

Rationale, secondary outcome scores and 1-year follow-up of a randomised trial of platelet-rich plasma injections in acute hamstring muscle injury: the Dutch Hamstring Injection Therapy study

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ABSTRACT

Background Platelet-rich plasma (PRP) injections are an experimental treatment for acute muscle injuries. We examined whether PRP injections would accelerate return to play after hamstring injury. The methods and the primary outcome measure were published in the *New England Journal of Medicine (NEJM)* as 'Platelet-rich plasma injections in acute muscle injury' (2014). This article shares information not available in the *NEJM* letter or online supplement, especially the rationale behind the study and the secondary outcome measures including 1 year re-injury data.

Methods We performed a multicentre, randomised, double-blind, placebo-controlled trial in 80 competitive and recreational athletes with acute hamstring muscle injuries. Details can be found in the *NEJM* (<http://www.nejm.org/doi/full/10.1056/NEJMc1402340>). The primary outcome measure was the time needed to return to play during 6 months of follow-up. Not previously reported secondary outcome scores included re-injury at 1 year, alteration in clinical and MRI parameters, subjective patient satisfaction and the hamstring outcome score.

Results In the earlier *NEJM* publication, we reported that PRP did not accelerate return to play; nor did we find a difference in the 2-month re-injury rate. We report no significant between-group difference in the 1-year re-injury rate (HR=0.89; 95% CI, 0.38 to 2.13; p=0.80) or any other secondary outcome measure.

Conclusions At 1-year postinjection, we found no benefit of intramuscular PRP compared with placebo injections in patients with acute hamstring injuries in the time to return to play, re-injury rate and alterations of subjective, clinical or MRI measures.

BACKGROUND TO THE STUDY: WHY WE DID IT?

Muscle injuries account for one-third of all time-loss sports injuries, with the hamstring being the most commonly injured muscle in major sports such as soccer, Australian football, American football, and track and field athletics.¹⁻⁵ Each team can expect 7 muscle injuries per season in amateur soccer⁶ and up to 15 in professional soccer.³

Despite both a high prevalence and risk of recurrence, there is a lack of evidence for the effectiveness of any therapeutic intervention for muscle injuries.^{7,8} Since the World Anti-Doping Agency permitted the intramuscular injection of platelet-rich plasma (PRP) in 2011, this experimental treatment has been used

to treat acute muscle injuries.^{9,10} PRP is derived from autologous whole blood using centrifuge separation systems to provide growth factor release from the α -granules of the platelets. The growth factors released are assumed to stimulate myoblast proliferation and accelerate myofiber regeneration.¹¹⁻¹⁴ PRP has been studied for a number of musculoskeletal disorders.¹⁵⁻¹⁸ The use in muscle injury has been proposed recently. Whether the ratio of growth factors in PRP is appropriate for muscle healing remains unproven. Despite uncertainty about its effectiveness, there is a large commercial market for PRP, which is expected to increase from \$45 million in 2009 to \$126 million in 2016.^{15,19}

Two recent systematic reviews show uncertainty about the effectiveness of PRP injections for musculoskeletal indications.^{15,16} We designed the Hamstring Injection Therapy (HIT) study to examine the efficacy of PRP injections in patients with acute hamstring muscle injuries. The primary outcome measure; time needed to return to play and the 2-month re-injury rate has been published previously in the *New England Journal of Medicine (NEJM)*.²⁰ Our purpose here is to share information that is not available in the *NEJM* letter or its associated online supplementary material, especially the rationale behind the study. Previously unreported secondary outcome measures and the 1-year re-injury data are presented.

METHODS

The core methods including the study design, study population, randomisation, sample size calculation, blinding and intervention were published in the *NEJM*²⁰ and supplemented with online supplementary material: http://www.nejm.org/doi/suppl/10.1056/NEJMc1402340/suppl_file/nejmc1402340_appendix.pdf.²⁰

Design considerations

Rationale for age criteria

We set the lower boundary at 18 years because of legislation issues related to medical research in minors. We chose an upper limit of 50 years for generalisability of the results to the athletic population seen in the sports medicine clinical practice, and to have a study population that would be comparable to the previously published series in hamstring injuries.



