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Alsousou, J

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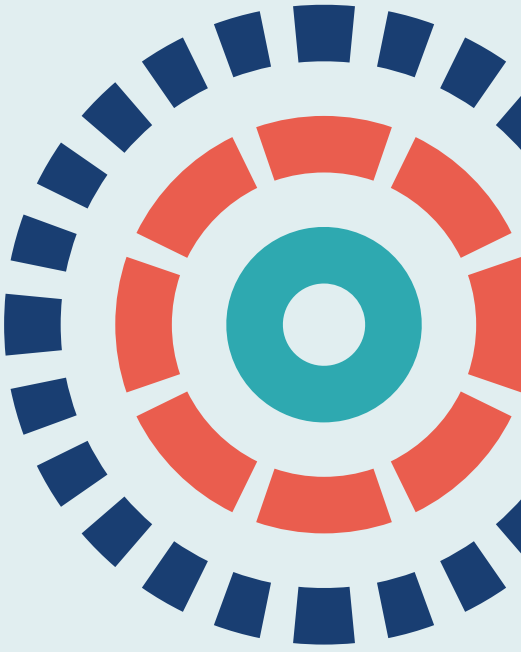

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Platelet-rich plasma injection for adults with acute Achilles tendon rupture: the PATH-2 RCT

Joseph Alsousou,[1*†] David J Keene,[2†] Paul Harrison,[3] Philippa Hulley,[4] Susan Wagland,[2] Jacqueline Y Thompson,[2] Scott R Parsons,[2] Christopher Byrne,[5] Michael M Schlüssel,[6] Heather M O'Connor,[6] Susan J Dutton,[6] Sarah E Lamb[2,6] and Keith Willett[2] on behalf of the PATH-2 study group

[1]Institute of Translational Medicine, University of Liverpool, Liverpool, UK
[2]Kadoorie Centre for Critical Care Research, John Radcliffe Hospital, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
[3]Institute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham Medical School, University of Birmingham, Birmingham, UK
[4]Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
[5]School of Health Professions, Faculty of Health and Human Sciences, University of Plymouth, Plymouth, UK
[6]Oxford Clinical Trials Research Unit, Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK

*Corresponding author
†Joint first authors

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Abstract

Platelet-rich plasma injection for adults with acute Achilles tendon rupture: the PATH-2 RCT

Joseph Alsousou,[1*†] David J Keene,[2†] Paul Harrison,[3] Philippa Hulley,[4] Susan Wagland,[2] Jacqueline Y Thompson,[2] Scott R Parsons,[2] Christopher Byrne,[5] Michael M Schlüssel,[6] Heather M O'Connor,[6] Susan J Dutton,[6] Sarah E Lamb[2,6] and Keith Willett[2] on behalf of the PATH-2 study group

[1]Institute of Translational Medicine, University of Liverpool, Liverpool, UK
[2]Kadoorie Centre for Critical Care Research, John Radcliffe Hospital, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
[3]Institute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham Medical School, University of Birmingham, Birmingham, UK
[4]Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
[5]School of Health Professions, Faculty of Health and Human Sciences, University of Plymouth, Plymouth, UK
[6]Oxford Clinical Trials Research Unit, Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK

*Corresponding author JosephAlsousou@doctors.org.uk
†Joint first authors

Background: Achilles tendon rupture (ATR) has a long healing period, which is challenging for patients and clinicians. Platelet-rich plasma (PRP) is an autologous concentration of platelets thought to improve tendon function recovery. Although preliminary research has indicated positive effects, there is, as yet, no evidence of clinical efficacy from adequately powered robust clinical trials.

Objectives: The objectives were to determine the clinical efficacy of PRP in patients with acute ATR using an objective mechanical muscle–tendon function measure and patient-reported outcome measures (PROMs), and to determine which PRP components contribute to its mechanism.

Design: This was a multicentre, parallel-group, participant- and outcome assessor-blinded randomised controlled trial (RCT) comparing PRP with placebo. Two embedded substudies investigated the PRP's quality and composition and its effects on healing tendon tissues.

Setting: This trial was set in trauma and orthopaedic surgery departments in 19 NHS hospitals in England and Wales.

Participants: Adults with acute ATR presenting within 12 days of injury to be treated non-surgically were eligible. Patients with platelet dysfunction or leg functional deficiency were excluded.

Interventions: Participants were randomised 1 : 1 to the PRP injection group or the placebo group (dry needle in the rupture gap) by central computer-based randomisation using minimisation, stratified by centre and age.

Main outcome measures: The primary outcome measure was the Limb Symmetry Index (LSI) of work during the heel-rise endurance test at 24 weeks. Secondary outcomes measures, collected at 4, 7, 13 and 24 weeks, were repetitions, maximum heel-rise height, Achilles tendon Total Rupture Score (ATRS), quality of life (as measured using the Short Form questionnaire-12 items version 2), pain and participant goal attainment. Needle biopsies of the affected tendon zone were taken under ultrasound guidance at 6 weeks from 16 participants from one centre. Whole blood was analysed for cell count. PRP was analysed for cell count, platelet activation and growth factor concentration. The primary analysis was intention to treat.

Results: A total of 230 participants were randomised: 114 to the PRP group (103 treated) and 116 to the placebo group (all treated). One participant withdrew after randomisation but before the intervention. At 24 weeks, 201 out of 230 participants (87.4%) completed the primary outcome and 216 out of 230 participants (93.9%) completed the PROMs. The treatment groups had similar participant characteristics. At 24 weeks, there was no difference in work LSI (mean difference –3.872; 95% confidence interval –10.454 to 2.710; $p = 0.231$), ATRS, pain or goal attainment between PRP- and placebo-injected participants. There were no differences between the groups in any PROM at any time point or in complication rates, including re-rupture and deep-vein thrombosis. There was no correlation between work LSI and platelet activation in PRP, or erythrocyte, leucocyte or platelet counts in whole blood or PRP. Biopsies showed similar cellularity and vascularity between groups.

Conclusions: This trial design and standardised PRP preparation gives the first robust RCT evidence about PRP's role in managing ATR, which suggests that PRP offers no patient benefit. Equally robust evidence to investigate PRP application in tendon and soft tissue injuries is required. The 24-month follow-up will be completed in April 2020.

Trial registration: Current Controlled Trials ISRCTN54992179.

Funding: This project was funded by the Efficacy and Mechanism Evaluation programme, a Medical Research Council and National Institute for Health Research (NIHR) partnership. The trial was supported by the NIHR Biomedical Research Centre, Oxford, and the NIHR Fellowship programme.

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List of abbreviations

AE	adverse event
ATR	Achilles tendon rupture
ATRS	Achilles tendon Total Rupture Score
bFGF	basic fibroblast growth factor
BMI	body mass index
CACE	complier-average causal effect
CI	confidence interval
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CV	coefficient of variation
DSMC	Data and Safety Monitoring Committee
DVT	deep-vein thrombosis
ELISA	enzyme-linked immunosorbent assay
ETC	excess treatment cost
FGF	fibroblast growth factor
GCP	Good Clinical Practice
G/NG	go/no-go
G/NG 1	go/no-go decision point 1
G/NG 2	go/no-go decision point 2
GP	general practitioner
HRET	heel-rise endurance test
ICC	intraclass correlation coefficient
IGF	insulin-like growth factor
IGF-1	insulin-like growth factor 1
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	intention to treat
LoA	limits of agreement
L-PRP	leucocyte-rich platelet-rich plasma
LSI	Limb Symmetry Index
MCS	mental component score
MFI	mean fluorescence intensity
mITT	modified intention to treat
NIHR	National Institute for Health Research
OCTRU	Oxford Clinical Trials Research Unit
OTUG	Oxford Trauma User Group
PATH-2	PRP in Achilles Tendon Healing
PCS	physical component score
PDGF	platelet-derived growth factor
PI	principal investigator
PIS	participant information sheet
PLT-F	platelet fluorescence
PP	per protocol
PPI	patient and public involvement
PROM	patient-reported outcome measure
PRP	platelet-rich plasma
PSFS	Patient-Specific Functional Scale
RCT	randomised controlled trial
SAE	serious adverse event
SD	standard deviation
SEM	standard error of measurement
SF-12	Short Form questionnaire-12 items
TGF-β	transforming growth factor beta
TMG	Trial Management Group
TSC	Trial Steering Committee
USB	Universal Serial Bus
VAS	visual analogue scale
VEGF	vascular endothelial growth factor

Plain English summary

Achilles tendon rupture (ATR) is a common injury and leads to months of difficulty with walking. The tendon attaches calf muscle to the heel. Most ATRs in the UK are treated by immobilising the lower leg in a plaster cast or boot, followed by months of exercises to restore calf muscle strength. Absence from work often lasts 2–3 months.

Platelets are the smallest blood cells and contain proteins that promote healing. Platelet-rich plasma (PRP) is a concentrate of a patient's own blood. Laboratory experiments suggest that it could improve tendon healing. The effects of PRP on ATR healing in adults were investigated and recovery using patient-reported measures was measured.

Using a computer, 230 patients from 19 hospitals were randomly allocated to receive either a PRP injection or an imitation injection (placebo). Patients having surgical repair of the tendon were not included. Participants were assessed before treatment and at 4, 7, 13 and 24 weeks after treatment. Information was collected on calf muscle strength, quality of life, pain and whether or not participants recovered the ability to do activities important to them. Any problems with their recovery were monitored. Participants' blood was tested for proteins known to help healing. In 16 participants, tiny samples of tendon tissue were taken to assess the healing.

There were no differences between participants injected with PRP and participants receiving the placebo in calf muscle strength or in the patient-reported measurements. This meant that PRP did not improve tendon healing during the 24 weeks. Complications were similar, with one out of 20 participants in each group having a further tear of the tendon. The number of platelets in PRP did not influence the outcome. The biopsies showed similar healing between the PRP and placebo groups.

It is concluded that PRP does not improve recovery from ATR over 24 weeks. Participants will be reassessed at 2 years. PRP is widely used for other musculoskeletal problems and should be tested just as rigorously in those contexts.

Scientific summary

Background

Achilles tendon rupture (ATR) accounts for 20% of all tendon ruptures, and leads to significant health-care and societal costs. The current treatment strategies are (1) surgical repair or (2) immobilisation in a cast or boot. The mechanical and biological properties of healed tendons appear to never match those of the original intact tendons, leading to a high risk of re-rupture (3–5%) or reduced function and a loss of, on average, 63–108 days of work.

Platelet-rich plasma (PRP) is an autologous, supraphysiological concentration of platelets that also contains other blood cells. Platelets play an important role at various stages of the repair process of tendon injury. On activation, platelets release an ordered sequence of growth factors, cytokines and an array of bioactive proteins over the lifespan of the platelets. Subsequently, this leads to recruitment of leucocytes, local stem cells and tenocytes to initiate the healing process. Different methods of PRP preparation result in biological component variability, which may influence its efficacy.

In published studies, there is substantial variation in the validity and type of outcomes measured, as well as inconsistency in the observed effect size of PRP. The underpowered and inadequately designed studies suggest that no definite conclusions can be made on PRP application as an adjunct to standard care in the management of ATR. Prior to the PRP in Achilles Tendon Healing (PATH-2) trial, the authors of a meta-analysis of PRP for orthopaedic conditions concluded that there was a need for adequately powered studies using disease-specific and patient-important outcomes to investigate the effect of PRP (Sadoghi P, Rosso C, Valderrabano V, Leithner A, Vavken P. The role of platelets in the treatment of Achilles tendon injuries. *J Orthop Res* 2013;**31**:111–18).

Objectives

- To evaluate the clinical efficacy of PRP among patients with acute ATR using an objective measure of mechanical muscle–tendon function.
- To evaluate the secondary outcome measures of patient-reported function, pain, participant goal attainment and quality of life.
- To determine the key components of PRP that may contribute to its mechanism of action.
- To identify the tissue-level parameters that PRP may alter to exert its effects in an exploratory biopsy substudy.

Methods

Design

A multicentre, parallel-group, participant- and outcome assessor-blinded randomised controlled trial comparing PRP with a placebo (imitation) injection in adults with acute ATR. Two substudies were embedded in the main study to contribute to the understanding of the PRP mechanism in tendon healing:

- substudy 1 – PRP and whole-blood analysis
- substudy 2 – immunohistochemistry analysis of ultrasound-guided needle biopsies from 16 participants at one centre (Oxford).

Setting

The trial was conducted in the trauma and orthopaedic surgery departments of 19 NHS hospitals in England and Wales.

Participants

Patients aged ≥ 18 years with an acute ATR attending an outpatient trauma or orthopaedic clinic within 12 days of sustaining the injury and suitable for non-surgical management were eligible for the trial.

The following patients were excluded:

- those with insertional or musculotendinous junction rupture
- those with previous tendon or ankle injury
- those with deformity to either lower leg
- those with a history of diabetes mellitus
- those with known platelet or haematological disorder
- those using systemic cortisone or anticoagulant treatment
- those with lower-limb gangrene/ulcers or peripheral vascular disease or hepatic or renal impairment
- pregnant or breastfeeding females
- those receiving radiotherapy or chemotherapy
- those with inadequate venous access
- those unable to participate in the trial or attend follow-up.

Interventions

Participants were individually randomised to receive either PRP injection or placebo (dry-needle insertion to the tendon rupture gap), preceded by local anaesthetic, in a 1 : 1 allocation ratio. A central computer-based randomisation system utilising minimisation, stratified by centre and age group (< 55 years or ≥ 55 years), with a probabilistic element of 0.8 to reduce predictability, was provided by the Oxford Clinical Trials Research Unit. Immediately after randomisation, up to 55 ml of venous blood was taken from participants in the PRP group and up to 5 ml was taken in the placebo group. Both interventions were delivered using the same technique by a surgeon or extended-scope physiotherapist while maintaining a participant's blinding. Post injection, the remaining blood and PRP samples were sent to a central laboratory for substudy 1 analysis. Sixteen participants (nine in the PRP group and seven in the placebo group) in one centre (Oxford) received an ultrasound-guided biopsy for substudy 2 assays. All participants received standardised rehabilitation in terms of the duration of ankle immobilisation and non-weight-bearing, and all were referred for physiotherapy.

Follow-up

Blinded outcome assessments were carried out at 4, 7, 13 and 24 weeks post randomisation. Following signed consent being obtained, baseline data were collected and the participant was randomised; in most cases, the injection treatment took place on the same visit. Primary outcome data were collected at a 24-week face-to-face appointment. At every time point, trial follow-up was carried out wherever possible by blinded assessors unaware of treatment allocation.

Outcome measures

Muscle–tendon function assessed by the Limb Symmetry Index (LSI) of work (joules) during the heel-rise endurance test (HRET) at 24 weeks was the primary outcome. Movement of the heel during the HRET in each leg was captured using a computer-controlled linear encoder. The work LSI was calculated as follows: (injured limb measurement/uninjured limb measurement) × 100.

Secondary outcomes were the maximum heel-rise height and number of repetitions during the HRET and the patient-reported outcomes of function and symptoms [measured using the Achilles tendon Total Rupture Score (ATRS)], quality of life [measured using the Short Form questionnaire-12 items (SF-12) version 2 acute], pain (measured using the visual analogue scale and subscale from ATRS) and participant goal attainment [measured using the Patient-Specific Functional Scale (PSFS)].

In substudy 1, whole-blood and PRP samples were analysed for cell count, platelet activation and growth factor concentrations [i.e. platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and transforming growth factor beta (TGF-β)]. In substudy 2, 16 participants in one centre had needle biopsy under ultrasound guidance

at 6 weeks. Analysis included tissue morphology, proliferation, apoptosis, vascularity, metabolic indicators and collagen ratio.

Analysis

The target sample size was 230 participants to provide 80% power. For the primary outcome, analysis included a modified intention-to-treat (mITT) population, defined as all randomised intention-to-treat participants with available work LSI data. Multivariate linear regression was used to investigate the effect of PRP on ATR recovery. Sensitivity analyses were carried out using imputation of values for missing HRET data to examine the robustness of the conclusions made from the analyses to address the primary aims of the trial.

A mITT population was also used for secondary outcome analyses. Linear mixed-effects regression models were used to allow the data collected at all follow-up time points to be taken into account, adjusting for pre-injury baseline scores when applicable. Data quality and effect of treatment received were assessed using complier-average causal effect (CACE) analysis in place of the originally planned per-protocol analysis. Complication events reported by participants were explored at two levels: serious adverse events and adverse events (AEs).

For the two substudies, analyses were primarily descriptive, and the relationship between various biomarkers and clinical outcomes was explored.

Results

A total of 230 participants were recruited between July 2015 and September 2017. Of these, 114 were randomised to receive the PRP injection and 103 (90%) of these received the allocated treatment; 116 were allocated to, and received, placebo. At 24 weeks, 201 out of 230 participants (87.4%) completed the HRET to provide the work LSI primary outcome, and 216 out of 230 (93.9%) completed the patient-reported outcomes. One participant withdrew from the trial. The average age of participants was 45 years; 75% were male, with 69% of injuries occurring during sporting activity. The baseline characteristics of the participants in the intervention groups were well matched.

Clinical trial results

There was no difference between the PRP and placebo groups at 24 weeks in the work LSI. In the PRP group ($n = 101$), the work LSI was 34.9%, compared with 38.3% in the placebo group ($n = 100$) [adjusted mean difference –3.872, 95% confidence interval (CI) –10.454 to 2.710; $p = 0.231$]. Statistical model adjustment by stratification factors and the predefined prognostic variables had no impact on the results attained. Sensitivity analyses accounting for participants with zero measurements for the uninjured limb (unable to lift the heel at all) in the HRET, individuals missing heel-rise repetitions, individuals missing the entire HRET data sets and compliance (i.e. CACE) showed that the results were robust.

There was no difference in secondary outcome results at 24 weeks: ATRS [PRP ($n = 107$), mean 64.9; placebo ($n = 109$), mean 65.6; adjusted mean difference –0.543; 95% CI –4.899 to 3.813; $p = 0.807$] and PSFS (PRP, $n = 109$, mean 7.198; placebo, $n = 107$, mean 7.495; adjusted mean difference –0.297; 95% CI –0.868 to 0.274; $p = 0.291$). ATRS-related pain scores were not different between the two groups in the follow-up period (PRP, $n = 109$, mean 7.661; placebo, $n = 107$, mean 7.449; adjusted mean difference 0.212; 95% CI –0.563 to 0.987; $p = 0.592$). Although no differences in the SF-12 physical component score were identified between the treatment groups (adjusted mean difference 0.805, 95% CI –1.269 to 2.879; $p = 0.447$), mean SF-12 mental component scores were lower in the PRP group than in the placebo group at 24 weeks (adjusted mean difference –2.714, 95% CI –5.242 to –0.187; $p = 0.035$). There was no difference between the PRP group and the placebo group in any of the patient-reported secondary outcomes at 4, 7 and 13 weeks. Daily pain over the 2 weeks after injection was not different between the groups (PRP, $n = 87$, mean 9.5; placebo, $n = 93$, mean 13.6; adjusted mean difference –4.019; 95% CI –10.302 to 2.265; $p = 0.210$). The two groups had similar AE rates related to their Achilles rupture or injection. The number of participants reporting at least one complication of any type related to their Achilles rupture or injection was 84 out of

113 (74%) for the PRP group and 90 out of 116 (78%) for the placebo group. The numbers of participants experiencing a re-rupture [PRP, 6/113 (5.3%); placebo, 4/116 (3.5%)] and deep-vein thrombosis [PRP, 6/113 (5.3%); placebo, 5/116 (4.3%)] were also similar.

Substudy 1 results

Whole-blood cell counts (red blood cells, white blood cells and platelets) showed that the two groups were relatively well matched at baseline. Cell count analysis of PRP samples showed wide variation in cell counts. The mean platelet count was 852.6 × 10^9/l [standard deviation (SD) 439.0 × 10^9/l], with a wide range from 6.0 to 2903.0 × 10^9/l. The mean white blood cell count was 15.1 × 10^9/l (SD 10.3 × 10^9/l), with a range of 1.7 to 65.3 × 10^9/l. Red blood cells were reduced remarkably (0.9 × 10^{12}/l, SD 1.5 × 10^{12}/l, range 0.1 to 9.0 × 10^{12}/l). The quality of the PRP samples in the majority of preparations was high, with low levels of basal activation, and they were capable of activation and degranulation. TGF-β, VEGF, PDGF, IGF-1 and FGF mean concentrations (133.4 ng/ml, 0.984 ng/ml, 55.49 ng/ml, 78.2 ng/ml and 112.5 pg/ml, respectively) were high, as expected. Overall, PRP samples were therefore shown to be functional, with the majority of platelets in the PRP preparations shown to be capable of activation and degranulation. Parameters of baseline whole blood taken before intervention in both groups did not correlate with the primary outcome measure at 24 weeks. PRP cell counts did not correlate with the primary outcome measure. None of the growth factor concentrations showed any correlation with the work LSI.

Substudy 2 results

All biopsy results except one showed evidence of healing at 6 weeks; collagen fibre density was lower in the PRP group. This did not correlate with differences in cellularity or vascularity as these parameters were similar in both groups, suggesting equivalent healing processes.

Conclusions

Implications for health care

The main finding of the PATH-2 trial is that there was no evidence of benefit for PRP application in acute ATR in terms of objective and subjective efficacy outcomes. The effect size estimates of the primary outcome and end point and the consistency with patient-reported secondary outcomes during the follow-up strongly support the validity of the conclusion that PRP does not improve the outcome of ATR management. Although a health economic analysis was not carried out, applying PRP in ATR management would add to the cost of standard care for no clinically measured improvement in the outcome. It is a no-value intervention in ATR management.

Recommendations for research

The implication of the PATH-2 trial is that the indication for PRP application in other soft-tissue injuries should be validated by similar robust clinical trials. The extent of functional asymmetry between injured and uninjured legs in this trial was substantial. Optimising recovery of tendon–muscle function during rehabilitation is therefore a recommended area of future investigation. An extended follow-up of PATH-2 participants at 2 years has started to evaluate longer-term patient-reported outcomes.

Trial registration

This trial is registered as ISRCTN54992179.

Funding

This project was funded by the Efficacy and Mechanism Evaluation programme, a Medical Research Council and National Institute for Health Research (NIHR) partnership. The trial was supported by the NIHR Biomedical Research Centre, Oxford, and the NIHR Fellowship programme.

Chapter 1 Introduction

In this chapter, we provide a background to the current management of Achilles tendon rupture (ATR), summarise the evidence base for the use of platelet-rich plasma (PRP) as a treatment and outline the objectives of the PRP in Achilles Tendon Healing (PATH-2) trial.

Background

Achilles tendon

The Achilles tendon is the largest and strongest tendon in the human body. It is the tendon of the gastrocnemius–soleus calf muscle complex. The Achilles tendon inserts to the calcaneum (heel bone), forming the major plantar flexor and stabiliser of the ankle joint.[1] The tendon is rounded and narrow in shape at its midpoint and finally fans out at the insertion (*Figure 1*). The tendon fibres rotate around 90° as they insert into the calcaneum. The narrowest part of the tendon is approximately 4 cm above the insertion.

The structure of the muscle–tendon unit allows for effective force to be generated when lifting the heel during locomotive activities such as walking and running.[1,2] The human Achilles tendon also stores and returns energy.[3] The tendon stretches in proportion to the force applied during the downwards motion of the body and then recoils to release most of the energy stored (74%) during the upwards movement.

Blood supply to the tendon is variable but is least in the middle (mid-portion). The mid-portion is the narrowest and a relatively avascular region within the tendon that corresponds to the most frequently injured area. Degenerative changes, common with advancing age and metabolic and chronic diseases,

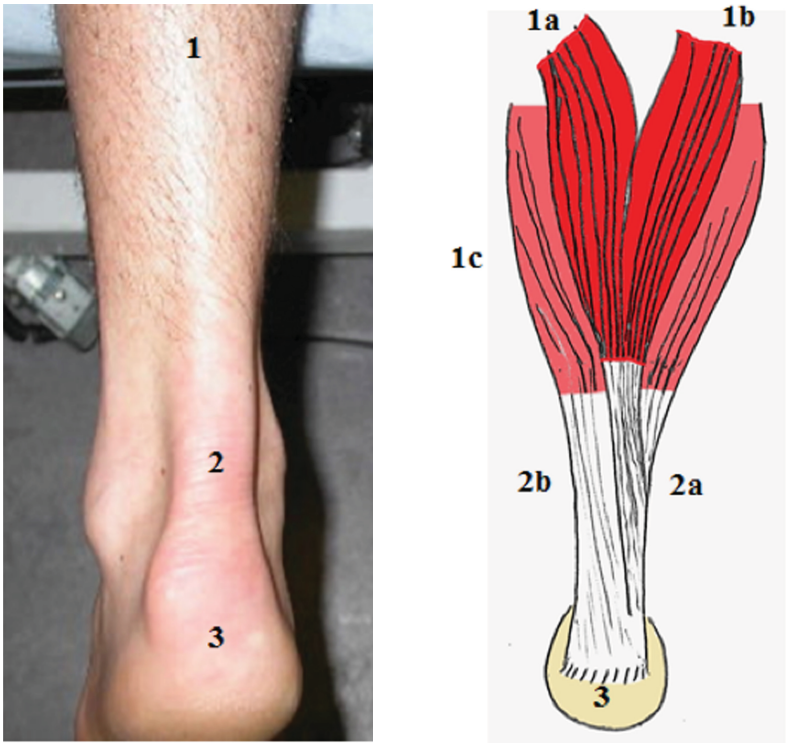

FIGURE 1 Anatomy of the Achilles tendon. The image on the left shows the left Achilles tendon projection at the posterior aspect of the calf and heel (1, calf muscles; 2, Achilles tendon; 3, Achilles tendon insertion to heel); and the image on the right is an illustration of the muscle–tendon unit (1a and 1b, gastrocnemius muscle medial and lateral heads; 1c, soleus muscle; 2, Achilles tendon fibres; 2a, gastrocnemius Achilles fibres; 2b, soleus Achilles fibres; 3, tendon insertion to the calcaneum).

further compromise tendon vascularity and structure.[4] Degenerative changes in the tendon decrease collagen cross-linking and small repetitive tears weaken the tensile strength of the tendon. The Achilles tendon ruptures when it is subjected to a load that exceeds its mechanical capacity.

Achilles tendon rupture epidemiology

The Achilles tendon is the most commonly injured tendon in the human body, accounting for 20% of all tendon ruptures.[5] The incidence of this injury is rising, due to increased engagement in high-impact recreational sport, common particularly among men in the third and fourth decades of life.[6,7] Other factors that might increase the risk of injury include previous injury to the contralateral leg, direct injection of steroids or administration of systemic corticosteroids and fluoroquinolones.[8] In the UK, 11,000 injuries are reported each year.[9] In Denmark, an increase in incidence from 22.1 ATRs per 100,000 person-years in 1991 to 32.6 per 100,000 person-years in 2002 was reported by Gulati *et al*.[10] The incidence of ATR is 12–18 cases per 100,000 people per year for sedentary professionals.[11] A Cochrane review[12] reported rehabilitation and work absence of 63–108 days, highlighting the socioeconomic burden of the injury.

The injury mechanism of ATR is mostly related to loading the tendon during weight-bearing physical activity. Typical acute injuries result from rapid force shifts to the lower leg during sports. This can result in partial or complete tear from direct (acute) trauma. During clinical assessment, patients often describe a history of feeling a direct blow localised to the posterior aspect of the ankle with a sudden onset of pain, swelling, bruising and difficulty with walking, usually during a sport activity. Physical examination of the area commonly shows indentation at the locations of the tendon rupture, soft-tissue swelling and tenderness, but these signs can be masked at the acute stage.

Spontaneous ruptures without a notable trauma, more common in ageing tendons, are typically associated with pre-existing chronic, pathological changes in the tendon. Degenerative changes, such as poor vascular supply, calcification, tendolipomatosis (replacement of tendon tissue with fat), necrosis (cell or tissue death), hypoxia and mucoid degeneration, have been shown to be present in ruptured tendons not related to sports.[13] Once an acute tear to the tendon occurs, the structural, biochemical and functional properties of the tendon are disrupted. Histopathological changes in the injured tissues have been reported. These include high vascularity, collagen disorganisation and hypercellularity relatively close to the ruptured site. There is a reduction in the number and diameter of type I collagen fibres, which are replaced with larger type III fibres.[8]

Clinically, a common method of examination to assess the integrity of the Achilles tendon is to lay the patient prone on a plinth with their foot hanging over the edge. In this position, the tendon gap is usually palpable and easy to observe, and on squeezing the calf muscle bulk, ankle plantarflexion can be anticipated if the tendon is intact (Simmonds–Thompson test).[14–16] These methods of assessment may result in misdiagnosis when the tendon gap is filled with oedema or when a plantarflexion response is generated by other intact muscles that also plantarflex the ankle (i.e. tibialis posterior and the peronei).[17] However, significant plantar flexion weakness on directed push-off, abnormal or poor gait pattern and inability to perform a heel rise on the affected leg are often indicative of a complete tendon tear.[18] Diagnosis is usually made clinically from the history and physical assessments, but some clinical centres use ultrasound and magnetic resonance imaging to support diagnosis. There are also treatment protocols that direct management using imaging findings,[10,18,19] but this is not currently routine practice in the NHS.

Healing of the tendon (repair and regeneration)

The healing of an injured tendon takes place in three overlapping, successive stages: inflammation, repair and remodelling. These cellular responses are directed by numerous growth factors that are upregulated and activated at various stages of the healing process.[13,20] In the inflammatory stage, haematoma forms and platelets release growth factors and bioactive proteins to attract inflammatory cells, including neutrophils. This haematoma and the inflammatory cells eventually organise into granulation and scar tissue.[13,20] In the repair stage, recruitment, activation and proliferation of tenocytes and fibroblast begins in the injury site. During this phase, neutrophil levels drop while macrophages continue to release growth factors that direct cell activity. In addition, intrinsic and extrinsic tenocytes synthesise and establish a network of extracellular

matrix composed of type III collagen fibres. The remodelling stage starts 1–2 months post injury and persists for ≥ 12 months. Tenocytes and collagen mature and become aligned with the direction of stress. Over time, the synthesis of collagen type III fibres decreases, while type I fibres become dominant and fibrous scarring tissue remodel towards the original tendon tissue.[13,20]

Although tendons have the ability to heal after injury, the natural process is slow and incomplete.[20] This limited healing capacity of tendons may result from a combination of relatively poor vascularisation and cellular turnover, changes to the matrix structure and the demanding mechanical environment of this connective tissue.[21–24] Poor vascularisation negatively affects the inflammation that is essential for the restoration of the mechanical and biological properties of tendons during the healing process.[25,26] Ageing tenocytes – the dominant cell type in tendons – tend not to be able to facilitate the proper differentiation of progenitor cells, resulting in ineffective healing. This leads to scar formation rather than tendon tissue regeneration, which results in reduced tensile strength.[13,20]

The mechanical properties of the healed tendon do not fully recover, leading to weakness of the muscle tendon unit and risk of re-rupture. Even with the best contemporary management, tendon injuries give rise to substantial morbidity that lasts for many months and poses considerable challenges for clinicians and patients during the lengthy healing and recovery period.[27]

Management of Achilles tendon rupture

The current treatment strategies for acute ATR are either surgical repair using sutures or non-surgical management by immobilisation of the ankle using a boot or cast.[19] Whether surgical or non-surgical management of ATR is the optimal treatment approach is uncertain.[7,19] The decision for the type of management is often based on patient-related factors such as timing of presentation, comorbidities, activity demands and previous tendon function.[28] Notably, there is a decline in the proportion of tendons treated surgically worldwide.[7]

Open, minimally invasive or percutaneous techniques for operative repair seem to show quicker return to work and greater heel-rise height during physical assessments.[29] However, surgical complications including sural nerve injury, keloid formation, wound breakdown or infection are risks to be considered, although they may be reduced when percutaneous surgical repair is used.[29,30]

Non-surgical management involves short periods (6–8 weeks) of ankle immobilisation starting in an equinus (plantarflexed) ankle position in a cast, rigid boot or ankle orthoses.[29] Recent developments of early functional rehabilitation and range of motion protocol with early weight-bearing – using controlled ankle motion boots – during non-surgical treatment have demonstrated similar surgical outcomes in terms of re-rupture rates (3–5%) and function. However, non-surgical management does not increase the rate of re-rupture or other complications compared with surgery.[31–33] The inclusion of functional rehabilitation as an adjunct to non-surgical management has not demonstrated improvement in biomechanical tendon properties or tendon elongation, which influence plantarflexion strength.[29,34] Although different surgical techniques and non-surgical approaches can be used, evidence shows similar outcomes.[35] These approaches are not known to alter the existing biological regenerative pathway of the tendon, so the lengthy rehabilitation, reduced function and re-rupture risk (3–5%) all remain.[36] Functional impairments identified by calf atrophy, heel-raise power compared with the opposite side, poor walking pattern (gait) and lower levels of physical activity are reported as outcomes of these management strategies.[37]

Platelet-rich plasma

Although significant advancements in the clinical management of ATR have resulted in some improvements in functional outcomes,[38] there has been little progress made with regard to the restoration of the structural and biomechanical integrity of an injured tendon to its original state. Thus, it is unsurprising that novel therapies are being explored to diminish the degree and duration of morbidity. Biological adjuncts such as PRP may augment the regeneration of a torn tendon. For example, platelet-derived growth factor (PDGF), a derivative of PRP, seems to upregulate the synthesis of cells in tendons that direct optimal cellular repair

response, which results in less scar tissue formation, stimulates healing and improves biomechanics.[20] Evidence to support its clinical application in the management of musculoskeletal conditions remains inconclusive.[39]

Platelet-rich plasma is an autologous derivative of whole blood that contains a higher concentration of platelets and other bioactive compounds. PRP is produced by separating the red blood cells from the platelets and other constituents in whole blood. The method of PRP preparation, such as centrifugation or filtration; the concentration and volume of platelets and white blood cells (leucocytes); the activation method; and growth factor levels have all been implicated in the local effects of PRP.[39–41] Unsurprisingly, the nature of the target tissue – acute, subacute or chronic – seems to affect local or systemic effects of PRP.[42]

As part of the normal injury response to an acute tendon rupture, platelets immediately contribute to the blood clot that forms within the injured tissue. That response includes multiple growth factors released from the platelets' α-granules interacting with neutrophils, macrophages and other cells, working synergistically to provide a wound healing mixture. This is part of a cascade within the injured tissue over a period ranging from minutes to an average of several days.[43,44] Active growth factors in PRP in the rupture site promote cell motility and recruit undifferentiated stem cells and surviving tenocytes, which then proliferate to start the healing process.[45] The upregulation of growth factors such as vascular endothelial growth factors (VEGFs) contained within PRP attracts vascular endothelial cells, which stimulate angiogenesis to restore normal tissue conditions.[46] PDGF, insulin-like growth factor 1 (IGF-1) and fibroblast growth factor (FGF) facilitate proliferation of cells and hasten the start of the remodelling phase.[47] Transforming growth factor beta (TGF-β) promotes the formation of fibrous tissue and the expression, organisation and maturation of collagen fibrils, enhancing the biomechanical strength of the tendon.[48]

Although there is evidence suggesting that leucocyte-poor PRP may produce better outcomes than leucocyte-rich PRP (L-PRP), leucocytes contained in PRP seem to interact with the tissue of the tendon, promoting healing by stimulating tenocytes within the injured area to produce their own growth factors.[49] Furthermore, PRP assists the initiation of a cascade of steps that induce the synthesis of fibroblasts, collagen fibrils, matrix-degrading enzymes and extracellular matrix. This sequential release of proteins is amplified to recruit a diverse range of cell types to act on the injured tendon and disrupted blood vessels. So far, conclusions from basic laboratory testing have indicated that PRP could be a novel agent for tendon injury.[44] Augmenting the natural biological healing pathway to improve patient recovery in Achilles tendon injury may therefore have significant clinical implications on the health system at large by reducing demands for surgical repair, accelerating rehabilitation, improving physical activity participation and reducing re-injury rates.

