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Protocol for a multicentre randomised triple-blind controlled trial assessing the clinical efficacy of intra-articular platelet-rich plasma injections versus placebo in symptomatic knee osteoarthritis (PIKOA)

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ABSTRACT

Introduction Despite their exponential use, intra-articular (IA) injections of platelet-rich plasma (PRP) are not part of the recommended treatments for knee osteoarthritis (OA) by most international scientific societies. The most recent clinical trials have shown conflicting results, and some did not find any clinical benefit of PRP injections. The PRP In Knee OsteoArthritis (PIKOA) trial was designed to assess the clinical efficacy and structural benefit of IA injections of PRP vs saline solution (placebo) in symptomatic knee OA.

Methods and analysis PIKOA is an academic phase 3, superiority, triple-blind (patients, investigators and injectors), multicentre, randomised placebo-controlled trial (1:1 ratio). It compares the efficacy of 1 weekly IA injection of 5 mL PRP or placebo (saline solution) for 3 weeks with a 6-month follow-up. The trial will enrol 210 participants ≥40 years old with symptomatic and moderate radiographic knee OA (Kellgren and Lawrence grade 2 or 3). PRP is prepared with the A-CP-Kit-T (20 mL) kit and its cellular composition is characterised for each patient. The main objective is to compare change in pain on a 0 mm to 100 mm visual analogue scale (VAS) between W0 and W14. The secondary objectives are to compare the two groups in terms of decrease in VAS pain, Western Ontario and McMaster Universities Osteoarthritis Index total score and subscores, analgesics consumption, OMERACT-Osteoarthritis Research Society International responder rate and improvement in quality of life measured by the EQ-5D-5L score. All these criteria are assessed at W8, W14 and W26. The decrease in serum Coll2-1 and Coll2-1 NO_2 levels (catabolic markers, reflecting cartilage destruction or joint inflammation) and increase in N-propeptide of cartilage IIA level (reflecting cartilage formation) are assessed at W8 and W14. Adverse events and study withdrawals are collected during the study.

Ethics and dissemination Ethics approval was obtained from the Nord Ouest ethical committee (2021-A00742-39). All participants need to provide written informed consent. The findings will be published in peer-reviewed journals.

Trial registration number NCT05378815 (ClinicalTrials.gov); pre-results.

Protocol version and number: V.3 of 17 July 2023.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Platelet-rich plasma (PRP) In Knee OsteoArthritis (PIKOA) is a new academic multicentre randomised triple-blind controlled trial with a very rigorous methodology that will limit bias and evaluate the efficacy of PRP intra-articular (IA) injections in knee osteoarthritis with a high level of evidence.

⇒ PIKOA compares 3 weekly IA PRP injection prepared with a new kit manufactured by a laboratory specialising in PRP and widely distributed worldwide to 3 weekly IA saline solution (1:1 ratio).

⇒ All PRP are precisely characterised in accordance with Minimum Information for studies evaluating Biologics in Orthopaedics guidelines.

⇒ The study criteria do not include objective performance testing as the focus is on patient-reported outcome measures of pain, function and quality of life.

⇒ The authorisation to use all analgesics and non-steroidal anti-inflammatory drugs, in addition to IA injections following the 3-month primary outcome, represents a potential limitation, which was deemed necessary to minimise the number of withdrawals and to restrict the duration of care limitations associated with the study.

INTRODUCTION

Platelet-rich plasma (PRP) injections are increasingly used in several musculoskeletal diseases such as chronic tendinopathies or muscle injuries. Another rapidly expanding indication is osteoarthritis (OA), particularly knee OA. Indeed, intra-articular (IA) PRP injection is now part of the injectable therapeutic arsenal alongside corticosteroids (CS) and hyaluronic acid (HA). Several therapeutic trials and meta-analyses have been conducted to assess the efficacy of IA PRP injections. Most studies have

compared PRP and HA and demonstrated that PRP has a symptomatic effect that is at least equivalent to HA in terms of symptom relief and potentially more prolonged in duration.[1 2] However, the efficacy of HA injections in knee OA is regularly questioned. Some authors consider that the improvement after HA injections may be attributed to a placebo effect or what has been termed a 'contextual' effect.[3] Consequently, HA does not appear to be an optimal comparator for assessing a new IA therapy in OA. Consequently, PRP trials should include a gold-standard control arm, such as saline solution.

To the best of our knowledge, fewer than 15 trials have compared PRP and saline in knee OA. The first trials reported that PRP exhibited superior efficacy to saline in alleviating pain and functional symptoms at various follow-up intervals (3, 6 and 12 months). However, these studies had some methodological flaws including a few number of patients, heterogeneous protocols in terms of number and frequency of injections, and unspecified PRP characteristics. Two recent studies, including a large randomised placebo-controlled trial with low risk of bias, did not demonstrate any benefit of PRP. Nevertheless, in this study, the primary clinical endpoint (knee pain score on 11-point numerical rating scale) was assessed at 12 months, which is not an optimal time point for IA injections.[4] However, the evaluation of symptomatic endpoints at 2 months revealed no statistically significant differences. The second double-blind placebo-controlled trial including three parallel groups (one or three IA PRP injections vs three IA saline solution injections) enrolled a smaller number of patients, with symptomatic assessment (primary outcomes: Knee Injury and Osteoarthritis Outcome Score (KOOS)[5] and European Quality of Life, 5-dimension (EQ-5D-5L))[6 7] at 6 weeks and 3, 6 and 12 months. All results were negative.[8] Recent results of a third multicentre randomised placebo-controlled trial demonstrated a significantly superior clinical response (primary outcome: Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC))[9] following PRP injection than saline in a very large population (610 participants) with a 60-month follow-up.[10] PRP injections were very well tolerated in these studies and no serious adverse events related to the treatment were reported.