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Rationale for including MRI inclusion criteria

Patients with a clinical diagnosis of an acute hamstring injury without lesions on MRI (commonly diagnosed as grade 0 injuries) were not included, as there are no macroscopic signs of tissue damage and the location of the lesion cannot be determined. Furthermore, these injuries are associated with a short recovery time,^{21–24} limiting the clinical relevance of hastening recovery with an invasive intervention.

Complete muscle ruptures/tendon avulsions (commonly indicated as grade III injuries) were excluded, as these are rare, severe injuries that may require surgical intervention.²⁵

Therefore, we only included MRI-positive injuries that are not complete ruptures (often diagnosed as grade I/II injuries²¹). It could be argued that PRP injections would have more potential in injuries with signs of macroscopic muscle tissue disruption (MRI grade II) than in injuries without (grade I). However, it has been shown previously that there is no significant difference in recovery time between MRI grade I and II injuries,²¹ suggesting that tissue healing may require the same time in grade I and II injuries. It is therefore questionable to what extent MRI grade I and II injuries distinguish between the presence and absence of tissue disruption. We hypothesise that in grade I injuries the tissue damage does not result in a visible disruption due to the limited resolution of MRI. As there is evidence that there is no difference in injury severity, we included both grade I and II injuries in our study.

Rationale for the number and timing of injections

The timing and the number of injections have been the subject of debate, as the tissue environmental milieu and the effect of growth factors change over time during the healing process.²⁶ However, it remains unclear whether the timing and number of injections are important factors for the effect of PRP on muscle regeneration.¹⁰ In usual clinical practice, the first injection is performed shortly after the injury and repeated injections are performed at several days to 1 week later.^{27–29} Concerns have been raised that during the biological healing phase of fibrosis, which starts 2–3 weeks after injury, TGF- β activity may be preferentially upregulated, thereby promoting fibrosis over regeneration.^{10 26} There is therefore a theoretical contraindication to inject PRP 2–3 weeks after a muscle injury. Taking into account the possible pro-fibrotic effect of PRP and the generally used procedures, we performed the first injection within 5 days of injury and a second injection 5–7 days later.

Description of clinical examination

Clinical examination was performed at baseline, 1 week and 26 weeks follow-up.

Manual muscle palpation

With the patient in a prone position, the complete posterior thigh was carefully palpated from the hamstring origin at the ischial tuberosity to the insertions medially at the pes anserinus and laterally at the fibula head. The total longitudinal length of the painful area and the distance between the point of maximal pain on palpation and the ischial tuberosity were recorded.

Hamstring flexibility testing

Hamstring flexibility was assessed with both the active knee extension^{30 31} and the passive straight leg raise test.³² Participants were tested in a supine position with an inclinometer placed on the anterior tibial border. For the active knee extension test, participants positioned the tested leg hip in 90°

flexion and were instructed to extend the knee until maximal tolerable stretch, with the contralateral leg remaining flat on the table. At the end point of maximal tolerable stretch, the absolute knee angle was measured.

For the passive knee extension test, participants were instructed to completely relax the leg, while the researcher lifted the leg with the knee in full extension until maximal tolerable stretch. The contralateral leg remained flat on the table. At the end point of maximal tolerable stretch, the angle between the leg and the table was measured. For both tests, the absolute flexibility deficit was calculated by subtracting the recorded angle of the injured leg from that of the uninjured leg. Participants were also asked if they experienced normal stretch or localised pain during the tests.

Isometric knee flexion force

Isometric knee flexion force was measured using handheld dynamometry.³³ Participants were tested in a prone position with the knee in 15° and 90° flexion. The researcher placed the dynamometer on the participant's heel and applied force to the heel, which was gradually increased over 3–5 s. Participants were instructed to resist the force applied by the researcher (break test). At the point that the participant could not resist the force anymore, the test was terminated and the reading taken. Each leg was tested three times in 15° and 90° knee flexion. For each angle, the highest force value was recorded. The relative strength deficit was calculated by dividing the recorded maximal force value of the injured leg by the maximal force value of the uninjured leg. Additionally, participants were asked to rate the hamstring pain during testing on a 0–10 numeric rating scale.

MRI

MRI was performed at inclusion within 5 days of injury and within 7 days after return to play.

