Platelet Rich Plasma in Achilles Tendon Healing

Study Title: A pragmatic multi-centre, blinded, randomised placebo-controlled trial comparing Platelet Rich Plasma injection (PRP) to placebo (imitation) injection in adults with Achilles tendon rupture. Two sub-studies are embedded within the main study to contribute to the understanding of the PRP mechanism in tendon healing.

Short Title: PATH-2 Study

Ethics Reference: 14/SC/1333
Date and Version Number: 21 April 2017– Version 5.0

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Funder	**MRC-NIHR partnership: EME Programme**

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MRC Medical Research Council

NHS National Institute for Health Research

Funding Acknowledgement

This project funding was awarded by the Efficacy and Mechanism Evaluation (EME) Programme. This grant is funded by the Medical Research Council (MRC) and managed by the National Institute for Health Research (NIHR) on behalf of the MRC-NIHR partnership.

Funding for the extended follow-up was awarded from the Kadoorie Centre Trauma Research Charitable Fund.

Disclaimer:

The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NHS, NIHR or the Department of Health.

Conflict of interest declaration

There is no conflict of interest to declare.

TABLE OF CONTENTS

1 SYNOPSIS

Study Title	A pragmatic multi-centre, blinded, randomised placebo-controlled trial comparing Platelet Rich Plasma injection (PRP) to placebo (imitation) injection in adults with Achilles tendon rupture (ATR). Two sub-studies are embedded within the main study to contribute to the understanding of the PRP mechanism in tendon healing
Short Title	PATH-2: Platelet Rich Plasma in Achilles Tendon Healing
Study Design	A prospective, multi-centre, blinded, randomised, placebo-controlled trial with two embedded mechanistic sub-studies
Study Participants	Adult participants with acute ATR suitable for standard (non-surgical) treatment
Sample Size	214 participants (main study)
Planned Trial Period	Total length of project 62 months. Each participant is in the trial for 24 weeks . If they agree to participating in the extended follow-up they remain in the study for 24 months
Primary Objective	Evaluate the clinical efficacy of PRP in acute ATR in terms of mechanical muscle-tendon function
Primary Endpoint	24 weeks post treatment
Primary outcome	Heel-Rise Endurance Test (HRET) 24 weeks post treatment
Secondary Objectives	1. Evaluate the clinical efficacy of PRP in acute ATR in terms of participant reported functional recovery, pain and quality of life 2. Determine the key components of PRP that contribute to its mechanism of action. 3. Further understand in an immunohistochemical sub-study the mechanisms of PRP which may account for its clinical efficacy. 4. Identify the histological pathways that PRP may alter to exert its effects. Using these results and that from the PRP biological component sub-study, to inform future targeted manipulation of PRP properties to maximise its efficacy in tendon healing.
Secondary outcomes	Participant reported: 1. Achilles Tendon Rupture Score (ATRS) 2. Patient Specific Functional Scale (PSFS) 3. SF-12 (acute) 4. Pain diary (Week 0-2): completed daily for 2 weeks
Secondary Endpoints	Secondary outcomes 1-4 are measured at 4, 7, 13 and 24 weeks post treatment.

	Secondary outcomes 1-3 are measured at 24 months for the subset of participants who agree to participate in the optional extended follow-up.
Sub-study 1 – All sites	
Sample size	214 participants
Sub-study 1 Objective	To determine the key components of PRP that contribute to its mechanism of action
Sub-study 1 Outcomes	Central analysis of samples: PRP components and blood sample analysis: cell count, growth factor analysis, and platelet activation
Sub-study 1 Endpoint	Blood sample taken immediately prior to treatment (after randomisation)
Sub-study 2 – Selected sites only	
Sample size	16 participants
Sub-study 2 Objective	1. To further understand the mechanisms of PRP immunohistochemical effects, which may account for its clinical efficacy 2. Identify the histological pathways that PRP may alter to exert its effects 3. Using these results and those from Sub-study 1 to inform future targeted manipulation of PRP properties to maximise its efficacy in tendon healing
Sub-study 2 Outcomes	Immunohistochemical analysis: pain receptors, angiogenesis, collagen and proliferation markers
Sub-study 2 Endpoint	Needle biopsy 6 weeks post treatment

PATH-2 is defined as a Clinical Research Study.

2 ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
ATR	Achilles Tendon Rupture
ATRS	Achilles Tendon Rupture Score
CI	Chief Investigator
CRF	Case Report Form
CRN	Clinical Research Network
CSM	Centre for Statistics in Medicine in Oxford
CTRG	Clinical Trials & Research Governance, University of Oxford
DSMC	Data and Safety Monitoring Committee
EME	Efficacy and Mechanism Evaluation (EME) Programme
FAOS	Foot and Ankle Outcome Score
FUSE	Functional UltraSound Elastography
GCP	Good Clinical Practice
GP	General Practitioner
HRET	Heel-Rise Endurance Test
HTA	Health Technology Assessment
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
ICMJE	International Committee of Medical Journal Editors
ISF	Investigator Site File
LSI	Limb Symmetry Index
MHRA	Medicines and Healthcare products Regulatory Agency
MRC	Medical Research Council
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
OCTRU	Oxford Clinical Trial Research Unit
PI	Principal Investigator
PIS	Patient Information Sheet
PRC	Platelet rich concentrate
PRGF	Preparation rich in growth factors

PROM	Patient Reported Outcome Measure
PRP	Platelet Rich Plasma
PSFS	Patient Specific Functional Scale
RCT	Randomised Controlled Trial
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SFQ	Site Feasibility Questionnaire
SOP	Standard Operating Procedure
TA	Tendo Achilles
TSC	Trial Steering Committee
UK	United Kingdom
UKCRN	United Kingdon Clinical Research Network
VISA-A	Victoria Institute of Sport Activity for Achilles

3 BACKGROUND:

Existing research

3.1.1 Achilles Tendon Rupture

Achilles tendon is the most commonly injured tendon in the human body, it accounts for 20% of all tendon ruptures. The prevalence of ATR is 12-18/10000/year and it is on the increase due to an upsurge in recreational sport(1) in patients in sedentary or professional occupations. In the ageing but physically active subjects such tendon injuries are incapacitating for many months.

Although tendons have the ability to heal after injury, the process is slow and the mechanical and the biological properties of healed tendons never appear to match those of the original intact tendons leading to higher risk of further injury.(2) This limited healing capacity of tendons appears to be caused by a combination of poor vascularisation, resultant changes to the matrix structure and alteration in the mechanical environment of this connective tissue.(3-6) Even with the best available clinical management, tendon injuries give rise to substantial morbidity that lasts for several months and poses considerable challenges for clinicians and patients, especially during that lengthy healing and recovery period.(7)

A Cochrane review reported mean rehabilitation and work absence of 63-108 days leading to major NHS and societal costs.(8) The current treatment strategies are either mechanical augmentation with surgical suture, or immobilization in cast. Neither of these are recognised to alter the existing biological regenerative pathway of the tendon so the lengthy rehabilitation, the reduced function and re-rupture risk (5-15%) all remain.(8)

3.1.2 Platelet Rich Plasma

Platelet Rich Plasma (PRP) is an autologous derivative of whole blood that contains a supraphysiological concentration of platelets.

Platelets are the smallest of blood cells, formed during megakaryocytes maturation, and contain granules packed with bioactive proteins. Platelets have evolved over millennia to deliver a combinatorial wound healing cocktail that

comprises over 1100 active soluble and membrane bound components, delivered sequentially over a timeframe from minutes to the lifespan of the platelets.(9, 10) This ordered sequence of release is optimized to recruit a diverse range of cell types, acting on the injured tendon and disrupted blood vessels as well as promoting chemotaxis and homing of distant white blood cells (leucocytes) and local or circulating stem cells as part of the healing cascade.

In the resulting haematoma after acute tendon rupture, platelets immediately contribute to the formation of a clot. Stem cells and surviving tenocytes are then recruited by chemotaxis and motogenic stimulation into that rupture site, and then proliferate to start the healing process.

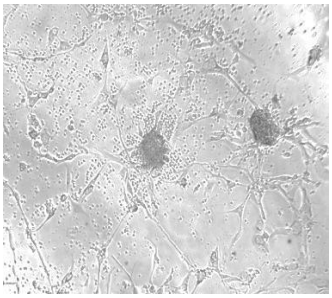
In our own laboratory studies we found PRP to be a potent mitogen for human tenocytes, dose-dependently enhancing the rate of proliferation.(11) PRP also protects tenocytes against the toxic effects of hypoxia and a range of drugs, indicating potent pro-survival activity.(12) Using phospho-kinase arrays we identify activation of a wide range of activated intracellular kinases, including the mitogen activated protein kinase ERK and the pro-survival kinase PKB.

The active growth factors in PRP act as both motogenic and chemotaxis agents for tenocytes. Tenocyte monolayers subjected to scratch assay fill in the wound area two days ahead of controls, through a combination of mitosis and migration. When tenocytes are placed in a transwell suspended above a clot of PRP, tenocytes move avidly through the membrane towards the PRP while control cells do not cross it. While PRP contains many active growth factors and chemokines we found that a key effect is the stimulation of tenocytes to begin their own production of these growth factors.(13) Thus the cells which are attracted by PRP rapidly begin to contribute to the tendon healing cocktail.

It is well known that tissue hypoxia, resulting from disrupted blood vessels, drives expression of VEGF in the tissue which chemo-attract vascular endothelial cells resulting in angiogenesis to restore normoxia. Our studies demonstrate that human tenocytes upregulate HIFα and produce VEGF under hypoxic conditions.(12) This has known relevance to tendon pathology in the torn rotator cuff(14) and in the ruptured Achilles tendon.(15) Similarly invading WBC will add their agents to the milieu. Our experiments also show that the cellular

components of PRP, i.e. platelets and leucocytes, may also interact directly with tenocytes and other regenerative response cells (see Figure 1). The presence of leukocytes has a great impact on the biology of PRP products, not only because of their immune and antibacterial properties, but also because they are turntables of local factor regulation.(10, 15, 16) Our findings are supported by rich evidence of PRP positive effect on tendon in cellular, tissue and animal studies.(9, 10)

Figure 1: Light microscopy image of tenocytes cultured with PRP in our study.Platelet clots seem to attract congregations of tenocytes around it.



PRP is prepared from autologous blood using either table-top centrifugation or filtration methods; both are simple and inexpensive. This facilitates its use in the clinic or operating theatre and its application as an autologous therapy. We have tested various PRP preparation methods in our pilot study and selected a technique that offers consistently viable and active PRP with high concentration of platelets. Our laboratory experiments confirm the synergistic effect of this preparation of PRP product on human Achilles tendon cells and tissues.(15, 17) From our research findings to date, and an international consensus body, it suggests PRP is a novel orthobiologic agent for tendon injury.(10) Improving Achilles tendon recovery may have major socioeconomic benefit and reduce demands on surgical and physiotherapy services.

3.1.3 Achilles tendon injury and PRP

We have conducted an extensive literature search of the application of PRP in Achilles tendon rupture in both animal and human studies. Nine studies present results for the use of platelets in Achilles tendon rupture treatment; seven from animal experiments and two from human trials.(18,19) Six of the animal studies used a rat model, and one used an ovine model. All animal studies, using biomechanical and histological assessments, are in agreement showing a

beneficial effect of PRP. Only two human studies have tested the effect of platelets on the treatment of Achilles tendon rupture based on imaging techniques and clinical results (Table 1). Sanchez et al(18) found positive effects in a group of 12 athletes treated with PRP suture repair at 32 months in a case-control study. They reported less tendon thickening, higher concentrations of TGF-β and other growth factors and the intervention patients regained range of motion faster and returned to gentle running earlier. Schepull et al(19), in a randomised study of 30 participants, found no effect of platelets on radioisometrical tendon contraction. Both of these small clinical studies used PRP as an adjunct to open surgical repair and this may have obscured any effect of PRP on healing.