In light of these conflicting results and methodological flaws, most scientific societies such as the American College of Rheumatology (ACR),[11] Osteoarthritis Research Society International (OARSI)[12] or the French Society of Rheumatology (SFR)[13] does not currently recommend PRP as a treatment for knee OA, considering insufficient evidence. The American Academy of Orthopaedic Surgeon (AAOS) is the only organisation to have published a guideline in favour of the use of PRP in knee OA, with a strength of recommendation considered to be limited.[14] Despite the aforementioned considerations, the use of PRP in routine care is increasing significantly worldwide.[15]

Moreover, many experts in the field advocate for the necessity to conduct new placebo-controlled trials with a high-quality methodology and sufficient statistical power to determine whether PRP may be regarded as an efficacious therapeutic modality for symptomatic knee OA.

Concerning the potential structure-modifying effect of PRP, several in vitro studies have confirmed its anabolic effect on synoviocytes and/or chondrocytes.[16 17] Furthermore, its beneficial impact on cartilage thickness and synovial inflammation has been demonstrated in various in vivo animal models.[18] However, human studies reported conflicting results on the structural benefit of IA PRP injections. Indeed, only one high-quality study reported a positive impact of PRP on cartilage thickness[10] while a few low-quality trials showed a positive effect on synovial inflammation.[19] The evaluation of a structural effect in OA is limited by the poor sensitivity to change. Consequently, several blood or urine biomarkers have been identified and subsequently validated as anabolic markers that reflect cartilage formation, such as serum N-propeptide of cartilage IIA (PIIANP), or catabolic markers that reflect cartilage destruction or inflammation, such as Coll2-1 (a 9-amino acid sequence-specific of type II collagen released during cartilage degradation) and Coll2-1 NO_2 (nitrated form of Coll2-1, reflecting oxidative-related type II collagen degradation and then the local inflammatory reaction).[20] To the best of our knowledge, few studies have focused on these biomarkers as a proxy of the structural effect of PRP injection.[21]

The PRP In Knee OsteoArthritis (PIKOA) trial is designed to assess the clinical efficacy and structural effect of IA injections of PRP versus saline placebo in symptomatic knee OA with moderate radiographic severity. The primary objective is to compare the change in pain between week (W)0 and W14 after three weekly IA injections of PRP versus saline placebo in patients with moderate femorotibial knee OA (Kellgren and Lawrence (KL)[22] grade 2 or 3). Secondary objectives include a comparison of changes in knee symptoms and quality of life, responder rate, analgesics consumption and tolerance in each group at W8, W14 and W26. Additionally, the trial will assess changes in mean cartilage biomarker values (such as serum Coll2-1, Coll2-1 NO_2 and PIIANP or others depending on scientific advancement by the end of the study) in each group at W8 and W14.

METHODS AND ANALYSIS

Study design

PIKOA is a phase 3, superiority, triple-blind, multicentre randomised placebo-controlled trial (1:1 ratio) comparing the efficacy of 1 weekly IA injection for 3 weeks of PRP or saline. In total, 18 centres (16 departments of rheumatology and 2 departments of rehabilitation and sport medicine) from 13 university hospitals, 3 regional hospitals, 1 private non-profit hospital and 1 private clinic from France and Monaco are participating in this trial.

This is an academic study sponsored by the Assistance Publique–Hôpitaux de Paris (Delegation for Clinical Research and Innovation). The study is conducted in

accordance with the Declaration of Helsinki and has been approved by an ethics committee (Ethical committee Nord Ouest, no. 2021-A00742-39). The protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (online supplemental file 1) and Consolidated Standards of Reporting Trials (CONSORT) (online supplemental file 2) guidelines. PIKOA is registered at ClinicalTrials.gov. The study started in October 2024 and is expected to be completed in October 2026.

Study participants and recruitment strategies

A total of 210 participants >40 years old with painful and radiographic knee OA will be enrolled in the trial. In patients with bilateral knee OA, only the most symptomatic knee is included in the study (target knee). Outpatients are recruited by investigators during consultations. Communication about the study is facilitated through multiple channels, including the Société Française de Rhumatologie (SFR) and the Association Française de Lutte Anti-Rhumatismale . Additionally, the study is disseminated through publications on social networks.

