Study protocol for a randomised placebo controlled trial of platelet-rich plasma injection to prevent post-traumatic knee osteoarthritis after anterior cruciate ligament reconstruction

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ABSTRACT

Introduction The elevated cytokine levels in patients suffering from anterior cruciate ligament (ACL) rupture may lead to acute post-traumatic arthritis (APTA) and post-traumatic osteoarthritis (PTOA). Due to its chondrogenic and anti-inflammatory effect, platelet-rich plasma (PRP) therapy is expected to show a positive outcome in APTA and PTOA. The proposed trial aims to quantitatively measure the efficacy of PRP injection in arresting post-traumatic cartilage degeneration among patients after ACL reconstruction.

Methods and analysis This will be a single-blind, randomised, prospective clinical trial designed following the Consolidated Standards of Reporting Trials guidelines. After ACL reconstruction, 80 patients will be randomised to receive either leucocyte-poor PRP injection after joint aspiration or a placebo control group receiving only joint aspiration. Participants (age 20–49 years) will be those who have undergone ACL reconstruction within the past 2 weeks with a body mass index<35 and Kellgren Lawrence osteoarthritis grade<2. The primary outcome will include MRI-T2 values of knee cartilage at 6 months. The secondary outcomes will include pain assessment by Visual Analogue Scale, Knee injury and Osteoarthritis Outcome Score, blood and urine test, physical findings, measurements for muscle strength and joint stability.

Ethics and dissemination The study was approved by the Independent Ethics Committee for Clinical Trials of the Japanese Association for the Promotion of State-of-the-Art Medicine. Results of the trial and each of the outcomes will be shared via conferences and publication in a peer-reviewed journal.

Trial registration number jRCTb030200391.

INTRODUCTION

Post-traumatic osteoarthritis of the knee (PTOA) is secondary osteoarthritis that develops after joint trauma such as ligament injury, meniscus injury, or intra-articular fracture. In the case of anterior cruciate ligament (ACL) injury, the risk of total knee arthroplasty is about seven times higher relative to the uninjured knee.1,2 Joint injuries, with or without associated disruption of the articular surface, frequently lead to a severe debilitating condition known as acute post-traumatic arthritis (APTA). With regard to knee injuries, approximately 40% are believed to progress to PTOA so it is especially important to suppress APTA in order to prevent progression to PTOA.3

The main causes of PTA are structural damage, joint instability and mechanical stress, but in recent years, biological factors have attracted attention as a possible contributor. In addition to the initial mechanical injury sustained by articular chondrocytes, inflammatory chemokines are released following injury, which have both immediate and longer lasting effects. Following ACL rupture, a common injury in athletes participating in contact or jumping sports, numerous inflammatory cytokine levels immediately...

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Elevated inflammatory cytokines can promote the development of PTOA via several different metabolic pathways. When the balance between inflammation and anti-inflammatory response is broken, this can lead to chronic inflammation, which in turn leads to APTA and eventually progression to PTOA. Although in the case of ACL rupture, reconstructive surgery and rehabilitation may stabilise the knee and improve the condition in many patients, some patients still progress to PTOA.

One potential treatment to counteract the inflammatory process is the augmentation of standard surgical and rehabilitation therapies with platelet-rich plasma (PRP) injection. This is the most frequently used cell-based therapy in which platelets are purified from venous blood. PRP is believed to have tissue repair and anti-inflammatory potential due to growth factors released from the platelets and cytokines. Previous experimental studies have suggested that PRP promotes extracellular matrix formation in human articular chondrocytes and inhibits inflammatory processes in osteoarthritic chondrocytes. Thus, intra-articular injection of PRP may possess both chondroregenerative and anti-inflammatory effects. Previous studies of PRP effectiveness are based on not objective outcome measures, but on patient-reported outcomes, such as the Knee Injury and Osteoarthritis Outcome Score (KOOS). We suspect that the anti-inflammatory effects of PRP may help arrest the progression of APTA. At present, there are no approved therapies to address APTA and prevent the onset of PTOA. Therefore, we hypothesise that PRP will suppress inflammation in APTA and prevent the transition.

