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Operative Techniques in
Orthopaedics

Platelet-Rich Plasma in Muscle and Tendon Healing

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Platelet-rich plasma (PRP) products represent advanced regenerative therapies for acute and chronic muscle and for tendon injuries because they can exploit the regenerative capabilities of the musculoskeletal system. PRP injections are used in clinical practice, but there is a need to evaluate the claims made about PRP therapies. Herein, we review current published clinical studies and focus on PRP formulations and application procedures. This article also describes the authors' clinical experience with PRP therapy in muscle and tendon conditions during the past decade. Treatment effects and the primary conclusions of clinical studies may be affected by procedures of PRP administration, and estimates of PRP treatment effect may deviate from its true value. To better define the conditions of clinical trials, we need to know more about the differences not only between PRP formulations but also among technical procedures in surgery and injection protocols, including applied volumes, target areas to treat, treatment schedules, and patient selection criteria.

KEYWORDS healing, injuries, muscle, platelet-rich plasma, tendinopathy, tendon

Soft-tissue disorders, including muscle, tendon, ligament, and joint capsular injuries, represent more than 50% of all the musculoskeletal injuries reported each year in the United States.[1] Primary care studies have shown that 16% of the general population suffers from shoulder pain, and elbow tendinopathy affects 1%-2% of the population. In 2002, an estimated US $15.8 billion in total health care expenditures was used for the medical management of these injuries.[2] The importance of this problem is substantial and represents a significant burden to society in terms of health care resources, personal disability, and activity restriction.

Platelet-rich plasma (PRP) technology represents an advanced regenerative therapy for acute and chronic injuries, and it is commonly used for the repair, reconstruction, or supplementation of a recipient's tissues. The management of musculoskeletal injuries with PRP therapies has been advocated since 2003,[3] and is a promising, innovative technology that can address diverse musculoskeletal conditions. The straightforward preparation protocols (minimally manipulated blood products) and the biosafety and versatility of PRP preparations have stimulated translational research and interest among both the scientific and medical communities, and have widened PRP applications to several musculoskeletal problems.[4] Several disciplines, including surgery, regenerative biology, and medicine, have converged toward the development of various PRP products suitable for harnessing the known regenerative capabilities of the musculoskeletal system. However, despite intensive research, there is a gap in the basic knowledge necessary[5] to ascertain the best PRP product for each clinical problem, as well as the guidelines for clinical applications.

These regenerative medical products pose novel challenges that may have significant bearing on the different PRP formulations and how application procedures are developed. There is preliminary evidence to suggest a link between PRP formulation (number of platelets, balance between platelet-secreted and plasma proteins, mechanism of plasma activation) and/or application procedures (ie, number of doses, volume, activation, and injection procedures) and clinical effect.[6,7] Some relevant concepts are needed to understand why the formulations and procedures for PRP application should be carefully analyzed. First, these products combine at least 3 established product paradigms: pharmaceuticals (biologically active substances), grafts (platelets and leuko-

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cytes embedded in a fibrin scaffold), and devices (PRPs combined with biomaterials). Second, PRP is a biological product that is critically different from conventional, chemically synthesized drugs: it is a complex mixture that is not readily characterizable in terms of its principal critical components or the dose–response relationship. Finally, in the absence of guidelines, physicians must use a strong rationale and their understanding of basic tissue and cellular responses to establish procedures that maximize the healing properties of PRP. Because of the unique biological activity and characteristics of PRP therapies, they require new preclinical paradigms to bring PRP products to clinical trials. Thus, the predominant approaches to designing application procedures should be based on tissue engineering ideas, rather than on pharmacologic concepts.

Indeed, PRP technologies could address unmet clinical needs; however, their complexity has introduced challenges for practitioners, and specific procedural steps should ensure that biological concepts and principles are implemented. In this article, we briefly review the clinical studies of PRPs in muscle and tendon conditions, with a special emphasis on injection treatments. Finally, we describe our procedures and the main applications of PRP therapies in soft-tissue pathology.

Summary of Clinical Data

Muscle Injuries

The vulnerability of athletes to strains and contusions represents a substantial problem for professional athletes and their teams. Such injuries involve significant time lost from training and competition. Current treatment modalities include physical therapy, ice, nonsteroidal antiinflammatory drugs, ultrasound technologies, and cortisone injections. However, given the increasing demands of training and competition, a clear need exists for novel treatments that are able to accelerate recovery from muscle injuries, do not adversely affect recurrence rates, and minimize scarring. The rationale for injection treatment with PRP products involves substituting blood clots with PRP clots, and in doing so, concentrating healing factors released by platelets at the injured site.