MRI protocol

The protocol used was a modified version of that described by Askling *et al.*³⁴ To locate the area of the injury, the entire hamstring of the injured limb was visualised by obtaining coronal and sagittal short tau inversion recovery (STIR) images from the ischial origin of the hamstring muscles to insertion on the fibula and the tibia (repetition time/echo time (TR/TE) of 3500/31 ms, field of view (FOV) of 300 mm and a 256×320 matrix). Subsequently, transverse STIR (TR/TE of 3500/31 ms, FOV of 300 mm and a 205×256 matrix), T1-weighted (TR/TE of 500/12 ms, FOV of 300 mm and a 355×448 matrix) and T2-weighted (TR/TE of 4080/128 ms, FOV of 300 mm and a 355×448 matrix) images were obtained from the injured area. The thickness of the slices for all sequences was 5 mm. MRIs were obtained with a 1.5-T magnet system (Magnetom Essenza, Siemens) with the use of a body matrix coil.

MRI assessment

Each MRI was assessed by a single radiologist specialised in musculoskeletal radiology. For assessment of the MRIs, we used standardised scoring forms.^{21 23 34–36} We recorded the involved muscle(s) and performed grading of the injury using the three-graded classification of Hancock: *et al.*³⁶ grade 1 increased signal intensity on fluid sensitive sequences without evidence of a macroscopic tear; grade 2 increased signal intensity on fluid sensitive sequences with a partial tear and grade 3 total muscle or tendon rupture. We measured the increased T2 signal intensity for the affected hamstring muscle in craniocaudal, transverse and anteroposterior dimensions on the fluid sensitive

sequences (STIR). We recorded the longitudinal length (cranio-caudal) and calculated the involved cross-sectional area as a percentage of the total muscle cross-sectional area in the transversal plane. We measured the distance of the most cranial pole of the intramuscular increased signal intensity to the distal tip of the ischial tuberosity. Increased signal intensity was defined as an abnormal intramuscular increased signal compared with the unaffected surrounding muscle tissue. Good to excellent inter-observer and intraobserver reliability for these MRI parameters has been reported.³⁷

Secondary outcome measures

Re-injuries during 1-year follow-up

Participants were followed up for re-injuries until 1 year after the initial injury. Players were instructed to immediately contact the coordinating researcher in the event of a suspicion of re-injury and re-injury occurrence was monitored at 4, 8, 16, 26 and 52 weeks with phone calls to the participants. Acute onset of posterior thigh pain that occurred on the same side as the initial injury and caused absence from play was counted as a re-injury.³

Other secondary outcome measures

Other previously unreported secondary outcome measures were: the subjective patient satisfaction, perceived recovery, a numeric rating scale for posterior thigh pain at rest (0–10, where a higher score indicates more pain), pain and flexibility deficit measured with the active knee extension test³⁰ and the passive straight leg raise test,³² isometric knee flexion force deficit measured with handheld dynamometry in 15° and 90° knee flexion,³³ hamstring outcome score (0–100, where a higher score indicates better hamstring function),³⁸ adherence to the rehabilitation programme and the amount of oedema on MRI at return to play.

Statistical analysis of the secondary outcome measures

We analysed the difference in re-injury rate between the treatment groups with a Cox proportional hazards model. In this

model, the time (days) from return to play to the event (re-injury) or the end of the follow-up is the dependent variable. Participants who sustained a severe injury (causing absence from training and matches >28 days^{3 39}) during follow-up that was not considered a hamstring re-injury were censored at the time of this injury. Participants lost to follow-up were censored at the time of their last available follow-up. Participants completing the 1-year follow-up were censored at the time of the last follow-up measure. We adjusted for ipsilateral hamstring injuries in the preceding 12 months, as a history of hamstring injury is previously reported as a predictor for re-injury.^{40 41} Time-to-re-injury curves were calculated with the Kaplan-Meier method.

The Hamstring Outcome Score was tested with a linear regression model. Continuous secondary outcome measures with repeated measures in time were tested with linear mixed models and binary secondary outcome measures with repeated measures in time were tested with generalised estimating equations. Secondary outcome measures were adjusted for the baseline measures. Adherence to the rehabilitation protocol was tested with an independent t test.