Achilles tendon injury and platelet-rich plasma

Although PRP shows some promise as a treatment for promoting faster healing in tendons,[20,50,51] the potential enhancement of healing demonstrated in laboratory studies is yet to be evidenced in the patient setting. The mechanisms of action through which PRP exerts its regenerative effects in humans are not completely understood. A systematic review has summarised the evidence from animal experiments that evaluated the use of platelets in the treatment of ATR.[52] The authors reported a moderate beneficial effect of PRP in the treatment of ATR in animal models. The observed effects were attributed to accelerated and enhanced scar tissue maturation.[52] An early systematic review of clinical trials of PRP in humans by Taylor *et al*.[50] reported faster healing and better function in the management of tendon and ligament injuries. However, a more recent review by Gholami *et al*.[51] reported no beneficial effects of PRP in the management of sports injuries for pain or function compared with other management options.

A number of studies using different postoperative protocols have investigated the effect of platelets on the treatment of ATR in humans. Two case reports, one of an injured athlete[53] and the other of a complete tear in an active 71-year-old male,[54] showed positive effects of quick return to play after non-surgical management. In a case–control study by Sánchez *et al*.,[55] 12 athletes with Achilles tendon injury were treated with either PRP and surgery, or surgery alone; the PRP and surgery group (six athletes) achieved

significantly quicker recovery and better functional outcome measures of greater range of motion and return to play than those treated with surgery alone. However, this effect was seen only at 22 weeks, and a difference was not found at the 1-year follow-up.[56] Similarly, one small clinical trial (15 participants per group) demonstrated no significant difference in structural and functional outcomes between surgery with PRP and surgery alone.[57] However, Zou *et al*.[58] reported some improvement in isokinetic muscle strength, ankle range of motion and measures of quality of life in a clinical study of 36 people with ATR comparing surgery and PRP with surgery alone.

A randomised controlled trial (RCT) ($n = 30$) by Schepull *et al*.[56] showed no difference for functional outcomes or biomechanical tendon properties at 1-year follow-up when PRP with surgery was compared with surgery alone.[59] However, the authors also stated that a limitation in their platelet preparation technique and storage of up to 20 hours resulted in only a 20% release of growth factors from the platelets. The level of activation to release growth factors may have an impact on the regenerative process and could alter the clinical outcome. We observed good activation rates of 69% as a measure of the quality of PRP used in a clinical pilot study,[57] in which 20 patients were randomised to receive non-surgical treatment with or without PRP injection or operative management with PRP gel, with standard rehabilitation for both groups. Achilles tendon Total Rupture Score (ATRS) and the Victoria Institute of Sport Activity for Achilles (VISA-A) score showed significantly better PRP group outcomes starting from week 3 to week 24 of follow-up.

All of these small clinical studies used PRP as an adjunct to surgical repair, which may have influenced the effect of PRP on the tendon as there is additional surgical trauma and the mechanical effects of having a suture across the tendon rupture gap. Overall, the results from RCTs with small numbers of participants that have assessed PRP in ATR highlight that the current evidence is inconclusive.

Rationale for the PATH-2 study

Despite significant advances in the management of musculoskeletal injuries (e.g. novel operating techniques),[27] slow recovery, morbidity and complication rates persist. PRP as an alternative, regenerative orthobiological agent has gained popularity on the basis that it could augment the management of traumatic musculoskeletal injuries. It is estimated that PRP is used to treat 86,000 tendon, ligament and muscle disorders annually in the USA and Europe.[60] There is also evidence to suggest that PRP injections are being administered in the NHS and private medical practices in the UK.[59]

Platelet-rich plasma was initially used only as an adjunct during orthopaedic surgery, but in recent times it has been applied in other areas of sports medicine. Basic experimental research has provided a biological rationale for PRP, with encouraging results from histological and biomechanical assessments; however, its clinical efficacy is yet to be confirmed by findings from studies with robust designs. Although PRP remains an attractive treatment option, owing to its simplicity, patient acceptability, affordability and practicality, the lack of proven clinical effects means that its use is controversial.

Although PRP has been shown to be relatively safe, preparation of PRP has yet to be standardised.[61] For example, some authors have suggested that administration during the early inflammatory stage of healing may disrupt normal underlying physiological processes. This may result in the downregulation of certain growth factors, such as TGF-β, which may promote the formation of fibrous tissue.[61] Others did not find a link with injection stage. Although injecting PRP concentrates may, in theory, increase the risk of infection or exacerbate the underlying microbial activity within spontaneous ruptured tendons,[23] no clinical impact on outcome has been reported.[36,50]

There is substantial variation in the validity and type of outcomes measured, as well as inconsistency in the effect size of PRP observed from published studies. The underpowered and inadequately designed studies to date suggest that no definite conclusions can be made on PRP as a superior (or inferior) treatment option over standard care in the management of acute ATR. A recent meta-analysis of PRP for orthopaedic conditions[62] stated the need for adequately powered studies using disease-specific and patient-important outcomes to investigate the effect of PRP.

We conducted a multicentre, pragmatic, parallel-group, blinded, placebo-controlled RCT to assess the efficacy of PRP. Patients presenting with acute ATR receiving non-surgical management at orthopaedic trauma surgery departments in the NHS in the UK were randomised to receive PRP or placebo (dry-needle injection). PRP and rehabilitation were standardised. Two substudies of blood and tendon biopsy were integrated into the main trial design to assess the components of PRP and to further explore the mechanism of action on injured Achilles tendons.

Research objectives

- To evaluate the clinical efficacy of PRP among patients with acute ATR using an objective measure of mechanical muscle–tendon function.
- To evaluate secondary outcome measures of patient-reported function, pain and quality of life.
- To determine in an exploratory substudy the key components of PRP that may contribute to its mechanism of action.
- To identify the tissue-level parameters that PRP may alter to exert its effects in an exploratory biopsy substudy.

Chapter 2 Clinical trial methods

Summary of study design

The PATH-2 trial was a multicentre, parallel-group, participant- and outcome assessor-blinded, randomised, placebo-controlled trial with two embedded mechanistic substudies. In NHS hospitals in England and Wales, patients with acute ATR for non-surgical treatment were randomised 1 : 1 to receive either an injection of PRP into the rupture gap or a placebo injection (dry-needle insertion). In substudy 1, whole blood and PRP were sent to a central specialised laboratory (Institute of Inflammation and Ageing, University of Birmingham) for analysis. In substudy 2, immunohistochemical analysis was carried out on tendon biopsy material from participants at one site (Oxford).

Settings and locations

Nineteen NHS hospital orthopaedic or trauma clinics in England and Wales screened and recruited participants for this study:

1. Oxford University Hospitals NHS Foundation Trust
2. University Hospitals of Leicester NHS Trust
3. Taunton and Somerset NHS Foundation Trust
4. North Bristol NHS Trust
5. Cardiff and Vale University Health Board
6. Shrewsbury and Telford Hospital NHS Trust
7. Barts Health NHS Trust
8. University Hospitals Coventry & Warwickshire NHS Trust
9. Warrington and Halton Hospitals NHS Foundation Trust
10. Basildon and Thurrock University Hospitals NHS Foundation Trust
11. Royal Liverpool and Broadgreen University Hospitals NHS Trust
12. Peterborough and Stamford Hospitals NHS Foundation Trust
13. Abertawe Bro Morgannwg University Health Board
14. Aintree University Hospital NHS Foundation Trust
15. University Hospital of South Manchester NHS Foundation Trust
16. Sheffield Teaching Hospitals NHS Foundation Trust
17. Royal Devon and Exeter NHS Foundation Trust
18. Mid Cheshire Hospitals NHS Foundation Trust
19. Royal Surrey County NHS Foundation Trust.

The principal investigator (PI) at each site was a trauma and orthopaedic surgeon. Sites were selected after completion of a site feasibility questionnaire, signed by the PI, which was used to assess whether or not each site had the appropriate resources to deliver the project and meet recruitment targets. The PI supervised implementation of the trial protocol at the site and co-ordinated with local physiotherapy services to provide a standardised rehabilitation protocol and to arrange a blinded physiotherapist to be the assessor for the primary outcome measurement. Confirmation of collaboration was provided in writing to the PI.

Participants

Participant screening and eligibility assessment

All patients attending orthopaedic or trauma clinics with a suspected acute ATR were screened for inclusion in the trial. Patients were identified for screening for the trial at this clinic visit, normally in the 12 days after the initial visit to hospital, where the attending surgeon (or an extended-scope physiotherapist) confirmed appropriateness for non-surgical treatment and trial eligibility.

Patients with acute ATR were eligible for the trial if they met all of the inclusion criteria and none of the exclusion criteria.

Patients were eligible for inclusion if:

- they were willing and able to give informed consent for participation in the trial
- they were aged ≥ 18 years
- they were ambulatory prior to injury without the use of walking aids or assistance of another person
- they were diagnosed with an acute, complete ATR
- they presented and received trial treatment within 12 days post injury
- the decision had been made for them to receive non-surgical treatment
- they were able (in the investigator's opinion) and willing to comply with all trial requirements
- they were able to attend a PATH-2 trial hospital site for the 24-week follow-up.

Patients were excluded if they:

- had an Achilles tendon injury at the insertion to the calcaneum or at the musculotendinous junction
- had a previous major tendon or ankle injury or deformity to either lower leg
- had a history of diabetes mellitus
- had a known platelet abnormality or haematological disorder
- were currently using systemic cortisone or a treatment dose of an anticoagulant (i.e. a prophylactic dose for preventing thrombosis was not an exclusion criterion)
- had evidence of lower-limb gangrene/ulcers or peripheral vascular disease
- had a history of hepatic or renal impairment or dialysis
- were female and pregnant or breastfeeding
- were receiving, or had received within the previous 3 months, radiotherapy or chemotherapy
- had inadequate venous access for drawing blood
- had any other significant disease or disorder that, in the opinion of the investigator, might put the participant at risk because of participation in the trial or might influence the result of the trial or the patient's ability to participate in the trial.

Members of the local research team informed the patient of the trial and carried out the informed consent process, baseline data collection and randomisation.

Standard treatment for this non-surgical population is usually application of a plaster cast, orthopaedic brace or splint during the clinic visit. The PATH-2 trial treatment options required the intervention to be delivered before the definitive immobilisation method was applied. Therefore, the time frame between the informed consent process and treatment was relatively short. To help raise awareness of the trial while patients waited in the clinic, sites displayed trial posters and distributed participant information sheets (PISs) in clinic waiting areas.

The attending clinician conducted a clinical examination and decided with the patient whether management would be surgical or non-surgical. If non-surgical management was appropriate, and the clinician confirmed the patient's eligibility for the trial, the patient was informed of the trial and given a PIS. The potential participants were allowed as much time as practically possible in this type of acute injury

to consider the information, and had the opportunity to ask questions of the attending clinical team and a member of the research team.

Consent was obtained by a member of the local research team who was trained in Good Clinical Practice (GCP) and authorised by the PI to take consent: this could be a research nurse, physiotherapist or surgeon at the local NHS trust, or a nurse assigned from the local National Institute for Health Research (NIHR) clinical research network. The person taking consent presented the PIS and consent form to the participant by means of a verbal discussion. The PIS detailed the exact nature of the trial, the implications and constraints of the protocol, the known side effects and any risks involved in taking part. It was clearly stated that the participant was free to withdraw from the trial at any time for any reason without prejudice to future care and with no obligation to give the reason for withdrawal. The consent form was personally signed and dated by the participant and by the person who obtained consent.

A copy of the signed consent form was given to the participant, and one copy was sent to the trial co-ordinating team in Oxford to facilitate central monitoring. The original signed consent form was retained in the medical notes, and a copy was held in the investigator site file. Consent forms were held in a secure location separately from trial data. Permission was obtained to inform the participant's general practitioner (GP) of trial participation.

The PIS specified that a blood sample of up to a maximum of 55 ml may be taken. Only a maximum amount was stated, as detailing the exact amount of blood withdrawn per treatment group would have revealed treatment allocation; the amount drawn varied depending on treatment. The PIS also outlined that the sample remaining after treatment for the PRP injection group and the sample for the placebo group would be dispatched to a member of the central research team in the Institute of Inflammation and Ageing, University of Birmingham, for analysis. Samples were anonymised before dispatch, and identified using only the participant's unique trial number. No laboratory results were reported back to participants or the recruiting centre.

The PIS stated that name and contact details (including mobile phone number, telephone number and e-mail address) would be collected to facilitate follow-up, full data collection and reporting of results. A copy of the contact details would be sent to the trial co-ordinating team in Oxford. These details were used by the trial team to check contact details through NHS Digital and to provide other basic trial-related information that was needed for follow-up.

Permission was sought to allow access to participant data by responsible members of the University of Oxford or the NHS trust for monitoring or audit of the trial to ensure that regulations were complied with.

The 24-week follow-up visit included the heel-rise endurance test (HRET) (primary outcome data collection). The participant was asked to consent to video-recording (without audio) of their ankle and leg movements at the time of the test. Consent for the video-recording was given on a second consent form so that the participant could consent to participation in the trial without consenting to video-recording. The participant personally signed and dated the consent form for video-recording, followed by the person who obtained consent, and the procedures for handling the form were the same as for the intervention consent form, detailed previously. Filming did not include the participant's face; it focused on the legs, thus reducing the risk of participants being identifiable from the film. The video file (or any still photographs from it) was labelled with the unique trial number, and no identifying details were used. Permission was sought to send the video file to the trial team in Oxford, where it was held and viewed by members of the research team.

At one site (Oxford) where participants were also asked to consent to the biopsy sample collection for substudy 2, a version of the PIS was used that, in addition to the content described previously, invited the patient to attend a separate trial visit during which a small sample of tendon tissue would be removed from the injured tendon. A separate consent form that included consent to giving a tissue sample was provided for these participants. The participant was under no obligation to give a tissue sample.

Baseline assessments

Following signed consent, baseline data were collected and the participant was randomised. The injection treatment took place during the same visit in most cases. A GCP-trained member of the local research team oversaw the participant's completion of the paper baseline questionnaire, which included:

- background information and demographics
    - participant-reported questions including date of injury, which leg was injured, general health, current medication, allergies, smoking status, alcohol use, sport activities, age, date of birth, sex, employment status, type of employment, activities related to standing/walking/driving, any medication taken for pain or inflammation, recreational activities prior to injury, the activity that led to the torn tendon, previous rupture history, height and weight
- the Patient-Specific Functional Scale (PSFS)[63,64]
    - participant reported – three important activities the participant was having difficulty with as a result of their ATR (range is 0–10; a higher score indicates that the participant is closer to achieving their goal)
- the ATRS
    - participant reported – questions specific to ATR (range is 0–100; a higher score indicates better function)
- the Short Form questionnaire-12 items (SF-12) version 2 acute
    - participant reported – without injury (recall of pre-injury function and health state) (range is 0–100 for each of physical health and mental health; a higher score indicates a higher level of health)
- the SF-12 version 2 acute
    - participant reported – with injury (current function/health state) (range as for pre injury)
- pain visual analogue scale (VAS)
    - participant reported – baseline report of pain prior to treatment using a VAS (range of scores is 0–100; a higher score indicates greater pain).

The following events took place in the clinic during the baseline data-collection period:

- Blood sample – following randomisation, a blood sample was taken and the intervention was prepared by a member of the local team according to allocated treatment.
- Participant contact details – contact details, including NHS number, were collected with a preferred time to be contacted to organise trial follow-up.
- Pain diary – a pain diary was provided to the participant before leaving the clinic, to be completed at home during the first 2 weeks following injection treatment, and to be returned to the trial office by post using a Freepost account.
- General practitioner letter – the participant's GP was informed of their participation in the PATH-2 trial.

Randomisation

Participants were randomly allocated (1 : 1) to receive either the PRP injection or placebo. Randomisation was carried out by a GCP-trained member of the local research team, either by telephone or via a secure central computer-based system provided by the Oxford Clinical Trials Research Unit (OCTRU) randomisation service. A randomisation method with minimisation algorithm using site and age group (< 55 years and ≥ 55 years) as strata and variable block sizes was used to ensure that sites and age groups were balanced across the treatment groups. A probabilistic element of 0.8 was introduced to reduce predictability of allocation. The service was accessible by telephone (during normal office hours: 08.00 to 17.00) and via a secure randomisation website (24 hours per day/7 days per week). Details of the block sizes and allocations were confidential and were known only by the trial statistician and OCTRU programmer.

Interventions

Once randomisation had taken place and the allocation was known to the research team, a blood sample was taken from the participant and prepared as appropriate for the treatment to be delivered by an unblinded research nurse (*Figure 2*).

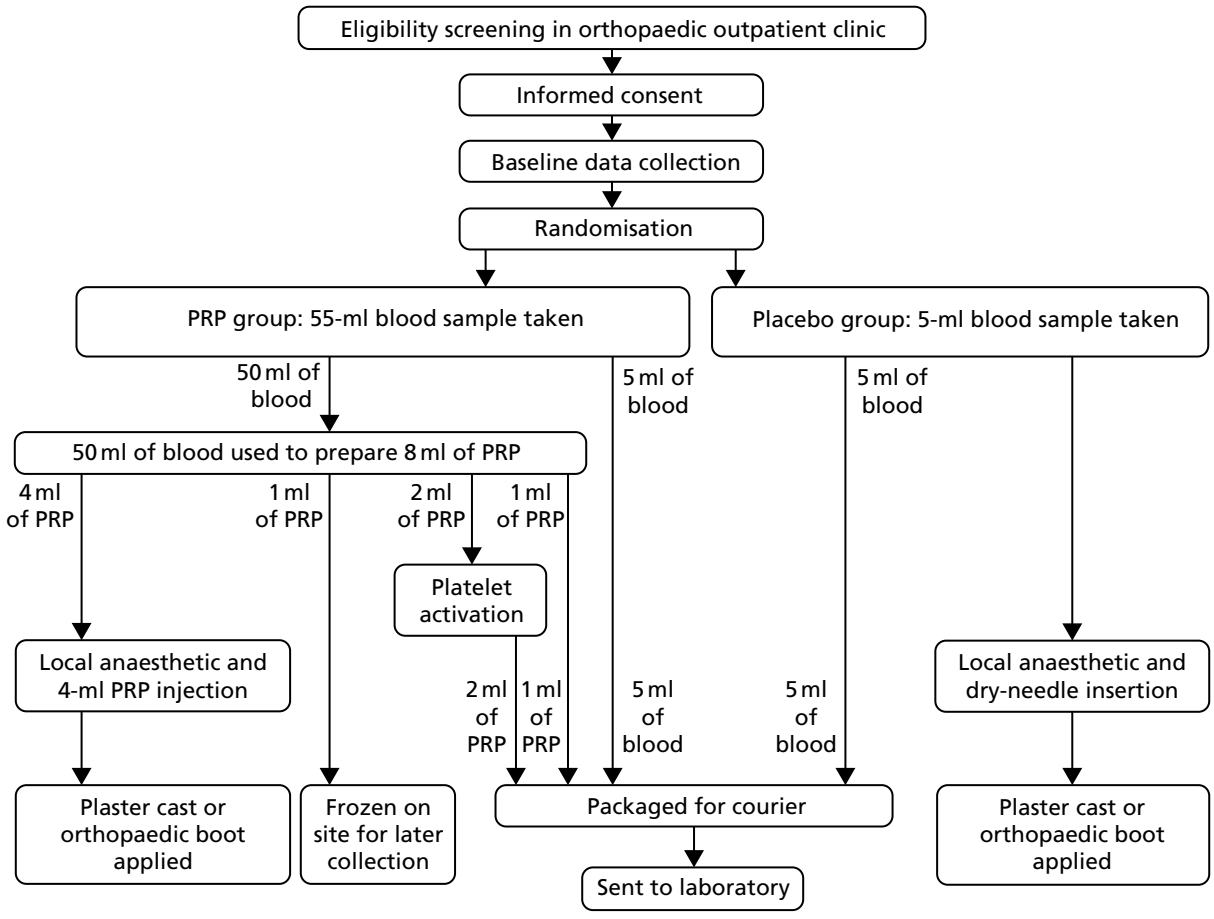

FIGURE 2 Activities taking place on day of randomisation.

Injection treatment was delivered in the outpatient clinic by a surgeon (consultant, registrar or clinical fellow) or an extended-scope physiotherapist who was appropriately qualified, as delegated by the PI (an orthopaedic surgeon). Full training in preparing and administering the intervention was given by central trial office staff and detailed instructions were provided in an illustrated intervention and blood processing manual, which was accessible to local staff when preparing the intervention and managing samples.

For participants in the PRP group, 5 ml of venous blood was drawn off into a 5-ml syringe, followed by 50 ml of venous blood into a 60-ml syringe that contained 8 ml of anticoagulant. The blood was drawn slowly to avoid platelet agitation and early activation. The participant was asked to wait in an adjoining room while the intervention was prepared. The 60-ml syringe was fitted into the trial-specific centrifuge provided to all sites (MAG-200 MAGELLAN® Autologous Platelet Separator, Arteriocyte Medical Systems, Hopkinton, MA, USA). A sterile, disposable PRP kit (MDK 300/MDK 300-1 platelet separation chamber, Arteriocyte Medical Systems, Hopkinton, MA, USA) was placed in the centrifuge, and the centrifuge was set to run to produce 8 ml of PRP, which was fed into a 10-ml syringe.

For participants in the placebo group, 5 ml of venous blood was drawn off into a 5-ml syringe. The participant was asked to wait in an adjoining room while the intervention was prepared. An empty syringe was prepared. The participant was not approached with the placebo intervention until a length of time had passed, similar to the time taken to produce PRP.

For both groups, the participant was asked to return to the treatment room and to lie prone on a treatment bed with the tendon exposed. The tendon gap was palpated clinically to determine the injection site and the area was cleaned. Local anaesthetic (1–2 ml) was administered to the skin.

Participants in the PRP group were given a 4-ml injection of PRP into the tendon rupture gap. Participants in the placebo group received a placebo injection: a dry needle of the same size was introduced via the skin into the tendon tissue, held in the skin briefly and withdrawn without injecting anything so that the biological haematoma was minimally disturbed.

For both groups, the injection area was covered with a dressing and the participant had application of a plaster cast or an orthopaedic boot with the ankle immobilised in the equinus (plantarflexed) ankle position.

If a participant randomised to the PRP group was unable to receive a PRP injection for any technical reason or if blood withdrawal failed, they received a placebo injection. This was recorded, and, whenever possible the participant remained blinded to knowledge of their treatment group. Trusts followed their own policy to manage deep-vein thrombosis (DVT) prophylaxis.

After injection delivery

The centrifuge produced 8 ml of PRP, of which 4 ml was injected into the participant's Achilles tendon; 4 ml remained for laboratory analysis (see *Chapter 4* for further details of the preparation of these samples). Immediately after the intervention, a member of the local team prepared the remaining PRP (PRP group) and the 5-ml whole-blood sample (PRP and placebo groups) for storage or dispatch. Samples were sent to a member of the central team at the University of Birmingham research laboratory. A single, specialised courier (Davies International, Hampshire, UK) was used for this purpose and a pre-paid account was established with the courier to facilitate ease of sample transport.

The 4 ml of PRP that remained after injection (PRP group only) was prepared for dispatch to the laboratory; 1 ml of PRP was transferred to a microtube and stored at the site in a –70 °C freezer within 2 hours of PRP production. These samples were collected by the dedicated courier at the end of recruitment and transported to the central laboratory for analysis for substudy 1.

In the PRP group, 1 ml of PRP was transferred to a microtube and placed in a trial-specific courier pouch; 2 ml of PRP was transferred into activation vials from a proprietary platelet-activation kit (CB kits, Platelet Solutions Ltd, Nottingham, UK) supplied by the central trial office for platelet activation and fixation. The platelet activation and fixation took around 5 minutes and full instructions were given in the intervention and blood processing manual. When the process of platelet activation and fixation was complete, these two tubes were placed in the courier pouch.

For both groups, the 5-ml venous whole-blood sample was transferred to a tube containing ethylenediaminetetraacetic acid, an anticoagulant, and placed in the courier pouch. Thus, the courier pouch contained four tubes if the participant was randomised to PRP (three tubes containing PRP and one tube containing blood) and one tube if the participant was randomised to placebo (blood only).

The courier pouch was packaged in accordance with instructions in the intervention and blood processing manual and the site contacted the courier for collection and next-day delivery service. Until collection, the courier package was stored at room temperature.

Preparation of PRP and samples and courier dispatch were recorded on a blood tracking sheet. The original version of this form was enclosed with the courier pouch for dispatch to the laboratory. A copy was sent separately to the central trial office and a copy was retained at the site. Blood and PRP analysis results were not reported back to the recruiting centre as they did not have an impact on future treatment.

Injection training

Training in the delivery of both the PRP injection and the imitation injection was provided by the trial team and recorded on a PATH-2 trial training form (treatment related), which was signed by the clinician receiving training and retained at the site. Training consisted of the provision of a training manual for all sites. At most sites, a training video or training session by a PATH-2 trial trainer using the trial kit and trial procedures was provided in addition to this manual. The PI at each site identified surgeons (or extended-scope physiotherapists) to be trained, and recorded those who completed training on the site delegation log. Only those individuals who were trial trained and listed on the delegation log were able to carry out trial treatments. When the delegation log was updated, a copy was sent to the trial co-ordinating office in Oxford. The PI at each site identified local staff who were responsible for preparing the blood and PRP samples for treatment and for later dispatch. Only those individuals who were listed on the delegation log as having this responsibility were able to carry out preparation of the blood and PRP and to manage blood and PRP samples.

Monitoring intervention delivery

Intervention delivery was monitored centrally by PATH-2 trial staff. Site staff documented the intervention, including preparation and processing of samples, using a blood sample and treatment case report form (CRF) and a blood tracking sheet. Site staff were trained in completion of these forms by central trial staff. The original blood tracking sheet was sent with the samples to the Birmingham laboratory as supporting documentation for substudy 1. Copies of these completed forms were returned to the trial office and copies were kept at the site.

On receipt of the two forms, trial office staff checked that the forms were fully and correctly completed, that the intervention had been carried out by an individual whom the PI had identified on the delegation log as suitably qualified and that the procedures had been completed within the time frame specified in the intervention and blood processing manual. If any problems had occurred during the preparation of the samples, intervention delivery or sample processing, the site was contacted to investigate whether or not a protocol deviation had occurred and if additional training was required.

Rehabilitation

All participants received standard care for their injury in accordance with local site procedures. Immediately after treatment, the participant's ankle was placed in a splint, orthopaedic brace or plaster cast. Rehabilitation was prescribed by local members of the clinical and physiotherapy teams. However, it was necessary to standardise key elements of rehabilitation in order to reduce the risk of efficacy interference due to substantial variation in rehabilitation protocols. The standardisation for PATH-2 participants involved:

- ankle immobilisation in the equinus ankle position in a splint, orthopaedic brace or plaster cast for a minimum of 3 weeks
- referral to physiotherapy for rehabilitation
- avoiding more than 6 weeks of rigid full-time immobilisation without ankle motion or weight-bearing.

Adherence to these guidelines was monitored by asking participants questions about their progress with rehabilitation. Standardisation of specific elements of rehabilitation, such as the splinting method, when weight-bearing commenced or specific exercises, was not mandated.

Outcomes

Primary outcome

The primary objective of the trial was to evaluate the clinical efficacy of PRP in acute ATR in terms of muscle–tendon function. The primary outcome measure at 24 weeks post randomisation was the Limb Symmetry Index (LSI) of the work performed by each lower limb in joules (J) during the HRET. The HRET is a validated objective measure of Achilles tendon muscle unit function.[65]

The HRET involves repetitive concentric–eccentric muscle actions of the plantar flexors in a single-leg stance until exhaustion, with performance quantified as (1) total work in J, (2) number of repetitions and (3) maximum heel-rise height in cm.[65] Total work was selected as the primary outcome measure because this index provides a measure of plantar flexor muscle endurance and Achilles tendon function as it incorporates the maximum height of each repetition. Total work (J) was computed as the product of body mass (kg), total vertical displacement (m) and the constant 9.807 converting kilopond metres to joules. To quantify performance, a linear displacement sensor (MUSCLELAB™, Ergotest Innovation A.S., Porsgrunn, Norway) was attached to the participant's heel during the test and recorded on software on a laptop computer. A PATH-2-specific user interface version of the MUSCLELAB software was developed with the manufacturer for ease of use in a multicentre trial. Key features of this software and more background on the HRET development are described in further detail in *Appendix 2*.

Heel-rise endurance test procedure for PATH-2

At the 24-week follow-up visit, the HRET procedure was explained to each participant by the blinded outcome assessor. Standardised participant information and instructions for the HRET involved the participant watching a video demonstration of the HRET and reading standardised written instructions detailing their expected conduct during the test and the test termination criteria. The standardised warm-up involved 5 minutes of usual-pace walking followed by 10 double-leg heel raises. Before testing each leg, the participants were asked to stand on the box being used and were allowed to familiarise themselves with the expected timing of heel raises by lifting both heels together.

The HRET was first carried out on the uninjured limb and then on the injured limb. The test started with the participant standing on one leg on a 10° incline box (so that the ankle was in a dorsiflexed position) with the cord from the linear encoder strapped to the participant's heel (*Figure 3*).

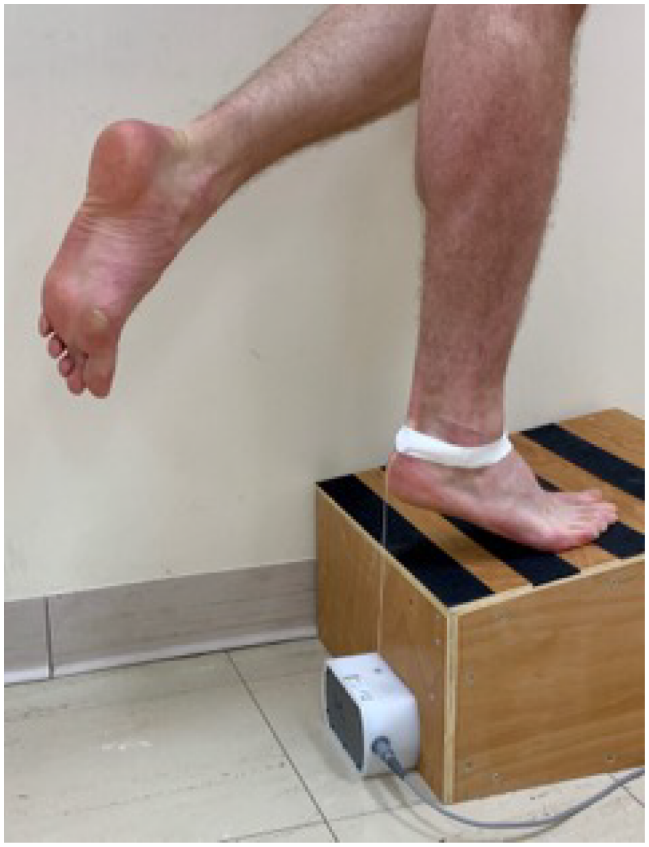

FIGURE 3 A participant performing the HRET. The participant stands on 10° incline box with a cord attached to their heel and connected to a linear encoder, and raises and lowers their heel repeatedly until fatigued.

