The Effect of Platelet-Rich Plasma on Recovery in Athletes with a Grade III Lateral Ankle Sprain

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THE EFFECT OF PLATELET-RICH PLASMA ON RECOVERY IN ATHLETES
WITH A GRADE III LATERAL ANKLE SPRAIN

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the Degree of
Master of Medical Science

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# Table of Contents

**LIST OF TABLES** ........................................................................................................ iv

**ABSTRACT** ....................................................................................................................... v

**CHAPTER 1: INTRODUCTION** ........................................................................................ 1

- Background: ...................................................................................................................... 1
  - Epidemiology of Ankle Sprains: ................................................................................... 1
  - Ankle Sprain Pathophysiology and Grading: ............................................................... 1
  - Diagnosis and Current Treatment: .............................................................................. 2
  - Platelet-rich Plasma: ...................................................................................................... 3

- Problem Statement: ........................................................................................................... 4

- Goals and Objectives: ....................................................................................................... 6

- Hypothesis: ....................................................................................................................... 6

- Definitions: ....................................................................................................................... 6

- Abbreviations: ................................................................................................................... 7

- References: ......................................................................................................................... 8

**Chapter 2: Literature Review** .......................................................................................... 10

- Introduction: ..................................................................................................................... 10

- Review of The Efficacy of Platelet-rich Plasma in The Literature: ................................ 10
  - Platelet-rich Plasma Use in the General Population .................................................... 10
  - Platelet-rich Plasma Use in Athletes ........................................................................... 12
  - Platelet-rich Plasma in Foot and Ankle Pathologies .................................................... 14
  - Platelet-rich Plasma in Lateral Ankle Sprains ............................................................. 16

- Review of Confounding Variables in the Literature ....................................................... 18
  - Platelet-rich Plasma Preparation ................................................................................. 18
  - Platelet-rich Plasma Administration ............................................................................ 19
  - Antiplatelet and Non-steroidal Anti-inflammatory Drugs (NSAID) ................................ 21
  - Rehabilitation Protocols .............................................................................................. 22

- Review of Relevant Methods ............................................................................................ 23
  - Participant Selection ....................................................................................................... 23
  - Return to Play .................................................................................................................. 24
  - Visual Analog Scale (VAS) ............................................................................................ 26
  - American Orthopedic Foot and Ankle Society Scale (AOFAS) .................................... 26
  - Blinding .......................................................................................................................... 27
  - Platelet-rich Plasma Kit and Dosage ............................................................................ 28
  - Follow-up ....................................................................................................................... 28
  - Statistical Analysis and Statistical Significance ............................................................ 29
  - Sample Size ................................................................................................................... 30

- Conclusion ......................................................................................................................... 31

- References ......................................................................................................................... 32

**Chapter 3: Study Methods** ............................................................................................. 36

- Study Design: ..................................................................................................................... 36
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Population and Sampling:</td>
<td>36</td>
</tr>
<tr>
<td>Subject Protection and Confidentiality:</td>
<td>38</td>
</tr>
<tr>
<td>Recruitment:</td>
<td>39</td>
</tr>
<tr>
<td>Baseline Characteristics:</td>
<td>39</td>
</tr>
<tr>
<td>Study Variables:</td>
<td>40</td>
</tr>
<tr>
<td>Assignment of Intervention:</td>
<td>41</td>
</tr>
<tr>
<td>Platelet-rich Plasma Preparation and Administration:</td>
<td>42</td>
</tr>
<tr>
<td>Blinding of Intervention:</td>
<td>42</td>
</tr>
<tr>
<td>Rehabilitation Protocol:</td>
<td>43</td>
</tr>
<tr>
<td>Adherence:</td>
<td>44</td>
</tr>
<tr>
<td>Data Collection:</td>
<td>44</td>
</tr>
<tr>
<td>Monitoring Adverse Events:</td>
<td>45</td>
</tr>
<tr>
<td>Analysis:</td>
<td>46</td>
</tr>
<tr>
<td>Timeline and Resources:</td>
<td>47</td>
</tr>
<tr>
<td>References</td>
<td>47</td>
</tr>
<tr>
<td>Chapter 4: Conclusion</td>
<td>49</td>
</tr>
<tr>
<td>Advantages:</td>
<td>49</td>
</tr>
<tr>
<td>Limitations:</td>
<td>50</td>
</tr>
<tr>
<td>Clinical Significance:</td>
<td>51</td>
</tr>
<tr>
<td>References</td>
<td>51</td>
</tr>
<tr>
<td>Appendix A: Authorization and Consent Form</td>
<td>53</td>
</tr>
<tr>
<td>Appendix C: American Orthopedic Foot and Ankle Society Scale</td>
<td>60</td>
</tr>
<tr>
<td>Appendix D: Visual Analog Scale</td>
<td>61</td>
</tr>
<tr>
<td>Appendix E: PAASS Framework</td>
<td>62</td>
</tr>
<tr>
<td>Appendix F: Sample Size Calculation</td>
<td>63</td>
</tr>
<tr>
<td>Appendix G: Baseline Characteristics/ Inclusion &amp; Exclusion Survey</td>
<td>64</td>
</tr>
<tr>
<td>Bibliography</td>
<td>65</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. Inclusion and exclusion criteria

Table 2. Baseline characteristics, variable type, and statistical analysis

Figure 1. Timeline for individual participant data collection
ABSTRACT

Lateral ankle sprains are the most common cause of missed athletic participation. One emerging therapy that has been used to hasten recovery from orthopedic injuries is injection with platelet-rich plasma. However, the effect of platelet-rich plasma on recovery from lateral ankle sprains has only been studied in the context of less severe injuries (grades one and two). **We hypothesize that a platelet-rich plasma injection will decrease the mean days to return to play in athletes with a severe (grade three) lateral ankle sprain.** We will conduct a multicenter randomized controlled trial to investigate the mean days to return to play in athletes with a grade three lateral ankle sprain who receive a platelet-rich plasma injection compared to a placebo injection. The study findings may indicate an adjuvant therapy that can improve outcomes and reduce unnecessary surgical intervention in a very common athletic injury.
CHAPTER 1: INTRODUCTION

Background:

Epidemiology of Ankle Sprains:

Ankle sprains are one of the most common musculoskeletal injuries, with approximately two million people presenting to the emergency room with acute ankle sprains per year in the United States and the United Kingdom.\(^1\) A specific population that is even more prone to ankle sprains is athletes. Ankle sprains account for up to forty percent of all sport-related injuries.\(^2\) Because of this, ankle sprains are the most common reason for missed athletic participation in college athletes.\(^2,3\) Athletes who play indoor or court sports such as basketball, volleyball, tennis, and wrestling were found to be at the highest risk for ankle sprains, with an incidence of 7 per 1,000 exposures.\(^4\) On average, an athlete with an ankle sprain will miss between 16-24 days of participation, with more severe sprains keeping athletes off the field for up to four to six weeks.\(^5,6\)

Ankle Sprain Pathophysiology and Grading:

An ankle sprain can be defined as a stretching or tearing of any of the ankle ligaments. Depending on the mechanism of injury, a variety of different ankle ligaments may be injured. Of the potential ligaments injured, one study found 73\% of ankle sprains were classified as lateral ankle sprains affecting the anterior talofibular ligament (ATFL) and or the calcaneofibular ligament(CFL), while the other 27\% of sprains were either medial or syndesmotic injuries.\(^1\) The anterior talofibular ligament prevents anterior translation of the talus and is frequently injured when the ankle joint is plantar flexed and inverted. On the other hand, the calcaneofibular ligament resists inversion of the talus and
is commonly injured by excessive dorsiflexion and inversion of the ankle joint.\textsuperscript{3}

Depending on the injury severity, ankle sprains are classified as grade I, grade II, or grade III. The grade of the injury is determined by the anatomical injury and the clinical symptoms. A grade I sprain is a stretched ligament with minor swelling, minor function loss, and no change in stability. A grade II sprain is a partial ligament tear with moderate swelling, increased instability, and moderate functional disability. A grade III sprain is a complete tear of the ligament with severe pain and swelling, significant loss of function, and the inability to bear weight.\textsuperscript{5}

\textit{Diagnosis and Current Treatment:}

Following an ankle injury, emergency room clinicians will often refer to the Ottawa Ankle Rules to determine if x-rays are needed to rule out a fracture of the foot or ankle. Once a fracture is ruled out, the majority of ankle sprain diagnoses can be made using history and physical exam. The patient will report a twisting injury, the ATFL region is tender to palpation, and inspection shows pain, swelling, and bruising in the ankle joint region. In more difficult to diagnose sprains, magnetic resonance imaging (MRI) is the imaging method of choice as it was found to have a sensitivity of 93\%-96\% and a specificity of 100\%.\textsuperscript{7} Due to the cost and limited accessibility, MRI is generally reserved for situations where an occult fracture, cartilage injury, or peroneal tendon tear is suspected. Once a diagnosis is made, the current treatment recommendation for a grade III lateral ankle sprain is rest, ice, compression, and elevation (RICE) for the first four to five days to reduce inflammation\textsuperscript{8} with three to five days of immobilization.\textsuperscript{5} Following immobilization, early weight bearing in a controlled ankle mobility (CAM) boot is recommended. One study found that in severe lateral ligament tears, a below-knee
or removable boot (CAM) led to a faster recovery when compared to a compression bandage. Lastly, in conjunction with RICE and immobilization, early physical therapy is also recommended as it has been shown to be associated with quicker time to recovery and improved outcomes. Yet, much controversy still exists on what is the best treatment of grade III lateral ankle sprains in athletes; it is debated whether they should be managed conservatively or with surgical intervention as they are at high risk for reinjury.