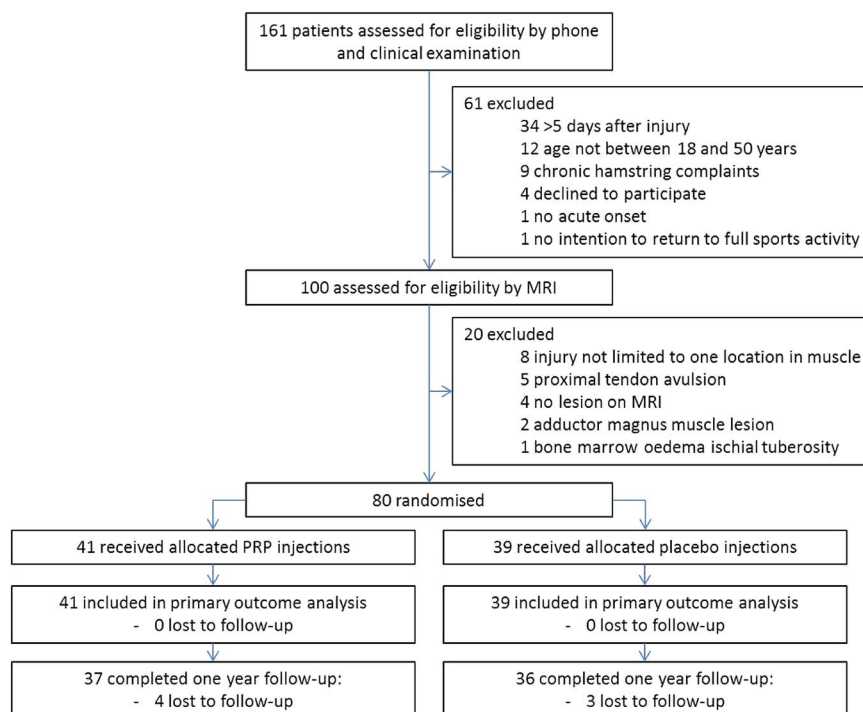
The coordinating researcher and the independent statistician, who performed the analysis, were blinded for the allocated treatment. The analysis was performed using SPSS V.21.0.1 (SPSS Inc, Chigaco, Illinois, USA). All p values are two sided.

PRP samples analysis

Platelet and leucocyte counts

We assessed the number of thrombocytes (platelets), leucocytes and leucocyte differentiation in whole blood and in PRP. Whole blood obtained from the cubital vein and 2 mL of PRP were collected in EDTA blood collection tubes. Directly after collection of the whole blood and PRP, the collection tubes were transported to the Clinical Chemistry Laboratory. Platelet and leucocyte counts were performed using the Sapphire blood analysis machine (Abbott Diagnostics, Hoofddorp, The Netherlands).

Figure 1 Flow diagram of patients through the study (PRP, platelet-rich plasma).



Microbial contamination

We tested the PRP samples for the presence of microorganisms. One millilitre of PRP was collected in a BACTEC Peds Plus/F culture vial. Before injection of the PRP into the vial, the top was disinfected using disinfection alcohol. Directly after collection, the vial was transported to the Microbiology laboratory and stored in a stove at 35°C for 7 days.

RESULTS

Between February 2011 and November 2012, 80 patients were enrolled and randomly assigned to either the PRP (N=41) or placebo (N=39) group (figure 1). All patients sustained their injury while participating in sports. All randomised patients received the allocated injections. The baseline characteristics of the patients have been published previously at <http://www.nejm.org/doi/full/10.1056/NEJMc1402340>.²⁰

Primary outcome measures

The primary outcome measure, time to return to play, has been published previously at <http://www.nejm.org/doi/full/10.1056/>

NEJMc1402340.²⁰ There was no significant difference between the study groups (figure 2, reprinted with permission).

Secondary outcome measures

Re-injuries during 1-year follow-up

Four patients in the PRP group and two in the placebo group were not included in the re-injury analysis: four patients sustained another injury before they returned to play, one patient in the PRP group did not achieve return to play within the study period and one patient in the placebo group was lost to follow-up after he returned to play. In the PRP group, 10 of the 37 patients (27%) and in the placebo group 11 of the 37 (30%) sustained a re-injury during the 1-year follow-up period. The adjusted HR for the PRP group was 0.89 (95% CI 0.38 to 2.13; $p=0.80$) (figure 3).

