Supplemental Online Content

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Supplement 1. Trial protocol and statistical analysis plan

This supplemental material has been provided by the authors to give readers additional information about their work.

Supplement

Study Protocol and Statistical Analysis Plan

Supplement to:

Liam D.A. Paget, M.D., Gustaaf. Reurink, M.D., Ph.D., Robert-Jan de Vos, M.D., Ph.D., et al Platelet-Rich Plasma Injections for Ankle Osteoarthritis

This supplement contains the following items:

1.
    a. Original protocol (IRB approved protocol version 4.0 27-07-2018; inclusion first patient 21-04-2020): Adobe pdf page 2-26
    b. Final protocol (Amendment due to COVID-19 pandemic: IRB approved protocol version 5.0 21-04-2020), Adobe pdf page 27-53
    c. Summary of changes, Adobe pdf page 54

2. Original statistical analysis plan, final statistical analysis plan, summary of changes.
    a. Original Statistical Analysis Plan (date): Adobe pdf page 55-70
    b. Final Statistical Analysis Plan (date): Adobe pdf page 71-91
    c. Summary of changes, Adobe pdf page 92

Platelet Rich plasma Injection Management for Ankle osteoarthritis study (PRIMA): A multi-center, stratified, block-randomized, double-blind, placebo-controlled trial

Version 4

This submission is based on the formerly approved studies (same PI) evaluating the efficacy of platelet rich plasma in musculoskeletal injuries:

- Chronic achilles tendinopathy by de Vos et al. JAMA 2010 (PRICT-study, registration NTR1420, ABR NL 22805.098.08).[1]
- Acute hamstring injuries by Reurink et al. NEJM 2014 (HIT-study registration NTR2771, NL34660.098.10 / 10-163; ABR / METC Zuidwest Holland).[2]


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PROTOCOL TITLE: Platelet Rich plasma Injection Management for Ankle osteoarthritis study (PRIMA): A multi-center, stratified, block-randomized, double-blind, placebo-controlled trial

Protocol ID	NL64160.018.18
Short title	**PRIMA**
Version	**4**
Date	**13-06-2018**
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
NSAID's	**Non-Steroidal Anti-Inflammatory Drugs**
OA	**Osteoarthritis**
OCD	**Osteochondral Defect**
PRP	**Platelet-Rich Plasma**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Pain is the cardinal symptom of ankle osteoarthritis (OA) and is a complex phenomenon with limited understanding of its pathomechanisms. The main objectives in the clinical management of OA are to reduce inflammation and cartilage degeneration processes as well as relieve pain. Platelet-rich plasma (PRP) is a high concentrate of platelets derived from patient's whole blood, centrifuged to remove red blood cells. PRP has been used to encourage a healing response across several specialties, in particular dentistry, orthopaedics and dermatology. Growth factors stored in the platelets are assumed to facilitate an anti-inflammatory and analgesic effect.

A recent review concluded that in animal models PRP can diminish multiple inflammatory IL-1 mediated effects, and can also positively influence the collagen network of the cartilage and subsequently reduce pain and improve function.[3]

Our recent and other systematic reviews showed that compared to placebo injections, hyaluronic acid or corticosteroid injections, PRP injections significantly decrease pain and improve function in knee OA patients.[4–6] Given the clinical effect on pain reduction in knee OA and safety, PRP might serve as a promising non-surgical therapy for ankle OA. PRP might potentially delay the irreversible surgical options like arthrodesis and joint replacement. No significant adverse advents have been reported for any PRP trials regarding acute hamstring injuries, Achilles tendinopathy, knee OA and specifically not ankle OA. [1,2,4,5,7–9]. Until present, there is no RCT conducted on the efficacy of PRP in the management of ankle OA.

Hypothesis

We hypothesize that:

PRP injections are efficacious for symptom reduction and functional improvement compared to placebo injections in the treatment of ankle (talocrural) OA.

Workplan

Study design

A multi-center, stratified, block-randomized, double-blind, placebo-controlled trial comparing two treatment groups.

Study population

Patients with ankle (talocrural) OA will be included if they meet the following 3 inclusion criteria:

1. Severity of Ankle OA pain on visual analogue scale (VAS) (0–100 mm) ≥ 40 during daily activities
2. X-rays (AP and lateral view) indicating ≥ grade 2 on the Van Dijk classification [10]
3. Age ≥ 18 years

Intervention

Patients will be randomised into two treatment groups: PRP injection or placebo (saline) injection. Both groups will receive two injections of PRP or placebo at an interval of 6 weeks.

Main study parameter/endpoint

American Orthopaedic Foot and Ankle Society (AOFAS) score at 26 weeks follow-up, validated scale for ankle OA (0-100) measuring three subdomains (pain, function and alignment).
After 26 weeks, the principal investigator will be unblinded after the analysis of the primary outcome. The patients will remain blinded to the therapy until 52 weeks follow-up.

Power analysis

Based on previous and ongoing studies, the study protocol of the randomised controlled trial is designed to detect a difference of 12 points (0-100) on the AOFAS score (minimal clinical relevant difference) between the groups.[11] Based on a previous placebo controlled RCT on injection therapy (hyaluronic acid) in ankle OA of DeGroot et al. a standard deviation of 16.3 can be expected. Taking into account a two-sided level of significance of 5%, a power of 90% and a dropout rate of 15%, approximately 50 (40 plus 15%) patients per group will be needed (N=100).[11]

STATISTICAL ANALYSIS

To test for the effect of treatment on the between-group difference in primary outcome, we will use a repeated measurements general linear model. The effect of potential confounders (varus/valgus) will be evaluated and a correction will be performed.

Expected results

We will provide evidence for the (potential) efficacy of PRP for symptom reduction and functional improvement in the treatment of ankle OA. A positive outcome will have an effect on the economical and disease burden. The relatively simple content and widespread availability of the PRP intervention and previously reported good safety will contribute to simple and optimal nationwide implementation.

Conclusion

Our project will provide conclusions on the efficacy of PRP in ankle OA.

1. INTRODUCTION AND RATIONAL

1.1 Clinical relevance of ankle OA

The reported incidence of symptomatic ankle OA is estimated at 1% to 4% in the general adult population.[12] Due to its onset at a relatively young age, the duration of ankle OA in the life of a patient is significantly longer than those with hip or knee OA and the available surgical intervention (arthrodesis) is associated with significant functional limitations. In contrast to hip and knee OA, where there is an excellent surgical alternative (joint replacement) for severe cases, there is a clear need for non-surgical successful interventions in ankle OA.

Health related quality of life and physical function limitations are severe and comparable with hip OA and end-stage kidney disease or congestive heart failure.[14]

1.2 Pathomechanisms of OA

Following pathological or traumatic injury of the hyaline cartilage, progressive catabolic chondrocyte activity leads to degenerative osteoarthritic joint changes. Two key characteristics of OA are the lack of regenerative activity of the chondrocytes and the inflammatory joint reaction.

1.3 Pain pathomechanism

Pain is the cardinal symptom in OA and it is a complex phenomenon of which we understand only a fraction of the pathomechanisms.[15] One of the major peripheral factors considered as being an important source of pain is joint inflammation. Previous studies showed that local inflammation, with the release of phospholipases, cyclooxygenases and leukotrienes, is involved in pain mechanisms.[15] These factors not only result in increased intra-articular pressure due to edema, but also induce the amount of nociceptors present with subsequent excitation of peripheral nerves.[15,16]

1.4 Non-surgical interventions for ankle-OA

The main objectives in the clinical management of OA are to reduce the inflammatory and cartilage degeneration processes, and to relieve pain.

At this time there is no evidence-based treatment algorithm for non-surgical management of ankle OA.[2,5,17,18] Several pain relief options, such as non-steroidal anti-inflammatory drugs, opioids and corticosteroid as well as hyaluronic acid injections, are available but there is a lack of evidence from high quality studies to assist in clinical decision-making.

1.5 PRP in OA – animal models

Platelet Rich Plasma (PRP) is defined as plasma containing a concentration of at least 1,000,000 platelets/µl. Growth factors (GF) are stored in α-granules within platelets, and are released in a selective manner upon activation. GF released from the α-granules of platelets are assumed to provide the regenerative and anti-inflammatory benefits of PRP.

Recent reviews concluded that in animal models, PRP can diminish multiple inflammatory IL-1 mediated effects.[3] Due to this local anti-inflammatory response, PRP might have an indirect analgesic effect. The second suggested effect might be an increased mRNA expression of proteoglycan core protein in the articular cartilage and decreased chondrocyte apoptosis.[3] Consequently, PRP could also positively influence the collagen network of the cartilage.

1.6 PRP in OA – clinical studies

Several systematic reviews have shown that compared to placebo injections, hyaluronic acid or corticosteroid injections, PRP injections significantly decrease pain, improve function and are simple and safe.[5,18] In the majority of studies, the patients received 2-3 injections at fixed intervals. Given its clinical effect on pain reduction in OA and safety, PRP therapy in ankle OA might serve as a distinct non-surgical therapy for reducing pain and improving function. Clinical studies on the use of PRP in ankle OA are limited to a single report of 5 cases, which showed significant reduction of the VAS-FA score at a mean of 16 months follow-up.[17] Our preliminary results on the outcome of PRP injection in 14 patients with van Dijk Grade 2 ankle OA, revealed that 64% reported improvement of their symptoms. A lack of validated outcome scores, low quality study designs and small sample sizes are the main limitations of these previously conducted studies.

1.7 Why this proposal will have a great impact

The strength and uniqueness of our project is that: We will evaluate the promising findings from animal studies and positive clinical observations of PRP in ankle OA patients in a Level 1 study.

2. Study Goal

The goal of this study is to determine the efficacy of PRP injections in the management of ankle osteoarthritis by comparing 2 groups, both receiving 2 injections of either: PRP or a placebo saline solution.

2.1 Hypothesis

We hypothesize that: PRP injections are efficacious for symptom reduction and functional improvement compared to placebo injections in the treatment of ankle (talocrural) OA

3. STUDY DESIGN

A multi-center, stratified, block-randomized, double-blind, placebo-controlled trial comparing two treatment groups.

4. STUDY POPULATION

4.1 Population (base)

Patients with ankle OA in two University Medical Centres (Erasmus MC, AMC), teaching hospital (OLVG), general hospital (Flevo Hospital) and focus clinic (Bergman Clinic) will be informed about the study.