Platelet-rich Plasma:

One potential adjuvant therapy that may be added to the conservative management of an ankle sprain is platelet-rich plasma (PRP). Platelet-rich plasma is a sample of autologous blood that contains a high concentration of platelets and related growth factors that have been proven to increase the stimulation of the healing processes in laboratory-based studies. The treatment is done by drawing a patient’s blood and separating out the platelets via centrifuge. In the centrifuge, the highest weight substances like red blood cells sink to the bottom, the lightest weight substances like water and electrolytes stay at the top, and in the middle are the protein and platelets that will be separated and called PRP. PRP is rich in growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), and basic fibroblast growth factor (bFGF). It has been found that these growth factors stimulate cell proliferation and neovascularization. A lack of blood supply in areas such as tendons, ligaments, and cartilage will cause them to heal much slower. However, by manually introducing high concentrations of the growth factors, the notion is this will stimulate quicker healing. PRP has become more utilized in the field of orthopedics over the two last decades as high-quality evidence supports its use in lateral
epicondylitis and osteoarthritis, and moderate high-quality evidence supports its use in patellar tendinopathy, patellar tendon graft ACL reconstruction, and plantar fasciitis.\textsuperscript{12} More specifically in foot and ankle pathologies, a recent review of the literature found platelet-rich plasma to be a promising treatment option with potential benefit, but there was not enough clinical evidence to make a definitive treatment recommendation.\textsuperscript{13} Yet, PRP use remains controversial, and in many cases clinicians tout it claims to be a miracle cure, whereas other clinicians claim it is overused and potentially fraudulent.

**Problem Statement:**

For athletes, ankle sprains are a frequent problem, they are the most common reason for keeping an athlete off the field. Of these ankle injuries, the majority of them affect the same ligaments, with about 90\% percent of sprains being inversion injuries that affect the lateral ligament complex.\textsuperscript{14} The current standard of care calls for RICE, immobilization, and physical therapy. The current treatment guidelines will keep athletes off the field for three to four weeks. A novel therapy known as platelet-rich plasma has shown to be effective in aiding in the healing of soft tissue injuries in various parts of the body. However, there is a lack of research on utilizing platelet-rich plasma injections in the treatment of ankle ligament sprains.

In the existing research on platelet-rich plasma injections in lateral ankle sprains, one randomized clinical trial looked at the effectiveness of platelet-rich plasma injections in grade II lateral ankle sprains and showed a reduction of pain and improved function in the experimental group at eight weeks.\textsuperscript{15} Another double-blind, randomized study evaluated the use of platelet-rich plasma for acute ankle sprains presenting to the
emergency room but did not specify the type or grade of the ankle sprain\(^{16}\). There have not been any studies that have looked at the effectiveness of platelet-rich plasma injections on grade III lateral ankle sprains. The severity of a grade III sprain is what will keep athletes off the field the longest and has the potential to show the most benefit from an adjunct therapy like PRP injections.

One population in the clinical research that has shown more consistent benefit from platelet-rich plasma is athletes. One study focused on platelet-rich plasma injections in elite athletes with grade III high ankle sprains and found that those who received PRP injections returned to play 19 days sooner than the control group.\(^{17}\) A different study looked at return to play time in rugby athletes with a high ankle sprain treated with a single PRP injection and found the experimental group to have a mean difference of 20.7 days less for return to play than the control group.\(^{18}\) A final study focusing on National Football League (NFL) players who had grade 2 hamstring injuries treated with PRP injections found that the experimental group missed a mean of 1.3 games while the control group missed a mean of 2.9 games.\(^{19}\) The existing data on the efficacy of PRP within the athlete population shows promise, indicating that it may also be effective in treating lateral ankle sprain in athletes.

In the existing literature, there is no evidence of any randomized controlled trials testing the effectiveness of platelet-rich plasma on the recovery from a grade III lateral ankle sprain in athletes. There is just one case study on a runner who had an MRI confirmed grade III lateral ankle sprain. At eight weeks, ultrasound confirmed a fully healed ligament, and MRI showed the ATFL was twice the normal thickness indicating increased stability.\(^{20}\)
Goals and Objectives:

The high incidence of lateral ankle sprains, lack of research in grade III sprain treatment with PRP, and the positive results of PRP injections in athletes give rise to the need for a study examining the effectiveness of PRP injections on recovery in athletes with a grade III lateral ankle sprain.

To test this, a double-blind, randomized controlled trial will be performed. The experimental group will receive a single platelet-rich plasma injection within 72 hours of injury, while the control group will receive a blinded placebo injection. Both groups will follow the recommended guidelines of RICE, immobilization via CAM boot, and physical therapy. The objective of the study will be to see if there is a mean difference in the return to play time between athletes who receive platelet-rich plasma versus those who do not. The goal of this study is to determine if platelet-rich plasma injections have utility in the treatment of grade III lateral ankle sprains in athletes.

Hypothesis:

Athletes with an MRI confirmed grade III lateral ankle sprain who receive a single injection of three milliliters (mL) of platelet-rich plasma into the anterior talofibular ligament will have a statistically significant reduction in days to return to play compared to athletes who receive a single placebo injection of three milliliters (mL) of saline into the anterior talofibular ligament.

Definitions:
Athletes- Men and women over the age of 18 years old competing in a sport at the professional or collegiate level.
Return to play- The date at which an athlete is cleared to return to competitive play by a clinician. Clinicians will make their decisions for return to play based on the PAASS framework (Appendix E).

Grade III Lateral ankle sprain- complete tear of the anterior talofibular ligament that is confirmed on 3.0 Tesla MRI imaging.

Platelet-rich plasma injection- 3 mL of platelet-rich plasma produced via the Zimmer Biomet Mini GPS III device.

**Abbreviations:**

AITFL- Anterior Inferior Tibiofibular Ligament  
AOFAS- American Orthopedic Foot and Ankle Society Score  
ATFL- Anterior Talofibular Ligament  
bFGF- Basic Fibroblast Growth Factor  
CAM- Controlled Ankle Motion  
CFL- Calcaneofibular ligament  
mL- Milliliters  
MRI – Magnetic resonance imaging  
NSAIDs- Non-Steroidal Anti-inflammatory Drugs  
NFL – National Football League  
PDGF- Platelet Derived Growth Factor  
PRP- Platelet-rich Plasma
PAASS- Pain, Ankle Impairment, Athlete perception, Sensorimotor control, Sport performance

RICE- Rest, Ice, Compression, Elevation

TGF-β - Transforming Growth Factor-β

VAS – Visual Analog Scale

VEGF- Vascular Endothelial Growth Factor

References:


Chapter 2: Literature Review

Introduction:

Literature Search Criteria

A review of the literature was conducted between June 2021 and May 2022 via PubMed, Ovid, Scopus, and Cochrane Database. The MeSH terms used to search these databases were “platelet-rich plasma,” “PRP,” “ankle sprain,” “lateral ankle sprain,” “anterior talofibular ligament,” “athlete,” “return to play,” and “return to sport.” The search was confined to articles published after 2016 initially but was later expanded to include more relevant studies. The search was limited to only articles published in the English language. The search included randomized controlled trials, cohort studies, case studies, and systematic reviews. The following literature review will evaluate the existing literature on the topic.

Review of The Efficacy of Platelet-rich Plasma in The Literature

Platelet-rich Plasma Use in the General Population

Platelet-rich plasma is an emerging therapy that is being used in Orthopedics to hasten the recovery of a variety of orthopedic injuries. Nevertheless, the actual effectiveness of platelet-rich plasma in the general population is still heavily debated. One systematic review and meta-analysis done by Massimo et al. compared platelet-rich plasma injections to various other conservative non-surgical treatments for orthopedic injuries and found it to have marginal effectiveness compared to the controls.¹But the evidence included in this study was ranked as low quality due to the risk of bias and inconsistency. Although it may have shown no difference when compared to other non-operative treatments, the efficacy of PRP may vary based on the type of injury. Chen et
al. performed a metanalysis and systematic review of the efficacy of PRP on tendon and ligament healing. The results showed that PRP was associated with less pain in lateral epicondylitis and rotator cuff injuries. There was insufficient evidence to conclude PRP effectively treated anterior cruciate ligament (ACL) injuries or tendinopathies. The study found the main limiting factor for PRP use was the lack of standardization of the PRP.\textsuperscript{2} In contrast, a literature review done by Le et al. found the existing literature to show that PRP is safe, but the evidence of its efficacy is highly variable based on the injury site. Some injury locations that the literature contained high-quality evidence for the use of PRP were lateral epicondylitis, osteoarthritis of the knee, patellar tendinopathy, and plantar fasciitis.\textsuperscript{3} Based on the current evidence, the use of platelet-rich plasma remains highly variable and lacks high-quality evidence to support its abundant use in the general public.

Even though there is no conclusive evidence to support the use of platelet-rich plasma, it is still often used by the general public. A possible explanation for the unsupported use is the potential financial gains from platelet-rich plasma. Most insurances do not cover the cost of platelet-rich plasma, and as a result, there is little price regulation. One epidemiological study evaluating the cost of PRP injections found the cost ranged from 175 dollars to 4973 dollars, with a mean of $707 \pm 388$ dollars.\textsuperscript{4} There is potential for financial exploitation as the public may view PRP as a fix-all because many famous athletes have used it. In reality, the cost burden is much less for an athlete, and there are more significant financial implications if an athlete can return to their sport sooner. Rajan et al. conducted a study evaluating the cost-effectiveness of platelet-rich plasma injections for knee osteoarthritis. The findings were that PRP injections were not
cost-effective. However, the main factor was not the price per injection but rather the minimal clinical efficacy in pain reduction and function improvement. While the platelet-rich plasma use in the public remains understudied and heavily debated, it is clear that more concrete evidence of its efficacy is needed to justify the high cost per injection for the general public. Whereas in athletics, the high cost can be justified by the financial benefit a player can offer the organization by returning to the team sooner.

