukocyte presence plays an essential role in forming this network [19].

Platelet concentrates containing leukocytes may additionally be classified as different types. They include neutrophils, monocytes/macrophages and lymphocytes. They play different roles in tissue healing.

Neutrophils are phagocytes and contain more than 40 hydrolytic enzymes. Their activation leads to phagocytosis of debris and release of free oxygen radicals and proteases. This release of toxic molecules from neutrophils may be conducive to secondary muscle damage [17, 20]. It is not yet known what effects neutrophils have on soft tissue injury, whether acute or chronic.

Macrophages are the tissue form of circulating monocytes and their job is debris clearance, mainly phagocytic in nature. They also have a role in reflections concerning the pro-inflammatory and anti-inflammatory aspects of healing [21]. Since it is only possible to fractionate different kinds of white cells within PRP, it is likely that the absence of macrophages is more unfavorable to healing than any eventual neutrophil-induced damage.

PRP and PH activation of the concentrate are some other parameters currently being discussed in the literature. Bovine thrombin, collagen, autologous thrombin and calcium have been utilized to activate platelets previously activated by

anti-coagulants. Many current clinicians are not activating PRP and achieving equal clinical results.

This combination results in the formation of a gel, which can be utilized in open surgery and in wounds, but cannot be injected, even with a high-caliber needle. Bovine thrombin, collagen and calcium activation produce intense PRP activation and consequently the rapid release of platelet growth factors. This event is still being discussed in the literature because it is not yet known without a doubt whether it is ideal to promote such activation and consequent early release of growth factors. Ehrenfest et al. (2012) [19], concluded from an *in vitro* study that if PRP is activated intensely with calcium or bovine thrombin, the fibrin network will be an unstable one. If PRP is activated in a more physiological manner, a stable, tetramolecular network forms. Because of this, autologous thrombin use intended to promote a more physiological environment is being increasingly encouraged.

When considering the actual PRP procedure we have to address the use of co-administered analgesics, as isolated PRP is quite painful when applied to tissue. Most clinicians employ some sort of anesthetic, i.e. local block with lidocaine or direct mixing of the PRP product with anesthetic. New research, pending publication, suggests a possible negative affect of combining high dose anesthetics, i.e. lidocaine with PRP. This negative anesthetic affect is very much a dose dependent phenomena, lower doses are not seen as cytotoxic at this time. Further research in this area is ultimately required.

Injection Technique

Ultrasound-Guided Peripheral Blocks

The use of ultrasound to guide peripheral nerve blocks has grown more frequent. Some neurostimulation-related benefits of this technique have been demonstrated in the literature. The leading ones are: lower failure rate, shorter procedure

time, shorter latency time and longer duration of block with lower risk of accidental vascular puncturing [22–27].

Common injections are extremely inaccurate when done "blind", with failure rates for shoulder joint injections around 73 % [28], and failure rates for hip joint injections estimated at around 20–40 % [29].

Some authors believe that image guidance is an absolutely crucial point towards ensuring adequate intra-articular needle placement. Accuracy of palpation-guided needles is highly variable (50–93 %) in various different techniques. Moreover, 100 % accuracy was demonstrated in ultrasound-guided intra-articular injections into cadaver knees, performed by professionals with limited experience, versus 55 % in palpation-guided injections. These data support the benefits of injecting biomaterial to the exact site of the condition, whether in a ligament, tendon or muscle.

With its lower probability of causing vascular injury, ultrasound has become an interesting tool for peripheral block guidance. Association of the two techniques, ultrasound and neurostimulation, though it may make the procedure more costly, enhances safety and further reduces the risk of failure [30, 31].

Today, with the advances in ultrasound equipment and methods, it is possible to identify vascular and neural structures highly accurately. Therefore, ultrasound-guided injections enable greater accuracy, both in reaching the spot to be treated and in minimizing the risk of accidental vascular injury. It is worth mentioning that success and complication rates are tied to the clinician's experience, as is the case with any other technique [32].

