Efficacy of autologous platelet-rich plasma for the treatment of muscle rupture with haematoma: a multicentre, randomised, double-blind, placebo-controlled clinical trial

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Background. The goals of the treatment of muscle injuries are to shorten the time of healing and to avoid relapses. The aim of this study was to assess the efficacy of autologous platelet-rich plasma (PRP) in the healing of muscle injuries.

Materials and methods. A multicentre, randomised, double-blind, parallel, controlled clinical trial was conducted in 71 patients (81.8% males) aged 45.6 (SD=10.0) years with muscle tears in the legs and haematoma. The haematoma was evacuated in all patients. Thirty-three patients were randomised to a single dose of autologous PRP and 38 patients to simulation of PRP administration. The primary end-point was time to complete recovery of muscle injury. Secondary end-points were pain, relapses, ultrasound parameters, and adverse events. The total follow-up per patient was 12 months.

Results. Time to complete recovery after the treatment was 31.63 days (SD=15.38) in the PRP group, and 38.43 days (SD=18.58) in the control group (p=0.261). Pain decreased over time in both groups without statistical differences between them. Eight patients relapsed (seven in the control group, and one in the PRP group). There were no adverse effects related to the interventions.

Discussion. Autologous PRP did not significantly improve the time to healing compared to that in the control group.

Keywords: muscle injuries, haematoma, tennis leg, platelet-rich plasma, growth factors.

Introduction

Muscular injuries account for one-third of sports injuries, and most of them are caused by excessive muscle stretching[1,2]. These lesions lead to muscle weakness and pain, and sometimes to recurrences[1]. The injuries cause the loss of days of training and sporting competitions which, in professional athletes, has economic consequences[3,4].

The rupture of the medial head of the gastrocnemius muscle is commonly known as "tennis leg" because of its association with tennis players. However, this tear is frequently found in middle-aged people who practice sport in a non-competitive way. Sudden activities in daily life or sporadic extra exercises can also result in a tear of the medial head of the gastrocnemius[5,6]. The symptoms are an abrupt sharp severe pain in the calf, sometimes accompanied by an audible sound.

The distal posterior fascia of the rectus femoral muscle is the part of the quadriceps muscle that is frequently affected by contusion due to its location. This injury is prevalent in soccer, basketball, and rugby players.

What both lesions have in common is that they are myofascial tears, and the ultrasound image of the resulting haematoma is very similar in these two injuries. There is a well-defined, hypo-echoic area in the union of the medial head of the gastrocnemius with the soleus muscle in tennis leg rupture[7], and in the deepest part of the rectus femoris in contact with the vastus intermedius, in the case of rectus femoral muscle lesions[8,9].

Generally, a muscle injury with haematoma is conservatively treated with rest, local ice, compression and elevation of the limb (RICE), analgesics, and evacuation of haematoma[7]. However, other concomitant therapeutic interventions, which might improve the healing process, are available.

Autologous platelet-rich plasma (PRP) is a biological blood product obtained from the patient. PRP is assumed

to have anti-inflammatory[10] and pro-regenerative functions[11], which is why its use in various medical and surgical procedures has increased over the past years[12-16]. However, the lack of evidence so far regarding the efficacy and safety of PRP in musculoskeletal injuries does not justify its increasing use[14-16]. It is, therefore, important to evaluate the efficacy and safety of PRP in muscle injuries. We performed a randomised clinical trial to assess autologous PRP in muscle injuries, specifically in moderate gastrocnemius and distal posterior rectus femoral injuries.

Our main hypothesis was that PRP would improve muscle regeneration and repair by shortening the time to complete recovery with few or no adverse effects. The primary objective was to compare PRP to standard care, to assess the former's ability to shorten the time to heal gastrocnemius or posterior distal rectus femoral injuries with haematoma. The secondary objectives were to evaluate the risk of injury recurrence, the quality of the lesion during the recovery process, and the safety of PRP.

Materials and methods

Research design

This was a multicentre, double-blind, placebo-controlled, parallel, randomised clinical trial. The protocol was approved by the local Research Ethics Committee and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before their inclusion in the study.

Patients

We recruited patients with a leg muscle injury with haematoma from November 25, 2010 to December 20, 2012.

Inclusion criteria for patients were: (i) age 18 or older, (ii) an evacuable haematoma at the gastrocnemius muscle or the lower portion of the rectus femoral muscle, (iii) surgery not recommended, and (iv) consent to participation in the study.