Subjective patient-related outcome measures

There were no significant differences between the study groups on the subjective patient satisfaction, perceived recovery and the numeric rating scale for posterior thigh pain at rest at 1, 4 and 10 weeks follow-up (table 1).

Physical examination

At 1 and 26 weeks, there were no significant differences between the study groups on pain and flexibility deficit measured with the active knee extension test and the passive straight leg raise test, except for the active knee extension deficit at 1 week follow-up. There were also no significant differences on pain and isometric strength deficit measured with handheld dynamometry at 1 and 26 weeks (table 2).

Hamstring outcome score

At 26 weeks, there were no significant differences between the study groups on the overall hamstring outcome score and the subscale symptoms soreness, pain, function in sports and quality of life (table 3).

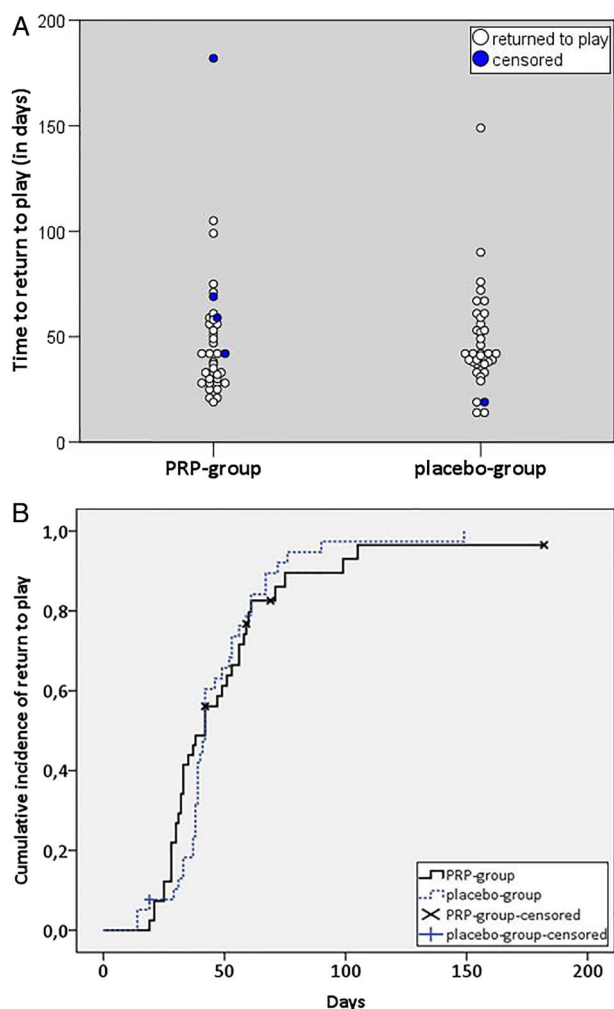


Figure 2 Return to play (reprinted with permission²⁰). (A) A scatter plot of number of days that were required for patients to return to sport in the group receiving injections of platelet-rich plasma (PRP) and the placebo group. (B) Kaplan-Meier curves for the cumulative probability of a return to sport. Data for patients who had a non-hamstring injury before they returned to sport were censored at the time of this injury.

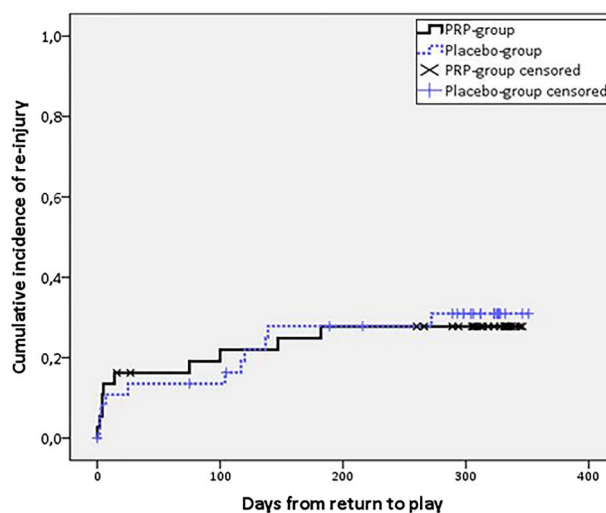


Figure 3 Kaplan-Meier curves for the cumulative incidence of re-injury. Data for patients who sustained a severe injury (causing absence from training and matches >28 days) during follow-up that was not considered as a hamstring re-injury were censored at the time of this injury. Participants that did not sustain a re-injury were censored at the time of their last available follow-up (PRP, platelet-rich plasma).