Ultrasound-Guided Anesthetic Blocks for Differential Pain Diagnoses

Ultrasound-guided anesthetic nerve blocks, using local anesthetic, associated or not with corticosteroids, opioids and other agents, may be performed for the purposes of diagnoses,

prognoses and/or therapy. Ultrasound-guided anesthetic blocks relieve pain by interrupting the sensitive pathways that carry information to the central nervous system. They may be used in association to other kinds of anesthetic blocks, such as trigger point injections, joint injections or integrated to treatments that may involve, according to disease stage, other therapeutic measures for the same patient, such as systemic medication (painkillers, anti-inflammatory drugs, muscle relaxants, opioids) and physical methods (physical therapy, hydrotherapy, RPG, Pilates) [33].

Ultrasound-guided anesthetic blocks are included in treatments of interdisciplinary nature. They are indicated in keeping not only with pain characteristics, but also with patient profile with respect to acceptance of such method [34].

Ultrasound-guided anesthetic blocks for diagnostic purposes may be employed to determine the anatomical source of pain and to guide differential diagnosis of peripheral or central, local or referred and visceral or somatic pains. With relation to chronic pain, after undergoing several imaging, laboratory and functional tests, it is common for patients, to remain doubtful about the cause of their pain. In this case, when we block the sensory pathway with local anesthetic, promoting a temporary interruption of the pain, we are able to define the anatomical source of pain and, thus, define the cause of the pain, enabling us to better direct the course of treatment [35–37] (Figs. 2.1, 2.2, 2.3 and 2.4).

Diagnostic and therapeutic interventional techniques have developed greatly in the past few years and, together with pharmacological and non-pharmacological treatment, constitute one of the mainstays of chronic pain treatment. The efficacy of interventional techniques in pain treatment depends on correct placement of needles and other devices in known nerve structures, guided by well-established anatomical landmarks [38, 39].

Intra-articular knee injections are generally performed by orthopedic surgeons and rheumatologists and, with the expanding role of physicians in chronic pain management, common injections are frequently done in primary care

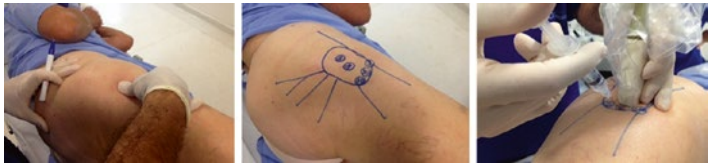

Fig. 2.1 Hip. Drawing of "pain map" based on VAS – (Visual Analogue Scale) – Ultrasound-guided diagnostic anesthetic block

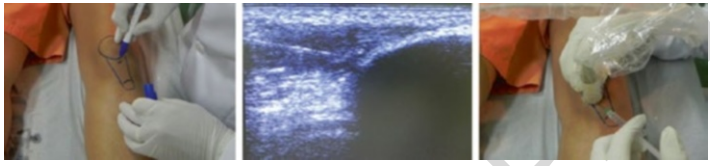

Fig. 2.2 Knee. Drawing of "pain map" based on VAS – (Visual Analogue Scale) – Ultrasound-guided diagnostic anesthetic block

settings. This trend highlights the need for process standardization to ensure patient safety and comfort by employing the most accurate injection techniques possible [40–42].

A number of imaging methods may be used to help the physician identify the correct path for the diagnostic and/or therapeutic intra-articular injection, including those guided by radiograph, ultrasound, computer tomograph and magnetic resonance. However, ultrasound presents one of the most practical options because it is safe, fast, relatively low-cost, and does not emit radiation [43].

Though a number of studies have reported intra-articular accuracy of common injections using images or anatomical landmark guidance, few controlled studies comparing the accuracy of these methods have been carried out. Results from available analyses show that utilization of imaging guidance improves the accuracy of intra-articular injections into large joints, including the knee. Furthermore, ultrasound guidance, specifically in the knee, greatly increases the probability of correct needle placement [43].