The following standardisation parameters were adopted:

- Ankle starting position of 10° dorsiflexion, produced by conducting the HRET on a custom-made 10° incline box.
- Knee starting position of full extension.
- Height of each repetition to be as high as possible.
- Pace of 30 raises per minute, guided by a digital metronome.
- Balance support by the fingertips only.
- Strictly defined test termination criteria – participants either stopped (i.e. volitional task failure) or were audibly instructed to stop with both feet flat on the box whenever any of the following test termination criteria were observed: (1) inability to keep pace with the metronome, (2) inability to maintain full knee extension of the standing leg or (3) using more than fingertip support. The desired end point was volitional task failure; however, outcome assessors were encouraged to use verbal prompts whenever the termination criteria were observed and to stop the test if the participant did not respond to two consecutive prompts.

Secure data transfer and confidentiality were assured by copying the HRET data from the encrypted laptop to an encrypted Universal Serial Bus (USB) drive that was sent to the trial office. Sites retained a copy of the entire site HRET data set on the dedicated trial laptop.

Assessor training material consisting of high-quality training videos, which were made by the PATH-2 trial team and produced by Oxford Medical Illustration (Oxford University Hospitals NHS Foundation Trust, Oxford, UK), and a training and reference manual. Face-to-face training was delivered by a member of the PATH-2 trial team to each outcome assessor prior to their first participant's 24-week follow-up appointment.

The linear encoder, a very sensitive device, recorded minimal movements that might not represent actual heel rises; it could also pick up any additional movements of the heel (e.g. participants tend to step off the box or lift the leg up at the end of the test). To dismiss potential measurement errors, two members of the trial team, who were blinded to treatment allocation, independently reviewed videos of all assessments for which participants consented to recording. The invalid heel-raise repetitions in the HRET data were identified so that could they could be dealt with in the analysis (see *Statistical methods*).

Secondary outcome measures

The secondary outcome measures were as follows:

- The ATRS,[66] a validated patient-reported outcome measure (PROM) incorporating 10 questions relating to muscle strength, fatigue, pain and function at 4, 7, 13 and 24 weeks post treatment. The score range is 0–100.
- The SF-12 version 2 acute, a health-related quality-of-life PROM[67] at 4, 7, 13 and 24 weeks post treatment. The scale consists of a 12-item short questionnaire that assesses eight different health domains reflecting both the physical health (physical functioning, role participation with physical health problems, bodily pain and general health) and the mental health (vitality, social functioning, role participation with emotional health problems and mental health) of the evaluated participants.
- A daily pain diary, reported by the participant for 14 days beginning on the day of treatment, using a VAS. The participant was asked to place a vertical mark on a 100-mm horizontal line between the extremes of 'no pain' and 'worst pain imaginable'. Trial office staff measured the distance to the vertical mark and converted the distance to a daily measure (0–100) of pain.
- The pain component of the ATRS at 4, 7, 13 and 24 weeks post treatment. In the ATRS, the participant was asked 'Are you limited due to pain in the calf/Achilles tendon/foot?' and invited to circle an integer from 0 to 10 inclusive, 0 indicating major limitations/symptoms and 10 indicating no limitations/symptoms.
- The PSFS,[63] a PROM indicating progress on self-selected recovery goals relating to daily activities affected by the injury on an 11-point scale (0–10), at 4, 7, 13 and 24 weeks post treatment.
- The maximum number of repetitions (heel rises) recorded during the HRET[68] at 24 weeks post treatment.
- The maximum vertical displacement (cm) recorded during the HRET[68] at 24 weeks post treatment.

Adverse events

Complication events reported by participants were explored at two levels: serious adverse events (SAEs) and adverse events (AEs).

Serious adverse events

A SAE is an untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is another important medical event.

Adverse events

Adverse events were events that were related to the trial treatment or conditions that did not require specific time-critical reporting but were collected as part of standard data collection in the PATH-2 trial. AEs were broken down further into foreseeable and unforeseeable AEs.

Foreseeable AEs:

- bruising and discomfort at the venesection site
- mild discomfort or minor bleeding from the ATR site following injection
- technical complications of the lower-leg casting and splinting
- consequences of depending on walking aids
- syncopal (fainting) episode associated with venesection or tendon injection
- discomfort at the ATR site during rehabilitation
- swelling or bruising of the lower leg and foot
- deep-vein thrombosis in a lower limb
- re-rupture of the treated Achilles tendon (including any surgery on the Achilles tendon treated in the trial).

Unforeseeable AEs:

- serious infection at the ATR injection site
- skin breakdown or ulceration of the treated lower leg other than 'plaster sores'
- severe pain requiring more than simple analgesia beyond 10 days after injection.

Events that were related to trial treatment and that were either foreseeable or unforeseeable were defined as AEs.

Serious adverse events were reported to the chief investigator by the local research team using a SAE form within 24 hours of their becoming aware of the event, the PI having assessed causality and relatedness.

Adverse events were reported to the central trial office on an AE form by site staff. In addition, participants were asked in follow-ups to report any type of AE that they had experienced.

Blinding

Blinding during treatment preparation and intervention

This was a double-blinded (participant and primary outcome assessor) trial. Participants remained blind to allocation throughout the trial. Those staff involved in treatment delivery were aware of treatment allocation because of the nature of the intervention. The primary outcome assessors were not aware of the allocation or treatment received.

Site staff were trained consistently to maintain blinding. Care was taken not to draw the participant's attention to the amount of blood taken as this could have indicated the intervention group. To facilitate treatment blinding, preparation of the intervention took place in a location away from the treatment room, or the participant waited outside, as there was a difference in the number of consumables handled depending on treatment allocation. When the injection was prepared, it remained out of the participant's view.

If a participant randomised to the placebo group was within listening distance of the centrifuge, the centrifuge was run on a dummy cycle. The wait time for the participant was approximately 17 minutes; this was the time taken to run the centrifuge to produce PRP. During the interventions, the participant was requested to lie face down with their foot and ankle slightly off the edge of the bed, and there was an option of placing a pillow on the back of the participant's shoulders below the neck to help visually shield the intervention procedure. The verbal cues used by site staff at the time of treatment delivery did not refer to allocation and were the same for both groups.

Full guidance on ensuring that the correct amount of blood was withdrawn into the appropriate tube or syringe while ensuring that the participant remained blinded to allocation was provided in the PATH-2 trial intervention and blood processing training materials, which were retained in an area where they were not visible to the public.

Facilitating blinding immediately post treatment

The PATH-2 blood sample and treatment CRF collected confirmation that allocated treatment was received. When a treatment other than that allocated was indicated on the CRF, the trial co-ordinating team contacted the site for further details and recorded the details. Additional training or troubleshooting of any issues was instigated when necessary.

In hospital notes and in the letter to the participant's GP, it was recorded that an injection was delivered according to the random allocation assigned, but the type of injection was not recorded.

To assess the completeness of participant blinding, after the 24-week trial questionnaire and HRET assessments were completed, participants were asked which treatment they believed they received ('PRP injection', 'imitation injection' or 'don't know') and why they believed this, along with questions around their experience of taking part in the trial. These data were used to compute two blinding indices with 95% confidence intervals (CIs) (James *et al.*[69] and Bang *et al.*[70] blinding indices).

Blinded follow-up assessors

Throughout the trial, follow-up was carried out wherever possible by blinded assessors unaware of treatment allocation. A physiotherapist or assessor who was blind to allocation carried out the 24-week trial follow-up assessment, including the HRET.

Sample size

Sample size

The trial was powered on the work LSI from the HRET at 24 weeks post randomisation and on the ATRS. There were no formal interim analyses of the outcomes planned for the PATH-2 trial. However, the Data and Safety Monitoring Committee (DSMC) agreed to check the sample size assumptions when at least half of the originally planned number of participants had completed the 24-week primary end-point assessment because they were based on the findings of a single previous study conducted in a single centre.[65] This decision was documented in the statistical analysis plan version 1.0.

Original calculation of sample size

It was calculated that 214 participants (107 per treatment group) would provide 90% power to detect a standardised difference of 0.5 in the HRET work measured by the work LSI at 24 weeks post randomisation and with 5% (two-sided) significance allowing for 20% loss to follow-up. This was based on previous data from the non-surgical arm of the 2010 study by Nilsson-Helander *et al.*,[65] in which a clinically important difference of 10 points with a standard deviation (SD) of 20 points was observed.

This sample size was also to provide 90% power and 5% (two-sided) significance to detect a standardised effect size of 0.5 in the ATRS between the two treatment groups, based on a difference of 11% and a SD of 21.4%.

Sample size review and recalculation

The DSMC agreed at their first meeting that the assumptions for the sample size should be reviewed after approximately half of the participants had been recruited. This review would be a blinded estimate of work LSI variability only. The first variability analysis was undertaken in October 2016 when half of the participants had been recruited ($n = 104$). It was observed that the SD of the work LSI was smaller than that used in the sample size calculation so no changes to the sample size were recommended (observed SD 17.37, versus sample size SD 20). However, at this time only 27 participants had completed the primary end-point assessment.

As of June 2017, approximately half of the participants had reached their primary end point and recruitment was still ongoing. Therefore, to provide a more robust estimate of the actual variability of the work LSI,

a further sample size assumption review was carried out. Using cleaned and validated data from 75 participants, the observed SD was 24, which was higher than that used in the sample size calculation. Based on this observed SD of 24 (being aware that this could go up or down with the addition of further participant data), we recalculated the sample size based on 80% power, which, allowing for 20% loss to follow-up, required a sample size of 226 patients. The DSMC advised the Trial Steering Committee (TSC) and trial management team during this process.

The trial continued to recruit to an agreed minimum of 230 participants (overshooting to account for further minimal fluctuations in sample size assumptions), to ensure that there would be a minimum of 80% power for the primary outcome analysis. These approaches, and the reduction in trial power, were supported by the TSC and by the funder.

Statistical methods

Software employed

All analyses outlined here and reported in *Chapter 4* were undertaken using Stata® version 15.0 (StataCorp LP, College Station, TX, USA).

Blinded analysis

Initial exploratory analyses were conducted on a blinded data set (not separated by treatment group) to identify the presence of missing values and to clarify the distribution of continuous variables. These analyses were also used to finalise trial populations and to aid in the identification of key prognostic variables to be included in the adjusted analysis. All subsequent analyses described were conducted on an unblinded data set. Following this blinded analysis, the statistical analysis plan was updated to incorporate any changes.

Data validation

Initial analytical steps assessed the validity of the final, blinded data set. The first of these steps was to manually check, when possible, the use of Stata for importing data and merging data sets for at least 20 participants who were randomly sampled. Once accurate importation was confirmed, data in the data set were validated by checking for duplicate records, checking the values of the range of variables and validating potential outliers against CRFs. Any discrepancies that could not be rectified were referred back to the trial sites. Finally, the production of calculated variables using Stata was manually checked for at least 20 participants who were randomly sampled.

Trial populations

Intention-to-treat population

This required the inclusion of all randomised participants, to be analysed in the groups to which they were allocated. For the primary outcome, analysis included a modified intention-to-treat (mITT) population, defined as all randomised intention-to-treat (ITT) participants with available work LSI data (i.e. at least one valid repetition for each lower limb after HRET data validation by the blinded reviewers of the assessment video files). For the secondary outcome, analysis also included a mITT population, defined as all randomised participants who completed their 24-week follow-up questionnaires, analysed in the groups they were allocated to.

Complier-average causal effect population

Complier-average causal effect (CACE) is an analytic approach that provides a robust estimate of the treatment effect among compliant participants.[71,72] Participants who received a 'poor-quality' PRP injection (e.g. received PRP with concentrations of platelets lower than the concentration in their whole blood or did not receive the PRP) in the PRP group were classified as not having received their allocated treatment.

Descriptive analysis

All participants were included in descriptive analyses. Recruitment into the trial was explored, including the numbers of individuals assessed, recruited, randomly assigned to the PRP injection or placebo, receiving treatment, completing the trial protocol and analysed for the primary outcome. Any protocol deviations and violations were investigated and issues with the screening data, including potential incidents of ineligible patients being randomised, were explored. The proportion of unblinded assessors for each assessment was assessed, and the James *et al.*[69] and Bang *et al.*[70] indices were calculated to determine whether or not participants were successfully blinded to the intervention they received.

The baseline comparability of participant-level data for each of the treatment groups was summarised. For baseline and follow-up data collected manually through the use of forms or questionnaires, data compliance was explored; all available data collected from these forms were summarised and the proportion of missing items from completed questionnaires was examined. Data-availability patterns for individual variables were assessed both overall and for the two treatment groups separately to explore missing information. Missing values were checked for consistency and the proportion of missing values per variable was assessed. Differentiations were made between partially completed and fully missing outcome data. For measures comprising multiple items, for example SF-12, pro-rata estimation of total and subscale scores was employed for each treatment group, using the appropriate scale-specific scoring guidelines. Imputation of data was utilised for sensitivity checking and is described in further detail in *Primary outcome*. Imputation was carried out as recommended for each relevant questionnaire, with the imputation value determined by the distribution of the underlying data. Methods utilised for imputation were assessed manually, when possible, for at least 20 participants who were randomly sampled. Sensitivity analyses were conducted to ensure that the missing-at-random assumption for imputation was met.

Comparisons of losses to follow-up were carried out. The proportions of participants defaulting or withdrawing from the trial over the whole period of trial follow-up, and at each analysis time point, were compared between the PRP and placebo groups. The importance of differences identified were assessed using a chi-squared test. For all analyses, a p-value of < 0.05 was considered indicative of a significant difference.

Quality assurance and compliance with the intervention, including any deviations from protocols prior to and during the trial, were assessed. Treatment received was summarised by treatment groups, and time frames for the steps with blood processing were explored to ensure that all participants received their PRP injection within 2 hours of their blood sample being taken. The grade of the health-care professional conducting the procedure was summarised. The importance of apparent differences in compliances was assessed using a chi-squared test.

Finally, data quality and the effect of the treatment received were assessed using CACE analysis in place of the originally planned per-protocol (PP) analysis, as this was deemed the optimal approach. As per the PP analysis, CACE analysis allows for adjustment to account for participants whose received treatment either complied or did not comply with that allocated to them; however, CACE analysis does so without assuming that compliers are the same as non-compliers, as would be the case with a PP analysis. CACE analysis retains information on the original treatment allocation that participants received, which allows us to forgo the core assumption of a PP analysis – that receipt of treatment is random with respect to the outcome predictors – and to enhance our understanding of the effect of PRP on ATR recovery. The decision to use CACE was taken post finalisation of the statistical analysis plan but ahead of the end of follow-up and, therefore, ahead of unblinding.

For the CACE analysis, compliance was defined as participants who received the intervention allocated to them who had sufficient platelets present in their PRP sample to classify it as platelet rich (i.e. higher platelet concentration in PRP than in whole blood). The results from the CACE analysis were reported with standard errors, p-values and 95% CIs, and assessments were made to ensure that the primary outcome estimation was captured within this range.

Standard care for ATR for all trial participants was unaffected by this trial. All participants should therefore have had their injured lower limb immobilised, received a referral for physiotherapy, received delivery of venous thromboembolism prophylaxis and received advice about when to start weight-bearing and ankle-motion exercises. Compliance with these different aspects of standard care was compared between treatment groups using a chi-squared test for categorical data and a Student's *t*-test for continuous data.

Analysis of the primary outcome

The primary outcome for this trial was muscle–tendon function, measured by the LSI of maximum work, performed during the HRET (see *Primary outcome* for more detail). The work LSI was calculated for all trial participants, at 24 weeks (varying by –2 to +8 weeks) post randomisation, as follows:

$$\text{LSI} = \frac{\text{Injured limb HRET measurement}}{\text{Uninjured limb HRET measurement}} \times 100. \quad (1)$$

The proportions of individuals missing data for outcome and explanatory variables were explored, and work LSI data for all participants were assessed for normality. Missing HRET data were defined and handled as follows:

- Participants with true missing data – participants who were lost to follow-up and participants for whom technical errors were experienced during HRETs. The participants who were lost to follow-up were any participants who either did not attend their 24-week follow-up at all or did not remain at their appointment to complete their HRET. The measurements for these participants were kept as missing.
- Participants with true zero measurements – participants who attempted to complete their HRET assessment but their attempts were insufficient for the encoder to record any results. Zero measurements for these participants were included for analysis.
- Participants with potential zero measurements – participants who did not attempt to complete their HRET in at least one leg, despite attending the follow-up appointment. Zero measurements for these participants were included for analysis.

The data followed a near-normal distribution, with a small elevation around zero due to participants defined as having true and potential zero measurements. However, work LSI data did not require transformation prior to inclusion in the final model as post-estimation assessments indicated that the distribution was suitable for the regression technique employed. The impact of zero inflation of the primary outcome measure was assessed using a two-parts model to (1) identify differences between participants with positive work LSI results and participants with zero measures, and (2) identify the impact of the inclusion of participants with zero measures on the primary outcome results. Mean work LSI differences, robust standard errors, *p*-values and 95% CIs were reported and compared with the core primary outcome results for deviation or interpretation changes.

An unpaired Student's *t*-test was used to explore the unadjusted effects of PRP compared with those of placebo.

Multivariate linear regression was used to investigate the effect of PRP on ATR recovery. The base-unadjusted regression model was built using work LSI as the dependent variable and treatment as the key independent variable. The principal analysis model built on this model by adjusting for participant age group and clustering by trial site. Supplementary regression models were built, which adjusted for the effects of sex, body mass index (BMI) and smoking status.

Sensitivity analyses were carried out using imputation of values for missing HRET data to examine the robustness of the conclusions made from the analyses to address the primary aims. Missing HRET data were handled using two approaches. The first was to employ simple imputation of the average concentric displacement (upwards movement); this method was used for missing data associated with specific repetitions of heel rise. The second method to handle missing data was to employ multiple imputation

by chained equations; this was used to calculate work LSI for participants with data missing for entire assessments. The multiple imputation using chained equations procedure created a series of complete data sets (observed plus imputed data) in which analyses were carried out individually. The regression parameter estimates plus corresponding standard errors obtained from the analyses of each of the 50 imputed data sets were then combined using the Rubin's rule approach. When models did not converge during the multiple imputation process, some predictor preselection was carried out based on clinical expertise and subject matter. Sensitivity analyses were employed to assess the assumptions made in preselecting these predictor variables. The method utilised for multiple imputation inclusion was to use the stratification variables already in the principal adjusted model and to further account for any variables with an R^2 value of ≥ 0.2 following correlation assessments. This resulted in three additional variables being selected: whether or not the participant jogged or ran, undertook weight training or participated in squash before their injury.

For all analyses, a two-sided *p*-value of 0.05 (5% significance level) was used to indicate evidence of statistical significance.

Analysis of secondary outcomes

The number of repetitions and the maximum heel-rise height recorded during the HRET assessment were explored as secondary outcome measures. Furthermore, four PROMs [pain as measured by the VAS (daily over the 14 days immediately post treatment), ATRS, PSFS and the SF-12 mental and physical health scores] were explored.

The ITT population was used for secondary outcome analyses.

Repeated-measures linear mixed-effects regression models were used to allow the data collected at all follow-up time points to be taken into account. Time elapsed from the intervention to the outcome measurements was included in the models as a random effect factor, considering that not all participants had their follow-up assessments at exactly the same time. Mean differences and 95% CIs were examined. All analyses were adjusted for the stratification factors included in the adjusted analysis of the primary outcome. As pre-injury scores had been collected for the SF-12 data, the models for this PROM were built so that the principal and supplementary analyses adjusted for these scores.

Sensitivity analyses were carried out on the overall ATRS data sets as this was the key secondary outcome. The first of these analyses used the mITT population used in the primary outcome analysis to assess the impact of selecting the mITT population used in the primary outcome analysis. The second applied multiple imputation by chained equations for those participants with data missing with missing ATRSs. The multiple imputation by chained equations technique that was applied to the ATRSs was the same as for the primary outcome, although the additional variables selected for inclusion to inform the imputation were different. The six additional variables used to build the imputation data sets in this situation were BMI as a continuous variable, the date of the ATR, the date when the treatment was received, the date when a participant started carrying out rehabilitation exercises (as recorded at week 13), whether or not a participant was jogging or running by week 24 and whether or not a participant was carrying out 'do it yourself' (DIY), heavy housework or gardening by week 24.

As with the primary outcome analysis, data quality was assessed using CACE on the overall ATRS data, to examine the robustness of the conclusions made from the analyses.

Adverse events

The number of participants experiencing one or more AE of any type over the 24 weeks was explored. The number of participants experiencing each specific type of AE over the 24 weeks was also analysed. The number of separate complication events was not explored owing to the opportunity for re-reporting by participants across the different follow-up time points and the nature of the events of interest.

Patient and public involvement

The Efficacy and Mechanism Evaluation programme recognises the value of active involvement of public contributors in research. In our patient and public involvement (PPI) work, we consulted trauma injury patients at John Radcliffe Hospital. Our aim was to ensure that:

- interventions were acceptable to patients
- follow-up procedures reduced the patient burden while obtaining meaningful scientific data
- patient information sheets and questionnaires were easy to read and user-friendly.

The protocol[73] was designed following a small-scale pilot study with the help of Achilles tendon injury patients at the John Radcliffe Hospital and the Oxford Trauma User Group (OTUG), which is composed of patients who have experienced the John Radcliffe Hospital orthopaedic trauma service and who volunteer to engage on clinical and research issues. Recommendations of OTUG members were incorporated in the original research proposal.

A questionnaire was sent to the OTUG members to explore opinions regarding the trial aims and procedures; 75 out of 105 replied to the survey. All responders indicated that the project might benefit patients with ATR. Ninety per cent were happy with the trial design; some felt that the number of follow-up contacts could be inconvenient. Taking that feedback, and experience of the pilot study,[74] into account, follow-up procedures were refined to reduce the number of follow-up contacts, thereby reducing participant burden.

Participant satisfaction with the intervention was explored in the pilot study. All 20 patients tolerated the injection well and no side effects were reported. The use of needle biopsy under local anaesthetic, as in substudy 2, was also reported during the pilot study to be acceptable.

A patient representative joined the TSC as a PPI representative to contribute to management of the trial. One of the co-applicants acted as a mentor to the PPI representative, being available by telephone before and after the meetings to help with interpretation of trial briefing documents. This provided an opportunity for the PPI representative to raise issues prior to meetings, which she did, and if necessary the mentor could raise issues on her behalf. All committee members were requested to use plain English in meetings.

The PPI representative was also actively involved in designing the approach to patients and the text of patient-facing materials (questionnaire and letters) and for the 2-year extended follow-up; the latter is ongoing and not reported as part of this trial.

Members of the trial management team attended the INVOLVE conference and PPI research meetings.

Outcomes for patient and public involvement

In the main trial, participants reported satisfaction at the end of the trial in a post-assessment questionnaire. We asked participants to rate their satisfaction with the trial on a five-point scale of 'very dissatisfied' to 'very satisfied'. Eighty-nine per cent of participants declared themselves 'satisfied' or 'very satisfied'. We invited comments on what could have improved their experience; the vast majority who answered ($n = 37$) said 'nothing' and none asked for fewer forms, although five said that some questions were not relevant and three asked for more information.

An adjustment to the statistical analysis related to how the pain score would be reported is an example of how feedback from our PPI representative improved the conduct of the trial.

The PPI representative reviewed a graphical information slide to be used in future public-facing presentations.

Overall, from participant feedback, we believe that our PPI involvement improved trial conduct and participant experience.

Ethics approval and monitoring

Ethics approval

The trial was given a favourable opinion by the South Central – Oxford A Research Ethics Committee on 11 November 2014 (reference number 14/SC/1333) and each site was granted site-specific approval from its NHS trust research and development department before trial commencement.

Data and Safety Monitoring Committee

The DSMC was established to safeguard the interests of trial participants, monitor the main outcome measures including safety and efficacy, and monitor data quality and completeness. In accordance with the DSMC charter, the DSMC received and reviewed information on the progress and data collection of the trial and advised the TSC on the conduct of the trial.

Trial Steering Committee

The TSC provided expert oversight of the trial on behalf of the sponsor and funder. Through its independent chairperson, the TSC also provided advice to the Trial Management Group (TMG), the funder and OCTRU on all aspects of the trial.

Trial Management Group

The day-to-day management of the trial was overseen by a TMG comprising the chief investigator, the lead research nurse at the lead site (John Radcliffe Hospital), the co-applicants, the trial statistician, research physiotherapists and the trial manager.

Trial registration

This trial is registered with the International Standard Randomised Controlled Trial Number (ISRCTN) Registry (ISRCTN54992179) and ClinicalTrials.gov (NCT02302664).

Summary of changes to the trial protocol

The changes to the project protocol are summarised in *Table 1*.

TABLE 1 Changes to the protocol during the trial by version number

Protocol version	Date issued	Details of changes made
1.0	10 September 2014	Not applicable as this was the first issue
2.0	12 February 2015	• Reference to 'Blood Sample Handling Manual' removed. Reader directed to the trial intervention and blood sample training materials • Registration details added (ISRCTN/ClinicalTrials.gov)
3.0	8 March 2016	• Addition of extended-scope physiotherapists, to reflect current NHS practice in some trusts, to assess and treat ATRs and to conduct injections under the supervision of the orthopaedic surgeon • Changes to eligibility criteria: patient is eligible if within 12 days, not 7 days, of injury; upper age limit removed • Clarified some aspects of eligibility criteria: location of acute Achilles tendon injury clarified; use of anticoagulant clarified • Change to randomisation process to correct error in randomisation system: from randomisation with stratification by strata (centre and age group) to using minimisation, a dynamic computer-generated allocation system based on the two strata. The change to randomisation was required because of imbalance in participants' age group stratum following a systems issue. The underlying systems issue was fixed and a change to the randomisation strategy was implemented to avoid this imbalance being preserved throughout the trial. The randomisations allocated prior to the change were not altered. This approach was reviewed and approved by the sponsor, DSMC, TSC and the ethics committee • Collection of current medications data at baseline • Inclusion in protocol of questions asked at 24 weeks on participant's experience of trial • Clarification on excess treatment costs for trial-specific consumables • DVT and re-rupture changed from 'unforeseeable' to 'foreseeable' AEs
4.0	8 March 2016	Removal of 'draft' from filenames, removal of draft watermark and removal of tracked changes from documents for version 3.0
5.0	21 April 2017	• Inclusion of 2-year extended follow-up • Dates changed to include 9-month extension, as agreed with the funder
6.0	24 July 2017	• Recruitment target changed from 214 to 230 after blinded review of variability in primary outcome data to date • Dates changed to include 2-month extension, as agreed with funder

Chapter 3 Clinical trial results

Trial participants

A total of 230 eligible participants were recruited from July 2015 to September 2017 from 19 NHS hospitals across England and Wales. Participants attended clinic visits at the time of randomisation (baseline) and at 24 weeks post intervention, with the last 24-week visit taking place in March 2018. Participants were also contacted by the trial team via telephone to complete follow-up questionnaires at 4, 7 and 13 weeks post intervention. The trial ended when the recruitment target of 230 was reached.

One participant withdrew from the trial. This participant withdrew following randomisation but prior to receiving any intervention. We therefore do not have baseline or follow-up data relating to this participant. The reason given for withdrawal was that the participant had decided that they would prefer a surgical intervention for their ATR. This participant had been randomised to the PRP injection group.

The number of participants at each stage of the PATH-2 trial, from assessment for eligibility through to analysis, is presented in the Consolidated Standards of Reporting Trials (CONSORT) flow diagram in *Figure 4*.

Comparisons of losses to follow-up

A total of 27 participants (27/229, 11.8%) were lost to follow-up by the point of completing their HRET assessment; these participants therefore did not contribute to the primary outcome analysis. Of these participants, 24 (24/229, 10.5%) were true losses to follow-up, with 12 in the placebo group and 12 in the PRP group. Of the 24 participants who failed to complete their HRET assessment:

- Eleven participants (11/24, 45.8%) completed all follow-up questionnaires up to and including their 24-week questionnaire but failed to remain to complete the assessment.
- Two participants (2/24, 8.3%) completed their 4-, 7- and 13-week follow-up questionnaires but not their 24-week questionnaire.
- Four participants (4/24, 16.7%) completed their 4- and 7-week follow-up questionnaires but did not complete their 13- and 24-week questionnaires.
- Two participants (2/24, 8.3%) completed only their 4-week follow-up questionnaires.
- One participant (1/24, 4.2%) completed only their 24-week questionnaire.
- Four participants (4/24, 16.7%) completed no follow-up past the baseline questionnaire.

These participants were spread across 10 sites and there were no discernible differences between them in relation to their age, sex, BMI or smoking status.

In addition to these 24 participants, there were three participants (3/229, 1.3%) who were missing data for their HRET assessments following a technical error. These participants therefore do not contribute to the primary outcome analysis. However, it is worth noting that, although one of these participants did not complete their 13-week follow-up questionnaire, all other questionnaires were completed.

There were no differences between treatment groups in whether or not a participant was lost to follow-up, irrespective of whether participants who experienced a technical error were included in this cohort [PRP injection, 12/113 (10.6%), vs. placebo, 15/116 (12.9%), difference –2.3%; $p = 0.588$] or not [PRP injection, 12/113 (10.6%), vs. placebo, 12/116 (10.3%), difference 0.3%; $p = 1.000$].

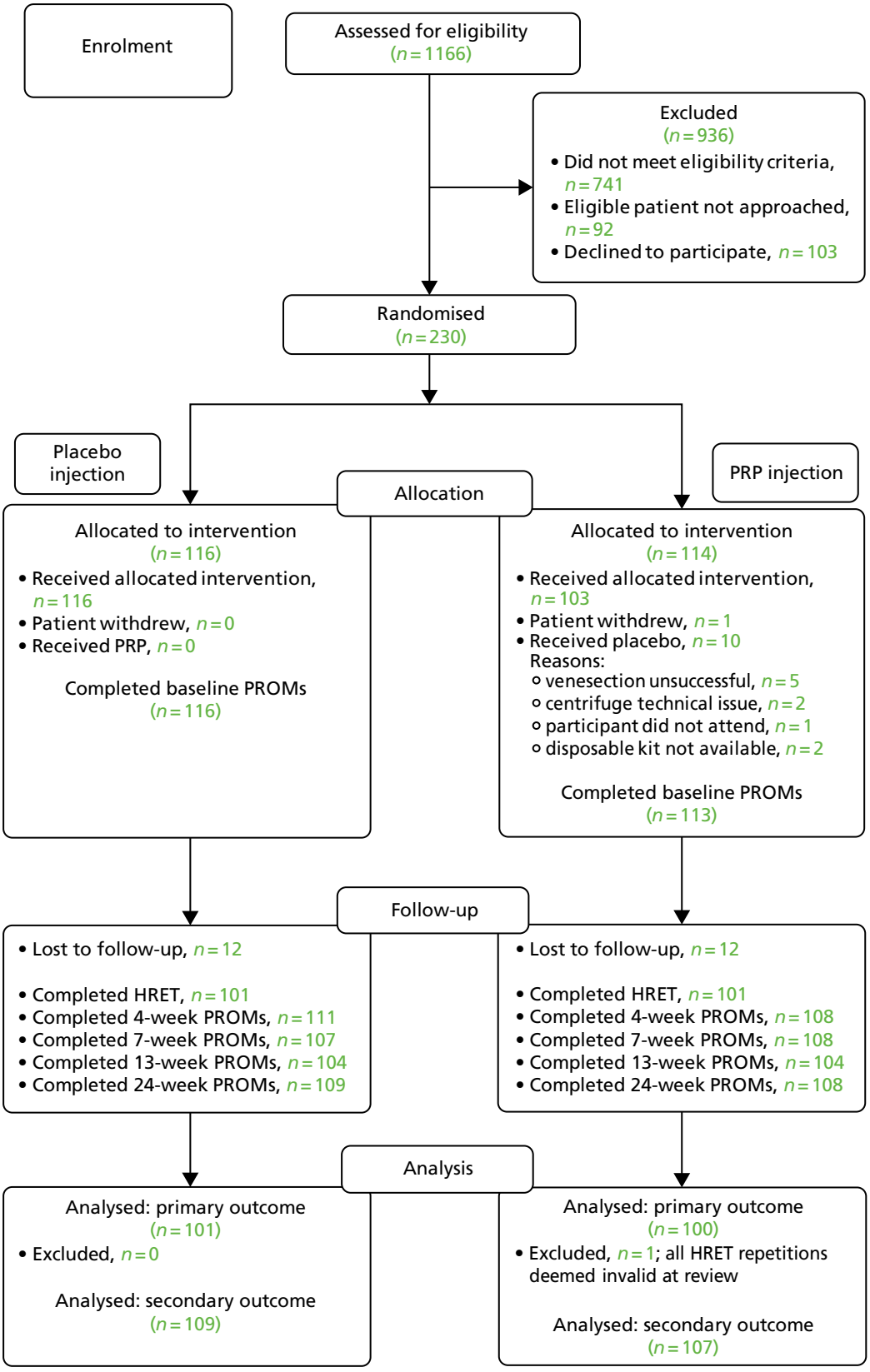

FIGURE 4 The PATH-2 trial CONSORT flow diagram.