*Platelet-rich Plasma Use in Athletes*

A more specific population where platelet-rich plasma may be more effective and more financially rational is with athletes. A randomized controlled trial done by Rossi et al. studied the return to play time in athletes with a grade 2 muscle injury. The study found that athletes who received a single PRP injection and a rehabilitation program had a mean time to return to play of 21.1 ±3.1 days, while the control group who received only rehabilitation had a mean of 25±2.8 days to return to play (p=.001). This study was limited because the patients were not blinded from their allocation, which could lead to bias. Also, the return to play decision was made by a therapist’s clinical impression which is subject to bias. Bradley et al. performed a retrospective cohort study that focused on the return to play time in NFL athletes with hamstring injuries. This study showed that athletes treated with PRP missed an average of 1.3 games, while athletes who did not receive PRP missed an average of 2.9 games (p<.05). This study did have several limitations. It could only detect a statistically significant difference in games; it could not detect a statistically significant difference in days and practices missed due to being underpowered. Additionally, the researchers did not control the number of PRP injections. Lastly, players both in and out of season were included, which could influence
the return to play decisions. A more reliable study focusing on hamstring injuries treated with PRP was done by Trunz et al. This retrospective study found that athletes with a grade 2 hamstring injury treated with hematoma aspiration and PRP returned to play in 23.5±5.4 days. In contrast, the conservatively managed group returned to play in 32.4 ± 81 days (p<.001). Moreover, one retrospective cohort study examined the outcomes of baseball players who received a PRP injection for an elbow ulnar collateral ligament injury and found it to be very effective in preventing surgery in the less severe ulnar collateral ligament injuries. Of the athletes with a grade I, II, or III UCL injury, 59.5% of them were able to return to play without needing surgery. However, the results of this study are questionable as the study had a small sample size which led to a non-statistically significant calculated odds ratio. Lastly, one case study of 15 varsity collegiate athletes promotes the use of PRP in the treatment of patellar and hamstring tendinopathies as a conservative treatment to avoid surgery. Of the 15 athletes included in the case study, 12 of the athletes could return to their sport without surgery. But, this study is lower quality evidence due to it being a case study rather than an experimental study. All in all, it is clear that the current literature shows favorable evidence for the use of PRP in athletic injuries.

Along with being beneficial in hastening return to play in athletes, PRP has also been shown to improve pain and function in athletes. Of 48 athletes who presented to a sports medicine center for PRP injections with a mean pre-procedure VAS score of 7.25±.70, at four weeks following injection, they had a mean VAS score of 2.42 ±.74 p=.0001. Although this was an observational study; the results show a promising reduction in pain among athletes who received PRP. One randomized controlled trial
comparing PRP to low dose radiation for athletes with plantar fasciitis found no significant difference between the two treatments but found both were effective for reducing pain scores and improving American Orthopedic Foot and Ankle Score (AOFAS). For the group that received PRP, their mean VAS score was reduced from 6.65 at baseline to 2.45 at three months (p<.0001), and their AOFAS scores went from 51.5 at baseline to 89.1 at six months (p<.0001). Additionally, positive effects on athletes’ pain and function were observed in the prospective cohort study done by Charousset et al. In this cohort of 28 athletes with chronic patellar tendinopathy, their Victorian Institute of Sport Assessment–Patella (VISA-P) scores improved from 39 to 94 (P<.001) and VAS scores improved from 7 to 0.8 (P<.001). Although this was a very long follow-up period, twenty-one of the twenty-eight athletes were able to return to their pre-injury sporting level by three months, which indicates promise for short-term improvement. While the evidence for the use of PRP in the general public is indifferent, there is evidence that its use may be beneficial in the athletic population.

Platelet-rich Plasma in Foot and Ankle Pathologies

There is debate on what type of injury PRP is most effective in treating, but one area that has shown promise in the literature is in foot and ankle pathologies. A review of the literature on the use of platelet-rich plasma in the foot and ankle found it to have a possible benefit in the treatment of Achilles pathologies, chronic plantar fasciitis, osteochondral lesions of the talus, ankle osteoarthritis, and diabetic foot ulcers. Nonetheless, due to the inconsistency in preparation and delivery of the PRP, there are inconsistent results in the literature. Despite the lack of conclusive evidence, there still is an abundant amount of promising data. Plantar fasciitis is one pathology that may
benefit from PRP. A systematic review by Hurley et al. found that PRP use in chronic plantar fasciitis was more beneficial than corticosteroids in reducing pain in the short term and improving function in the long term.\(^\text{15}\) A randomized trial by Peerbooms et al. measured the pain and function of 115 patients with chronic plantar fasciitis treated with either a PRP injection or a corticosteroid injection. The results of the study showed that at the one year follow up, the PRP group had significantly lower pain scores (mean difference, 14.4; 95% CI, 3.2-25.6) and significantly lower Foot Function Index Disability scores (mean difference, 12.0; 95% CI, 2.3-21.6) than the corticosteroid group. This study was the first randomized controlled trial to test PRP on plantar fasciitis in a sample size greater than 100 participants. Yet, it still had limitations. There was a variation between a 30mL and 60 mL PRP kit, and the injections were not given under ultrasound guidance which leaves more room for error.\(^\text{16}\) A similar study comparing platelet-rich plasma, corticosteroids, and a placebo in the treatment of plantar fasciitis found both PRP and corticosteroids to be effective in reducing VAS scores and improving function, but PRP was more effective in the long. At 18 months, PRP showed the most significant reduction in VAS from 8.2 to 6.1 \((p=.005)\) and the most significant improvement in Roles and Maudley score, a measure of function, from 1.7 to 3.7 \((p=.05)\).\(^\text{17}\) While the study attempted to limit bias by blinding at many levels, there was a degree of self-bias as the data was measured by their own researchers.

Another ankle pathology that has minimal but promising data is high ankle sprains. A high ankle sprain is an injury to the anterior inferior tibiofibular ligament (AITFL). Though much less common than a lateral ankle sprain, these injuries have a much longer recovery time. Laver et al. conducted a randomized controlled trial
examining the effect of PRP injection on return to play and pain in athletes with a high ankle sprain. The study found that the athletes treated with PRP had a mean days to return to play of 40.8 ± 8.9 days versus 59.6 ± 12.0 days (p=.006). This study recorded a notable effect size, but it had a number of limitations. First, none of the participants were blinded to their intervention which could have created performance bias. Furthermore, there was variability in the time to PRP administration in the treatment groups. Some athletes received injections up to 11 days after the injury. Despite these limitations, the study still displayed a significant improvement in return to play in the athletes treated with PRP. Samra et al. also examined the effects of PRP on AITFL injuries in a cohort of rugby players. The findings of this study were that the athletes treated with PRP returned to play in an average of 48.6 ± 11.7 days, while the control group had a mean time to return to play of 69.3 ± 29.1 days (p=.048). The results of this study are consistent with the findings of the Laver et al. study. Nevertheless, this study was limited because it used a historical control group preventing any randomization or blinding.

Platelet-rich Plasma in Lateral Ankle Sprains

Of the existing foot and ankle literature, it seems evident that PRP may be effective in ligamentous injuries. Both the plantar fascia and the anterior inferior tibiofibular are ligaments in the foot. But the most commonly injured ligament of the foot is the anterior talofibular ligament (ATFL). There is a lack of studies examining the efficacy of PRP in the treatment of ATFL injuries. There is evidence of only two randomized controlled trials examining the efficacy of PRP in lateral ankle sprains in the literature. In 2020, Blanco-Rivera et al. conducted and randomized controlled trial to
evaluate the effect of PRP on patient outcomes with a grade II lateral ankle sprain. The study found that at eight weeks follow up, the group who received the PRP intervention had lower VAS scores 0.3± 0.5 versus 1.4± 0.5 (p<.0001), higher AOFAS scores 98.2 ± 4.0 versus 89.8 ± 0.6 (p<.0001), and higher Foot and Ankle Disability Index scores (FADI) 133.1 ± 1.0 versus 129.5 ± 4.0 (p=.0003) when compared to the control group.20 While this study showed promising results; it had multiple limitations. No placebo injections were used which prevented the participants from being blinded to their intervention. The grading of the ankle sprain was by clinical classification, which may have led to observer bias. Moreover, the outcomes were all measured from the patient’s point of view which may have led to reporting bias. Another randomized controlled trial done by Rowden et al. opposed the findings of the Blanco-Rivera et al. trial. The double-blinded randomized controlled trial evaluated the use of PRP for acute ankle sprains in the emergency room. The study found no significant difference in VAS scores or Lower Extremity Functional Scale values between the intervention group and the control group.21 The study had a very small sample size of only 37 patients which resulted in a low power that limited the ability to detect a significant difference. Another issue with the study was that it did not control the pain medication regimen among participants. The variability in pain medication could have significantly affected patients’ pain and function scores. And lastly, the study had an extremely short follow-up of only eight days. The final data for PRP use in latera ankle sprains came from a case study by Lai et al. The case examined was a 39-year-old runner who suffered an MRI confirmed grade III lateral ankle sprain treated with PRP. The athlete returned to running in 8 weeks.22 Though this is only a case study of one patient, it does suggest there is potential
for PRP to aid in the recovery of a complete tear of the ATFL. Of the current literature, there has never been a randomized controlled trial done evaluating the use of PRP in a grade III lateral ankle sprain.