Table 1: PRP and Achilles tendon rupture studies

Author	Year	Random-ised	Blinded	Power Calculation	PRP Type	Follow-up (month)	Platelet Group	Control Group
Schepull et al[19]	2007	Yes	Yes	No	PRC	12	12 4 lost FU	14
Sanchez et al[18]	2011	No	No	No	PRGF	12	6	6

Three other studies report on the use of platelets in human Achilles tendinopathy. This disease however has a different pathology, tissue regenerative properties and treatment pathways. We cannot infer the results of these trials are applicable to acute injury Achilles tendon rupture. In summary, to date, there is only one underpowered randomised controlled trial (RCT) that has assessed PRP in Achilles tendon rupture treated surgically.(19) The authors also recognised that a limitation in their platelet preparation technique and storage of up to 20 hours resulted in only a 20% release (activation) of growth factors from the platelets. In our 20-participant pilot study, preceding this trial, we achieved 69% activation.(20)

Recently published systematic reviews(21, 22) concluded that there are encouraging signs that PRP could be developed as an effective tendon therapy. Sadoghi et al(21) concluded that there is evidence in support of a positive effect of platelet concentrates in the treatment of ATR in vivo in animal models and

human application, consistent with a medium to large sized effect. This effect is most likely attributed to an accelerated and enhanced scar tissue maturation. In another systematic review, Taylor et al(22) concluded that PRP use in tendon and ligament injuries has several potential advantages, including faster recovery and, possibly, a reduction in injury recurrence, with no adverse reactions described. Both reviews emphasised the need for an adequately-powered RCT to establish PRP efficacy with disease-specific outcome measures.

We describe a multi-centre UK wide RCT to evaluate the efficacy and mechanism of PRP in patients with acute ATR. Adequate power and robust validated objective and participant-reported outcomes will ensure successful efficacy evaluation. Two sub-studies are embedded in the trial design to investigate PRP components and the mechanism of its effect on the tendon connective tissue.

3.1.4 Pilot Study: PRP in Accelerated Tendo-Achilles Healing (PATH)

We have already undertaken a prospective two-arm participant-blinded randomised controlled pilot trial. ATR patients were randomised into PRP treatment or control groups. In one arm of the study, participants who had already been selected for non-operative treatment received a PRP or control injection in the outpatient clinic. In the other arm, participants selected for operative treatment, at the time of surgery received PRP gel instilled in the rupture gap during percutaneous repair. A standard rehabilitation protocol was used and participants were followed up for 24 weeks. The pilot study outcome measures were: the Achilles Tendon Rupture Score (ATRS), the Victoria Institute of Sport Activity for Achilles (VISA-A) score and the Foot and Ankle Outcome Score (FAOS) scores were collected as patient reported outcome measures (PROMs). In addition a Functional UltraSound Elastography (FUSE) examination was performed at each follow-up to assess the mechanical properties of tendons. PRP analysis was undertaken to measure the bioactive protein release and tendon needle-biopsy was performed to study the histological differences during healing in both groups.

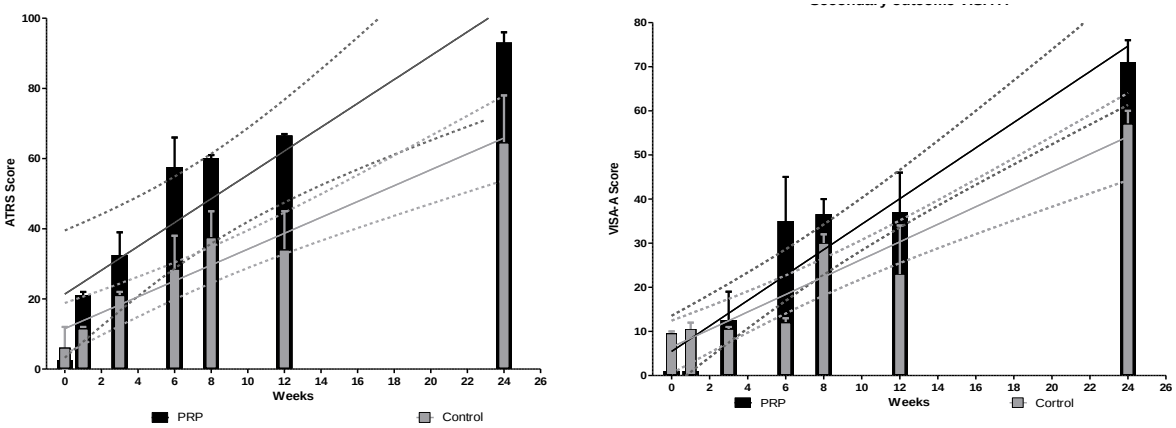
20 participants were recruited with a mean standard deviation (SD) age of 37.5 (8.8). The mean SD PRP platelet count was 1044 (320) x1000/µL. The platelet concentration in PRP increased 4.75-fold in comparison to blood. Interestingly,

white blood count concentration increased 2.16 fold in the PRP product. This confirmed that the PRP we used was also Leukocyte-rich (L-PRP) according to the Ehrenfest classification.

CD62p expression analysis using flow cytometry confirmed that 92.5% of platelets in PRP were viable and resting. Thrombin stimulation resulted in 68.4% of platelets being activated producing growth factor release. The results show that PRP growth factor concentrations were significantly higher in PRP comparing to whole blood ($p < 0.001$). Our methods resulted in an increase in the growth factor concentrations by between 5.42 and 8.66 fold.

Linear regression analysis of ATRS showed that the PRP group had a significantly better ATRS score from week 3 until week 24 ($P < 0.001$). The VISA-A analysis showed that the outcome for the PRP group was significantly better from week 6 onwards ($p<0.001$) (see Figure 2).

Figure 2: Pilot study results: linear regression analysis of ATRS (left) and VISA-A (right) over the follow-up period 0-24 weeks.



Linear regression analysis of FAOS demonstrated a significant association in the pain score ($p<0.05$); the other components were not significantly different between PRP and control groups. (This outcome measure, designed for chronic foot and ankle conditions, has not been validated in acute ATR). Strain mapping using FUSE scan showed larger, stiffer tendons in the PRP group.

The findings of our pilot study showed that PRP application in Achilles tendon acute rupture may lead to faster regeneration, improved pain and faster return to function. The feasibility of applying PRP in operative and non-operative groups was established. We found this method easy to use in both outpatients and the operating theatres. PRP was viable and produced a high concentration of growth factors upon activation.

3.1.5 Rationale for the current study

Platelet Rich Plasma has gained increasing attention in both the scientific literature and the wider media for its potential application in the treatment of traumatic musculoskeletal injury. The clinical use of PRP has been largely confined to the last two decades and initially centred on its application in surgery. Recently, developments in basic science research of PRP in a range of tissues have attracted interest in orthopaedic surgery and sports medicine where effective healing of damaged tissues is a critical determinant of successful clinical outcome. A recent meta-analysis of PRP for orthopaedic conditions(23) stated the need for adequately powered studies using disease-specific and patient-important outcomes and to investigate the effect of PRP.

Despite that lack of high quality trial data, PRP administration remains a potentially attractive strategy to explore given it is of relatively low cost and minimally invasive. There has been a recent steep growth in PRP use for musculoskeletal conditions. It is estimated that PRP is used to treat 86,000 tendon, ligament and muscle disorders annually in the United States.(24)

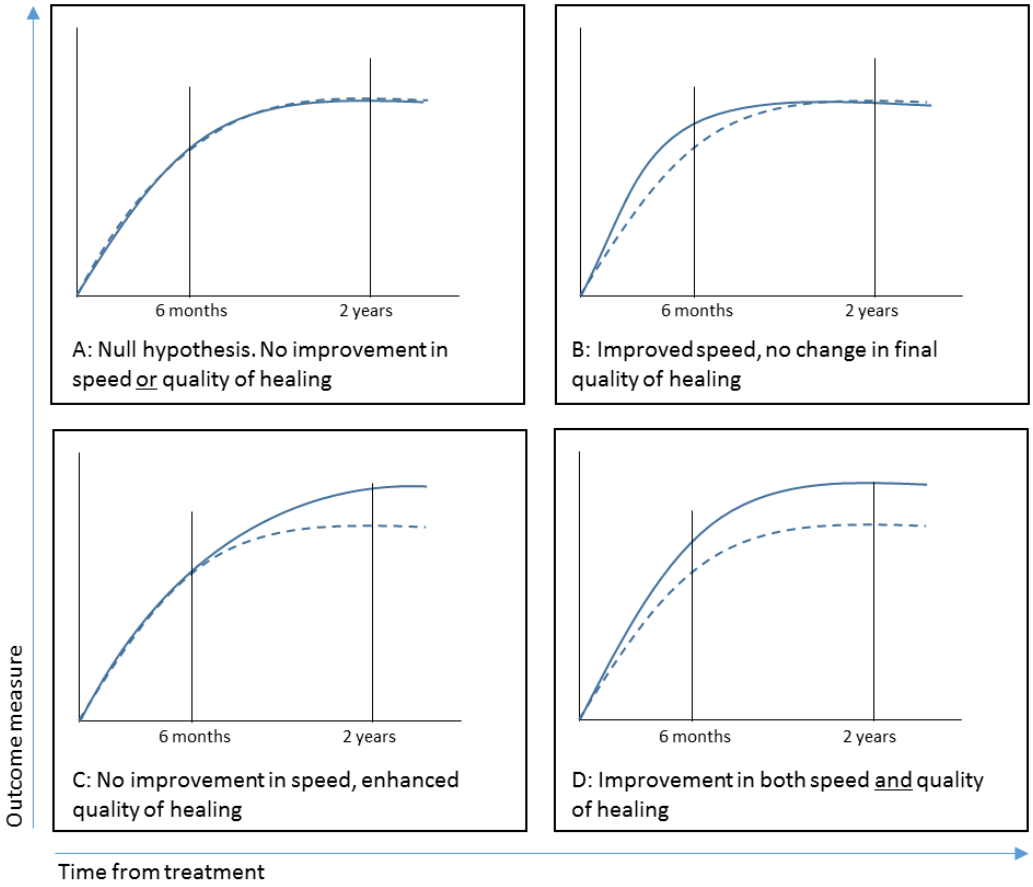
There is also evidence that PRP injections are being introduced in NHS clinical practice, in addition to a wider use in private medicine within the United Kingdom. We surveyed the declarations of use of PRP in NHS hospitals and discovered four offering PRP for tendon injuries. Without evidence of efficacy the consequences range from the NHS incurring extra costs for a treatment with unproven clinical effects to the non-deployment of an effective autologous intervention. There is therefore a pressing need to undertake this study before the use of PRP becomes widely adopted. The results of this study may inform the design of a potential future clinical trial of optimal PRP therapy versus surgical repair for ATR in restoring patient function.

The NICE review of PRP use (IPG 438) states that specialist advisers noted that this was an established practice(25), and it is of concern that participants are being exposed to these intervention techniques without adequate clinical investigation into their efficacy and validity. NICE encourages further research comparing autologous blood injections (with or without techniques to produce PRP) against established non-surgical methods.

3.1.6 Rationale for the extended follow-up

Mechanistically, we view the intervention, if effective, as improving speed of healing, final quality of recovery, or both. A difference in speed of healing, if evident, will be seen when the tendon is in recovery phase and our PATH-2 primary outcome measure at 24 weeks post injury is timed to capture this data. However, Achilles tendon ruptures prevent full return to function, sport and work over a long period, with overall recovery time of greater than 2 years post injury (26, 27). If PRP affects the quality of the repaired tendon, we would expect to see this at 2-3 years post injury. We therefore envisage recovery as having one of the four profiles in Figure 3. Our extended follow-up at 24 months will confirm whether there is any beneficial effect of PRP on the quality of the repaired Achilles tendon at 2 years post injury.

Figure 3: Possible repair outcomes from a single injection of Platelet Rich Plasma (PRP), if PRP enhances recovery. Solid line: PRP treatment. Dotted line: placebo treatment.



4 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

Primary Objective

Evaluate the clinical efficacy of PRP in acute ATR in terms of mechanical muscle-tendon function.