Table 1 Secondary outcome measures obtained by questionnaire at 1, 4, and 10 weeks*

	1 Week			4 Weeks			10 Weeks		
	PRP (n=41)	Placebo (n=39)	Between-group difference (95% CI)	PRP (n=40)	Placebo (n=39)	Between-group difference (95% CI)	PRP (n=40)	Placebo (n=38)	Between-group difference (95% CI)
Good/excellent patient satisfaction, %	93	82	11 (−4 to 25)	93	95	−2 (−13 to 8)	93	100	−7 (−16 to 7)
Perceived full recovery, %	0	3	3 (−2 to 8)	28	31	3 (−17 to 23)	80	76	−4 (−22 to 15)
Pain score in rest—0–10 rating scale (SD)	0.7±1.5	0.5±1.2	0.2 (−0.2 to 0.6)	0.2±0.8	0.2±0.8	0.0 (−0.4 to 0.4)	0.1±0.4	0.2±0.7	−0.1 (−0.5 to 0.3)

*Plus-minus values are means±SD.
PRP, platelet-rich plasma.

Oedema on MRI

There were no significant differences between the study groups on the extent of oedema on MRI at return to play (table 4).

Adherence to the rehabilitation programme

In the PRP group, 49% and in the placebo group, 51% of the patients kept and returned their daily logs of the rehabilitation programme. There were no significant differences in reported adherence to the rehabilitation programme between the study groups (table 5).

PRP samples analysis

Mean platelet concentration in whole blood was within expected ranges (232, SD $48 \times 10^3 \mu\text{L}$) and increased with a factor 1.9 in PRP (433, SD $125 \times 10^3 \mu\text{L}$) (table 6).

Two of the 160 collected PRP samples were positive for microbial growth (*Micrococcus luteus* and *Staphylococcus aureus*), suggestive of contamination of dermal microbes. There were no clinical signs of infection after the PRP injections of these samples.

Adverse events

There were no serious adverse events. One patient in the PRP group developed painful dermal hyperaesthesia of the area at the posterior thigh, which prevented return to play within the follow-up period.

DISCUSSION

In the earlier *NEJM* letter, we reported that PRP did not accelerate return to play, nor did we find an effect on the 2-month re-injury rate.²⁰ In the present report, we found no differences

in the 1-year re-injury rate, the subjective and functional secondary outcome measures and the extent of oedema on MRI at return to play.

Comparison with existing literature

Previous clinical evidence of the effectiveness of PRP in muscle injuries was limited to one case series²⁸ and two retrospective case-control studies^{27 29} with major methodological flaws, including the lack of a proper control group, no blinding and insufficient power. The clinical use of PRP was often supported by animal model results,^{11–14} the assumption of a safe autologous therapy and the absence of reported complications and side effects.¹⁰

After publication of our primary outcome, one randomised, non-blinded controlled trial examining PRP in acute hamstring injuries was published. The authors reported a significant reduction in time to return to play in the PRP group compared with the control group.⁴² In this study, all 28 patients were prescribed a rehabilitation programme. The patients in the PRP group received a single PRP injection within 7 days of the injury. The patients in the control group did not receive an injection. The mean time to full recovery was 26.7 (±7.0) days in the PRP group and 42.5 (±20.6) days in the control group.