At present, no randomized trials have studied the merits of PRP therapies for muscle healing, and only 4 clinical reports, all of them level 3 or 4 observational studies, have been published. Wright-Carpenter et al[8] assessed the effects of several autologous conditioned serum (ACS) injections in lower limb muscle tears of moderate severity, in a nonblinded, nonrandomized, case-control study. ACS is prepared by incubating whole blood with glass beads and then centrifuging the mixture to obtain serum, which contains signaling proteins, including interleukin 1β (IL-1β), tumor necrosis factor α, IL-7, fibroblastic growth factor (FGF)-2, IL-1 receptor antagonist (IL-1Ra), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF-AB), transforming growth factor (TGFβ-1), and insulin-like growth factor (IGF)-1. In this study, 17 patients were treated with ACS, whereas the control group, which was analyzed retrospectively, included 11 patients who had received Traumeel/Actovegin (3:2) (homeopathic formulation/deproteinized calf blood hemodialysate). The RICE (rest, ice, compression, elevation) protocol was used for initial care in both groups. The mean number of injections per patient was 5.4 in the ACS group and 8.3 in the control group. The experimental group returned to competition after 16.6 days, whereas the control group took 22.3 days; in addition, magnetic resonance imaging scans taken at 16 days in both groups confirmed that the regression of edema/bleeding was faster in the ACS group. At the 2nd World Congress of Regenerative Medicine, Sanchez et al[9] reported the application of pure PRP in 21 muscle injuries of different severities and at different anatomical locations; small tears progressed well with a single application, whereas more severe tears required 2-3 ultrasound (US)-guided injections. The injected volume depended on tear severity. These athletes, who played in first division teams of the Spanish soccer league, resumed normal training activities in half the time needed by matched historical controls. Using the same pure PRP preparation in a single case study, Wee et al[10] reported a good outcome (1 week to return to preinjury activities) after 3 weekly US-guided injections to treat adductor longus strain. Recently, Hamilton et al[11] reported on a buffered L-PRP injection in a grade II hamstring strain injury, with a daily physiotherapy program in a single case study. Seventeen days after injury, the patient had full range of motion and was pain free in maximal contraction, consistent with a magnetic resonance imaging scan demonstrating complete resolution.

PRP injection is a form of management of muscle injuries that can be considered in clinical practice. However, it is difficult to recommend it as a best practice, both because clinical management is based on only a few studies at different anatomical locations and because PRP therapies are unclear regarding their best formulation, the technical procedure, the timing, the doses, and the repetitions.[12]

Tendon Pathology

The current concept of tendinopathy is that the underlying pathology is progressively degenerative due to a failed healing response and/or insufficient healing capacity of the tendon.[13] Current injection treatments, in addition to PRP, include dry needling, high-volume normosaline with local anesthetics and hydrocortisone or aprotinin, autologous blood, sclerosis agents, steroids, and aprotinin. At this time, surgical and conservative management of tendon conditions with PRP injections and research attention to this management are increasing. The rationale for the application of blood products in tendon pathologies is the providing of blood-derived healing factors to otherwise deprived tissue. Table 1 shows the clinical studies performed in tendon pathology at various anatomical locations.

Upper Limb Conditions

Epicondylar Tendinopathies

Medial and lateral epicondylar problems are common in people who perform repetitive wrist motions and strong grip-

Table 1 Platelet Rich Plasma Therapies to Treat Tendon Pathology: Clinical Studies

