Title: Effect of Platelet Rich Plasma Injections on Inflammatory and Chondrodegenerative Biomarkers in Patients with Acute Anterior Cruciate Ligament Tears

Principal Investigator: Adam Anz, M.D.

Co-Investigators: Steve Jordan, M.D.

Brett Kindle, M.D.

Roger Ostrander, M.D.

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Institution(s): Andrews Institute
1040 Gulf Breeze Pkwy.
Gulf Breeze, FL 32561
(850) 916- 8570

1 Background / Scientific Rationale

In the United States, 80,000 anterior cruciate ligament (ACL) ruptures are estimated to occur annually, with an increased likelihood of these injuries involving individuals participating in sports and aged between 15-25 years [7]. A potential long-term consequence of ACL injuries is the development of posttraumatic osteoarthritis (PTOA) in the years following injury [8,9]. There are no curative treatments for osteoarthritis, increasing the importance of minimizing the occurrence of PTOA following ACL injuries. Current literature has begun to indicate that biochemical changes in the knee joint cartilage, such as chondrocyte death, following injury can contribute to the development of PTOA [10-12].

A non-surgical treatment option for the management of osteoarthritis include injectables such as corticosteroids and platelet rich plasma (PRP). These injectables work by positively affecting cartilage cells, also known as chondrocytes, and the cells of the joint lining tissue, also known as synoviocytes. PRP is an autologous derived blood product, i.e. a joint injectable made from the patient's own blood at the time and location of injection with simple blood centrifugation. Studies in the bench-top laboratory setting have provided in-vitro evidence that PRP decreases synoviocyte production of metallometal proteases, an inflammatory protein with negative effects on cartilage, decreases the effects of inflammatory proteins such as IL-B on chondrocytes, and has an anti-inflammatory effect upon the gene expression of chondrocytes and synoviocytes[14-16]. While these are mechanisms that can help manage osteoarthritis after it arises, they also are mechanisms that could help prevent osteoarthritis before it forms. Recent comparative studies of PRP to other injectables in the management of osteoarthritis suggest a greater, long-lasting effect of PRP than other injectables [1-6].

Orthopedic research has focused on methods to prevent PTOA after ACL injury. Recent study involving the post-injury ACL joint milieu suggested that an early intervention of joint aspiration and corticosteroid injection has positive effects, including a decrease in biomarkers responsible for chondral degeneration [13]. This combined with in vitro study on the effects of PRP on chondrocyte and synoviocyte activity suggest that PRP has potential to affect the ACL-injured joint. However, current literature is lacking in regard to this assertion, and in vivo study of PRP to positively affect the joint after following ACL injury is warranted.

2 Objectives

The main objective of this study is to determine if an early intervention of joint aspiration and PRP injection will positively affect the biomarkers representative of chondral degeneration in patients with ACL injuries. We hypothesize that the intervention will reduce the volume of inflammatory and chondrodegenerative biomarkers following ACL injury.

3 Participant Eligibility

Inclusion Criteria: Patients between the ages of 14 and 40 years with closed growth plates as visualized on plain radiography, no history of previous traumatic ipsilateral knee injury, and no

clinical evidence of posterior cruciate ligament injury with no more than grade 1 medial or lateral collateral ligament injury.

Exclusion criteria: Patients without a palpable knee effusion, an injury occurring more than 10 days before enrollment, previous ipsilateral knee surgery, intra-articular cortisone or PRP injection into either knee within 3 months of injury, participation in another clinical drug trial within the 4 weeks before injury, and a history of any inflammatory disease or immune-comprised. Any patient who will have difficulty obtaining internet access, does not have an active e-mail address, or is unable to comprehend study documents or give informed consent will be excluded.

4 Participant Enrollment

60 patients will be recruited through the Andrews Institute physician practices. Potential participants will be prescreened for inclusion and exclusion criteria through standard of care medical evaluations. Once a potential participant has agreed to be involved in the study, they will go through the described informed consent process. Patients meeting the inclusion criteria will have the study explained to them by one of the members of the investigating team, and they will be given an opportunity to participate if they are interested. No specific advertising or recruitment material will be utilized.

The participants will then be randomly placed in one of the two study groups and be scheduled to receive either an injection of PRP or no injection in their involved knee.

Participants will not be billed for the joint aspirations or PRP injections.

