# Use of platelet-rich plasma in the care of sports injuries: our experience with ultrasound-guided injection

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**Background.** Platelet-rich plasma is being used more frequently to promote healing of muscle injuries. The growth factors contained in platelet-rich plasma accelerate physiological healing processes and the use of these factors is simple and minimally invasive. The aim of this study was to demonstrate the efficacy of ultrasound-guided injection of platelet-rich plasma in muscle strains and the absence of side effects.

**Materials and methods.** Fifty-three recreational athletes were enrolled in the study. The patients were recruited from the Emergency Room in the University Hospital at Parma according to a pre-defined protocol. Every patient was assessed by ultrasound imaging to evaluate the extent and degree of muscle injuries. Only grade II lesions were treated with three ultrasound-guided injections of autologous platelet-rich plasma every 7 days. Platelet concentrate was produced according to standard methods, with a 10% variability in platelet count. The platelet gel for clinical use was obtained by adding thrombin to the concentrates under standardised conditions. Outcomes assessed were: pain reduction, muscle function recovery and return to sports activity, ultrasound-imaging tissue healing, relapses, local infections, and any side effect during the treatment.

**Results.** In all cases muscle lesions healed fully on ultrasound-imaging, the pain disappeared, and muscle function recovery was documented with a return to sports activity. A single patient had a relapse 1 year after treatment.

**Discussion.** Platelet-rich plasma injected into the injury site is one of the most important factors rendering the treatment effective. To maximise its efficacy the preliminary ultrasound must be done accurately to localise the lesion and guide the needle into the corresponding lesion. According to the current results, which document full muscle recovery and no relapse except for one case, platelet-rich plasma ultrasound-guided injection represents a valid mini-invasive treatment for muscle injuries.

**Keywords:** platelet-rich plasma (PRP), muscle injuries, US-guided injection, sport rehabilitation.



## Introduction

Muscle strains are one of the most common injuries induced by sporting activities. According to the severity and location of the lesion different treatments can be delivered, including growth factor therapy through administration of autologous platelet-rich plasma (PRP) during the early period following the injury. PRP is easily obtained from centrifugation of the patient's own blood and contains high concentration of platelets, which release growth factors and cytokines by adding thrombin[1]. This process improves tissue repair in cartilage, tendons, ligaments, muscles and bones[2] by down-regulation of inflammatory mediators and synthesis of regenerative proteins[3-7]. Furthermore, PRP has antimicrobial properties that may contribute to reduce pain[8] and to prevent infections[9]. PRP is administered by local injection or applied directly in the form of gel into the site of injury. Initially many PRP injections were done without imaging guidance by either palpating the site of tenderness or using a peppering technique to distribute the gel uniformly. The use of ultrasound (US)-guided injection has led to a more precise and direct visualisation of the exact site of the pathology and of the injected blood products in the region of lesion. Furthermore, sonography allows the position of the needle position to be adjusted in real time[10-13]. Considering these properties, local US-guided injection of PRP may improve the repair of tendons, muscles, ligaments, cartilage and bone injuries[3]. The aim of this study was to demonstrate the efficacy of US-guided injections of PRP in muscle strains and their absence of side effects.

## Materials and methods

### Recruitment and treatment of patients

The patients were recruited from the University Hospital Emergency Room (ER) of Parma. Criteria for inclusion in the study were grade II muscular or myotendinous lesions according to the American Medical Association classification[14] which occurred within 3 days of admission to the ER. US imaging with a 7.5-12 MHz linear probe was always performed by an experienced radiologist who determined the precise size, extent, and location of the lesion. Only in few selected cases was magnetic resonance imaging (MRI) also carried out. At the same time a medical rehabilitation specialist evaluated the clinical condition of the patients. Fifty-three recreational athletes (36 men, 17 women) were included in the study.