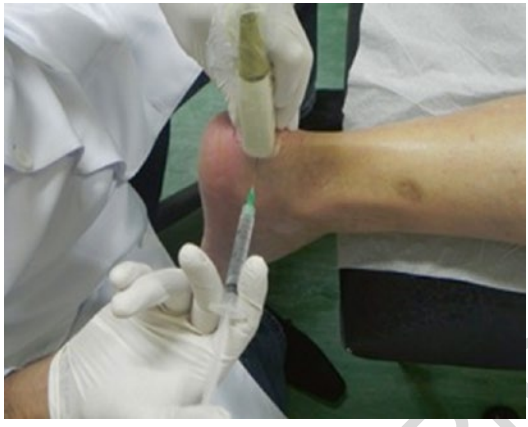

Fig. 2.3 Diagnostic anesthetic block to Achilles tendon

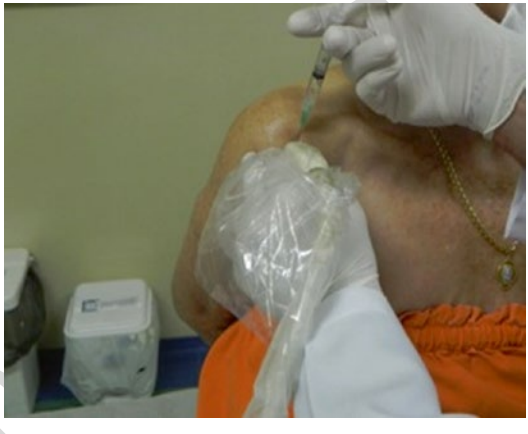

Fig. 2.4 Diagnostic anesthetic block to supraspinatus tendon

Accurate ultrasound-guided intra-articular injections have led to positive results and preliminary clinical evidence suggest that these patient benefits may result in reduction of health costs in the long term [44].

In the U.S.A., only one in five rheumatologists regularly utilize ultrasound in their musculoskeletal practice, even though three out of four agree that it should be a standard

clinical tool, for diagnosis, injection guidance, and evaluation of treatment response [44].

PRP in Orthopedics and Pain Medicine

In pain medicine, the primary goal is pain relief and increase in functional capacity of the injured tissue. Therefore, treatment should not only target local pain reduction, but also provide an environment where healing is stimulated, promoting tissue regeneration.

Several authors have reported successful use of Platelet-Rich Plasma (PRP) therapy in treating musculoskeletal injuries, with positive and promising results in pain reduction and injury healing. Several studies describing PRP use have emerged in which there were no complications.

There have been frequent reports of significant reduction in pain, risk of amputation, decrease in bleeding and consequent improvement of quality of life among patients.

The analgesic effect of PRP in the post-operative period was observed by Gardner et al. [45] in patients who had undergone total knee arthroplasty, who had lower pain intensity and who needed a smaller amount of painkillers. The same was observed in the post-operative period immediately after dental surgeries [46]. Besides the growth factors situated in the alpha-granules, platelets contain other substances stored in the dense granules, such as serotonin, histamine and dopamine, which possibly have an analgesic effect and justify these results [47].

Civinini et al. (2011) [48] reviewed *in vivo* and *in vitro* clinical studies in which PRP was used and they concluded it was a safe, easily preparable and relatively low-cost procedure for treatment of subacute and chronic injury.

Studies suggest that PRP has beneficial effects on post-operative inflammation, blood loss, infections, medication reduction, osteogenesis promotion, and soft tissue healing.

Besides the hemostasis provided at the vascular injury site, platelets contain a large amount of growth factors and cytokines that are fundamental in healing bone and soft tissue

[49]. This leads to greater awareness regarding the role platelets play in the healing process and has also led to the concept of their therapeutic application [50].

Autologous PRP injections are being used and documented in many areas, including orthopedics, sports medicine, dentistry, neurosurgery, cardiology, ophthalmology, urology and injury healing, as well as in the fields of cosmetics, cardiothoracic surgery and maxillofacial surgery, with positive and promising results.

With the growing benefits being provided in these areas, PRP gradually started to be studied and utilized in several branches of orthopedic surgery, mainly because it perfects and accelerates healing [51].

Tendons and Ligaments

Several studies have supported PRP use in the treatment of tendinopathies and ligament injuries.

Tendons and ligaments have slower healing potential compared to the majority of tissues because of their sparse vascularization. In a wound healing process, this may result in new tissue which does not possess the same structural and functional properties as those of the original tissue. One possible explanation is that their sparse vascularization results in an inadequate release of growth factors and healing cells at the site of injury. That is why it is possible that PRP, held to be a good source of growth factors and healing cells can improve the speed and quality of healing in these tissues.