Participants are eligible for the study if they meet all of the following inclusion criteria:

- Age 40–79 years (inclusive).
- Symptomatic OA in target knee according to ACR criteria,[23] evolving for >3 months.
- Pain predominantly in femorotibial compartment of the target knee.
- Moderate radiographic OA involving at least one femorotibial compartment (KL stage 2 or 3) in the target knee on X-rays dated <6 months (KL scale includes five stages: 0, normal; 1, doubtful narrowing of the joint space with possible osteophyte formation; 2, possible narrowing of the joint space with definite osteophyte formation; 3, definite narrowing of joint space, moderate osteophyte formation, some sclerosis and possible deformity of bony ends, 4, large osteophyte formation, severe narrowing of the joint space with marked sclerosis and definite deformity of bone ends[22]).
- Pain in the target knee according to visual analogue scale (VAS) score ≥40/100 (with or without usual analgesic treatments).
- Failure or contraindications to conventional treatments (analgesics, non-steroidal anti-inflammatory drugs (NSAIDs)).
- Able to understand the requirements of the trial and sign informed consent before inclusion
- Able to read and understand written instructions.
- Able to complete self-questionnaires and a diary.
- Use of effective contraception in non-menopausal women.

Exclusion criteria are as follows:

- Other pathologies of the lower limbs interfering with the evaluation of knee OA (eg, symptomatic hip OA, lumbar radiculopathy, tendinosis).
- Contralateral symptomatic knee OA with VAS score ≥40/100 (with or without usual analgesic treatments).
- Radiographic lesions predominantly in patellofemoral compartment in the target knee.
- Previous instrumental surgery in target knee.
- Trauma in the target knee within the last 3 months leading to painful or functional symptom modifications.
- History of inflammatory rheumatism or crystal-induced arthritis.
- Previous diagnosis of fibromyalgia.
- Morbid obesity (body mass index (BMI) >40 kg/m^2).
- Inflammatory congestive flare-ups in the target knee (Knee Osteoarthritis Flare-Ups Score (KOFUS)[24] ≥7).
- Treatment with morphine in the previous month
- Refusal to discontinue NSAIDs between the inclusion visit and W14 visit and to stop analgesic treatments 48 hours before each visit
- Previous infection of the target knee regardless of the date of infection
- Presence of significant chondrocalcinosis on the front view X-ray in the target knee.
- Previous injection of PRP in the target knee.
- Injection of HA or CS into the target joint within the last 3 months.
- Hemostasis disorders or curative dose of anticoagulant medication.
- Severe metabolic or systemic disorders
- Haemodynamic instability.
- Haematological disease (eg, haematological malignancies, myelodysplasia, autoimmune thrombocytopenia) in progress or in remission for <5 years.
- Thrombocytopenia (< 150 000 platelets/mm^3).
- Chemotherapy or immunosuppressive drugs.
- Infective disease at inclusion (bacterial infection and/or presence of fever and/or antibiotics use).
- Participation in a clinical trial of knee OA within the last year.
- Participation in any clinical trial ongoing or completed within the last 3 months.
- Mental disease preventing the understanding of the nature, objectives and possible consequences of the study.
- Under legal protection.
- Pregnant woman or planning to become pregnant during the study.
- Breastfeeding woman.

Randomisation procedure

After signing the consent form (online supplemental file 3), eligible patients are randomised to one of two treatment groups (PRP or saline). The randomisation process is initiated by the investigator via the e-CRF between the inclusion visit and the day of the first injection (period from W-4 to W0).

Randomisation process managed by the Henri Mondor Clinical Research Unit integrates a computer minimisation algorithm, with a random element not disclosed to investigators to limit predictability. The minimisation criteria considered are centre, initial VAS pain level (4–6,

6–8, 8–10), KL grade (2 or 3), BMI (≥30 or <30 kg/m^2), failure of the last HA or CS IA injection in the target joint (<30% efficacy within 1 month of injection according to the patient) and use of antiplatelet agents. Indeed, initial pain level, KL stage, BMI, failure of previous IA injections and use of antiplatelet agents are potential predictors of PRP response, and the minimisation balances these criteria between the two groups.

Blinding

Our trial is designed as a triple-blind study in which none of the patients, investigators or physicians performing the IA injections is informed of the treatment arm.

Several methodological precautions help maintain this blinding. Only the nurse involved in the injection procedure is informed of the randomisation results via the Cleanweb software. A 20 mL sample of venous blood is obtained from each patient using the A-CP-Kit-T kit, followed by centrifugation. The distinction in the visual appearance of the saline solution and the PRP is obscured by the nurse utilising a syringe that has been covered with two MEPORE dressings (6×7 cm). It is noteworthy that the viscosity of these two products is not significantly different and not perceptible by the clinician. An opaque screen is placed not only between the nurse and the physician performing the injection but also the patient during treatment preparation and between the patient and the physician during injection. The injected volume of PRP and saline solution is the same (5 mL). Clinical assessment is performed by an investigator not involved in the injection process at W8 and W14 and also involves self-questionnaires at W26, without a face-to-face visit. Finally, investigators ask the patients which group they thought they were in. The biomarkers are measured with blinding to clinical data or information related to the type of treatment received.