Reference	Tendon Pathology/Intervention	Type of PRP (Platelet Concentration) Activation-Clotting	Study Design/Level Evidence	Results
Epicondylitis				
Mishra and Pavelko[14]	Epicondylitis/1 injection	Buffered L-PRP (platelets: 4-8×; leukocytes: 5×; no activation)	Cohort study/III N = 15 PRP N = 5 controls	Improved pain (VAS) and Mayo elbow performance scores up to 6 mo; >90% resuming normal activities at 26 mo in PRP group; 60% drop-out in controls
Peerbooms et al[15] Gosens et al[16]	Epicondylitis/1 injection	Buffered L-PRP (platelets: 4-8×; leukocytes: 5×; no activation) vs corticosteroids 4-mL injections	RCT/I N = 51 PRP N = 49 corticosteroid	VAS and DASH score improved in both groups but much better in PRP group 49% in corticosteroid group vs 73% success in PRP 2-Year follow-up PRP more successful than corticosteroids
Craney et al[17]	Epicondylitis/2 injections	1.5 mL L-PRP(2.8×) vs 1.5 mL autologous blood	RCT/I	No differences in pain and physical function (PRTE score) 1, 3, and 6 months
Thanasas et al[18]	Epicondylitis/1 injection peppering technique, US guidance	3 mL PRP vs 3 mL autologous blood L-PRP 5×, no activation	RCT/I N = 14 PRP N = 14 autologous blood	PRP: VAS better at 6 weeks (61.47% vs 41.6% improvement) No differences (Liverpool elbow score)
Shoulder				
Zavadil et al[19]	Arthroplasty/open surgery	L-PRP/PPP vs controls	RCT/I N = 20 PRP-PPP N = 20 controls	Less pain and higher internal rotation index in the PRP/PPP group
Everts et al[20]	Subacromial decompression/open surgery	L-PRP (4-8×) thrombin clotted vs controls	RCT/I	Less pain medication, earlier functional recovery, greater range of motion
Randelli et al[21]	Rotator cuff/ arthroscopic surgery	L-PRP (4-8×) autologous thrombin clotted Platelets: 9.3×; leukocytes: 5×	Case series/IV N = 14 cases	Less pain (VAS), improvement in function (UCLA, Constant scores) at 6, 12, and 24 mo
Randelli et al[22]	Rotator cuff/arthroscopic surgery	L-PRP (4-8×) Autologous thrombin clotted 6 mL L-PRP injected in dry subacromial space	RCT/I PRP group N = 26 control N = 27	Earlier healing in PRP group than in controls and decreased postop pain; better functional outcome at 3 mo (VAS, UCLA, and Constant), no differences at 2 years
Castricini et al[23]	Rotator cuff/arthroscopic surgery	Pure PRP (1-2×) $CaCl_2$ clotting (PRFM); clot from 8-10 mL plasma; PRFM sutured under supraspinatus	RCT/I N = 45 PRFM and 43 controls	No improvement, not applicable to small and medium tears
Barber et al[24]	Rotator cuff/arthroscopic surgery	Pure PRP (1-2×) $CaCl_2$ clotting (PRFM); clot from 8-10 mL plasma PRFM sutured into repair site	Case-control N = 20 each group, matched/III	50% vs 86% healing in PRFM, tears <3 cm lower retear rates (MRI) with PRFM no differences in the clinical outcome
Achilles tendon				
Sánchez et al[25]	Achilles tears/surgery	Pure PRP (2-3×) $CaCl_2$ (22 mM) activated and clotted	Case-control/III N = 10 each group	Enhanced healing and functional recovery, less cross-sectional area in PRP group
Gaweda et al[26]	Achilles tendinopathy/injection number tailored to patient	L-PRP (4×), 3 mL into hypoechogenic areas	Case series/IV N = 14 patients, 15 tendons	Size reduction or regression of hypoechogenic foci clinical and functional improvement (AOFAS and VISA)
Schepull et al[27]	Achilles tendon ruptures/ Tantalum beads implanted proximal and distal to the rupture	10 L L-PRP 10× 10-mL injection ruptured site vs controls 250 mM $CaCl_2$	RCT/level II PRP N = 16 control N = 14	No differences in e-modulus (RSA) or heel raise index. Detrimental effect of PRP as measured with Achilles tendon total rupture score
De Vos et al[28] De Jonge et al[29]	Achilles tendinopathy/1 injection at 3 different needle locations, 5 aliquots injected	4 mL buffered L-PRP (4-8×) (no activation) vs saline both groups eccentric exercise program	RCT/I N = 54	Improvement (VISA-A) in both groups but no differences between groups for VISA score, satisfaction or return to sports 1-Year follow-up; no clinical or ultrasonographic differences
Patellar tendon				
Volpi et al 2007[30]	Patellar tendon/1 injection	L-PRP	Case series/level IV N = 8	Improved VISA and MRI in (=% of the injected tendons at 120 days)
Kon et al[31]	Patellar tendon/3 injections	L-PRP (4-8×) $CaCl_2$ activated, stored at −30°C	Case series/level IV N = 20	Improvement in Tegner, EQ, VAS, SF 36 scores; 80% were satisfied, 70% complete or marked resolution at 6 mo

Table 1 Continued

Reference	Tendon Pathology/Intervention	Type of PRP (Platelet Concentration) Activation-Clotting	Study Design/Level Evidence	Results
Filardo et al[32]	Patellar tendon/3 injections, 3 wks apart	L-PRP (6×) $CaCl_2$ activated (22 mM), stored at −30°C and thawed before application + physical therapy vs physical therapy	Nonrandomized controlled trial PRP N = 15 control N = 16/III	Improvement in Tegner, EQ, VAS scores at the end of treatment
Others				
Barret et al[33]	Chronic plantar fasciitis	Ultrasound-guided PRP	Case series/IV N = 9	Complete pain resolution for up to 1 year in 77.8% of patients. Reduced thickness of plantar bands
Finnoff et al[34]	Diverse tendons recalcitrant tendinopathy	Percutaneous needle tenotomy and PRP injection	Case series/IV N = 51	68% functional improvement 68% pain improvement 80% patient satisfaction 64% resolution intratendinous calcification, 82% decrease neovascularity
Volpi et al[35]	Diverse tendons/1 injection	Buffered L-PRP (platelets: 4-8×; leukocytes: 5×; no activation)	Case series/IV N = 15	Improvement in clinical symptoms (VISA), for 2 years

L-PRP, leukocyte and platelet-rich plasma; PRTE, patient-related tennis elbow evaluation; RCT, randomized clinical trial; VAS, visual analog scale for pain; EQ and SF-36, general health questionnaires; PRFM, platelet-rich fibrin matrix; DASH, disabilities of the arm, shoulder and hand score; AOFAS, American orthopedic foot and ankle society; VISA-A, Victorian Institute of Sport Assessment—Achilles.

ping. A pioneering observational study[14] reported a significant reduction in pain after 1 intratendinous injection of buffered PRP in refractory tendinopathy. Corticosteroid injections are frequently used to treat tendon pain and were compared with PRP injections in a double-blind, randomized clinical trial.[15] PRP injections were more efficacious in pain reduction and functional improvement, and their beneficial clinical effects were maintained for at least 1 year.[16] However, corticosteroid injections produced worse long-term outcomes than a wait-and-see policy. Autologous blood injections for refractory lateral epicondylitis have been advocated since 2003,[37] and recently, they have been compared with PRP injections in 2 randomized clinical trials. Creaney et al[17] evaluated two 1.5-mL injections administered with a

