Title: A Single Blind, Randomized Controlled Trial Evaluating Platelet Poor Plasma Versus Corticosteroid Injections as A Control for Thigh Muscle Injuries

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1. Abbreviations

- BAMIC – British Athletics Muscle Injury Classification
- CBC – Complete Blood Count
- CTCAE – Common Terminology for Adverse Events
- EGF – Epithelial Growth Factor
- FGF – Fibroblast Growth Factor
- IGF – Insulin-like Growth Factor
- IGF-1 – Insulin-like Growth Factor 1
- IGF-2 – Insulin-like Growth Factor 2
- IKDC - Subjective International Knee Documentation Committee
- IL-1β – Interleukin-1 Beta
- IL-6 – Interleukin-6
- IL-10 – Interleukin-10
- IL-12 – Interleukin-12
- LDU – Long Duration Ultrasound
- L-PRF - Leukocyte-and Platelet-Rich Fibrin
- L-PRP - Leukocyte-and Platelet-Rich Plasma
- MiLB – Minor League Baseball
- MLB – Major League Baseball
- MRI – Magnetic Resonance Imaging
- NCAA – National Collegiate Athletic Association
- PAW – Platelets, Activation, White blood cells
- PDGF - Platelet-Derived Growth Factor
- PPP – Platelet-Poor Plasma
- P-PRF - Pure Platelet-Rich Fibrin
- P-PRP - Pure Platelet-Rich Plasma
- PROMIS - Patient-Reported Outcomes Measurement Information System
- PRP - Platelet-Rich Plasma
- RICE – Rest, Ice, Compression, Elevation
- TGF-β - Transforming Growth Factor- Beta
- TNF-α – Tumor Necrosis Factor-Alpha
- TRAP– Thrombin Receptor Activating Peptide
- VAS – Visual Analog Scale

- VEGF - Vascular Endothelial Growth Factor

2. Background

Platelet-Rich Plasma

An autologous orthobiologic therapy showing promise for the recovery of thigh muscle and tendon injuries is Platelet-Rich Plasma (PRP). PRP is formally defined as plasma consisting of supraphysiologic concentrations of platelets that may range from 3 to 6 times greater than the physiologic concentration.[1,5] That is, the concentration of thrombocytes found within the plasma are higher in proportion than they would be if studied in normal blood under general conditions.

The reason why PRP has been extensively studied is due to its high concentration of growth factors.[40] Growth factors are proteins that produce a certain action based on their binding to receptors on the surface of specific target cells.[31] Among these growth factors, are platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 2 (IGF-2). It should be noted that the intensity of the action and effects thereof can be based on the concentration of the proteins, as well as the sensitivity of the binding receptors.[31] Studies have supported the notion that PRP is effective in assisting with wound healing, bone and soft tissue repair and regeneration, and survival of fat grafts in soft tissue augmentation.[4,30,31,40,43,44] *In vitro* PRP applications have proven to increase cell proliferation, increase the differentiation of satellite cells, as well as increase factors necessary for the formation of new blood vessels.[31] Moreover, it has been clinically proven that PRP as a whole has aided in the reduction of swelling and pain in muscle injuries.[31]

Pertaining specifically to orthopedics and sports medicine, PRP has also been studied as a means of decreasing the rate of injury recurrence. Unfortunately, while some studies have suggested a decrease in recurrence of muscle strains, some studies suggest that PRP injections may actually increase the risk of recurrence due to the higher potential of initiating the formation of fibrotic tissue in skeletal muscle.[4,5,31]

Platelet-Poor Plasma

With the inconclusive results of PRP for acute muscle injuries, the alternative of platelet poor plasma (PPP) is gaining attention within orthobiologics.[1] PPP is defined as plasma

consisting of infraphysiologic concentrations of platelets. In other words, the platelet concentration levels found in PPP are significantly lower than that of whole blood.[1,43,46] Although PPP has been viewed as a waste product stemming from obtaining PRP from anticoagulated blood, researchers have discovered that PPP can be used for considerable physiological benefits.[1,46,52]

Although it is still in its early stages of understanding, such activation potentials have been attributed to PPP's content of bioactive molecules. This reservoir has shown an extensive impact within the future of orthopedics. So far, PPP has been identified as evoking biological responses in key healing processes such as promoting bone maturation and bone formation, regeneration of mucosa, tenocyte proliferation, and collagen production.[43,46] As it relates to muscle, clinical findings have shown that PPP is a contributing factor toward the progression of myogenesis (formation of skeletal muscle).[46,73] Specifically, studies have shown that PPP reduces the rate in which myoblasts proliferate but significantly induces differentiation of myoblasts into the muscle development pathway and formation of myotubes.[1,46] The importance of this function is highlighted in the end products of proliferation versus differentiation. With myoblast proliferation, the end results are more myoblasts that do not necessarily have to go down the pathway of myogenesis. However, with myoblast differentiation, they are included in the pathway which either produces myotubes as single products, or myotubes that continue on to become multinucleated muscle cells.[1]