Interventions

In line with most published trials,[4 8 10] the PRP and saline groups receive three IA knee injections at weekly intervals. First, effusion is sought by ultrasonography. If present, aspiration is performed with a needle inserted in the suprapatellar bursa by a lateral patellofemoral approach, and its volume is noted. Then, a nurse prepares an occluded syringe with 5 mL normal saline solution or PRP, which are injected into the knee under ultrasound guidance. Following the injection, the patient performs passive knee flexion and extension five times and remain under surveillance for 10 min.

The second and third injections are performed approximately 1 week and 2 weeks later with the same protocol (figure 1).

PRP preparation

Autologous PRP is prepared with the tube A-CP-Kit-T (20 mL) (reference: A-CP-T-20; Regen Lab SA). This is a class IIb medical device that meets the General Safety and Performance Requirements of all relevant European Medical Device Regulations (European Commission marking). It contains 1 mL sodium citrate anticoagulant and a separator gel. The PRP extracted is leukocyte-poor, has a 1.5-fold to 2.0-fold platelet concentration as

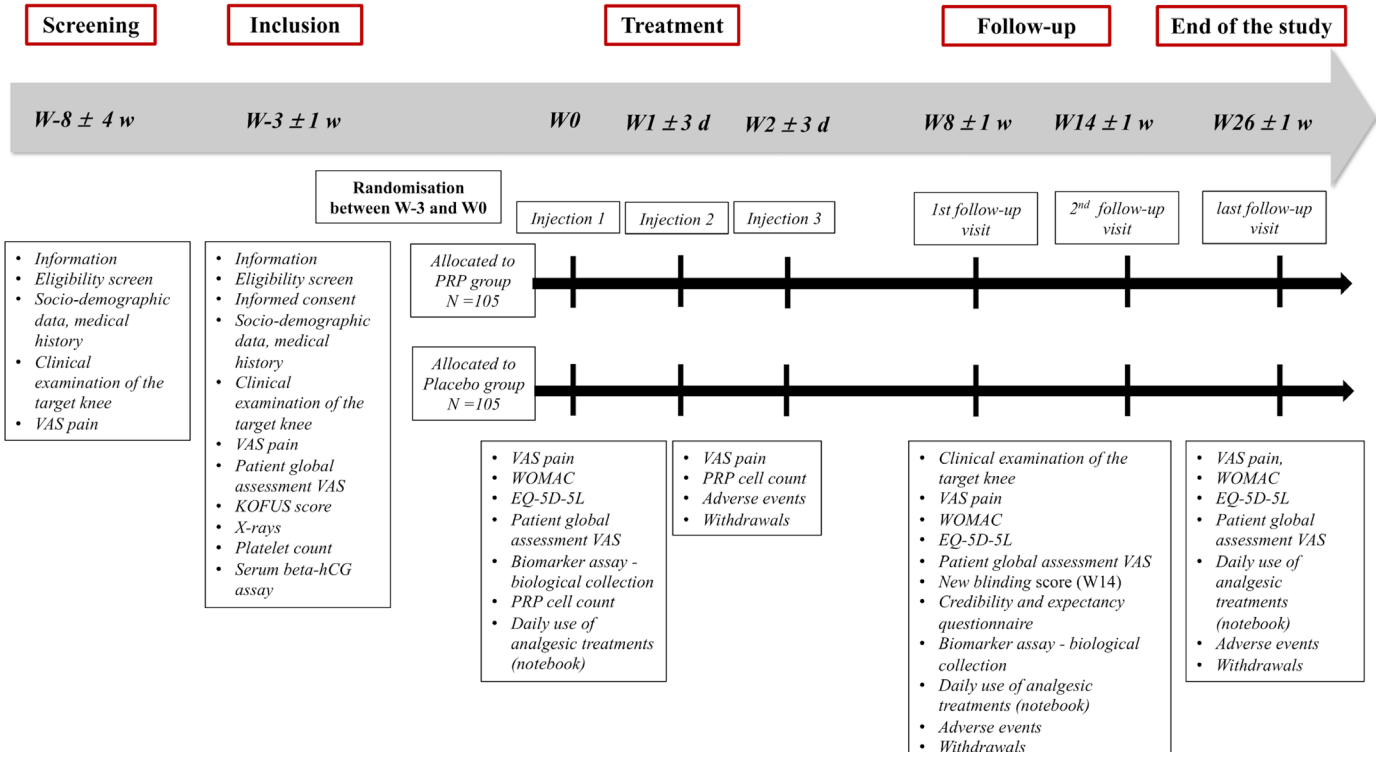

Figure 1 Flow diagram of the study protocol. Beta-hCG, beta-human chorionic gonadotropin; d, day; EQ-5D-5L, EuroQol 5-Dimension, 5-Level Quality of Life Questionnaire; KOFUS, Knee Osteoarthritis Flare-Ups Score; PRP, platelet-rich plasma, VAS, visual analogue scale; W, week; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

compared with whole blood and is not activated (P2B according to PAW classification[25]; 3B according to Mishra classification[26]).

To obtain the PRP, the nurse collects 20 mL blood by venous puncture usually in the cubital fossa or posterior part of the patient's hand, directly into the A-CP-Kit-T tube under strict sterility conditions. The tube is then centrifuged at 1500 g for 5 min. Centrifugation separates the plasma containing the platelets (PRP) from the red and white blood cells isolated from the PRP by the separator gel.