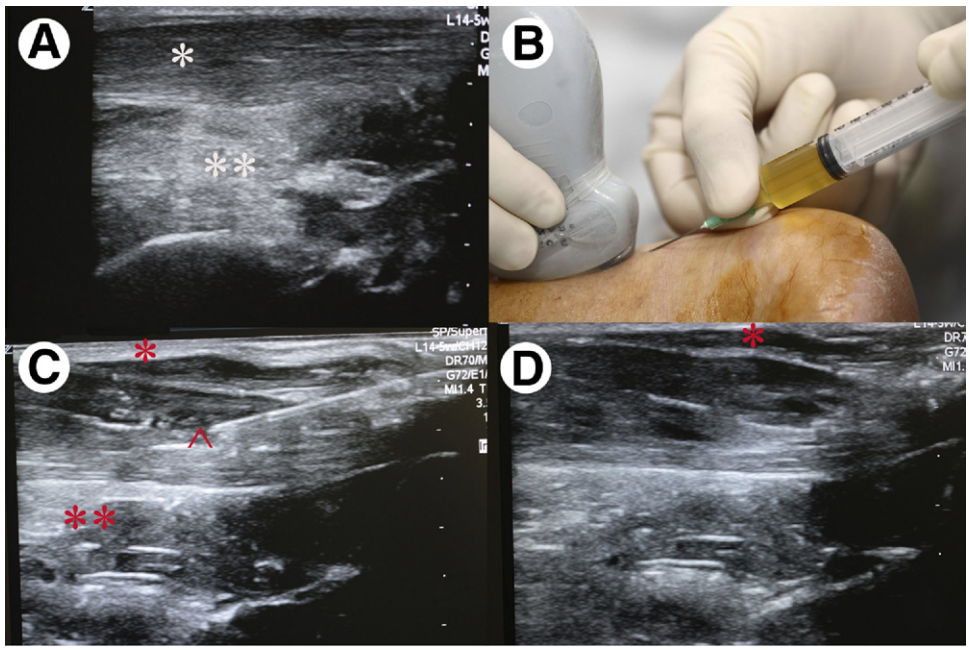

Figure 1 PRP injection for the treatment of Achilles tendinopathy. Ultrasound images show tendon, white asterisk; fat, double white asterisk; paratenon, red asterisk: (A) Ultrasound view of Achilles tendon (*) and associated fat (**) immediately before PRP injection; (B) The needle is inserted under ultrasound guidance from distal to proximal, in a parallel track to the collagen fascicles; (C) Intratendinous (*) injection of plasma, ^needle tip immediately adjacent to injected plasma; (D) Ultrasound image shows PRP injected between the tendon and paratenon (red asterisk) and plasma injected within the Achilles-associated fat. Plasma spreads when injected between the tendon and paratenon due to the laxity of the extracellular matrix. The goals are to inject the maximum volume that can be confined within the area of degeneration and to deliver some volume of PRP to the nearby tissue to activate tenocytes.

1-month interval between them, whereas Thanasas et al[18] treated epicondylitis with a single 3-mL injection. Both protocols improved pain and function, but only the former study[17] found a superior effect of PRP over autologous blood on pain reduction at 6 months.

Overall, studies on the management of tennis elbow with PRP have shown promising results that have been better than corticosteroids, albeit similar to autologous blood injections. The PRP product in all these studies consisted of high platelet and leukocyte concentrations, and the PRP was buffered before injections in 2 of the 4 studies.

Surgical Applications in Shoulder Pathology

Initially, observational studies reported significant improvement in pain and function after arthroscopic rotator cuff surgery assisted with PRP.[21] Likewise, Zadavil et al[19] and Everts et al[20] reported better functional recovery and less pain in arthroplasty and open subacromial decompression, respectively, using L-PRP in prospective, randomized, double-blind trials. Likewise, a 2-year follow-up prospective randomized study in arthroscopic rotator cuff repair showed reduced pain in the first postoperative months and significantly higher strength in external rotation in the L-PRP group.[22] In contrast, PRFM (a pure PRP-clotted *ex vivo* with $CaCl_2$) augmentation in the surgical treatment of rotator cuff tears did not show any clinical advantage compared with controls,[23] as reported in 2 level I clinical trials, although it resulted in lower retear rates (tears <3 cm) in 1 study.[24]

The treatment effects and primary conclusions of these clinical trials may have been substantially affected by suboptimal procedures of PRP administration, and estimates of PRP treatment effects may have deviated from their real value. To define the conditions of clinical trials better, we need to know more about differences not only between PRP formulations but between technical descriptions of surgery or injection protocols, including applied volumes, target areas to treat, treatment schedules, and patient selection criteria.

Lower Limb Conditions

Management with PRP injections is becoming more widespread in lower limb conditions, and the injections are used both in surgery and in conservative management.