Primary Outcome Measure/Endpoint

Heel-Rise Endurance Test (HRET): a validated objective measure of calf-muscle Achilles tendon capacity to work at 24 weeks, which is measured in the unit joules (J).(28) The HRET involves the participant standing on one leg and raising and lowering the heel

repeatedly until fatigued. The work during the HRET for each lower limb is measured. The performance of each limb is then converted into a limb symmetry index (LSI), which is the primary outcome metric from the HRET. The LSI is calculated as follows:

$$LSI = \frac{injured\ limb\ measurement}{uninjured\ limb\ measurement} \times 100$$

Data from the movements will be obtained using a computer-controlled linear encoder to calculate work during the heel rise test. The calibrated instrument that enables the calculation of the work performed during the HRET is a linear encoder. The linear encoder measures the height of each heel-rise during the HRET. This information is used in conjunction with the participant's body weight to calculate the work performed during the HRET. We will also record the number of heel rises performed and the maximum displacement (cm) during the HRET. The test will be standardised through training the blinded assessors and by offering trial specific guidance notes and videos.

Secondary Objectives

1) Patient reported outcomes: to evaluate the clinical efficacy of PRP in acute ATR in terms of patient reported functional recovery, pain and quality of life
2) Blood samples: to determine the key components of PRP that contribute to its mechanism of action. Baseline blood from both groups will be analysed.
3) Needle biopsy: to further understand in a sub-study the mechanisms of PRP immunohistochemical effects, which may account for its clinical efficacy. This includes tendon tissue regeneration, cell differentiation, collagen formation, tissue morphology and pain neuroreceptors count in the healing tendon.
4) Blood samples and needle biopsy: to identify the histological pathways that PRP may alter to exert its effects. Using these results and that from the PRP biological component sub-study, to inform future targeted manipulation of PRP properties to maximise its efficacy in tendon healing.

Secondary Outcome Measures/Endpoints

1) Achilles Tendon Rupture Score (ATRS): a validated patient reported outcome measure (PROM) incorporating questions relating to muscle strength, fatigue, pain and function.(29) ATRS has been identified in a systematic review of

PROMs for Achilles tendon rupture as the only tool to have undergone appropriate validation with positive consistency, convergent validity and responsiveness.(30)

2) Daily Pain Diary for 2 weeks (daily Visual Analogue Score (31) [0-100, 0=no pain, 100=worst pain imaginable]): records pain in the early phase of healing (patient reported)
3) Patient Specific Functional Scale (PSFS): a PROM indicating participants' progress with own functional recovery goals on an 11-point scale.(32, 33)
4) SF-12 (34): a health-related quality of life scale (PROM)
5) Blood sample: PRP components analysis, and analysis of cell count, relevant tendon active growth factors, concentrations and platelet activation
6) Sub-study participants only. 16 participants, 8 in each arm, selected sites: A needle biopsy of the healing Achilles tendon under ultrasound guidance during an outpatient visit will take place and samples will undergo immunohistochemistry analysis. Our pilot study demonstrated the safety and acceptability of this technique.(20)

Secondary outcomes will be assessed at key time-points:

1) **Baseline:** ATRS, PSFS, SF-12 acute version (pre-injury and post injury), VAS (pre-treatment), and blood sample taken immediately prior to treatment. (Central analysis of blood at later date.)
2) **For 2 weeks, starting on day of treatment**: Daily record of post-treatment pain using Daily Pain Diary (VAS).
3) **At 6 weeks post treatment:** Tendon needle biopsy under ultrasound guidance during an outpatient visit. Later analysis carried out centrally by the research team. (Sub-study participants only. 16 participants, 8 in each arm, selected sites.)
4) **Weeks 4, 7 and 13**: ATRS, PSFS and SF-12 will be recorded by telephone call or during outpatient visit.
5) **Week 24:** ATRS, PSFS, SF-12 and HRET will be conducted via face-to-face assessment at outpatient visit.
6) **Month 24:** ATRS, PSFS and SF-12 will be conducted via postal questionnaire or over the telephone.

Participants will be asked to report any complications at each of the study data collection points. In addition, progress with rehabilitation will be recorded.

Site staff will report adverse events according to the guidance in this protocol, see 'Safety Reporting' section.

5 STUDY DESIGN

PATH-2 is a pragmatic prospective multi-centre, blinded, randomised, placebo-controlled trial with two sub-studies embedded within the main study. The study aims to evaluate the clinical efficacy of PRP in acute ATR in terms of mechanical muscle-tendon function.

The PATH-2 study will take place in a minimum of 15 NHS hospitals across the UK. Patients will be identified in the orthopaedic/trauma outpatient clinic, usually following an emergency hospital attendance for an Achilles tendon rupture. We anticipate in most sites the surgeon will confirm appropriateness for non-surgical treatment and study eligibility. A member of the local research team will carry out the informed consent process, baseline data collection and randomisation. Participants will be randomised via a telephone or website randomisation service, and will be allocated to receive either 'PRP injection' or 'Imitation (placebo) injection'. Treatment will be administered by a surgeon or an extended scope physiotherapist who is appropriately qualified, as delegated by the Principal Investigator (an orthopaedic surgeon). This will take place during the outpatient visit.

Those involved in treatment delivery will be aware of treatment allocation due to the nature of the intervention. Participants should remain blind to allocation throughout the study (see 'Randomisation, blinding and unblinding' section).

There are four blinded outcome assessments at 4, 7,13 and 24 weeks after treatment and one at 24 months after treatment. For the first three (4, 7,13 weeks) responses will be collected over the telephone or during a hospital outpatient visit (where this coincides with local follow-up). The fourth follow-up requires a hospital attendance at 24 weeks, with a face-to-face interview and assessment by a physiotherapist/assessor. At this visit,

the primary outcome will be collected, the HRET. See Study Flowchart, Appendix A. The follow-up data at 24 months will be collected via a postal questionnaire or over the telephone.

The participant remains in the study until data related to their 24 week follow-up have been collected. The majority of data outcomes are patient reported, collected on paper CRFs (questionnaires). The outcome measure questions will be combined and presented to participants as one questionnaire per timeframe (Baseline Questionnaire, 4/7/13/24 week / 24 month Questionnaire) .

The two embedded sub studies are:

1) Sub-study 1: Blood component (all participants, all sites) and PRP component analyses (PRP intervention arm only). A blood sample will be taken from each participant after consent and randomisation but prior to treatment. A non-centrifuged blood sample from all participants in both study arms will be analysed to compare its composition by blood cell type. PRP samples will be prepared according to treatment allocation and a portion will remain after treatment. This will be sent to a central laboratory for later analysis.
2) Sub-study 2: Immunohistochemstry analysis (16 participants from selected sites only who have given consent to undergo the sample collection procedure). A needle biopsy of the healing Achilles tendon under ultrasound guidance will be taking during an outpatient visit. Samples will undergo immunohistochemistry analysis centrally in a specialist laboratory.

6 PARTICIPANT IDENTIFICATION

Study Participants

All patients with acute Achilles tendon rupture attending outpatient trauma/orthopaedic clinic within 12 days of sustaining the injury will be eligible for inclusion in the trial.

Inclusion Criteria

- Patient is willing and able to give informed consent for participation in the study
- Aged 18 years or over

- Ambulatory prior to injury without the use of walking aids or assistance of another person
- Diagnosed with an acute, complete, Achilles tendon rupture
- Presenting within and receiving study treatment within 12 days post injury
- Patients in whom the decision has been made for non-operative treatment
- Able (in the Investigator's opinion) and willing to comply with all study requirements
- Able to attend a PATH-2 study hospital site for the 24-week follow-up

Exclusion Criteria

The patient may not enter the study if ANY of the following apply:

- Achilles tendon injuries at the insertion to the calcaneum or at the musculotendinous junction
- Previous major tendon or ankle injury or deformity to either lower leg
- History of diabetes mellitus
- Known platelet abnormality or haematological disorder
- Current use of systemic cortisone or a treatment dose of an anticoagulant (i.e. a prophylactic dose for preventing thrombosis would not be an exclusion)
- Evidence of lower limb gangrene/ulcers or peripheral vascular disease
- History of hepatic or renal impairment or dialysis
- Female patients who are pregnant or breast feeding
- Is currently receiving or has received radiation or chemotherapy within the last 3 months
- Has inadequate venous access for drawing blood
- Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participant at risk because of participation in the study, or may influence the result of the study, or the patient's ability to participate in the study

7 STUDY PROCEDURES

Recruitment

7.1.1 Recruitment centres

A minimum of 15 NHS hospital orthopaedic trauma/outpatient clinics will participate to recruit 214 participants with acute ATR.

Each site will identify a surgeon to act as PATH-2 Principal Investigator (PI). The PI will need to utilise links with local physiotherapy departments to facilitate communication regarding the standardised rehabilitation used in the PATH-2 Study, and for identifying physiotherapists who may act as blinded assessors for the primary outcome measurement.

Sites will be selected based on suitability. An invitation pack which includes a Site Feasibility Questionnaire (SFQ) will be provided to potential sites. The SFQ may be completed by an individual with adequate, authoritative knowledge of the site (where a site is known to the study office through previous research enterprises the SFQ may be part-completed in advance). The PI or an appropriate deputy must confirm participation and the accuracy of any SFQ submitted to the study coordinating office in Oxford.

The coordinating team will evaluate returned SFQs to ensure a site is equipped with appropriate resources to deliver the project and meet recruitment targets. Confirmation of collaboration will be provided in writing to the PI.

7.1.2 Participant recruitment

Patient will be identified in the outpatient trauma/orthopaedic clinic. We anticipate in most sites the attending surgeon will confirm appropriateness for non-surgical treatment and study eligibility. A member of the local research team will carry out the informed consent process, baseline data collection and randomisation. Those patients suitable for inclusion will be given information about the study and invited to discuss the study further with a member of the research team. Patients who are willing to participate will be given the opportunity to ask questions and the appropriate informed consent will be gained. Baseline outcome data will be

collected prior to injection treatment and it is anticipated that baseline data collection and randomisation will take place during the same timeframe.

Randomisation may be carried out by telephone or website option provided by the Oxford Clinical Trials Research Unit (OCTRU) randomisation service. Participants will be allocated to receive one of two treatment options, either 'PRP injection' or 'Imitation (placebo) injection' and **once this allocation is known**, a blood sample will be taken from the participant and prepared as appropriate for the treatment to be delivered. Injection treatment will then be delivered in clinic by a surgeon or an extended scope physiotherapist who is appropriately qualified, as delegated by the Principal Investigator (an orthopaedic surgeon).

After treatment remaining blood samples for both groups are dispatched to a central laboratory for analysis (Sub-study 1), see full guidance in the PATH-2 intervention and blood processing training materials. Blood analysis results are not reported back to sites as they do not impact on future treatment.

Participants should remain blind to allocation throughout the study (see 'Randomisation, blinding and unblinding' section). Those involved in treatment delivery will be aware of treatment allocation due to the nature of the intervention.

To facilitate telephone follow-up at 4, 7, 13 and 24 weeks, before the participant leaves clinic they will be asked for appropriate contact details and the preferred time of day to be contacted.

See Study Flowchart in Appendix A.

Before sending an extended follow-up questionnaire at 24 months, study office staff will obtain postal addresses from NHS Digital. The consent form for the main study includes consent for these details to be obtained.

Informed Consent

Due to the very acute nature of the injury, ATR patients usually attend the outpatient trauma clinic following an emergency hospital attendance. Standard treatment for this non-surgical population is usually a cast application during that initial clinic visit. The PATH-2 study treatment options require the PRP or imitation injection to be delivered before the cast is applied. Therefore although clinic waiting times can be fairly lengthy due to the emergency nature of the attendance, the timeframe between the informed consent process and treatment is relatively short. To help raise awareness of the study during the clinic wait, sites will be provided with written study participant information (including posters), to display in clinic where potential participants are waiting to be seen by the clinical team.

The attending surgeon (or attending clinician) will meet with the participant for the clinical exam, discuss the study and the written information, the Patient Information Sheet (PIS). A member of the research team will continue the informed consent process and take consent. The participant will be allowed as much time as practically possible in this type of acute injury to consider the information, and have the opportunity to ask questions of the surgeon (or attending clinical team) and a member of the research team.

The participant must personally sign and date the latest approved version of the Informed Consent Form before any study-specific procedures are performed. A written version and a verbal discussion of the Patient Information Sheet and Informed Consent Form will be presented to the participants which details the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

Written informed consent will then be obtained by means of participant dated signature, and dated signature of the person who presented and obtained the informed consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Principal Investigator; we anticipate in most sites this will be a research nurse/physiotherapist who will be a part of the local NHS Trust or the local NIHR Clinical Research Network.