Approximately 11–12 mL PRP is obtained. The first 5 mL of the surface layer of the PRP (poor in platelets) is discarded. The A-CP-Kit-T tube is then inverted several times to homogenise the remaining PRP (approximately 6–7 mL). An amount of 5 mL is recovered in a syringe covered with opaque dressing and immediately injected in the patient's knee joint under ultrasound control, and 0.5 mL is sent to the haematology laboratory for cell counting. 0.5 mL is sent to the haematology laboratory for cell counting. This allows accurate characterisation of PRP according to Minimum Information for studies evaluating Biologics in Orthopaedics guidelines.[27]

Saline injections

A total of 20 mL blood is collected by venous puncture from patients in the placebo group and PRP is extracted by using the A-CP-Kit-T kit with the same procedure for each weekly injection. The nurse, who is aware of the randomisation, fills a syringe covered with opaque dressing with 5.0 mL saline, and most of the PRP is discarded except 0.5 mL, which is sent to the haematology laboratory for cell counting.

Objectives and outcome measures

The primary objective is to compare the change in pain level assessed by a VAS at W14 between the PRP and saline groups.

The secondary objectives are to compare the two groups in terms of the following:

- Decrease in pain level assessed by the VAS at W8 and W26.
- Decrease in WOMAC pain score at W8, W14 and W26.
- Decrease in items 1 and 2 of the WOMAC pain score at W8, W14 and W26.
- Decrease in WOMAC function score at W8, W14 and W26.
- Decrease in total WOMAC score at W8, W14 and W26.
- OMERACT-OARSI responder rate at W8, W14 and W26
- Improvement in quality of life measured by European Quality of Life, 5-dimension, 5-level (EQ-5D-5L) score at W8, W14 and W26.
- Decrease in analgesic consumption at W8, W14 and W26.
- Changes in serum cartilage biomarkers such as Coll2-1, Coll2-1 NO_2 and PIIANP levels at W8, W14 and W26.
- Assessment of severe and non-severe adverse events and study withdrawals at W1, W2, W8, W14 and W26.
- Assessment of associations between PRP composition and the different assessment criteria (VAS, WOMAC, OMERACT-OARSI response, EQ-5D-5L, adverse events etc).
- Assessment of the quality of blinding by the new Blinding Index[28] at W14.
- Assessment of the credibility of the interventions and expectancies of the participants by the Credibility/Expectancy Questionnaire[29] at W14.

The VAS consists of a 100 mm horizontal line with no pain on the left and maximum pain on the right. The patient draws a vertical line corresponding to the mean pain of the target knee during the previous 48 hours considering both pain at rest and during various activities. The WOMAC score is a self-administered composite score validated and widely used in international clinical trials of knee OA.[9] It includes three subscores assessing pain (5 items), function (17 items) and stiffness (2 items). Each item is measured on a scale from 0 to 100 (100 corresponding to the most severe symptoms). A mean out of 100 is calculated for each subscore and then for the total WOMAC score. The WOMAC score is completed before the first injection (W0) and at W8, W14 and W26.

The OMERACT-OARSI response is defined by the following[30]: an improvement in the WOMAC pain or function score of at least 50% and at least 20/100 points or an improvement of at least 20% and at least 10/100 points for two of the three following criteria: WOMAC pain score, WOMAC function score and patient's global assessment (measured on a scale from 0 to 100). This response is evaluated at W8, W14 and W26.

The EQ-5D-5L score is a composite quality-of-life score assessing five domains (mobility, self-care, daily activities, pain and anxiety). It has been validated in patients with OA and chronic pain.[6 7] Each item is measured on a scale from 1 (most favourable state) to 5 (most unfavourable state) according to the severity of symptoms. The EQ-5D-5L score is calculated before the first injection (W0) and at W8, W14 and W26.

Analgesic consumption is assessed at W0 (first PRP injection), W8, W14 and W26. For the W8 and W14 visits, the number and dose of analgesics consumed during the week preceding the visit are recorded (day (D)−7 to D-2 before each assessment because patients are not allowed to take analgesics in the 48 hours preceding the assessment). The consumption of analgesic treatments and NSAIDs between W14 and W26 which are authorised during this period (up to D-2), as well as the administration of IA injections in the knee (HA, CS and PRP) are collected. A notebook is provided to the patient to record each treatment taken with the dosage and daily frequency. The number of study withdrawals in each group is assessed at W1, W2, W8, W14 and W26. Blood biomarkers are assayed in serum collected on the day of the first IA injection (W0) and at W8 and W14 visits.