Trial populations

A total of 229 participants are included in the descriptive statistics, 113 of whom were in the PRP group and 116 of whom were in the placebo group.

Intention-to-treat population

- Primary outcome (work LSI): a total of 201 participants were included, 100 of whom were in the PRP group and 101 of whom were in the placebo group.
- Key secondary outcome (ATRS): a total of 216 participants were included, 107 of whom were in the PRP group and 109 of whom were in the placebo group.

Compliance

Baseline, randomisation and treatment delivery forms

There were no differences between treatment groups in compliance with baseline, randomisation and treatment delivery forms. Completion rates for all of these forms were 100% for both treatment groups.

Follow-up diaries and questionnaires

There were no differences between treatment groups in compliance with follow-up questionnaires and diaries. A total of 180 out of 229 participants (78.6%) returned their pain diary for the first 2 weeks post intervention. Completion of all follow-up questionnaires at weeks 4, 7, 13 and 24 was $\geq$ 90% in both intervention groups.

Treatment allocation compliance

Nine per cent (10/113) of PRP group participants did not receive their allocated treatment, whereas all placebo group participants received the intervention assigned to them. Treatment for participants in the placebo group was dependent on the availability of the trial kit for this intervention. However, treatment for participants in the PRP group was further dependent on the ability to withdraw sufficient whole blood to produce the required 8-ml PRP sample. Therefore, it is not unexpected that a higher number of participants in the PRP group did not receive their allocated treatment.

The reasons for participants in the PRP group not receiving their allocated treatment were:

- site staff being unable to take a blood sample from the participant (3/10, 30.0%)
- site staff not being able to take a blood sample of sufficient volume to produce PRP (2/10, 20.0%)
- technical issues encountered with centrifuge (2/10, 20.0%)
- participant not remaining on site to receive treatment (1/10, 10.0%)
- in-date PRP disposable kit not available (1/10, 10.0%)
- site staff unable to obtain PRP disposable kit (1/10, 10.0%).

In all PRP injection participants who were able to have blood samples drawn (103/113, 91.15%), the mean time from taking the blood sample to their PRP injection was 42 minutes (range 12–105 minutes). This breaks down to a mean of 28 minutes from taking the blood sample to producing the PRP sample (range 1–80 minutes) and a mean of 14 minutes from producing the PRP sample to receiving the injection (range 0–45 minutes). All PRP intervention participants who were able to have blood samples drawn received their injection within the expected 2-hour time frame.

Grade of the clinician administering the treatment

A total of 75.5% of participants [total, 173/229; PRP group, 86/113 (76.1%), vs. placebo group, 87/116 (75.0%)] had their treatment administered by a consultant surgeon; 19.2% had their treatment administered by a surgical registrar or research fellow [total, 44/229; PRP group, 19/113 (16.8%),

vs. placebo group, 25/116 (21.5%)], 1.3% had it administered by an extended-scope physiotherapist [total, 3/229; PRP group, 1/113 (0.9%), vs. placebo group, 2/116 (1.7%)] and 1.7% had it administered by any other grade of physiotherapist [total, 4/229; PRP group, 2/113 (1.8%), vs. placebo group, 2/116 (1.7%)]. Although we do not have any information for 2.2% of participants (5/229), there were no discernible differences between groups in relation to the grade of clinician administering the treatment ($p = 0.706$).

Compliance with standard care

Compliance with standard care is reported in *Table 2*. There were no discernible differences between treatment groups in compliance or in the use of venous thromboembolism prophylaxis [PRP group, 56/113 (49.5%), vs. placebo group, 58/116 (50.0%), difference –0.4%, $p = 0.891$]; the use of a below-knee cast [PRP group, 47/113 (41.6%), vs. placebo group, 55/116 (47.4%)] or splint/brace [PRP group, 64/113 (56.6%), vs. placebo group, 61/116 (52.6%)] to immobilise the ankle ($p = 0.443$); or in the receipt of a physiotherapy consultation within 7 weeks [PRP group, 64/113 (56.6%), vs. placebo group, 68/116 (58.6%)] or 13 weeks [PRP group, 100/113 (88.5%), vs. placebo group, 100/116 (86.2%)] ($p = 1.000$).

The date a participant started weight-bearing was recorded at the 4-week and 7-week follow-ups, and the date they started ankle-motion exercises was recorded at 7 and 13 weeks. It was assumed that participants remembered the date they were weight-bearing or exercising their ankle better if the time point was closer to it. The time to start weight-bearing and the time to start ankle exercises was calculated as the date reported minus the date the ATR occurred. The date reported was determined to be the earliest date reported by the participant that this activity took place. To allow for the fact that participants may have stopped either weight-bearing or exercising their ankle between questionnaires, participant binary responses as to whether or not they were carrying out an activity were taken into account, and if participants answered 'yes' in the earlier questionnaire and 'no' in the later questionnaire, their reported date was not included.

TABLE 2 Summary of treatment received, by intervention group

	Intervention group		
Treatment received	PRP ($N = 113$)	Placebo ($N = 116$)	Total ($N = 229$)
Venous thromboembolism prophylaxis use, *n* (%)			
No	54 (47.79)	58 (50.00)	112 (48.91)
Yes	56 (49.56)	58 (50.00)	114 (49.78)
Missing	3 (2.65)	0 (0)	3 (1.31)
Method of ankle immobilisation, *n* (%)			
Below-knee cast	47 (41.59)	55 (47.41)	102 (44.54)
Splint/brace	64 (56.64)	61 (52.59)	125 (54.59)
Missing	2 (1.77)	0 (0)	2 (0.87)
Physiotherapy consultation received, *n* (%)			
Within 7 weeks of injury	64 (56.64)	68 (58.62)	132 (57.64)
Within 13 weeks of injury	100 (88.50)	100 (86.21)	200 (87.34)
None reported by 13 weeks	4 (3.54)	4 (3.45)	8 (3.49)
Missing at 13 weeks	9 (7.96)	12 (10.34)	21 (9.17)
Time to starting weight-bearing (days), *n*; mean (SD)	96; 27.04 (24.12)	93; 27.70 (16.10)	189; 27.36 (15.24)
Time to starting ankle-motion exercises (days), *n*; mean (SD)	104; 47.40 (19.57)	103; 48.48 (19.97)	207; 47.94 (19.73)

Following from the creation of the described time to weight-bearing and time to ankle-exercising variables, assessments of their distributions demonstrated that they did not significantly differ from normal. Therefore, differences in time taken to complete these tasks between treatment groups were assessed using Student's *t*-test; there were no differences in the time to weight-bearing between the PRP group (mean 27.0 days, 95% CI 24.1 to 30.0 days) and the placebo group (mean 27.7 days, 95% CI 24.4 to 31.0 days) (PRP group vs. placebo group mean difference –0.7 days, 95% CI –3.7 to 5.0 days; $p = 0.768$) or in the time taken to start ankle-motion exercises between the PRP group (mean 47.4 days, 95% CI 43.6 to 51.1 days) and placebo group (mean 48.5 days, 95% CI 44.7 to 52.4 days) (PRP group vs. placebo group mean difference –1.1 days, 95% CI –4.2 to 6.5 days; $p = 0.672$).

Descriptive analyses

The baseline characteristics of participants according to their intervention group are presented in *Table 3*. Participants in both intervention groups are remarkably similar in all characteristics, with no observable between-group differences.

Information on the stratification factors (age group and trial site) and other prognostic factors of interest by intervention group are presented in *Table 4*. This table demonstrates that there are clear differences between the levels of the subgroups; for example, more participants in this trial were aged < 55 years than ≥ 55 years and there were notably more male participants than female participants in this trial. However, there are no noteworthy differences between the intervention groups.

On exploration, it was found that only around 8.0% (13/163) of participants questioned were regularly taking medications that could have an impact on their platelet function, with similar low proportions of participants taking such medications in each intervention group [PRP group, 8/86 (9.3%)a, vs. placebo group, 5/91 (5.5%)].

Pain levels at their injection site as reported by participants – relating to the need for more than simple pain relief – were explored. Only 2.6% of participants (6/229) reported experiencing severe pain at their injection site, with too-small numbers of participants to detect any difference between the PRP group (4/113, 3.5%) and the placebo group (2/116, 1.7%). For more details, see *Appendix 3*.

The academic and employment characteristics of participants prior to their injuries were explored. These explorations related to participants' employment or academic status, the type of work undertaken and the time participants spent on their feet or driving. There were no differences between the two intervention groups in relation to any of the academic or employment-related characteristics investigated. For more information, see *Appendix 3*.

TABLE 3 Baseline characteristics of participants, by intervention group

	Intervention group								
	PRP ($N = 113$)			Placebo ($N = 116$)			Total ($N = 229$)		
Baseline characteristics	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
BMI (kg/m^2)[a]	113	27.69	5.29	114	27.25	4.22	227	27.47	4.78
Age (years)	113	45.90	13.74	116	45.16	12.43	229	45.53	13.07
Alcohol consumption (units)	113	9.90	11.33	116	10.47	10.37	229	10.19	10.83
Days since injury	113	5.35	2.95	116	5.20	3.08	229	5.27	3.01

a Two participants who were missing baseline measurements of weight had measurements for this variable taken at later follow-up dates that were used to calculate BMI for the advanced analyses, to prevent exclusion.

TABLE 4 Stratification factors and sociodemographic characteristics of participants, by intervention group, as used in adjusted analyses

Participant characteristics	Intervention group, *n* (%)		
	PRP (*N* = 113)	Placebo (*N* = 116)	Total (*N* = 229), *n* (%)
Centre[a,b]			
John Radcliffe Hospital, Oxford	21 (18.58)	22 (18.97)	43 (18.78)
Musgrove Park Hospital, Taunton	9 (7.96)	8 (6.90)	17 (7.42)
Southmead Hospital, Bristol	7 (6.19)	9 (7.76)	16 (6.99)
Llandough Hospital, Cardiff	0 (0)	1 (0.86)	1 (0.44)
Royal London Hospital	7 (6.19)	8 (6.90)	15 (6.55)
Leicester Royal Infirmary	22 (19.47)	21 (18.10)	43 (18.78)
University Hospital Coventry	5 (4.42)	5 (4.31)	10 (4.37)
Warrington Hospital	3 (2.65)	2 (1.72)	5 (2.18)
Basildon University Hospital	9 (7.96)	9 (7.76)	18 (7.86)
Royal Liverpool Hospital	6 (5.31)	7 (6.03)	13 (5.68)
Peterborough City Hospital	6 (5.31)	5 (4.31)	11 (4.80)
Morriston Hospital, Swansea	3 (2.65)	4 (3.45)	7 (3.06)
University Hospital Aintree	2 (1.77)	1 (0.86)	3 (1.31)
Wythenshawe Hospital, Manchester	3 (2.65)	5 (4.31)	8 (3.49)
Northern General Hospital, Sheffield	3 (2.65)	4 (3.45)	7 (3.06)
Royal Devon and Exeter Hospital, Exeter	1 (0.88)	1 (0.86)	2 (0.87)
Royal Surrey Hospital, Guildford	1 (0.88)	1 (0.86)	2 (0.87)
Leighton Hospital, Crewe	5 (4.42)	3 (2.59)	8 (3.49)
Age (years)[a,b]			
< 55	86 (76.11)	88 (75.86)	174 (75.98)
≥ 55	27 (23.89)	28 (24.14)	55 (24.02)
Sex[b]			
Female	25 (22.12)	32 (27.59)	57 (24.89)
Male	88 (77.88)	84 (72.41)	172 (75.11)
Lower limb injured			
Right	49 (43.36)	48 (41.38)	97 (42.36)
Left	64 (56.64)	68 (58.62)	132 (57.64)
BMI (kg/m²)[c]			
Normal weight (18.5–24.99)	40 (35.40)	39 (33.62)	79 (34.50)
Overweight (25–29.99)	43 (38.05)	46 (39.66)	89 (38.86)
Obese (30–39.99)	30 (26.55)	31 (26.72)	61 (26.64)
Smoking status[b]			
Smoker	14 (12.39)	13 (11.21)	27 (11.79)
Ex-smoker	28 (24.78)	38 (32.76)	66 (28.82)
Never smoked	71 (62.83)	65 (56.03)	136 (59.39)

TABLE 4 Stratification factors and sociodemographic characteristics of participants, by intervention group, as used in adjusted analyses (*continued*)

Participant characteristics	Intervention group, *n* (%)		
	PRP (*N* = 113)	Placebo (*N* = 116)	Total (*N* = 229), *n* (%)
Use of antiplatelet medication[d]			
Yes	8 (7.08)	5 (4.31)	13 (5.68)
No	78 (69.03)	86 (73.27)	163 (71.18)
Not asked[e]	27 (23.89)	26 (22.42)	53 (23.14)

a Used as a stratification factor in adjusted analyses.
b Included in fully adjusted analyses.
c Included in fully adjusted analyses as a continuous variable.
d Medications taken: apixaban, aspirin, clexane, clopidogrel, dalteparin, dipyridamole, fragmin and pradaxa.
e Participants who had their baseline questionnaire administered before antiplatelet medication use was included in the form.

The activity levels of participants before they experienced their rupture and the activity being undertaken when the rupture occurred were explored (*Table 5*). The majority of participants (157/229, 68.6%) injured themselves during sport. As with previous baseline variables, there were no clear differences between intervention groups in relation to their pre-injury activity levels or the activity being undertaken when the injury occurred.

Analyses to address primary aims

Missing data assessments

Missing data explorations were carried out to maximise inclusion in the final analysis. The final classification of participants with missing data following these explorations is given in *Table 6*. (For information on how missing data were classified, see *Chapter 2*, *Analysis of the primary outcome*.)

Primary analysis outputs

There were three data sets available for the analysis of the primary outcome:

1. all available HRET assessments
2. all available HRET assessments that were deemed valid on review of video footage (see *Chapter 2*, *Primary outcome*, for more information on the validation review)
3. all available HRET assessments that were deemed valid on review and included only participants with assessments available in both legs.

The primary outcome analysis focuses on data set 3 (all valid HRETs available on both legs).

Explorations of the mean work exhibited during the HRET assessment in participants' injured and uninjured legs on all three data sets were carried out. Basic explorations of the subsequent measure for work LSI in participants with measurements in both legs were undertaken. These explorations demonstrated that the mean work LSI and subsequent HRET measurements for both intervention groups were very similar.

Focusing on the third data set, the mean work LSI in the injured leg was lower in both the PRP group (675.2, SD. 622.3) and the placebo group (748.2, SD 630.3) than the mean work LSI in the uninjured legs in the PRP (1783.8, SD 838.8) and placebo (1825.5, SD 796.2) groups. Comparing the subsequent work LSI measurement in the PRP group (mean 34.9) with the work LSI measurement in the placebo group (mean 38.3) using the Student's *t*-test demonstrated no difference between these two groups (unadjusted PRP group vs. placebo group difference –3.467, 95% CI –10.979 to 4.246; $p = 0.384$). These explorations demonstrate that the work LSI measurements for both groups are very similar.

TABLE 5 Physical activity of participants before and during their injury, by intervention group

Activity type	Intervention group, *n* (%)		Total (*N* = 229), *n* (%)
	PRP (*N* = 113)	Placebo (*N* = 116)	
Pre-injury activities			
Fitness[a]			
More than once/week	100 (88.50)	105 (90.52)	205 (89.52)
Less than once/week	10 (8.85)	10 (8.62)	20 (8.73)
Never	3 (2.65)	1 (0.86)	4 (1.75)
Ball sports[b]			
More than once/week	24 (21.24)	25 (21.55)	49 (21.40)
Less than once/week	22 (19.47)	21 (18.10)	43 (18.78)
Never	67 (59.29)	70 (60.34)	137 (59.83)
Racquet sports[c]			
More than once/week	8 (7.08)	15 (12.93)	23 (10.04)
Less than once/week	21 (18.58)	20 (17.24)	41 (17.90)
Never	84 (74.34)	81 (69.83)	165 (72.05)
Non-sporting activity[d]			
More than once/week	65 (57.52)	67 (57.76)	132 (57.64)
Less than once/week	28 (24.78)	34 (29.31)	62 (27.07)
Never	20 (17.70)	15 (12.93)	35 (15.28)
Mechanism of injury			
During sports	81 (71.68)	76 (65.52)	157 (68.56)
Heavy DIY, housework, gardening, etc.	1 (0.88)	3 (2.59)	4 (1.75)
Other	31 (27.43)	37 (31.90)	68 (29.69)

DIY, do it yourself.
a Fitness activities = cycling, jogging/running, walking, weight training, aerobics/keep-fit and athletics.
b Ball sports activities = football, rugby, hockey and netball.
c Racquet sports activities = tennis, squash and badminton.
d Non-sporting activities = heavy DIY, housework, gardening and other activities not included above.

TABLE 6 Classifications of participants with missing HRET data, by intervention group

Missing data classification	Intervention group, *n* (%)		Total, *n* (%)
	PRP	Placebo	
True missing data	12 (10.62)	15 (12.93)	27 (11.79)
Loss to follow-up	12 (10.62)	12 (10.34)	24 (10.48)
Technical errors	0 (0)	3 (2.59)	3 (1.31)
Participants with true zero measurements	9 (7.96)	11 (9.48)	20 (8.73)
Participants with potential zero measurements	4 (3.54)	1 (0.86)	5 (2.18)

Analyses using multivariate linear regression adjusting for stratification factors (adjusted analysis and principal analysis) and other confounding factors (fully adjusted analysis) were undertaken to assess the robustness of conclusions based on these results (*Table 7*). It is clear from these analyses that there is no evidence of any difference between the intervention groups in relation to work LSI, recorded at 24 weeks post recovery. Taking account of stratification factors and the defined prognostic variables has no impact on the results attained. Sensitivity analyses were also undertaken accounting for participants with zero measurements (two-parts model), with individual missing HRET repetitions (simple imputation) and missing entire HRET data sets (multiple imputation) and for compliance (CACE), and these showed that the results were robust and, therefore, they had no impact on the interpretation of the results.

Post-estimation assessments of the fully adjusted primary outcome regression were carried out to ensure that the results reported by the regression analyses were valid. There were no major issues identified during these assessments and, therefore, the model was kept as simple as possible.

No formal subgroup analyses were carried out; however, results were explored visually using forest plots to examine whether or not the effect of PRP compared with placebo was consistent across the stratification factors (age group and trial site) and other prognostic variables (BMI categories, smoking status and sex) (*Figure 5*). Although the variability was wide for some categories, such as trial site, as was expected owing to the low number of participants randomised in some sites, the treatment effect was consistent across subgroups, showing that the results are robust.

Analysis to address secondary outcomes

Overall Achilles tendon Total Rupture Score

An overall ATRS of 0 is the lowest (i.e. poorest) score a participant can attribute to their recovery and an overall ATRS of 100 is the highest. At baseline, the unadjusted mean overall ATRS in the PRP group was 14.0, compared with 11.7 in the placebo group. By week 24, this had risen to 64.9 in the PRP group, compared with 65.6 in the placebo group (*Table 8*).

A repeated-measures mixed-effects regression analysis accounting for the time elapsed since the intervention was received was undertaken (see *Table 8* and *Figure 6*). As with the primary outcome analysis, sensitivity analyses were conducted to assess the robustness of conclusions based on these results. These analyses found no evidence of any difference between the intervention groups in relation to average overall ATRS recorded throughout the recovery period. Taking account of stratification factors had no impact on the results attained. In addition, accounting for participants missing ATRS data (multiple imputation) and for compliance (CACE) had no impact on the interpretation of the results. These analyses have shown that participants' overall ATRS increased from baseline to 24 weeks post treatment at an almost identical rate in the PRP and the placebo groups, irrespective of adjustment for stratification variables.

Post-estimation assessments of the fully adjusted primary outcome regression were carried out to ensure that the results reported by the regression analyses were valid. There were no major issues identified during these assessments and, therefore, the model was kept as simple as possible.

As before, no formal subgroup analyses were carried out; however, results for the principal secondary outcome (ATRS) were explored visually using forest plots to examine whether or not the effect of PRP compared with placebo differed based on stratification factors (age group and trial site) and other prognostic variables (BMI categories, smoking status and sex) (*Figure 7*).

As for the work LSI, this shows that the treatment effect was consistent across subgroups, showing that the results are robust.

TABLE 7 Primary outcome analysis on subjects with assessments for both legs (valid repetitions only), accounting for participants with true and potential zero measurements, with primary analysis and sensitivity analyses presented

		Intervention group, LSI							
		PRP		Placebo					
Analysis	*n*	Mean	95% CI	Mean	95% CI	Mean difference	Robust standard error	*p*-value	95% CI
Unadjusted[a]									
Primary analysis	201	34.925	29.528 to 40.321	38.292	32.922 to 43.661	–3.367	3.860	0.384	–10.979 to 4.246
Sensitivity: two-parts model	170	42.078	36.786 to 47.371	44.454	39.284 to 49.623	–2.375	3.747	0.527	–9.773 to 5.023
Sensitivity: simple imputation	201	34.930	29.536 to 40.325	38.299	32.931 to 43.667	–3.369	3.859	0.384	–10.979 to 4.241
Sensitivity: MICE	229	34.596	29.132 to 40.060	38.457	33.128 to 43.787	–3.861	3.897	0.323	–11.549 to 3.826
Sensitivity: CACE	201	34.551	28.599 to 40.502	38.292	32.988 to 43.595	–3.741	4.642	0.380	–12.095 to 4.614
Adjusted[b]									
Primary analysis	201	34.671	30.945 to 38.397	38.543	33.753 to 43.333	–3.872	3.120	0.231	–10.454 to 2.710
Sensitivity: two-parts model	170	41.464	37.754 to 45.174	45.040	41.224 to 48.855	–3.576	2.741	0.209	–9.348 to 2.206
Sensitivity: simple imputation	201	34.676	30.946 to 38.407	38.551	33.768 to 43.334	–3.875	3.122	0.231	–10.462 to 2.712
Sensitivity: MICE	229	34.583	30.016 to 39.150	38.526	33.362 to 43.690	–3.943	3.312	0.256	–11.120 to 3.234
Sensitivity: CACE	201	34.235	30.242 to 38.228	38.548	34.241 to 42.854	–4.313	3.413	0.206	–11.003 to 2.377
Fully adjusted[c]									
Primary analysis	201	34.360	30.438 to 38.282	38.851	34.161 to 43.541	–4.491	3.249	0.185	–11.345 to 2.364
Sensitivity: two-parts model	170	41.614	37.552 to 45.675	44.897	41.177 to 48.617	–3.283	2.886	0.271	–9.372 to 2.805
Sensitivity: simple imputation	201	34.365	30.438 to 38.293	38.859	34.176 to 43.541	–4.493	3.254	0.185	–11.358 to 2.371
Sensitivity: MICE	229	34.378	29.740 to 39.017	38.594	33.361 to 43.827	–4.216	3.400	0.237	–11.572 to 3.140
Sensitivity: CACE	201	33.862	29.725 to 37.999	38.850	34.673 to 43.027	–4.988	3.515	0.156	–11.877 to 1.901

MICE, multivariate imputation by chained equations.
a LSI and treatment group.
b LSI, treatment group and age category, clustered by trial site.
c LSI, treatment group, age category, BMI, sex and smoking status, clustered by trial site.

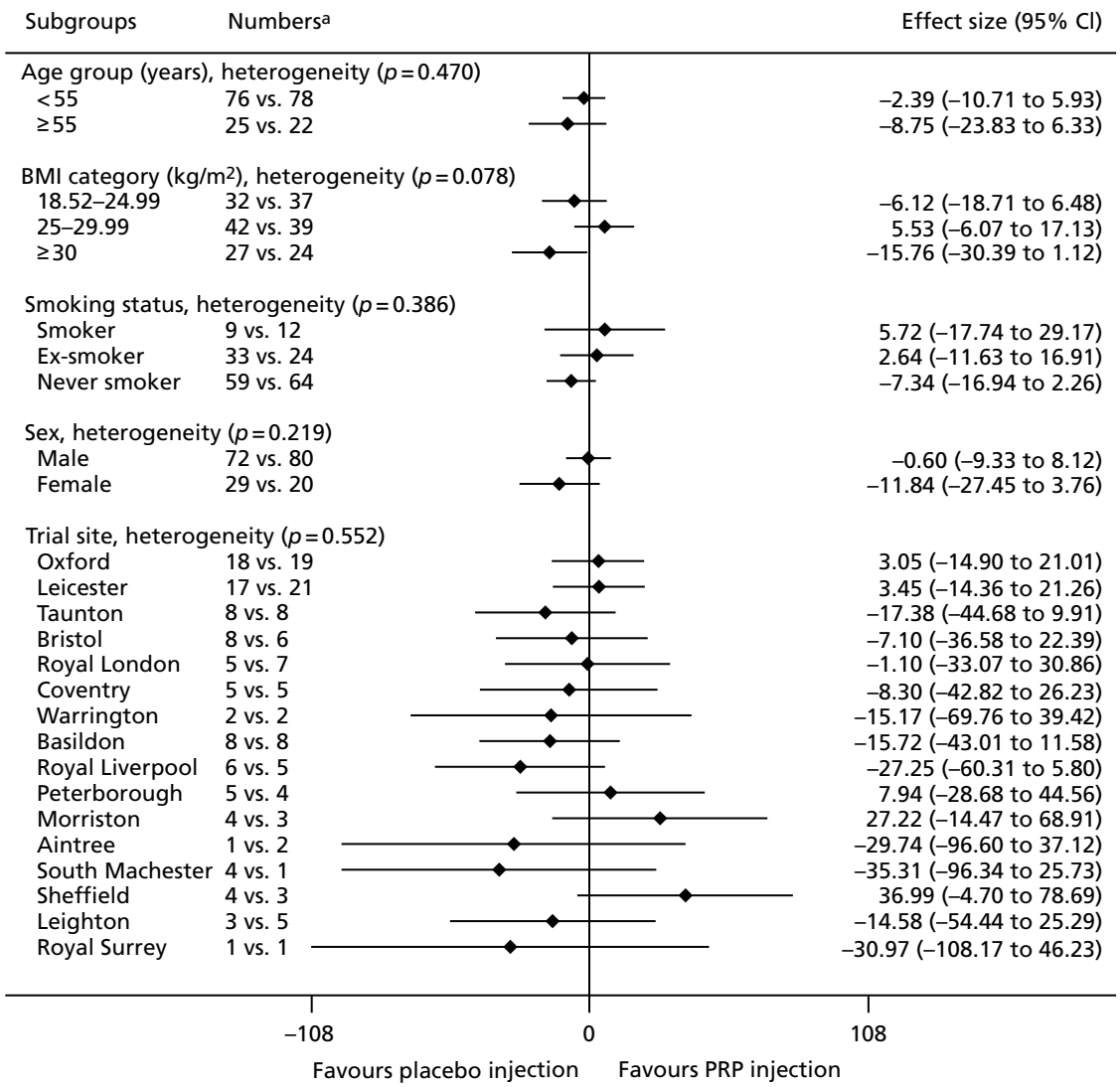

FIGURE 5 Forest plot of work LSI effect demonstrating the effect of the intervention on subgroups of defined stratification and prognostic factors. a, Number in the placebo arm vs. number in the PRP arm. The Cardiff and Royal Devon and Exeter sites were excluded as neither had patients in the treatment arm completing HRET assessment.

Pain-related Achilles tendon Total Rupture Score

Participants' pain-related ATRSs, a subset of the overall ATRS, increased at a relatively steady rate from baseline to 24 weeks post treatment, with similar rates in the PRP and placebo groups, irrespective of adjusting for stratification variables (*Table 9* and *Figure 8*).

Patient-specific functional scale

Participants' overall PSFS score increased from baseline to 24 weeks post treatment at an almost identical rate in the PRP and placebo groups, irrespective of adjusting for stratification variables (*Table 10* and *Figure 9*).

Maximum concentric displacement and maximum number of repetitions

As with the primary outcome analysis, there was no difference between the intervention groups in the maximum concentric displacement LSI achieved and the maximum number of repetitions achieved (*Table 11*). After adjusting for all stratification variables, the average maximum concentric displacement LSI [the LSI calculated as (maximum displacement in the injured leg/maximum displacement in the uninjured leg) × 100, to account for potential ability differences] achieved by participants in the PRP group was 55.1 (95% CI 51.4 to 58.8), compared with 55.4 (95% CI 49.6 to 61.3) in the placebo group. This difference was not significant (mean difference –0.3, 95% CI –6.1 to 5.4; $p = 0.898$). Similarly, after adjusting for stratification variables,

TABLE 8 Principal adjusted secondary outcome analysis results for overall ATRS reported by participants over time, with primary analysis and sensitivity analyses presented

Analysis	*n*	Intervention group, ATRS				Mean difference	Robust standard error	*p*-value	95% CI
		PRP		Placebo					
		Mean	95% CI	Mean	95% CI				
Baseline									
Adjusted analysis	216	14.090	10.974 to 17.205	11.668	8.637 to 14.699	2.422	2.218	0.275	–1.925 to 6.769
Sensitivity: HRET mITT	201	14.082	10.904 to 17.260	11.309	8.192 to 14.426	2.773	2.273	0.222	–1.682 to 7.227
Sensitivity: MICE	229	14.899	11.566 to 18.232	13.085	9.781 to 16.389	–1.814	2.401	0.450	–2.892 to 6.723
Sensitivity: CACE	216	14.480	12.139 to 16.821	11.918	9.789 to 14.047	2.562	1.534	0.095	–0.446 to 5.569
Week 4									
Adjusted analysis	216	28.461	25.286 to 31.637	30.609	27.561 to 33.657	–2.148	2.246	0.339	–6.550 to 2.255
Sensitivity: HRET mITT	201	28.441	25.212 to 31.670	31.304	28.168 to 34.441	–2.864	2.298	0.213	–7.369 to 1.641
Sensitivity: MICE	229	28.849	27.688 to 34.498	31.093	27.688 to 34.498	–2.244	2.476	0.365	–7.098 to 2.609
Sensitivity: CACE	216	28.175	24.881 to 31.469	30.499	27.527 to 33.471	–2.325	2.177	0.286	–6.591 to 1.942
Week 7									
Primary analysis	216	37.579	34.432 to 40.727	38.619	35.537 to 41.702	–1.040	2.248	0.644	–5.446 to 3.366
Sensitivity: HRET mITT	201	37.369	34.170 to 40.568	38.381	35.207 to 41.555	–1.012	2.301	0.660	–5.521 to 3.497
Sensitivity: MICE	229	37.454	34.014 to 40.894	38.523	35.083 to 41.962	–1.069	2.490	0.668	–5.951 to 3.813
Sensitivity: CACE	216	37.407	33.671 to 41.144	38.832	35.491 to 42.173	–1.424	2.640	0.589	–6.598 to 3.749

Analysis	*n*	Intervention group, ATRS: PRP Mean	PRP 95% CI	Placebo Mean	Placebo 95% CI	Mean difference	Robust standard error	*p*-value	95% CI
Week 13									
Primary analysis	216	51.663	48.483 to 54.844	53.114	50.016 to 56.213	–1.451	2.266	0.522	–5.892 to 2.990
Sensitivity: HRET mITT	201	53.091	49.858 to 56.324	53.432	50.254 to 56.611	–0.342	2.315	0.883	–4.878 to 4.195
Sensitivity: MICE	229	50.344	46.846 to 53.842	51.656	48.135 to 55.178	–1.312	2.498	0.599	–6.210
Sensitivity: CACE	216	51.635	47.801 to 55.468	53.236	49.855 to 56.618	–1.601	2.723	0.557	–6.939 to 3.736
Week 24									
Primary analysis	216	64.987	61.864 to 68.111	65.530	62.495 to 68.565	–0.543	2.222	0.807	–4.899 to 3.813
Sensitivity: HRET mITT	201	66.321	63.135 to 69.507	65.815	62.694 to 68.936	0.506	2.277	0.824	–3.956 to 4.968
Sensitivity: MICE	229	62.697	59.303 to 66.092	63.890	60.520 to 67.261	–1.193	2.434	0.624	–5.963 to 3.577
Sensitivity: CACE	216	65.143	60.681 to 69.604	65.738	61.769 to 69.708	–0.595	3.013	0.843	–6.500 to 5.310

MICE, multivariate imputation by chained equations.