A copy of the signed Informed Consent Form will be given to the participant, and one copy will be sent to the study coordinating team in Oxford. The original signed Consent Form will be retained in the medical notes, and a copy held in the Investigator Site File (ISF). Holding a copy in the coordinating office will facilitate central monitoring. Consent forms will be held in a secure location separately from any study data.

Permission will be obtained to inform the participant's GP of study participation.

The PIS will specify that a blood sample up to a maximum amount of 55ml may be taken. Only a maximum amount will be stated, as detailing the exact amount of blood withdrawn per treatment group would reveal treatment allocation: the amount drawn varies depending on treatment. The PIS will also outline that the sample remaining after treatment for PRP injection group, and the sample of the imitation injection group, will be dispatched to a member of the central research team in the Birmingham University Research Laboratory for analysis. Samples will be anonymised before dispatch using only the participant's unique study number. No results will be reported back to participants or the local team. The consent form will record appropriate consent from the participant for these activities.

The PIS will outline that name, and contact details (including mobile, phone and email) will be collected to facilitate follow-up, full data collection and results reporting. A copy will sent to the study coordinating team in Oxford. These details may be used by the study team to check contact details using NHS Digital, and to provide other basic study-related information that may be needed for follow-up.

Permission will be sought to allow access to participant data by responsible members of the University of Oxford or the NHS Trust for monitoring and/or audit of the study to ensure we are complying with regulations

The 24 week follow-up visit includes the HRET (primary outcome) and participants will be asked to consent to the filming of their ankle and leg movements at the time of the test. Filming will not include the face so participants will not be identifiable from the film. The video file (or any still photographs from it) will be labelled with the unique study number, and no identifying details will be used. Permission will be sought to send and hold the file with the study coordinating team in Oxford where it may be viewed by members of the research team.

Sites collaborating in the needle biopsy sub-study will have a separate PIS and ICF. .

Screening and Eligibility Assessment

Patients have usually attended the Emergency Department with a suspected ATR and are referred to the trauma/orthopaedic outpatient clinic for treatment. Patients who meet the eligibility criteria can be considered for the trial and randomised and receive treatment up to (and including) the 12th day post injury, as indicated in the inclusion criteria. However, due to the severity of injury it is likely early treatment will be required.

We anticipate in most sites the surgeon will confirm appropriateness for non-surgical treatment and study eligibility during the clinical exam.

After signed informed consent, baseline data will be collected and randomisation will take place.

Randomisation, blinding and code-breaking

Participants will be randomly allocated (1:1) to the two treatment groups via a central computer-based allocation randomisation system provided by the Oxford Clinical Trials Research Unit (OCTRU) with minimisation using site and age group as strata and variable block sizes. This ensures that sites and age groups are balanced across the treatment arms. The service may be accessed by both telephone (during normal office hours, 8 am to 5 pm), or via a secure randomisation website (24 hours / 7 days a week). Details of the block sizes and allocations will be confidential and known only by the trial statistician and OCTRU programmer.

Participants will remain blind to their allocated treatment throughout the study including the extended follow-up.

Facilitating blinding at treatment delivery

To facilitate treatment blinding, the research team will ask the participant to wait outside the immediate consulting area when preparing the injection consumables. This is because there is a difference in the number of consumables handled depending on treatment allocation. Once the injection is prepared it should remain out of view and the patient asked to return for treatment. They should be asked to lie face down on the

examination couch with the foot and ankle slightly off the edge of the bed. Once the patient is in place they will not be able to see the injection being given.

Both treatment and control procedures are performed after local anaesthetic injection and with the same size needles. In both the PRP injection and imitation injection procedures the centrifuge should be activated to reinforce blinding of the participant if the centrifuge is located in an area where the participant may become aware of its use or lack of use.

The clinical team delivering the injection will be aware of allocation to ensure the appropriate treatment is delivered.

Facilitating blinding immediately post treatment

The PATH-2 Treatment CRF will collect confirmation that allocated treatment was delivered but will not specify the treatment delivered. Where a treatment other than that allocated was received, this should be noted on the CRF and the study coordinating team will contact the site for further details once the CRF has been received and processed in the study office.

When any hospital notes are updated relating to treatment or GP letters dictated, it should be recorded that an injection was delivered as per the random allocation assigned by the PATH-2 study. The type of injection should not be recorded. Stickers for the hospital notes stating the patient's involvement in the PATH-2 study, without specifying allocation, will be provided to sites. A study GP letter will also be provided.

We will conduct an assessment of participant blinding at 24 weeks after all required study assessements are completed. The participant will be asked which treatment they believe they received (PRP injection, imitation injection or don't know) along with questions around their experience of taking part in the study. This data will be used to compute two blinding indices with 95% confidence intervals (James' and Bang's blinding indices).(35, 36)

Blinded follow-up assessors

Wherever possible, study follow-up will be carried out by blinded assessors unaware of treatment allocation.

A physiotherapist/assessor who is blind to allocation will meet with the participant and carry out the 24-week study follow-up assessment.

Code-break, Unblinding and Emergency unblinding

Code-break: is the term used for revealing treatment allocation. For PATH-2 there will be a list of treatment allocations for all participants embedded in the OCTRU PATH-2 database held in Oxford. The PATH-2 randomisation service is also provided by OCTRU and the allocation data will be transferred internally from the randomisation service to the database therefore allocation information remains secure within OCTRU.

Unblinding: The team delivering the injection treatment will be aware of the allocation. In general, unblinding of participants during the conduct of a clinical trial is not allowed unless there are compelling medical or safety reasons to do so.

Emergency unblinding: The treatments in this study are considered at low risk for the need for unblinding. However, if it is considered necessary to request unblinding after the treatment period, the request should be directed to the Chief Investigator via the central co-ordinating team in Oxford with full details of the reason for the request. The Chief Investigator will work with OCTRU and a decision made regarding the unblinding request. See ISF for central co-ordinating team contact details.

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Unblinding after completion of the trial: The participants recruited to this trial may not be informed of their allocation until after the end of the study. If a request is made, participants may be informed of their allocation after the end of the study.

Baseline Assessment

Following signed consent, baseline data will be collected prior to injection treatment. A member of the local research team will oversee the participant's completion of the paper CRF, which will include:

1. Background Information and demographics
    - Participant reported questions including: general health, current medication, allergies, smoking, alcohol use, sport activities, age, date of birth, sex, employment status, type of employment, activities related to standing/walking/driving, any medication taken for inflammation, recreational activities prior to injury, the activity that led to the torn tendon, previous rupture history, height and weight

2. Patient-specific functional scale (PSFS)
    - Participant reports three important activities they are having difficulty with as a result of their Achilles tendon rupture
3. Achilles Tendon Rupture Score (ATRS)
    - Participant reported: Questions specific to ATR
4. SF-12 acute version
    - Participant reported: without injury (recall of pre injury function/health state)
5. SF-12 acute version
    - Participant reported: With injury (current function/health state)
6. Visual analogue score (VAS)
    - Baseline collection of pain prior to treatment using VAS

In clinic during baseline data collection period

- Blood sample: Following randomisation, blood sample taken and prepared by a member of the local team according to allocated treatment.
- Participant contact details: collected alongside a preferred time to be contacted to organise study follow-up. Includes NHS number.

- Pain diary: provided to participant before leaving clinic, completed at home during the first two weeks following injection treatment. Returned to the study office by post using a Freepost account.
- GP Letter: The participant's GP will be informed of their participation in the PATH-2 study.

Subsequent Assessments

Study outcomes will be assessed at key time points following treatment:

- **Week 0-2: Daily Pain diary**
  - Participant reported pain diary: completed at home during the first two weeks following injection treatment.

- **Weeks 4, 7 and 13**:
  - Participant reported: ATRS, PSFS and SF-12 and questions related to progess with rehabilitation will be obtained by a telephone call to the participant (or during an outpatient visit as appropriate). Carried out by a member of the research team who will be blind to treatment allocation. See Appendix D.

- **Week 6**: 16 participants, 8 in each arm, (sub-study, selected sites only)
  - Needle biopsy of healing Achilles tendon under ultrasound guidance (requires additional hospital outpatient visit). Carried out by a member of the research team who will be blind to treatment allocation.

- **Week 24**:
  - Participant reported: ATRS, PSFS, SF-12, HRET and questions related to progess with rehabilitation. Obtained at an additional hospital visit: a face-to-face assessment carried out by a trained blinded physiotherapist/assessor.
  - The lower leg is filmed during performance of the HRET if the participant agrees. Filming is required to capture the participant's performance of the HRET to (1) enable quality control of the testing protocol regarding participant positioning, attachment of the linear encoder, start/stop of

recorded measurements – this will prompt extra assessor training where required (2) provide a separate/back-up record of performance should there be a failure of the linear encoder (using a reference object on screen to estimate height of heel raises and counting the number of repetitions).

- An up-to-date email address is collected for communicating results of the study to participants who wish to receive them
- After all mandatory assessments have been completed for the study the participant is asked about their experience of trial participation and which treatment they believe they received.

- **Month 24**:
  - Participant reported: ATRS, PSFS and SF-12 and questions related to re-rupture will be obtained by postal questionnaire or during a telephone call to the participant.

Participant-reported complications are collected at each of the 4, 7, 13 and 24 week follow-up time-points, and re-rupture events will be collected at at 24 months.

In total, five main study follow-up assessments take place, at 4, 7, 13 and 24 weeks and at 24 months. Three (4, 7, 13 weeks), are via telephone or face-to-face during a normal care outpatient visit (See Appendix D). One (24 weeks) requires an additional hospital out-patient visit (See Appendix E). The 24 month follow-up will be conducted by a combination of post and telephone (see Appendix F).

It is anticipated that site research staff blind to allocation carry out the follow-up at 4, 7 and 13 weeks. However, due to the relatively close data collection time-points, where necessary a member of the research team in Oxford may carry out telephone data collection. Extended follow-up telephone calls will be carried out by a member of the central research team. Where possible, this person will be blind to treatment allocation. See Schedule of Study Procedures, Appendix B.

Sample Handling

Following randomisation and prior to treatment, a blood sample (up to 55ml) is taken and prepared according to the treatment allocation. Guidance on how to prepare the samples for treatment is provided in the PATH-2 intervention and blood processing training materials.

Following treatment, remaining samples are prepared for dispatch, which includes anonymising the sample using the participant's unique study number and initials. Samples are sent to a member of the central research team at Centre for Translational Inflammation Research at the Birmingham University Research Laboratory. Only those involved in the research process have access to the sample. The results of the blood analysis are not reported back to participants or the local clinical team and will not inform future treatment. See Appendix G and full guidance in the PATH-2 intervention and blood processing training materials.

Standardised Rehabilitation

Standardisation of key elements of rehabilitation is required for this study to reduce the risk of efficacy signal interference from substantial variations in rehabilitation. Loading of the Achilles tendon during recovery is likely to influence the healing of the injured tissues.(37) There is significant heterogeneity in rehabilitation protocols after Achilles rupture and limted evidence regarding the effectiveness of different approaches.(38, 39) The results from an informal survey of potential collaborators indicated it should be feasible to standardise key aspects of rehabilitation.

The following will be standardised for the PATH-2 trial:

- duration of intial externally applied ankle splinting of 3 weeks after intervention
- position of the ankle in equinus during the initial immobilsation
- referral to physiotherapy for rehabilitation
- avoidance of rigid full-time immobilisation without ankle motion or weight bearing of >6 weeks
- same rehabilitation program for both intervention groups at each centre

Standardisation will not be required for the ankle splinting method or device, when weight bearing is commenced or the specific exercise prescription. We will standardise rehabilitation by providing guidance to surgeons and physiotherapists in written form. Monitoring adherence with these guidelines will be assessed by asking participants questions relating to progress with rehabilitation during follow-up.