The quality of blinding is assessed not only directly by the new Blinding Index (questioning the patient about the treatment they thought they had received during the study)[28] but also indirectly by the Credibility/Expectancy Questionnaire.[29]

Adverse effects

All trials and meta-analyses provide reassuring data concerning the tolerance of PRP injections.[31–36] Only one recent publication reported septic arthritis after IA PRP injection.[37]

Adverse events are collected in the patient's notebook before the second (W1) and third (W2) IA injection as well as during W8, W14 and W26 visits. The description, duration and consequences of adverse events and their treatments are recorded. The severity of adverse events is defined according to the Common Terminology Criteria for Adverse Events V.5.0 (CTCAE V.5.0) classification. Serious adverse events are defined as any unexpected medical event that results in death, persistent or significant disability or incapacity or that induces a congenital anomaly or birth defect or any other important medical condition that may require medical or surgical intervention or requires hospitalisation or prolongation of hospitalisation. In case of serious adverse event, the treatment will be interrupted, and the allocated intervention will be unblinded immediately from the e-CRF.

Sample size calculation

From the available data,[38 39] we postulate a mean change in VAS score of 20 points (SD=25) between W0 and W14 in the saline placebo arm. The analysis of 99 participants per arm will allow to show, with a power of 80% and a two-sided alpha risk of 5%, a minimal difference of 10 points in change in VAS score between the two groups between W0 and W14 (ie, change in VAS score of 30 points (SD=25) in the PRP arm). Considering 5% drop-outs, we aim to include 210 patients.

Data collection and management

Data are collected in an e-CRF, devised by the study coordinator. Data are completed by the investigators with the help of a clinical research technician. All information required by the protocol are recorded in physical or electronic report files, and an explanation must be provided for any missing data. All questionnaires used are validated. A clinical researcher associate (CRA) will be responsible for the good completion of the study, for collecting, documenting, recording and reporting all handwritten data, in accordance with the standard operating procedures applied within the clinical research and innovation department. To ensure that the protocol is respected in each centre, the CRA conducts regular audits throughout the study period. A steering committee with a coordinator, coinvestigators and a methodologist meets every 12 months to resolve unexpected situations. Source documents are kept for 15 years by the investigator or by the hospital in the case of a hospital medical file. During and after the research involving human participants, all data collected concerning the participants are rendered anonymous. Only the participant's initials are recorded, accompanied by an encoded number specific to the study indicating the order of enrolment. An audit can be carried out at any time by individuals appointed by the sponsor and independent of those responsible for the research. Only the sponsor will have access to the final trial dataset (table 1)

Screening visit

The screening visit takes place between 8 and 2 weeks before the inclusion visit and is performed in consultation with a physician from an investigating centre and from private practice. The diagnosis of symptomatic knee OA, meeting the ACR criteria,[23] with radiographic OA predominantly in the femorotibial compartment (KL grade 2 or 3) is confirmed and a differential diagnosis is eliminated. The physician assesses the indication for IA injection (failure of oral and/or local analgesic and/or anti-inflammatory treatments) and the absence of CS or HA injection in the last 3 months or a previous PRP injection. Pain intensity is assessed by the VAS. The patient is informed about the PIKOA trial. If the patient is interested in participating in the study, the physician gives the patient the telephone number of the nearest investigating centre to contact the investigator or clinical research associate. With the patient's consent, the physician can also contact the investigating centre directly and then the investigator or clinical research associate will call the patient in order to organise the inclusion visit. Then, the date of the first visit is set.

Inclusion visit

The inclusion visit is performed in consultation with one of the investigators. The first step consists of checking inclusion and exclusion criteria. It takes place between 2 and 4 weeks before the W0 visit (first IA injection). The patient is again informed about the study and the benefits and risks of IA injections of PRP, and written informed consent is obtained.

During this visit, the patient evaluates the intensity of the knee pain by the VAS (inclusion criterion VAS score ≥4/10). The KOFUS score,[24] an exclusion criterion, is also assessed by the investigator. The various treatments administrated to the patient are recorded. Failure of conventional treatment is at the discretion of the investigator enrolling the patient, but his decision is based on the lack of sufficient improvement in pain or functional limitation with the usual pharmacological treatment of knee OA including analgesics and NSAIDs, unless contraindicated. The target knee flexion is measured, and the investigator assesses the presence of joint effusion, active and passive flessum, pain in each knee compartment and varum or valgum misalignment. The predominance of femorotibial pain will be confirmed primarily through the palpation of the various knee compartments (medial and lateral femorotibial compartments and

Table 1 Schedule of enrolment, interventions and assessments for the PIKOA trial according to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 guidelines

	Screening	Allocation	Injection 1	Injection 2	Injection 3	Postallocation		End of the study
Trial steps	**Week −8±4**	**Week −3±1**	**Week 0**	**Week 1±3 days**	**Week 2±3 days**	**Week 8±1 week**	**Week 14±1 week**	**Week 26±1 week**
Enrolment								
Information	X	X						
Eligibility screen	X	X						
Informed consent		X						
Randomisation		X*						
Interventions								
Intra-articular injection of PRP or saline solution			X	X	X			
Assessment								
Socio-demographic data, medical history	X	X						
Clinical examination of the target knee	X	X				X	X	
VAS pain (primary outcome)	X	X	X	X	X	X	X	X
WOMAC			X			X	X	X
EQ-5D-5L			X			X	X	X
Patient global assessment VAS		X	X			X	X	X
New Blinding Index							X	
Credibility and Expectancy Questionnaire							X	
X-rays		X (*if>6 months*)						
Platelet count		X						
Serum beta-hCG assay		X (*in non-menopaus-al women*)						
Blood sample 2 x 9 mL (biomarker assay - biological collection)			X			X	X	
Cell count on PRP			X	X	X			
Daily use of analgesic treatments (notebook)			X			X	X	X
Adverse events				X	X	X	X	X
Withdrawals				X	X	X	X	X