Sánchez et al. (2007) [52] evaluated the recovery of 12 athletes who had undergone surgical repair for total rupture of the Achilles tendon, 6 of them jointly with PRP therapy. Results showed a significant difference in recovery between patients treated with PRP and the control group, such as shorter time for recovery of range of movement, significant pain relief and shorter time before resuming training.

In a case report, Maniscalco et al. (2008) [53] described the use of PRP in its gel form for treatment of rotator cuff injury

as an alternative to tendon transposition. The researchers observed complete wound healing after 6 months of treatment.

Knoet et al. (2009) [54] used three separate PRP injections, at 15-day intervals, to treat patellar tendinopathy and the study showed a significant improvement in physical pain relief, improvement in function and quality of life. Patients were followed up for 6 months.

In 2010, de Vos et al. [55] evaluated the efficacy of PRP in the treatment of chronic tendinopathy of the calcaneus tendon. In a random, double-blinded, prospective study, the researchers compared 27 patients who received PRP injections with 27 patients who received saline injections. The groups were equally treated with eccentric exercises. No statistically significant differences were observed between groups relative to pain and activity. In the study conclusion, the researchers did not recommend PRP for treatment of chronic tendinopathy of the calcaneus tendon.

The first randomized clinical study on PRP use in complete calcaneus tendon ruptures was published in 2011 by Schepull et al. [56]. The results evaluated were elasticity after 7 weeks and functional assessments after 1 year. No difference was observed between groups relative to elasticity capability and one potential detrimental effect of PRP was observed in relation to functional results after 1 year. It should be noted that in this study they utilized average platelet concentration, about 10 times the amount found in peripheral blood. Compared to similar studies, this amount is much higher.

In a recent controlled, randomized, prospective clinical trial, Almeida et al. (2012) [57] selected 27 patients who were later divided at random into groups to receive (n – 12) and not to receive (n – 15) PRP injections to the harvesting site of patellar tendon during ACL reconstruction surgery. The results were evaluated by magnetic resonance imaging (MRI) of the patellar tendon after 6 months. The researchers observed that the recovery of the patellar tendon tear zone/cleft was significantly better in the PRP group than in the

control group. The Visual Analogue Scale (VAS) was also used and post-operative pain scores were significantly lower in the group that received PRP. In conclusion, they confirmed the hypothesis that PRP could improve tissue healing at the patellar tendon harvesting site. PRP also reduced post-operative pain.

Several studies have highlighted the beneficial effects of PRP on ligament injury. There have been frequent reports of reduction in post-operative pain and decrease in length of time before resuming previously compromised activities, such as that of Sánchez et col (2003) who reported fewer complications and better healing after PRP injections in 100 patients who had undergone ACL reconstruction, in a retrospective clinical trial [58].

In a randomized, prospective study, Vogrin et al. (2010) [59] evaluated the use of platelet gel and leukocytes for reconstruction of the anterior cruciate ligament with tendon graft in 25 patients, having observed significant improvement in antero-posterior knee stability in patients treated with the gel, as compared to the control group. Also, they observed significant reduction in post-operative pain.

Radice et al. (2010) [60] evaluated data from a prospective, non-randomized, blind assessment study regarding PRP use in ACL reconstruction. Fifty patients who had undergone ACL reconstruction with autologous graft of the patellar or ischio-tibial ligaments were included in this study, and divided into two groups, in which PRP had been utilized or not in the graft. The evaluation included a series of magnetic resonance tests, done between 3 months and 1 year after surgery, and researchers evaluated graft homogeneity. It was seen that in the group that received PRP, the graft became homogeneous within a period of time 48 % shorter than that recorded for the control group.

Muscle

Muscle injury often leads to pain and significant impairment and is frequently a cause of disability among athletes and non-athletes. Even though there are many treatment options,

pain and the long duration of current treatments have underpinned the search for new therapies.

The basic science of muscle healing has therefore focused therapeutic attention on the use of autologous biological products as alternative treatment for muscle injury. The role of diverse growth factors in natural muscle repair is evident and is based on the increase of cytokine levels found in the muscle tissue healing process. PRP is known to contain many of these bioactive proteins and growth factors, leading to their utilization also in this kind of injury.