Discontinuation/Withdrawal of Participants

Each participant has the right to withdraw from the study at any time. The attending investigator may also request the participant is withdrawn from receiving study treatment for clinical reasons, however as treatment is an injection at one point in time we anticipate very few investigator requests for a participant to be withdrawn. An intention-to-treat analysis will be carried out therefore all participants remain in the study irrespective of whether their allocated treatment was received (unless the participant themselves withdraws). The PI will record any reason for any withdrawal on the study Withdrawal CRF and the participant will be asked if the study team may use the data collected to the point of withdrawal and/or contact them for collection of patient-reported follow-up data.No additional participants will be recruited to replace withdrawn participants.

Reporting a death

In the event of the PI becoming aware of a death the collaborating site should contact the central co-ordinating team in Oxford as soon as possible. This early notification will ensure no participant is contacted for study follow-up such as to cause distress to other family members.

See also Safety Reporting section in this protocol to evaluate whether the death should be reported as a study Serious Adverse Event.

Definition of End of Study

The end of the main study is 21 days after the the date of the 24 month follow-up of the last participant.

8 INTERVENTIONS

Intervention groups

Participants will be randomly assigned to one of two groups by the OCTRU randomisation service, either:

Group 1 (Treatment): **PRP injection**: local anaesthetic injection to skin, followed by PRP injection to the tendon rupture gap

or

Group 2 (Placebo): **Imitation Injection:** local anaesthetic injection to skin, followed by needle insertion into tendon rupture gap

Participants must remain blind to allocation (see 'Randomisation, blinding and unblinding' section). Both groups will follow study-standardised rehabilitation. Trusts should follow their own policy to manage DVT prophylaxis.

Blood sample following randomisation

The blood sample **must** be taken **after** randomisation. Up to 55 ml of venous blood may be withdrawn from the participant. The exact amount will be dependent on the random allocation. To ensure the correct amount of blood is withdrawn into the appropriate tube/syringe see full guidance in the PATH-2 intervention and blood processing training materials. To facilitate blinding detailed guidance is not provided in this protocol. The intervention and blood processing training materials should be retained in an area where they are not visible to the public.

PRP injection preparation

PRP kits will be used for the preparation and delivery of the PRP treatment. A portion of the blood sample will be placed in the kit and the kit linked to a centrifuge which will be set to spin the blood to produce a specific amount of PRP in a sterile syringe ready for use. See full guidance in the PATH-2 intervention and blood processing training materials.

A study-specific centrifuge is required as are study-specific PRP kits. PRP kits should not be used outside of the PATH-2 study. If a participant is unable to receive a PRP injection for any technical reasons they will receive an imitation injection and this will be recorded.

Placebo (imitation) injection preparation

When administering the imitation injection the needle is introduced via the skin into the tendon tissue, held in the skin briefly and withdrawn without injecting so the biological haematoma is not disturbed.

After injection delivery

Immediately post treatment a member of the local team will prepare the remaining blood sample (PRP or Imitation Injection Group) for dispatch. See full guidance in the PATH-2 intervention and blood processing training materials. Samples will be sent to a member of the central research team at Birmingham University Research Laboratory. A pre-paid account will be established by the study to facilitate ease of postage.

Surgical training

Training in delivery of both the PRP injection and imitation injection will be provided by the trial team. The PI at each site will identify surgeons (or extended scope physiotherapists) to be trained and will record those who have completed training on the site Delegation Log. Only those study-trained and listed on the Delegation Log are able

to carry out trial treatments. New staff should be trained and added to the log as the study progresses. When the Delegation Log is updated, a copy should be sent to the study coordinating office in Oxford.

Staff training: blood samples

The PI at each site will identify local staff who will be responsible for preparing the blood sample for treatment and for later dispatch where these duties are delegated to other members of staff. The Delegation Log should be updated accordingly. New staff should be trained and added to the log as the study progresses. When the Delegation Log is updated, a copy should be sent to the study coordinating office in Oxford.

The Delegation Log is part of the ISF and must be updated when any responsibilities are delegated locally. A copy of the updated version of the log must be sent to the study office.

Excess treatment costs

Excess treatment costs for this study are:

1) a study-specified centrifuge: used to prepare the blood sample ready for treatment injection
2) study-specified PRP kits: attached to the centrifuge and used to deliver the treatment injection

The equipment will be standardised across all sites; full details of equipment supply will be provided by the study office.

9 SAFETY REPORTING

Definitions

9.1.1 Adverse Events and Serious Adverse Events

An adverse event (AE) is considered a serious adverse event (SAE) if it is an untoward medical occurrence that:

- Results in death,
- Is life-threatening,

  NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Requires in-patient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Other important medical events*

  *Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

9.1.2 Foreseeable Adverse Events/Reactions

Because PRP is prepared from autologous blood, it is inherently safe, and any concerns of disease transmission such HIV, hepatitis, or Creutzfeldt-Jacob disease or immunogenic reactions that exist with allograft or xenograft preparations are eliminated. There have been no serious adverse reactions related to using PRP reported in the literature.

Foreseeable occurrences (adverse events), related to study treatment that **do not** require specific time-critical reporting but may be collected as part of standard data collection are:

- Bruising and discomfort at the venesection site
- Mild discomfort or minor bleeding from 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 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

9.1.3 Unforeseeable Adverse Events/Reactions

Unforeseeable events related to the study treatments **should be reported as an SAE if** they take place within 24 weeks of receiving trial treatment/placebo **and** fulfil the SAE criteria in 9.1.1 (see also Figure for guidance). Other Unforeseeable events, even if not fulfilling the SAE criteria, should be reported as part of the adverse event data collection on the study CRFs.

The examples below are unforeseeable events:

- Serious infection of ATR injection site
- Skin breakdown or ulceration of treated lower leg other than “plaster sores”
- Severe pain requiring more than simple analgesia beyond 10 days after injection

Adverse events will be collected during the study treatment episode and staff should report any events they become aware of up to and including the 24 week follow-up appointment. Participants will be asked if they have experienced any complications during follow-up data collection.

Reporting Procedures for Serious Adverse Events

Serious adverse events must be reported to the Chief Investigator within 24 hours of the local research team becoming aware of the event even if full information is not

available at that time; the local PI should be informed at the earliest possible opportunity.

Collaborating sites – PI assessment of an event prior to reporting

The PI or a delegated health care professional **must assess causality** of any suspected SAEs **before** the event is reported to the study coordinating office in Oxford. Where the event is considered **both** 'related' to a study treatment **and** 'unforeseeable' (see Figure 4), the individual should complete the appropriate section on the paper copy PATH-2 Serious Adverse Event Form.

The SAE form must be completed as fully as possible, and sent to the study coordinating office in Oxford. Full contact details are provided on the SAE Form.

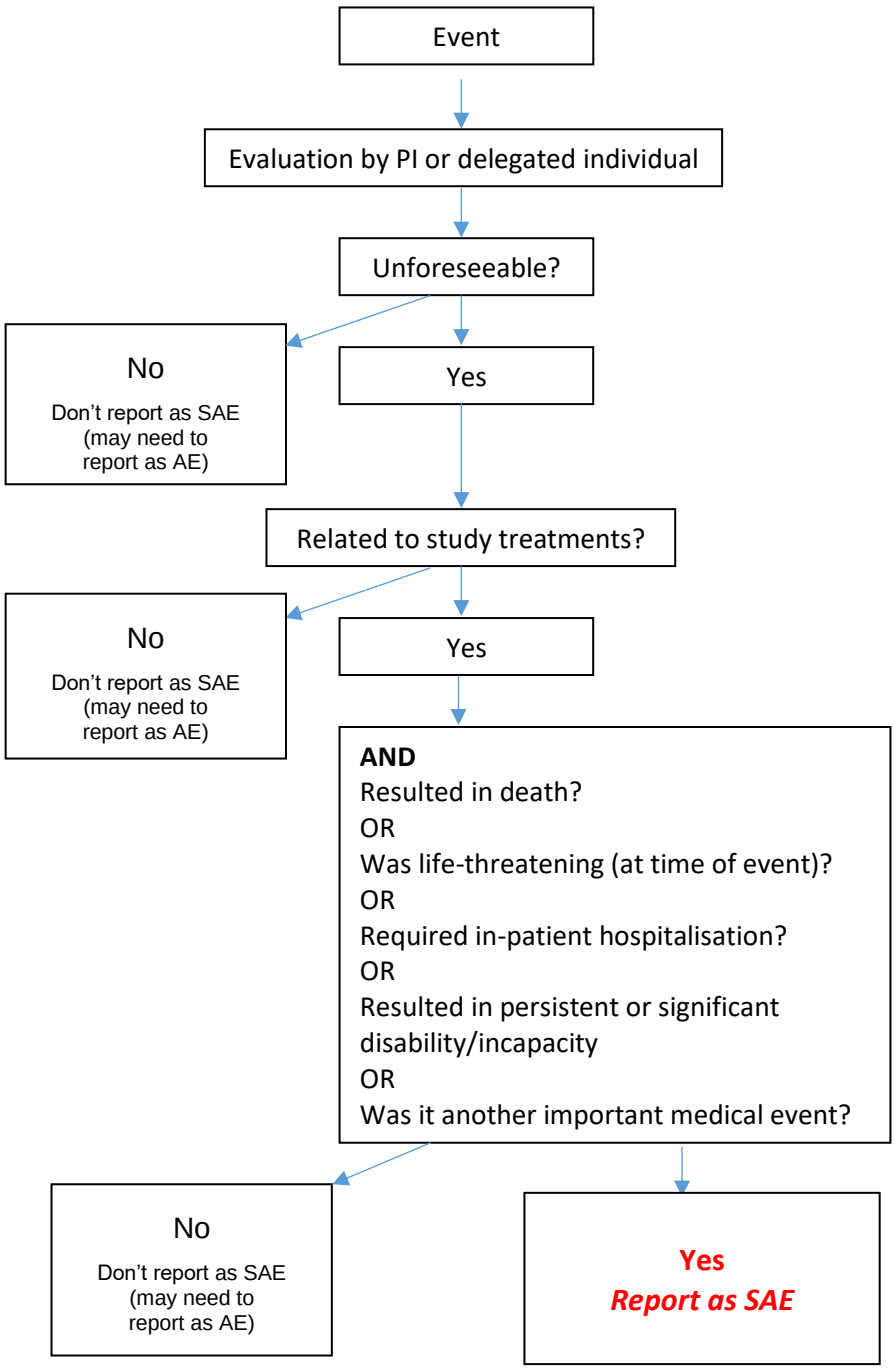
Any outstanding information should be provided as soon as possible.

Where the assessment indicates the event is **not** an SAE, the event will be classed an adverse event and should be reported to the study coordinating office in Oxford on the appropriate PATH-2 CRF during normal data collection.

CI and independent SAE Assessor

SAEs reported to the CI, once investigated, will be assessed either by the CI or an independent assessor (SAE Assessor) who is a health care professional. They will consider the SAE and give an opinion on whether the event is: 'related' – that is, it resulted from administration of any of the research procedures; and 'unforeseeable' – that is, the type of event is not listed in the protocol as a foreseeable occurrence. The assessor will inform the CI of their decision within 10 days of being notified of the event.

Figure 4: Collaborating sites: evaluating an event



Reporting SAEs to REC: The CI will be responsible for reporting all study SAEs occurring to a participant to the REC that gave a favourable opinion of the study where, in the opinion of the SAE Assessor, the event is confirmed as 'related' and 'unforeseeable'.

Confirmed SAEs will be sent by the CI to the REC within 15 days of the CI becoming aware of the event. The information provided to the REC will be unblinded.

No separate annual safety report in addition to the information provided through the annual progress report to the REC is required for this study.

10 STATISTICS AND ANALYSIS

A senior medical statistician from OCTRU was involved in designing this study and developing the statistical methods.

The primary statistical analysis will be carried out on the basis of intention-to-treat (ITT), with all participants being analysed according to their allocated treatment group irrespective of which treatment they actually receive.

A separate Statistical Analysis Plan (SAP) will contain full details of all statistical analyses and will be prepared early in the trial and finalised before the primary analysis database lock. A summary of the planned statistical analysis is included here.