4.2 Inclusion criteria

1. Severity of Ankle OA pain on a visual analogue scale (VAS) (0–100 mm) ≥ 40 during daily activities
2. X-rays (AP and lateral view) indicating ≥ grade 2 on the Van Dijk classification[10]
3. Age ≥ 18 years

4.3 Exclusion criteria

1. Patient has received injection therapy for ankle OA in the previous 6 months
2. Patient does not want to receive one of the two therapies
3. Patient has clinical signs of concomitant OA of one or more other major joints of the lower extremities that negatively affects their daily activity level
4. Previous ankle surgery for OA or Osteochondral defects (OCD) < 1 year (not including surgery for an ankle fracture in the past)

4.4 RADIOGRAPHS

AP and lateral X-rays of the ankles will be scored according to the Van Dijk classification:[10]

- 0 Normal joint or subchondral sclerosis
- I Osteophytes without joint space narrowing
- II Joint space narrowing with or without osteophytes
- III (Sub)total disappearance or deformation of the joint space

4.5 Sample size calculation

Based on previous and ongoing studies, the study protocol of the randomised controlled trial is designed to detect a difference of 12 points (0-100) on the AOFAS score (minimal clinical relevant difference) between the groups.[11] Based on a previous placebo controlled RCT on injection therapy (hyaluronic acid) in ankle OA of DeGroot et al. a standard deviation of 16.3 can be expected. Taking into account a two-sided level of significance of 5%, a power of 90% and a dropout rate of 15%, approximately 50 (40 plus 15%) patients per group will be needed (N=100).[11]

5. INTERVENTION

5.1 Intervention

In this study, patients will be randomised into two treatment groups: PRP injection or placebo saline injection. Treatment allocation will be concealed. One syringe of 15ml autologous blood will be collected twice from the cubital vein: at inclusion and at a time interval of approximately 6 weeks. All participants will receive a second injection, regardless of the effect of the first injection. This blood will be prepared according to the instructions of the manufacturer (see appendix F4 Protocol bloedafname - PRP bereiden - intra-articulair injectie enkel), and the injection will be given within 30 minutes following venipuncture. For each injection 2 ml will be injected into the affected ankle joint under ultrasonographic guidance. The control group will follow the exact same protocol of venipuncture and preparation of the PRP, but instead of PRP, 2ml physiological saline will be injected on both occasions. To guarantee blinding for the allocated treatment of the patient, treatment assessor and treating physician, blood will be drawn and PRP will be prepared for each patient during both injections (at inclusion and at a time interval of 6 weeks after the first injection). An unblinded research assistant will prepare an injection with either PRP or physiological saline. The injection will be blinded by a specially manufactured covering sheath in order to conceal randomisation. Following the intra-articular injection, the sheath (containing either the remnants of the PRP or saline), will be directly handed to the unblinded research assistant, who will immediately dispose of the syringe, therefore keeping the physician and coordinating researcher blinded.

5.2 PRP preparation

This blood will be prepared according to the instructions of the manufacturer (see appendix F4 Protocol bloedafname - PRP bereiden - intra-articulair injectie enkel). The same method was used in a RCT on acute hamstring injuries by Reurink et al, 2014 NEJM.[2] For each patient the coordinating researcher will prepare a PRP and a placebo injection (isotonic saline: 0.9% sodium chloride).
Prior to commencement of the study, the coordinating researcher was trained by a representative of Arthrex, as well as two experienced members of the PRIMA trial research group (dr. G Reurink en dr. RJ de Vos) with a vast experience regarding PRP preparation and injection. The PRP will be prepared using a widely used and commercial available system (Arthrex double syringe PRP system, Arthrex Medizinische Instrumente GmbH, Garching, Germany). Quality of the system meets internationally accepted standards according to the international organization for standardization standard (ISO13485 medical devices). Further CE certificates are in the appendix (D2). One syringe of approximately 15ml of venous blood will be collected from the cubital vein. After blood collection the syringe will undergo 5 minutes of centrifugation. Further reference regarding injection instructions and sterility procedure to be found in appendix F4 Protocol bloedafname - PRP bereiden - intra-articulair injectie enkel.

5.3 Use of co-intervention

Patients are instructed to avoid the use of co-interventions and NSAID's 24 hours prior to the intervention and during the follow-up period. Throughout the study, any co-interventions (including usual care: exercise therapy and healthy life style advice) used by participants will be registered, such as NSAID's, other analgesic drugs, intra-articular injections or inlays.

6. METHODS

6.1 Main study parameter/endpoint

1. American Orthopaedic Foot and Ankle Society (AOFAS) score at 26 weeks follow-up, validated scale for ankle OA (0-100) measuring three subdomains (pain, function and alignment).

6.2 Secondary study parameters/endpoints

Main secondary outcome:

1. Pain scores: (VAS 0-100) during activities of daily living and the pain sub-scale of AOFAS (0-40)
2. Ankle activity score (0-10)
3. Subjective patient satisfaction (4 categories)
4. Health related quality of life (SF-36 scale)

5. The Global Attainment Scaling (GAS)
6. EQ-5D-3L utility score
7. Ankle Osteoarthritis Score (AOS)
8. Foot and Ankle Outcome Score (FAOS)

6.3 Randomization, blinding and treatment allocation

In line with our previously conducted multicenter RCT, patients will be included at the centre of their first outpatient clinic appointment.[2] For each patient the coordinating researcher prepared a syringe with PRP and a syringe with placebo (isotonic saline: 0.9% sodium chloride). CASTOREdc will be used to perform a computer generated block randomisation scheme with patients stratified to center with a variable block size of two, four or six. The coordinating researcher remains blinded to the allocated intervention. An independent researcher from the coordinating location will have access to the randomization result and the allocated intervention. This will be relayed to a research assistant. The research assistant then selects one of the two syringes based on the allocated intervention and blinds the syringe with a covering sheath.The patients, physicians, and coordinating researcher will all blinded to the allocation of the intervention and to the contents of the syringe. The success of blinding will be assessed by asking participants which injection they think they have received, this will then be registered accordingly. In the event of questions or problems that cannot be answered or solved by the treating physician or coordinating researcher, patients may contact the independent physician.

After the 26 weeks follow-up of the last patient in the study, the principal investigator and coordinating researcher will be unblinded only after the analysis of the primary outcome. The patients will remain blinded to the therapy until the 52 weeks follow-up (online questionnaire) of the last patient in the study. At 52 weeks follow-up, a second blinded researcher will evaluate the patients using the outcome measurements.

6.4 Study procedures

Following x-ray imaging performed under usual patient care, in the event the patient meets the criteria for inclusion and exclusion, he or she will be informed in more detail about the study procedure. At that time the patient can ask questions about the study and decide whether they will participate. The patient has no maximum time limit to consider participation and may proceed to sign the informed consent form. Subsequently the patient will proceed to inclusion and the randomization procedure.

6.5 Inclusion

Patients are recruited for inclusion by their treating physicians at location. An AP and lateral view X-ray will be performed at baseline prior to inclusion to the PRIMA trial. Following inclusion PRP will be prepared according to the PRP system instructions of the manufacturer (see supplement 1). During the first two consultations a total of two intra-articular injections will be documented. The patient will have no additional costs as a result of taking part in this study.

Follow-up will be at 6, 12, 26, 39, 52 weeks and 5 years (Questionnaires will be managed and distributed digitally using a GCP approved data management system (Castor EDC). The time points and outcome measurements are described below:

6 weeks:

− Standard follow-up questionnaires, physical examination (see appendix F1 & F4)

− Second PRP or normal saline placebo injection

12 weeks:

− *Standard follow-up questionnaires and PRODISQ questionnaire* (see appendix F1 & F4)

26 weeks:

− *Standard follow-up questionnaires, PRODISQ questionnaire and physical examination* (see appendix F1 & F4)

39 weeks:

− *PRODISQ questionnaire* (see appendix F1)

52 weeks:

− *Standard follow-up questionnaires, PRODISQ questionnaire* (see appendix F1 & F4)

6.6 Replacement of individual subjects after withdrawal

In the sample size calculation we compensated for an expected loss of 10% of patients to follow-up. No patients will be replaced after withdrawal.

6.7 Statistical analysis

The statistical analysis of the primary outcome measure will be blinded using only the blinded codes of the randomisation groups. To test for the effect of treatment on the between-group difference in primary outcome, we will use the repeated measurement general linear model. Changes from baseline to all follow-up time points will be included in the model. Adjustments will be made for those variables that influenced the primary outcome with $P < 0.10$. However, both adjusted and unadjusted results will be presented. Logistic and linear regression analyses will be used for

respectively binary and numerical secondary outcome parameters. Our analysis will include imputation for missed data and sensitivity analysis.

6.8 Economic analysis

In the event of a positive significant outcome, an economic analysis is needed to support a possible change of practice. An economic analysis (costs) will be performed in order to determine cost-effectiveness. Consequently, the amount of symptom reduction may be related to cost-effectivity of PRP injection treatment. The analysis will be based on indirect costs and direct costs and will be determined using the PRODISQ questionnaire. The PRODISQ questionnaire is taken at baseline and every 3 months thereafter up until 1 year. The cost-effectivity analysis occurs at 1 year. The PRODISQ questionnaire is submitted in the Appendix (F1).

7. ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the Declaration of Helsinki and the Personal Data Protection Act (Wbp). The AMC medical ethical committee will judge whether the study meets the criteria for the Medical Research involving Human subjects act (WMO).

7.1 Informed consent

When patients wish to participate in the trial, he or she will be asked to fill in a written informed consent form.

7.2 Benefits and risk assessment, group relatedness

When compared to OA of the knee and hip, ankle OA is more common in the relatively young and active population, with the highest incidence in females. Health related quality of life and physical functioning is comparable with hip OA and end-stage kidney disease or congestive heart failure.[14] This relatively young (female) ankle OA population is at increased risk for decreased work participation and family care. In the absence of evidence-based non-surgical interventions, a positive outcome will have an impact on the economical (if cost-effective) and disease burden of this prevalent disease. The relatively simple content and widespread availability of the intervention and previously reported good safety will contribute to simple and optimal nationwide implementation.

Complications have not been observed in previous studies with PRP injections having been performed on different muscle and tendon injuries as well as intra-articular injections of the knee and ankle. Although no adverse effects have been previously reported, no guarantee can be given.

Experiences form experts in clinical practise using intra-articular PRP injections indicate that approximately 10% of participants experience some mild joint pain up to 3 weeks following the PRP injection. On inclusion, participants will undergo ankle x-rays as would normally have been the case had they not participated in the study.
A negative outcome (no effect of PRP) will prevent the widespread use of a non-efficacious treatment on patients. Our previous PRP RCTs have shown that initially one high quality study will have an enormous impact on clinical application and negates the need for starting low quality studies (as it evolved in knee OA PRP studies).

7.3 Incentives

In the event of additional visits related to the study, travel compensation will be granted.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 1, of the WMO (http://www.ccmo.nl/attachments/files/wmo-engelse-vertaling-29-7-2013-afkomstig-van-vws.pdf), the sponsor (AMC) will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC.

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.3 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

9.1 Handling and storage of data

After giving permission for participating in this study, participants will receive a link to fill in surveys and their informed consent in Castor EDC. All data gained outside Castor EDC will be stored on the AMC secured hard drive. All data will be coded and stored in the Castor EDC online database which meets the AMC safety criteria and good clinical practice guidelines. The primary investigator and project leader will safeguard the coded data through password secured access. All participant's data will be archived for at least 15 years and handled with in accordance with the Dutch Personal Data

Protection Act (Wbp). Data protections is provided through the safety protocol of Castor EDC with automated backups and SSL security.

9.2 Monitoring and Quality Assurance

Throughout the trial, 5 monitoring visits will take place.