This Malaysian study has several methodological flaws. The study is at great risk of bias because neither participants nor treating medical staff were blinded to the intervention. The study failed to assess for re-injury after the completion of treatment. Furthermore, it is remarkable that return-to-play criteria included a less than 10% side-to-side difference in isokinetic strength testing. This conflicts with existing evidence which indicates that at return to play after a hamstring injury, 67% of the

Table 2 Secondary outcome measures obtained by clinical examination at 1 and 26 weeks*

	1 Week	Placebo (n=39)	Adjusted	26 weeks	Placebo (n=39)	Adjusted
	PRP (n=41)		between-group difference† (95% CI)	PRP (n=41)		between-group difference† (95% CI)
Active knee extension deficit, degrees	3±10	7±9	−4 (−7 to −1)‡	−1±5	1±5	−2 (−5 to 1)
Passive straight leg raise deficit, degrees	2±5	2±3	0 (−2 to 3)	−2±6	0±6	−1 (−3 to 1)
Isometric knee flexion strength testing						
Strength deficit in 15° knee flexion, %	13±21	13±20	−1 (−10 to 7)	−1±18	1±14	−2 (−11 to 6)
Strength deficit in 90° knee flexion, %	11±17	7±18	2 (−5 to 9)	3±14	1±13	2 (−5 to 9)
Pain score in 15° knee flexion	1.6±1.9	1.7±2.2	−0.1 (−0.8 to 0.7)	0.4±1.3	0.5±1.6	−0.1 (−0.9 to 0.7)
Pain score in 90° knee flexion	1.3±1.7	1.9±2.3	−0.6 (−1.3 to 0.2)	0.4±1.3	0.5±1.6	0.1 (−0.7 to 0.9)

*Plus-minus values are means±SD.

†Between-group differences are adjusted for the baseline measure.

‡Statistical significant difference (p=0.01).

PRP, platelet-rich plasma.

Table 3 Hamstring outcome score at 26 weeks follow-up*

	26 Weeks		Between-group difference (95% CI)
	PRP (n=41)	Placebo (n=39)	
Overall score (0–100)	86±19	88±21	–3 (–12 to 7)
Symptoms (0–100)	79±28	86±26	–7 (–20 to 6)
Soreness (0–100)	89±18	91±19	–2 (–11 to 7)
Pain (0–100)	91±18	90±20	1 (–9 to 10)
Function in sports (0–100)	95±14	92±22	4 (–6 to 13)
Quality of life (0–100)	77±27	82±26	6 (–18 to 7)

*Plus-minus values are means±SD.
PRP, platelet-rich plasma.

participants tested had a >10% side-to-side isokinetic strength difference.⁴³

The time to return to play in our study is within the range of the mean of 22–51 days reported in previous high-quality randomised controlled trials in hamstring injuries,^{44–46} but longer than in other previously reported case series.^{21–23 35 40 47 48} There are several factors that may contribute to this discrepancy. First, the inferior study methodology of the majority of previously published series may lead to bias towards a quicker return to play, as methodological quality is often negatively correlated with reported outcome success.^{49 50} Second, patients with more severe injuries may be more willing to participate in research and receive an injection, which is reflected by the proportion of patients with severe injuries with macroscopic muscle fibre disruption on MRI. Third, the majority of previously published series were performed in professional athletes, compared with our study, which had a large number of competitive amateur athletes. It may be that professional athletes are more likely to seek and receive medical care for less severe injuries than amateur athletes, and thus progress faster through rehabilitation.

Strengths and limitations

The methodological strengths of our study include the minimisation of bias by the placebo controlled double-blind design, no loss to follow-up for the primary outcome measure and identical measurements for all patients performed by one physician. To minimise the influence of subjective judgements, all patients performed a predefined criteria-based rehabilitation programme with strict functional criteria to progress through the programme. The nationwide recruitment in three different clinical settings (academic clinic, general clinic and specialised high-level athlete clinic) contributes to the generalisability of the results.

Our study has some limitations. First, there are some uncertainties about the adherence of the patients to the rehabilitation programme, and there was no assessment of the adherence of the supervising physiotherapists in following the recommended physiotherapy protocol. As the rate of missing adherence data is

Table 4 Oedema on MRI at return to play*

	PRP (n=33)	Placebo (n=30)	Between-group difference (95% CI)
Cross sectional area, % of total muscle	15±22	14±20	1 (–9 to 12)
Longitudinal length, cm	5.3±5.2	5.5±5.4	–0.2 (–2.8 to 2.4)

*Plus-minus values are means±SD.
PRP, platelet-rich plasma.