Patients were excluded if they: (i) had a history of bleeding disorders, (ii) were unable to attend follow-ups, and (iii) had received corticosteroids, acetylsalicylic acid, or steroidal anti-inflammatory drugs within a week prior to their proposed inclusion in the study.

Ten Spanish centres participated in the study. Seven private outpatient clinics recruited and followed the included patients. One private centre checked whether the patients met all inclusion criteria, asked them to sign the informed consent form, contacted the coordinating centre to obtain the allocation of the patients, and administered the interventions. In this centre, a technician was responsible for the preparation of the autologous PRP or for the implementation of the sham. Ultimately, an independent centre was responsible for the allocation of the patients and the coordination of the study (Figure 1).

Randomisation and masking

The random sequence for treatment allocation was generated by computer software to which researchers were blinded. To ensure a balanced number of patients in the two groups, a block randomisation process of ten patients was performed, stratified by type of lesion (gastrocnemius or rectus femoral).

The treatment allocation was centralised from the coordinating centre. The centre that administered the interventions obtained the patient's assignment to treatment via phone call to the coordinating centre. The researchers involved in this process knew the assignment at the moment when the patient was included in the study. The researchers who assessed the patient after treatment were blinded to the administered treatments.

Patients were randomised to receive either autologous PRP into the muscle wound after evacuation of the haematoma (experimental or PRP group), or evacuation of the haematoma alone (control group).

Preparation of the platelet-rich plasma

Autologous PRP was prepared from the patients' whole blood and was administered immediately. The preparation took between 10 and 15 minutes. A dose of platelets was obtained through a process of plateletphaeresis, using a multicomponent cell separator (MCS+, Haemonetics, Braintree, MA, USA).

Platelets were automatically separated from other blood components by centrifuging (4,800 rpm and 1,290 *g*) the whole blood in a centrifuge chamber, and collecting the platelets in a sterile single-use bag of polyvinyl chloride. Red blood cells and plasma were returned to the patient. In one cycle we obtained approximately 40-50 mL of PRP.

The autologous PRP was activated by the researcher, just before administering it. The PRP was activated using a standard procedure, by adding 0.05 cc of calcium chloride ($CaCl_2$), as described by Anitua *et al.*[17] Activated PRP was immediately injected in liquid form, under aseptic conditions. A single dose of 4 to 8 mL was administered depending on the size of the gap.

Study intervention

After randomisation, about 40 mL of venous blood was extracted from each of the patients of both the active treatment group and the control group, and baseline analyses (coagulation tests and a blood count), testing for viruses (hepatitis B and C, human immunodeficiency virus), and syphilis serology were performed.