**Review of Confounding Variables in the Literature**

*Platelet-rich Plasma Preparation*

The most common limiting factor noted in the majority of systematic reviews about the efficacy of platelet-rich plasma has been the lack of standardization in the preparation of platelet-rich plasma. As of 2019, there were more than 16 different kits available for providers to use to produce platelet-rich plasma. Each kit varies based on the volume of blood required, the volume of PRP produced, processing time, centrifugation speed, platelet capture efficiency, and many other factors. There has been limited data comparing different kits and the concentrations of platelets and growth factors produced. Fitzpatrick et al. compared GPS III, Smart-Prep2, Arteriocyte Magellan, and ACP and found that the GPS III, Smart-Prep2, and Arteriocyte Magellan were associated with increases in platelet concentration 3 to 6 times the basal values. At the same time, ACP only produced a concentration of 1 to 1.7 times the basal value. As a result of the differences between the kits, it makes it very hard to reproduce and generalize the findings of each study. Not only is there variation from kit to kit, but studies also vary based on the number of times the sample was centrifuged. Double centrifugation was found to produce a greater number of platelets but came with the risk of damaging or altering the platelets from excessive centrifugation force. As a result, after a systematic review of the literature, Croisé et al. recommend singular or simple centrifugation for day-to-day use of PRP. Furthermore, there has not been formal
guidance on how long and at what force the samples should be centrifuged. Eren et al. compared a single centrifuge at 400 g for 10 or 12 minutes. They concluded that the 12-minute 400 g produced high VEGF concentrations but did not change the PDGF or TGF-B concentrations. Clearly, there is extreme variability in the process of preparing PRP, which can significantly affect the results of studies using it as an intervention. Though there have not yet been standardized guidelines published for PRP preparation, it is crucial to consider the existing evidence on the most effective preparation methods in the literature when designing a study. The proposed study considers this data in the methods section.

**Platelet-rich Plasma Administration**

Extreme variability exists when preparing PRP, but there is also extreme variability in PRP administration. For instance, the Laver et al. study was criticized for ranging between 1 to 11 days between injury and PRP administration. But, there is minimal data to support the ideal time after an injury to receive an injection. A meta-analysis by Seow et al. noted that the injection should be given in the post-inflammatory phase, but there is no standardized protocol for the number of days. The reason for this is there is a lack of studies on the timing of PRP injections in humans. One study performed on rats found that when PRP was administered three days following an injury, it had greater cell proliferation when compared to the administration at day 7. Many studies have also shown variability in the number of PRP injections given. In knee osteoarthritis, a systematic review of the evidence compared singular versus multiple injections of PRP. Based on the existing literature, it was shown that there was no significant difference between singular and multiple injections when it comes to pain
scores like VAS. Although, multiple injections were found to be more beneficial in functionality improvement.\textsuperscript{29} Similar findings can also be seen in rotator cuff injuries as Xiang et al. found that a single PRP injection was more effective in short-term reduction of pain relief when compared to multiple injections.\textsuperscript{30} These results favor the treatment of an acute injury with a single PRP injection, while there may be more functional benefits to treating chronic injuries with multiple injections.

As stated earlier, the final concentrations of platelets and growth factors vary considerably with each platelet preparation kit. Due to this lack of similarity, there is no actual evidence on the ideal concentration of platelets and growth factors to be used. Although it has been found that the minimum platelet concentration should be more than $1 \times 10^6/\mu L$ or an approximately five-fold increase in platelets from baseline.\textsuperscript{31} Additionally, Oudelaar et al. performed a literature review and recommended that the choice for platelet concentration should be determined based on clinical application. He found that most of the ideal concentrations of PRP vary by field, and there is still a need for studies to focus on what PRP is most suitable for specific fields.\textsuperscript{32} The final difference in the application of PRP observed in studies is in ultrasound-guided injections versus non-guided injections. Although some studies did use a blind injection method like in the Peerbooms et al. study.\textsuperscript{16} The decision to use a blind injection came from a 2001 study done by Kane et al., which found that ultrasound-guided injections and palpation-guided injections were equally effective in managing plantar fasciitis.\textsuperscript{33} In the time since this study, ultrasound technology has improved tremendously. A systematic review by Soh et al. found that patients who underwent ultrasound-guided corticosteroid injections had statistically significant improvement in shoulder pain than those who received landmark
guided corticosteroid injections.\textsuperscript{34} As a result, in future studies an ultrasound-guided injection will most likely lead to more accurate injections and less room for error. It is essential to control for the differences in administration of PRP to make studies more generalizable and limit confounding.

*Antiplatelet and Non-steroidal Anti-inflammatory Drugs (NSAID)*

Besides PRP preparation and administration, another factor that can significantly impact the results of any study using PRP is non-steroidal anti-inflammatory drug (NSAID) or antiplatelet drug use. The ideology behind PRP injections is that manually injecting a high concentration of platelets and growth factors into an area of injury will stimulate healing. NSAIDs and antiplatelet medications directly contradict this idea as they work to inhibit growth factor release by competitively and sometimes irreversibly inhibiting COX 1 and or COX 2. Frey et al. discovered that antiplatelet medications might decrease the growth factor release profile in a COX 1 and COX 2 dependent manner following a systematic review of the literature.\textsuperscript{35} But the existing data seems somewhat inconclusive. A controlled laboratory study by Jayaram et al. found that 14 days of aspirin intake had no significant effect on any of the growth factor concentrations in PRP.\textsuperscript{36} Yet, in a study by Zhao et al., volunteers were given aspirin, clopidogrel, dipyridamole, or a combination of those medications, and PDGF levels were measured. In the group receiving aspirin, there was a significantly decreased concentration of PDGF.\textsuperscript{37} Antiplatelet medications may also limit the effects of PRP by inhibiting platelet aggregation which can be seen in the Schipperinger et al. study. This study concluded that subjects who were taking NSAIDs in the two weeks prior to having their blood used for
PRP had significantly impaired platelet aggregation via light transmission aggregometry testing.\textsuperscript{38}

While the impact of NSAIDs and antiplatelet medications on the efficacy of PRP remains somewhat inconclusive, they should also be controlled for in studies because they could impact subjective outcomes related to pain. Many studies examining the efficacy of PRP use VAS or another measure of pain as an outcome. Pain is a subjective outcome that varies by person. For instance, the Rowden et al. study that evaluated the use of PRP in the evaluation of acute ankle sprains in the emergency room failed to control for pain management between subjects.\textsuperscript{21} Most likely, these patients took various medications that impacted their pain levels. As a result, it is very hard to draw conclusions about PRP’s effect on pain if there is no control for NSAID or pain medication use. Clearly, it is necessary to control for NSAID use in PRP studies because they can impact the efficacy of the treatment and the subjective outcomes related to pain.

\textit{Rehabilitation Protocols}

In orthopedics, the outcomes following an injury heavily depend on the rehabilitation after the injury. Following a lateral ankle sprain, the recommended rehabilitation protocol is RICE (rest, ice, compression, elevation) for the first 4-5 days, immobilization, early weight bearing in a CAM boot, and early physical therapy.\textsuperscript{39-41} While this protocol was shown to improve outcomes for patients with ankle sprains, patient adherence and variability in physical therapy are the most significant factors that limit outcomes. There is always variability in the structure of physical therapy regimens. Some studies utilized an unsupervised physical therapy program while others used supervised physical therapy. The majority of studies show that a supervised physical
therapy program leads to improved functional outcomes. For example, a randomized controlled trial comparing supervised versus unsupervised physical therapy for lumbar spinal stenosis found that supervised physical therapy produced greater improvement in symptom severity, physical function, and decreased likelihood of surgery.\textsuperscript{42} Partially, the variability in outcomes could be due to low patient adherence to unsupervised therapy. As far as patient adherence to rehabilitation protocols goes, a systematic review by Peek et al. found that, on average, only about 67\% percent of patients adhere to their at-home rehabilitation programs.\textsuperscript{43} An adherence rate this low may lead to extreme variability in outcomes related to the recovery following an injury. Moreover, there is variability in the specific physical therapy program between training flexibility, strength, proprioception, joint mobilization, and pain control. A consistent therapy program between subjects is vital in a study because it will lead to more reproducible outcomes. For instance, if one program includes manual joint mobilization and another does not, then this might cause confounding. Evidence of this can be seen in a systematic review by Loudon et al., where manual joint mobilization was found to improve ankle dorsiflexion, pain reduction, and improved stride length.\textsuperscript{44} Patient adherence, the structure of rehabilitation, and specificity of rehabilitation are imperative factors that must be controlled to limit confounding in any trial involving physical therapy.

**Review of Relevant Methods**

*Participant Selection*

In order to ensure adequate reliability and validity between studies, it is important to consider the inclusion and exclusion criteria used to select a population. To begin, in the literature reviewed, the grade of injury was frequently included in the inclusion
criteria. When applicable, the grades varied from a grade one injury to a grade three injury. Since, the grading of an injury affects the expected healing time, grading accuracy is extremely critical. There was great variability between clinical diagnoses of grade,\textsuperscript{16,18,20,21} MRI graded\textsuperscript{7,9,12,19,22,45}, and ultrasound graded\textsuperscript{46} injuries. The imagining modality shown to have the most specificity and sensitivity was MRI. Furthermore, it is recommended that 3.0 Tesla MRI be used in the imaging as it provides more detailed imaging and better diagnostic specificity and sensitivity. One study comparing 1.5 Tesla and 3.0 Tesla MRI imaging of ankle ligament pathology found that 3.0 Tesla imaging had a statistically significant higher average sensitivity of 0.69 compared to 0.50 with the 1.5 Tesla imaging.\textsuperscript{46}

Another area studies differ is in the inclusion of athletes versus the general public. As seen by the existing literature, there is evidence that PRP may be more effective in athletes. Conversely, it is also imperative to set strict exclusion criteria to limit confounding. Some crucial factors seen in the exclusion criteria in the existing literature were prior ankle injuries, prior ankle surgery, prior injection to the site, use of NSAIDs or antiplatelet medication, and history of diabetes. Many of these criteria were discussed in the confounding section above, which explains why they were used as exclusion criteria. A more detailed list of inclusion and exclusion criteria is discussed in chapter three.