Visit no.	Selected Sites	Planning*
Initiation Visit	All sites	Before enrolment of the first subject, but after Ethics Committee approval has been obtained.
First Monitoring Visit	All sites	After 3 enrolled subjects, irrespective of (e)CRF completion.
Second Monitoring Visit	All sites	After approximately 10 -15 enrolled subjects at site have completed the 26 weeks follow-up visit.
Third Monitoring Visit	AMC	After 70 enrolled subjects.
Remote Close Out	All sites	After database lock

More details is to be found in the monitoring plan enclosed in the appendix K6.

9.3 Amendments

Amendments are defined as changes made to the protocol after it has been approved by the study group. Considering that this study might meet the criteria of the WMO (Medical Research Involving Human subjects Act) the METC will be notified of any amendments made if there is a question that effects the WMO criteria.

9.4 Publication

The results of this project study and new knowledge will be disseminated through the Dutch Arthritis Foundation (Reumafonds), presentations, news publications, blogs, websites social media and professional organisations (rheumatology, orthopaedics, primary care medicine, sports medicine, public health).

10. References

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Platelet Rich plasma Injection Management for Ankle osteoarthritis study (PRIMA): A multi-center, stratified, block-randomized, double-blind, placebo-controlled trial

Version 5

This submission is based on the formerly approved studies (same PI) evaluating the efficacy of platelet rich plasma in musculoskeletal injuries:

- Chronic achilles tendinopathy by de Vos et al. JAMA 2010 (PRICT-study, registration NTR1420, ABR NL 22805.098.08).[1]
- Acute hamstring injuries by Reurink et al. NEJM 2014 (HIT-study registration NTR2771, NL34660.098.10 / 10-163; ABR / METC Zuidwest Holland).[2]

PROTOCOL TITLE: Platelet Rich plasma Injection Management for Ankle osteoarthritis study (PRIMA): A multi-center, stratified, block-randomized, double-blind, placebo-controlled trial

Protocol ID	NL64160.018.18
Short title	**PRIMA**
Version	**5**
Date	**21-04-2020**
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
NSAID's	**Non-Steroidal Anti-Inflammatory Drugs**
OA	**Osteoarthritis**
OCD	**Osteochondral Defect**
PRP	**Platelet-Rich Plasma**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**

WMO **Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

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SUMMARY

Pain is the cardinal symptom of ankle osteoarthritis (OA) and is a complex phenomenon with limited understanding of its pathomechanisms. The main objectives in the clinical management of OA are to reduce inflammation and cartilage degeneration processes as well as relieve pain. Platelet-rich plasma (PRP) is a high concentrate of platelets derived from patient's whole blood, centrifuged to remove red blood cells. PRP has been used to encourage a healing response across several specialties, in particular dentistry, orthopaedics and dermatology. Growth factors stored in the platelets are assumed to facilitate an anti-inflammatory and analgesic effect.

A recent review concluded that in animal models PRP can diminish multiple inflammatory IL-1 mediated effects, and can also positively influence the collagen network of the cartilage and subsequently reduce pain and improve function.[3]

Our recent and other systematic reviews showed that compared to placebo injections, hyaluronic acid or corticosteroid injections, PRP injections significantly decrease pain and improve function in knee OA patients.[4–6] Given the clinical effect on pain reduction in knee OA and safety, PRP might serve as a promising non-surgical therapy for ankle OA. PRP might potentially delay the irreversible surgical options like arthrodesis and joint replacement. No significant adverse advents have been reported for any PRP trials regarding acute hamstring injuries, Achilles tendinopathy, knee OA and specifically not ankle OA. [1,2,4,5,7–9]. Until present, there is no RCT conducted on the efficacy of PRP in the management of ankle OA.

Hypothesis

We hypothesize that:

PRP injections are efficacious for symptom reduction and functional improvement compared to placebo injections in the treatment of ankle (talocrural) OA.

Workplan

Study design

A multi-center, stratified, block-randomized, double-blind, placebo-controlled trial comparing two treatment groups.

Study population

Patients with ankle (talocrural) OA will be included if they meet the following 3 inclusion criteria:

1. Severity of Ankle OA pain on visual analogue scale (VAS) (0–100 mm) ≥ 40 during daily activities
2. X-rays (AP and lateral view) indicating ≥ grade 2 on the Van Dijk classification [10]
3. Age ≥ 18 years

Intervention

Patients will be randomised into two treatment groups: PRP injection or placebo (saline) injection. Both groups will receive two injections of PRP or placebo at an interval of 6 weeks.

Main study parameter/endpoint

American Orthopaedic Foot and Ankle Society (AOFAS) score at 26 weeks follow-up, validated scale for ankle OA (0-100) measuring three subdomains (pain, function and alignment).
After 26 weeks, the principal investigator will be unblinded after the analysis of the primary outcome. The patients will remain blinded to the therapy until 52 weeks follow-up.

Power analysis

Based on previous and ongoing studies, the study protocol of the randomised controlled trial is designed to detect a difference of 12 points (0-100) on the AOFAS score (minimal clinical relevant difference) between the groups.[11] Based on a previous placebo controlled RCT on injection therapy (hyaluronic acid) in ankle OA of DeGroot et al. a standard deviation of 16.3 can be expected. Taking into account a two-sided level of significance of 5%, a power of 90% and a dropout rate of 15%, approximately 50 (40 plus 15%) patients per group will be needed (N=100).[11]

STATISTICAL ANALYSIS

To test for the effect of treatment on the between-group difference in primary outcome, we will use a repeated measurements general linear model. The effect of potential confounders (varus/valgus) will be evaluated and a correction will be performed.

Expected results

We will provide evidence for the (potential) efficacy of PRP for symptom reduction and functional improvement in the treatment of ankle OA. A positive outcome will have an effect on the economical and disease burden. The relatively simple content and widespread availability of the PRP intervention and previously reported good safety will contribute to simple and optimal nationwide implementation.

Conclusion

Our project will provide conclusions on the efficacy of PRP in ankle OA.

1. INTRODUCTION AND RATIONAL

1.1 Clinical relevance of ankle OA

The reported incidence of symptomatic ankle OA is estimated at 1% to 4% in the general adult population.[12] Due to its onset at a relatively young age, the duration of ankle OA in the life of a patient is significantly longer than those with hip or knee OA and the available surgical intervention (arthrodesis) is associated with significant functional limitations. In contrast to hip and knee OA, where there is an excellent surgical alternative (joint replacement) for severe cases, there is a clear need for non-surgical successful interventions in ankle OA.

Health related quality of life and physical function limitations are severe and comparable with hip OA and end-stage kidney disease or congestive heart failure.[14]

1.2 Pathomechanisms of OA

Following pathological or traumatic injury of the hyaline cartilage, progressive catabolic chondrocyte activity leads to degenerative osteoarthritic joint changes. Two key characteristics of OA are the lack of regenerative activity of the chondrocytes and the inflammatory joint reaction.

1.3 Pain pathomechanism

Pain is the cardinal symptom in OA and it is a complex phenomenon of which we understand only a fraction of the pathomechanisms.[15] One of the major peripheral factors considered as being an important source of pain is joint inflammation. Previous studies showed that local inflammation, with the release of phospholipases, cyclooxygenases and leukotrienes, is involved in pain mechanisms.[15] These factors not only result in increased intra-articular pressure due to edema, but also induce the amount of nociceptors present with subsequent excitation of peripheral nerves.[15,16]

1.4 Non-surgical interventions for ankle-OA

The main objectives in the clinical management of OA are to reduce the inflammatory and cartilage degeneration processes, and to relieve pain.

At this time there is no evidence-based treatment algorithm for non-surgical management of ankle OA.[2,5,17,18] Several pain relief options, such as non-steroidal anti-inflammatory drugs, opioids and corticosteroid as well as hyaluronic acid injections, are available but there is a lack of evidence from high quality studies to assist in clinical decision-making.

1.5 PRP in OA – animal models

Platelet Rich Plasma (PRP) is defined as plasma containing a concentration of at least 1,000,000 platelets/µl. Growth factors (GF) are stored in α-granules within platelets, and are released in a selective manner upon activation. GF released from the α-granules of platelets are assumed to provide the regenerative and anti-inflammatory benefits of PRP.

Recent reviews concluded that in animal models, PRP can diminish multiple inflammatory IL-1 mediated effects.[3] Due to this local anti-inflammatory response, PRP might have an indirect analgesic effect. The second suggested effect might be an increased mRNA expression of proteoglycan core protein in the articular cartilage and decreased chondrocyte apoptosis.[3] Consequently, PRP could also positively influence the collagen network of the cartilage.

1.6 PRP in OA – clinical studies

Several systematic reviews have shown that compared to placebo injections, hyaluronic acid or corticosteroid injections, PRP injections significantly decrease pain, improve function and are simple and safe.[5,18] In the majority of studies, the patients received 2-3 injections at fixed intervals. Given its clinical effect on pain reduction in OA and safety, PRP therapy in ankle OA might serve as a distinct non-surgical therapy for reducing pain and improving function. Clinical studies on the use of PRP in ankle OA are limited to a single report of 5 cases, which showed significant reduction of the VAS-FA score at a mean of 16 months follow-up.[17] Our preliminary results on the outcome of PRP injection in 14 patients with van Dijk Grade 2 ankle OA, revealed that 64% reported improvement of their symptoms. A lack of validated outcome scores, low quality study designs and small sample sizes are the main limitations of these previously conducted studies.

1.7 Why this proposal will have a great impact

The strength and uniqueness of our project is that: We will evaluate the promising findings from animal studies and positive clinical observations of PRP in ankle OA patients in a Level 1 study.

2. Study Goal

The goal of this study is to determine the efficacy of PRP injections in the management of ankle osteoarthritis by comparing 2 groups, both receiving 2 injections of either: PRP or a placebo saline solution.

2.1 Hypothesis

We hypothesize that: PRP injections are efficacious for symptom reduction and functional improvement compared to placebo injections in the treatment of ankle (talocrural) OA

3. STUDY DESIGN

A multi-center, stratified, block-randomized, double-blind, placebo-controlled trial comparing two treatment groups.

4. STUDY POPULATION

4.1 Population (base)

Patients with ankle OA in two University Medical Centres (Erasmus MC, AMC), two teaching hospitals (OLVG, Spaarne Gashtuis, ageneral hospital (Flevo Hospital) and focus clinic (Bergman Clinic) will be informed about the study.