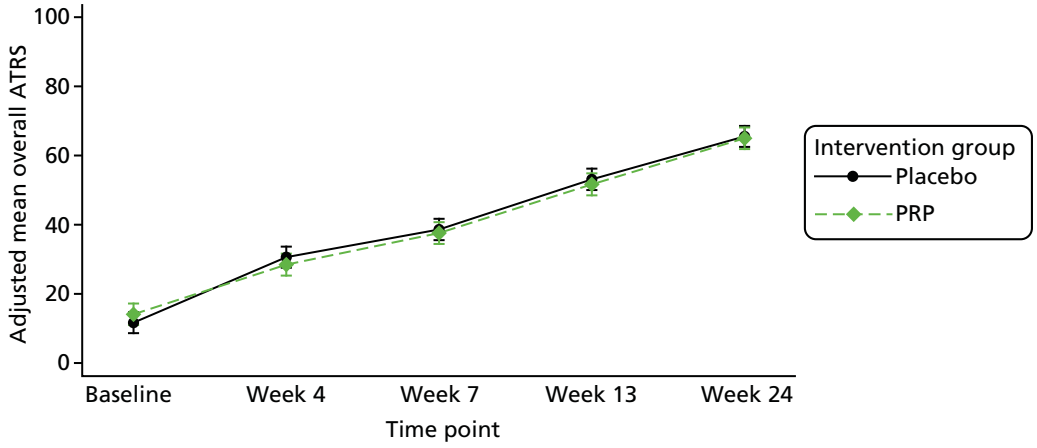

FIGURE 6 The ATRS principal adjusted repeated-measures mixed-effects regression model demonstrating the change in ATRS in PRP and placebo group participants over time. 0 = totally limited because of ATR; 100 = not limited at all because of ATR.

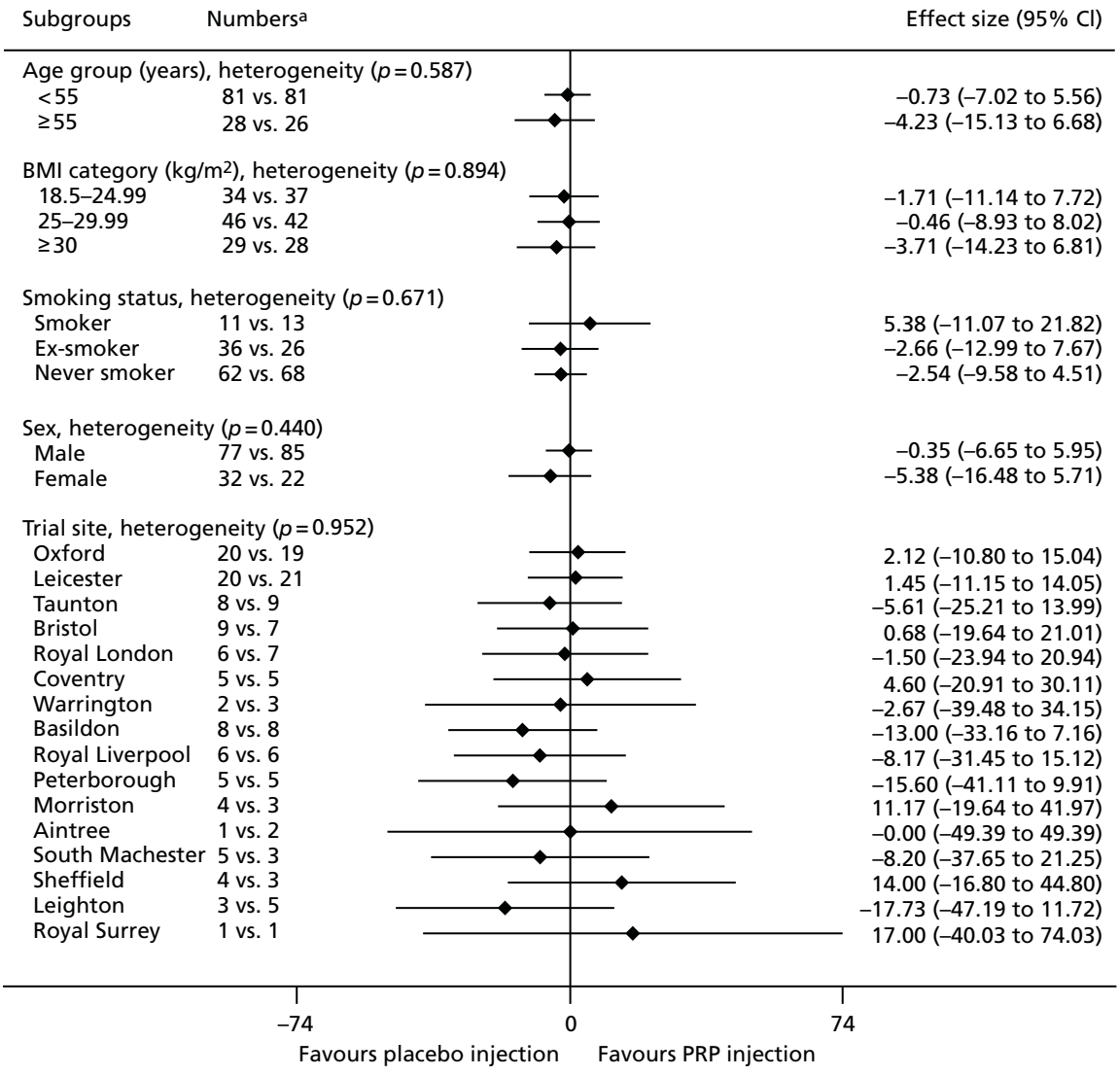

FIGURE 7 Forest plot of ATRS demonstrating the effect of the intervention on overall ATRS in subgroups of defined stratification and prognostic factors. a, Number in the placebo arm vs. number in the PRP arm. The Cardiff and Royal Devon and Exeter sites were excluded as neither had patients in the treatment arm completing the assessment.

TABLE 9 Adjusted secondary outcome analysis results for pain-related ATRS reported by participants over time

		Intervention group, ATRS pain							
		PRP		Placebo					
Time point	*n*	Mean	95% CI	Mean	95% CI	Mean difference	Standard error	*p*-value	95% CI
Baseline	216	3.678	3.124 to 4.233	3.051	2.511 to 3.590	0.628	0.395	0.112	–0.146 to 1.401
Week 4	216	6.255	5.690 to 6.819	6.277	5.734 to 6.821	–0.023	0.400	0.954	–0.806 to 0.761
Week 7	216	7.006	6.445 to 7.568	6.679	6.129 to 7.230	0.327	0.401	0.415	–0.459 to 1.113
Week 13	216	7.489	6.921 to 8.057	7.235	6.681 to 7.788	0.255	0.405	0.529	–0.538 to 1.048
Week 24	216	7.661	7.106 to 8.217	7.449	6.909 to 7.990	0.212	0.395	0.592	–0.563 to 0.987

Adjusted for ATRS, treatment group, study site and age category, with time elapsed included as a random effect.

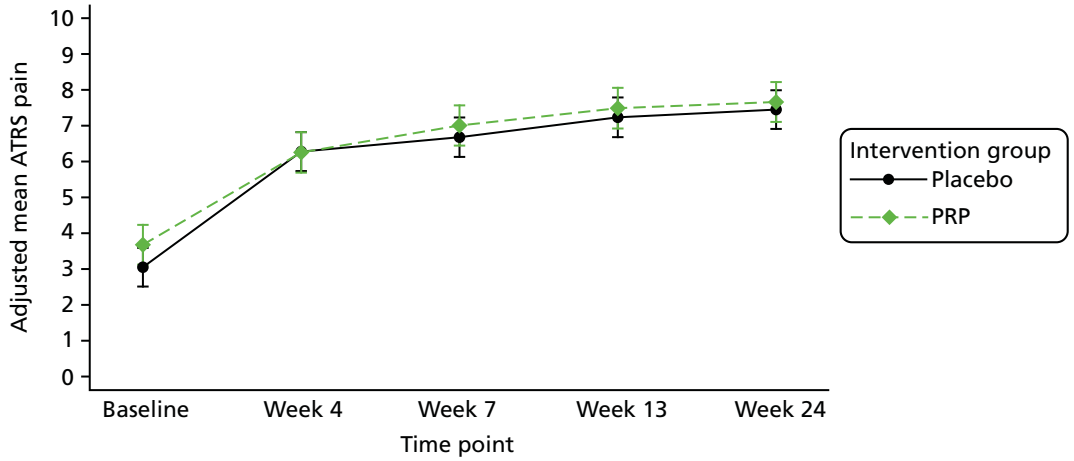

FIGURE 8 The ATRS pain mixed-effect regression model. Results from the adjusted repeated-measures mixed-effects regression model demonstrating the change in ATRS pain measure in PRP and placebo group participants over time. 0 = totally limited because of pain; 10 = not limited at all because of pain.

TABLE 10 Adjusted secondary outcome analysis results for PSFS reported by participants over time

		Intervention group, overall PSFS							
		PRP		Placebo					
Time point	*n*	Mean	95% CI	Mean	95% CI	Mean difference	Standard error	*p*-value	95% CI
Baseline	216	0.833	0.425 to 1.241	0.846	0.448 to 1.244	–0.013	0.291	0.965	–0.583 to 0.557
Week 4	216	2.021	1.608 to 2.433	2.030	1.632 to 2.429	–0.009	0.293	0.975	–0.583 to 0.564
Week 7	216	3.128	2.717 to 3.539	3.357	2.955 to 3.759	–0.229	0.293	0.435	–0.804 to 0.346
Week 13	216	5.810	5.395 to 6.225	5.776	5.372 to 6.180	0.034	0.296	0.909	–0.546 to 0.614
Week 24	216	7.198	6.788 to 7.608	7.495	7.097 to 7.893	–0.297	0.291	0.308	–0.868 to 0.274

Adjusted for PSFS, treatment group, study site and age category, with time elapsed included as a random effect.

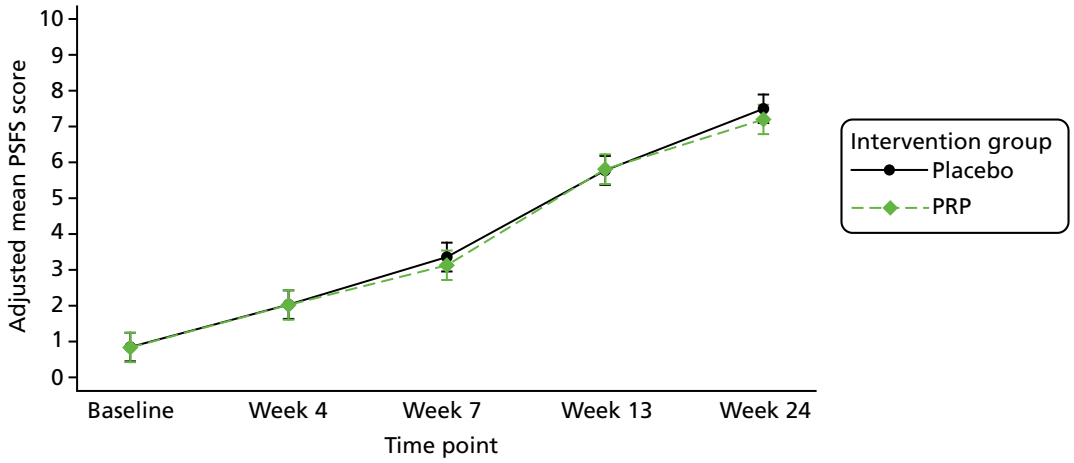

FIGURE 9 The PSFS mixed-effects regression model. Results from the adjusted repeated-measures mixed-effects regression model demonstrating the change in PSFS in PRP and placebo group participants over time. 0 = unable to carry out activities; 10 = able to carry out activities at same levels as before ATR.

TABLE 11 Adjusted analysis results for maximum displacement LSI and maximum number of repetitions proportion achieved by participants during their HRET, on subjects with assessments for both legs (valid repetitions only), accounting for participants with true and potential zero measurements

HRET measure	*n*	Intervention group, maximum displacement				Mean difference	Robust standard error	*p*-value	95% CI
		PRP		Placebo					
		Average	95% CI	Average	95% CI				
Maximum displacement	201	55.099	51.436 to 58.762	55.453	49.610 to 61.296	–0.354	2.717	0.898	–6.087 to 5.378
Maximum number of repetitions	201	50.080	43.735 to 56.417	60.752	52.841 to 68.662	–10.671	5.325	0.061	–21.906 to 0.564

the average maximum number of repetitions proportion [calculated as (maximum number of repetitions in the injured leg/maximum number of repetitions in the uninjured leg) × 100, to account for potential ability differences] achieved by participants in the PRP group was 50.1% (95% CI 43.7% to 56.4%), compared with 60.7% (95% CI 52.8% to 68.7%) in the placebo group. This difference was not statistically significant (mean difference –10.7, 95% CI –21.9 to 0.6; $p = 0.061$).

Visual analogue scale

Participants' pain levels as recorded in their pain diaries in the first 2 weeks post intervention demonstrate that the pain experienced by participants decreased at a relatively steady rate from day 1 to day 14 (*Figure 10*).

Accounting for the stratification factors, participants in the PRP group reported a mean overall VAS score of 37.3 (95% CI 33.0 to 41.7) at day 1, with the placebo group reporting a similar level of 31.2 (95% CI 26.7 to 35.7). By day 14, participants in the PRP group were reporting a mean VAS score of 9.5 (95% CI 5.2 to 13.9) and participants in the placebo group were reporting a mean VAS score of 13.6 (95% CI 9.0 to 18.1). This difference at day 14 was not significant (mean difference –4.0, 95% CI –10.3 to 2.3; $p = 0.210$). This demonstrates that the decline in reported pain was at similar rates in the PRP and placebo groups.

Short Form questionnaire-12 items quality of life

Participants' SF-12 physical component scores (PCSs) increased gradually over time to near-normal levels at 24 weeks post treatment (*Figure 11*) at an almost identical rate in the PRP and placebo groups, irrespective of stratification variable and accounting for participants' pre-injury PCSs (*Table 12*).

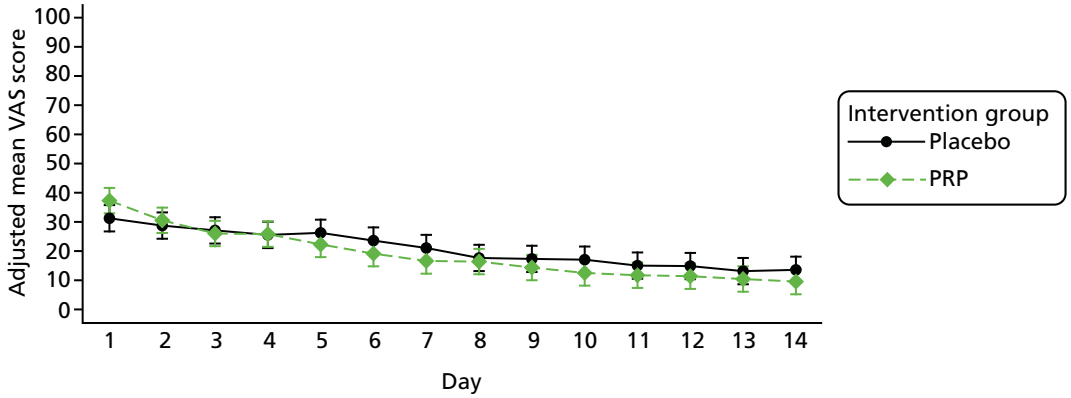

FIGURE 10 Pain VAS mixed-effects regression model. Results from the adjusted repeated-measures mixed-effects regression model demonstrating the change in VAS pain measure in PRP and placebo group participants in the first 2 weeks post intervention. 0 = no pain; 100 = severe pain.

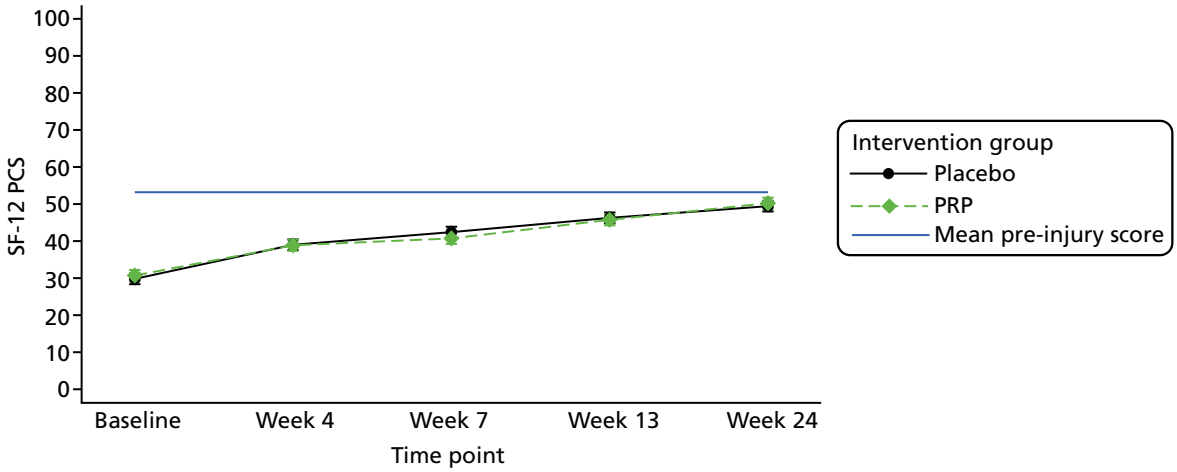

FIGURE 11 The SF-12 PCS mixed-effects regression model. Results from the adjusted repeated-measures mixed-effects regression model demonstrating the change in PCS in PRP and placebo group participants over time, with mean pre-injury score (with 95% CIs) shown.

TABLE 12 Adjusted secondary outcome analysis results for SF-12 PCS reported by participants over time

Time point	*n*	Intervention group, overall PCS				Mean difference	Standard error	*p*-value	95% CI
		PRP		Placebo					
		Mean	95% CI	Mean	95% CI				
Baseline: post injury	213	30.720	29.250 to 32.191	29.799	28.367 to 31.230	0.922	1.048	0.379	–1.133 to 2.976
Week 4	213	38.865	37.376 to 40.354	39.001	37.565 to 40.438	–0.136	1.057	0.897	–2.208 to 1.935
Week 7	213	40.731	39.249 to 42.214	42.422	40.971 to 43.872	–1.691	1.059	0.110	–3.767 to 0.386
Week 13	213	45.760	44.260 to 47.259	46.272	44.812 to 47.731	–0.512	1.069	0.632	–2.606 to 1.584
Week 24	213	50.240	48.751 to 51.729	49.436	47.996 50.876	0.805	1.058	0.447	–1.269 to 2.879

Adjusted for PCS, treatment group, study site, age category and PCS pre-injury score, with time elapsed included as a random effect.

Participants' SF-12 mental component scores (MCSs) remained relatively stable and close to their pre-injury scores over the entire trial follow-up period (*Figure 12*). At 24 weeks post treatment, participants in the PRP group had a MCS that was nearly 3 points lower than that of the MCS in the placebo group after adjusting for stratification variables and accounting for participants' pre-injury MCSs ($p = 0.035$) (*Table 13*).

Success of blinding

Participants were questioned following their 24-week HRET assessment as to which treatment they believed they received; 210 participants responded to this question (*Table 14*).

Using participant responses to this question, assessments of blinding were carried using the James *et al.*[69] and Bang *et al.*[70] blinding indices to determine whether or not participants were successfully blinded to the intervention they received. Using the information generated from both of these assessments, we can be confident that there is no evidence to suggest that blinding in this trial was unsuccessful. For further details on the outcome of these assessments, see *Appendix 3*.

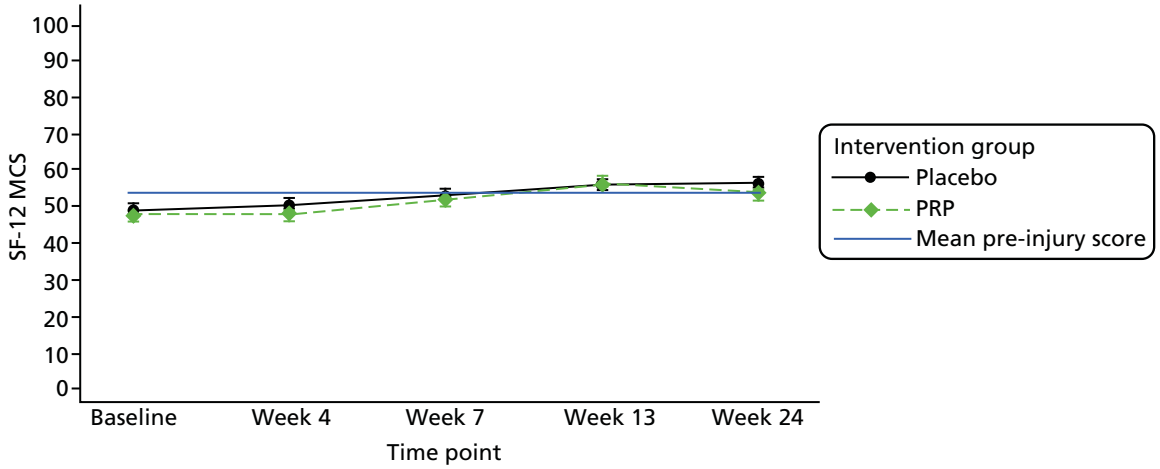

FIGURE 12 The SF-12 MCS mixed-effects regression model. Results from the adjusted repeated-measures mixed-effects regression model demonstrating the change in MCS in PRP and placebo group participants over time.

TABLE 13 Adjusted secondary outcome analysis results for SF-12 MCS reported by participants over time

Time point	*n*	Intervention group, overall MCS				Mean difference	Standard error	*p*-value	95% CI
		PRP		Placebo					
		Mean	95% CI	Mean	95% CI				
Baseline: post injury	213	47.802	46.003 to 49.601	49.105	47.356 to 50.855	–1.303	1.281	0.309	–3.813 to 1.207
Week 4	213	48.287	46.460 to 50.114	50.686	48.925 to 52.446	–2.398	1.295	0.064	–4.936 to 0.139
Week 7	213	52.054	50.235 to 53.873	53.420	51.639 to 55.200	–1.365	1.299	0.293	–3.911 to 1.180
Week 13	213	56.415	54.580 to 58.251	55.673	53.885 to 57.462	0.742	1.308	0.571	–1.822 to 3.305
Week 24	213	53.788	51.972 to 55.604	56.503	54.745 to 58.260	–2.714	1.289	0.035	–5.242 to –0.187

Adjusted for MCS, treatment group, study site, age category and MCS pre-injury score, with time elapsed included as a random effect.

TABLE 14 Participant prediction of treatment received, by intervention group

Participant prediction of treatment	Intervention group, *n* (%)		
	PRP	Placebo	Total, *n* (%)
PRP injection	32 (30.77)	28 (26.41)	60 (28.57)
Imitation injection	17 (16.35)	9 (8.49)	26 (12.38)
Don't know	55 (52.88)	69 (65.09)	124 (59.05)
Total	104 (100.00)	106 (100.00)	210 (100.00)

Adverse events

Adverse events are reported in *Table 15*. Only one participant (1/229, 0.4%) reported a SAE during the study. There were similar AE rates in the treatment groups [PRP, 84/113 (74%); placebo, 90/116 (78%)].

TABLE 15 Complications reported by participants during the 24 weeks after injury and treatment

Complication type	Intervention group, *n* (%)	
	PRP (*N* = 113)	Placebo (*N* = 116)
Participants experiencing at least one AE of any type[a]	84 (74.34)	90 (77.59)
SAEs[a]	1 (0.88)	0
ST elevation myocardial infarction	1 (0.88)	0
AEs[a]	84 (74.34)	90 (77.59)
Foreseeable AEs[a]	83 (73.45)	87 (75.00)
Mild discomfort or minor bleeding following injection	22 (19.47)	8 (6.90)
Technical complications of lower leg casting and splinting	38 (33.63)	28 (24.14)
Consequences of depending on walking aids	1 (0.88)	1 (0.86)
Syncopal episode related to venesection or tendon injection	0	0
Discomfort at rupture site during rehabilitation	9 (7.96)	10 (8.62)
Swelling or bruising of the lower leg and foot	68 (60.18)	77 (66.38)
DVT in a lower limb	6 (5.31)	5 (4.31)
Re-rupture of treated Achilles tendon	6 (5.31)	4 (3.45)
Unforeseeable AEs[a]	16 (14.16)	19 (16.38)
Serious infection of injection site of ATR	0	0
Skin breakdown or ulceration of treated lower leg	13 (11.50)	13 (11.21)
Severe pain (more than simple analgesia) > 10 days after injection	6 (5.31)	6 (5.17)
Other AEs related to treatment or ATR[a]	13 (11.50)	13 (11.21)
Frequent discomfort at injection site	5 (4.42)	6 (5.17)
Infection at injection site confirmed by doctor	0	3 (2.59)
Infection at non-injection site[b]	0	3 (2.59)
Other problem[c]	9 (7.96)	6 (5.17)

a Participants could have experienced multiple AEs so number of participants reporting foreseeable and unforeseeable AEs may not add up to the overall total reporting.
b Infections included cellulitis, pneumonia, and an infected insect bite on the treated leg.
c Participant-specific complications associated with treatment or rupture not covered by other complication types.

Note
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The overall re-rupture rate among study participants was 4.4% (10/229), with similar rates in the PRP group (6/113, 5.3%) and the placebo group (4/116, 3.5%). Nine of the ten participants with a re-rupture were then treated with surgery. DVT rates were also similar (PRP group: 6/113, 5.3%; placebo group: 5/116, 4.3%).

There were some complications at the injection site, with 11 out of 229 (5%) participants reporting frequent discomfort and 3 out of 229 (1%) participants reporting clinically diagnosed infections. However, no clinically serious infections were reported.

Summary of key findings

- The results of the analyses of the primary end point allow us to conclude that there was no evidence of a difference between PRP injection and placebo for the treatment of acute ATR in adults in terms of muscle–tendon function at 24 weeks.
- There was no evidence of any differences between the intervention groups in the PROMs (ATRS, PSFS, pain and SF-12 physical function) across all time points assessed. There was a small statistically significant difference in SF-12 mental function, with the PRP group doing worse than the placebo group. This was probably due to chance.
- There was no difference in complications and AEs between the two groups.
- The results were robust to adjustment for stratification factors (age and centre) with/without other confounding factors and for the sensitivity analyses.
- The overall re-rupture rate was low, at 4.37%, and no significant difference was seen between the groups.

Chapter 4 Substudy 1: platelet-rich plasma and blood analysis

Introduction

Autologous platelet preparations are increasingly being used in many areas of regenerative medicine. However, there are few properly controlled RCTs and the preparation, content and definitions of many platelet preparations are generally poorly defined, standardised and controlled. Most platelet products therefore have varying concentrations of blood cells [platelets, white blood cells (leucocytes) and red blood cells (erythrocytes)], plasma and fibrin. Studying the potential role(s) of all the cells and their bioactive factors is, therefore, essential to understand the variables associated with the biological activity and efficacy of any platelet preparation.

Although our pilot study demonstrated efficacy signal of a PRP (L-PRP) preparation in ATR healing, as a pilot study it was underpowered to study the variables within PRP preparations that may be associated with efficacy of healing. The PATH-2 trial, powered on the work LSI as the primary outcome, randomised a total of 230 participants, 103 of whom received the PRP product. This provided the opportunity to not only study the variation in cellular and bioactive components within the PRP but also to determine the reliability of using a single device to prepare an autologous sterile product for ATR healing across 19 trial centres.

The aim of substudy 1 was to determine the cellular content of the PRP product (platelets, erythrocytes and leucocytes) and the quality and growth factor content of the PRP preparations used. This provided one of the most comprehensive studies of the within- and between-centre performance of a single preparation device of PRP in a clinical trial setting. The data also provided insight into the key variables within PRP that were associated with the biological efficacy of the product as determined by the primary and secondary outcomes.

Methods

Blood and platelet-rich plasma samples

Venous blood (1–5 ml) was taken from all participants recruited into the trial and anticoagulated within ethylenediaminetetraacetic acid vacutainers (Becton, Dickinson and Company, Plymouth, UK). In the PRP treatment group, an additional 50 ml of venous blood was taken for automated sterile PRP preparation in the centrifuge; 8 ml of sterile PRP was then harvested and 4 ml was used for injection into the Achilles tendon, and the remaining 4 ml was divided into four 1-ml aliquots. One microtube was immediately frozen at –70 °C for storage until the end of the trial when it was used for batch measurement of growth factor levels (see below). The remaining PRP aliquots (each 1 ml) were stimulated at room temperature for 5 minutes by addition to two tubes (Platelet Solutions Ltd, Nottingham, UK) containing either (tube B) saline alone, to provide an unstimulated baseline, or (tube C) adenosine diphosphate and U46619, to fully activate the platelets. Both samples were then fixed using 1 ml of PAMFix (Platelet Solutions Ltd, Nottingham, UK). The whole blood, unfixed PRP and tubes B and C were then transported at room temperature by courier to the Institute of Inflammation and Ageing at the University of Birmingham and processed as soon as possible on arrival.

Cell counting

Whole-blood and unfixed PRP cell counts were conducted using the Sysmex XN-1000 haematology analyser (Sysmex UK, Milton Keynes, UK). The analyser utilises three primary analysis principles: fluorescence flow cytometry, direct current detection with hydrodynamic focusing and sodium lauryl sulfate haemoglobin detection. The WNR (white cell nucleated) channel evaluates white blood cell count, basophils and nucleated

red blood cells. An impedance red blood cell count is also reported. The WDF (white blood cell differential) channel also provides a differential count of the neutrophils, lymphocytes, eosinophils, monocytes and immature granulocytes. The platelet fluorescence (PLT-F) channel is a specialised fluorescence optical analysis exclusively for platelets. The PLT-F parameter utilises traditional fluorescence flow cytometry in which platelets are stained with oxazine (a ribonucleic acid binding dye that eliminates any interference mediated by cellular debris). A measurement of platelet production (immature platelet fraction) is also in this channel. The analyser thus reports three platelet counts (platelet impedance, platelet optical and PLT-F), mean platelet volume and the immature platelet fraction. Quality control material (XN Check™, Sysmex UK, Milton Keynes, UK) was tested on a daily basis to ensure instrument performance throughout the trial. The instrument was also enrolled in a national external quality assurance scheme (UK NEQAS, Watford, UK) and maintained on a service contract (Sysmex UK). To determine normal ranges for each cellular parameter, blood samples from 40 healthy volunteers (control cohort) were analysed and normal ranges are represented as the mean value ± 2 SDs.

Measurement of platelet quality

Tubes B and C containing fixed resting and activated platelets, respectively, were analysed for the expression of a platelet-specific activation marker P-selectin (CD62P) by flow cytometry as a measure of platelet quality. After gentle mixing, 5 µl of fixed PRP from tubes B and C was incubated with 5 µl of test antibody [CD62P conjugated to flourescein isothiocyanate (FITC); Beckman Coulter Inc., Brea, CA, USA] or its corresponding isotype control (IgG conjugated to FITC; Beckman Coulter Inc.) with 40 µl of 0.2-µm filtered phosphate buffered saline (pH 7.4) for 20 minutes at room temperature. The samples were then further diluted by the addition of 4 ml of phosphate-buffered saline. The platelets were then analysed by flow cytometry (Accuri™ Flow cytometer, Becton, Dickinson and Company). Platelets were identified according to their characteristic size and granularity (using log-forward scatter and log-side scatter), with a forward-scatter threshold of 25,000 to eliminate noise. An amorphous gate was placed around the platelet population and 10,000 events were collected. Analysis regions were set using the isotype control so that 0.5% of the platelet population was positive. CD62P positivity was then measured and both percentage expression and median fluorescent intensity of all platelet events were recorded.

Measurement of growth factor levels within platelet-rich plasma

At the end of participant recruitment, all frozen aliquots of PRP were shipped to Birmingham for analysis of growth factor levels. Appropriate batches of samples were thawed at 37 °C for 10 minutes. One-fortieth volume of 20% Triton™ X-100 (Roche Diagnostics, Mannhein, Germany) was added to ensure full lysis prior to a further incubation at 37 °C for 10 minutes. Samples were mixed then centrifuged at 1500 g for 10 minutes at room temperature. Supernatants were then removed and simultaneously assayed for five different growth factors (TGF-β1, FGF, VEGF, IGF-1 and PDGF-AB) by commercial enzyme-linked immunosorbent assay (ELISA) kits (Bio-Techne Ltd, Abingdon, UK). Optimal sample dilutions for each growth factor were predetermined by assaying L-PRP samples prepared from control volunteers using the identical method of preparation as in the trial. Diluted samples, blanks and standards were pipetted in duplicate into 96 well plates coated with capture antibodies and incubated as instructed within each growth factor ELISA kit. Plates were then washed four times with the washing buffers provided and the peroxidase-conjugated secondary antibodies provided within each kit were added to each well and incubated as instructed. Plates were then washed four times with washing buffer before addition of substrate solution to each well. The plates were then incubated for 20–30 minutes at room temperature in the dark before the addition of the stop solution. The optical density of each well was then measured immediately using a microplate reader and readings taken at 450 nm. A further reading was taken at 540 nm and the values subtracted from the readings at 450 nm to correct for optical imperfections in the plate. The duplicate readings were averaged for each standard, control and sample, and the zero standard optical density was subtracted. Standard curves were generated by plotting the mean absorbance for each standard on the *y*-axis against the concentration on the *x*-axis and the best-fit line generated by regression analysis. Unknown sample values were then read off the regression line and final concentrations were determined by multiplying using the dilution factors used in each assay. The concentration for each growth factor per ml of lysate was reported.