Once each patient had been enrolled in the study, signed consent was obtained and the course for the PRP treatment was established and the transfusion specialist evaluated the patient's suitability for autologous blood donation according to the Regional guidelines on autologous blood collection (Guidelines on Autologous Blood, Emilia-Romagna Region, 31/10/2005). Autologous blood components underwent serological validation according to haemovigilance rules. Patients were treated with three PRP injections (one treatment every 7 days) and each of them required an extemporaneous preparation of PRP. The authors considered as relevant outcomes the following: pain reduction (assessed using a visual analogue scale, VAS), muscle function recovery, noticeable US-imaging tissue healing, absence of local infection during the treatment and of any other side effects. These parameters were assessed before each injection and 2 weeks after the end of the treatment. In all patients return to regular sport activity was also noted. All patients were analysed 1 year after treatment in order to detect relapses of the muscle lesion.

### Preparation of the platelet-rich plasma[15]

Platelets are key components in haemostasis, and stimulate the construction of new connective tissue and revascularisation. They are derived from the fragmentation of precursor megakaryocytes and have a lifespan of 5-9 days. Once activated, by the action of thrombin, they secrete the contents of their alpha and dense granules which facilitate different stages of healing. The autologous platelets were obtained from an autologous whole blood unit of 350 mL collected in a quadruple blood bag (Compoflex, Fresenius Kabi, Bad Homburg, Germany) and separated by centrifugation to extract 50-60 mL of PRP. The platelet units produced were maintained under continuous agitation for 24 hours at 22 °C. Following serological validation, the platelet units were separated into three aliquots of 20 mL under a sterile closed circuit with Terumo Sterile Tubing Welder (TSCD®, Terumo Medical Corporation, Tokyo, Japan). Each aliquot was identified with labels complying with UNI standards and showed the writing "autologous platelet concentrate" and frozen at −40 °C. The platelet content of each aliquot was evaluated in order to determine whether the minimum therapeutic dose of $1\times10^6$ platelets/μL had been reached. The platelet count in the aliquots of the platelet concentrates used ranged from $960\times10^5$ to $1.35\times10^6$ platelets/μL.

### Preparation of the thrombin

Autologous thrombin was obtained by collecting 20 mL of whole blood from the patient into four Vacutainer test-tubes (Vacuette®, Greiner, Interconsult s.r.l. Medical Division, Caravaggio, Bergamo, Italy). The test-tubes were centrifuged at 3,200 *g* for 10 minutes, the serum was separated under a flow hood and 0.2 mL of 10% calcium gluconate were added (Bioindustria L.I.M., Novi Ligure, AL, Italy) before incubation at 37 °C for 15-30 minutes. Finally, the supernatant, containing thrombin precursors, was divided into two or three aliquots and labelled in order to ensure correspondence with the platelet concentrates from the patient. The aliquots of thrombin were then stored at −40 °C.

### Activation of the platelet-rich plasma

The aliquots of platelet concentrate were thawed at 37 °C for 15 minutes. The product was activated at the patient's bedside. This was done by withdrawing 5 mL of the platelet concentrate with a syringe, then adding 1 mL of autologous thrombin and 1 mL of 10% calcium chloride. The solution thus obtained was mixed gently four or five times. Subsequently the activated PRP was injected into the exact site of the tissue lesion under US guidance, using an 18-21 gauge needle (Figure 1).

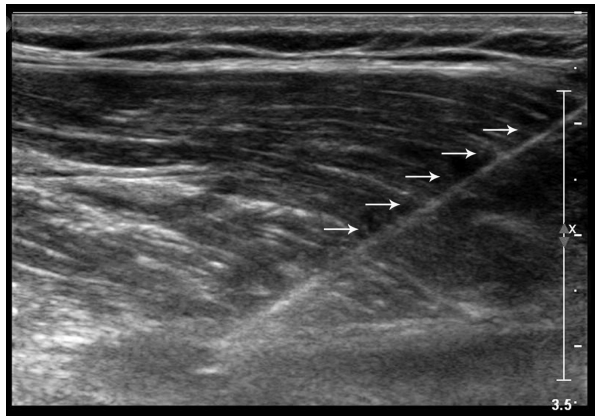


**Figure 1 -** Needle in the site of the lesion (arrows); US-image.

## Results

The mean age of the 36 men included in the study was 26 years, while that of the 17 women was 23 years. All of the participants were recreational athletes involved in different sports: volleyball, soccer, basketball,

dancing, trekking and skiing. All injuries occurred while the athletes were practising their respective sport (competition or training activities). Table I shows which muscle was involved in relation to the sport they practised when the muscle injury occurred. Injuries treated in this study were 50 grade II muscle strains and three myotendinous lesions. Muscle injuries were classified into grade I (mild wound), grade II (moderate wound), and grade III (severe wound) as shown in Table II[14-17].