Return to Play and Pain

While all of these results based around molecular biology have helped to shape our understanding of PPP, there are very limited studies that include the aspect of return to play.[1,76] One case report that was found showed that a PPP injection under the guidance of ultrasound did reduce the time to return to play in a NCAA Division 1 football placekicker that suffered an acute quadriceps grade 2 strain.[77,78] This evidence regarding thigh injuries is supported by a case presented of a collegiate baseball player that was found to have a grade 2 strain of the biceps femoris. With a PPP injection into the long head of the biceps femoris muscle, the return to play time was reduced in comparison to the average MLB/MiLB duration for such an injury.[77]

PRP on the other hand, has had several studies prove or disprove its efficacy regarding return to play. A key point mentioned surrounding PRP is the importance of dosage. This stems from the conclusion that, when compared to rehabilitation alone, a single PRP injection combined with an intensive rehabilitation regimen does not provide a reduction in the return to

play duration.[2] Another important consideration highlighted in the study of PRP for return to play is ultrasound guidance. Rossi et al. found that with ultrasound guidance, a single injection of autologous PRP in combination with rehabilitation does reduce the amount of time it takes to return to play from an acute grade 2 muscle injury of the lower extremity.[4,5,74] This statement is supported by Hamid et al., in which twenty-eight patients with grade 2 hamstring muscle injuries were randomized to either follow a rehabilitation program or receive a single PRP injection under ultrasound guidance in combination with a rehabilitation program.[4,5,75]

When directly comparing the efficacies of both PPP and PRP for return to play, the number of published findings is minimal. Coupled with standardized rehabilitation, in a study that carried out direct comparison between PRP injections, PPP injections, and no injection for MRI-positive hamstring injuries, Hamilton et al. found that the PRP injection group had the lowest return to play time of the three groups.[5] Kruse et al. added a contrast to this finding when they published the largest dataset currently available comparing PRP and PPP.[77] Within their study of one hundred athletes with acute thigh muscle injuries, it was found that PPP had a faster return to play time than PRP.[1,77] One noticeable factor observed within their study that has not been mentioned prior is post-procedural pain. The pain post-procedure was lower in the PPP injection group. Also, the PPP injection group also had the shortest duration of pain post-procedure compared to the PRP injection group.[1,77] However, there was no statistical significance found between the groups as it related to injury recurrence up to one-year post-procedure.[1]

It is important to note that there have been other considerations for injection therapy within the arena of musculoskeletal conditions, namely that of controlled injections such as corticosteroid injections, local anesthetics, and whole blood. Some of the common lower extremity sites of injection for local anesthetics include the knee, hamstring and ankle. However, these injection sites are deemed largely unsafe.[9] Prior to the recent innovations within orthobiologics, corticosteroid injections have been traditionally used to treat pain and the inflammation associated with musculoskeletal injuries.[6,7,86] For professional athletes, both corticosteroids and local anesthetics have been regulated as safe to use by anti-doping agencies.[9,80] However, some argue that local anesthetics have the potential to be “performance-enhancing”.[80]

Another part of the aversion from corticosteroids is the possibility of complications. One study from Fernandes et al. found that such injections were linked to the risks of knee discomfort or knee replacement surgery.[9] Levine et al. highlighted the possibility of incomplete healing and possible rupture in those with hamstring injuries.[86] However, UK practices have found other side effects for corticosteroid injections such as septic arthritis, tendon rupture, steroid-induced

arthropathy, and dermal/subcutaneous tissue atrophy.[6] As it relates to professional athletes returning to play, these findings prove inconclusive. Levine et al. presented that corticosteroid injections allowed 58 professional football players to avoid reinjury, as well as return to their previous strength and activity levels after suffering a hamstring injury.[86]

The last type of injection alternative highlighted has been autologous whole blood. In a study observing chronic lateral elbow epicondylitis, it was found that PRP significantly improved the VAS pain score post-injection compared to autologous whole blood.[81,83] However, this does not negate the fact that significant pain and improvement of function can also be observed in the patients that receive autologous whole blood for the condition.[82] When compared to corticosteroid injections, autologous whole blood has been shown to have greater pain reduction long-term.[82] One hypothesis regarding these outcomes is linked to the concentration of platelets.[81-83] Corticosteroid injections typically do not contain platelets, autologous whole blood does contain platelets, and PRP contains the highest concentration of platelets of the three groups.[81-83]

3. Objectives

3.1. Primary Objective

The primary objective of this study is to compare the duration of days it takes to return to play between ultrasound guided intramuscular corticosteroid injections compared to PPP injections for thigh muscle injuries.