MATERIALS AND METHODS

Study design

The present study is a single-blind, randomised, prospective, controlled comparative clinical trial at Juntendo University, Tokyo, Japan. The study was approved by The Independent Ethics Committee for Clinical Trials of the non-profit organisation, the Japanese Association for the Promotion of State-of-the-Art Medicine. It was registered with Japan Registry of Clinical Trials (trial registry number: jRCTb030200391). We will compare an active treatment group of patients injected with PRP after joint aspiration and a placebo control group of joint aspiration only. We will conduct our randomised controlled trial in accordance with the guidelines of the Consolidated Standards Of Reporting Trials (CONSORT) and will provide a CONSORT checklist on publication our results.

Patients

Eligible patients will be those ages 20–49 years of age who have had a primary ACL reconstruction using a single bundle semitendinosus graft within 2 weeks prior to recruitment with a body mass index (BMI)<35, and a Kellgren Lawrence osteoarthritis grade<2. Patients will be excluded if they are diagnosed with a multiligament injury of the knee (posterior cruciate ligament injury, posterolateral corner injury, and/or medial collateral ligament injury requiring repair), a lateral femorotibial angle of 10° or more, or one of the following comorbidities: malignancy, hepatitis B, C, Human T-cell leukemia virus type 1 (HTLV-1), syphilis, HIV infection, autoimmune rheumatological disease, blood disorder, drug addiction, end-stage renal disease) patients whose communication cannot be measured; patients judged by researcher to be inappropriate. At the time of recruitment, eligible patients will provide informed consent and must be judged by the recruiting researcher to be committed to comply with all study procedures and have no obvious communication limitations or lack of dedication to participation.

Patient and public involvement

Patients and general public are not involved in the design of this study, participant recruitment, or in the determination of the outcome measures of the current trial.

Procedure

The summary of this trial is outlined in figure 1. In-hospital patients who have undergone ACL reconstruction within the past 2 weeks will be screened for eligibility. Once written informed consent is obtained, patients will complete the pretreatment assessment reporting the Tegner Activity Scale (TAS). As standard of care, all ACL reconstruction patients at this institution complete pain Visual Analogue Scale (VAS), and KOOS. On completion of informed consent, all study patients will be randomised to either group.

PRP preparation

For patients randomised to PRP injection, the PRP preparation will be obtained by a single centrifugation of whole blood using the MyCells autologous platelet preparation system (Kaylight, Ramat HaSharon, Israel) from the 22 mL of whole blood aspirated from the median cubital vein. Approximately, 4.0–5.0 mL of PRP will be obtained according to the manufacturer’s instructions. The PRP obtained using this method is classified as P2-B PRP (LP-PRP) based on the Platelets Activation White Blood cells (PAW) classification system. In brief, 22 mL of whole blood will be aspirated into two sets of MyCells kit syringes containing 1 mL of anticoagulant dextrose solution A and separation gel. The samples will be centrifuged for 7 min at 2000-g. After aspirating the supernatant platelet-poor plasma, the residual 2.0–2.5 mL of plasma will be pipetted to peel off the platelets from the surface of the separation gel. The filter column will then be inserted into the separation syringe to remove the debris and filtered PRP. Blood draws from control patients will only be used for blood analysis.

Intervention protocol

All patients will have 22 mL of whole blood aspirated from the median cubital vein. All intra-articular injections will be administered by an orthopaedic surgeon using the lateral suprapatellar approach with patients’ eyes shielded by an assistant to prevent the patient from
knowing the type of injection received. All knees will be aspirated using a 23-gauge needle. Even with a 23-gauge needle, the synovial fluid can be sufficiently aspirated.