The authors noted a progressive improvement of pain during treatment as shown in Figure 2. At baseline the mean VAS score was 7.1 (range, 6-8). One week after the first injection the mean pain VAS score was 2.6 (range 2-4), 2 weeks after the first injection it was 1.1 and 2 weeks after the end of the treatment the score was 0.3. Following each injection pain progressively disappeared within days and did not necessitate non-steroidal anti-inflammatory drugs except in two patients after the first injection. All patients reported a decrease in pain after the first PRP injection and in 45 patients (85%) an improvement of function was observed at the same time. After injury patients presented with limited motion (flexion, internal and external rotation, abduction and adduction) which was fully restored after treatment. Before the initial injection ultrasound examinations always showed muscle tissue breakdown, haemorrhagic formation, and hypoechoic gaps of various sizes at the site of injury or haematoma which infiltrated the muscle or collected around the lesion (Figure 3A).

Healing processes occurring in an injured muscle (necrosis/degeneration, inflammation, repair, and scar-tissue formation) are all interrelated and time-dependent. Acute muscle degeneration and inflammation occur immediately after injury and last up to 7 days, whereas tissue proliferation generally begins 7 to 10 days after the injury. The proliferative process usually peaks at 2 weeks and moves towards scar maturation at 3 to 4 weeks post-injury, and can last up to 1 year. This scar tissue formation (fibrosis) is the final product of muscle repair which begins between the second and third weeks after the injury and increases in size over time[14,18].

In our study after injections of PRP it was always possible to assess the progressive parenchymal recovery of the muscle, the development of superficial hyperechoic scar tissue at the site of the injury**,** and the reabsorption of the surrounding haematoma as determined by US (Figure 3B) and MRI (Figures 4 and 5). All athletes began, at a mean of 20 days (±2 SD; range, 16-28) after the first injection, a personalised rehabilitation and training programme based on a physiatrist's physical assessment. This programme consisted in gradual muscle strengthening initially in a controlled environment (a gym) and ultimately in the field.

All patients returned completely to their regular sporting activity after a mean period of 30 days (±1.2 SD; range, 28-35). Since there is limited evidence on the procedure to ascertain the timing for returning to sport activity[19], the authors chose criteria based on absence of pain on direct palpation and during muscular contraction with good symmetrical muscle function. No infections, major side effects, or complications related to the procedure were observed. At the 1 year follow-up three patients had had a new muscular injury in a different muscle and only one reported a new injury in the previously treated muscle (all lesions of the hamstrings). This last patient re-injured himself playing

**Table I -** Localisation of lesions related to type of sport.

| Type of muscles involved | Type of sport | | | | | | Patients/site of injury |
|---|---|---|---|---|---|---|---|
| | volleyball | soccer | basketball | dancing | trekking | skiing | |
| Femoral rectus | 5 | 7 | 3 | 1 | 0 | 2 | 18 |
| Femoral biceps | 4 | 3 | 2 | 0 | 2 | 1 | 12 |
| Calf muscles (medial and lateral gastrocnemius) | 3 | 1 | 2 | 1 | 0 | 3 | 10 |
| Long adductor | 2 | 2 | 2 | 1 | 1 | 0 | 8 |
| Abdominal rectus | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Peroneal muscles | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Femoral rectus myotendinous junction | 1 | 1 | 0 | 0 | 0 | 1 | 3 |
| **Patients/sport** | 15 | 15 | 9 | 3 | 3 | 8 | **53** |