Despite the importance of this type of injury, there are few clinical studies to evaluate treatment options. Conventional treatments seek to reduce injury-associated bleeding and swelling. The administration of anti-inflammatory drugs may relieve pain, nonetheless, there is evidence that these medications interfere with the healing capacity of muscle tissue. Anti-inflammatory drugs may inhibit precursor myogenic cell fusion, thereby hampering muscle healing [61].

Sánchez et al. (2005) evaluated the clinical benefits of ultrasound-guided injections of growth factors in 20 patients with sports-related muscle injury [62]. Results demonstrated reduction in pain and swelling, total recovery of functional capacity before the expected time and muscle tissue regeneration, observed by ultrasound. There was no evidence of fibrosis in any of the cases treated and there was no recurrence of injury among all athletes after they resumed their normal sports activities.

In a review of the literature, Mishra et al. (2009) [63] evaluated studies on PRP injections to muscles and tendons. The authors postulate that when PRP is injected in its inactive form, it is activated by conjunctive tissue collagen. PRP then releases its growth factors and cytokines. These bioactive proteins, on their part, stimulate local stem cells and increase expression of the extracellular matrix gene. From this point, repair cells from bone marrow or local circulation are recruited. At the same time, PRP inhibits excess inflammation, apoptosis, and metalloproteinase activity. Interaction between these pathways results in tissue repair of the muscle, which allows it to endure effort or sporting activities, thus reducing the pain.

There are few publications involving PRP injections for treatment of muscle injury. Up to the moment, there are no publications of randomized clinical studies involving PRP injection for muscle injury treatment.

Hamid et al. (2012) [64] published a protocol where a blinded, controlled, randomized study will be undertaken. Twenty eight patients, 18 years and over, with recent Grade II ischiotibial muscle injury will be invited to participate. Participants will be distributed at random either to receive autologous PRP injection and take part in a rehabilitation program, or only to take part in a rehabilitation program. Participants will be followed up on the third day post injection and afterwards on a weekly basis for 16 weeks. At each follow-up visit, participants will be evaluated with respect to their capacity to resume playing, using a set of criteria. The primary endpoint is when participants have met the criteria for return to their game or at the end of 16 weeks. The main outcome of this study will be the time taken to resume playing after injury. This study protocol proposes rigorous assessment and with significant potential for utilization for Class 2 muscle injuries. If PRP efficacy is proven, such findings may greatly benefit patients with similar injuries.

Cartilage

The social impact of degenerative diseases such as joint cartilage conditions is increasing, and it is a consequence of the rise in the average age of the active population [65, 66]. Joint cartilage injuries have limited potential for repair, they are difficult to treat and remain a problem for doctors and orthopedic surgeons. Capacity for cartilage regeneration is limited because of its isolation from systemic regulation and because of vascular deficiency [67–69]. Unlike the majority of tissues, in an inflammatory process, chondrocytes do not migrate to the joint injury from a healthy, intact site [41,43]. Biomechanical, metabolic, and biological changes, as well as trauma and isolated chondral injuries, may lead to loss of tissue homeostasis,

resulting in faster degeneration of joint surfaces, leading to cartilage injury or osteoarthritis (OA). These pathologies are generally known to be crippling diseases, for they are constantly associated to severe pain and mobility difficulties.

Numerous growth factors are present in joint cartilage. They work together to regulate the development and maintenance of joint cartilage homeostasis throughout life [70]. In this manner, PRP has been frequently proposed as a promising treatment for cartilage regeneration. Various studies assessing PRP use for treatment of knee osteoarthritis [71–74] have reported promising clinical results with pain reduction, functional improvement, speedier return to daily and sporting activities and consequent improvement of quality of life.

A case report was published in 2003 by Sánchez et al., describing PRP use to treat cartilage avulsion. In the patient treated, 2 ml activated PRP was injected into the space between the fragment and its bone bed. Treatment was considered successful, with the patient evolving without pain and resuming sporting activities 18 weeks after PRP injection. The authors concluded that the addition of PRP strengthened cartilage healing, given the normal prognosis [13].

The positive and promising results of PRP treatment led Sánchez et al. (2008) to carry out a cross-sectional, observational study in which 30 patients with knee pain received PRP intra-articular injections so they could be compared to a group that received hyaluronic acid (HA) [72]. The patients received three PRP injections per week, or HA, and were followed up over a 6-month period. According to the authors, the PRP injection boosted the success rate by 33.4 %, in comparison with a 10 % success rate in the HA group. Furthermore, PRP significantly improved the percentage of physical pain relief in the assessment questionnaires utilized.