Return to Play

When evaluating the literature, it is essential to keep in mind that the findings of any study come with a series of limitations. Many sports medicine studies will look at the time to return to play as a key outcome to evaluate the efficacy of a treatment. While time to return to play is important, we must remember that there is an inconsistency between
return to play criteria across studies limiting the study’s reliability. The Laver et al. study used four functional tests to designate a player was ready to return to play; there is no evidence to back the test they selected are the best indicator that an athlete was ready to return to play.\textsuperscript{18} Moreover, the researchers were not blinded to the athlete’s treatment allocation, which could lead to a substantial amount of bias. In contrast, the Samara et al. study, which is another study that focused on the return to play time, used a graded in-house sport-specific physical fitness test. Besides the potential for bias using in-house, non-blinded physical therapists, there was no explanation of what tests were part of the physical fitness test. While these two studies examined the same primary outcome in the same injury, it is difficult to conclude that these results would be reproducible. The need for a consistent return to play criteria was highlighted in a systematic review titled “Lack of Consensus on Return-to-Sport Criteria Following Lateral Ankle Sprain,” which concluded a need to determine assessments and cutoff thresholds to minimize recurrent LAS risk.\textsuperscript{47} But in 2021, Smith et al. made the first attempt to create a framework for return to play after a lateral ankle sprain. After surveying 155 health professionals involved with elite sports teams. They determined that the return to play decision should depend on five domains. The domains are “Pain (during sports participation and over the last 24 hours), Ankle impairments (range of motion; muscle strength, endurance, and power), Athlete perception (perceived ankle confidence/reassurance and stability; psychological readiness), Sensorimotor control (proprioception; dynamic postural control/balance), Sport/functional performance (hopping, jumping and agility; sport-specific drills; ability to complete a full training session)” which create the acronym PAASS for the framework.\textsuperscript{48} While the PAASS is useful for guiding decisions because it
considers a broad spectrum of factors such as functional performance, athlete perspectives, and clinical impressions it lacks standardized evaluations for these factors. Therefore, for the proposed study, we have included suggested methods of evaluating each factor to limit variability see Appendix E. Ultimately, the decision to return to play will be made by a blinded clinician to limit bias, and decisions will be guided by the PAASS framework to make the results more consistent and generalizable.

Visual Analog Scale (VAS)

A common and universal measure of pain that has been consistent throughout the literature is the Visual Analog Scale (VAS). Despite the type of injury examined, the VAS was used to describe the pain felt by a patient. The issue with VAS is that it is a subjective measure that can vary significantly from person to person, but that is because pain is subjective. There is no objective measure of pain that exists or has been used in any of the literature examined. Although one study did use the Foot Function Index pain score\textsuperscript{16} and not VAS, the proposed study will be using VAS as a secondary outcome. This will be most consistent with the literature and provide more generalizability in its results.

American Orthopedic Foot and Ankle Society Scale (AOFAS)

There is much more heterogeneity in the measurements of function in the literature. Depending on the injury, a variety of scales have been developed to measure functionality. In the randomized controlled trials evaluating PRP in foot and ankle pathologies, various functional measures were used. The measures used were the American Orthopedic Foot and Ankle Society scale (AOFAS)\textsuperscript{11,16,20}, Lower Extremity Function Scale\textsuperscript{21}, Roles and Maudsley score\textsuperscript{17}, and non-standardized study-specific
measurements\textsuperscript{18,19}. The AOFAS is one of the most commonly used measures of outcome in patients with an ankle injury. One of the reasons this tool has gained popularity is that it is not solely a patient-reported outcome, but rather a combination of physician-reported and patient-reported outcomes. This makes the measure less biased and increases its reliability and validity. Due to the high frequency of use in ankle injuries and the superiority over other functional outcome measures, the proposed study will measure functionality with the AOFAS.

\textit{Blinding}

In the literature, various blinding methods were implemented to limit performance bias within the study. It is proven that the most effective study design for limiting bias is a double-blind design where both the participant and examiner are blinded to the allocations. Of the studies reviewed, only a handful were double-blind designs\textsuperscript{16,17,21,45}. All of these studies withdrew a set amount of blood from all the participants to keep the participant blinded. In those that received the non-PRP injection, their blood was discarded. The more significant challenge came with blinding the researchers. Many of the studies just stated that they had a researcher who was blinded to the allocation give the injection, but this may not be effective if the researcher could visualize the syringe contents. Rowden et al. had the most effective method of blinding the researchers. An unblinded research assistant prepared the platelet-rich plasma solutions and the placebo syringes containing saline and then taped the syringe to block the contents. The research assistant then gave the investigator the appropriate taped syringe, blinding both the participant and investigator from the allocation\textsuperscript{21}. The proposed study will use a similar
double-blind design as the one detailed in the Rowden et al. study as it seemed to be the most effective method.

*Platelet-rich Plasma Kit and Dosage*

As discussed previously, there is significant variation between PRP preparation kits and the adequate amount of PRP to be used. The literature review found extreme variation between preparation methods, with many studies giving very little detail on how the PRP was produced. Of the studies reviewed, the most frequently used kit was the GPS III. And, based on a systematic review comparing the kits, the GPS III was one of the kits capable of producing 3 to 6 times the baseline concentration of platelets and growth factors which was found to be an effective therapeutic concentration. The proposed study will use the GPS III kit to be consistent with the trends in the field.

The studies also fluctuated in the amount of PRP injected into the injury site. Anywhere from one ml to seven ml of PRP was used in the studies reviewed. Since there are no guidelines to direct what amount of PRP should be used, the proposed study will use the mean amount of all the reviewed studies examining PRP use in ankle injuries. These studies used anywhere from 1.5 ml to 5 ml of PRP with the mean amount being three mL.

*Follow-up*

The average recovery time from a grade III lateral ankle sprain is around 42 days. Since it is an acute injury, the follow-up time will be very different from some other chronic injuries where the recovery time is much longer. Of the studies studying PRP in lateral ankle sprains, the Blanco-Rivera et al. study had follow-up at 3, 5, 8, and 24 weeks. This is surprising as this study included only grade II lateral ankle sprains which
have an even shorter recovery period than a grade III sprain. It is crucial to have a long-term follow-up to evaluate for function, but 24 weeks for an acute injury is unnecessary. The study found no difference in function or pain between either group at 24 weeks. The other study evaluating lateral ankle sprains was done by Rowden et al. In contrast, this study had a much shorter follow-up. The study evaluated LEFS and VAS at days 0, 3, and 8 and had a phone call follow-up on day 30 to obtain VAS and LEFS scores. This study’s follow-up time was much too short to detect any noticeable difference which is most likely why it found no significant difference between the groups. While the existing literature does not give a reasonable estimate of the follow-up time, it is reasonable to believe that the appropriate follow-up should be somewhere in between that of the Blanco-Rivera et al. study and the Rowden et al. study. As a result of this, the proposed study will have a follow-up at 2, 4, 5, 6, 8, and 12 weeks.

Statistical Analysis and Statistical Significance

The primary outcome of time to return to play could be operationalized in multiple ways. The way the outcome is operationalized affects the statistical test used to analyze the data. In the literature, the majority of the studies operationalized the return to play time as mean days, but few studies operationalized it as a time to event. The Cox proportional hazard test would analyze time to event data, but that does not seem necessary for the proposed study. Cox proportional hazard analysis is often used to analyze survival data and give the probability of an event happening in a given population. Since lateral ankle sprains are an acute injury where everyone ends up returning to play eventually, the proposed study will not be analyzing the return to play time with the cox proportional hazards model. Instead, the proposed study will use an
independent t-test to analyze the two groups’ mean days to return to play. This method is more consistent with how the data has been analyzed in similar literature.

The majority of the literature on the effect of PRP in ankle injuries calculated statistical significance using an alpha of 0.05 and power of 80%. Our study will utilize the same alpha and power to determine statistical significance and calculate our sample size; see Chapter Three and Appendix F for more details.

Sample Size

While ankle sprains are a frequent injury, all of the studies evaluating PRP had a small sample size. Most likely, the reason for this is that platelet-rich plasma is not frequently used to treat such an acute injury. Since the literature did not contain any evidence evaluating the return to play time in athletes with a lateral ankle sprain treated with PRP, other studies needed to be used to calculate the sample size.

Laver et al. evaluated the return to play time in athletes with a high ankle sprain. The study had a sample size of 16 athletes, with 8 in the intervention group and 8 in the control group. The findings were that the PRP intervention group had a mean-days to return to play of 40.8 ± 8.9 days, while the control group had a mean-days to return to play of 59.6 ± 12.0 days. We used this information to calculate the effect size for the proposed study. The effect size in the Laver et al. study was 35%. This study was selected to estimate the effect size in the proposed study because it looked at the intervention in athletes, and all the syndesmotic sprains included were grade III. Even though high ankle sprains have a much lengthier recovery period than lateral ankle sprains, we expect a similar effect size due to the similarity of the populations and the similarity in the grade of the injury. Secondly, the Malliaropoulos et al. study found that
track athletes with a grade III lateral ankle sprain, managed with standard of care
treatment, had an average time to return to sport of 42 days. This study subdivided
grade III injuries into grade IIIa and grade IIIb injuries based on clinical features. For the
sample size calculation, we used the average return to play of both subcategories of grade
III injuries. We used this information as our baseline expected mean return to play time in
the control group. Of the current literature reviewed, the majority of the studies used an
alpha of .05 and a power of 80%. To be consistent with the current literature, we also
used an alpha of .05 and a power of 80% when calculating our sample size. Based on the
effect size from Laver et al., the baseline data from Malliaropoulos et al., and the alpha
and power parameters, the proposed study needs to have a sample size of 24 participants.
To account for a loss to follow up of 15%, we will recruit a total of 28 participants, with
14 in each group. Details of the calculation can be found in chapter 3 and Appendix F.