**Table II -** Degree, description, and sonographic appearance of the muscle lesions.

| Degree | Description | Sonographic appearance |
|---|---|---|
| Grade I | Very small laceration involving less than 5% of side of muscles. No losses of strength or limitation in movements. | Patchy zone with hypoechoic area (diameter <1 cm) |
| Grade II | Laceration with loss of muscle strength involving 5 to 70% of muscle fibres. Oedematous imbibition and blood effusion. | Patchy zone with hypoechoic area. (diameter <3 cm) |
| Grade III | More than 70% of muscle fibres involved (subtotal lesion) or a complete rupture of muscle belly (total lesion). | Muscle structure disruption, with retraction and hypoechoic area. (diameter ≥3 cm) |

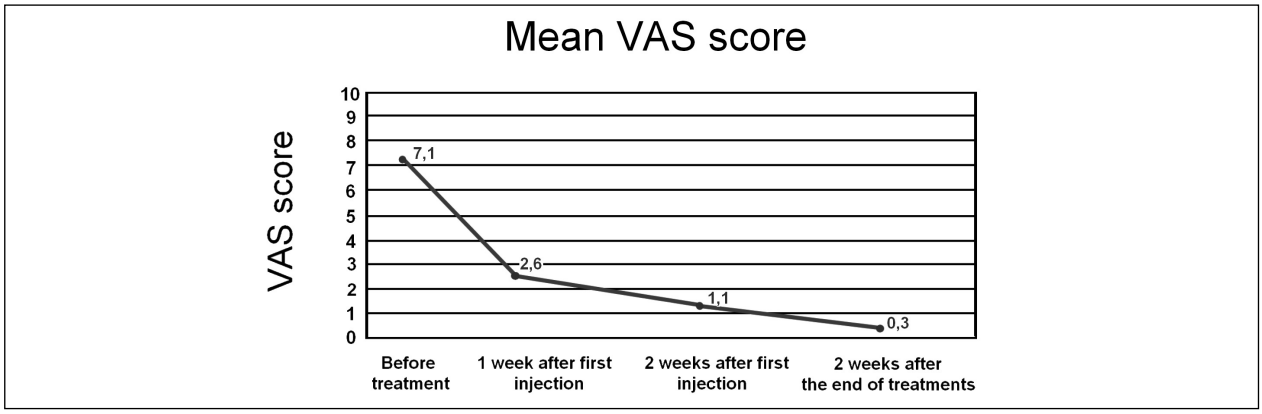


**Figure 2 -** Mean VAS score before treatment, 1 and 2 weeks after the first injection, and 2 weeks after the end of the treatment.

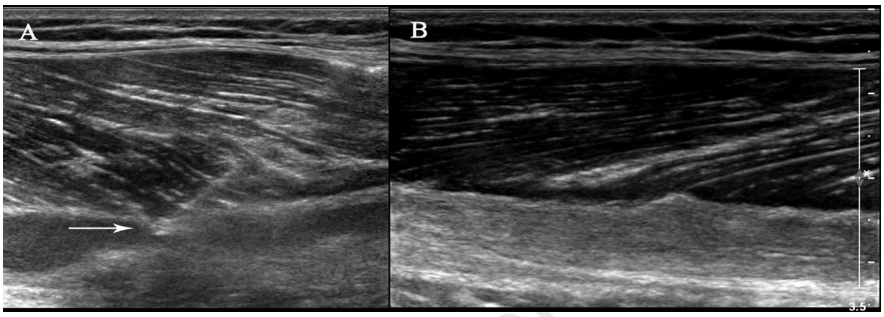


**Figure 3 -** A 26-year old man with a grade II muscle injury. (A) Ultrasound image showing the position of the needle (arrow) and a haematoma that has infiltrated the muscle. (B) Ultrasound image showing hyperechoic scar tissue in the site of the injury 2 weeks after the end of the treatment.