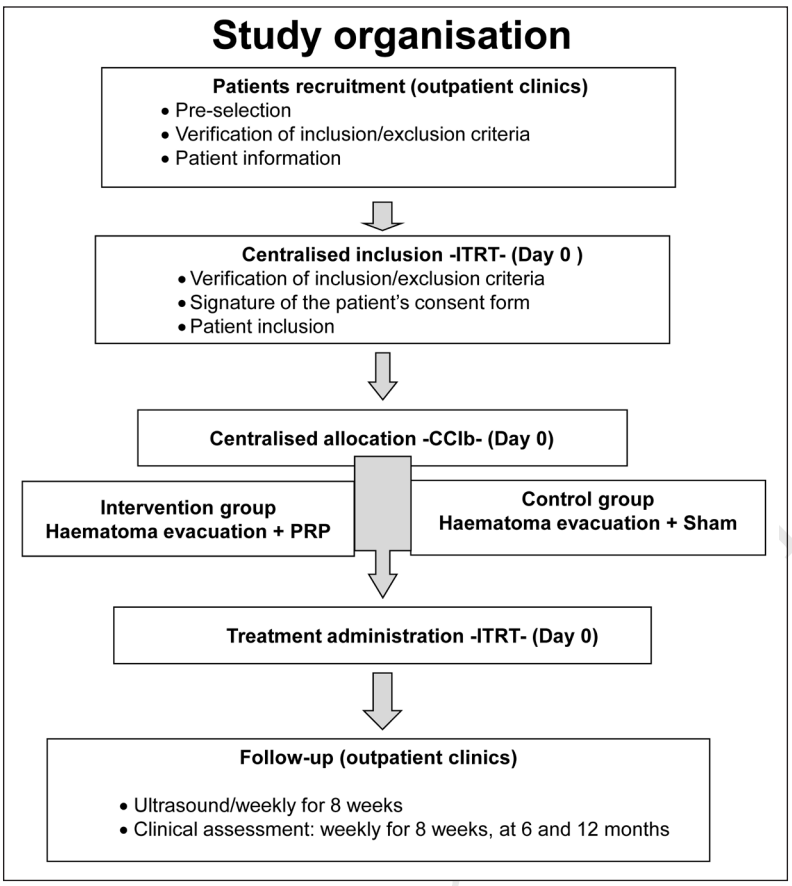


Figure 1 - Study organisation.
ITRT: Institut de Teràpia Regenerativa Tissular; CCIb: Centro Cochrane Iberoamericano.

The active treatment group underwent plateletphaeresis, whereas the control group underwent a simulation. Patients of both groups had a peripheral venous catheter. In the experimental group, the catheter was connected to the plateletphaeresis machine. A curtain prevented patients from seeing what the peripheral venous catheter was connected to. The medical personnel monitoring these procedures did not communicate the intervention to the patient (Figure 2).

Guided by ultrasonography, the haematoma was evacuated using a syringe through a 21 g×11/2 needle in all patients. The autologous PRP group received activated PRP in the area from where the haematoma had been evacuated. A single dose of PRP was administered, as this was sufficient to cover the empty site after the evacuation of the haematoma.

The usual treatment -rest, local ice, inelastic compression and leg elevation- was recommended in both groups. When necessary, an oral analgesic, such as paracetamol or opiate codeine, was prescribed to relieve pain. The follow-up care consisted of giving the patient a sheet with recommendations of a specific recovery protocol for calf/rectus femoris injuries[18].

End-point measures

The primary end-point was the time to healing. This end-point was assessed weekly during the first 8 weeks after the treatment. We defined time to healing as the time to return to usual pre-injury activities with absence of pain when walking, jumping or practicing sports. This information was obtained from the patient's history and affirmed by a physician.

Secondary end-point measures were: (i) rate of patients with complete healing at 8 weeks; (ii) rate of lesion recurrence during the 12 months of follow-up, with recurrence defined as a relapse of symptoms (pain, functional disability) and a confirmation of a change in the previously injured area by ultrasound; (iii) intensity of pain, determined using a visual analogue scale (VAS) of 100 mm, measured every week for the first 8 weeks of the study, and then at 6 and 12 months; pain was assessed while walking, doing daily activities and during the practice of sports; (iv) quality of the regenerated area at the end of the follow-up, measuring the lesion gap and fibrosis by ultrasonography, and presence of hypervascularisation; (v) adverse events assessed weekly for the first 8 weeks of the study, and then at 6 and 12 months.