Statistical analysis

The comparability of key blood parameters and bioactive factors for each treatment group was summarised with means and SDs. For whole-blood parameters, a Student's *t*-test was used to explore differences between treatment arms.

Correlation between the primary outcome and both key blood parameters and bioactive factors was explored using Pearson's correlation.

As per analyses in the main study, a *p*-value of < 0.05 was seen as indicative of a statistically significant difference.

Results

Cell counts within whole blood and platelet-rich plasma

Summary statistics for key blood parameters in whole-blood and PRP samples are given in *Table 16*. A comparison of the whole-blood counts in the PRP and placebo groups is shown in *Figure 13*. There was no significant difference in the whole-blood samples between the treatment groups for red blood cell count (mean difference –0.002, 95% CI –0.144 to 0.139; $p = 0.974$), white blood cell count (mean difference –0.259, 95% CI –0.784 to 0.264; $p = 0.330$) or platelet count (as PLT-F) (mean difference –19.045, 95% CI –38.310 to 0.040; $p = 0.051$). *Figure 14* illustrates the cell counts within the PRP samples showing the

TABLE 16 Summary statistics for key blood parameters in whole-blood and PRP samples, by intervention group

	Intervention group								
	PRP (*N* = 113)			Placebo (*N* = 116)			Total (*N* = 229)		
Parameter	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Whole-blood									
Erythrocytes (10^{12}/l)	107	4.825	0.589	114	4.827	0.475	221	4.826	0.532
Leucocytes (10^9/l)	107	6.742	2.049	114	7.001	1.904	221	6.875	1.975
Platelets (10^9/l)[a]	104	208.183	77.729	110	227.227	63.537	214	217.972	71.264
PRP									
Erythrocytes (10^{12}/l)	101	0.907	1.489	–	–	–	–	–	–
Leucocytes (10^9/l)	101	15.130	10.275	–	–	–	–	–	–
Platelets (10^9/l)[a]	98	852.551	438.958	–	–	–	–	–	–
IGF-1 (ng/ml)	103	78.178	23.180	–	–	–	–	–	–
TGF-β1 (ng/ml)[b]	97	131.915	74.372	–	–	–	–	–	–
PDGF-AB (ng/ml)[c]	100	55.339	27.640	–	–	–	–	–	–
VEGF (ng/ml)[d]	103	0.981	0.721	–	–	–	–	–	–
bFGF (pg/ml)[e,f]	103	111.038	76.970	–	–	–	–	–	–

bFGF, basic fibroblast growth factor.
a Using PLT-F measure.
b Participants with a value of < 10.02 ng/ml ($n = 2$) included in data as 0 ng/ml.
c Participants with a value of < 5.1 ng/ml ($n = 3$) included in data as 0 ng/ml.
d Participants with a value of < 0.125 ng/ml ($n = 2$) included in data as 0 ng/ml.
e Participants with a value of < 10 pg/ml ($n = 3$) included in data with as 0 pg/ml.
f Participants with a value of < 20 pg/ml ($n = 3$) included in data as 10 pg/ml.

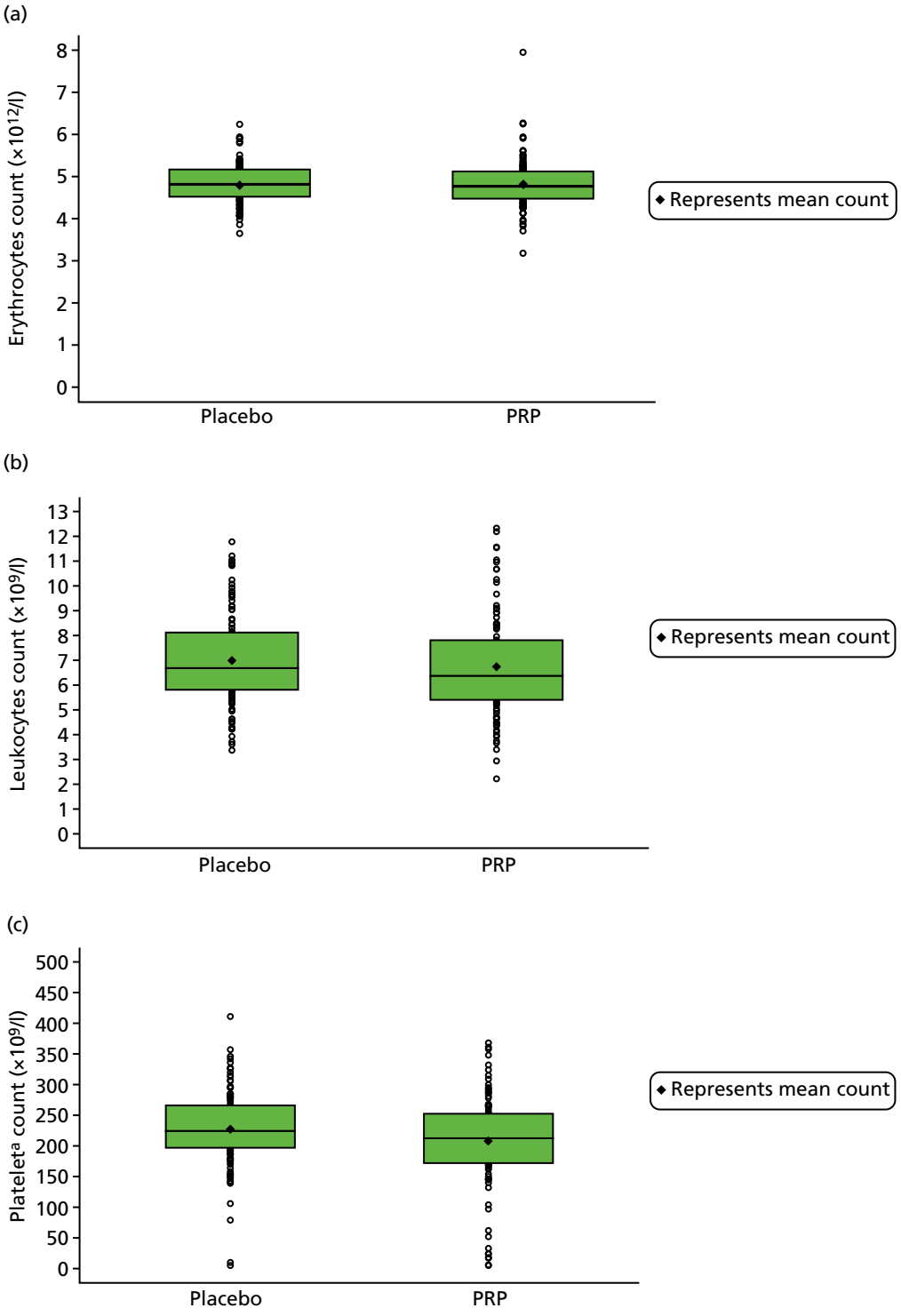

FIGURE 13 Cell counts in whole-blood samples, by intervention group. (a) Erythrocytes; (b) leucocytes; and (c) platelets. a, Using the PLT-F measure.

variation in platelet, red blood cell and white blood cell counts, with the whole-blood sample summaries given in the same figure for reference. The mean platelet count (PLT-F) in the PRP sample was 852.5 × 10^9/l, but with a wide range (6.0–2903.0 × 10^9/l). Although a small number of PRP platelet counts were lower than original whole-blood counts due to some unforeseen centrifuge problems, the overall mean increase in platelets was 4.1-fold. The mean red blood cell count was 0.9 × 10^{12}/l (range 0.1–9.0 × 10^{12}/l). As expected, the red blood cell count decreased on average in the PRP by a factor of 5.3 of the original count in whole blood. The mean white blood cell count was 15.1 × 10^9/l (range 1.7–65.3 × 10^9/l).

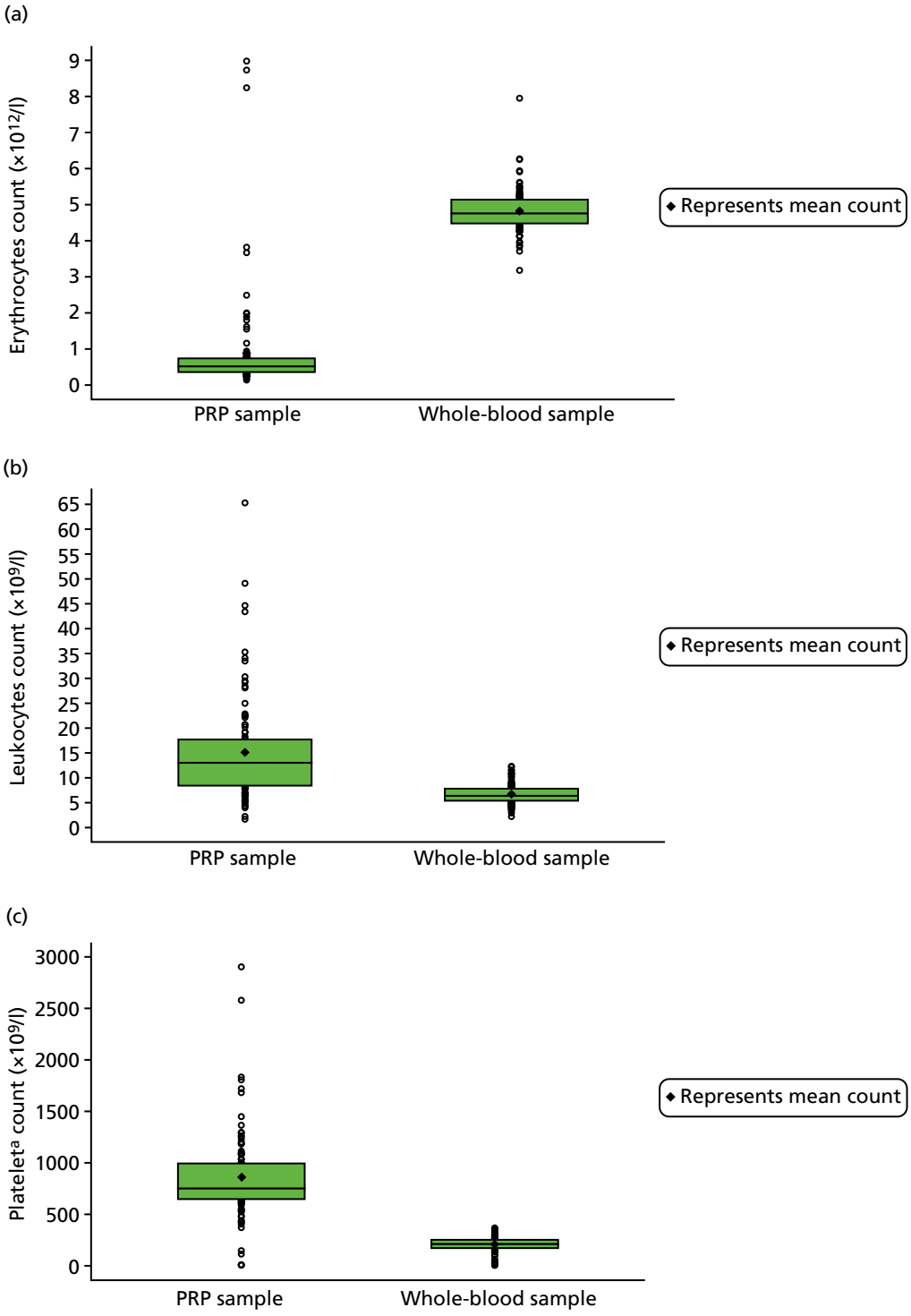

FIGURE 14 Cell counts in PRP samples, represented against counts in whole-blood samples. (a) Erythrocytes; (b) leucocytes; and (c) platelets. a, Using the PLT-F measure.

As this preparation is a L-PRP, the mean white blood cell count increased by a factor of 2.2, as expected. The white blood cell differential count was 14.6% monocytes (mean 2.2 × 10^9/l, range 0–21.1 × 10^9/l), 45.2% lymphocytes (mean 6.8 × 10^9/l, range 0.1–18.7 × 10^9/l), 39.2% neutrophils (mean 5.9 × 10^9/l, range 0.2–30.4 × 10^9/l), 0.1% basophils (mean 0.02 × 10^9/l, range 0–0.5 × 10^9/l) and 0.82% eosinophils (mean 0.1 × 10^9/l, range 0–1.5 × 10^9/l).

Platelet quality

The basal levels of CD62P expression [percentage and mean fluorescence intensity (MFI)] within the platelets of resting basal PRP and activated PRP samples are shown in *Table 17* and *Figure 15*. In resting PRP, the mean CD62P expression was 4.3% with a MFI of 248.6. The majority of PRP samples were therefore of good quality with low levels of activation, with a few exceptions. In the activated PRP samples, the mean CD62P expression was 60.1% with a MFI of 1208.0. The majority of PRP samples were functional, with the majority of platelets in the PRP preparations being shown to be capable of activation and degranulation, with a few exceptions.

TABLE 17 Summary statistics for key blood parameters in PRP samples

	PRP samples					
	Activated ($N = 103$)			Resting ($N = 103$)		
Parameter	*n*	Mean	SD	*n*	Mean	SD
CD62P expression (%)	102	60.092	22.264	103	4.274	5.046
MFI	102	1208.046	556.599	103	248.568	50.941

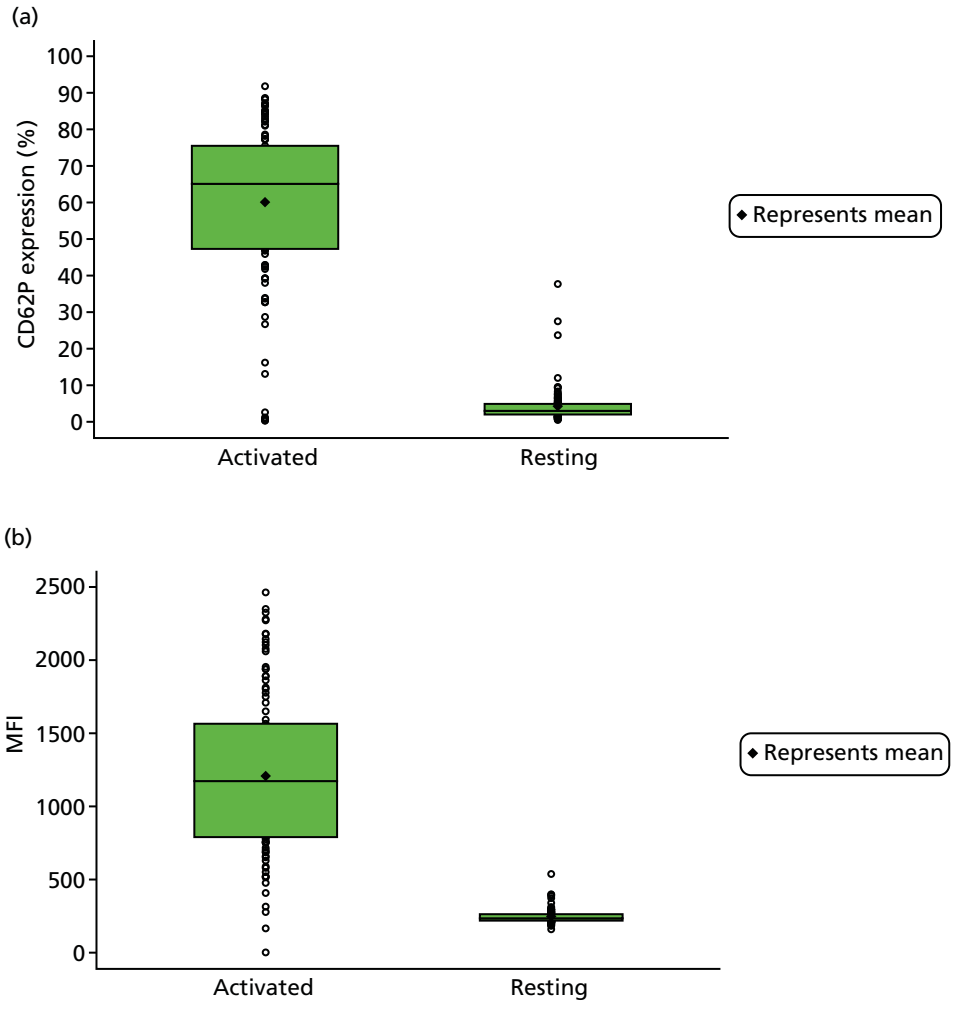

FIGURE 15 Platelet quality in PRP samples, demonstrating both (a) CD62P expression and (b) MFI in activated and resting (unactivated) PRP.

Growth factor levels within platelet-rich plasma

The levels of each growth factor are shown in *Table 16* and *Figure 16*.

Blood and platelet-rich plasma variables and clinical outcomes

Baseline blood and the primary outcome measure

Parameters of baseline whole blood taken before the intervention did not correlate with the primary outcome measure at 24 weeks (*Figure 17* and *Table 18*), irrespective of intervention group.

Platelet-rich plasma and the primary outcome measure

The PRP cell count and growth factor concentration correlation with the primary outcome measure were analysed to assess if the variability in PRP had any effect on the outcome. The results are displayed in *Figures 18–20* and *Table 19*.

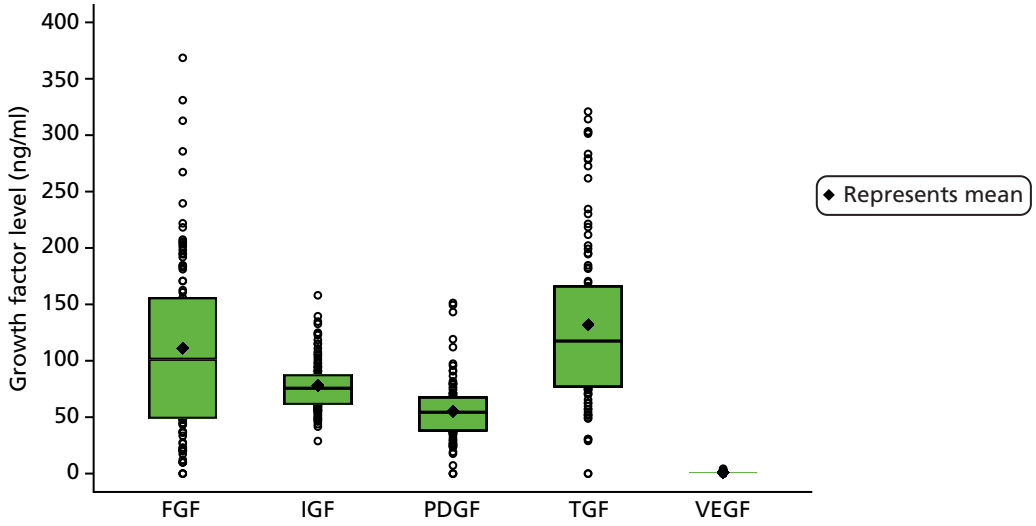

FIGURE 16 Growth factor concentrations in PRP samples.

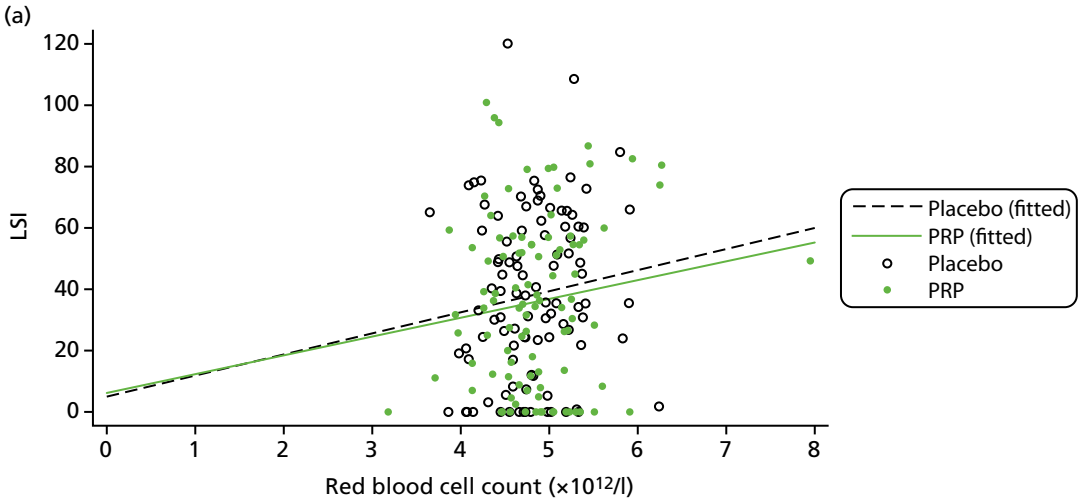

FIGURE 17 Correlation between whole-blood sample blood cell counts and the primary outcome (work LSI) at 24 weeks. (a) Erythrocytes; (b) leucocytes; and (c) platelets. a, Using the PLT-F measure. (*continued*)

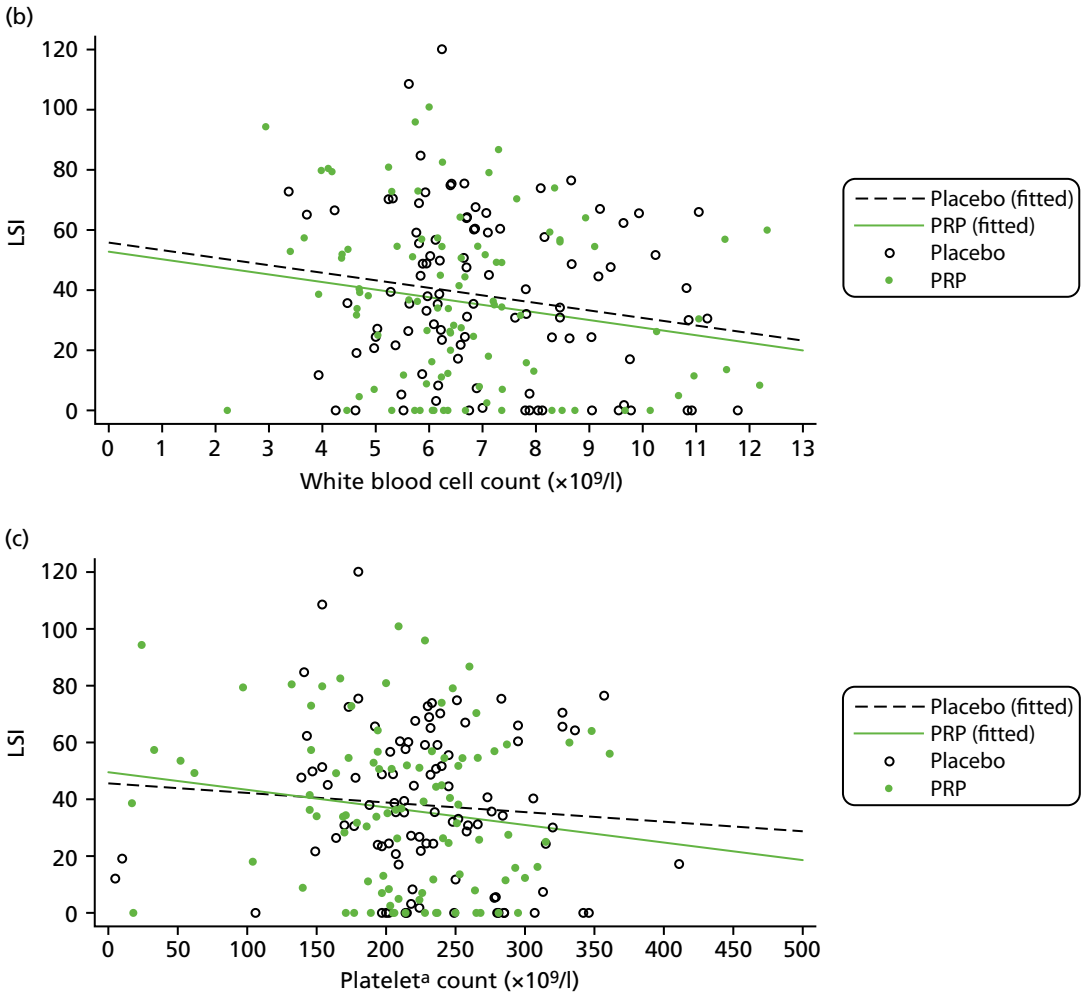

FIGURE 17 Correlation between whole-blood sample blood cell counts and the primary outcome (work LSI) at 24 weeks. (a) Erythrocytes; (b) leucocytes; and (c) platelets. a, Using the PLT-F measure.

TABLE 18 Results from primary outcome correlation assessments overall and by intervention group, for whole-blood sample key blood parameters

Parameter by group	*n*	*r*	% variance[a]	*p*-value
Erythrocytes				
PRP	97	0.133	1.780	0.193
Placebo	99	0.122	1.496	0.228
Leucocytes				
PRP	97	–0.184	3.393	0.071
Placebo	99	–0.172	2.976	0.088
Platelets[b]				
PRP	94	–0.153	2.329	0.142
Placebo	96	–0.080	0.634	0.440

r, Pearson's product-moment correlation coefficient.
a Proportion of variance in LSI explained by key blood parameter, calculated as ($R^2 \times 100$).
b As PLT-F.

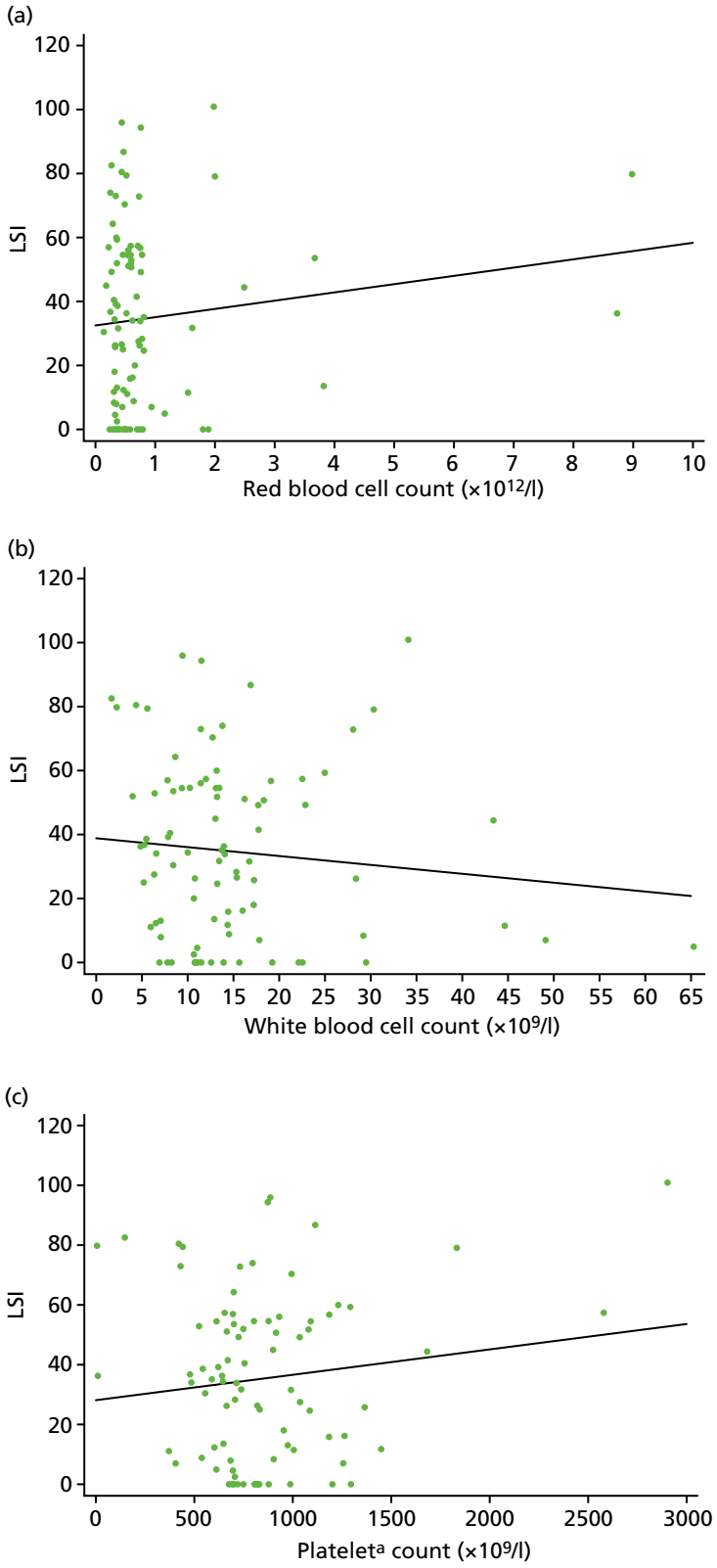

FIGURE 18 Correlation between PRP sample blood cell counts and the primary outcome (work LSI) at 24 weeks. (a) Erythrocytes; (b) leucocytes; and (c) platelets. a, Using the PLT-F measure.

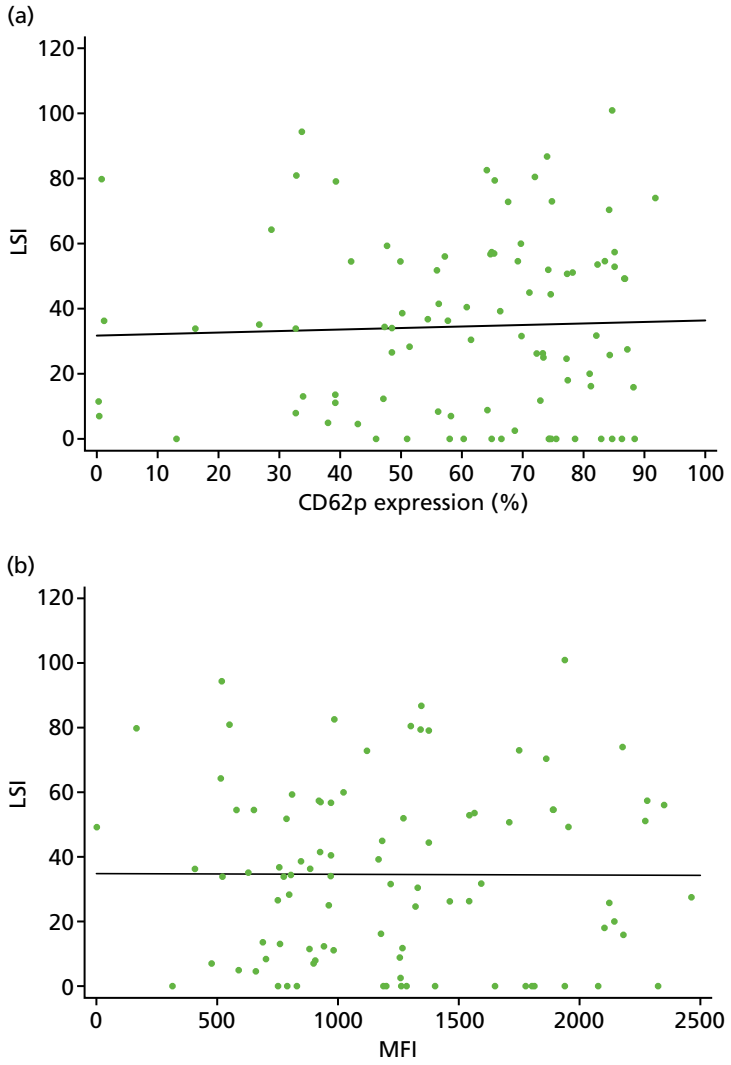

FIGURE 19 Correlation between platelet quality and the primary outcome (work LSI) at 24 weeks. (a) Activated CD62P expression (%); and (b) activated CD62P MFI expression (%).