Conclusion

The literature review illustrates why there is a need for the proposed study. There
is promising evidence for the use of platelet-rich plasma injections to hasten recovery
time in athletes. Furthermore, platelet-rich plasma is likely not effective in every type of
injury, but in the current evidence, there are suggestions of positive results for its use in
various foot and ankle pathologies. To date, there has never been a randomized controlled
trial evaluating the efficacy of platelet-rich plasma in grade III lateral ankle sprains.
Therefore, the study proposed in chapter 3 will evaluate if platelet-rich plasma is
effective in reducing the return to play time for athletes with a grade III lateral ankle
sprain. Ultimately, uncovering if PRP used as an adjunct therapy will help treat one of the
most common injuries in athletes.
References


Chapter 3: Study Methods

Study Design:

The proposed study will be a multicenter, double-blind, randomized, placebo-controlled trial. We will be evaluating the effect of a single platelet-rich plasma injection on the mean days to return to play in athletes with a grade III lateral ankle sprain. When athletes are evaluated at their team medical facilities and determined to have a grade III lateral ankle sprain via 3.0 Tesla MRI imaging, they will have the option to be enrolled in the trial. Enrolled athletes will be randomized to receive the intervention of a single PRP injection or a placebo injection of saline. The primary hypothesis of the study is athletes with a grade III lateral ankle sprain who receive a single injection of platelet-rich plasma will have fewer days to return to play than athletes who receive a placebo injection.

Study Population and Sampling:

The study will include athletes over the age of 18 years old who present to a sports medicine facility with an MRI confirmed grade III (complete tear of ATFL) lateral ankle sprain with the injury occurring within the last three days. We will include the following five sports medical programs: Yale Sports Medicine, Hartford Healthcare Sports Medicine, Hospital for Special Surgery Sports Medicine Institute, New York-Presbyterian Sports Medicine, and New York University Langone Sports Medicine Center. Each of these groups is associated with multiple professional and collegiate sports teams and will have access to the necessary equipment to carry out the study. Convenience sampling will be used to find participants who are presenting to these centers with a grade III lateral ankle sprain. A medical provider and research assistant
will be placed at each site to conduct the care necessary for the trial. A clinical research coordinator will oversee that proper protocol is followed and provide the necessary support to each research site. Each site will receive the necessary medical equipment to conduct the research trial. A complete proposal of the study will be submitted to the central Institutional Review Board (IRB). Upon approval from the IRB, participants will begin to be recruited for the trial.

To be included in the study, participants must be older than 18 years of age, actively participate in sports at the collegiate or professional level, and have an MRI confirmed complete tear (grade III) of the anterior talofibular ligament that occurred in the last 72 hours. Participants will be excluded from the study if they have an associated fracture, have had previous ankle injections, have had surgery on their affected ankle, are currently using NSAIDs or antiplatelet medication, have prior sprains to the affected ankle, have diabetes, are pregnant, or are not mentally capable of participating. See detailed inclusion and exclusion criteria in Table 1. If the participant meets all the inclusion criteria and none of the exclusion criteria, then they will be invited to participate in the trial.

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
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<td>• Greater than 18 years of age</td>
<td>• Less than 18 years of age</td>
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<tr>
<td>• Actively participating in a sport at the collegiate or professional level</td>
<td>• Associated fracture involved in the ankle injury</td>
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<tr>
<td>• MRI confirmed grade III lateral ankle sprain</td>
<td>• Had previous injections or surgeries to affected ankle</td>
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</table>
- Ankle sprain occurred within the last 72 hours
- Had prior sprains to affected ankle
- Used antiplatelet medication or NSAIDs in the last ten days
- Have diabetes
- are currently pregnant
- not mentally capable of participating in the study

<table>
<thead>
<tr>
<th>Table 1: Inclusion and exclusion criteria</th>
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<td><strong>Subject Protection and Confidentiality:</strong></td>
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Prior to the start of the study, an application will be reviewed by the Institutional Review Board. If approved by the IRB, participants will be required to sign a consent form describing the study, explain the risks and benefits, economic considerations, information about voluntarily withdrawing, and confidentiality and privacy agreements (Appendix A).

To protect the patient’s identity, it will be required that all researchers involved in conducting the trial will be certified through Health Insurance Portability and Accountability Act (HIPAA). All patient data will be password protected and stored on encrypted devices. Only members of the research team will have access to patient information. For added security, patient information will be de-identified and assigned to randomized identification numbers. After analyzing patient information, all patient records will be destroyed appropriately and disposed of.
Recruitment:

Subjects will be recruited for the study via referral from the selected sports medical centers participating in the trial. When an athlete presents to the medical center with an MRI confirmed grade III lateral ankle sprain, the clinicians at the participating center would then refer that athlete to be enrolled in the trial. If a patient agrees to participate in the trial, they will be asked to fill out the baseline demographics survey (Appendix G) and sign a consent form (Appendix A). All participants will be treated at the sports medicine centers they were referred from. The clinical sites will receive financial compensation for their participation and use of facilities. The athletes will not receive compensation; intrinsic motivation to return to their sport will entice them to participate.

Baseline Characteristics:

In order to ensure similarity between the intervention and control group, we will control for several baseline characteristics. The baseline characteristics being evaluated will be age, gender, race, body mass index, affected ankle, sport, level of sport, baseline VAS, and baseline AOFAS. Baseline characteristics will be assessed via the baseline characteristics survey patients will receive at enrollment (Appendix G). Multiple linear regression will be used to identify statistically significant differences in baseline characteristics between study groups. See Table 2 for a description of baseline characteristics and statistical analysis method.
<table>
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<tr>
<th>Characteristic</th>
<th>Variable type</th>
<th>Analysis method</th>
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<td>BMI</td>
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<tr>
<td>Baseline AOFAS</td>
<td>Continuous</td>
<td>Student T-test</td>
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Table 2: Baseline characteristics, variable type, and statistical analysis

**Study Variables:**

The independent variable will be a single injection of three mL of PRP plasma injected into the anterior talofibular ligament under ultrasound guidance. The control will be a single injection of three mL of saline injected into the anterior talofibular ligament under ultrasound guidance. Athletes will receive their allocated injection within three days of a grade III lateral ankle sprain. The primary outcome will be the number of days until the athlete is able to return to play. Blinded clinicians will make the return to play decision based on the PAASS framework (Appendix E). According to the PAASS guidelines, to be cleared for return to play, athletes should exhibit confidence in their readiness to return, have minimal pain, have minimal ankle impairment, exhibit adequate
sensorimotor control, and exhibit adequate functional performance on sport-specific activities. Athletes must meet all five domains of the PAASS framework to be cleared to return to competitive play.

The secondary outcomes that will be measured are pain and ankle function. Pain will be measured via the Visual Analog Scale (VAS) (Appendix D), and ankle function will be via the American Orthopedic Foot and Ankle Score (AOFAS) (Appendix C). Return to play, VAS, and AOFAS will be measured at 2, 4, 5, 6, 8, and 12 weeks from the date of injection. Athletes will also have the opportunity to request return to play evaluations as they feel ready; they will not need to wait until the set follow-up periods. Return to play evaluations do not need to be continued once the athlete is cleared to return to play. Pain and function must still be measured until the 12-week mark.

**Assignment of Intervention:**

As athletes present to the enrolled athletic centers and meet the required criteria to participate in the study, they will be randomized in a 1:1 ratio to the intervention (platelet-rich plasma injection) or the control (saline injection) group. The randomization will be done by a computer program that will use subjects from all five sites. The online program will use block randomization to randomly assign participants into a block of 14 for the intervention and a block of 14 for the control. Participants and physician investigators will be blinded to the allocations. The research assistants will not be blinded as they must know whom to prepare PRP injections for and whom to prepare saline injections for. All participants will have blood drawn, receive the same information on the risks of PRP, and follow the same rehabilitation protocol despite their allocation.
Platelet-rich Plasma Preparation and Administration:

After consenting to the study, the athlete will have 55 mL of whole blood drawn from the median cubital vein using an 18-gauge needle. This is the specified amount of whole blood required for the Zimmer Biomet GPS III system. The research assistant will be notified of the participant’s treatment allocation via the computer software that will randomize allocations. At this time, the research assistant will either prepare three mL of platelet-rich plasma or three mL of saline based on the participant’s treatment allocation. The assistant will use the Zimmer Biomet GPS III machine for all platelet-rich plasma preparation. Platelet-rich plasma will be prepared according to the manufacturer’s instructions. The assistant will place 55 mL of the participant’s blood into the container and place it into the centrifuge. They will then counterbalance the centrifuge with 55 mL of saline in another container. As per the manufacturer’s guidelines, a single centrifugation at 3200 rpm for 15 minutes will be performed. First, the research assistant will remove the platelet-poor plasma via the yellow port on the container. Then, the research assistant will draw up three mL of platelet-rich plasma via the red port into a ten mL syringe. The assistant will then hand the correct allocated syringe to the clinician to administer the injection in the injured anterior talofibular ligament under ultrasound guidance. This will be day zero. All baseline measurements of pain and function will be done.

Blinding of Intervention:

As a means of limiting bias, both the researchers and the subjects will be blinded from the intervention. To achieve this, all participants will have 55 mL of blood drawn at the start of the trial despite their assignment to the intervention or control group. Those
assigned to the control group will have their blood properly discarded by a research assistant. Those assigned to the intervention group will have the PRP injection prepared by a research assistant as stated in the guidelines of the previous section. All syringes, despite intervention or control, will be wrapped in tape to hide the contents inside. The syringes will contain either three milliliters of PRP (intervention) or three milliliters of saline (control). Once the syringe is prepared for the correct patient based on their allocation, the research assistant will give the syringe to the clinician to perform the injection under ultrasound guidance. Both the clinician and the participant will be unable to see the contents of the syringe and thus will be blinded from knowing the intervention allocation.