3.2. Primary Endpoints

The primary endpoint of this study is the number of days required for a patient to resume full, unrestricted, and asymptomatic participation in sports following the injection. In addition, Visual Analog Scale (VAS) scores will be collected and analyzed at the following time points: immediately post-procedure, 24 hours, 2 weeks, 6 weeks, 12 weeks, and 1 year after the procedure.

3.3 Secondary Endpoints

The secondary endpoints of this study include characterization of the thigh injury using a modified version of the British Athletics Muscle Injury Classification (BAMIC) system.

Although originally designed for MRI, the same classification variables will be applied using musculoskeletal ultrasound, which, while commonly utilized in clinical practice, has not been formally validated for this purpose. Additional secondary endpoints include documentation of the specific thigh muscle group involved, patient-reported satisfaction with the procedure, and comparison of peak strength between the injured and uninjured thigh using a digital handheld dynamometer at the time of return-to-play clearance. Reinjury incidence will also be tracked and analyzed throughout the one-year follow-up period.

4. Study Design

4.1. Study Overview

The presented study is a prospective comparison of the duration of return to play and re-injury rate in patients that have received either PPP or a corticosteroid injection after sustaining a thigh muscle injury.

4.2. Study Duration

The duration of the presented study will be approximately two years, and study procedures will take place over the span of a maximum of five in-person visits. The exception to this will be based on the availability of the principal investigator, as screening/informed consent and the injection procedure can be done on the same day and combined into one visit.

5. Treatment Plan

5.1. Visit 1: Screening/Informed Consent

Potential participants will be identified by the clinical staff or by the research team. Once identified, a pre-screening assessment will be conducted. If eligible, the potential participant will be referred to the research team to complete the informed consent process. No study assessments will be completed until the participant signs the informed consent form. The informed consent will be collected on paper and a copy will be provided to ensure the volunteer understands the details of the study, including the benefits and risk factors. The participant will be provided with

sufficient time to consent and sign the informed consent form (ICF). The research team will be available to answer any questions or provide clarifications during the informed consent process.

5.2 Visit 2: Injection Procedure

After completing the informed consent process, participants will be blinded and randomized to receive either a corticosteroid or PPP injection guided by ultrasound. Peak force of the non-injured thigh will be measured using the Hoggan MicroFet 2 digital handheld dynamometer. Blood will be drawn from the participant's preferred extremity, and for those randomized to the PPP group, the sample will be processed using the Arthrex Angel centrifuge system. To ensure quality control and rule out thrombocytosis, a complete blood count (CBC) will be performed on both the whole blood and PPP samples. While blindfolded, the participant will receive the assigned injection. Immediately following the procedure, the participant will complete a Visual Analog Scale (VAS) for pain. The British Athletics Muscle Injury Classification (BAMIC) will be completed by a clinician based on ultrasound findings. Twenty-four hours post-procedure, the VAS score will be collected again via telephone.

5.3 Visit 3: Two-Week Post-Injection Visit

Two weeks following the day of the procedure, the patient will be assessed in-clinic. The site of the injection will be observed for any abnormalities, in which they will be documented. During this visit, the participant will also complete a VAS for pain.

5.4 Telephone Follow-ups

Following the two-week post-injection visit, VAS scores will also be collected at 6-week and 12-week marks post-procedure via telephone.

5.5 VAS Assessments

The VAS assessment will be done 24 hours (±6 hours) after the injection. The

subsequent VAS assessments will be done within ±3 days of their follow ups at 2 weeks, 6 weeks, 12 weeks and 1 year post-injection.

5.5 Return to Play Visit

If patient returns to play prior to the patient's one-year mark post-injection, the patient will notify the research team and schedule an in-person appointment at return to play (+3-7 days) to collect the patient's satisfaction of procedure survey, VAS assessments, and peak force of both injured and uninjured legs.

5.6 Visit 4: Assessment/End of Study (One Year)

During the one-year visit, the patient will be asked to complete the satisfaction of procedure survey (if not returned to play already) and VAS assessment for a final time. Lastly, the patient's previously injured thigh will then be analyzed to exude peak force using the Hoggan MicroFet 2 dynamometer.