Surgical Treatment of Achilles Tendon Tears

PRP improved functional recovery after surgery for Achilles tendon tears. In this study, fresh PRP (2-3×) was applied in several configurations, that is, PRP-fibrin constructs (PRP jellified *ex vivo*) and liquid-activated PRP (jellified *in vivo*). The effect induced by PRP therapy had long-term results, such as decreased cross-sectional area after 18 months.[25] More recently,[27] open repair of the Achilles tendon was not enhanced by an L-PRP product that consisted of leukocytes and a high concentration of platelets (14×). This L-PRP was maintained for up to 20 hours at room temperature, with constant rotation before use. Moreover, the protocol for application differed from the Sánchez protocol in that 6 mL of L-PRP was injected through a cannula at the rupture site after tendon suture, and the remaining 4 mL was injected transdermally at the ruptured site. The patients wore casts for 7 weeks, in contrast to the Sánchez protocol, in which the patients started physiotherapy after 2 weeks. Together, these observations lead to the notion that differences in PRP products, surgical procedures, and PRP application protocols have consequences for clinical outcomes.

Conservative Management of Achilles Tendinopathy

US-guided pure PRP injections did not improve pain and activity,[28,29] compared with saline injections, in a double-blind, randomized clinical trial. In this study, the PRP treatment consisted of a single injection of 4 mL of buffered L-PRP and eccentric exercises. Five small aliquots were injected at 3 different needle locations under US guidance. Notably, the patients received a single PRP application. All patients improved, but there were no differences between PRP and saline injections, either in the short- or long-term (1 year) follow-up. Gaweda et al[26] injected 3 mL of L-PRP (4×) into hy-

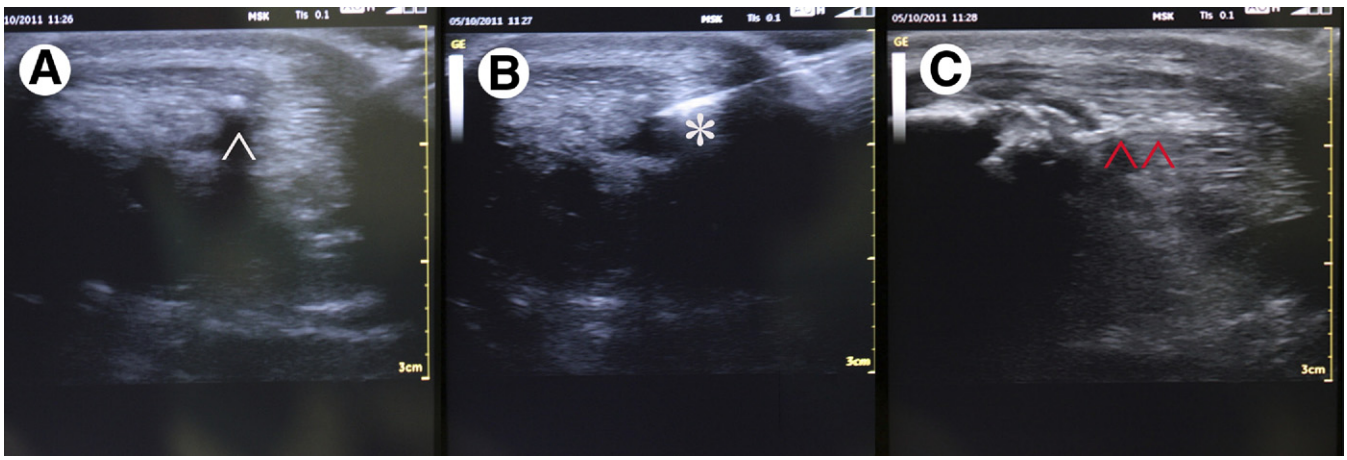

Figure 2 Ultrasound views of patellar tendon during PRP injection. (A) Calcification in the proximal pole (white arrowhead); (B) needle tip immediately adjacent to calcification; (C) image acquired immediately after infiltration shows clotted PRP.