Description of Statistical Methods

The primary outcome will be the HRET measured by the LSI at 24 weeks post randomisation. The time window for collecting the HRET will be 24 weeks ±2 week to allow some flexibility. If the data on the primary outcome is normally distributed then the two groups (placebo x treatment) will be compared using an unpaired Student's t-test to compare the unadjusted mean scores, by multivariate linear regression adjusting for the minimisation factors (primary analysis) and by multivariate linear regression adjusting for additional prognostic factors. For all continuous secondary outcomes if there are severe departures from normality the first approach will be data transformation. If the data cannot be transformed to normality then the Mann-Whitney U test will be used and in this case no adjustment will be made. The analysis will be by ITT.

A sensitivity analysis will assess the internal validity of the trial results by performing a per-protocol analysis on all subjects who adhere to the major criteria in the protocol, as determined by a blinded analysis immediately prior to the primary analysis database lock.

Safety will be summarised by treatment arm for all patients who started treatment.

The patient reported outcome measures (PROMs) ATRS, PSFS, SF-12 and pain scores will be analysed in a linear mixed model longitudinal framework to allow all data collected at all time-points to be taken into account. This is a robust procedure that deal with some missing values; however, missing data imputation will be carried out if necessary. Full details will be available in the SAP.

For the sub-studies primary analyses will primarily be descriptive with, additionally, the relationship between various biomarkers and clinical outcomes being explored.

The Number of Participants

214 patients (107 per arm) will provide 90% power to detect a standardised difference of 0.5 in the HRET work measured by the LSI at 24 weeks post randomisation and with 5% (2-sided) significance allowing for 20% loss to follow-up. This is based on previous data from the non-surgical arm of the Nillson-Helander 2010 study (28) who observed a clinically important difference of 10% with an SD of 20.

This sample size will also provide 90% power and 5% (2-sided) significance to detect an effect size of 0.5 in the ATRS (patient reported outcome) between the two treatment arms, based on a difference of 11 and an SD of 21.4.

A 58% response rate to the extended follow-up (n=124) will provide 80% power at a 5% significance level to detect an effect size of 0.5 in the ATRS. With a more optimistic response rate of 79% (n=168), the study would have 90% power to detect a similar effect size (0.5) at the same significance level (5%).

The Level of Statistical Significance

All the tests will be done at a 5% two-sided significance level. All comparative results will be presented as summary statistics with 95% confidence intervals and reported in

accordance with the Non-Pharmacological extension to the CONSORT statement.(40, 41)

Procedure for Accounting for Missing, Unused, and Spurious Data

Missing data will be reported and summarised by treatment arm. The distribution of missing data will be explored in order to assess the assumption of data being missing at random. Multiple-imputation will be utilised, if appropriate. Full details will be provided in the SAP.

For the PROMs a linear mixed longitudinal model will be used to analyse all available data. This method can take account of missing observations either due to missed visits or to a patient leaving the study prematurely, and can also be used when patients are not all assessed at exactly the same time point as the exact time for each observation is used in the analysis.

In the optional 24 month follow-up, missing data constitutes data items that remain incomplete after the questionnaire has been partly completed, either by post or by phone, and attempts have been made to contact the patient for clarifications. Missing data at this point will be treated as for the main study.

Procedures for Reporting any Deviation(s) from Original Statistical Plan

The SAP will be drafted early in the trial and finalised prior to the primary analysis datalock and before unblinding of the data. Any changes at this time will be incorporated into the final SAP and signed off as per current OCTRU SOPs. Any changes/deviations from the original SAP will be described and justified in protocol and/or in the final report, as appropriate.

Inclusion in Analysis

All available data from both treatment arms will be used in data analysis. Safety will only be assessed in patients who underwent the intervention.

Data and Safety Monitoring Committee

An independent Data Safety Monitoring Committee (DSMC) will be established to safeguard the interests of trial participants, potential participants and future patients, to assess the safety and efficacy of the interventions during the trial, and to monitor the overall conduct of the trial, protecting its validity and credibility

The DSMC will adopt a DAMOCLES charter which defines its terms of reference and operation in relation to oversight of the trial. They will not be asked to perform any formal interim analyses of effectiveness. They will, however, see copies of data accrued to date, or summaries of that data by treatment group. They will also consider emerging evidence from other trials or research on PRP. They may advise the chair of the Trial Steering Committee at any time if, in their view, the trial should be stopped for ethical reasons, including concerns about participant safety. DSMC meetings will be held at least once a year during the recruitment phase of the study.

Go/No go Assessment

The funder will carry out an assessment of study feasibility after the 10th month following the start of recruitment. The predicted site and participant recruitment rates, intervention compliance and safety will be assessed and discussed with the DSMC. The DSMC will make recommendations about the continuation or otherwise of the study to the Trial Steering Committee. The TSC will make the final decision and report to the funder.

11 DATA MANAGEMENT

Access to Data

Direct access will be granted to authorised representatives from the Sponsor or host institution for monitoring and/or audit of the study to ensure compliance with regulations.

Data Recording and Record Keeping

All data will be processed according to the Data Protection Act 1998, and all documents will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent and the contact details used for follow-up, the participant is referred to by the study participant number/code, not by name. Identifiable information is stored separately from study data.

Patient contact details are entered online by site staff through the secure randomisation service RRAMP at, or shortly after, randomisation. Site staff are requested to send consent forms by secure email to a study secure email address.

All other data is collected from participants and site personnel via paper CRFs which are returned to the central trial office by post using a Freepost address (pre-paid). In some cases CRFs may be collected during a site visit or face-to-face meeting with a member of the local team.

Data collected at the 4, 7 and 13 week follow-up time-points will be by telephone call or during a face to face outpatient appointment with responses recorded directly onto the CRF by the participant (or by the research team member who is making the telephone call), see Appendix D. Where necessary a member of the wider research team in Oxford will carry out telephone follow-up. A postal option of data collection may be used as necessary.

At the 24 month timepoint, extended follow-up data will be collected by post via paper CRF sent directly to trial participants by the central trial office, completed by participants, and returned to the central trial office by post using a Freepost address. Participants who do not respond will be contacted by central office staff by telephone. Participants who wish to opt out of the optional 24 month follow-up will be asked to return their blank questionnaire in the prepaid envelope.

Blood samples and needle biopsy samples sent for analysis will be anonymised at source and only identified using the unique study number and participant initials. Blood samples will be stored at the Centre for Translational Inflammation Research at the Birmingham University Research Laboratory, disposed of at the end of the study. Needle biopsy samples for those participants taking part in the sub-study (n=16), will be stored in the Oxford Musculoskeletal Biobank. Any data provided from the blood sample

analysis or biopsy samples analysis may be entered into the study database in Oxford. Data transferred will use appropriate password protected and/or encrypted files.

The HRET data will be collected from the participant during a face to face visit at 24 weeks, this includes tendon movement data. Movement data is transferred via the linear encoder linked to a study-dedicated laptop then transferred to the study-dedicated database in Oxford. MuscleLab software (Ergotest Innovation AS, Norway) will be used to run and record the HRET data. A video file will be made of the ankle/leg movements associated with the HRET, where a participant provides consent, filming concentrating on the ankle/leg area only. The video file will contain no identifying details and only the unique study number will be used. The file will be sent to the central coordinating team in Oxford where it may be viewed by members of the study team and will aid interpretation of the HRET data.

The central study office will receive the CRFs and the coordinating team will carry out appropriate data quality and validation checks, and the data will be entered into a study-dedicated database which is developed and maintained by the Oxford Clinical Trials Research Unit, a UKCRN Registered Clinical Trials Unit. OpenClinica software will be used. Central study office staff may phone participants to resolve data queries on the 24-month questionnaire, if the participant has answered “yes” on the 24–month questionnaire to give permission to this.

To identify manual entry errors a 10% double entry check of follow-up questionnaires will be carried out at regular intervals during the data collection phase of the study. A Data Management Plan will be produced for the trial.

Trial documentation must be retained for 5 years after completion of study-related activities. Collaborating sites are delegated the responsibility of archiving local essential documents in an appropriate secure environment locally. The central Trial Master File and associated documents in Oxford will be archived according to University of Oxford policy and this may include the use of an external professional archiving site.

12 QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

A Monitoring Plan will be developed according to OCTRU's SOPs which involves a risk assessment. The monitoring activities will be based on the outcome of the risk assessment and may involve central monitoring or site monitoring.

13 ORGANISATION

Trial Steering Committee

The Trial Steering Committee (TSC) provides overall supervision of the trial on the behalf of the funder and is chaired by an Independent Member. The TSC abides by the OCTRU SOP and the OCTRU TSC Charter which is based on the MRC Clinical Trials Unit template. The TSC will monitor trial progress and conduct and advise on scientific credibility. The TSC will consider and act, as appropriate, upon the recommendations of the DSMC or equivalent and carries ultimate responsibility for deciding whether a trial needs to be stopped on grounds of safety or efficacy. Meetings of the TSC will take place at least one a year during the recruitment phase of the study. This is typically 6-8 weeks after the DSMC meeting to allow the TSC to consider the recommmendations of the DSMC.

Study Central Co-ordinating Team

This team will oversee the day-to-day running of the trial, the majority of the team based in Oxford. Contact details are provided in ISFs.

For project timeframe see Gantt Chart in Appendix C.

Local Co-ordination

Each participating site will have a local surgical co-ordinator who will take on the role of Principal Investigator and oversee the study activities and compliance with the protocol.

14 ETHICAL AND REGULATORY CONSIDERATIONS

Declaration of Helsinki and Good Clinical Practice

This trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice and the applicable requirements as stated in the Research Governance Framework for Health and Social Care (2nd edition 2005). Local investigators must ensure the study is conducted in accordance with relevant regulations and with Good Clinical Practice.

Approvals

The PATH-2 study can only start after approval from one of the Health Research Authority Research Ethics Committee, and once local Trust management approval and a local contract (Clinical Trials Agreement) is in place. This study does not fall under MHRA guidelines.

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and host institution(s) for written approval.

The Sponsor will also review the documents. The Sponsor for the PATH-2 study is: University of Oxford.

The Chief Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

The REC has the purpose to look after the rights, well-being and dignity of patients. The REC reference number is given on the front page of this protocol. The REC that reviewed this study was the **South Central - Oxford A REC, Ref: 14/SC/1333.**

Local approvals

The study office will assist collaborating sites with the necessary approvals to allow the study to take place within their Trust. Typically this involves the submission of a Site Specific Information electronic form via the on-line Integrated Research Application System, and a signed contract between the Sponsor and the local site's Research and Development Office. Once these approvals are in place the study coordinating office in Oxford will inform the local Principal Investigator of the date the study can open to recruitment at their site.

Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

Funder reports

The funder requires regular Progress Reports throughout the main study period and a final report at the end of the main study.

Trial registration

The PATH-2 study is registered on publically available databases on the internet:

1. The International Standard Randomised Controlled Trial Number (ISRCTN) Registry: The ISRCTN for PATH-2 is ISRCTN54992179 http://www.isrctn.com/search?q=ISRCTN54992179

2. Clinicaltrials.gov: The identifier for PATH-2 is NCT02302664 https://clinicaltrials.gov/ct2/show/NCT02302664?term=path-2&rank=1

3. UK Clinical Trials Gateway. The identifier is NCT02302664. https://www.ukctg.nihr.ac.uk/trials/trial-details/trial-details?trialId=14850

Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participant study number on the CRF and any electronic database. All documents will be stored securely and only accessible by the central trial team and authorised personnel. The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so.

Personal data held by the central coordinating office in paper format to facilitate follow-up and results reporting will be stored separately from any data collected and only accessed by authorised personnel. The consent form includes consent for this data to be held.

Expenses and Benefits

Reasonable travel expenses for the 24 week follow-up visit, which is additional to normal care, will be reimbursed on production of receipts or a mileage allowance applied. The study coordinating team in Oxford will process the request for reimbursement following receipt of a completed claim form and production of receipts (University of Oxford policy will be followed). Participants taking part in the needle biopsy will also be reimbursed for travel to the biopsy appointment. Participants who have given consent to being contacted for the 24-month follow-up will be sent, enclosed with their questionnaire, a £5 shopping voucher and a pen, which they may keep whether or not they participate in the optional 24-month follow-up.

Other Ethical Considerations

Short timeframe to consider participation: Due to the nature of the injury and the treatment being evaluated there is a short time frame for the participant to consider taking part in the study. Patients attend clinic after an unexpected event that typically requires a cast to be fitted, usually within the first clinic following their injury. The injection being evaluated in this study is given before the cast is applied.