4.2 Inclusion criteria

1. Severity of Ankle OA pain on a visual analogue scale (VAS) (0–100 mm) ≥ 40 during daily activities
2. X-rays (AP and lateral view) indicating ≥ grade 2 on the Van Dijk classification[10]
3. Age ≥ 18 years

4.3 Exclusion criteria

1. Patient has received injection therapy for ankle OA in the previous 6 months
2. Patient does not want to receive one of the two therapies
3. Patient has clinical signs of concomitant OA of one or more other major joints of the lower extremities that negatively affects their daily activity level
4. Previous ankle surgery for OA or Osteochondral defects (OCD) < 1 year (not including surgery for an ankle fracture in the past)

4.4 RADIOGRAPHS

AP and lateral X-rays of the ankles will be scored according to the Van Dijk classification:[10]

- 0 Normal joint or subchondral sclerosis
- I Osteophytes without joint space narrowing
- II Joint space narrowing with or without osteophytes
- III (Sub)total disappearance or deformation of the joint space

4.5 Sample size calculation

Based on previous and ongoing studies, the study protocol of the randomised controlled trial is designed to detect a difference of 12 points (0-100) on the AOFAS score (minimal clinical relevant difference) between the groups.[11] Based on a previous placebo controlled RCT on injection therapy (hyaluronic acid) in ankle OA of DeGroot et al. a standard deviation of 16.3 can be expected. Taking into account a two-sided level of significance of 5%, a power of 90% and a dropout rate of 15%, approximately 50 (40 plus 15%) patients per group will be needed (N=100).[11]

5. INTERVENTION

5.1 Intervention

In this study, patients will be randomised into two treatment groups: PRP injection or placebo saline injection. Treatment allocation will be concealed. One syringe of 15ml autologous blood will be collected twice from the cubital vein by the coordinating researcher or a trained research assistant or a BIG registered person with significant experience with the procedure: at inclusion and at a time interval of approximately 6 weeks. All participants will receive a second injection, regardless of the effect of the first injection. This blood will be prepared according to the instructions of the manufacturer (see appendix F4 Protocol bloedafname - PRP bereiden - intra-articulair injectie enkel), and the injection will be given within 30 minutes following venipuncture. For each injection 2 ml will be injected into the affected ankle joint under ultrasonographic guidance. The control group will follow the exact same protocol of venipuncture and preparation of the PRP, but instead of PRP, 2ml physiological saline will be injected on both occasions. To guarantee blinding for the allocated treatment of the patient, treatment assessor and treating physician, blood will be drawn and PRP will be prepared for each patient during both injections (at inclusion and at a time interval of 6 weeks after the first injection). An unblinded research assistant will prepare an injection with either PRP or physiological saline. The injection will be blinded by a specially manufactured covering sheath in order to conceal randomisation. Following the intra-articular injection, the sheath (containing either the remnants of the PRP or saline), will be directly handed to the unblinded research assistant, who

will immediately dispose of the syringe, therefore keeping the physician and coordinating researcher blinded.

5.2 PRP preparation

This blood will be prepared according to the instructions of the manufacturer (see appendix F4 Protocol bloedafname - PRP bereiden - intra-articulair injectie enkel). The same method was used in a RCT on acute hamstring injuries by Reurink et al, 2014 NEJM.[2] For each patient the coordinating researcher a trained research assistant or a BIG registered person with significant experience with the procedure, will prepare a PRP and a placebo injection (isotonic saline: 0.9% sodium chloride). Prior to commencement of the study, the coordinating researcher was trained by a representative of Arthrex, as well as two experienced members of the PRIMA trial research group (dr. G Reurink en dr. RJ de Vos) with a vast experience regarding PRP preparation and injection. Prior to delegating the PRP and placebo injection syringe preparation procedure to a research assistant, the research assistant will be trained by a representative of Arthrex or a BIG registered person with significant experience with the procedure for the PRP injection syringe preparation. These tasks will be delegated only after approval by the local PI and registration in the delegation log.
For the placebo injection syringe preparation procedure, the research assistant or a BIG registered person with significant experience with the procedure will be trained by the coordinating researcher through a video manual or videoconsult.
The PRP will be prepared using a widely used and commercial available system (Arthrex double syringe PRP system, Arthrex Medizinische Instrumente GmbH, Garching, Germany). Quality of the system meets internationally accepted standards according to the international organization for standardization standard (ISO13485 medical devices). Further CE certificates are in the appendix (D2). One syringe of approximately 15ml of venous blood will be collected from the cubital vein by the coordinating researcher or a research assistant. After blood collection the syringe will undergo 5 minutes of centrifugation. Further reference regarding injection instructions and sterility procedure to be found in appendix F4 Protocol bloedafname - PRP bereiden - intra-articulair injectie enkel.

5.3 Use of co-intervention

Patients are instructed to avoid the use of co-interventions and NSAID's 24 hours prior to the intervention and during the follow-up period. Throughout the study, any co-interventions (including usual care: exercise therapy and healthy life style advice) used by participants will be registered, such as NSAID's, other analgesic drugs, intra-articular injections or inlays.

6. METHODS

6.1 Main study parameter/endpoint

1. American Orthopaedic Foot and Ankle Society (AOFAS) score at 26 weeks follow-up, validated scale for ankle OA (0-100) measuring three subdomains (pain, function and alignment).

6.2 Secondary study parameters/endpoints

Main secondary outcome:

1. Pain scores: (VAS 0-100) during activities of daily living and the pain sub-scale of AOFAS (0-40)
2. Ankle activity score (0-10)
3. Subjective patient satisfaction (4 categories)
4. Health related quality of life (SF-36 scale)
5. The Global Attainment Scaling (GAS)
6. EQ-5D-3L utility score
7. Ankle Osteoarthritis Score (AOS)
8. Foot and Ankle Outcome Score (FAOS)

6.3 Randomization, blinding and treatment allocation

In line with our previously conducted multicenter RCT, patients will be included at the centre of their first outpatient clinic appointment.[2] For each patient the coordinating researcher or research assistant or a BIG registered person with significant experience with the procedure prepared a syringe with PRP and a syringe with placebo (isotonic saline: 0.9% sodium chloride). CASTORedc will be used to perform a computer generated block randomisation scheme with patients stratified to center with a variable block size of two, four or six. The coordinating researcher remains blinded to the allocated intervention. An unblinded research assistant will have access to the randomization result and the allocated intervention. The unblinded research assistant then selects one of the two syringes based on the allocated intervention and blinds the syringe with a covering sheath. The patients, physicians, and coordinating researcher (or research assistant or a BIG registered person with significant experience with the procedure) will all be blinded to the allocation of the intervention and to the contents of the syringe. The success of blinding will be assessed by asking participants which injection they think they have received, this will then be registered accordingly. In the event of questions or problems that cannot be answered or solved by the treating physician or coordinating researcher, patients may contact the independent physician.

After the 26 weeks follow-up of the last patient in the study, the principal investigator and coordinating researcher will be unblinded only after the analysis of the primary outcome. The patients will remain blinded to the therapy until the 52 weeks follow-up (online questionnaire) of the

last patient in the study. At 52 weeks follow-up, a second blinded researcher will evaluate the patients using the outcome measurements.

6.4 Study procedures

Following x-ray imaging performed under usual patient care, in the event the patient meets the criteria for inclusion and exclusion, he or she will be informed in more detail about the study procedure. At that time the patient can ask questions about the study and decide whether they will participate. The patient has no maximum time limit to consider participation and may proceed to sign the informed consent form. Subsequently the patient will proceed to inclusion and the randomization procedure.

6.5 Inclusion

Patients are recruited for inclusion by their treating physicians at location. An AP and lateral view X-ray will be performed at baseline prior to inclusion to the PRIMA trial. Following inclusion PRP will be prepared according to the PRP system instructions of the manufacturer (see supplement 1). During the first two consultations a total of two intra-articular injections will be documented. The patient will have no additional costs as a result of taking part in this study.

Follow-up will be at 6, 12, 26, 39, 52 weeks and 5 years (Questionnaires will be managed and distributed digitally using a GCP approved data management system (Castor EDC). The time points and outcome measurements are described below:

6 weeks:

- Standard follow-up questionnaires, physical examination (see appendix F1 & F4)
- Second PRP or normal saline placebo injection

12 weeks:

- *Standard follow-up questionnaires and PRODISQ questionnaire* (see appendix F1 & F4)

26 weeks:

- *Standard follow-up questionnaires, PRODISQ questionnaire and physical examination* (see appendix F1 & F4). *Since trial patients may not be able to come to the investigational site for protocol-specified visits (due to local regulations) due to the COVID-19 pandemic, video consultations will be implemented when necessary and feasible, and will be sufficient to assure the safety of trial patients."*

39 weeks:

- *PRODISQ questionnaire* (see appendix F1)

52 weeks:

– *Standard follow-up questionnaires, PRODISQ questionnaire* (see appendix F1 & F4)

6.6 Replacement of individual subjects after withdrawal

In the sample size calculation we compensated for an expected loss of 10% of patients to follow-up.

No patients will be replaced after withdrawal.

Post randomisation replacement during the Covid-19 related regulations:
To prevent potential immediate hazard to the patients and in compliance with the institutional and national Covid-19 -related clinical research regulations, we deviated from the protocol and replaced patients following Institutional Review Board (IRB) (in Dutch: Medisch Ethische Toetsingscommissie) *approval (submission date 14-4-2020).*(1,2)

During the COVID-19 pandemic, 12 received their first intervention (intra-articular injection), but these patients had no access to receiving their second injection at the pre-defined 6 week time-interval. Following consultation with the head of the department and/or local principal investigators, considering the risks and descaling of elective patient bound activities, we found the COVID-19 associated potential risks to outweigh the potential damage due to the disease for which they had no access to the intervention.

As in-person visits are required for administration of the investigational products (intra-articular injections), protection of a participant's safety, welfare, and rights is best served by discontinuing the administration or use of the investigational product and subsequent participation in the trial.(1,2) *In order to minimise protocol deviations, maintain the previously calculated sample size of 100 patients, we asked IRB approval for replacing them with 12 new inclusions. Following IRB approval we will start including patients as soon as (1) out-patient non-COVID-19 care is scaled up, (2) there is approval from the local head of the department and/or local principal investigator and (3) the local regulations permitted. By doing this, we accounted for the potential risks for patients and documented this accordingly in an emergency risk management plan.*
At the start of the trial we did not anticipate that a substantial number of patients who were randomised into the trial would subsequently be unable to undergo the intervention (due to the Covid-19 crisis).
By asking the IRB approval for replacing these patients that had no access to the intervention, due to COVID-19 regulations, we minimize potential bias, as:

- *Allocation to the treatment or control arm will not have influenced the discontinuation of trial participation (internal validity).*
- *We will follow the similar recruitment procedure of consecutive patients after the COVID-19 ban has been stopped and therefore the newly included patients are expected to be representative for the same population as the patients for whom the trial participation has been discontinued (external validity).*

Participation of these 12 patients will be discontinued and they will be informed by written letter, e-mail and/or telephone call. The monitoring body will be informed within 48 hours following IRB approval.

The Sponsor and clinical investigators will document how restrictions related to COVID-19 led to the changes in study conduct and duration of those changes and indicate which and how trial patients will be impacted. We will capture specific information in the case report form that explains the basis of potential missing data, including the relationship to COVID-19 for missing protocol-specified information (e.g., from missed study visits or study discontinuations due to COVID-19). This information, will be summarized in the clinical study report.

The proposed IRB amendment (submitted on 14-4-2020) with changes in the protocol will be updated in the data management and/or statistical analysis plan amendments. Prior to locking the database, we will address in the statistical analysis plan how protocol deviations related to COVID-19 will be handled for the pre-specified analyses.

In these extreme circumstances, we are confronted with a crisis and are forced to think of solutions in order to maintain the quality of the study. The European Committee for Human Medicinal Products, recommend collection of as much data as possible. In the current situation we find the trial load for patients no longer participating too heavy and thus unethical. Consequently, we will limit data collection in these patients to the primary outcome measure, AOFAS at 26 weeks (1x 10 min by videoconsult).