*Randomisation can be done from the inclusion visit to the first injection visit (from W4 to W0).
Beta-hCG, beta-human chorionic gonadotropin; EQ-5D-5L, EuroQol 5-Dimension, 5-Level Quality of Life Questionnaire; PRP, platelet-rich plasma; VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities Arthritis Index.

anterior patellofemoral compartment). Furthermore, a patellofemoral syndrome will be investigated based on the patient's description of knee pain. It will be suspected if pain is located in the front of the knee and is predominantly experienced when ascending or descending stairs, when squatting, or when sitting for a long period with less pain when walking on flat ground. Additionally, a differential diagnosis is excluded through examination of the homolateral hip as well as spine to seek a radicular origin.

The moderate radiographic severity of knee OA (KL score 2 or 3) predominating in the medial or lateral tibial-femoral compartment on radiographs <6 months should be confirmed by the investigator. If there are no recent X-rays or if they are incomplete, radiographs are performed during this visit and include a weight-bearing front view in extension and profile view at 20° of flexion as well as patellofemoral incidence at 30° of flexion. The normality of platelet count is also verified by a blood test performed at the inclusion visit. In non-menopausal women, investigators ensure the use of effective contraception and perform a serum beta-human chorionic gonadotropin assay. A notebook is given to the patient to record each painkiller taken the week before each evaluation, noting the dosage and daily frequency (at W0, W8, W14 and W26) as well as adverse events. The patient is informed to not take any analgesic in the 48 hours preceding the first injection (W0) as well as before the W8, W14 and W26 visits. After validation of the selection criteria, the patient is randomised.

Treatment and follow-up visits

The protocol includes three visits (W0, W1 and W2) after the inclusion visit, during which IA injections of PRP or saline are administered, followed by three follow-up visits at W8, W14 and W26. During the W0 visit, the patient completes the self-reporting questionnaires (WOMAC, EQ-5D-5L) and evaluates not only the intensity of the knee pain but also the global impact of knee OA (patient's global assessment) by using the VAS. The consumption of analgesics during the last week (D-7 to D-2, patients not being allowed to take painkillers in the 48 hours preceding the visit) is documented. Then, PRP or saline is injected under ultrasound guidance by a blinded investigator. A fraction of the PRP (0.5 mL) is sent to the biological haematology laboratory for cell counting (red and white blood cells and platelets). In parallel, a blood sample is taken for determining levels of cartilage biomarkers such as Coll2-1, Coll2-1 NO_2 and PIIANP or others depending on the state of scientific knowledge at the end of the study and for biological collection. The W1 and W2 visits include the collection of adverse events and the second or third IA injection of PRP or saline. Again, a fraction of the PRP (0.5 mL) is sent to the haematology laboratory. In case of a serious adverse event after the previous injection of PRP or saline, the treatment is interrupted, but the patient's follow-up is maintained if the patient agrees and if their clinical condition allows it. In case of occurrence of a bacterial and/or viral infectious requiring or not oral antibiotics (grade 2 according to the CTCAE V.5.0 classification) and unrelated to the knee (eg, angina, bronchitis, urinary tract infection), the next IA injection is not performed but could be postponed for a maximum of 2 weeks. Mean target knee pain (VAS) in the 48 hours after the IA injection is noted in the patient's notebook.

W8 and W14 visits are conducted by a blinded investigator not involved in injection process. During these visits, the occurrence of adverse events is also reported. In addition, the patient completes the self-reporting questionnaires (WOMAC, EQ-5D-5L) and evaluates not only the intensity of the knee pain but also the global impact of the disease by the VAS. The consumption of analgesics during the last week (D-7 to D-2) is quantified. Patients are permitted to take non-opioid and weak opioid medications until W14; however, they are not allowed to take NSAIDs or strong opioids. A blood sample of 18 mL is obtained for determining levels of the cartilage biomarkers and the biological collection (see visit W2). From visit W14, patients are allowed to use NSAIDs, all analgesics including strong opioids and to receive IA injections in the target knee treated in the study (HA, CS and PRP). During the W26 visit, the occurrence of adverse events is reported. In addition, the patient completes the self-reporting questionnaires (WOMAC, EQ-5D-5L) and evaluates the intensity of the knee pain but also the global impact of the disease by using the VAS. Treatments received (level 1, 2 and 3 analgesics; NSAIDs and IA HA, CS and PRP injections) between W14 and W26 (up to D-2) are recorded in the patient's notebook.

Premature discontinuation visit

In case of treatment discontinuation, follow-up visits at W8, W14 and W26 are maintained with patient agreement. In case of withdrawal, a clinical data collection identical to that of W8 or W14 is performed with the patient's consent.