After joint aspiration, the surgeon will change the syringe to determine whether additional fluid can be drained or to inject PRP. All aspirations will be performed between 2 and 7 weeks post-ACL reconstruction. Postoperative cytokines released from bone hole, intra-articular hematoma and synovium are supposed to have a strong effect in both groups after surgery particularly in the acute phase, so we aspirated joint fluid between 2 and 7 weeks. Aspiration will be performed in both groups. However, it can be a dry needle in case of insufficient amount of joint fluid.

Sample size
There is no previous study from which to estimate the sample size needs for this trial of PRP for the prevention of post-traumatic cartilage degeneration. A statistical power analysis was performed based on a prior study of adipose stromal vascular fraction injection. This sample size calculation (G-power, Germany) aimed for a power of 80%, based on a prespecified significance level of $\alpha<0.05$, and assuming a medium effect size (effect size = 0.35) with a dropout rate of 10%. This power analysis yielded an estimated sample size of 80 patients with a 1:1 allocation between PRP (40 patients) and control (40 patients).

Randomisation and blinding
Eligible patients will be randomly assigned into two groups: PRP or Control (joint aspiration only). age, sex, BMI and TAS will be set as a priori allocation factors, which will be adjusted for in multivariable analysis even if sufficient balance is achieved through randomisation. Randomisation will be performed by Research Electronic Data Capture, REDCap (Vanderbilt University, Vanderbilt, Tennessee, USA).

All patients will be blinded to the treatment to which they are allocated. Study staff who collect pre-treatment and post-treatment information (eg, PROMs scores) will also be blinded to the treatment. Finally, the orthopaedic
surgeon or musculoskeletal radiologist who calculates the T2 mapping scores will be blinded to the treatment allocation. Further, the treating surgeon will not be involved in postoperative assessment or T2 mapping evaluation. This triple blinding should prevent bias in assessment of outcomes from the patient and clinician perspective.

OUTCOMES

Patient characteristics

Patients’ information including sex, date of birth, BMI, TAS, radiographic assessments and medical history will be recorded. Additionally, information related to the injury such as date of operation, presence of meniscus and cartilage damage, preoperative MRI-T2 value of cartilage will be documented. Meniscus tear pattern is routinely documented intraoperatively during the ACL reconstruction. Cartilage injury will be graded by the International Cartilage Research Society Cartilage Defect Classification system.

Study outcome assessment

Study assessments will include MRI T2 values, blood test, urine test, joint fluid test, pain VAS, KOOS, physical findings, muscle strength measurement and joint stability measurement.

Primary outcome measures

Qualitative changes rather than morphological changes in MRI will be made the primary outcome for evaluation due to the relatively short time course of 6 months. T2 mapping sequences are quantitative cartilage imaging techniques which has been used to evaluate the early cartilage degeneration of the knee joint. T2 mapping values using a 3T MRI unit (Discovery MR750w 3.0T; GE Healthcare) will be assessed. The method of calculating the T2 mapping value is as follows; we will select medial and lateral slices that pass through the centre of the weight-bearing cartilage surrounded by the anterior and posterior margins of the meniscus on a sagittal slice with reference to the regional subdivision of the articular surfaces in Whole-Organ MRI Score. The region of interest (ROI) will be set at the weight-bearing full-thickness cartilage of the medial and lateral femoral condyle and medial and lateral tibial plateau on the central slice of the sagittal image. Overall, the T2 mapping values of 14 ROIs will be measured. According to this analysis, the lower the T2 mapping values, the lower the degree of articular cartilage degeneration.

Other outcomes

Patient reported outcome measures

Clinical outcomes will be assessed based on change in the the pain VAS and KOOS scores.

The KOOS is a validated self-administered patient-reported outcome measure with individual items graded on a 5-point Likert scale from 0 to 4, which comprises the following five subscales: pain, symptoms, activities of daily living, sport and recreation function, and knee-related quality of life. Quantification of growth factors and cytokines in blood, joint fluid and urine

All growth factors and cytokines in blood, joint fluid and urine will be quantitated using an ELISA kit.