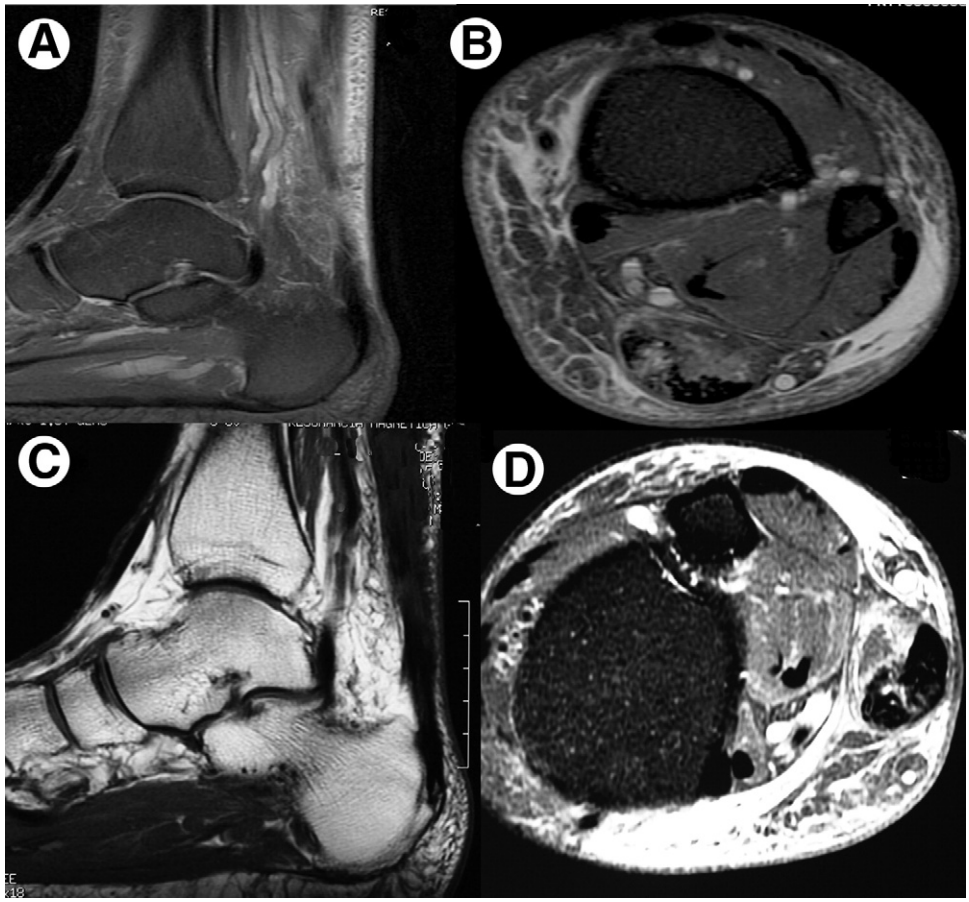

Figure 3 Achilles tendinopathy attributed to the oral use of corticosteroids in a 60-year-old man; after informed consent, the patient received 3 injections of PRP weekly. MRI showing (A-B) the pathologic area of tendon degeneration before PRP management; (C-D) Achilles tendon 2 months posttreatment.

poechogenic areas under US guidance. Patients avoided full loading for 3 days and used crutches for the next 2 weeks, with full loading 6 weeks after injection. The number of injections was personalized to the patient's condition. Patients showed significant overall improvement and improved tendon structure, as assessed by echography. This finding suggests that the results of clinical studies cannot be generalized without differentiating the characteristics of PRP products and the protocols for administration (doses, volumes). If the desired effect is an account of application procedures, then providing information about the clinical results of different protocols is crucial to the designing of clinical trials, ideally for different clinical conditions. Assuming that tendinopathy is a consequence of a failed healing response process aggravated by mechanical factors, it seems logical that the optimal volume and number of injections should be tailored to each patient, taking into account the severity of the pathology.

Patellar Tendinopathy

Patients with chronic patellar tendinopathy who received 3 L-PRP injections reported improved function and reduced pain.[31,32] The L-PRP (6×) was stored at −30°C, was thawed before application, and was activated with $CaCl_2$ (22 mM) before injection.

Barrett and Erredge demonstrated that PRP could be safely injected for plantar fasciitis. At 1 year, 7 of 9 patients had complete resolution of pain.[33] Finnoff et al[34] and Volpi et al[35] confirmed the beneficial effects of L-PRP injections at various anatomical locations, but these studies were limited to a case series.

No PRP formulation has yet provided solid evidence for the stimulation of tendon healing; nevertheless, the treatment is safe, and no complications have been reported after PRP treatments in any study, although the evidence for or against the beneficial effects of PRP therapy in chronic tendon problems needs to be clarified. Moreover, the number of doses should be tested because it seems improbable that a single injection could reverse a chronic, degenerative pathology. The administration of repeated doses seems more reasonable.

Authors' Experience

As mentioned earlier in the text, results have been controversial; thus, the need is great to understand better the relationships between PRP formulations, indications, and procedures for application in different muscle and tendon conditions. Optimal formulations and protocols that take individual conditions into account are needed; for example,