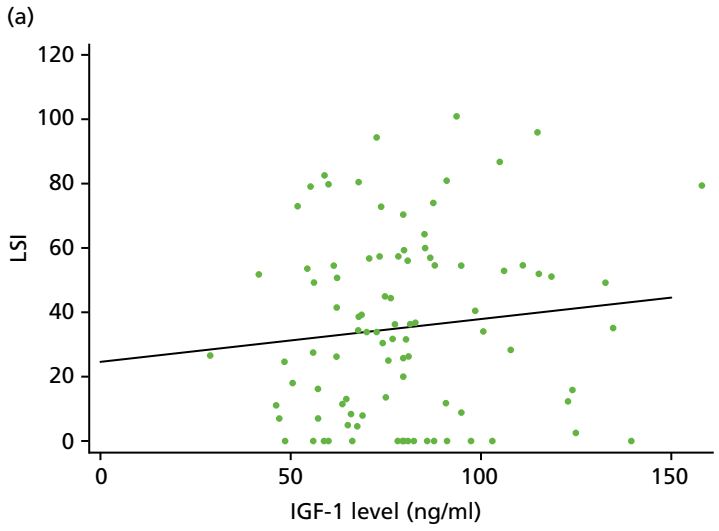

FIGURE 20 Correlation between growth factors and the primary outcome (work LSI) at 24 weeks. (a) IGF-1; (b) TGF-β1; (c) PDGF-AB; (d) VEGF; and (e) bFGF. bFGF, basic fibroblast growth factor. (*continued*)

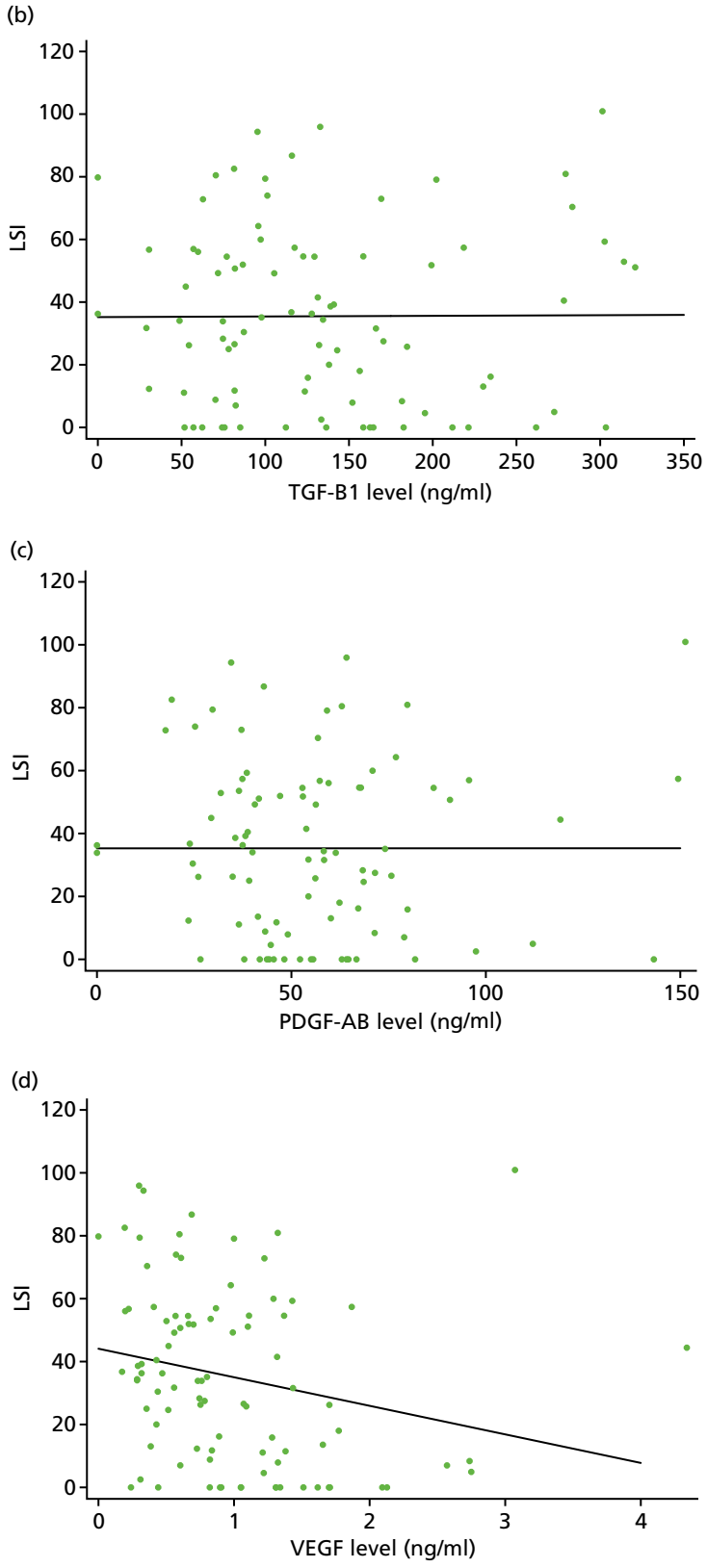

FIGURE 20 Correlation between growth factors and the primary outcome (work LSI) at 24 weeks. (a) IGF-1; (b) TGF-β1; (c) PDGF-AB; (d) VEGF; and (e) bFGF. bFGF, basic fibroblast growth factor. (*continued*)

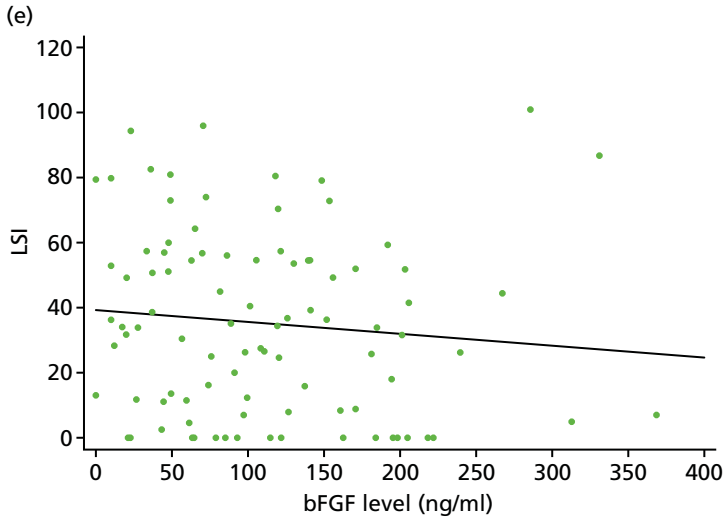

FIGURE 20 Correlation between growth factors and the primary outcome (work LSI) at 24 weeks. (a) IGF-1; (b) TGF-β1; (c) PDGF-AB; (d) VEGF; and (e) bFGF. bFGF, basic fibroblast growth factor.

TABLE 19 Results from primary outcome correlation assessments overall and by intervention group, for PRP sample key blood parameters

Parameter	*n*	*r*	% variance[a]	*p*-value
Blood cell counts				
Erythrocytes	91	0.126	1.590	0.234
Leucocytes	91	–0.103	1.053	0.333
Platelets[b]	88	0.128	1.649	0.233
Platelet quality				
Activated CD62P expression (%)	92	0.038	0.142	0.721
MFI	92	–0.004	0.002	0.966
Growth factors				
IGF-1	93	0.124	1.248	0.287
TGF-β1	88	0.005	0.003	0.960
PDGF-AB	90	< 0.001	< 0.001	0.998
VEGF	93	–0.231	5.355	0.026
bFGF	93	–0.102	1.049	0.329

bFGF, basic fibroblast growth factor; *r*, Pearson's product-moment correlation coefficient.
a Proportion of variance in LSI explained by key blood parameter, calculated as ($R^2 \times 100$).
b As PLT-F.

The red blood cell, white blood cell and platelet (as PLT-F) counts and work LSI at 24 weeks linear regression analysis showed no significant correlation (see *Figure 18*). This was also the case for the platelet-quality measures of interest (see *Figure 19*) and for all growth factors (see *Figure 20*) with the exception of VEGF, for which the evidence suggests a negative correlation between PRP VEGF concentration and week 24 LSI performance ($p = 0.026$), with around 5% of the variance in LSI being explained by this growth factor.

Discussion

The results showed that the whole-blood parameters in participants' venous blood samples were identical in both groups immediately prior to injection of PRP or placebo. As it is possible that variation in platelets and leucocytes in whole blood may play a role in the healing process of tendons, these results confirmed that there was no baseline blood variation between the groups.

To our knowledge, this is the largest analysis of PRP in a multicentre RCT in a musculoskeletal disease or injury setting. The results of this substudy showed that the centrifuging method in this population produced L-PRP. The average platelet count in PRP was > 4 times that in whole blood. The leucocyte concentration was 2.2 times the whole-blood leucocyte concentration. This may have an influence on the healing process as both cells play a crucial role in the initial tissue response to an injury.

The growth factors IGF-1, TGF-β1, PDGF-AB and VEGF are reportedly the greatest contributors to tissue healing in PRP. Previous work has identified a need for growth factor analysis so that the interpatient variation in different parameters can be properly explored. Here, the results of such an analysis on 103 samples are presented. The hypothesis that there would be a significant positive relationship between growth factor concentrations and platelet and leucocyte concentrations was mostly supported in this study. Most of the growth factors had a significant or moderate association with platelet concentrations. The lack of association with IGF-1 supports the opinion that platelets are not the major contributors of that factor.

An important finding of the growth factors analysis was the large degree of variation exhibited between participants, even when corrected for platelet concentration. Using correlation and regression analysis, statistically significant associations have been identified between the concentrations of TGF-β1, PDGF-AB, VEGF, basic fibroblast growth factor (bFGF), platelets and leucocytes. These relationships may contextualise the understanding of the biological mechanisms at work in PRP tissue response.

The relationship between product-variable biological content and clinical effect was presented. The key finding was that there was no association of either the cellular or growth factors' content with the primary outcome measure. The level of platelet activation as a measure of platelet quality at baseline also did not correlate with the clinical outcome. Baseline blood was also assessed to make sure that there was no variation between the two groups. There was no correlation of the cellular component of whole blood on the primary outcome.

The results of substudy 1 confirm that variability in this biological product did not change the outcome of Achilles tendon injury management.

Chapter 5 Substudy 2: immunohistochemistry study of needle biopsies from Achilles tendon rupture lesion site

Introduction

Torn and healing tendons progress through stages of scar tissue formation and remodelling, in which processes of cell death, inflammation, matrix breakdown, stem cell migration, proliferation, revascularisation, tenocyte differentiation, matrix synthesis and matrix remodelling are evident.[76,77] Although increased cellularity and vascular invasion are essential features of initial scar tissue formation, successful remodelling requires a marked reduction in both to levels that are normal for healthy tendon. Critically, the collagen matrix must resolve from small, randomly aligned fibres to large-diameter fibres with high packing density, aligned with tensile loading forces. In addition, existing undamaged collagen bundles must be knitted back into the new tissue. At the same time, a return to normal loading is essential in restoring normal structure and, therefore, mechanical function.[34,78] Why some tendons heal fully but many remain permanently in a scar tissue or fibrotic stage is the necessary focus of much current research, as the dosing and timing of potentially beneficial growth factor, stem cell and mechanical therapies require considerable optimisation.[79] In this needle biopsy substudy, the effects of PRP were investigated 6 weeks after rupture and treatment using relevant cellular markers.

Methods

Sixteen participants (nine in the PRP group and seven in the placebo group) were enrolled from a single selected trial site and gave consent to undergo the sample collection procedure at 6 weeks post rupture. Both the ultrasound radiologists and the tissue investigators were blinded to treatment allocation until analysis was completed. A biopsy from the mid-lesion site was taken under guided ultrasound using a 14-gauge needle (Temno Evolution soft tissue biopsy needle; Merit Medical Systems, South Jordan, UT, USA) slanted diagonally through the mid-line of the Achilles tendon, as previously developed by the trial team.[57] Tissue was collected into ice-cold phosphate-buffered saline and dissected immediately. Two-thirds of the biopsy was fixed in 10% formalin, embedded in paraffin wax and sectioned longitudinally at 4-µm thickness. One-third of the biopsy was snap frozen at –80 °C for follow-up studies.

Sections were stained by hand with routine haematoxylin and eosin or with picrosirius red (10 minutes in 0.1% sirius red dissolved in 1% picric acid). Blood vessels were stained using a Dako Autostainer Link 48 for an angiogenesis marker [i.e. CD31 (Abcam clone C31.3; ab187377)] (Dako Agilent, Santa Clara, CA, USA). A relevant mouse isotype control was undertaken and the detection system was horseradish peroxidase/DAB (3,3′-diaminobenzidine) with haematoxylin counterstain (Envisage FLEX; Dako Agilent, Santa Clara, CA, USA).

Immunohistochemistry sections were imaged to include most of the biopsy area for CD31 (six viewing fields at 200× magnification). Total cell count was obtained from haematoxylin-stained sections, using automatic nuclear counts from six random fields at 400× magnification (ImageJ version 1.51s; http://imagej.nih.gov/ij; accessed February 2019). Collagen density was assessed in four fields of view per section. In all fields of view, large blood vessels and adipose tissue were avoided. Images were scored by a blinded assessor. A modified Bonar scale[81] was used and included analysis of the following components relevant to healing: (1) cellularity, (2) collagen fibre density and (3) vascularity. Each variable was scored on a 4-point scale of 0–3 (0, normal; 1, slightly abnormal; 2, abnormal; and 3, markedly abnormal). The samples were scored according to whether or not there was an abnormal appearance (i.e. hypercellular, hypervascular, low collagen). The total score

varied between 0 (normal tendon) and 9 (severe abnormality).[80,81] Treatment groups were compared using two-tailed Mann–Whitney *U*-tests, with significance taken as a *p*-value of < 0.05.

Results

The bulk of tissue in all biopsies comprised abnormal tendon tissue in the process of active remodelling. There were very few intact mature collagen bundles, while cellularity and vascularity were typically high compared with normal tissue. One of the biopsies was severely compromised in cellularity and vascularity, with minimal intact collagen. It is not possible to assess whether this was prevalent ahead of the rupture injury or developed subsequently, but tissue repair could be challenging with few matrix-secreting cells and absence of vascular remodelling, and this biopsy was scored as 3 (or abnormal) for all three Bonar categories, as previously suggested.[81] The participant in question did not have a re-rupture, suggesting that the biopsy was not representative of the whole lesion.

In exploratory analysis, there were no detectable differences between placebo and PRP-treated groups in either cellularity or vascularity (*Table 20*). However, the PRP group had lower collagen fibre density. Overall, the combined Bonar score was higher for the PRP group.

Platelet-rich plasma quality was measured in substudy 1 for all participants randomised to receive PRP injection. The nine participants from whom biopsies were obtained all received good-quality PRP with low platelet activation on collection and containing high growth factor levels after activation.

Discussion

Biopsies were obtained from 16 participants from one centre who consented to this component of the study. This substudy was intentionally exploratory so the sample size was accordingly limited and not powered for detecting between-group differences in specific measurements.

Tendon healing at 6 weeks after an acute rupture should have significantly progressed but will not be complete. Marked increases in both cellularity and vascularity must happen in order for healing to occur but should then reduce to normal tendon levels. In humans, collagen synthesis and remodelling can be expected to continue for months after the injury. Although Bonar and Movin scores have been developed to assess tendinopathy rather than recovery from injury, they are frequently used for both.[81,82] It is, in fact, difficult to separate the two processes in a biopsy as many tendons will have ruptured because of prior tendinopathy.[8] In this study, the centre of the rupture lesion was biopsied and the main difference in interpretation of scores is that an injured tendon in the process of healing has high cellularity, reduced and more-diffuse collagen and elevated vascularity, which are also common signs of tendinopathy in an uninjured tendinopathic biopsy. A badly compromised injured tendon will have very few cells (low score), massive collagen loss (high score) and few blood vessels (low score), which, overall, could score quite low or 'good' on a tendinopathy scale, but is clearly abnormal. It is therefore useful to consider relative differences between groups rather than absolute score.

Although all biopsies except one showed evidence of healing at 6 weeks, there was less collagen fibre density in the PRP group. This did not correlate with differences in cellularity or vascularity, as these parameters were similar in both groups, suggesting equivalent healing processes. There are several possible explanations. The collagen may be forming more slowly in the PRP-treated group or may have been subjected to an early phase of breakdown and is still catching up at 6 weeks. This was a small study of 16 biopsies, which might be prone to chance allocation. Ultimately, the speed of return to full function and the long-term re-rupture rate will indicate whether this difference in healing process is beneficial, negative or unimportant.

TABLE 20 Statistical analysis of the groups according to Bonar scoring

	Intervention group, Bonar score								
	Placebo				PRP				
Bonar score	Mean (SD)	Median (IQR)	Frequency ($n = 7$)	Relative frequency	Mean (SD)	Median (IQR)	Frequency ($n = 9$)	Relative frequency	*p*-value
Cellularity[a]	1.71 (0.49)	2 (1–2)	5	0.71	1.89 (0.60)	2 (1.5–2)	6	0.67	0.665
Collagen stain[b]	1.14 (0.38)	1 (1–1)	6	0.86	2.00 (0.50)	2 (2–2)	7	0.78	0.007
Vascularity[c]	0.42 (0.53)	0 (0–1)	4	0.57	1.11 (1.05)	1 (0–2)	3	0.33	0.201
Bonar score total[d]	3.28 (0.76)	3 (3–4)	3	0.43	5.00 (1.87)	5 (3.5–6)	2	0.22	0.038

IQR, interquartile range.
a Cellularity score: 0 = low normal, 1 = moderate, 2 = high, 3 = abnormal.
b Collagen-density score: 0 = dense, 1 = good, 2 = dispersed, 3 = low/abnormal.
c Vascularity score: 0 = low normal, 1 = moderate, 2 = high, 3 = abnormal.
d Bonar total: 0 = normal tendon, 1 or 2 = healing tendon, 3 = abnormal tendon.

Chapter 6 Discussion

In this chapter, we summarise the main findings of the clinical trial and both substudies before considering the internal and external validity of the trial. Interpretation of the results and their relationship with other literature are discussed, followed by consideration of the implications for clinical practice and future research.

Aim and overview of clinical trial findings

Our primary objective was to evaluate the clinical efficacy of PRP among patients with acute ATR using an objective measure of muscle–tendon function, the LSI of work performed during the HRET at 24 weeks post randomisation. PROMs were used to study function, symptoms, pain and quality of life. An analysis of whole-blood and PRP biological components was embedded in the trial in order to determine key components of PRP that may contribute to its mechanism of action and to enhance quality assurance during the trial. A second substudy to explore the possible tissue and immunohistochemical effects of PRP on the healing tendon tissue compared with those of the control was also embedded in the trial and included 16 participants. Investigating efficacy and mechanism together in the present trial provides robust information on PRP application in ATR and offers a rigorous platform for future research into PRP applications in musculoskeletal problems.

This was a prospective, multicentre, parallel-group, participant- and outcome assessor-blinded, randomised, placebo-controlled superiority trial. A total of 230 patients presenting with acute ATR within 12 days of injury were recruited in 19 NHS hospitals in England and Wales.

We found that PRP was not effective in improving muscle–tendon function 24 weeks after acute ATR. There were also no differences in the secondary outcome measures of patient-reported function, symptoms, quality of life and pain at 24 weeks. There were no statistical differences in all secondary outcome measures between the PRP group and the placebo group throughout the follow-up period, indicating that there was no early acceleration of recovery. Including stratification factors and other predefined prognostic variables in the analysis, when relevant, had no impact on the attained primary and secondary outcome results. CACE and sensitivity analyses demonstrated that the conclusions were robust under a range of assumptions.

The PRP group and the control group had nearly the same rate of re-rupture, surgical repairs, swelling, DVT, skin breakdown or ulceration, swelling and casting-related complications. Secondary surgical intervention to repair the Achilles tendon was required by 4% (9/229) of participants; this was carried out on participants who failed non-surgical treatment and had re-rupture of the tendon.

Blood and platelet-rich plasma substudy findings

An analysis of whole-blood cellular composition showed that the two groups had similar baseline blood parameters. PRP analysis showed that the preparation method resulted in a mean 4.1-fold increase in platelet count and a 2.2-fold increase in white blood cell count compared with whole blood. The majority of platelets in the PRP were of good quality with low levels of activation. The PRP produced using the trial procedures was therefore functional, with the majority of platelets in the PRP preparations being shown to be capable of activation and degranulation, with a few exceptions. As would be expected, there was correlation between platelet and leucocyte counts and some growth factor concentrations.

Despite using a standardised preparation method to manage operator error, there was wide variation in platelet count, white blood cell count and growth factor concentrations; this indicates the variable nature of this biological product. However, this did not have an effect on the clinical outcome.

Clinical outcomes and platelet-rich plasma analysis correlation

Parameters of baseline whole blood taken before intervention in both groups did not correlate with the primary outcome measure at 24 weeks.

Overall, there was no association between PRP cellular or growth factor content on the primary outcome measure. The level of platelet activation and white blood cell concentration at baseline also did not correlate with the primary outcome. The results of this mechanistic analysis confirmed that any variability in this biological product did not change the outcome of ATR management.

Immunohistochemistry study findings

In substudy 2, with 16 participants, all except one biopsy showed evidence of healing at 6 weeks. There was less collagen fibre density in the PRP group. This did not correlate with differences in cellularity or vascularity, as these parameters were similar in both groups, suggesting equivalent healing processes.

The findings of this substudy could indicate that collagen was forming more slowly in the PRP group or was subjected to early breakdown at this early 6-week stage. However, this was a small-scale study, meaning that cautious interpretation is required.

The findings of this study could not be linked to the clinical outcome because of the small number of biopsies undertaken.

Internal validity and methodology

The trial was powered to be a definitive superiority trial and had lower than expected loss to follow-up. Blinding of participants and outcome assessors and low levels of missing data across the outcome measures strengthen the trial results. Allocation concealment was ensured through random computer allocation, stratified by site and age, on participant registration via a remote computer randomisation service. Analyses were pre-planned and agreed by the DSMC.

Platelet-rich plasma quality control and measurements in substudy 1 enabled ongoing monitoring during the trial so that any issues could be resolved. Measuring PRP activation and viability allowed us to ensure that viable PRP was injected and also allowed the correlation with the clinical primary outcome measure. During the initial phase of the trial, four PRP samples showed very low platelet counts. The trial was temporarily halted and preparation methods, devices and kit were tested. This was thought to be due to faulty sensors in some devices. Those devices and kits were replaced and the trial continued without any further issues of that nature.

Platelet-rich plasma products vary according to preparation techniques and devices.[83] In this trial, standardising the preparation method and providing the same kit and PRP preparation devices to all centres ensured that any significant variability due to different preparation techniques and devices was eliminated. The results of substudy 1 still show variability in PRP content and activation; however, this is probably due to the variable nature of this biological product rather than preparation methods.

Complier-average causal effect analysis was used to estimate the treatment effect while accounting for whether participants complied or did not comply with the treatment allocated to them (without assuming that compliers were the same as non-compliers as would be the case with PP analysis). The consistency between the conclusions of the mITT findings and CACE analysis improves the confidence in the findings of the PATH-2 trial.

The validation checks on the HRET (see *Appendix 3*) identified invalid data on a small number of participants and it was reassuring that the conclusions from the estimates with raw HRET data and those with valid data after the checks were consistent. To dismiss potential measurement errors, two members of the trial team who were blinded to treatment allocation reviewed the videos of all assessments independently and any invalid heel-raise repetitions included in the HRET data were discounted. The test was standardised through training the blinded assessors and by offering trial-specific guidance notes and videos.

In the context of the PATH-2 trial, the conclusions drawn from the work LSI are supported by the high precision of estimates and the consistency with the secondary outcomes (ATRS, PSFS, SF-12 and VAS).

On formal analysis, the success of the outcome assessor blinding at the end point was adequate. Both the James *et al.*[69] and Bang *et al.*[70] blinding indices gave evidence to support the assumption that, overall, participants in this trial were blinded. This demonstrates that the findings of this trial are extremely unlikely to be due to participants' expectations of treatment received.

External validity and generalisability

The trial was conducted in acute hospitals, from major trauma centres through to smaller district general hospitals, and, therefore, is representative of the range of settings for ATR management in the UK. The PRP and placebo interventions were delivered by NHS trauma and orthopaedic teams with relatively little trial-specific training. Rehabilitation guidelines were set to augment some standardisation of care for trial participants. All participants should therefore have had their injured lower limb immobilised for at least 3 weeks after injection, avoided more than 6 weeks of rigid full-time immobilisation without ankle motion or weight-bearing and received a referral for physiotherapy. Compliance with these different aspects was measured and was good, with no difference between treatment groups.

The demographics of the trial participants were representative of the age and sex profile expected of adults with ATR,[9] with the majority being male and aged < 55 years. Excluding patients with rupture of the Achilles tendon at the insertion or musculotendinous junction limits generalisability. However, the healing in these areas is usually better than that of mid-substance rupture.[8]

The primary end point at 24 weeks could be considered too early for definitive assessment of outcome. However, a difference in speed of healing should have been seen when the tendon is in the recovery phase, and our PATH-2 trial primary outcome measure at 24 weeks post injury is timed to capture this. In addition, the theoretical mode of action of PRP by enhancement of cellular response in the early inflammatory healing phase would be expected to peak in the early stages of Achilles tendon repair. A meta-analysis[32] demonstrated that improvement of patient outcomes from previously published studies of ATR showed that most improvement occurs in the first 6 months, which, therefore, supports this as the definitive assessment point.

There were no discernible differences between groups in relation to the grade of the clinician administering treatment. Notably, the clinicians were mostly consultant surgeons (75%) and surgical registrars (17%). A number of extended-scope physiotherapists and research fellows have applied the intervention, reflecting the range of clinicians in contemporary orthopaedic clinical practice.

Implications for clinical practice

Achilles tendon rupture leads to a major impairment in function, irrespective of the treatment received. The findings demonstrate that participants recovered < 40% of the muscle–tendon unit function at 24 weeks. This observation is also supported by the secondary outcome measures ATRS and PSFS. The majority of participants continued to experience pain at the end point, although of significantly less

severity than at baseline. This underlines the impact of this injury on individuals in terms of day-to-day activities, as well as the wider socioeconomic impact.

The PATH-2 trial results are important for clinicians practising in this field who are aware of PRP use and who may receive requests from patients to administer PRP by injection. These results challenge the growing use of PRP in ATR. For patients, who are increasingly being involved in decision-making about their treatments, these findings are an important contribution to that discussion. The similarity in the primary outcome findings at 24 weeks and for the patient-reported secondary outcomes throughout follow-up strongly support the validity of the conclusion that PRP does not improve the outcome of ATR management. Therefore, the application of PRP for this indication appears unjustified and without benefit.

The trial results also showed that injection is associated with some AEs at the injection site, with 11 out of 229 (5%) participants reporting frequent discomfort and 3 out of 229 (1%) participants reporting clinically diagnosed infections. However, no clinically serious infections were reported.

Re-rupture rates were low in both groups, but it should be noted that the trial was not powered to detect a between-group difference for this outcome.

Platelet-rich plasma application in clinical settings has a significant cost implication for health-care providers. Although we did not carry out a health economic analysis, applying PRP in ATR management will add to the cost of standard care. The cost of applying PRP is variable, but includes (1) a centrifuge, (2) a PRP disposable kit per patient, (3) an injection kit, (4) blood withdrawal and preparation time (15–30 minutes) and (5) surgeon or practitioner injection time (10 minutes).

In addition, PRP injection is usually administered in a fracture or trauma clinic in NHS hospitals, which are busy, with limited time and space allocation for each patient. The time taken to withdraw blood and prepare the PRP and the injection may have an impact on patient flow in those clinics. The injection is usually administered in a clinic room or cubicle and adds to the burden on clinic staff.

A recent report estimated that the value of the PRP market was US$214.3M in 2016, which is expected to reach US$625M globally by 2025.[84] This market estimate does not include the cost to health-care providers in terms of staff and clinic space; however, it does include PRP applications in all specialties. PRP application in sport injuries in Europe is rapidly increasing.[85] Current PRP cost in ATR is unknown but likely to be growing.[85] The findings of this trial will help inform clinical decision-makers, commissioners and payers for health-care services and may limit the cost burden of PRP in ATR management.

Limitations

In the PATH-2 trial, we used and assessed a single PRP preparation system based on a previous study[86] to standardise the procedure. There are three methods to prepare PRP: (1) gravitational platelet sequestration or centrifugation, (2) standard cell separators and (3) selective filtration technology or plateletpheresis. Clinically approved devices use one of these methods to produce PRP from autologous blood with varying concentrations and platelet yields. We selected a fully automated system that utilises infrared sensors to detect platelet layers in the centrifuged blood and automatically load it to a syringe, ready to be injected. Our tests of this device and the PRP method in the pilot study importantly demonstrated a viable product and a reliable preparation system. All PRP preparation devices produce varying concentrations of platelets and white blood cells; however, we have shown in this trial that there was no link between platelet count or white blood cell count and the clinical effect.

Nine per cent of participants in the PRP group did not receive the allocated treatment. The allocated intervention for participants in the PRP group was dependent on the ability to withdraw sufficient whole blood to produce the required 8-ml PRP sample, a fully working centrifuge, an available in-date PRP

preparation kit and patients willing to wait the time for the preparation. This reflects the reality of this intervention in usual acute NHS hospital settings.

Standardising the rehabilitation protocol across sites can hinder the pragmatic approach of the trial. Within an agreed common approach, we allowed rehabilitation to be tailored to the participant and local provision. All participants had to be referred for physiotherapy but then it was the responsibility of the therapy service to provide what was their usual care. This pragmatic approach may have resulted in some site-to-site variation in provision, but we stratified randomisation and adjusted primary treatment estimates on centre to help manage this.

Documentation of medication that may have an effect on platelet function, such as aspirin or clopidogrel, was not requested until after the trial had started; therefore, information on this was available for only 65% of patients. Only 5.7% of patients who were asked about this were on antiplatelet medication, and this was similar between the two treatment arms; therefore, it was unlikely to have an influence on outcomes.

The timing of the primary end point (24 weeks) may be considered early for final functional recovery of the tendon. However, the results indicate no effect at this early stage when PRP was expected to exert its maximum effect in enhancing recovery. A 2-year follow-up of patient-reported data is being conducted and the results will be reported in due course.

The number of histological study biopsies was too small to secure statistically significant results of the effect of PRP on the tendon tissues. The substudy findings were intentionally considered exploratory and indicative rather than confirmative of the changes in the healing tendon. The limited number of biopsies is within the generally accepted range for similar studies on human participants.

Further research

The PATH-2 trial found no evidence of efficacy for PRP in the treatment of acute ATR. The implications are that similarly robust clinical trials are recommended for investigating the efficacy of PRP applications for other musculoskeletal disorders.

The extent of muscle–tendon impairment identified in the HRET in the injured limb, irrespective of treatment group, highlights the degree of ongoing musculotendinous unit compromise 6 months after tendon rupture. Although musculotendinous impairment has been found in other ATR cohorts,[35] the extent of asymmetry between injured and uninjured legs in this trial was substantial. Optimising recovery of neuromuscular function during rehabilitation is therefore a recommended area of future investigation.

There were some variations in the non-surgical management of acute ATRs within the range permitted in the standardised rehabilitation guidelines for the PATH-2 trial. An ongoing clinical trial funded by the NIHR Health Technology Assessment programme (project number 13/115/62) will provide additional evidence regarding the clinical effectiveness and cost-effectiveness of two commonly used protocols: plaster casting versus a rigid functional brace with immediate weight-bearing.[87]

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Contributions of authors

Joseph Alsousou (NIHR Academic Clinical Fellow in Orthopaedics) developed the trial conception, and intervention, designed the trial, developed substudies 1 and 2 and wrote and reviewed the report.

David J Keene (NIHR Postdoctoral Research Fellow, University of Oxford) developed the trial conception, and intervention, was clinical co-ordinator, designed the trial, delivered intervention training and wrote and reviewed the report.

Paul Harrison (Senior Lecturer, University of Birmingham) designed and led substudy 1, conducted the blood and PRP analysis and wrote the report.

Philippa Hulley (Associate Professor, University of Oxford) designed and led substudy 2, conducted the immunohistochemistry analysis and wrote the report.

Susan Wagland (Clinical Trials Manager, University of Oxford) managed and supervised the trial, and wrote and reviewed the report.

Jacqueline Y Thompson (Research Physiotherapist, University of Oxford) delivered the HRET training and wrote the report.

Scott R Parsons (Trial Co-ordinator, University of Oxford) provided administrative and project co-ordination support, and reviewed the report.

Christopher Byrne (Lecturer in Physiotherapy, University of Plymouth) developed the HRET assessment, delivered the HRET training and wrote the report.

Michael M Schlüssel (Medical Statistician, University of Oxford) was trial statistician, conducted the statistical analysis and wrote the report.

Heather M O'Connor (Medical Statistician, University of Oxford) was trial statistician, conducted the statistical analysis and wrote the report.

Susan J Dutton (University Lecturer and OCTRU Lead Statistician, University of Oxford) was senior trial statistician, conducted the statistical analysis and wrote the report.

Sarah E Lamb (Professor in Rehabilitation and Director of Centre for Statistics in Medicine, University of Oxford) developed the trial conception and design, and reviewed the report.

Keith Willett (Professor in Orthopaedic Trauma Surgery, University of Oxford; Medical Director for Acute Care, NHS England) was chief investigator, led the funding application, trial conception and design, and development of interventions, provided overall trial supervision and wrote and reviewed the report.

The PATH-2 trial team

The following members of staff were members of the central trial team:

- Ramona Barbu, Clinical Trial Support Officer
- Christopher Byrne, Research Physiotherapist
- Daryl Hagan, Clinical Trial Administrative Co-ordinator
- David Keene, Research Physiotherapist, Research Fellow
- Lesley Morgan, Clinical Trial Manager
- Scott Parsons, Clinical Trial Administrative Co-ordinator
- Emma Roberts, Clinical Trial Administrative Co-ordinator
- Jacqueline Y Thompson, Research Physiotherapist
- Susan Wagland, Clinical Trial Manager
- Keith Willett, Chief Investigator.

Trial Management Group

- Chief Investigator: Professor Keith Willett.
- Trial Co-investigators: Mr Joseph Alsousou, Dr David J Keene, Dr Paul Harrison, Dr Philippa Hulley, Mrs Susan J Dutton, Professor Sarah E Lamb.
- Trial Manager: Dr Susan Wagland.
- Trial Co-ordinator and Administrator: Dr Scott Parsons.
- Trial Statisticians: Heather M O'Connor, Dr Michael M Schlüssel, Mrs Susan J Dutton.