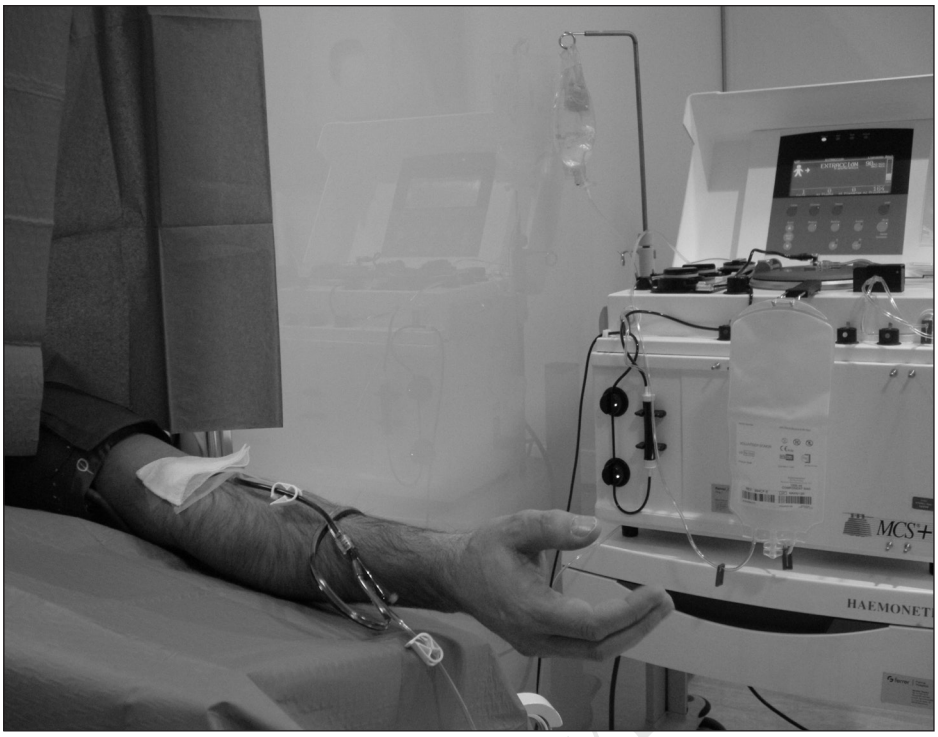


Figure 2 - Preparation of platelet-rich plasma and blinding of patient.

Blinding

Both clinical and ultrasound assessments were performed by researchers blinded to the intervention. Although all patients underwent an evacuation of their haematoma, they were unable to observe the process of evacuation and, therefore, remained blinded to the intervention.

The follow-up was conducted in the recruiting centres by researchers blinded to the administered treatment.

Statistical analysis

Accepting an alpha risk of 0.05, a beta risk of 0.2 in a two-sided test, 62 patients (31 subjects in each group) were necessary to detect a statistically significant difference in the time to healing equal to or greater than 8 days. The common standard deviation assumed was 11 days, based on results of the clinical trial by Wright-Carpenter *et al.*[19] However, a drop-out rate of 20% was anticipated and the final sample size was 76 patients. We used the GRANMO programme, version 7.10, June 2010 (Institut Municipal d'Investigació Mèdica, Barcelona, Spain).

The main analysis was per protocol, since some patients withdrew their consent to participate after being randomised, and others had been unavailable for follow-up and assessment. When possible, we also analysed data by an intention-to-treat principle, including all randomised patients in the analysis. For missing values of the outcomes "pain" and "ultrasonography measures", we considered the value of the last observation carried forward.

For categorical data we calculated frequency counts. For quantitative data, when appropriate, we calculated the mean, standard deviation (SD), standard error (SE), and 95% confidence interval (CI). The Mann-Whitney and Pearson's chi-square tests were used for ordinal and categorical data, respectively. When the sample size of categorical data was small, we applied Fisher's exact test.

To analyse the primary end-point (time required for complete healing) we calculated survival curves according to the Kaplan-Meier method. Survival was calculated from the date of randomisation until the end of the follow-up (8 weeks) or until the lesion healed, whichever happened first. We used a proportional hazards regression analysis to adjust for gender, type of muscle lesion (gastrocnemius or rectus femoral), and time between lesion occurrence and administration of study treatments.

Pain was analysed by calculating the mean difference between the values during the follow-up (weekly during the first 8 weeks after the intervention) and at baseline by a two-way ANOVA analysis. Ultrasound measurements were analysed calculating the mean of the last measurement obtained at follow-up.

Statistical significance was set at $p \leq 0.05$. The software used for data analysis was SPSS 21 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Distribution of participants

A total of 71 patients were randomised to one of the two treatment groups; 33 in the PRP group and 38 in the control group. After allocation, 12 patients (16.9%) did not receive the assigned intervention because they withdrew their consent to participate in the study (8 patients; 4 in each group), the size of their haematoma was not sufficient to be evacuated (3 patients), or there was a violation of the protocol (1 patient in the control group received PRP by mistake). Additionally, during the follow-up, two patients were lost in the first 8 weeks; one in the PRP group, and one in the control group. At the end of the trial, 57 (80.3%) patients had been followed for the whole 12 months, 27 in the PRP group and 30 in the control group (Figure 3).

Baseline characteristics

Table I shows the baseline characteristics of the study population. The mean age of the included patients was 45.6 (SD=10.0) years and 81.8% were men. No differences at baseline were found regarding the leg affected by the injury. The location of the lesion was principally in the gastrocnemius muscle, but three patients had a lesion in the rectus femoral muscle. The activity that caused the lesion was principally sport-related, mainly football, rackets sports (tennis or paddle tennis), athletics (running), cycling, skiing, gymnastics, basketball, golf, swimming, and judo; however, some lesions were caused by non-sport activities (Table I).