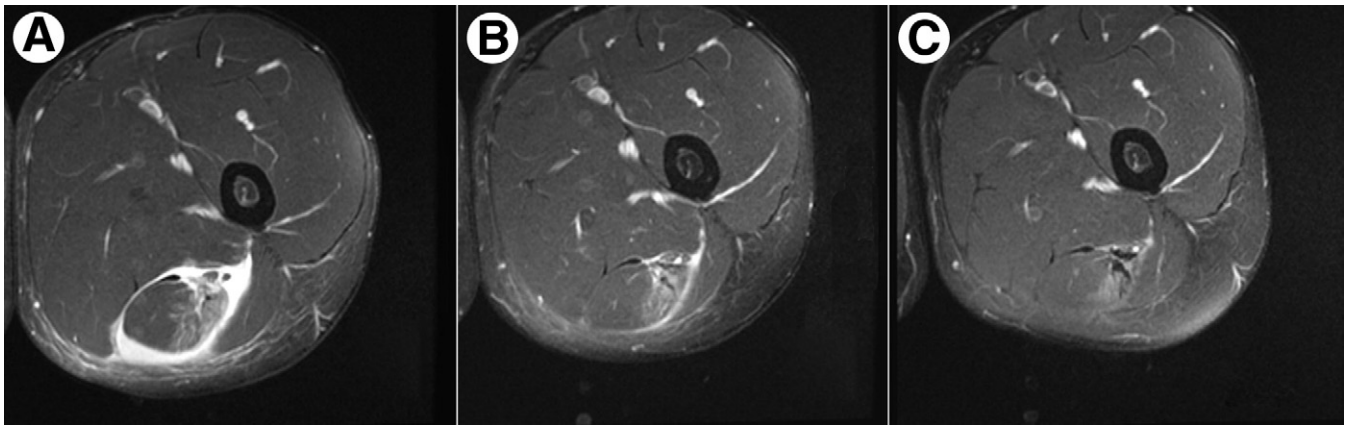

Figure 4 A young soccer player with a biceps femoris tear was treated every other week with 2 PRP injections. (A) Axial MRI reveals increased T2 signal intensity with disruption of the biceps femoris tendon and perifascial fluid, consistent with a grade II injury; (B) two weeks after the first PRP injection, the same axial level shows less fluid and muscle edema with some healing tissue; (C) outcome three weeks after the second injection (at five weeks from the beginning of the treatment): the same axial level shows no fluid, a faint edema and plenty of scar tissue in miotendinous unit of the biceps femoris tendon.

our clinical experience has involved PRP prepared through single-step centrifugation, containing 2-3 times the normal platelet concentration and no leukocytes. In contrast, PRPs obtained through double-centrifugation or filtration produce platelet-leukocyte concentrates 4-6 times those found in peripheral blood. Some protocols do not activate plasma because contact with collagen and tissue factors would activate clotting. Instead, we activate plasma with calcium chloride before injection; this process results in fibrin confinement and proteins secreted gradually from the matrix. Usually, these proteins must accumulate over time to reach the threshold set by the affinity of their receptors, a physiological mechanism found in many cellular processes *in vivo*. To reach these thresholds, further cells synthesizing additional amounts of growth factors and cytokines are therefore crucial. Hence, we also inject "healthy" peripheral tissue that targets fibroblasts.

The optimal volume and number of injections are still unclear. It seems logical that these properties should be tailored to each patient, taking into account the severity and location of the injury and the clinical response. High volumes have additional mechanical advantages.[36] When multiple injections are considered, the ideal period between injections is unknown; however, because PRP therapies influence early healing, 1 week may be adequate for monitoring individual outcomes and making decisions about further plasma injections.

Pure PRP Preparation

The patients are advised not to eat fatty foods in the 6 hours before blood extraction. Forty milliliters of peripheral venous blood is withdrawn, using a 21-G needle, to avoid damaging or partially activating the platelets, into 9-mL tubes that contain 3.8% (wt/vol) sodium citrate. Pure PRP, that is, PRP without leukocytes and with a moderate concentration of platelets (1.5-3×), is prepared by a single spin at 580*g* for 8 minutes at room temperature (PRGF-Endoret, Vitoria, Spain). The 2 mL of plasma fraction, located just above the sedimented red blood cells and above the buffy coat, is collected using a plasma transfer device in a sterile tube under vertical airflow conditions. Calcium chloride (10%) is added at a final concentration of 22.8 mM, shortly before the intratendinous or intramuscular injection. We use PRP soon after preparing it and never freeze it. Currently, we treat both tendon or muscle pathology using the same plasma formulation, prepared as described later in the text.

Technical Description of the Injection Techniques for Muscle and Tendon Pathology

Skin preparation is performed with an antiseptic solution in the usual manner. The patient is clinically examined to correctly identify and mark the area of maximum tenderness and/or swelling. After the examination, a sterile longitudinal 7.5-MHz transducer is used to confirm and image the precise location of the damage (hematoma or degenerated area). We do not administer anesthesia; instead, cold therapy is applied after injection. We choose the injection site based on the anatomical location of the pathologic lesion and always perform injection guided with US.

Muscle. When possible, we evacuate a fresh hematoma through an intramuscular 18-G needle attached to a 50-mm Luer-Lock syringe; next, without changing the position of the needle, we inject the pure liquid PRP ($CaCl_2$-activated) into the ruptured fibers under sonographic guidance; the goal is to replace the red blood clot with the yellow plasma clot, reducing the presence of erythrocytes, leukocytes, and especially neutrophils. We further inject plasma into the interfascicle spaces, redirecting the needle in all directions—ventral, lateral, medial, and dorsal. As a rule, we inject high volumes, approximately 8-15 mL.

Tendon. We insert the needle from distal to proximal, in a parallel track to the collagen fascicles; PRP is injected (shortly after $CaCl_2$ addition) within the focus of altered tendon sub-

stance using a 21-G needle attached to a Luer-Lock syringe. The intention is to inject the maximum volume that can be confined within the area of degeneration, commonly between 3 and 5 mL (depending on the specific tendon and clinical case). Next, at some point during the extraction of the needle, additional PRP is delivered to the healthy tendon. We also inject plasma around the tendon between the tendon and the paratenon, and finally, a smaller volume is delivered into the associated fat. The precise anatomical location of the clotted plasma can be observed as a hyperechogenic image during the injection procedure (Fig. 1A-D); in fact, $CaCl_2$-activated plasma clots rapidly because of body temperature (37 °C) and the presence of tissue factors, a situation that cannot be mimicked in cadaveric studies. Plasma spreads when injected between the tendon and paratenon because of the laxity of the ECM (Fig. 1D). Very often, we are able to observe the hyperechogenic image (attributed to fibrin) during the next week's injection.