**Rehabilitation Protocol:**

Once the participant has received their injection, they will receive the standard of care treatment for a grade III lateral ankle sprain. Athletes will be instructed to elevate the ankle above the level of the heart and apply ice to the area for 10-15 minutes as needed for the first five days. They will also have their ankle immobilized via a CAM boot, be given crutches to assist with movement, but instructed to weight bear as tolerated. This is the current standard of care and has been shown to improve functional outcomes. On day six, participants will begin to start their physical therapy program. All participants will follow this protocol, despite their intervention allocation. Patients will be able to remove the CAM boot after two weeks, at this time they may begin evaluations to return to sport. Each site will be given the same six-week physical therapy program for the participants to follow (Appendix B).
**Adherence:**

All participants will be asked to adhere to the standard of care RICE, immobilization, and a 6-week physical therapy program. In order to improve adherence to the rehabilitation program, all patients will undergo supervised physical therapy. Studies have shown that physical therapy supervision improves adherence and outcomes. All participants will be required to attend three physical therapy sessions per week and have the therapist sign off on their participation via the physical therapy program (Appendix B).

**Data Collection:**

The clinicians will collect data at 2, 4, 5, 6, 8, and 12 weeks from the injection date. At each of these benchmarks, the clinicians will use the PAASS framework (Appendix E) to evaluate whether the patient is eligible to return to play. Athletes will also be able to request return to play evaluations outside of these set dates based on how they feel. The secondary outcome measures will only be measured at the set dates. The measured secondary outcomes will be pain, based on the Visual Analog Scale (Appendix D), and function, based on the American Orthopedic Foot and Ankle Score (Appendix C). All clinicians will be trained to make measurements on the necessary scoring systems being used. See Figure 1 for more information on data collection.
**Figure 1.** Timeline for individual participant data collection

**Monitoring Adverse Events:**

In order to monitor for adverse events, patients will have frequent and close follow up with a clinician. With PRP, extreme adverse effects are very unlikely as there have not been any adverse effects observed outside of those expected with any injection.\(^3\)

With PRP or any injections, there is still a chance of adverse events like bleeding, tissue damage, infection, or nerve injury. As a result of this, when patient outcomes are measured at each follow-up date, clinicians will also be asking and examining for signs of adverse events. The acuity of the injury being tested requires frequent follow-up; thus, there will be frequent monitoring for adverse events.

**Sample Size Calculation:**

As outlined in Chapter Two, the Laver et al. study was used to calculate the effect size of 35% for platelet-rich plasma injections’ effect on return to play.\(^4\) We used the data from the Malliaropoulos et al. study of 42 days\(^5\) for the baseline for return to play time with the current standard of care. Return to play time is a continuous variable and will be operationalized as the number of days till an athlete can return to competitive play.
Assuming that a continuous normally distributed outcome would be observed in the intervention and control group, the sample size was calculated using the ClinCalc LLC, Sample Size Calculator. Using an alpha of .05 and with 80% power on a two-tailed hypothesis, a minimum sample size of 24 athletes is required (Appendix F). In order to account for loss to follow up at an estimated rate of 15%, an additional four athletes will be recruited. This will give us a final sample size of 28 athletes total, with 14 in the intervention group and 14 in the control group. See Appendix F for more information on sample size calculation.

**Analysis:**

The primary outcome of return to play time will be operationalized as the number of days till an athlete can return to competitive play after being cleared on the PAASS framework. The mean number of days to return to play will be measured for the intervention and control groups. A student t-test will be used to compare the mean days to return to play between the two groups. The secondary outcome of pain and function will be measured on the VAS scale and AOFAS scale, respectively. They will be operationalized as a mean score for each group. A student t-test will be used to analyze the secondary outcomes between the two groups. All baseline characteristics will be analyzed using the stated methods in Table 2 and multiple linear regression. A P-value less than .05 will be necessary to define a result as statistically significant. All statistical data will be analyzed using the intention-to-treat protocol.
**Timeline and Resources:**

Participants will be recruited over the course of 20 months or until the desired sample size is reached. At 20 months, participants will no longer be recruited because they need to have at least 12 weeks of follow-up following their injection. The entire timeline of the study will not exceed two years. Once enrolled, the patient will receive a single injection based on their allocation in the study. This will be day zero. A clinician will see the patients at 2, 4, 5, 6, 8, and 12 weeks from day zero. See Figure 1 for more details on the timeline.

The sites enrolled in the study will be required to have specific resources such as access to 3.0 Tesla MRI, access to ultrasound, and access to physical therapy equipment and physical therapists. Once a site is enrolled, a research assistant will be sent to the site to assist with following the study protocol. Each site will be provided with a platelet-rich plasma preparation kit (GPS III), phlebotomy supplies, and syringes. The research assistants will prepare the platelet-rich plasma injections based on the proposed guidelines to limit variability based on sites. A blinded site clinician will be administering the injections and measuring the outcomes at the specified follow-up dates.

**References**


Chapter 4: Conclusion

Advantages:

The proposed study has a number of strengths, the first being its novelty. This study would be the first randomized controlled trial to evaluate the effect of platelet-rich plasma in grade III lateral ankle sprains. The existing literature has only evaluated the effects in high ankle sprains or less severe lateral ankle sprains with low-quality evidence.\textsuperscript{1-4} The severity and longer recovery from grade III lateral ankle sprains makes them an optimal study candidate to see the effects of platelet-rich plasma injections. An additional strength of the study is the use of blinding and randomization as a means of increasing internal validity. The use of a computer program to randomly assign patients to their intervention limits selection bias within the sample. Furthermore, blinding both the researchers and the participants from their allocations by using opaque syringes for the injections helps limit confirmation bias and observer bias. Lastly, the proposed study has clearly stated guidelines on the exact preparation method and administration of the platelet-rich plasma. There is extreme variability in platelet concentration depending on the preparation, as a review found doses of platelets in injections can vary from 0.21 to 5.43 billion, corresponding to a 25-fold difference.\textsuperscript{5} The goal of clearly stating the preparation and administration method was to help limit the variability and make the study results more reproducible. Overall, the strengths of the proposed study were its novelty, internal validity, and reproducibility.
Limitations:

Along with the strengths, the proposed study also has various limitations. The first limitation is the cost of conducting this study. The study requires MRI usage, multiple platelet-rich plasma preparation kits, and multiple medical personnel (medical clinicians, research assistants, MRI technicians, and physical therapists). Ideally, the costs could be reduced by conducting the study at just one center over a more extended period of time to reach the required sample size. An additional limitation of the study is that it is targeted at a very niche population, limiting the generalizability of the results. The main reason for including only collegiate and professional athletes is that this is a population where the high cost of platelet-rich plasma injections for an acute injury is more justifiable. Largely because there is financial support from the organizations the athletes belong to, and there may be financial benefits for the organization if they can return an athlete to play sooner. It is tough to justify the high cost of platelet-rich injections for an acute injury in the general public as there are mixed results regarding its efficacy. Another limitation of the study is there may be difficulty recruiting subjects due to strict inclusion and exclusion criteria. The 72-hour window from the time of injury to the time of injection is a small window of time which may make it difficult to find subjects. Furthermore, excluding subjects who have taken NSAIDs or antiplatelet medication in the last ten days may also make recruitment difficult, as NSAIDs are commonly taken for lateral ankle sprains. Therefore, by making this study a multicenter design with a long recruitment time for a small sample size, we hope this will combat these recruitment difficulties. The final limitation of the proposed study is that the return to play decision is being made based on a clinician’s judgment. This may limit the study’s external validity as the decision is
subjective. In order to limit the subjectivity of the decision, we proposed that clinicians make their decision based on the PAASS guidelines, so all clinicians will be considering the same factors when making their decisions. Also, the reason the study is a double-blind design is so the clinicians will not be subject to as much bias when making their decisions. All in all, the proposed study has limitations regarding the cost of the study, recruitment, generalizability, and a subjective outcome measure.

Clinical Significance:

Ankle sprains are an extremely prevalent injury, with over 2 million ankle sprains treated annually in the United States and the United Kingdom in just emergency rooms, with countless more treated in other settings. Of all these ankle sprains, nearly half of them occur during sports. This is an extremely common athletic injury that keeps athletes off the field, can limit athletic performance, and even lead to chronic reinjury. Athletes with a grade III lateral ankle sprain treated with the standard of care usually miss an average of six weeks of play but, in some cases, may miss up to ten weeks. Ideally, PRP injections could be used in conjunction with the current standard of care to hasten the recovery time for athletes. Thus, the proposed study will evaluate if platelet-rich plasma has an effect on ankle sprain recovery times. Additionally, the study will also reveal the effects of platelet-rich plasma on other outcomes like pain and ankle function. The findings of the study could implicate an adjuvant therapy that has the potential to shorten recovery periods, decrease pain, and improve function for an extremely common injury. Or, it could reveal that PRP has no effect on recovery time, function, or pain which can help clinicians and patients make more informed decisions regarding this costly therapy.
Furthermore, there are certain instances where surgical intervention may be needed for severe grade III lateral ankle sprains. The findings of this study could implement an alternative method of treating grade III lateral ankle sprains and decrease the need for surgery. While PRP injections are still expensive, they would be a cheaper option compared to surgical intervention.