► Blood: matrix metalloproteinase 3,13 (MMP-3,13), tumour necrosis factor-α (TNF-α), interleukin 6 (IL-6), interleukin 1 receptor antagonist (IL-1Ra), platelet-derived growth factor BB (PDGF-BB), insulin-like growth factor-1, cartilage oligomeric matrix protein.

► Joint fluid: MMP-3,13, TNF-α, MCP-1, IL-1Ra, IL-6, hyaluronic acid, vascular endothelial growth factor, PDGF-BB, a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5).

► Urine: C-telopeptide fragments of type II collagen (CTX-II).

Adverse events

A data safety monitoring board (DSMB) will be established for evaluation of study safety and early stopping rules. The study staff will collect information on adverse events such as adverse tissue reaction at the injection site (swelling, pain, sensitivity). Further, haematoma, nerve injury and bacterial infection will be evaluated at each follow-up visit. The study PI will make a preliminary determination as to whether each adverse event is probably, possibly, unlikely, or not related to the intervention. The DSMB will evaluate each adverse event to adjudicate the PI’s determination. For biannual DSMB meetings, the study statistician will create a blinded safety report to allow the DSMB to recommend study continuation or early stopping based on criteria to be determined by the DSMB members once the board is established. For ancillary and post-trial care, we have clinical research insurance.

Data collection and management

Data will be collected until patients return to sports activity. All data collected will be stored in REDCap. REDCap excels not only in data processing but also in confidentiality. To ensure confidentiality, each patient will be given a trial identification number. Data entry and coding will be performed by a data manager who will be allowed to access to the

Statistical analysis

The principle of intention to treat (ITT) will be applied for the primary outcome. The ITT analysis includes data that are incomplete and data from patients who have deviated from the study protocol. Multiple imputation methods will be used in handling any missing data. The T2 value (average value) as a primary end point will be analysed using an unpaired t-test, 2 factor analysis of variance, and mixed-effect model. The mixed model will be used to adjust for the a priori allocation factors of age, sex, BMI and TAS. All statistical analysis will be performed using the SPSS V.27.0 (SPSSS), and a p value of <0.05 will be considered to be statistically significant.
Monitoring and auditing
Central monitoring will be conducted to confirm that the trial is properly conducted by the independent committee, clinical trial centre of Juntendo University. And the auditor of Clinical Research Compliance Promotion Office of Juntendo University also will conduct visit audit at the end of this study.

ETHICS AND DISSEMINATION
The study was approved by the Ethics Committee of Juntendo University Hospital, was registered at the Japan Registry of Clinical Trials (jRCTb030200391), and adheres to the Declaration of Helsinki. Participation is strictly voluntary, and potential patients will be made aware of all procedures involved in the trial during the informed consent process. Patients will be required to sign the informed consent form given by researcher before participation. At any time, patients may withdraw from the study. If the protocol needs to be changed, the researcher will immediately inform the patients and staff. Results from this trial will be communicated at scientific conferences, and we also intend to publish our findings in a peer-reviewed journal.

Perspectives of the study
In recent years, more and more clinicians have been using autologous blood products such as PRP which offers a minimally invasive, simple approach to acquire the growth factors concentrations. At present, there are no approved therapies to address APTA and prevent the onset of the chronic disease PTOA. Therefore, we are hopeful that PRP may be a treatment that mediates the transition from APTA to PTOA.

Trial status
Recruitment started on 9 February 2021, and the study recruitment is expected to be completed by March 2024. Data collection will continue until the last recruited patient has completed follow-up. We plan to continue with longer term follow-up of these patients if possible.

Contributors YM, MN and YS conceived the trial and led the development of intervention design, data management, statistical analysis and drafted the first version of the manuscript. YM, YS, MN, YK, RN, SU, TW, HN, SF, SW, JT, KK, KY, MK and MI are involved in patient recruitment and data collection. YM, MN and MI provided valuable feedback in preparing the manuscript. YS, MN and MI have read and accepted the final version of the manuscript and are accountable for all aspects of the work.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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