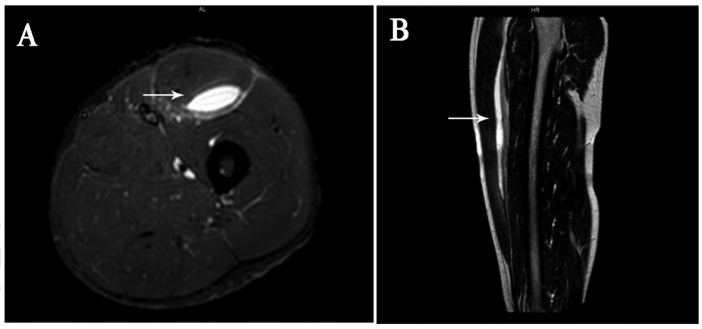


**Figure 4 -** A 29-year old man with a grade II muscle injury. (A) MRI axial view with haematoma (arrow). (B) MRI sagittal view with haematoma (arrow).

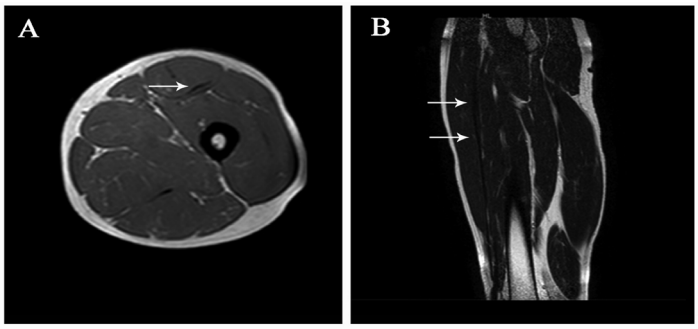


**Figure 5 -** The same patient as in Figure 4. (A) MRI axial view after healing and haematoma reabsorption (arrow). (B) MRI sagittal view after healing and haematoma reabsorption (arrows).

soccer 5 months after resuming his recreational sport. The relapse was a grade II lesion of the middle third section of the femoral biceps muscle whereas the first injury was localised in the upper third section of the same muscle. This patient was treated with PRP again with good results.

## Discussion and conclusion

Although the functions of all growth factors involved in tissue healing and regeneration are not yet fully understood potential benefits of some have been demonstrated[20]. Plasma becomes a vehicle of growth factors such as transforming growth factors beta, platelet-derived growth factor, epidermal growth factor, vascular endothelial growth factor[21,22], platelet-derived epidermal growth factor, bone morphogenetic protein, insulin-like growth factor, endothelial cell growth factor and basic fibroblast growth factor (bFGF)[11]. These factors play key roles in most tissue healing processes[23-25], allowing a more physiological and rapid healing of muscles lesions.

Despite the high incidence of muscle injuries the best method of their treatment has not yet been clearly defined, when a quick return to sporting activity is a primary goal[26]. US-guided injection of PRP has been gaining importance in the treatment of muscle injuries[5,17,18]. Understanding the physiological processes of muscular tissue repair is fundamental for establishing a therapeutic treatment focused on accelerating healing. Nevertheless, there is currently very limited scientific evidence of the clinical efficacy of PRP use for muscle strains in athletes[27]. This study describes a protocol to evaluate the clinical efficacy of three US-guided PRP injections after grade II muscle injury in recreational athletes in order to guide these patients' return to practising their sporting activity safety as soon as possible. The authors consider that the strengths of this study are the homogeneity of the sample (all grade II lesions treated with the same PRP treatment protocol) and a procedure that was always performed by the same physician under US-guide. Even if different muscles were treated in this study and there was no control group, the authors consider the results to be valid and reliable. All patients had complete healing of the muscular lesion without side effects, as testified by imaging, thus proving the efficacy of PRP and demonstrating that this procedure is well tolerated. Furthermore, the time of the return to sports activity was similar to that in other reports in the literature in which PRP treatment was described[20,26]. On the basis of the current results the authors concluded that US-guided PRP injections allow physiological, rapid and lasting healing of muscle lesion and represent a valid and safe mini-invasive treatment for grade II muscles injuries.

*The Authors declare no conflicts of interest.*

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