6. Participant Recruitment and Enrollment

6.1 Recruitment

Participants will be recruited through the investigator's orthopedic practice at the Andrews Institute. Approximately 30 participants will be recruited to participate in this research study. The principal investigator is responsible for ensuring the participants enrolled adhere to the inclusion/exclusion criteria. Participants will be identified as those that participate in a sport and have suffered a thigh muscle injury. Participants will be considered enrolled in the study after completing the informed consent.

6.2 Sample Size Determination

Based off study layout, a power analysis was completed to confirm that a total population size of 10 (5 per treatment group) needed to be the minimum number of patients enrolled. However, based off of patient availability and presentation of cases, the enrollment goal is 30 total patients (15 per treatment group).

6.3 Inclusion Criteria:

Participants will satisfy the following criteria to be included in the present study:

- 15-30 years of age (and)
- Participant in a sport (and)
- Confirmed thigh muscle injury

6.4 Exclusion Criteria:

Participants will be excluded from participating in the present study if they fall within the exclusion criteria:

- Patient is incapable or unwilling to provide informed consent.
- Patient is not within the age range
- Patient does not have a thigh injury
- Patient's thigh injury occurred more than 4 weeks prior to treatment
- Patient's thigh injury requires surgical intervention
- Patient has had an injection for their thigh injury within the past 30 days
- Patient has thrombocytosis or other platelet abnormality in whole blood

6.5 Informed Consent Process

In adherence to the 21 CFR 50, Protection of Human Participants Guidelines, the informed consent process will be performed by one of the study investigators or staff through the research office that has received training on the informed consent process. This may be completed in the research office or in the principal investigator's clinic. No aspects of the study will be conducted prior to obtaining informed consent from each participant. The purpose and methods of the study, along with the expected effects, will be reviewed with each potential participant. Each participant will be provided with a copy of the consent form and sufficient time will be given for the opportunity to read and ask any questions about the study. After signing the informed consent document, participants will be given a copy of their records.

In addition, the designee will review with each participant that they are free to refuse to participate in the study.

6.6 Consent Withdrawal

During the informed consent procedure, participants will be informed that at any point during the study, consent may be withdrawn. To withdraw consent, participants can request in writing to withdraw HIPAA authorization, and the research site will not use or provide any health information to researchers. At this time, the link between the participant's health information will be severed with the research team. Withdrawal of consent will also include no further study activities.

6.7 Participant Benefits

This study could provide information that allows clinicians to improve clinical methods and outcomes for the treatment of thigh injuries. Participants will not receive any monetary compensation for their participation.

6.8 Participant Risks

As with any research involving human subjects, there is the inherent risk of a breach in patient confidentiality. This will be minimized by using participant identification numbers and adhering to all HIPAA guidelines.. The possible risks to subjects are those that would be incurred for procedures related to platelet poor plasma injections or corticosteroid injections within the muscles of the thigh. Such risks include pain at the injection site, redness, tenderness, and swelling.

7. Study Procedures

7.1 General Considerations

Study data will include data captured through the participant's outcomes measures as outlined above. Additional AE/SAE data will be collected. Study source documentation will be maintained by Andrews Research & Education Foundation under strict HIPAA compliance. Data will be available for monitoring of protocol fidelity by the quality assurance team as needed. Study source documentation will be maintained by Andrews Research & Education Foundation under strict HIPAA compliance.

7.2 Data Capture

CRIO, an electronic data capture system, will be used to store patient data, as it adheres to 21 CFR 11 compliance measures. Each participant will be given a unique identifier, under which their data will be collected. All data collection will adhere to good clinical data management practices for clinical trials. Quality control checks will be completed by the study quality team to ensure that source documentation matches the EDC throughout the investigation.

8. Safety Analysis

A safety analysis will be completed throughout the study. The study team will assess AE/SAEs as appropriate due to the nature of the study. The seriousness and relatedness of each AE/SAE will be assessed by the investigator.

8.1 Adverse Events (AE)

An adverse event is any untoward or unfavorable medical occurrence in the human participant, including any abnormal sign, symptom, or disease, temporally associated with the participants' involvement in the research, whether considered related to the participants involvement in the research.

Adverse events will be classified as follows:

- **Mild:** Awareness of signs or symptoms but easily tolerated and are of minor irritant type causing no loss of time from normal activities. Symptoms do not require medical evaluation; signs and symptoms are transient.
- **Moderate:** Events introduce a low level of inconvenience or concern to the participant and may interfere with daily activities but are usually improved by simple therapeutic measures; moderate experiences may cause some interference with functioning.
- **Severe:** Events interrupt the participant's normal daily activities and generally require systemic drug therapy or other treatment; they are usually incapacitating.