6.7 Statistical analysis

The statistical analysis of the primary outcome measure will be blinded using only the blinded codes of the randomisation groups. To test for the effect of treatment on the between-group difference in primary outcome, we will use the repeated measurement general linear model. Changes from baseline to all follow-up time points will be included in the model. Adjustments will be made for those variables that influenced the primary outcome with $P < 0.10$. However, both adjusted and

unadjusted results will be presented. Logistic and linear regression analyses will be used for respectively binary and numerical secondary outcome parameters. Our analysis will include imputation for missed data and sensitivity analysis.

6.8 Economic analysis

In the event of a positive significant outcome, an economic analysis is needed to support a possible change of practice. An economic analysis (costs) will be performed in order to determine cost-effectiveness. Consequently, the amount of symptom reduction may be related to cost-effectivity of PRP injection treatment. The analysis will be based on indirect costs and direct costs and will be determined using the PRODISQ questionnaire. The PRODISQ questionnaire is taken at baseline and every 3 months thereafter up until 1 year. The cost-effectivity analysis occurs at 1 year. The PRODISQ questionnaire is submitted in the Appendix (F1).

7. ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the Declaration of Helsinki and the Personal Data Protection Act (Wbp). The AMC medical ethical committee will judge whether the study meets the criteria for the Medical Research involving Human subjects act (WMO).

7.1 Informed consent

When patients wish to participate in the trial, he or she will be asked to fill in a written informed consent form.

7.2 Benefits and risk assessment, group relatedness

When compared to OA of the knee and hip, ankle OA is more common in the relatively young and active population, with the highest incidence in females. Health related quality of life and physical functioning is comparable with hip OA and end-stage kidney disease or congestive heart failure.[14] This relatively young (female) ankle OA population is at increased risk for decreased work participation and family care. In the absence of evidence-based non-surgical interventions, a positive outcome will have an impact on the economical (if cost-effective) and disease burden of this prevalent disease. The relatively simple content and widespread availability of the intervention and previously reported good safety will contribute to simple and optimal nationwide implementation.

Complications have not been observed in previous studies with PRP injections having been performed on different muscle and tendon injuries as well as intra-articular injections of the knee

and ankle. Although no adverse effects have been previously reported, no guarantee can be given. Experiences form experts in clinical practise using intra-articular PRP injections indicate that approximately 10% of participants experience some mild joint pain up to 3 weeks following the PRP injection. On inclusion, participants will undergo ankle x-rays as would normally have been the case had they not participated in the study.

A negative outcome (no effect of PRP) will prevent the widespread use of a non-efficacious treatment on patients. Our previous PRP RCTs have shown that initially one high quality study will have an enormous impact on clinical application and negates the need for starting low quality studies (as it evolved in knee OA PRP studies).

7.3 Incentives

In the event of additional visits related to the study, travel compensation will be granted.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 1, of the WMO (http://www.ccmo.nl/attachments/files/wmo-engelse-vertaling-29-7-2013-afkomstig-van-vws.pdf), the sponsor (AMC) will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC.

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.3 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

9.1 Handling and storage of data

After giving permission for participating in this study, participants will receive a link to fill in surveys and their informed consent in Castor EDC. All data gained outside Castor EDC will be stored on the AMC secured hard drive. All data will be coded and stored in the Castor EDC online database which meets the AMC safety criteria and good clinical practice guidelines. The primary investigator and project leader will safeguard the coded data through password secured access. All participant's data will be archived for at least 15 years and handled with in accordance with the Dutch Personal Data

Protection Act (Wbp). Data protections is provided through the safety protocol of Castor EDC with automated backups and SSL security.

9.2 Monitoring and Quality Assurance

Throughout the trial, 5 monitoring visits will take place.

Visit no.	Selected Sites	Planning*
Initiation Visit	All sites	Before enrolment of the first subject, but after Ethics Committee approval has been obtained.
First Monitoring Visit	All sites	After 3 enrolled subjects, irrespective of (e)CRF completion.
Second Monitoring Visit	All sites	After approximately 10 -15 enrolled subjects at site have completed the 26 weeks follow-up visit.
Third Monitoring Visit	AMC	After 70 enrolled subjects.
Remote Close Out	All sites	After database lock

More details is to be found in the monitoring plan enclosed in the appendix K6.

9.3 Amendments

Amendments are defined as changes made to the protocol after it has been approved by the study group. Considering that this study might meet the criteria of the WMO (Medical Research Involving Human subjects Act) the METC will be notified of any amendments made if there is a question that effects the WMO criteria.

9.4 Publication

The results of this project study and new knowledge will be disseminated through the Dutch Arthritis Foundation (Reumafonds), presentations, news publications, blogs, websites social media and professional organisations (rheumatology, orthopaedics, primary care medicine, sports medicine, public health).

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Summary of Changes Protocol

Protocol version	**Approval Medical Ethics Review Committee Amsterdam Medical Center, the Netherlands (ABR 2018–042)**	**Description of and reason for changes** (Highlighted in yellow in version 5)
4.0	23-07-2018	
First patient included in the trial 24-08-2018		
5.0	21-04-2020	- Amendment due to COVID-19 pandemic: During the COVID-19 pandemic, 12 patients received their first intervention (intra-articular injection), but these patients had no access to receiving their second injection at the pre-defined 6 week time interval. Participation of these 12 patients was discontinued and they were replaced with 12 new inclusions.

Statistical Analysis Plan

Platelet-Rich plasma Injection Management for Ankle osteoarthritis study (PRIMA): protocol of a Dutch multicentre, stratified, block-randomised, double-blind, placebo-controlled trial				
Dutch Trial Register	NTR7261 (registered 2018-06-06)	METC ABR	2018-042	
SAP Version	1	SAP Version Date	10-2-2020	
Role of Contributor	Name	Affiliation	Signature	Date of Signature
Principal Investigator	Prof. J.L. Tol	Orthopaedic Surgery Amsterdam UMC		10 Feb 2020
Researcher who will perform the statistical analysis	L.D.A. Paget	Orthopaedic Surgery Amsterdam UMC		10 Feb 2020
TRIAL STATISTICIAN	Prof. S.M.A. Bierma-Zeinstra	General Practice, Erasmus University Medical Center		10 febr 2020
Contributor to Statistical Analysis Plan	G. Reurink	Orthopaedic Surgery Amsterdam UMC		10 Feb 2020
Contributor to Statistical Analysis Plan	R.J. de Vos	Orthopaedics and Sports Medicine, Erasmus University Medical Center		10 Feb 2020
Contributor to Statistical Analysis Plan	A. Weir	Orthopaedics and Sports Medicine, Erasmus University Medical Center	AW	10-2-2020

1c. Revision History of the statistical analysis plan

Update statistical analysis plan version	Protocol version	Section number(s) changed	Description of and reason for changes	Date of approval
1.0	4	0	0	31-01-2020

1.2. Planned period of observation

The study included its first patient in August 2018 and aims to include the last patient by March 2020, consequently allowing analysis and then de-blinding of the coordinating researcher, principle investigator and fellow project members, to commence after the last follow-up (26 weeks) of the last patient by September 2020.

2. Introduction

2.1. Background and rationale

Platelet-rich Plasma (PRP) is a potentially efficacious treatment for ankle OA but its use has not been examined in high quality studies. Systematic reviews show that platelet-rich plasma (PRP) injections significantly decrease pain and improve function in knee OA patients. Ankle OA is more common than hip or knee OA in the young active population; with a prevalence of 3.4%. PRP injections in ankle OA are shown to be safe and improve quality of life over time, but no randomised controlled trial has been conducted. Our randomised controlled trial will evaluate the efficacy of PRP injections for symptom reduction and functional improvement, compared to placebo, in the treatment of ankle (talocrural) OA. The PRIMA trial is registered in the Netherlands trial Register: NTR7261 and its protocol has been published.[1]

2.2. Study Objectives

We aim to determine the efficacy of PRP injections in the management of ankle OA by comparing 2 groups, both receiving 2 injections of either: PRP or placebo solution. We hypothesize that PRP injections are efficacious for symptom reduction and functional improvement compared to placebo in the treatment of ankle (talocrural) OA.

3. Study Methods

3.1. Study Design

The PRIMA study is a multi-center, stratified, block-randomized, double-blind, placebo-controlled trial design will be conducted in order to compare two treatment groups: PRP vs Placebo (saline). After the 26 weeks follow-up of the last patient in the study, the coordinating researcher, principal investigator and fellow project members will be unblinded only after the analysis of the primary outcome. A flow chart of the design and follow-up is shown in figure 1.

3.2. Randomisation, Blinding and Treatment Allocation

In this study, patients will be randomised into two treatment groups: PRP injection or placebo saline injection. For each patient the coordinating researcher will prepare a PRP and a placebo injection (isotonic saline: 0.9% sodium chloride).

We will include patients at the centre of their first outpatient clinic appointment. For each patient the coordinating researcher will prepare a syringe with PRP and a syringe with placebo (isotonic saline: 0.9% sodium chloride). A Good Clinical Practice (GCP) approved data management system (Castor EDC, based in Amsterdam, the Netherlands) will be used to perform a computer generated block randomisation scheme with patients stratified to centre with a variable block size of 2,4 or 6. This procedure will ensure treatment allocation concealment. The coordinating researcher, treating

physician and patient all remain blinded to the allocated intervention. An independent researcher from the coordinating location will have access to the randomization result and the allocated intervention. This will be relayed to a GCP-certified research assistant at the centre. The research assistant then selects one of the two syringes based on the allocated intervention and blinds the syringe with a covering sheath, ensuring concealment of the content of the syringe. The patients, treating physicians, and coordinating researcher will all be blinded to the allocation of the intervention and to the contents of the syringe. The success of blinding will be assessed by asking patients which injection they think they have received just after the injection procedure, this will then be registered accordingly.

3.3. Sample Size

Based on previous and ongoing studies, the study protocol of the randomised controlled trial is designed to detect a difference of 12 points (0-100) on the AOFAS score. There is no official agreement on the minimal clinical important difference for the AOFAS score regarding ankle OA. However in relatable musculoskeletal literature, 10% – 15% of the used scale was reported. [2–4] Our pre-defined minimal clinical important difference of 12 % is located within this range.[2–4] Based on a previous placebo controlled RCT on injection therapy (hyaluronic acid) in ankle OA of De Groot et al. a standard deviation of 16.3 can be expected.[5] Taking into account a two-sided level of significance of 5%, a power of 90% and a dropout rate of 10%, approximately 50 (45 plus 10% drop out) patients per group will be needed (N=100 in total).

3.4. Hypothesis testing framework

The PRIMA trial uses a superiority hypothesis testing framework for all primary and secondary outcomes.

3.5. Interim Analysis

No interim analyses will be performed as the study has been classified as low-risk. All adverse events reported spontaneously by the patient or observed by the investigator or his staff will be recorded. A variety of conditions have been treated with PRP ranging from muscle and tendon injuries to intra-articular injections of the knee and ankle. To date, no serious adverse events have been documented in the literature, concerning PRP intra-articular injections of the ankle. In accordance with the Central Committee on Research Involving Human Subjects (CCMO) guidelines, this study was classified as low-risk for adverse events. Therefore, the local Medical Ethical Commission will be notified of any serious adverse events. In the event this happens, the advice of the Medical Ethical Commission will be followed accordingly.