We have made the PIS easy to read while still providing full information. We will provide copies for reading during the clinic waiting time which can be up to several hours. We also provide our website details to allow the participant on-going ease of access to study information. Website: http://path2.octru.ox.ac.uk/

Unblinding after completion of the trial: The participants recruited to this trial will be invited to participate in longer term follow-up. Therefore participants may not become aware of their allocated treatment until after the official end of study date given in this Protocol (See Section 7 for further information).

15 FINANCE AND INSURANCE

Project Funding

Project funding for the study up to and including the 24 week follow-up was awarded by the Efficacy and Mechanism Evaluation (EME) Programme and is funded by the Medical Research Council (MRC) and managed by the National Institute for Health Research (NIHR) on behalf of the MRC-NIHR partnership.

Funding for the extended follow-up was awarded by the Kadoorie Centre Trauma Research Charitable Fund.

Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment which is provided.

Funding for participating sites

The PATH-2 study is a UK CRN portfolio study and as such collaborating sites may have access to resources within the local NIHR Clinical Research Network in England. The lead Network for PATH-2 is Thames Valley and South Midlands. For details of your local CRN see contact details on their CRN website (at time of writing the website is: http://www.nihr.ac.uk/about-us/how-we-are-managed/managing-centres/crn/).

Data collection at 4, 7 and 13 weeks

The PATH-2 study will reimburse collaborating sites for study-specific data collection.

The HRET at 24 weeks (primary outcome)

The HRET will be carried out by a blinded assessor who will be a member of the local team at a site. A fee will be paid per assessor trained in the study HRET by a member of the Oxford central coordinating team (to a maximum of 4 assessors per site), and a fee will be paid for each successful 24 week follow-up assessment carried out.

A laptop and equipment to carry out the HRET will also be provided.

All reimbursement figures are pre-set and include VAT, full details will be provided in the contract between the site and the Sponsor.

Discontinuing/withdrawal of a participating site

Recruitment and screening data will be monitored by the trial team. This will also be reviewed by the Trial Management Group, the Trial Steering Committee and the Data and Safety Monitoring Committee. Where necessary, after appropriate support, if a site has persistent low recruitment or is unable to facilitate the timely delivery of the intervention, a site may be required to close and resources linked to the study moved to another site (specifically equipment relating to the HRET).

16 PUBLICATION POLICY

Data from this study should not be presented in public or submitted for publication without requesting consent from the Trial Steering Committee.

Authors will acknowledge that the study was funded by the NIHR EME. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. The Chief Investigator will coordinate dissemination of data from this study. All publications using study data from the main analyses will be submitted to the TSC for review before release.

A summary of the trial outcome will be disseminated to trial participants on relevant websites, and by email, where an email address is provided.

In addition to the NIHR monograph report, the results will be published in peer-reviewed medical literature, and may be presented at relevant national and international conferences. The work may also contribute to any refresh of NICE guidance.

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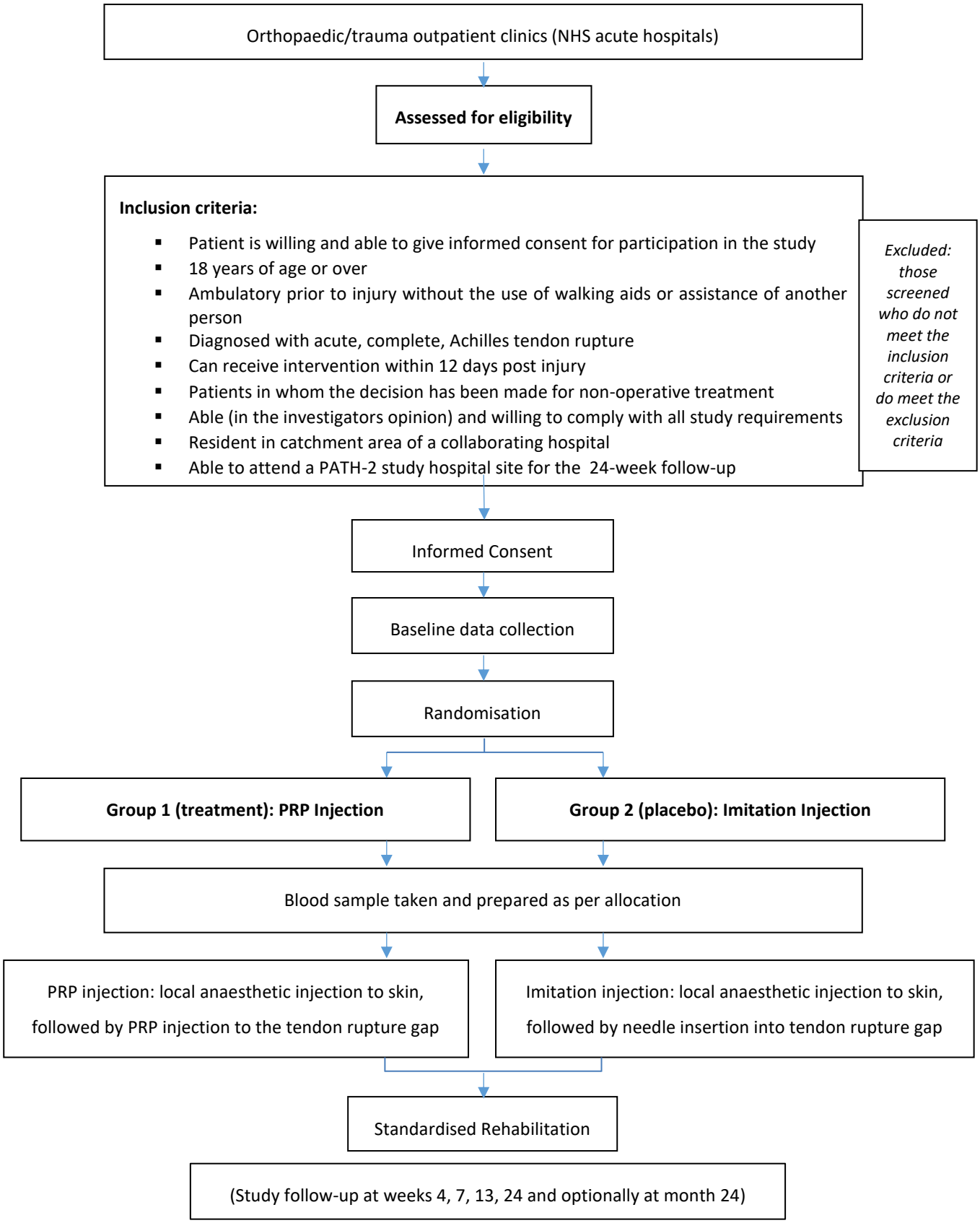
18 APPENDIX A: STUDY FLOW CHART



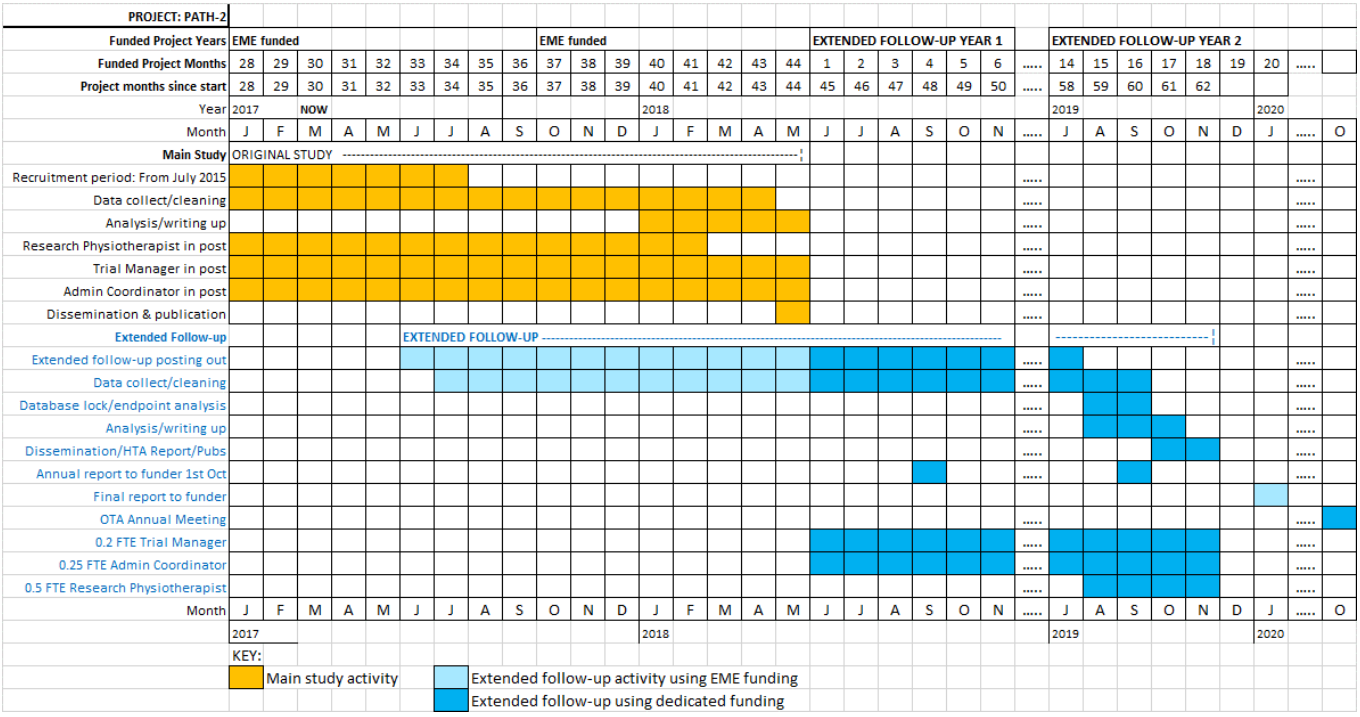
19 APPENDIX B: SCHEDULE OF STUDY PROCEDURES

Time point	Enrolment	Baseline	Allocation	Treatment	Post-allocation				
					Weeks 0-2	Week 6	Weeks 4,7, 13	Week 24	Month 24
Eligibility screen	X								
Informed consent	X								
Baseline questionnaire		X							
RANDOMISATION			X						
Blood sample				X					
Allocation A: PRP injection				X					
Allocation B: Imitation injection (placebo)				X					
ASSESSMENTS									
Baseline • Background data including medical history • SF-12 Pre-injury • SF-12 • ATRS • PSFS • VAS Pre-treatment		X							
Pain Diary: VAS completed daily at home first 2 weeks					X				
4,7,13, 24 week Follow-up • SF-12 • ATRS • PSFS • Rehabilitation questions							X	X	
24 week follow-up (Primary outcome) • HRET-related • HRET technical data • Employment/Activity/pain relief								X	
24 month follow-up (optional) • SF-12 • ATRS • PSFS									X
Adverse events				X	X	X	X	X	
Selected sites only:						X			

Needle biopsy (n=16) week 6									

Telephone or outpatient data collection: x 3 at 4, 7,13 weeks
Postal or telephone data collection at 24 months
Hospital outpatient visit: x 1, at 24 weeks
Selected sites only (n=16): 1 additional visit for biopsy participants

20 APPENDIX C: STUDY GANTT CHART



21 APPENDIX D: BLINDED FOLLOW-UP AT 4, 7, AND 13 WEEKS

The blinded follow-up at 4, 7, and 13 weeks may be undertaken by one of the local research team who is unaware of the participant's treatment allocation. No physical tests are involved in this follow-up. Data collection is via a pre-defined study follow-up questionnaire containing outcome measures and other relevant questions as outlined in this Protocol.

The PI at each site will identify local staff who will be responsible for carrying out blinded follow-up assessments. The Delegation Log should be updated accordingly to reflect the delegation of these duties. New staff should be trained and added to the log as the study progresses. When the Delegation Log is updated, a copy should be sent to the study coordinating office in Oxford.