Postinjection protocol. Because the procedure is not very painful, we do not administer anesthesia; instead, cold therapy is applied for approximately 10 minutes after the PRP injection. We instruct the patients to limit physical activities for 24 hours and to use cold therapy 2-3 times during the day.

Treatment schedule. In general, we perform 2-3 PRP injections weekly on an outpatient basis. These criteria are largely arbitrary and are based on our clinical experience. Moreover, because PRP therapies promote early healing, 1 week may be adequate for monitoring individual outcomes and making decisions about further plasma injections. Ultrasonographic monitoring drives our clinical decision regarding whether to perform additional PRP injections.

Rehabilitation. In both muscle and tendon management, we do not change rehabilitation protocols, including eccentric strengthening exercises, which are always personalized to the patient's condition. The only change is that we tend to move into different rehabilitation phases sooner.

Main Indications for Injection Treatment

Tendon Pathology

Tendinopathy is a complex disease with histologic features of hypoxic degeneration, mucoid or myxoid degeneration, hyaline degeneration, fatty degeneration, fibrinoid degeneration, fibrocartilaginous metaplasia, bony metaplasia, fiber calcification, or some combination of these entities. It seems obvious that such a challenging pathology would require multiple, repetitive interventions to reverse the pathologic process. Furthermore, it seems improbable that a single injection could stop or change the outcome of an ongoing degenerative process. Instead, repeated injections appear to be more efficacious in degenerative pathologies.

Initially, we recommend repetitive percutaneous injections for patients with painful tendinopathy and/or partial tears, in which other conservative management has proved ineffective. Most often, supplementary PRP injections are required in patients with partial tears. In those with chronic refractory tendinopathies, PRP therapies cannot fulfill all the heterogeneous needs of the pathologic condition, and we associate percutaneous needling with PRP injections. For example, we create fenestrations by needling the pathologic tissue in the distal or proximal pole of the patella, especially in the presence of small areas of ectopic calcification in the patellar pole (Fig. 2). The final results are still under evaluation, but the preliminary results indicate that there is remodeling, with more normal tendon structure.

We have been using PRP therapies routinely for more than 8 years in patients with refractory tendinopathy. For instance, we have treated more than 100 patients with tendinopathy in the past 2 years, in various tendons located in the lower limbs. A number of patients, however, have not adequately responded to injections (roughly 25%) and have been treated operatively. Indications may be different depending on multiple factors, including etiology, location of the injury, type of tendon, and mechanical demands. For example, oral corticosteroid or fluoroquinolone treatment may cause tendinopathy, an adverse effect that is not well characterized; in our experience, degenerative mucoid tendinosis and fluoroquinolone- or corticosteroid-associated tendinopathy respond very well to PRP injections (Fig. 3A-D), whereas a less efficacious PRP action has been observed in nodular tendinopathies. Additionally, we do not expect good results with PRP injections in tendinopathies in which mechanical factors have a principal role, such as in supraspinatus pathology. PRP treatment, addressing only biological factors, may be beneficial only for a subgroup of patients.

In our own experience, ie, using pure PRP and following our application procedures, midportion tendinopathy has an excellent prognosis (+++), whereas insertional tendinopathy has a good prognosis (++). We have also treated cases that presented with concomitant bursitis and osseous edema with good prognoses (++). Rarely, osseous edema may persist; in such cases, we inject PRP intraosseously.

Muscle Pathology

We treat both acute and chronic injuries, specifically cystic lesions and painful scar tissue.

Acute Muscle Injuries

When possible, it is better to treat acute injuries within the first 48 hours. PRP management is effective for most acute injuries (+++). Traumatic injuries in the anterior rectus muscle have poor prognoses when untreated, but PRP management is very helpful, although more injections and greater volumes may be needed, compared with other muscles. Figure 4 demonstrates a grade 2 tear of the left hamstring muscles in a young soccer player treated with 2 PRP injections.

Chronic Muscle Injuries

Painful scars. We inject activated PRP within the fibrotic tissue. This treatment is contraindicated when the fibrotic tissue is close to the main blood vessels.

Cysts. We aspirate cysts and fill the cavity with PRP; finally, we apply a compressive bandage. The success of PRP thera-

pies depends on clarifying the optimal procedure for administration, the characteristics of the plasma, its activation, and the volume used. However, this issue is challenging because PRP therapies influence the output of multiple mechanisms and also because we are far from a quantitative understanding of the issue.

Conclusions

There is a need to define more rigorously the value of the PRP therapies that we use and that we imagine optimizing in the near future. There is also a need to evaluate the therapeutic claims made about PRP therapies. However, to better define the conditions of clinical trials, we need to know more about differences, not only between PRP formulations but between technical procedures in surgery and between injection protocols, including applied volumes, target areas to treat, treatment schedules, and patient selection criteria.

The keys to both future advances in PRP science and to the application of PRP in the treatment of musculoskeletal disease and trauma lie in a better understanding of the repair processes that set the basis for thoroughly developing application procedures and identifying primary indications.

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