Principal investigators and research associates by centre

PI	Research associates
Oxford University Hospitals NHS Foundation Trust	
Robert Handley	Kathryn Lewis
	Louise Spoors
	Yuhan Zhang
	Maria Mestre
	Doreeen Muller
	Sangeetha Prasath
	Martin Austin
University Hospitals of Leicester NHS Trust	
Maneesh Bhatia	Manjit Attwal
	Carla Christie
	Tracy Brear
Taunton and Somerset NHS Foundation Trust	
Andrew Kelly	Keira Beacham
	Sarah Brown
	James Allen
	Alison Whitcher
	Kate James
	Eliza Foster

PI	Research associates
North Bristol NHS Trust	
Steve Hepple	Steven Barnfield
	Katherine Coates
	Ruth Halliday
	Lucille Hooper
Cardiff and Vale University Health Board	
Paul Hodgson	Helen Hodgson
	Cheryl Cleary
	Claire Nott
	Jessica Whiteman
Shrewsbury and Telford Hospital NHS Trust	
Michael Carmont	Louise Tonks
Barts Health NHS Trust	
Nima Heidari	Jamila Kassam
	Catherine Hilton
	Shanaz Ahmad
	Sophie Young
University Hospitals Coventry and Warwickshire NHS Trust	
Jonathan Young	Louise Clarkson
	Sylvia Turner
	Kerri McGowan
	Janet Lowe
Warrington and Halton Hospitals NHS Foundation Trust	
Gareth Stables	Kerry Bunworth
	Pascal de Feyter
Basildon and Thurrock University Hospitals NHS Foundation Trust	
Ranjith Ravindran	Mark Vertue
Royal Liverpool and Broadgreen University Hospitals NHS Trust	
Simon Frostick (co-PI: Joseph Alsousou)	Chris Walker
	Sivia Sirikonda
	Anjani Sighn
	Joanne Stokes-Denson
	Lisa Watson
	Deborah Scanlon
	Philip Walker
Peterborough and Stamford Hospitals NHS Foundation Trust	
James Carmichael	Susie O'Sullivan
	Deborah Butcher

PI	Research associates
Abertawe Bro Morgannwg University Health Board	
Claire Topliss (co-PI: Anne-Marie Hutchison)	Sharon Storton
	Amanda Cook
	Leanne Quinn
Aintree University Hospital NHS Foundation Trust	
Lyndon Mason (co-PI: Joseph Alsousou)	Phil Ellison
	Andrew Molly
	Joanne Earley
University Hospital of South Manchester NHS Foundation Trust	
Moez Ballal	Joanne Bradley-Potts
	Georgina Williamson
	Richard McCormick
Sheffield Teaching Hospitals NHS Foundation Trust	
Mark Davies	Julie Walker
	Joanne Badloe
Royal Devon and Exeter NHS Foundation Trust	
Adrian Hughes	Anna Grice
	Jayne Dark
Mid Cheshire Hospitals NHS Foundation Trust	
Simon Barnes (co-PI: Jagan Velpula)	Kelly Amor
	Janet Brown
Royal Surrey County NHS Foundation Trust	
Matthew Solan	

Trial Steering Committee

Professor Roger Smith (Chairperson), Dr Catriona Graham, Dr Dylan Morrissey, Professor John O'Byrne, Dr Gustaaf Reurink and Mrs Sarah Webb.

Data and Safety Monitoring Committee

Dr Chao Huang (Chairperson), Mr Andy Goldberg and Dr Carey McClellan.

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Publications

Keene DJ, Alsousou J, Willett K. How effective are platelet rich plasma injections in treating musculoskeletal soft tissue injuries? *BMJ* 2016;**352**:i517.

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Data-sharing statement

All data requests should be submitted to the corresponding author for consideration. Access to available anonymised data may be granted following review.

Patient data

This work uses data provided by patients and collected by the NHS as part of their care and support. Using patient data is vital to improve health and care for everyone. There is huge potential to make better use of information from people's patient records, to understand more about disease, develop new treatments, monitor safety, and plan NHS services. Patient data should be kept safe and secure, to protect everyone's privacy, and it's important that there are safeguards to make sure that it is stored and used responsibly. Everyone should be able to find out about how patient data are used. #datasaveslives You can find out more about the background to this citation here: https://understandingpatientdata.org.uk/data-citation.

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Appendix 1 The PATH-2 trial: trial management

Introduction

The PATH-2 trial completed recruitment on schedule against the plans agreed with the funder (*Figure 21*) and also obtained very good levels of follow-up and a relatively low number of missing outcome data. We believe that an experienced, dedicated, trial management team that could apply the same depth for both the scientific elements and the clinical elements of the study in addition to the dedication of the clinicians and researchers at the recruiting hospitals, was critical to the success of the PATH-2 trial.

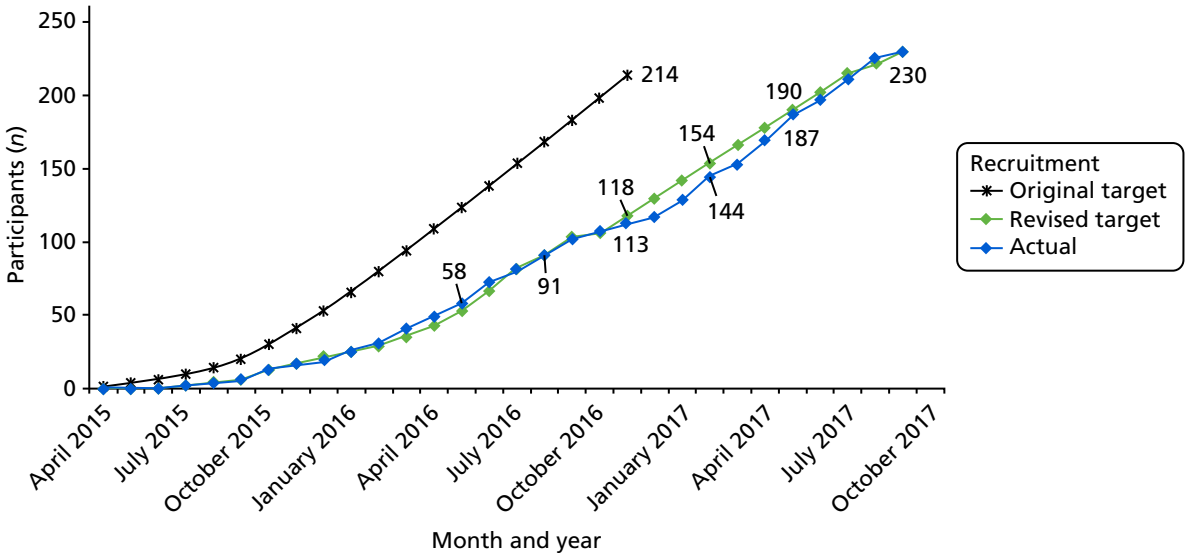

FIGURE 21 The PATH-2 trial recruitment against the original and revised targets.

Management milestones

TABLE 21 The PATH-2 trial management milestones

Planned activity to be completed	Project month in project management plan agreed with funder, version 2.0, August 2014	Project month in revised project management plan version 4.0, September 2017	Actual month or date
First TMG meeting, finalise protocol	1 (October 2014)		21 March 2014
Obtain ethics approval	3 (December 2014)		11 November 2014
First DSMC meeting	6 (March 2015)		First DSMC/TSC meeting, 31 July 2015
Second DSMC meeting	16 (January 2016)		First DSMC meeting, 22 January 2016
Second TSC meeting	17 (February 2016)		First TSC meeting, 4 March 2016

continued

TABLE 21 The PATH-2 trial management milestones (*continued*)

Planned activity to be completed	Project month in project management plan agreed with funder, version 2.0, August 2014	Project month in revised project management plan version 4.0, September 2017	Actual month or date
15 sites open to recruitment (sites recruitment ends)	18 (March 2016)	Superseded; recruitment target changed	
			Second DSMC meeting, 22 July 2016
214 patients recruited (recruitment closes)	26 (November 2016)	Superseded; recruitment target changed	
Substudy 2: 16 biopsies taken place (recruitment to substudy 2 closes)	26 (November 2016)	October 2016	11 August 2016
Substudy 1: 107/214 PRP/blood samples collected for analysis	27 (December 2016)	Superseded; recruitment target changed	
Substudy 1: platelet analysis finalised	27 (December 2017)	November 2017	March 2018
Third DSMC meeting	28 (January 2017)	January 2017	Third DSMC meeting, 3 February 2017
Third TSC meeting	29 (February 2017)	October 2016	Second TSC meeting, 14 October 2016
214 24-week follow-ups completed (primary outcome data collection ends)	32 (May 2017)	Superseded; recruitment target changed	
Substudy 2: biopsy analysis finalised	32 (May 2017)	May 2017	April 2018
Final data cleaning	32 (May 2017)	March 2018	June 2018
Fourth TSC meeting		July 2017	Third TSC meeting, 21 July 2017
Substudy 1: growth factor analysis finalised	34 (July 2017)	August 2017	March 2018
230 patients recruited (recruitment closes)		September 2017	18 September 2017
Substudy 1: 115/230 PRP/blood samples collected for analysis		September 2017	September 2017
			Second DSMC/TSC meeting, 29 June 2018
Site close-out completed		June 2018	Ongoing
Data analysis	34 (July 2017)	July 2018	July 2018
Write-up of draft final report and publications	35 (August 2017)	April 2018	14 August 2018
Dissemination of findings	35 (August 2017)	July 2018	August 2019

Recruitment target and site selection

The original application predicted a recruitment rate of one patient per participating centre per month, which required a total of 15 sites to be opened at the rate of one per month.

Some sites expressed interest in the trial after seeing a notice on the NIHR website or information disseminated through the clinical research networks. Sites that had participated in our previous AIM (Ankle Injury Management) trial[88] and sites with a surgeon who was a member of the British Orthopaedic Foot and Ankle Society were approached if it was thought that their catchment area was large enough.

Sites then completed a site feasibility questionnaire, which included their predicted recruitment rate. We continued negotiation with sites that predicted at least one participant per month after allowing for patients opting for surgery and patients who declined to participate. Site initiation visits were held, mostly in person, although some via teleconference, at which the remaining procedures were discussed and any potential problems were raised.

Establishing collaborating sites was hampered due to their inability to obtain excess treatment costs (ETCs), which were required to purchase trial-specific centrifuges for trial treatment. Sites could not obtain local NHS trust research and development approval until funds for the equipment were in place. The chief investigator purchased a small number of centrifuges, allowing two sites to open to recruitment, while addressing the ETC issue, and in December 2015 the Department of Health and Social Care agreed that the PATH-2 trial could apply for a subvention to purchase the equipment. There was further delay while the centrifuges were shipped from the US manufacturer. The start of patient recruitment was delayed from April 2015 to mid-July 2015. The funder was informed of every development throughout.

In February 2016, the recruitment forecast was re-profiled based on the delay to the start of recruitment and the rate of recruitment at that time (0.75 participants per site per month) and the go/no-go (G/NG) decision point 2 was pushed back from 1 May 2016 to 1 July 2016. One site closed early by mutual agreement with the PI as they had not recruited any participants and all patients were choosing surgery, which made them ineligible for the PATH-2 trial. It was agreed that four more sites would be opened to guard against lower than expected recruitment in some newly opened sites. Finally, 18 sites were opened to recruitment.

In July 2016, after the stop/go decision point, a 9-month extension was agreed with the funder, moving the expected end of recruitment to July 2017.

In June 2017, with approximately half of the sample having reached their 24-week follow-up point, variability was looked at for 75 participants with valid cleaned data (SD 24), and found to be greater than that assumed for the sample size calculation (SD 20). To maintain the integrity of the trial data, we proposed continuing recruitment to 229 participants, which would give 80% power to detect a 10% difference (standardised effect size of 0.5) with 20% loss to follow-up (although this was running at 15%). This meant extending recruitment into September 2017, instead of finishing at the end of July 2017 as planned. The DSMC assisted with the proposal and recommended this course of action, and it was supported by the TSC. The funder approved a 2-month no-cost extension and the recruitment target was raised to 230, which was reached in September 2017.

Go/no-go assessment

Go/no-go decision points formed part of the contract with the Department of Health and Social Care for the deliverability of the PATH-2 trial. The purpose of G/NG decision points is for the Efficacy and Mechanism Evaluation programme to review whether or not funding should continue at each identified stage of the project. Two G/NG decision points were specified as follows:

1. Go/no-go decision point 1 (G/NG 1). The milestone for G/NG 1 was ethics approval being sought and approved within 2 months of the project start date. As the project start date was 1 October 2014 and favourable ethics opinion was granted on 11 November 2014, this milestone was achieved.
2. Go/no-go decision point 2 (G/NG 2). The milestones for G/NG 2 were to be assessed in May 2016, 10 months into the recruitment stage, when approximately 50 participants were to have been recruited.

The DSMC was requested to report on the following milestones for G/NG 2:

- safety of the intervention
- site recruitment (consider stopping if seven out of a planned 15 sites were not recruited)
- participant recruitment (consider stopping if < 20% of eligible patients who were approached had consented)
- intervention compliance (consider stopping if < 80% of participants who started their allocated treatment were compliant)
- follow-up compliance (consider stopping if loss to follow-up at week 24 was > 30%).

Data on safety, trial conduct and feasibility were reported to the DSMC, which made recommendations about the continuation or otherwise of the trial to the TSC, which made the final decision about the continuation or otherwise of the trial.

In October 2016, the funder approved an amendment to extend recruitment by 9 months and the G/NG position was moved to 1 July 2016. The reasons for the extension were as follows:

- Delay in site recruitment – establishing collaborating sites was hampered due to sites' inability to obtain ETCs, which were required to purchase upfront trial-specific centrifuges for trial treatment. In January 2016, we received formal notice that a subvention would be provided by the Department of Health and Social Care to cover the ETCs (to purchase the centrifuges). We proceeded to order centrifuges for trial sites from the supplier.
- Sites halting recruitment for 1 month because of a quality issue with PRP samples that were produced in the trial-specific centrifuges. The issue was investigated with the help of the supplier and did not recur.

Following a database lock on 4 July 2016, the DSMC chairperson wrote to the funder to confirm that the milestones had been met as follows:

- Number of sites open to recruitment (seven expected) – 12.
- Proportion of patients consented/eligible (at least 20%) – 65% (75/116).
- Number of participants randomised (50 minimum) – 75.
- Compliance with intervention (80% minimum) – 96%.
- Losses to follow-up (< 30%) – 0%.
- Safety – no SAEs were reported by the randomised participants up to the stop/go point. Six AEs were reported.

The DSMC confirmed that the trial met the G/NG milestones and that the trial should continue.

Monitoring of trial recruitment

Sites completed a monthly screening log, declaring all patients who had presented to the emergency department or specialist fracture clinic or foot and ankle clinic with a suspected ATR. Reasons for not recruiting were monitored centrally and trends were identified. In response, certain eligibility criteria were revisited early in the trial: sites with a single weekly fracture clinic could not always randomise participants within the required 7 days from injury, so the criterion was changed to within 12 days from injury; some otherwise-healthy patients were excluded because they were aged > 70 years, so the age criterion was removed and a criterion of 'ambulatory prior to injury' was implemented. These two changes to eligibility criteria were implemented in a change to the protocol in March 2016.

We were alert to repeated reports at certain sites, such as patients being missed because they were treated at the weekend when there were no research staff present, or higher than expected rates of patients choosing surgery. This led to discussions with the sites to explore possible solutions, and identification of one site where all patients were having surgery and no patients were recruited. Once this was recognised, it was decided to close the site, by agreement with the PI. A minority of patients declined participation because they were uncomfortable with injections or blood being taken, and it was discussed with sites how this could be introduced to the patients in a less concerning fashion.

Data management

According to the standard operating procedures of the OCTRU, data management procedures were defined in a data management plan. This covered trial databases and data handling, definition of critical data fields, forms and questionnaires used, how protocol deviations were recorded, data rulings, handling data deviations, data security and confidentiality, data set closure, archiving and data sharing. Each data management plan version was signed off by the chief investigator and trial statistician.

The monitoring plan determined the need for central and on-site data monitoring. All sites were monitored centrally, and 12 sites underwent a site monitoring visit, either routine ($n = 8$) or triggered by a concern ($n = 4$).

Statistics on data collection, data entry and query management were presented to each TMG meeting for oversight.

Trial promotion

Promotion to patients and the public

Each recruiting centre displayed an ethics-approved patient poster in the fracture clinic, to make patients aware of the trial before they were approached to participate.

The PATH-2 trial has a public-facing web page on the OCTRU trials website (https://path2.octru.ox.ac.uk/; accessed August 2019), where trial information, current recruitment figures and news are publicised. An additional page called Reach Target Event was created in April 2018 with the aim of encouraging site staff to screen and recruit all eligible patients. This was promoted as an alternative to a study day for site staff, and included videos about the trial and the scientific objectives, discussion forums, competitions and cartoons. The Reach Target Event page is now available to the public and was entered into NIHR's 'Let's Get Digital' competition in June 2017.

Promotion within the trauma and scientific communities

The PATH-2 trial design has been presented at:

- the fifth NIHR Musculoskeletal Trauma Trials Annual Meeting, Warwick, 11 January 2017
- the Trauma Orthopaedic Research Collaboration, Oxford, 6 October 2017
- the sixth NIHR Musculoskeletal Trauma Trials Annual Meeting, Bristol, 10 January 2018
- the fourth Annual Celebrating Trauma Research in the Thames Valley, Reading, 28 February 2018
- Tendon UK, Oxford, 11–12 April 2018.

Appendix 2 Development of heel-rise endurance test outcome measures

Background

The HRET was introduced over 30 years ago as an objective outcome measure for assessing functional status following ATR.[68] The HRET involves repetitive concentric–eccentric muscle actions of the plantar flexors in a single-leg stance until exhaustion, with performance quantified by the number of raises (repetitions) performed.[89] Subsequently, the HRET has been consistently employed as an outcome measure in studies of ATR rehabilitation.[86,90–95] However, reporting of test standardisation procedures has been poor and, to our knowledge, no universally acceptable test parameters have been published to date.[89] A systematic review of the test identified six key testing parameters required for adequate standardisation of the test that have been reported inconsistently: (1) ankle starting position, (2) knee starting position, (3) height of heel raise, (4) pace (raises per minute), (5) balance support and (6) test termination criteria.[89] In addition, the use of repetitions as the primary outcome measure in the majority of studies represents a crude and insensitive measure of function as this outcome provides little information on the quality (height) of movements performed. This is important as tendon elongation and disproportionate strength loss at the end of plantar flexor range are two impairments in ATR that are not adequately assessed by a simple measure of repetitions. Therefore, standardisation of testing parameters and selection of outcome measures that are sensitive to ATR functional impairments were the two key areas identified for test development.

Nilsson-Helander *et al.*[65] advanced HRET methodology by introducing a measuring device in the form of a linear displacement sensor attached to the participant's heel, which enables the height of each repetition to be measured and three outcome measures quantified: (1) number of repetitions, (2) total work in J and (3) maximum heel-rise height in cm. Their results revealed that work as an endurance measure and maximum height as a measure of tendon function are more sensitive impairment outcome measures than repetitions following ATR. For example, repetitions classified the percentage of patients having normal function (defined as injured limb performance of $\geq 90\%$ of the uninjured limb) at 6 and 12 months after ATR as 38% and 63%, respectively. This compared with 9% and 23% for work and 6% and 22% for maximum height at 6 and 12 months, respectively. Work and maximum height (but not repetitions) also demonstrated significant positive associations at 6 months post ATR with the Achilles tendon Total Rupture Score, a validated PROM being employed in the PATH-2 trial. Deficits at the 6- and 12-month follow-ups were 39% and 24% for endurance (work), 28% and 20% for maximum height and 26% and 5% for repetitions, respectively.[65] In addition, the deficit in maximum height was significantly and highly correlated with Achilles tendon elongation at 6 months ($r = -0.94$; $p = 0.002$) and 12 months ($r = -0.74$; $p = 0.037$).[96] Further studies have demonstrated that work and maximum height are sensitive to, and predictive of, long-term residual impairments.[97] Both remain significantly impaired at 2 years post ATR, with the majority of recovery occurring in the first 6 to 12 months,[97] and deficits at 12 months correlate with abnormal ankle movement and force during walking, jogging and jumping activities at 6 years post ATR.[98] On the basis of current evidence, the use of a linear encoder measurement device during the HRET to provide outcome measures of work and maximum height represents the best available method of assessing ATR rehabilitation in a clinical setting and was adopted for the PATH-2 trial.

Heel-rise endurance test methodology development

The aims of the HRET development work were to (1) develop standardisation procedures for the HRET, (2) develop clinician training material and participant information, (3) develop easy-to-use HRET software and secure data-transfer procedures for clinician outcome assessors and (4) determine the test–retest reliability of HRET outcome measures when applying our procedures.

Standardisation procedures

A linear encoder, consisting of a spring-loaded cord connected to a linear displacement sensor with a measurement resolution of 0.019 mm and a sample rate of 200 Hz, and data-collection software were loaned for pilot testing from a commercial supplier (Encoder and MUSCLELAB™ software, Ergotest Innovation A.S., Porsgrunn, Norway). The key testing parameters identified by Hébert-Losier *et al.*[89] were used to aid standardisation of the HRET. In addition, we developed a standardised warm-up for participants that was suitable for a clinical environment, consisting of 5 minutes of usual-pace walking followed by 10 double-leg heel raises. The following standardisation parameters were adopted: (1) ankle starting position of 10° dorsiflexion produced by conducting the HRET on a custom-made 10° incline box, (2) knee starting position of full extension, (3) height of each repetition to be as high as possible, (4) pace of 30 raises per minute guided by a digital metronome, (5) balance support by the fingertips only and (6) strictly defined test termination criteria. The criteria for the end of the HRET were that participants either stopped (i.e. volitional task failure) or were audibly instructed to stop with both feet flat on the box whenever any of the following test termination criteria were observed: (1) inability to keep pace with the metronome, (2) inability to maintain full knee extension of the standing leg or (3) using more than fingertip support. The desired end point was volitional task failure; however, outcome assessors were encouraged to use verbal prompts whenever the termination criteria were observed and to stop the test if the participant did not respond to two consecutive prompts. In accordance with Nilsson-Helander *et al.*,[65] three outcome measures were established: (1) total work in J, (2) number of repetitions and (3) maximum heel-rise height in cm. Each could be expressed as a LSI, with the injured limb performance as a percentag of the uninjured limb performance. Total work LSI was selected as the primary outcome measure as this indexe provides a measure of plantar flexor muscle endurance and Achilles tendon function as it incorporates the maximum height of each repetition. Total work (J) was computed as the product of body mass (kg), total vertical displacement (m) and the constant 9.807, converting kilopond metres to J.

Software and training materials

The MUSCLELAB™ software was deemed too complex for use in a clinical environment and a simpler clinician-facing software was designed by the PATH-2 trial team incorporating our own standardised procedures and produced by a commercial partner (PATH-2, MUSCLELAB™, Ergotest Innovation A.S.). Key features of this software were step-by-step instructions for the conduct of the test and the addition and integration of a video-recording of each test, which enabled a post-HRET evaluation of the integrity of each test by the PATH-2 trial team. Standardised participant information and instructions for the HRET involved the participant watching a video demonstration of the HRET and reading standardised written instructions detailing their expected conduct during the test and the test termination criteria. At test completion, the software also guides the clinician on secure participant HRET data transfer by encrypted USB. The HRET equipment and software were packaged with clinician training material, consisting of high-quality training videos created by the PATH-2 trial team and produced by Oxford Medical Illustration, and a training and reference manual. Face-to-face training would be delivered by a member of the PATH-2 trial team to each clinician outcome assessor prior to their first participant's 24-week follow-up appointment.

Heel-rise endurance test reliability study

Following the establishment of our standardisation procedures, a reliability study was conducted on 38 healthy participants [18 males and 20 females; mean age 36 years (SD 9 years)], mean body mass 71.5 kg (SD 15.3 kg). The findings were reported in accordance with guidelines for reporting reliability and agreement studies,[99] and were published in 2017.[100] Reliability was assessed by the intraclass correlation coefficient (ICC) and agreement was assessed by a range of measures including the standard error of measurement (SEM), coefficient of variation (CV) and 95% limits of agreement (LoA). Reliability for repetitions (ICC 0.77, 95% CI 0.66 to 0.85) was equivalent to work (ICC 0.84, 95% CI 0.76 to 0.89) and maximum height (ICC 0.85,

95% CI 0.77 to 0.90). Agreement for repetitions (SEM 6.7, 95% CI 5.8 to 7.9; CV 13.9%, 95% CI 11.9% to 16.8%; 95% LoA –1.9% ± 37.2%) was equivalent to work (SEM 419 J, 95% CI 361 J to 499 J; CV 13.1%, 95% CI 11.2% to 15.8%; 95% LoA 0.1% ± 34.8%), with maximum height being superior (SEM 0.8 cm, 95% CI 0.6 cm to 1.0 cm; CV 6.6%, 95% CI 5.7% to 7.9%; 95% LoA 1.3% ± 17.1%). We found that work and maximum height demonstrated acceptable reliability and agreement that were at least equivalent to those of the traditional repetitions measure.

Appendix 3 Additional results

Participant reporting of pain

One potential issue of injecting PRP into an ATR site is that, by introducing fluid into this area, increased pain may be experienced immediately after. However, there is a possibility that, over time, any pain experienced by participants may reduce faster if PRP has been received, due to the assistance in healing that is offered. As a result, any severe pain experienced by participants was explored, alongside the information relating to pain as reported in their pain diaries, completed daily for the first 2 weeks. The results from these explorations are given in *Table 22* and offer no evidence to suggest any difference between intervention groups in pain experienced.

Participant employment and academic characteristics

The academic and employment characteristics of participants, as recorded at baseline, are presented in *Table 23*.

Participants' pre-injury clinical characteristics

The health of an individual not only affects their risk of experiencing an injury but can also affect their healing. At recruitment, participants were excluded if they were identified to have any medical conditions that would make them unsuitable for this trial. However, at baseline, information on additional medical conditions and medications taken by participants was collected. The summary of this information by intervention group is given in *Table 24*.

TABLE 22 Pain reported by participants 24 weeks after injury, by intervention group

Pain measure	Intervention group, *n* (%)		Total (*N* = 229), *n* (%)
	PRP (*N* = 113)	Placebo (*N* = 116)	
Severe pain at the injection site requiring more than simple pain relief			
No	102 (90.27)	106 (91.38)	208 (90.83)
Yes	4 (3.54)	2 (1.72)	6 (2.62)
Missing	7 (6.19)	8 (6.90)	15 (6.55)
Pain reduction in first 2 weeks[a]			
No	18 (15.93)	21 (18.10)	39 (17.03)
Yes	74 (65.49)	63 (54.31)	137 (59.83)
Missing	21 (18.58)	32 (27.59)	53 (23.14)

a Calculated as VAS pain score on day 14 compared with VAS pain score on day 1.

TABLE 23 Academic and employment-related characteristics of participants, by intervention group

Academic/employment status	Intervention group, *n* (%)		
	PRP (*N* = 113)	Placebo (*N* = 116)	Total (*N* = 229), *n* (%)
Academic status			
Full-time student	0 (0)	4 (3.45)	4 (1.75)
Part-time student	2 (1.77)	0 (0)	2 (0.87)
Not studying	111 (98.23)	112 (96.55)	223 (91.38)
Employment status[a]			
> 40 hours/week	40 (36.04)	39 (34.82)	79 (35.43)
25–40 hours/week	46 (41.44)	49 (43.75)	95 (42.60)
10–25 hours/week	5 (4.50)	9 (8.04)	14 (6.28)
< 10 hours/week	2 (1.80)	2 (1.79)	4 (1.79)
Unemployed, looking for work	3 (2.70)	1 (0.89)	4 (1.79)
At home/not looking for paid employment	2 (1.80)	4 (3.57)	6 (2.69)
Unable to work owing to illness or disability	1 (0.90)	0 (0)	1 (0.45)
Fully retired (no paid work)	12 (10.81)	8 (7.14)	20 (8.97)
Type of work[b]			
Office based	42 (45.16)	47 (47.47)	89 (46.35)
Shop work or similar	5 (5.38)	4 (4.04)	9 (4.69)
Classroom or equivalent	11 (11.83)	11 (11.11)	22 (11.46)
Physical outside work	6 (6.45)	10 (10.10)	16 (8.33)
Physical indoor work	8 (8.60)	15 (15.15)	23 (11.98)
Mainly travel/on the road	6 (6.45)	3 (3.03)	9 (4.69)
Other	15 (16.13)	9 (9.09)	24 (12.50)
Childcare	0 (0)	1 (1.01)	1 (0.52)
Driving	4 (4.30)	0 (0)	4 (2.08)
Manual	1 (1.08)	3 (3.03)	4 (2.08)
Office or standing	10 (10.75)	5 (5.05)	15 (7.81)
Time spent on feet			
Most of the day	37 (32.74)	42 (36.21)	79 (34.5)
> 4 hours/day	29 (25.66)	26 (22.41)	55 (24.02)
< 4 hours/day	24 (21.24)	24 (20.69)	48 (20.96)
Not much time (mostly sitting)	23 (20.35)	24 (20.69)	47 (20.52)
Time spent driving			
Most of the day	5 (4.42)	3 (2.59)	8 (3.49)
> 4 hours/day	7 (6.19)	3 (2.59)	10 (4.37)
< 4 hours/day	49 (43.36)	47 (40.52)	96 (41.92)
Just to/from work	46 (40.71)	47 (40.52)	93 (40.61)
Do not drive	6 (5.31)	16 (13.79)	22 (9.61)

a Includes all participants not studying.
b Includes all participants in employment, irrespective of working hours.

TABLE 24 Clinical characteristics of participants, by intervention group

Clinical characteristics	Intervention group, *n* (%)		
	PRP (*N* = 113)	Placebo (*N* = 116)	Total (*N* = 229), *n* (%)
Medical condition in addition to tendon rupture			
Heart disease	2 (1.77)	3 (2.59)	5 (2.18)
Hypertension	10 (8.85)	14 (12.07)	24 (10.48)
Asthma/COPD	22 (19.47)	13 (11.21)	35 (15.28)
Parkinson's disease	0 (0)	0 (0)	0 (0)
Epilepsy	1 (0.88)	1 (0.86)	2 (0.87)
Liver disease	0 (0)	0 (0)	0 (0)
Stroke/mini-stroke (TIA)	3 (2.65)	0 (0)	3 (1.31)
Peptic ulcer	1 (0.88)	4 (3.45)	5 (2.18)
Cancer	6 (5.31)	3 (2.59)	9 (3.93)
DVT/pulmonary embolism	0 (0)	0 (0)	0 (0)
Osteoarthritis	7 (6.19)	4 (3.45)	11 (4.80)
Rheumatoid arthritis	3 (2.65)	4 (3.45)	7 (3.06)
Allergies	33 (29.20)	25 (21.55)	58 (25.33)
Other[a]	31 (27.43)	36 (31.03)	67 (29.26)
Number of comorbidities			
0	44 (38.94)	56 (48.28)	100 (43.67)
1	36 (31.86)	28 (24.14)	64 (27.95)
2	20 (17.7)	23 (19.83)	43 (18.78)
> 2	13 (11.50)	9 (7.76)	22 (9.61)
Taking medication for pain or inflammation before injury			
Yes	7 (6.19)	8 (6.90)	15 (6.55)
Nothing regular	19 (16.81)	17 (14.66)	36 (15.72)
No	87 (76.99)	90 (77.59)	177 (77.29)
Missing	0 (0)	1 (0.86)	1 (0.44)

COPD, chronic obstructive pulmonary disease; TIA, transient ischaemic attack.
a Other medical conditions reported were cardiovascular (*n* = 5), endocrine (*n* = 5), gastrointestinal (*n* = 8), haematological (*n* = 4), mental health related (*n* = 9), neurological (*n* = 2), physical musculoskeletal impairment (*n* = 23), respiratory (*n* = 2), visual (*n* = 1) and other (*n* = 8).

Success of blinding

The results from the James *et al.*[69] blinding index are given in *Table 25* and indicate that, overall, participants in this trial were completely blinded. However, the James *et al.*[69] index automatically assumes that participants reporting that they do not know their treatment allocation are reporting so honestly rather than to give a response they perceive to be desired by their interviewer or to simply avoid making a judgement. Furthermore, this index does not distinguish between treatments.[69] To determine the effect of each treatment group and ensure that 'don't know' responses are taken into account, the Bang *et al.*[70] index is also used to understand blinding. The Bang *et al.*[70] blinding index for the PRP group is positive, indicating that participants were more

TABLE 25 The James *et al.* and Bang *et al.* blinding index results, including participants reporting 'don't know' for treatment allocation

Blinding index	Index	Standard error	95% CI
James *et al.*[69]	0.817	0.026	0.775 to 0.859
Bang *et al.*[70]			
PRP group	0.144	0.066	0.036 to 0.252
Placebo group	–0.179	0.055	–0.269 to –0.089

likely to guess that they received a PRP injection than that they received the placebo treatment. However, the absolute value for this is < 0.2, indicating that blinding was likely to have been successful in this treatment group. Conversely, the Bang *et al.*[70] blinding index for the placebo group is negative, indicating that participants were more likely than those in the control group to guess that they received the PRP injection. However, as with the PRP group, the absolute value for this is < 0.2, indicating that blinding was likely to have been successful in this group. Taking the information from both blinding indices into account, there is no evidence to suggest that blinding in this trial was unsuccessful.

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