3.6. Start statistical analysis of data

The current estimate is that the final patient will be included in the study in March 2020. We therefore expect to perform the statistical analysis for both primary and secondary outcomes at 26 weeks follow-up in September 2020.

3.7. Time points

Table 1. Follow-up

Baseline	- 1st intervention injections - Physical Examination - AOFAS - PROMs - PRODISQ cost-effectivity

6 weeks	- 2nd intervention injection - Physical Examination - AOFAS - PROMs
12 weeks	- AOFAS - PROMs - PRODISQ cost-effectivity
26 weeks	- Physical Examination - AOFAS - PROMs - PRODISQ cost-effectivity
39 weeks	- PRODISQ cost-effectivity
52 weeks	- AOFAS - PROMs - PRODISQ cost-effectivity
5 years	- AOFAS - PROMs

Table 1. In addition to the American Orthopaedic Foot and Ankle Society (AOFAS) score, the following PROMs will be taken: Foot and Ankle Outcome Score (FAOS), Ankle Osteoarthritis Score (AOS), Visual Analogue Scale (VAS), Ankle activity score (AAS), Subjective patient satisfaction, Short Form Health Survey (SF-36), The Global Attainment Scaling (GAS), EuroQol-5 dimensions-3 levels (EQ-5D-3L). These PROMs will be elaborated on further on. Furthermore the PROductivity and DISease Questionnaire (PRODISQ) will be used to perform a cost-effectivity analysis. These questionnaires can be found in appendix 1

4. Statistical Principles

A statistical significant difference between both treatment groups (placebo or PRP), regarding primary and secondary outcomes, will be determined if the two sided p-values are less than 0.05. A 95% confidence interval will be provided for primary and secondary outcome measures. No adjustment will be made for multiplicity as there is only one primary outcome measured at a single time point. Protocol deviations will be listed according to treatment group. This will also be presented as a percentage and number of patients in each treatment group having experienced a protocol deviation. The intention to treat population includes all participants randomized, regardless of protocol deviation.

After the 26 weeks follow-up of the last patient in the study, a standard operating procedure will be available to logically recode and clean the data. The data will be interpreted according to a blinded data interpretation scheme described by Järvinen et al.[6] A statistical expert (SB) is present among the authors. The authors will interpret the statistical results until a consensus is reached. Once the authors are in agreement, the two groups will be unblinded and no changes will be made to the interpretation of the results. Thus the principal investigator, coordinating researcher and other project members will be unblinded only after the analysis of the primary outcome. Patients will be unblinded 1 year after the 1 year follow-up of the last patient.

5. Study Populations

5.1. Inclusion and exclusion criteria

Patients with ankle OA in University Medical Centres, teaching hospitals, general hospitals and private specialist clinics will be informed about the study. In order to participate, patients must meet the eligibility criteria documented below.

Inclusion criteria

1. Severity of Ankle OA pain on a visual analogue scale (VAS) (0–100 mm) ≥ 40 mm during daily activities
2. X-rays (anteroposterior (AP) and lateral view) indicating ≥ grade 2 talocrural OA on the Van Dijk classification (clarified under section Radiographs)[7]
3. Age ≥ 18 years

Exclusion criteria

1. Patient has received injection therapy for ankle OA in the previous 6 months
2. Patient does not want to receive one of the two therapies
3. Patient has clinical signs of concomitant OA of one or more other major joints of the lower extremities that negatively affects their daily activity level
4. Previous ankle surgery for OA or Osteochondral defects (OCD) < 1 year (not including surgery for an ankle fracture in the past)

Radiographs

AP and lateral X-rays of the talocrural joints will be scored according to the Van Dijk classification:[7]

0) Normal joint or subchondral sclerosis
1) Osteophytes without joint space narrowing
2) Joint space narrowing with or without osteophytes
3) (Sub)total disappearance or deformation of the joint space

5.2. Planned information for flowchart

A flowchart will present the patients that were screened, met the inclusion criteria, were excluded, randomised and allocated to each study arm, withdrawing from the study (with reason and timing) and assessed for primary outcome.

5.3. Loss to follow-up

The coordinating researcher will attempt to limit loss to follow-up as much as possible by contacting every patient and being present at every patient visit. All digital questionnaires will be constantly monitored to ensure they are being filled in and otherwise followed up by the coordinating researcher. In the event of patient withdrawal, an analysis of demographic and prognostic characteristics will be done on these cases and the remaining patients. As previously described by Järvinen et al, we will document the patient eligible for and compliant with each follow-up.[6]

6. Analysis

6.1. Outcome measures

Baseline characteristics

Baseline characteristics including age, gender, weight, length, van Dijk classification at inclusion, duration of ankle symptoms and previous ankle injury (ipsilateral ankle), ankle with OA (left or right), level of sports, weekly sport participation, Ankle ROM and anterior drawer test, will be collected for all participants.

Primary study parameter/endpoint

The primary objective of this study will be to quantify pain or functional improvement using the American Orthopaedic Foot and Ankle Society (AOFAS) score at 26 weeks follow-up. Studies

evaluating the efficacy of PRP in knee OA maintained a follow-up between 3 and 12 months. We therefore opted to take 26 weeks for our primary outcome measure.[8] The AOFAS is a validated scale for ankle OA (0-100) measuring three subdomains (pain, function and alignment) which together total nine items.[9–12] The subdomain of pain is measured by one item where a maximal score of 40 indicates no pain. Function consists of 7 items where full function is indicated by the maximal score of 50 points. Similar to the pain subdomain, alignment has a potential maximum score of 10 points using one item, indicating good alignment.[9,10] The AOFAS questionnaire, having undergone forward and backward translation to Dutch by de Boer et al. 2017, has an excellent internal consistency (Cronbach's α 0.947) and an excellent test-retest reliability (ICC 0.93).[9]

Secondary study parameters/endpoints

Secondary outcome measures are a number of other Patient Reported Outcome Measures (PROMs). Specific time points of the secondary outcome measures can be found in Table 1.

1. Ankle Osteoarthritis Score (AOS) is a visual analog scale from 0 – 100 mm with 18 questions; 9 relating to pain and 9 relating to disability.[13]
2. Foot and Ankle Outcome Score (FAOS). Each question is assigned 0 – 4 points based on the answer given. The scale runs from 0 (extreme symptoms) to 100 points (no symptoms).[14]
3. In order to evaluate pain, the pain sub-scale of AOFAS (0-40 points) will be analysed. On this scale the lower the score the more pain the patient has. Additionally a VAS score (VAS 0-100 mm) is measured during activities of daily living, with 0 mm being no pain and 100 mm the worst pain imaginable.[10,15]
4. Total AOFAS score at the other time points than the primary one (at 6, 12 and 52 weeks as well as 5 years).[10,15]
5. Ankle activity score (0-10 points) is scored according to chart based on the performable activity level.[16]
6. Subjective patient satisfaction (4 categories) Poor, Fair, Good, Excellent

7. Short Form Health Survey SF-36 is a health-related quality of life score (0-100 points). The higher the patient scores, the higher the disability.[17]
8. The Global Attainment Scaling (GAS) is a method of scoring based on achievement related to pre-determined goals in agreement with the patient. Points are subtracted for not achieving the pre-defined goals or vice versa. Scores range from 100 (high functioning) to 0 (severely impaired).[18]
9. EuroQol-5 dimensions-3 levels (EQ-5D-3L) utility score allows a patient's health to be defined by a 5-digit number.[19]
10. PROductivity and DISease Questionnaire (PRODISQ) will be used to determine indirect costs and direct costs cost effectivity. The PRODISQ questionnaire is taken at baseline and every 3 months thereafter up until 1 year. This will be done in conjunction with the EQ-5D-3L.[20]

6.2. Analysis method

Baseline characteristics

Baseline characteristics will be reported between groups using descriptive statistics.

Primary outcome measure

Analysis will be performed using an intention to treat approach. To test for the effect of treatment on the between-group difference in primary outcome, we will use a repeated measurement general linear model. Changes from baseline to all follow-up time points will be included in the model. Adjustments will be made for those baseline variables that influenced the primary outcome with $p < 0.10$.

Secondary outcome measures

To test for the effect of treatment on between-group differences in secondary outcomes, we will use the repeated measurement general linear model. Changes from baseline to all follow-up time points will be included in the model.

Economic analysis

In the event of a positive significant outcome in favour of the PRP group, an economic analysis is needed to support a possible change of practice. An economic analysis (costs) will be performed in order to determine cost-effectiveness.

We will assess the differences in mean quality-adjusted life years (QALYs), costs, and net benefits between the PRP injection group and the placebo group using linear models. We express the cost-effectiveness by using cost-effectiveness acceptability curves from both a healthcare perspective and a societal perspective. With multiple bootstrap replicates of the average difference in costs and effects in the incremental cost-effectiveness plane we will express the uncertainty of our cost-effectiveness analysis.

The cost-effectivity analysis will be performed with a 1-year time horizon. We use the three-level EQ-5D questionnaire (Euroqol, Rotterdam, the Netherlands) to calculate QALYs as the area under the curve of the utility scores measured over 12 months, according to the Dutch pricing system. The analysis will be based on indirect costs and direct costs and will be determined using the PRODISQ questionnaire. The PRODISQ questionnaire is taken at baseline and every 3 months thereafter up until 1 year.

6.3. Missing Data

Missing items of a score will be handled according to the instructions of the specific scales. In the event of no instructions, we will calculate the percentage of missing items on a scale. Due to the

potential impact on trial conclusions, multiple imputation (if >10% missing items on a scale) will be applied. Multiple imputation will be based on age, sex, allocation and earlier scores in the appropriate scale. Single imputation by last observation carried forward (LOCF) will be applied if the missing data is within 10 weeks of the last observation. Argumentation for application of LOCF will be presented descriptively. Little's Missing Completely at Random (MCAR) test will be performed in order to allow us to assume that the missing data is "missing at random" (MAR). Due to the potential impact on trial conclusions, a sensitivity analysis will be performed if missing data is more than 5%.

6.3. Statistical software

Analysis will be performed in IBM SPSS statistics for windows.

7. References to literature, standard operating procedures and reporting guidelines

7.1. Data Management Plan

The current data management plan is called: "RDM F01 Data Management Plan_version 1_01102018" Version 1; dated 1-10-2018 in the digital trial master file (G:\divb\orthopedie\orca\PRIMA-study\PRIMA Trial\16. Data Management\16.1 Forms and documentation).

7.2. Data storage

Following extraction from CASTORedc, the syntax files will be stored at the digital location G:\divb\orthopedie\orca\PRIMA-study\7.2. Standard Operating Procedure

The Standard Operating Procedure (SOP) will be followed when using and analysing the data.