The average time between occurrence of the lesion and treatment was 1 week longer for the control group (p=0.036). There were no baseline differences between the groups regarding pain in sporting activities (p=0.347) and walking (p=0.539). However, the PRP group had more pain during daily activities (52.82; SD=38.91) than did the control group (35.62; SD=25.58) (p=0.040). All patients had negative test results for virus and syphilis serology, and coagulation tests were normal. At baseline, the mean blood platelet counts and the volume of evacuated haematoma were similar in the two groups.

Regarding treatment compliance, 28 patients received autologous PRP plus haematoma evacuation (PRP group), and 31 from the 59 treated patients received solely haematoma evacuation (control group). The volume of the evacuated haematoma was similar in the two groups (Table II). The platelet concentration in the PRP group was $1{,}381\times10^9$ (SD=430)/L, and the ratio of platelet concentration regarding blood platelets was 4.89 (SD=0.87). The leucocyte and erythrocyte levels in the PRP were 0.11×10^9 (SD=0.06)/L and 0.04×10^{12} (SD=0.04)/L, respectively.

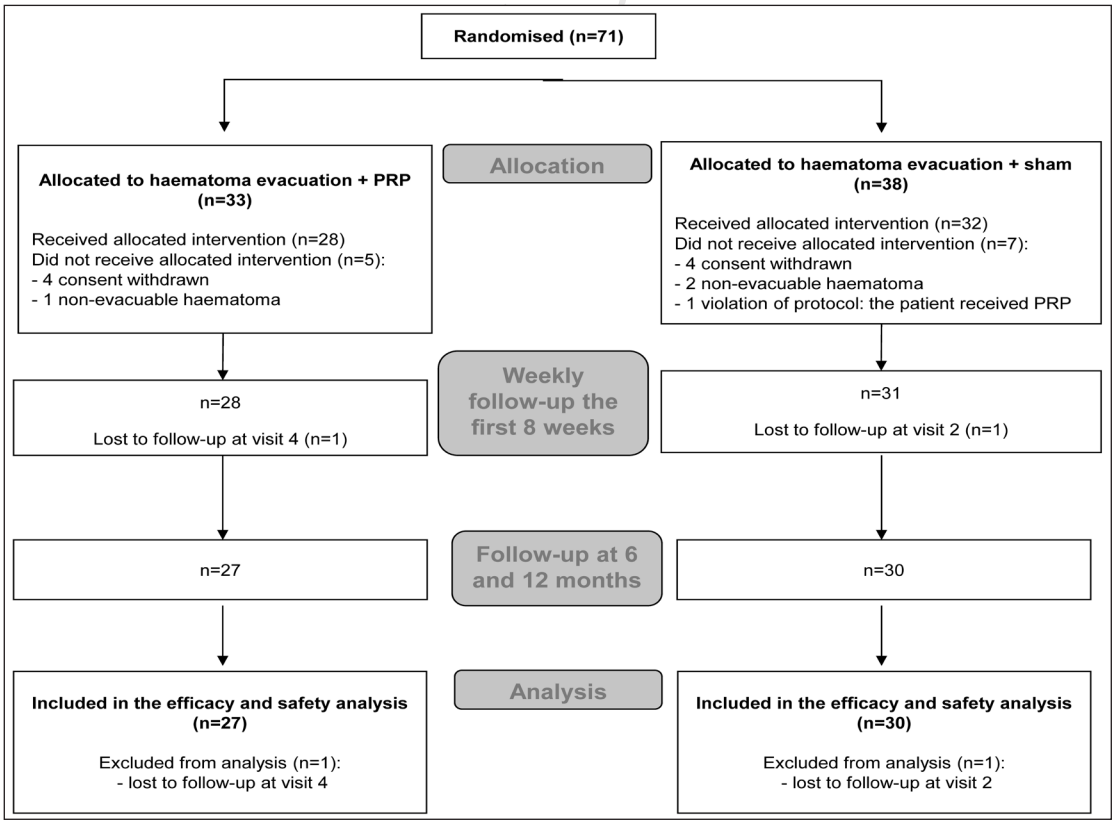


Figure 3 - Flow chart of the study patients.

Table I - Baseline characteristics of the included patients.