Table 5 Adherence to the rehabilitation programme*

	PRP (n=20)	Placebo (n=20)	Between-group difference (95% CI)
Supervised physiotherapy (% of performed sessions)	80±22	80±29	0 (–17 to 16)
Home exercise programme (% of performed sessions)	68±17	59±21	9 (–4 to 21)

*Plus-minus values are means±SD.
PRP, platelet-rich plasma.

comparable in both study groups, and the physiotherapists were blinded, it is unlikely that this introduces a potential bias in the treatment effect.

Generalisability

This study has several features that may limit the generalisability of the findings. In a letter to the editor, Anitua *et al*⁵¹ suggested that the timing and the dosage of the PRP injections in our study may have rendered the PRP injections ineffective. In a response letter, we indicated that there is no evidence that the optimal time window for injections is earlier than we used in the present study (median 3 days, IQR 2–4 days) and that the adjustment for the time between the injury and the injection did not change the treatment effect.⁵²

There are several autologous platelet-rich blood products commercially available that differ in their preparation procedure and composition of platelets and leucocytes. Although the generalisability to these other products remains unknown, the platelet concentration is comparable to several other separation systems.⁵³ The population in this study consisted primarily of male competitive athletes who played sport at least 3 times a week. The generalisability to other populations remains unknown.

Many unanswered questions

Our current scientific knowledge about PRP remains at a basic science level and there are many unanswered questions regarding its use in muscle injury.¹⁰ These include some very basic questions, such as what concentrations and ratio of growth factors are required for optimal muscle healing? Which specific growth factors are active? Is timing and number of injections important? Does the injected PRP remain at the injected site? Is the presence of leucocytes in the PRP beneficial or detrimental for muscle healing? In addition to these unanswered basic questions, no proven scientific mechanism is currently available for a therapeutic effect of PRP in muscle injury. Furthermore, no high-quality clinical trials exist that justify the use of PRP in acute muscle injury.

Table 6 Platelet and leucocyte count in whole blood and PRP (in PRP group)*

	Whole blood	PRP
Platelets	232±48	433±128
Leucocytes	6.5±3.6	1.9±2.1
Neutrophils	3.44±1.07	0.52±0.69
Lymphocytes	1.96±0.50	1.13±1.21
Monocytes	0.49±0.15	0.23±0.32
Eosinophils	0.16±0.13	0.02±0.06
Basophils	0.04±0.03	0.02±0.03

*All data is presented in 10³ μL; plus-minus values are means ±SD.
PRP, platelet-rich plasma.

High-quality randomised studies on PRP use in other soft tissue injuries, such as the tendon and ligament, also failed to find a beneficial effect.^{17 54 55}

CONCLUSION

In conclusion, as previously published in the *NEJM*, we found no benefit of intramuscular PRP injections compared with placebo injections in patients with acute hamstring injuries in the time to return to play. In addition, we found no difference in alterations of subjective, clinical and MRI measures between PRP and placebo injections.

What is known?

The primary results of our randomised controlled trial published in the *NEJM* research letter showed that there is no benefit of platelet-rich plasma (PRP) injections in acute hamstring injuries on the time to return to play and the re-injury rate within 2 months after return to play. There is no proven scientific mechanism for any therapeutic benefit of PRP and the ideal ratios and timing of growth factors are not known.

What are the new findings?

In this article, we provide additional results and background information that have not been shared previously. There is no benefit of platelet-rich plasma (PRP) injections in acute hamstring injuries on the secondary outcome measures, including the 1-year follow-up re-injury rate, and alterations in subjective, clinical and MRI measures.

How might it impact on clinical practice in the near future?

Platelet-rich plasma (PRP) injections are not recommended for the treatment of acute hamstring injuries. The scientific basis for PRP in muscle injuries is not established. There are no high-quality clinical trials that justify the use of PRP in acute muscle injury.

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Contributors GR designed the study, monitored the data collection, analysed and interpreted the data and drafted the paper. GJG, MHM and JLT designed the study, interpreted the data and revised the paper. AW, JANV and SMAB-Z interpreted the data and revised the paper. MM analysed the MRIs, interpreted the data and revised the paper. All authors gave final approval for the version to be published.

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Rationale, secondary outcome scores and 1-year follow-up of a randomised trial of platelet-rich plasma injections in acute hamstring muscle injury: the Dutch Hamstring Injection Therapy study

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