Follow-up should be planned in advance to ensure data is collected as close as possible to the 4, 7, and 13 week time frames, ideally **+/- one week** of the follow-up due date. Follow-up dates are calculated from the date the participant **received** their **study treatment**, the allocated treatment given by the randomisation service ('PRP injection' or 'Imitation (placebo) injection'). Staff should check any preferred contact time the participant has indicated regarding 'best time' to be contacted.

We anticipate telephone contact will be the main method of organising follow-up. Text and email may be used were appropriate to facilitate timely follow-up where these contact details have been provided. Due to the relatively short window between each follow-up, once a date has been agreed a text or email to the participant confirming the agreed time for follow-up may act as a useful reminder. Ethics approved text and email content has been approved, see ISF.

Prior to each follow-up the three activities the participant chose at baseline as activities they wish to improve should be written into the PSFS section of the current follow-up questionnaire. This will ensure the appropriate activities can be referred to during follow-up. These activities are unique to the participant and were originally listed on the PSFS section of the baseline questionnaire.

Follow-up may take place over the telephone using the contact details provided by the participant. Responses should be transcribed directly onto the paper questionnaire.

Where follow-up is carried out during a hospital visit, a member of the research team should oversee the completion of the questionnaire, checking completeness before the participant departs, following through on any missing data as required.

Once follow-up has taken place and the questionnaire fully completed, a photocopy should be taken. The original is posted (via a pre-paid account) to the central study office in Oxford for data entry, the copy is placed in the study ISF locally (where a carbonated copy is provided the copy should remain at the site and the top copy is sent to the study office).

The central study office in Oxford should be notified as soon as possible where there are difficulties contacting participants for follow-up.

Telephone follow-up carried out by the central study team in Oxford

Where necessary, after discussion with the local site, the study office may carry out telephone follow-up to facilitate timely data collection.

APPENDIX E: BLINDED FOLLOW-UP AT 24 WEEKS

The blinded follow-up at 24 weeks is a face-to-face assessment carried out by a trained blinded physiotherapist/assessor who is unaware of the participant's treatment allocation. A physical test, the HRET, is conducted at the 24 week follow-up and where appropriate separate consent will be sought to film the leg movements of this test at the time of the test. In addition to the HRET, data collection is via a pre-defined study follow-up questionnaire containing outcome measures and other relevant questions as outlined in this protocol.

The PI at each site will identify local staff who will be responsible for carrying out blinded follow-up assessments at 24 weeks. The Delegation Log should be updated accordingly to reflect the delegation of these duties. New staff should be trained and added to the log as the study progresses. When the Delegation Log is updated, a copy should be sent to the study coordinating office in Oxford.

Follow-up should be planned in advance to ensure follow-up data is collected as close as possible to the 24 week time frames, ideally within +/- two weeks of the follow-up due date. Follow-up dates are calculated from the date the participant received their study treatment, the allocated treatment given by the randomisation service ('PRP injection' or 'Imitation (placebo) injection'). Staff should liaise with the central study office in Oxford where necessary to report any difficulties contacting participants.

We anticipate telephone contact will be the main method of organising follow-up. Text and email may be used were appropriate to facilitate timely follow-up were these contact details have been provided. Once a date has been agreed a text or email to the participant confirming the agreed time for follow-up may act as a useful reminder. Ethics approved text and email content has been approved, see your ISF.

Prior to follow-up the three activities the participant chose at baseline as activities they wish to improve should be written into the PSFS section of the current follow-up questionnaire. This will ensure the appropriate activities can be referred to during follow-up. These activities are unique to the participant and were originally listed on PSFS section of the baseline questionnaire.

The physiotherapist/assessor should oversee the completion of the questionnaire, checking completeness before the participant departs, following through on any missing data as required.

Once follow-up has taken place and the questionnaire fully completed, a photocopy should be taken. The original posted (pre-paid account), to the central study office in Oxford for data entry, the copy placed in the study ISF locally (where a carbonated copy is provided the copy should remain at the site and the top copy sent to the study office).

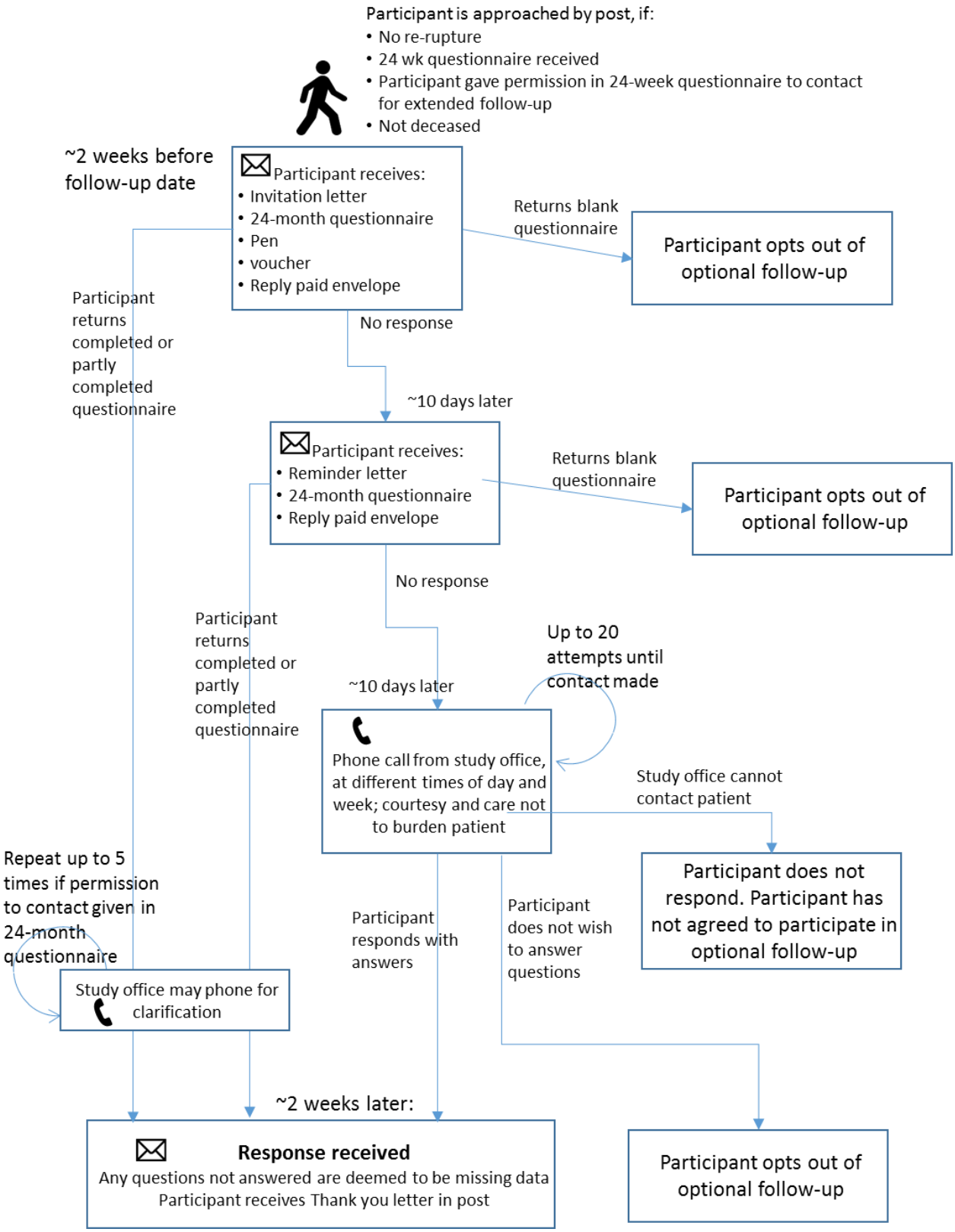
If due to unforeseen circumstances the participant is not able to attend for the 24 week face to face appointment, site staff should inform the study office as soon as possible. In such cases where all other options have been considered, the coordinating team may collect the secondary outcome measures by post.

APPENDIX F: OPTIONAL EXTENDED FOLLOW-UP AT 24 MONTHS

At 24 months after treatment, participants who agreed to being contacted for longer-term follow-up are contacted first by post and if no response is received, by telephone. This follow-up is conducted entirely by central study office staff.

Figure 5 describes the procedure for the optional 24-month extended follow-up.

Figure 5: Flow diagram of procedure for contacting participants for optional 24-month extended follow-up.



22 APPENDIX G: SUB-STUDIES

Two mechanism and efficacy sub studies are embedded in the main trial.

Sub-study 1: PRP components analysis

This will inform our understanding of the active biological components of clinically prepared PRP, their distribution in the study population and the correlation with clinical outcomes. We will measure:

1. Cell concentration: Platelets, white blood cells and red blood cells
2. The release from the platelets of the bioactive contents and activation (CD62P marker expression)
3. Tendon-related active growth factor concentrations

These components have been identified as key to the biological response to injury in previous cell and animal studies (6).

Sub-study 1 Method

Venous blood is obtained from all participants following consent and randomisation. Up to 55 ml of blood will be obtained.

The blood sample is processed according to allocated treatment. Detail here is purposely left brief. To detail the specifics of blood withdrawal and processing may hinder blinding of the participant as the protocol is publicly available. Details are provided in the PATH-2 intervention and blood processing training materials provided to collaborating sites.

Following treatment blood samples will be dispatched to an off-site laboratory led by Dr Paul Harrison (co-applicant) in Birmingham University which is collaborating with the PATH-2 study. Transfer to the laboratory is time critical for some samples, see full guidance in the PATH-2 intervention and blood processing training materials. Following completion of the study blood samples will be disposed of appropriately in line with laboratory policy.

A pre-paid mailing system will be used which will cover the cost and facilitate the dispatch of samples to the laboratory. Advice on how to organise the despatch of samples will be provided.

Sub-study 2: Immunohistochemistry analysis

This sub-study will investigate the biological characterisation of PRP and the immunohistochemical mechanisms which may account for its clinical efficacy. This will include measures of tendon tissue regeneration, cell differentiation and collagen formation, and examining tissue morphology and pain neuroreceptors in the healing tendon. In addition, it will help to identify the transcriptional or gene expression pathways that PRP may alter to exert its effects. Using these results and that from the PRP biological component sub-study (1), it may inform future targeted manipulation of PRP properties to maximise its efficacy in tendon healing.

Sub-study 2 method

16 participants (8 in each arm) will be invited to take part in this sub-study which will take place at the Oxford site (and may expand to include other sites if necessary to achieve full recruitment). The study will involve participants undergoing an ultrasound-guided needle biopsy at week 6 post treatment to retrieve a tissue sample from the healing Achilles tendon. This will be performed by a senior radiologist in the radiology outpatient department using a validated percutaneous ultrasound-guided needle biopsy technique, already developed by the study team.(42). This sterile method employs an automated biopsy needle to resect a small amount of tissue from the tendon. There have been no reports of side effects after using this method.

Biopsy samples will be divided into two sections for use in histological and transcriptomic analysis. The sections are:

1. RNA transcription section: this will be placed in RNAlater solution and stored at -80 °C in a designated repository shared with Oxford Musculoskeletal Biobank (HTA approved, Botnar Research Centre, NDORMS, University of Oxford) until the end of recruitment. RNA sequencing / transcriptomics is to be carried out by Dr Phillipa Hulley's (co-applicant), research group in Oxford.
2. Histology section: this will be fixed in 10% buffered formalin and wax embedded. Sections will be stained by an autostainer using an automated staining device for markers that may be involved in PRP mechanism: proliferation (KI-67), Collagen I & III, neuroreceptors, angiogenesis and hypoxia/ROS markers. Histology will be scored by a blinded assessor using a validated score (Bonar and Movin). Immunohistochemistry (IHC) markers

images will be anonymised and scored using validated automated image analysis software operated by a blinded assessor.

We have already demonstrated feasibility of this technique and its practical use in Achilles tendon rupture in a pilot study (20).

Travel expenses for participants attending for Needle Biopsy

Participants agreeing to take part in the needle biopsy sub-study will attend a designated hospital responsible for carrying out this procedure; this is an additional visit. Travel costs can be claimed and will be reimbursed according to the University of Oxford guidelines.

The needle biopsy is taking place in selected sites only, and involves 16 participants. Staff in those hospitals will be given further information regarding the process for reimbursing participants.