	PRP N=33	Control N=38	p
Age, mean ± SD, years	45.87±10.27	45.28±9.8	0.702
Gender, % men	84.8	78.9	0.521
Medical history, n			
Hepatic disease	0	1	
Renal disease	1	1	
Heart disease	0	3	
Neurological disease	1	0	
Digestive disease	3	2	
Allergic disease	2	2	
Surgical intervention	11	14	0.793
Muscle injury	10	3	0.014
Frequency of sporting activities, n			
Occasionally	9	6	0.400
Weekly	11	16	
Daily	7	11	
Location of the injury, n			
Right leg	13	16	0.599
Left leg	16	15	
Gastrocnemius muscle	32	36	0.900
Lower portion of rectus femoris muscle	1	2	
Physical activity at the time of the injury, n			
Athletics	3	7	0.706
Football	7	8	
Paddle tennis	2	6	
Tennis	2	1	
Walking	3	3	
Other sporting activities	7	8	
Non-sporting activity	3	1	
Baseline pain			
During sports, mean ± SD	93.93±16.52	87.57±31.96	0.347
During daily activities, mean ± SD	52.82±38.91	35.62±25.58	0.040
While walking, mean ± SD	33.13±25.45	29.06±26.36	0.539
Treatments received for the muscle injury, n			
Rest	23	23	0.462
Ice	23	22	0.314
Compression	17	21	0.702
Leg elevation	22	18	0.092
Analgesic	18	14	0.141
Leg massage	4	5	0.513
Hyperthermia	2	8	0.081
Other treatments	6	11	0.146
Interval from lesion occurrence to study intervention, mean ± SD, days	14.2±9.1	21.6±17.9	0.036
Blood platelets, 10^9/L, mean ± SD	289.32±126.85	260.70±109.28	0.344

PRP: platelet-rich plasma; SD: standard deviation.

Table II - Volume of haematoma evacuated and characteristics of the platelet-rich plasma (PRP).

	PRP N=28	Control N=31	p
Volume of haematoma evacuated, mm^3, mean ± SD	19.27±25.35	22.52±27.83	0.662
Volume of PRP administered, mm^3, mean ± SD	8.42±3.89	-	-
Platelet concentration in PRP, 10^9/L, mean ± SD	1,381±.430	-	-
Ratio platelet concentration in PRP/blood, mean ± SD	4.89±0.87	-	-
Leucocytes in PRP, 10^9/L mean ± SD	0.11±0.06	-	-
Erythrocyte concentration in PRP, 10^{12}/L mean ± SD	0.04±0.04	-	-

SD: standard deviation.

Healing

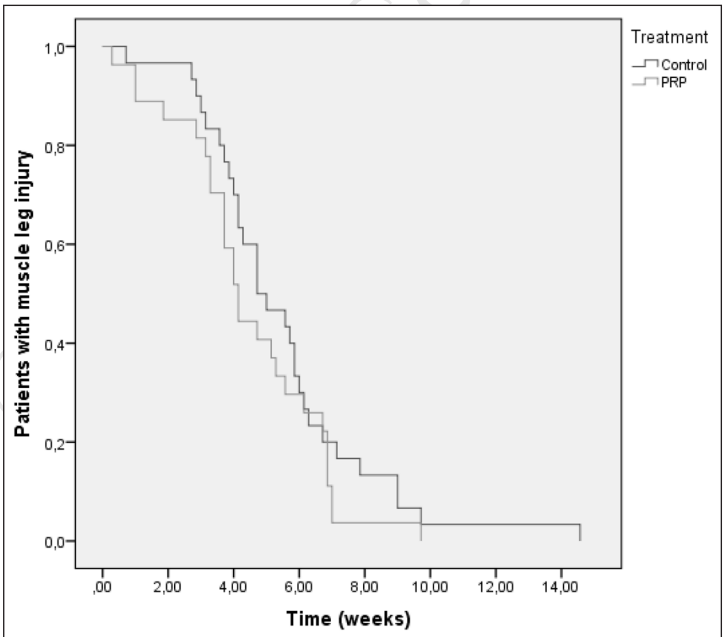
Regarding the primary end-point, time to healing, 27 patients were analysed in the experimental group and 30 in the control group. The time to healing was not significantly different between the two groups (p=0.261) (Figure 4). This result was not affected by gender, type of muscle lesion (gastrocnemius or rectus femoral), or time between the lesion occurrence and administration of the study treatments (p=0.131).

The rate of patients with complete healing at 8 weeks was 96.30% in the PRP group and 80.60% in the control group (p=0.075). At 12 months of follow-up, all lesions healed, except one gastrocnemius muscle lesion in the control group.