Adverse events will also be reviewed for the relatedness to the study intervention as follows:

- **Definitely Related:** The adverse event is clearly related to the investigational agent/procedure – i.e., an event that follows a reasonable temporal sequence from administration of the study intervention, follows a known or expected response pattern to the suspected intervention, that is confirmed by improvement on stopping and reappearance of the event on repeated exposure, and that could not be reasonably explained by the known characteristics of the participant's clinical state.
- **Possibly Related:** An adverse event that follows a reasonable temporal sequence from administration of the study intervention follows a known or expected response pattern to the suspected intervention, but that could readily have been produced by several other factors.
- **Not Related:** The adverse event is clearly not related to the investigational agent/procedure - i.e., another cause of the event is most plausible; and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the study intervention and/or a causal relationship is considered biologically implausible.

A standard grading scale from NCI CTCAE will be utilized for event grading (National Cancer Institute [NIH], 2020) (CTCAE v.6).

- **Grade 1 Mild;** asymptomatic or mild symptoms, clinical or diagnostic observations only, intervention not indicated.
- **Grade 2 Moderate;** minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental Adaptive Living Skills.
- **Grade 3 Severe or medically significant but not immediately life-threatening;** hospitalization or prolonged hospitalization indicated; disabling; limiting self-care daily living skills.
- **Grade 4 Life-threatening consequences;** urgent intervention indicated.
- **Grade 5** Death related to AE

8.2 Serious Adverse Events (SAE)

Serious adverse events are any events that:

- Result in death
- Is life-threatening or places the participant at immediate risk of death from the event as it occurred
- Requires or prolongs hospitalization
- Causes persistent or significant disability or incapacity
- Results in congenital anomalies or congenital disabilities
- Is another condition which investigators justify representing significant hazards

8.3 Unanticipated Problem (UP):

Defined by DHHS 45 CFR part 46 as any incident, experience, or outcome that meets the following criteria:

- Unexpected, in terms of nature, severity, or frequency, given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, and (b) the characteristics of the study population.
- Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4 Assessment of AE, SAE

Assessment of AE, SAE will occur before assessment of each participant. Source documentation will be completed and maintained in each participant's source file and communicated to the appropriate regulatory authority.

8.5 Reporting of AE, SAE, UP

Throughout the study, the research team will monitor the occurrence of AEs and SAEs. Data will be collected if an instance occurs, and the PI will be notified. All AE data, such as onset date, resolution date, outcome, and treatments given will be documented in the source documents and will be recorded and assessed by the investigators for severity and relatedness.

Follow-up will occur using the provided safety monitoring form if AEs or SAEs occur. The follow-up will end either when the symptoms are resolved or up to 30 days past the end of study participation. This data will be reported as a measure of complications across treatment arms as well.

9. Statistical Considerations

The variables of interest will be the duration of return to play for both groups and VAS scores for each injured participant. Additionally, the overall instances of AE/SAE events will be reviewed. Statistical analysis will be completed to assess the trends observed in the data set.

10. Regulatory Requirements

10.1 Institutional Review Board Approval

Institutional Review Board (IRB) approval of the study and study procedures will be obtained from the Baptist Hospital IRB.

10.2 Protocol Compliance

Conformance to the protocol will maintain the quality and integrity of the presented randomized clinical trial. However, when deviations occur, they will be documented. If a deviation results in an AE/SAE, refer to the IRB reporting guidance. All protocol deviations will be assessed as major or minor by the research team. Major protocol deviations include behaviors

such as nonconformance to informed consent procedures regarding eligibility, receiving incorrect treatment or treatment dosage.

10.3 Training

The study principal investigator will ensure appropriate training is completed and documented for each study personnel's designation of authority before the initiation of the study. The principal investigator will provide protocol training, inclusion/exclusion training, safety analysis training, and schedules of events review to all study team members.

Information to be collected include:

1) Age
2) Gender
3) Ethnicity/Race
4) Muscle groups involved in injury
5) VAS pain scale
6) BAMIC muscle injury classification
7) Sport(s) played (if multiple, list of primary and secondary sports)
8) Injection type
9) Location of injection (i.e. muscle belly, muscle origin, or muscle insertion)
10) Which thigh was injured and date of injury
11) If this is the first time that the thigh injury has occurred or if injury is a reoccurrence
12) Peak strength of injured thigh
13) Peak strength of uninjured thigh
14) Days to return to play (one year, more than one year, or if clearance is done prior to one-year post-procedure)
15) Reinjury occurrence
16) Patient satisfaction with procedure
17) Adverse events
18) Severe adverse events
19) Complete blood count (CBC) values from whole blood samples and platelet poor plasma
20) Musculoskeletal abnormalities at or near the site of injection (if any)

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