Kon et al. (2010) [75] treated 100 patients (115 knees) with four PRP injections over a 21 day period and accompanied these patients over a 12-month period. Of the patients evaluated in this study, 58 suffered from degenerative chondral injury with early-stage OA and 24 patients were diagnosed

with advanced-stage OA. A significant improvement in functional questionnaires and visual analogue scale (VAS) scores were observed. 80 % of patients were satisfied with the treatment, while older patients had a smaller response in comparison with younger patients. Patients with advanced-stage OA showed significant improvement in only 30 % of the cases. The authors concluded that PRP therapy is safe and efficient for pain relief, improvement in function and quality of life in patients with degenerative joint disease.

Sampson et al. (2010) [74] also reported diminished symptoms after three PRP injections for treatment of knee OA. In a prospective, preliminary study, 14 patients with primary and secondary knee osteoarthritis who had met study criteria received three PRP injections into the affected knee over a period of 4 weeks. To measure cartilage height, specific pain assessment and functional capacity questionnaires were utilized, as well as musculoskeletal echographies. The study showed significant and almost linear improvement in knee injuries, including pain reduction and symptom relief. The majority of patients expressed favorable results after 12 months of treatment. The researchers concluded that the positive trends and the safety profile that PRP demonstrates could be utilized in a larger blinded, random study, to determine if PRP is truly efficient in knee osteoarthritis treatment.

Recently, in a study published by Sánchez et al. (2012) [76], 40 patients suffering from severe monolateral hip OA received three PRP injections, which were administered once a week. The primary endpoint was significant pain relief, described as a reduction in pain intensity by at least 30 % from baseline, assessed by the WOMAC sub-scale at least 6 months after treatment. The Visual Analogue Scale (VAS) and the Harris sub-scale for hip pain, were also utilized to verify results. Secondary outcomes included at least 30 % improvement in pain and incapacity. Statistically significant reductions in grading on pain and functional capacity questionnaires were reported. 57.5 % of the patients reported clinically relevant pain reduction. The study supports the

safety and tolerability of PRP injections for pain relief and functional improvement in patients with hip OA.

The basic science, the pre-clinical studies and the clinical studies collectively indicate that PRP is a promising treatment for cartilage injury and joint pain. Even though the mechanism of action of PRP has still not been completely elucidated, at this moment, studies suggest that there is an anabolic effect on chondrocytes, synoviocytes, with a significant increase in cell proliferation and matrix production, as well as an anti-inflammatory effect by means of the regulation of the known catabolic signalling pathway.

Spinal Column

Spinal pain includes all painful conditions originating from the vertebral spine, whether from intervertebral disks, muscles, ligaments, joints or bones. The structures responsible for spinal pain are the vertebrae themselves, intervertebral discs, spinal cord, nerve roots, facet joints, muscles and ligaments 1-10. It is a public health problem in Western industrialized societies. Prevalence rates range from 12 to 35 % and around 10 % of these patients become chronically disabled. In the U.S.A., there are 1.5–4 million adults suffering from secondary lumbar pain to spinal degeneration for whom conservative treatment was ineffective, and many of these patients eventually undergo surgical procedures [77].

Back pain is closely related to intervertebral disk degeneration (IDD). This condition, though asymptomatic in many cases, is also associated with sciatica and intervertebral disk herniations or prolapses. Studies have shown that discogenic pain is the most common cause of chronic back pain, with rates ranging from 30 to 60 % in all cases. IDD alters disk height and the resting mechanisms of the spinal column, possibly adversely affecting the behavior of other spinal structures, such as muscles and ligaments. In the long run, it may lead to spinal stenosis, an important cause of

pain and disability in the elderly; its incidence is increasing exponentially with current demographic changes and increase in life expectancy.

Disks degenerate earlier than any other musculoskeletal tissue structures. There is a significant reduction in end-plate vascularization before the end of the first decade of life which marks the beginning of a structural disorganization of the intervertebral disc. Around the age of 20, the end-plate and the subchondral bone severs blood supply to the intervertebral disc [78, 79] and, after that, the intervertebral disc begins to depend on diffusion for nutrients [80]. About 20 % of people in their teenage years have discs with degenerative signs and this number can reach 60 % of severe disk degeneration around the age of 70.