Biological collection

For this purpose, 18 mL blood are collected in two 9-mL dry tubes allowing for the recovery of approximately 6 mL serum. The dry tubes containing the patient's blood are first left at room temperature for 1 hour for coagulation and then centrifuged at 1000 g at 4°C for 10 min. The serum obtained is divided into three cryotubes of 200 µL (for the three biomarkers) and two storage cryotubes (approximately 2.5–3 mL per storage cryotube). The tubes are stored ideally at −80°C. Each participating centre stores the cryotubes. At the end of the study, all samples will be sent simultaneously to the laboratory Artialis (Liege, Belgium) under the responsibility of Prof Yves Henrotin. They will be transported in a refrigerated truck for approximately 3–12 hours, depending on the location of the investigation centre. The two storage cryotubes will be kept at the Artialis laboratory (biological collection). After the end of the study, samples from the stored cryotubes may be used to study new biomarkers of interest in OA.

Data monitoring committee

A data monitoring committee (DMC), independent of the sponsor and without competing interest, has already been established. Its primary role is to monitor safety data. A preliminary DMC meeting was held prior to protocol submission to authorities and ethical committee.

Statistical analyses

The results of this study will be reported according to CONSORT recommendations for randomised trials. The study population will be analysed on an intention-to-treat (ITT) basis for the efficacy endpoints. The ITT analysis will consider all randomised patients, including patients for whom injections were interrupted or deferred. Sensitivity analyses will also be performed in the per-protocol population (including patients without major protocol deviations) and modified ITT population (excluding patients who did not receive any injections). No intermediate analysis is planned.

First, a descriptive analysis of baseline data will be performed within each arm (PRP and saline). Continuous variables will be reported with mean±SD or median (IQR), depending on the distribution of the variable. Categorical variables will be described with frequency (percentage).

The primary endpoint (change in VAS pain between W0 and W14) will be compared between the two arms using a Student's or Mann-Whitney test, depending on the data distribution. A linear regression model analysis with adjustment for minimisation criteria (baseline VAS pain, KL grade, BMI, failure of the last HA or CS injection in the target joint, use of antiplatelet agents) will also be performed.

Quantitative secondary endpoints will be compared between the two arms using the same methodology. Qualitative secondary endpoints will be compared by χ^2 or Fisher's exact tests, depending on the conditions of application and an analysis using a logistic regression model with adjustment for minimisation criteria will be performed. A mixed-effects linear regression model will be carried out for longitudinal quantitative secondary endpoints and a mixed-effects logistic regression model for qualitative endpoints. These analyses will be performed without and with adjustment for minimisation criteria. Results will be expressed as regression coefficients (beta coefficients) and their 95% CIs for quantitative endpoints and ORs and their 95% CIs for qualitative endpoints. The effect size will be estimated using the regression coefficient (beta coefficient) and its 95% CI.

Change in VAS score and composite scores (WOMAC, EQ-5D-5L) within each group at W8, W14 and W26 will be studied by Student's t-test or Wilcoxon test, depending on the normality of data distribution.

PRP cellular composition at W0, W1 and W2 will be described with mean±SD or median (IQR), according to the normality of their distribution. Associations between PRP composition and the different assessment criteria (VAS, WOMAC, OMERACT-OARSI response, EQ-5D-5L, adverse events, etc) will be evaluated with linear regression models for the quantitative criteria and logistic regression models for the qualitative criteria.

For the analysis of the quality of blinding, the new Blinding Index will be estimated as well as their 95% CIs. Results of the credibility/expectancy questionnaire will be expressed as mean±SD (or median (IQR)).

The frequency of adverse events will be summarised in each group and described by type and severity in the per-protocol analysis population and the modified ITT population.

Finally, analyses stratified according to the following criteria will be performed: baseline VAS pain, KL grade, BMI, failure of the last HA or CS infiltration in the target joint, use of antiplatelet agents. Interactions between these criteria and the treatment arm will be investigated. All analyses will be carried out using Stata V.17.0 (StataCorp, College Station, Texas) or later, in the Public Health Department of Henri Mondor Hospital under the responsibility of Dr Nadia Oubaya.

Patients and public involvement

Patients and public were not involved in the design, or conduct, or reporting or dissemination plans of our research.

Ethics and dissemination

Ethics approval was obtained from the Nord Ouest ethical committee (2021-A00742-39). All participants need to provide written informed consent for their participation and biological collection. Any substantial modification to the protocol by the coordinating investigator must be sent to the sponsor and then approved by the ethical committee before being implemented. The information and consent forms will be revised, if necessary, particularly in case of a substantial amendment to the study or if adverse events occur. Results of this study, whether positive or negative, will be presented at national and international congresses and published in a peer-reviewed journal. All principal investigators, study designers and methodologists will be listed as authors of the publication.

Trial protocol

The trial protocol (V.3.0 APHP200819 (17 July 2023)/ N° IDRCB: 2021-A00742-39) is available in online supplemental file S4.

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Contributors FE, NO, PO, JS, PR, XC conceived and designed the study and will participate in the implementation and data management. FE drafted the initial version of the manuscript. All the other authors provided critical revisions and approved the final revisions. FE is the guarantor.

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