5 Study Design and Procedures

Study design will be randomized control trial. Participants who meet the inclusion criteria will have the study explained in detail and informed consent will be obtained as outlined above.

Patients will be randomized to 1 of 2 groups. Both groups will have joint aspirations performed at an initial visit, within the first 10 days after injury, and at the time of surgery, within 4 weeks of injury. Group 1 will receive only joint aspirations as outlined above. Group 2 will undergo aspirations as outlined above and also receive a PRP injection at the initial visit and at a second visit 5-12 days after the initial visit. Joint aspirations and injections will be performed through the superolateral, suprapatellar approach Patients will be instructed not to take any prescription or over-the-counter nonsteroidal anti-inflammatory medications. Patients will be encouraged to rest, ice, and elevate their injured knee.

PRP Manufacturing: PRP preparation will involve Autologous Conditioned Plasma disposables (Arthrex, Naples, FL, USA), a commercially available kit for the production of PRP. Preparation will follow the manufacturer's instructions. The treating physician will then inject the PRP into the involved knee joint following standard aseptic technique per the physician's standard of care. This entire process will be completed in a single patient visit. A 0.1-0.5 mL sample of the PRP will be inserted into a vacutainer for analysis of cell count. Any unused portion of a sample in the physician's office will be disposed of through standard biohazard waste disposal systems as required by law.

Biomarker Analysis: Samples will be prepared and frozen at -80° C until all study sample have been obtained. Enzyme-linked immunosorbent assay kits will be used to assess chondrodegenerative & synovial fluid biomarkers.

6 Expected Risks and Benefits

Risks and Discomforts: Potential risks include those expected with any injection including syncope, dizziness, headache, nausea, tachycardia, infection (septic arthritis, phlebitis, and osteomyelitis), bleeding, or pain. Additionally, participants may experience knee stiffness or inflammation associated with the PRP injection. Knee aspiration and injection are standard medical procedures used routinely in the management of knee osteoarthritis and acute injury. As with any research involving patients there is the inherent risk of a breach in patient confidentiality though this will be minimized through the use of participant code numbers and adherence to all HIPAA guidelines.

Benefits: Direct benefits of the study include potential pain relief, improvement of knee function from joint aspiration, and the potential to decrease the risk of PTOA after ACL injury. It is also believed that the information obtained in this study will help advance treatment of PTOA through the use of regenerative medicine.

7 Data Management Procedures

All personal information is strictly confidential and no names will be disclosed except as required by law. All information and data collected during this research will be recorded in REDCap and exported into a spreadsheet. This spreadsheet will not contain protected health information. The spreadsheet will be stored in a secure password protected folder on a laptop that only the study Investigators will have access to and will be permanently deleted following publication of any and all manuscripts, if any, written as a result of this research. Records related to this study will be securely retained in a secure location for a period of 3 years after the

completion of the study or longer as required by law. At that time, all records will be properly destroyed.

8 Data Analysis

This study is anticipated to take 2 years to complete. All data will be entered into REDCap. The investigators will meet at appropriate intervals to evaluate and analyze the data.

9 Statistical Considerations

A power analysis was performed to determine sample size based upon recent clinical trials. Analysis utilized the average of standard deviation to detect average of group mean effect at 80% power. This determined that 25 patients per group would be needed to reach statistical significance. 10 additional patients will be account for potential patient withdrawal or lost-to-follow-up.

Ranges, means and standard deviations for all measures will be determined and calculated. Data will be analyzed for differences between groups using a t-test design, using a significant p-value of < 0.05 for rejecting the null hypothesis.

10 Quality Control and Assurance

All protocols will be monitored and analyzed data will be checked for accuracy by the principal investigator and /or a designated AREF research team member. All medical data will be kept in compliance with HIPAA guidelines.

11 Regulatory Requirements

Informed Consent:

The informed consent process will be performed by one of the study investigators or staff, in the office. All participants will have the study described to them and will give as much time as they require to read an approved, stamped version of the informed consent document. After signing of the informed consent document, participants will be given a copy for their records. This process will take place only after the patient has consented to proceed with the study.

Participant Confidentiality:

Participant confidentiality information is listed above in #7 (Data Management Procedures). All medical data will be recorded and stored in compliance with HIPAA guidelines.

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