Pain and ultrasound parameters

During the follow-up four patients received analgesia for pain: in the PRP group one p atient was given naproxen and two were given paracetamol, whereas in the control group one patient was given piroxicam. Pain differences compared to baseline values, measured weekly for the first 8 weeks, improved over time in both groups (p<0.001) during sports activities, daily activities, and when walking. Although the PRP group had greater improvements, no statistically significant differences with the control group were found (Online Supplementary Figures 1-3).

There were no significant differences in the ultrasonographic parameters between the groups at 8 weeks of follow-up (Online Supplementary Table I).



	PRP group N=27	Control group N=30
Mean (ES)	4.51 (0.42)	5.49 (0.48)
Median (CI 95%)	4.14 (3.60 to 4.68)	4.71 (3.33 to 6.09)

Figure 4 - Time to complete recovery of the muscle injury.
Log rank (Mantel-Cox): p=0.261.
PRP: platelet-rich plasma; ES: standard error; CI: confidence interval.

Adverse events

A total of 24 adverse events (11 in the PRP group and 13 in the control group) were reported in 17 patients (8 in the PRP group and 9 in the control group). None of the adverse events was related to the interventions.

Seven patients (6 in the PRP group and 1 in the control group) had mild and occasional unspecific discomfort at the healed lesion site. One patient in the PRP group and seven patients in the control group had recurrent haematoma that was resolved with standard treatment, except one patient in the control group who received PRP. Six patients had deep venous thromboses, two in the PRP group and four in the control group.

Furthermore, one patient in the PRP group had an Achilles' tendon rupture in the same leg as the initial muscle injury, diagnosed by magnetic resonance imaging 3 months after his inclusion, and was treated with PRP. One patient in the control group developed cellulitis in the contralateral leg, and another patient had a fracture of the calf bone of the same leg.

All the adverse events, except for the seven cases of occasional discomfort and haematoma recurrence in the control group, resolved during the 12 months of follow-up.

Discussion

This was a double-blind, randomised controlled clinical trial assessing the efficacy of autologous PRP in muscle lesions with haematoma. Our primary end-point of interest was time to healing, since this is one of the main contributions that PRP might make. The time to healing in the autologous PRP group and the control group was 4.51 weeks and 5.49 weeks, respectively, which was a non-significant 1-week difference. A previous pilot, non-randomised controlled study[19] in 29 sportsmen with muscle strains showed that patients receiving autologous conditioned serum returned to sporting activities earlier than the control group (16.6 *vs* 22.3 days). However, the study by Wright-Carpenter *et al*. differs from ours in its non-randomised nature and other methodological issues, PRP synthesis, and the dose and timing of the administration. In the study by Wright-Carpenter *et al.*[19] the autologous conditioned serum was obtained from 50 mL of patients' whole blood, centrifuged for 10 minutes at 3,500 rpm and stored at –20 ºC until it was used. Every 2 days a 2.5 mL dose was administered to the patient, with a mean of five doses per patient. In our study, we obtained autologous PRP by plateletphaeresis and administered a single dose to the patient a few minutes later without freezing it.

Recently, three prospective, randomised studies have been published, one single-blind study[20] and two double-blind studies[21,22]. The clinical trial by Hamid *et al*.[20] included 28 patients with a hamstring muscle injury and used a single injection of PRP prepared using a commercially available kit (Biomet GPS III, Biomet Inc.,Warsaw, IN, USA). The mean time to return to play was 42.5±20.6 days in the control group and 26.7±7.0 days in the PRP group. This difference of 2 weeks was statistically significant. However, the clinical trial by Reurink *et al*.[21], which included 80 patients with hamstring muscle injury who received two injections of PRP or isotonic saline placebo prepared using a commercially available kit (Arthrex ACP™ double syringe system), did not show any difference in the median time to return to play between groups (42 days). Similar results were published by Malavolta *et al*.[22], who studied 54 patients with supraspinatus tears given a single injection of PRP prepared by aphaeresis. At a 24-month follow-up, the score on the University of California at Los Angeles (UCLA) scale was similar in the PRP group and the control group (32.44 and 32.70, respectively).

We used plateletphaeresis to obtain PRP and the administered dose was five times higher than the blood platelet levels. There are different well-known procedures to obtain PRP, and each one makes a PRP concentrate with different levels of platelets and plasma, so that the platelet concentration varies from one- or two- to nine-fold the blood platelet concentrations[23-25]. Despite this heterogeneity, there are no studies that accurately assess which procedure and dose of PRP are the most effective. Moreover, published clinical trials often do not specify the platelet concentration contained in the PRP, making comparisons between trials difficult. However, a correlation between a higher platelet concentration and a higher concentration of growth factors such as insulin-like growth factor has been identified. Insulin-like growth factor recruits bone marrow-derived stem cells to the site of muscle damage[26,27].