Current treatments only attempt to reduce the pain, rather than repair the degenerated disc. Therefore, these approaches are only palliative. The more conservative treatments are rest (not recommended), muscle relaxants, corticosteroid or local anesthetic injections and manipulation therapies. Several minimally invasive interventions are also used, such as chemonucleolysis, intradiscal radiofrequency, annuloplasties and percutaneous decompression. Although some trials report successful outcomes, these interventional therapies have been effective in about 50 % of patients, and still constitute palliative treatments. Surgical treatment of IDD must always remain the last option because of the related complications and also because they do not provide a cure. Disk prostheses and arthrodeses are the most common.

Disk degeneration can lead to changes in adjacent tissue and is a risk factor for development of spinal stenosis in the long term. Biological therapies aim to restore disk height and its biomechanical function, therefore, they will not only serve as palliative treatment, but they will also change the natural history of the disease. Many studies have demonstrated the benefits of some biological therapies for the treatment of degenerative disk disease, such as those discussed below.

Glucosamine and Chondroitin

These agents have been used in several peripheral joint osteoarthritis studies. There is evidence that glucosamine and achondroitin synergistically increase the natural response of chondrocytic hypermetabolic repair and delay cartilaginous enzymatic degradation. Derby et al. conducted a pilot study utilizing intradiscal injections of glucosamine and chondroitin with DMSO (dimethyl sulfoxide) and hypertonic dextrose to promote a repair response in the intervertebral disc [81]. Clinical efficacy was similar to IDET procedures but more cost-effective. There is a need for comparative, controlled, randomized studies to establish the efficacy of intradiscal glucosamine and chondroitin injections.

Cell Therapies

The goal of cell therapies is to promote matrix regeneration in degenerated intervertebral disks. The disk cells can be negatively influenced by an increase in load (pressure), by hypoxia and nutrient deprivation. In response, they secrete lactate, cytokines and proteases that will induce acidification and disk degeneration. This degenerated matrix can cause end-plate sclerosis, sensitize nociceptors and exacerbate the adverse effects of load and decreased transport. Growth factors can increase intracellular disk matrix synthesis up to fivefold.

In 1991, Thompson et al. demonstrated strong effects of TGF-β 1, a growth factor, on disk cell proliferation and on proteoglycan synthesis [82]. In 2000, Gruber et al. showed that platelet-derived growth factors have an antiapoptotic effect on fibrous annulus cells [83], besides stimulating annular cell proliferation after 4 days of exposure [84]. In turn, PRP contains high TGF-β 1 concentrations and platelet-derived growth factors [3, 4].

In a study with rabbits, Obata et al. showed an increase in the number of chondrocytes in the nucleus pulposus after

intra-discal PRP injection. Radiological and histological management also demonstrated reparative effects on the degenerated disk, with an increase in disk height in compared to the control group [85]. Several other studies in animals have found promising results using growth factors to reduce disk degeneration [86–90].

In a trial in patients who had undergone anterior spinal fusion, Hartmann et al. demonstrated increased bone density in the fusion area in the group that had received PRP intra-operatively in comparison with the control group [91].

The use of cell therapy for spinal degenerative disease represents a great hope in the treatment of this complex condition. Randomized studies in humans should be conducted so that this therapeutic possibility can be evaluated.

Conclusion

Platelet-rich Plasma emerges as an autologous, non-immunogenic, therapeutic option capable of stimulating the supraphysiological release of cells responsible for wound healing, with the aim of augmenting healing potential, mediating inflammatory processes and relieving pain. The frequently reported reduction in pain and in healing time renders the PRP technique an important therapeutic tool in interventional pain treatment. Correct association with adjunctive therapies is also related to a good pain prognosis. It is important to remember that being an autologous biomaterial, the success of PRP therapy depends on the patient's general clinical conditions. The literature at current state has mixed reviews of the affect of PRP 92, therefore Careful assessment of the mechanical and biological factors involved in the wound healing process must be performed so as to focus treatment and guide precise indication of the technique. Assuredly, all these effects related to the use of growth factors will continue spurring research into the ideal role of PRP and its main indications in pain medicine and orthopedics.

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