File name: SOP RDM001 Research data management v3.0

Location: G:\divb\orthopedie\orca\PRIMA-study\PRIMA Trial\15. Monitoring-Audits

7.3. Reporting Guidelines

Results of the PRIMA trial will be presented in accordance with the CONsolidation Standards Of Reporting Trials (CONSORT) guidelines.

8. References

1. Paget LDA, Bierma-Zeinstra SMA, Goedegebuure S, et al. Platelet-Rich plasma Injection Management for Ankle osteoarthritis study (PRIMA): Protocol of a Dutch multicentre, stratified, block-randomised, double-blind, placebo-controlled trial. *BMJ Open*. 2019. doi:10.1136/bmjopen-2019-030961

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Statistical Analysis Plan

Platelet-Rich plasma Injection Management for Ankle osteoarthritis study (PRIMA): protocol of a Dutch multicentre, stratified, block-randomised, double-blind, placebo-controlled trial			
Dutch Trial Register	NTR7261 (registered 2018-06-06)	METC ABR	2018-042
SAP Version	3	SAP Version Date	26-11-2020

Role of Contributor	Name	Affiliation	Signature	Date of Signature
Principal Investigator	Prof. J.L. Tol	Orthopaedic Surgery Amsterdam UMC		30-11-2020
Researcher who will perform the statistical analysis	L.D.A. Paget	Orthopaedic Surgery Amsterdam UMC		30-11-2020
TRIAL STATISTICIAN	Prof. S.M.A. Bierma-Zeinstra	General Practice, Erasmus University Medical Center		18-12-2020
Contributor to Statistical Analysis Plan	G. Reurink	Orthopaedic Surgery Amsterdam UMC		30-11-2020
Contributor to Statistical Analysis Plan	R.J. de Vos	Orthopaedics and Sports Medicine, Erasmus University Medical Center		26-11-2020
Contributor to Statistical Analysis Plan	A. Weir	Orthopaedics and Sports Medicine, Erasmus University Medical Center		26-11-2020

1c. Revision History of the statistical analysis plan

Update statistical analysis plan version	Protocol version	Section number(s) changed	Description of and reason for changes	Date of approval
1.0	4	0	0	31-01-2020
2.0	5	3.8 6.3	Amendment due to COVID-19 pandemic	22-06-2020
3.0	5	6.1	Additional radiological baselines variables to check as potential confounders	26-11-2020

1.2. Planned period of observation

The study included its first patient in August 2018 and aims to include the last patient by March 2020, consequently allowing analysis and then de-blinding of the coordinating researcher, principle investigator and fellow project members, to commence after the last follow-up (26 weeks) of the last patient by September 2020.

2. Introduction

2.1. Background and rationale

Platelet-rich Plasma (PRP) is a potentially efficacious treatment for ankle OA but its use has not been examined in high quality studies. Systematic reviews show that platelet-rich plasma (PRP) injections significantly decrease pain and improve function in knee OA patients. Ankle OA is more common than hip or knee OA in the young active population; with a prevalence of 3.4%. PRP injections in ankle OA are shown to be safe and improve quality of life over time, but no randomised controlled trial has been conducted. Our randomised controlled trial will evaluate the efficacy of PRP injections

for symptom reduction and functional improvement, compared to placebo, in the treatment of ankle (talocrural) OA. The PRIMA trial is registered in the Netherlands trial Register: NTR7261 and its protocol has been published.[1]

2.2. Study Objectives

We aim to determine the efficacy of PRP injections in the management of ankle OA by comparing 2 groups, both receiving 2 injections of either: PRP or placebo solution. We hypothesize that PRP injections are efficacious for symptom reduction and functional improvement compared to placebo in the treatment of ankle (talocrural) OA.

3. Study Methods

3.1. Study Design

The PRIMA study is a multi-center, stratified, block-randomized, double-blind, placebo-controlled trial design will be conducted in order to compare two treatment groups: PRP vs Placebo (saline). After the 26 weeks follow-up of the last patient in the study, the coordinating researcher, principal investigator and fellow project members will be unblinded only after the analysis of the primary outcome. A flow chart of the design and follow-up is shown in figure 1.

3.2. Randomisation, Blinding and Treatment Allocation

In this study, patients will be randomised into two treatment groups: PRP injection or placebo saline injection. For each patient the coordinating researcher will prepare a PRP and a placebo injection (isotonic saline: 0.9% sodium chloride).

We will include patients at the centre of their first outpatient clinic appointment. For each patient the coordinating researcher will prepare a syringe with PRP and a syringe with placebo (isotonic saline: 0.9% sodium chloride). A Good Clinical Practice (GCP) approved data management system (Castor EDC, based in Amsterdam, the Netherlands) will be used to perform a computer generated block randomisation scheme with patients stratified to centre with a variable block size of 2,4 or 6. This procedure will ensure treatment allocation concealment. The coordinating researcher, treating

physician and patient all remain blinded to the allocated intervention. An independent researcher from the coordinating location will have access to the randomization result and the allocated intervention. This will be relayed to a GCP-certified research assistant at the centre. The research assistant then selects one of the two syringes based on the allocated intervention and blinds the syringe with a covering sheath, ensuring concealment of the content of the syringe. The patients, treating physicians, and coordinating researcher will all be blinded to the allocation of the intervention and to the contents of the syringe. The success of blinding will be assessed by asking patients which injection they think they have received just after the injection procedure, this will then be registered accordingly.

3.3. Sample Size

Based on previous and ongoing studies, the study protocol of the randomised controlled trial is designed to detect a difference of 12 points (0-100) on the AOFAS score. There is no official agreement on the minimal clinical important difference for the AOFAS score regarding ankle OA. However in relatable musculoskeletal literature, 10% – 15% of the used scale was reported. [2–4] Our pre-defined minimal clinical important difference of 12 % is located within this range.[2–4] Based on a previous placebo controlled RCT on injection therapy (hyaluronic acid) in ankle OA of De Groot et al. a standard deviation of 16.3 can be expected.[5] Taking into account a two-sided level of significance of 5%, a power of 90% and a dropout rate of 10%, approximately 50 (45 plus 10% drop out) patients per group will be needed (N=100 in total).

3.4. Hypothesis testing framework

The PRIMA trial uses a superiority hypothesis testing framework for all primary and secondary outcomes.

3.5. Interim Analysis

No interim analyses will be performed as the study has been classified as low-risk. All adverse events reported spontaneously by the patient or observed by the investigator or his staff will be recorded. A variety of conditions have been treated with PRP ranging from muscle and tendon injuries to intra-articular injections of the knee and ankle. To date, no serious adverse events have been documented in the literature, concerning PRP intra-articular injections of the ankle. In accordance with the Central Committee on Research Involving Human Subjects (CCMO) guidelines, this study was classified as low-risk for adverse events. Therefore, the local Medical Ethical Commission will be notified of any serious adverse events. In the event this happens, the advice of the Medical Ethical Commission will be followed accordingly.

3.6. Start statistical analysis of data

The current estimate is that the final patient will be included in the study in March 2020. We therefore expect to perform the statistical analysis for both primary and secondary outcomes at 26 weeks follow-up in September 2020.

3.7. Time points

Table 1. Follow-up	
Baseline	- 1st intervention injections - Physical Examination - AOFAS - PROMs - PRODISQ cost-effectivity

6 weeks	- 2nd intervention injection - Physical Examination - AOFAS - PROMs
12 weeks	- AOFAS - PROMs - PRODISQ cost-effectivity
26 weeks	- Physical Examination - AOFAS - PROMs - PRODISQ cost-effectivity
39 weeks	- PRODISQ cost-effectivity
52 weeks	- AOFAS - PROMs - PRODISQ cost-effectivity
5 years	- AOFAS - PROMs

Table 1. In addition to the American Orthopaedic Foot and Ankle Society (AOFAS) score, the following PROMs will be taken: Foot and Ankle Outcome Score (FAOS), Ankle Osteoarthritis Score (AOS), Visual Analogue Scale (VAS), Ankle activity score (AAS), Subjective patient satisfaction, Short Form Health Survey (SF-36), The Global Attainment Scaling (GAS), EuroQol-5 dimensions-3 levels (EQ-5D-3L). These PROMs will be elaborated on further on. Furthermore the PROductivity and DISease Questionnaire (PRODISQ) will be used to perform a cost-effectivity analysis. These questionnaires can be found in appendix 1

3.8. Amendment due to COVID-19 pandemic

To prevent potential immediate hazard to the patients and in compliance with the institutional and national Covid-19 -related clinical research regulations, we deviated from the protocol and replaced patients following Institutional Review Board (IRB) (in Dutch: Medisch Ethische Toetsingscommissie) approval date 6-5-2020.[6,7]

During the COVID-19 pandemic, 12 received their first intervention (intra-articular injection), but these patients had no access to receiving their second injection at the pre-defined 6 week time-interval. Following consultation with the head of the department and/or local principal investigators, considering the risks and descaling of elective patient bound activities, we found the COVID-19 associated potential risks to outweigh the potential damage due to the disease for which they had no access to the intervention.

As in-person visits are required for administration of the investigational products (intra-articular injections), protection of a participant's safety, welfare, and rights is best served by discontinuing the administration or use of the investigational product and subsequent participation in the trial.(1,2) In order to minimise protocol deviations, maintain the previously calculated sample size of 100 patients, we asked IRB approval for replacing them with 12 new inclusions. Following IRB approval we will started including patients as soon as (1) out-patient non-COVID-19 care was scaled up, (2) there was approval from the local head of the department and/or local principal investigator and (3) the local regulations permitted. By doing this, we accounted for the potential risks for patients and documented this accordingly in an emergency risk management plan. At the start of the trial we did not anticipate that a substantial number of patients who were randomised into the trial would subsequently be unable to undergo the intervention (due to the Covid-19 crisis). By asking the IRB approval for replacing these patients that had no access to the intervention, due to COVID-19 regulations, we minimize potential bias, as:

• Allocation to the treatment or control arm will not have influenced the discontinuation of trial participation (internal validity).

• We will follow the similar recruitment procedure of consecutive patients after the COVID-19 ban has been stopped and therefore the newly included patients are expected to be representative for the same population as the patients for whom the trial participation has been discontinued (external validity).

Participation of these 12 patients will be discontinued and they will be informed by written letter, e-mail and/or telephone call. The monitoring body will be informed within 48 hours following IRB approval.

The Sponsor and clinical investigators will document how restrictions related to COVID-19 led to the changes in study conduct and duration of those changes and indicate which and how trial patients will be impacted. We will capture specific information in the case report form that explains the basis of potential missing data, including the relationship to COVID-19 for missing protocol-specified information (e.g., from missed study visits or study discontinuations due to COVID-19). This information, will be summarized in the clinical study report.

The proposed IRB amendment (submitted on 14-4-2020, approved 6-5-2020) with changes in the protocol will be updated in the data management and/or statistical analysis plan amendments. Prior to locking the database, we will address in the statistical analysis plan how protocol deviations related to COVID-19 will be handled for the pre-specified analyses.

In these extreme circumstances, we are confronted with a crisis and are forced to think of solutions in order to maintain the quality of the study. The European Committee for Human Medicinal Products, recommend collection of as much data as possible. In the current situation we find the trial load for patients no longer participating too heavy and thus unethical. Consequently, we will

limit data collection in these patients to the primary outcome measure, AOFAS at 26 weeks (1x 10 min by videoconsult).