Our PRP had very low leucocyte concentrations. These blood cells promote inflammatory phenomena and are rich in metalloproteases that destroy growth factors[27-31]. Thus, our PRP was advantageous to the healing of the muscle lesions.

In our study, pain related to sports, walking or everyday activities was reduced significantly in both groups, but there were no statistical differences between the groups. Little is known about the risk of recurrences in the injuries included in the study. After a follow-up period of 12 months, we found a lower rate of recurrent haematoma in the PRP group than in the control group (1 *vs* 7 patients, respectively), but these incidences are too low to affirm some benefits associated with the use of PRP. No serious adverse events related to the interventions occurred and the number of total adverse events was similar in the two groups.

The major strengths of this study are its sound methodological design and the prolonged follow-up period of 12 months. This study did, however, have

some limitations. We experienced difficulties in obtaining the number of patients needed. We included 71 of a predetermined sample of 76 patients (calculated assuming a 20% rate of drop-outs). During the study there were difficulties with recruiting patients. In addition, patients in private medical centres were not willing to participate in a randomised study in which sham treatment was used as a control. To overcome these limitations, we prolonged the period of inclusion, we expanded the inclusion criteria by accepting patients with rectus femoral lesions because of similarities of the ultrasound characteristics of these lesions with those of gastrocnemius muscle injury and its relatively high incidence in athletes, and, finally, we removed the criterion that the injury occurrence time had to be within 15 days of the patient's inclusion in the study.

Although we finally achieved a sample size of 71 patients, 13 were lost to follow-up or withdrew their consent, resulting in a decrease of statistical power in the study. The number of evaluable patients for the main end-point was slightly inferior to the minimum calculated sample size (57 *vs* 62). Likewise, some baseline features were statistically different between groups, favouring the control group. The PRP group had significantly more patients with muscle lesions. The control group had a longer time interval between lesion occurrence and intervention (21.6 days *vs* 14.2 days). Although we adjusted the analysis by gender, type of muscle lesion (gastrocnemius or rectus femoral), and time interval from lesion occurrence to study treatment administration, the PRP group still healed 1 week earlier than the control group, but this difference was not statistically significant.

We were concerned that unblinded researchers who administered the intervention might have influenced the time from lesion to intervention between groups; however, we found that there was no difference between the groups in the number of days from the date that researchers knew the patients' assignment (randomisation date) to the date of administration of the intervention (mean of 1.6 days in the PRP group and 1.8 days in the control group; p=0.790). Thus, the difference in time between lesion and intervention could have been caused by chance, the relatively limited sample size, or the absence of restrictions related to the time of lesion occurrence when including patients in the study.

A possible explanation for the non-significant results in our study could be the delay in the administration of PRP after the muscle injury, which ranged from 2 to 3 weeks. According to Anitua *et al*., PRP exerts its effects during the first 2 hours after an injury and, for physiological reasons, would lose its efficacy after 2 days[32]. If this were indeed the case, a confirmatory study would only be possible if recruitment and treatment took place within the first 48 hours after the injury, implying greater need of resources to ensure the availability of equipment and experienced personnel in each recruiting centre, circumstances that were not present in our study.

Conclusions

This study shows that there are no statistically significant differences in time to healing between a control group and patients treated with PRP, containing a high concentration of platelets and low concentration of leucocytes, 2 to 3 weeks on average after injury. Due to methodological and performance limitations of this study, a well-powered clinical trial to confirm or refute our findings is required and, ideally, should test early administration of treatment after injury.

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Authorship contributions

MJM-Z, LlO, RB, RS, GR, LlT and XB conceived the clinical trial and developed the methodological aspects of the protocol. MJM-Z coordinated the clinical trial. Dr. Grifols and Mr. Bacuñana collaborated in preparing the PRP. LlO, RS and GR performed the interventions. RB, LlT, and XP, together with Drs. Sales, Permanyer, Rius, and Boffa included and followed-up the patients. GU and IG gave methodological support. IG performed the statistical analysis. All Authors participated in the production and revision of this paper.

The Authors declare no conflicts of interest.

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