4. Statistical Principles

A statistical significant difference between both treatment groups (placebo or PRP), regarding primary and secondary outcomes, will be determined if the two sided p-values are less than 0.05. A 95% confidence interval will be provided for primary and secondary outcome measures. No adjustment will be made for multiplicity as there is only one primary outcome measured at a single time point. Protocol deviations will be listed according to treatment group. This will also be presented as a percentage and number of patients in each treatment group having experienced a protocol deviation. The intention to treat population includes all participants randomized, regardless of protocol deviation.

After the 26 weeks follow-up of the last patient in the study, a standard operating procedure will be available to logically recode and clean the data. The data will be interpreted according to a blinded data interpretation scheme described by Järvinen et al.[8] A statistical expert (SB) is present among the authors. The authors will interpret the statistical results until a consensus is reached. Once the authors are in agreement, the two groups will be unblinded and no changes will be made to the interpretation of the results. Thus the principal investigator, coordinating researcher and other project members will be unblinded only after the analysis of the primary outcome. Patients will be unblinded 1 year after the 1 year follow-up of the last patient.

5. Study Populations

5.1. Inclusion and exclusion criteria

Patients with ankle OA in University Medical Centres, teaching hospitals, general hospitals and private specialist clinics will be informed about the study. In order to participate, patients must meet the eligibility criteria documented below.

Inclusion criteria

1. Severity of Ankle OA pain on a visual analogue scale (VAS) (0–100 mm) ≥ 40 mm during daily activities
2. X-rays (anteroposterior (AP) and lateral view) indicating ≥ grade 2 talocrural OA on the Van Dijk classification (clarified under section Radiographs)[9]
3. Age ≥ 18 years

Exclusion criteria

1. Patient has received injection therapy for ankle OA in the previous 6 months
2. Patient does not want to receive one of the two therapies
3. Patient has clinical signs of concomitant OA of one or more other major joints of the lower extremities that negatively affects their daily activity level
4. Previous ankle surgery for OA or Osteochondral defects (OCD) < 1 year (not including surgery for an ankle fracture in the past)

Radiographs

AP and lateral X-rays of the talocrural joints will be scored according to the Van Dijk classification:[9]

0) Normal joint or subchondral sclerosis
1) Osteophytes without joint space narrowing

2) Joint space narrowing with or without osteophytes

3) (Sub)total disappearance or deformation of the joint space

5.2. Planned information for flowchart

A flowchart will present the patients that were screened, met the inclusion criteria, were excluded, randomised and allocated to each study arm, withdrawing from the study (with reason and timing) and assessed for primary outcome.

5.3. Loss to follow-up

The coordinating researcher will attempt to limit loss to follow-up as much as possible by contacting every patient and being present at every patient visit. All digital questionnaires will be constantly monitored to ensure they are being filled in and otherwise followed up by the coordinating researcher. In the event of patient withdrawal, an analysis of demographic and prognostic characteristics will be done on these cases and the remaining patients. As previously described by Järvinen et al, we will document the patient eligible for and compliant with each follow-up.[8]

6. Analysis

6.1. Outcome measures

Baseline characteristics

Baseline characteristics including age, gender, weight, length, whether x-ray is weighted or non-weighted[10], Medial distal tibial angle,[11–13] talar tilt,[11–13] van Dijk classification,[9] Kellgren-Lawrence classification[14] and the Takakura classification[15,16] at inclusion, duration of ankle symptoms and

previous ankle injury (ipsilateral ankle), ankle with OA (left or right), level of sports, weekly sport participation, Ankle ROM and anterior drawer test, will be collected for all participants.

Primary study parameter/endpoint

The primary objective of this study will be to quantify pain or functional improvement using the American Orthopaedic Foot and Ankle Society (AOFAS) score at 26 weeks follow-up. Studies evaluating the efficacy of PRP in knee OA maintained a follow-up between 3 and 12 months. We therefore opted to take 26 weeks for our primary outcome measure.[17] The AOFAS is a validated scale for ankle OA (0-100) measuring three subdomains (pain, function and alignment) which together total nine items.[18–21] The subdomain of pain is measured by one item where a maximal score of 40 indicates no pain. Function consists of 7 items where full function is indicated by the maximal score of 50 points. Similar to the pain subdomain, alignment has a potential maximum score of 10 points using one item, indicating good alignment.[18,19] The AOFAS questionnaire, having undergone forward and backward translation to Dutch by de Boer et al. 2017, has an excellent internal consistency (Cronbach's α 0.947) and an excellent test-retest reliability (ICC 0.93).[18]

Secondary study parameters/endpoints

Secondary outcome measures are a number of other Patient Reported Outcome Measures (PROMs). Specific time points of the secondary outcome measures can be found in Table 1.

1. Ankle Osteoarthritis Score (AOS) is a visual analog scale from 0 – 100 mm with 18 questions; 9 relating to pain and 9 relating to disability.[22]
2. Foot and Ankle Outcome Score (FAOS). Each question is assigned 0 – 4 points based on the answer given. The scale runs from 0 (extreme symptoms) to 100 points (no symptoms).[23]
3. In order to evaluate pain, the pain sub-scale of AOFAS (0-40 points) will be analysed. On this scale the lower the score the more pain the patient has. Additionally a VAS score (VAS 0-100 mm) is measured during activities of daily living, with 0 mm being no pain and 100 mm the worst pain imaginable.[19,24]

4. Total AOFAS score at the other time points than the primary one (at 6, 12 and 52 weeks as well as 5 years).[19,24]
5. Ankle activity score (0-10 points) is scored according to chart based on the performable activity level.[25]
6. Subjective patient satisfaction (4 categories) Poor, Fair, Good, Excellent
7. Short Form Health Survey SF-36 is a health-related quality of life score (0-100 points). The higher the patient scores, the higher the disability.[26]
8. The Global Attainment Scaling (GAS) is a method of scoring based on achievement related to pre-determined goals in agreement with the patient. Points are subtracted for not achieving the pre-defined goals or vice versa. Scores range from 100 (high functioning) to 0 (severely impaired).[27]
9. EuroQol-5 dimensions-3 levels (EQ-5D-3L) utility score allows a patient's health to be defined by a 5-digit number.[28]
10. PROductivity and DISease Questionnaire (PRODISQ) will be used to determine indirect costs and direct costs cost effectivity. The PRODISQ questionnaire is taken at baseline and every 3 months thereafter up until 1 year. This will be done in conjunction with the EQ-5D-3L.[29]

6.2. Analysis method

Baseline characteristics

Baseline characteristics will be reported between groups using descriptive statistics.

Primary outcome measure

Analysis will be performed using an intention to treat approach. To test for the effect of treatment on the between-group difference in primary outcome, we will use a repeated measurement general linear model. Changes from baseline to all follow-up time points will be included in the model.

Adjustments will be made for those baseline variables that influenced the primary outcome with $p < 0.10$.

Secondary outcome measures

To test for the effect of treatment on between-group differences in secondary outcomes, we will use the repeated measurement general linear model. Changes from baseline to all follow-up time points will be included in the model.

Economic analysis

In the event of a positive significant outcome in favour of the PRP group, an economic analysis is needed to support a possible change of practice. An economic analysis (costs) will be performed in order to determine cost-effectiveness.

We will assess the differences in mean quality-adjusted life years (QALYs), costs, and net benefits between the PRP injection group and the placebo group using linear models. We express the cost-effectiveness by using cost-effectiveness acceptability curves from both a healthcare perspective and a societal perspective. With multiple bootstrap replicates of the average difference in costs and effects in the incremental cost-effectiveness plane we will express the uncertainty of our cost-effectiveness analysis.

The cost-effectivity analysis will be performed with a 1-year time horizon. We use the three-level EQ-5D questionnaire (Euroqol, Rotterdam, the Netherlands) to calculate QALYs as the area under the curve of the utility scores measured over 12 months, according to the Dutch pricing system. The analysis will be based on indirect costs and direct costs and will be determined using the PRODISQ questionnaire. The PRODISQ questionnaire is taken at baseline and every 3 months thereafter up until 1 year.

6.3. Data of excluded patients due to COVID-19

We are of opinion that in the current situation the trial load for patients no longer participating is too heavy and thus unethical. Therefore, in these patients, data collection will be limited to the

primary outcome measure, AOFAS at 26 weeks (1x 10 min by videoconsult). Baseline data, AOFAS at 26 weeks and any other data acquired while the verdict of the IRB was awaited will be presented descriptively. Data of the 12 new inclusions will be analysed according to protocol, as if they belonged to the original 100 inclusions.

6.4. Missing Data

Missing items of a score will be handled according to the instructions of the specific scales. In the event of no instructions, we will calculate the percentage of missing items on a scale. Due to the potential impact on trial conclusions, multiple imputation (if >10% missing items on a scale) will be applied. Multiple imputation will be based on age, sex, allocation and earlier scores in the appropriate scale. Single imputation by last observation carried forward (LOCF) will be applied if the missing data is within 10 weeks of the last observation. Argumentation for application of LOCF will be presented descriptively. Little's Missing Completely at Random (MCAR) test will be performed in order to allow us to assume that the missing data is "missing at random" (MAR). Due to the potential impact on trial conclusions, a sensitivity analysis will be performed if missing data is more than 5%.

6.5. Statistical software

Analysis will be performed in IBM SPSS statistics for windows.

7. References to literature, standard operating procedures and reporting guidelines

7.1. Data Management Plan

The current data management plan is called: “RDM F01 Data Management Plan_version 1_01102018” Version 1; dated 1-10-2018 in the digital trial master file (G:\divb\orthopedie\orca\PRIMA-study\PRIMA Trial\16. Data Management\16.1 Forms and documentation).

7.2. Data storage

Following extraction from CASTORedc, the syntax files will be stored at the digital location G:\divb\orthopedie\orca\PRIMA-study\7.2. Standard Operating Procedure

The Standard Operating Procedure (SOP) will be followed when using and analysing the data.

File name: SOP RDM001 Research data management v3.0

Location: G:\divb\orthopedie\orca\PRIMA-study\PRIMA Trial\15. Monitoring-Audits

7.3. Reporting Guidelines

Results of the PRIMA trial will be presented in accordance with the CONsolidation Standards Of Reporting Trials (CONSORT) guidelines.

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Summary of Changes Statistical Analysis Plan

Update statistical analysis plan version	Protocol version	Section number(s) changed	Description of and reason for changes (highlighted in version 3.0 in yellow)
1.0	4	0	0
2.0	5	3.8 6.3	Amendment due to COVID-19 pandemic: During the COVID-19 pandemic, 12 patients received their first intervention (intra-articular injection), but these patients had no access to receiving their second injection at the pre-defined 6 week time interval. Participation of these 12 patients was discontinued and they were replaced with 12 new inclusions.
3.0	5	6.1	Additional radiological baselines variables (whether x-ray is weighted or nonweighted, Medial distal tibial angle, talar tilt, van Dijk classification, Kellgren-Lawrence classification and the Takakura classification at inclusion) to weigh as potential confounders