If the study results were to show potential short-term benefits, then further studies should be done to evaluate the effects of PRP on long-term outcomes, as 40-50% of people will experience reinjury or residual symptoms following a lateral ankle sprain.⁸

References:
Appendix A: Authorization and Consent Form

CONSENT FOR PARTICIPATION IN A RESEARCH STUDY

YALE UNIVERSITY
YALE UNIVERSITY SCHOOL OF MEDICINE
YALE-NEW HAVEN HOSPITAL

Study Title: THE EFFECT OF PLATELET-RICH PLASMA ON RECOVERY IN ATHLETES WITH A GRADE III LATERAL ANKLE SPRAIN
Principal Investigator (the person who is responsible for this research): Matthew Attolino, PA-S2; Sean Peden, MD

Why is this study being offered to me?
You are invited to participate in a research study evaluating the effect of platelet-rich plasma injections on recovery from a grade III lateral ankle sprain. We are asking you to take part in this research study because you are a professional or collegiate athlete, older than 18 years old, who has been diagnosed with an MRI confirmed grade III lateral ankle sprain. We are looking for 28 participants to be part of this research study.

In order to decide whether or not you wish to be a part of this research study, you should know the study’s risks and benefits to make an informed decision. This consent form gives you detailed information about the research study. This form will go over all aspects of this research: the purpose, the procedures that will be performed, any risks of the procedures, possible benefits and possible alternative treatments. Once you understand the study, you will be asked if you wish to participate; if so, you will be asked to sign this form.

Who is paying for the study?
Yale University and Yale University School of Medicine

Who is providing other support for the study?
Yale Sports Medicine, Hartford Healthcare Sports Medicine, Hospital for Special Surgery Sports Medicine Institute, New York-Presbyterian Sports Medicine, and New York University Langone Sports Medicine Center.

What is the study about?
The purpose of this study is to investigate the effect of platelet-rich plasma injections on the recovery from a grade III lateral ankle sprain. The study will evaluate the impact a single platelet-rich plasma injection has on days to return to play, pain, and ankle function.

Platelet-rich plasma injections are a therapy where a subject has their whole blood drawn via phlebotomy. The whole blood is spun at high speeds to separate the various contents within the blood. The platelets and plasma are then removed from the whole blood and
injected into a site of injury. With the hope that by injecting the subject’s own platelets at higher concentration then in whole blood this will promote healing at that location.

**What are you asking me to do and how long will it take?**

If you agree to take part in this study, this is what will happen:

To begin you will receive an MRI of the injury ankle to confirm a grade III lateral ankle sprain if you have not already had one done.

You will be randomly assigned to receive an injection of platelet-rich plasma or saline. Randomization will be done using a computerized program and will not include any personal information. Both you and the clinicians giving the injection will not be aware of what treatment you will be receiving.

You will then have 55 mL of blood drawn to be used for the platelet-rich plasma injection. If you are assigned to receive the saline injection your blood will be properly discarded. If you are assigned to the platelet-rich plasma injection, a research assistant will prepare the injection with your 55 mL of blood you had drawn.

You will then receive 3 mL of your assigned injection into the injured ankle under ultrasound guidance by a clinician. The syringe will be taped to hide the contents of the injection from the participant and the clinician.

You will have your injured ankle placed into a supportive walking boot, be given crutches, and instructed to weight bear as tolerated. You will also be told to apply ice and elevation to the affected ankle as needed.

All participants will receive the same physical therapy program. It will be a 6-week program that will require 3 sessions per week. All physical therapy will be done by a certified physical therapist.

You will be required to attend 6 office visits following your visit today. You will need to be seen at 2, 4, 5, 6, 8, and 12 weeks from the date you received your injection. At each of these visits you will be examined by a clinician who will evaluate (1) if you are ready to return to play, (2) pain level via the Visual Analog Scale, and (3) ankle function via the American Orthopedic Foot and Ankle Scale.

Return to play decisions will be made based on the judgement of the clinician. All clinicians will be using the PAASS framework to make their decisions. The PAASS framework which considers pain, ankle impairment, athlete perception on readiness, sensorimotor control, and sport performance measures.

The length of time of the study from injection to conclusion will be 12 weeks.

All study participants will be kept informed of any major study developments as they occur. If for any reason we find that one treatment is superior to the other during the
course of the study, we will terminate the study and offer the superior treatment to all participants. Participants are encouraged to reach out to research staff if they have questions, concerns, or issues at any point in the trial. Patients will be able to withdraw from the study at any point with no penalty or consequences. This study and all relevant information will be listed on clinicaltrials.gov in accordance with national law. You are welcome to access information on this study at this site at any time.

**What are the risks and discomforts of participating?**
We do not anticipate any significant adverse effects to occur during the study. Both platelet-rich plasma and saline are considered to be safe therapies and there have been no significant adverse effects reported in other similar studies. The most probable adverse event would be bruising or pain at the injection site. Participants will be given ice packs if any of these events occur. Although unlikely, as with any injection there is a risk of bleeding, tissue damage, infection, or nerve injury. To minimize these risks injections will be done with sterile technique under ultrasound guidance.

**How can the study possibly benefit me?**
All participants will receive 6 weeks of physical therapy and the standard of care medical treatment for a lateral ankle sprain free of charge. Those assigned to the platelet-rich plasma group will also be receiving a platelet-rich plasma injection free of charge.

**Are there any costs to participation?**
You will not have to pay for taking part in this study. The only costs include transportation and your time coming to the study visits.

**Will I be paid for participation?**
Participants will not be compensated for their participation in the study.

**What are my choices if I decide not to take part in this study?**
If you do not wish to participate in the study, you may pay for treatment as you regularly would, you may pursue treatment from another provider, or you may continue conservative treatments on your own.

**How will you keep my data safe and private?**
Patient information will be de-identified and assigned to randomized identification numbers. After analyzing patient information, all patient records will be destroyed appropriately and disposed.

We will keep information we collect about you confidential. We will share it with others if you agree to it or when we have to do it because U.S. or State law requires it.
example, we will tell somebody if you we learn that you are hurting a child or an older person.

All subject's information or biospecimens collected as part of the research, even if identifiers are removed, will not be used or distributed for future research studies.

**What Information Will You Collect About Me in this Study?**

The information we are asking to use, and share is called “Protected Health Information.” It is protected by a federal law called the Privacy Rule of the Health Insurance Portability and Accountability Act (HIPAA). In general, we cannot use or share your health information for research without your permission. If you want, we can give you more information about the Privacy Rule. Also, if you have any questions about the Privacy Rule and your rights, you can speak to Yale Privacy Officer at 203-432-5919.

The specific information about you and your health that we will collect includes:

- Research study records
- Medical and laboratory records of only those services provided in connection with this study.
- Records about your study visits
- Information obtained during this research regarding
  - Physical exams
  - Laboratory, x-ray, and other test results
  - Records about any study drug you received

**How will you use and share my information?**

We will use your information to conduct the study described in this consent form. We may share your information with:

- The U.S. Department of Health and Human Services (DHHS) agencies
- Representatives from Yale University, the Yale Human Research Protection Program and the Institutional Review Board (the committee that reviews, approves, and monitors research on human participants), who are responsible for ensuring research compliance. These individuals are required to keep all information confidential.
- The study sponsor or manufacturer of study drug/device
- Health care providers who provide services to you in connection with this study.
- Laboratories and other individuals and organizations that analyze your health information in connection with this study, according to the study plan.
- Principal Investigator of the study
- Co-Investigators and other investigators
- Study Coordinator and Members of the Research Team
- Data and Safety Monitoring Boards and others authorized to monitor the conduct of the Study
We will do our best to make sure your information stays private. But, if we share information with people who do not have to follow the Privacy Rule, your information will no longer be protected by the Privacy Rule. Let us know if you have questions about this. However, to better protect your health information, agreements are in place with these individuals and/or companies that require that they keep your information confidential.

**What if I change my mind?**

The authorization to use and disclose your health information collected during your participation in this study will never expire. However, you may withdraw or take away your permission at any time. You may withdraw your permission by telling the study staff or by writing to Matthew Attolino at the Yale University, New Haven, CT 06520.

If you withdraw your permission, you will not be able to stay in this study but the care you get from your doctor outside this study will not change. No new health information identifying you will be gathered after the date you withdraw. Information that has already been collected may still be used and given to others until the end of the research study to ensure the integrity of the study and/or study oversight.

**What if I want to refuse or end participation before the study is over?**

Taking part in this study is your choice. You can choose to take part, or you can choose not to take part in this study. You also can change your mind at any time. Whatever choice you make, you will not lose access to your medical care or give up any legal rights or benefits.

We would still treat you with standard therapy or, at your request, refer you to a clinic or doctor who can offer this treatment. Not participating or withdrawing later will not harm your relationship with your own doctors or with this institution.

To withdraw from the study, you can call a member of the research team at any time and tell them that you no longer want to take part.

The researchers may withdraw you from participating in the research if necessary.
Who should I contact if I have questions?

Please feel free to ask about anything you don't understand.

If you have questions later or if you have a research-related problem, you can call the Co-Principal Investigator Matthew Attolino.

If you have questions about your rights as a research participant, or you have complaints about this research, you call the Yale Institutional Review Boards at (203) 785-4688 or email hrpp@yale.edu.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Authorization and Permission

I have read (or someone has read to me) this form and have decided to continue to participate in the project described above. Its general purposes, the particulars of involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent addendum form.

Your signature below indicates that you have read this consent document and that you agree to be in this study.

We will give you a copy of this form.

<table>
<thead>
<tr>
<th>Participant Printed Name</th>
<th>Participant Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Person Obtaining Consent Printed Name</th>
<th>Person Obtaining Consent Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

If after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at (203) 432-5919.

If you have further questions about this project or if you have a research-related problem, you may contact the Co-principal Investigator, Matthew Attolino. If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.
Appendix B: Physical Therapy Program

6 Week Physical Therapy Program
You are required to attend 3 physical therapy sessions per week. Please have physical therapist initial next to exercise each time you complete it (first session-initial next to 1. in Week 1).

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range-of-motion (dorsiflexion, plantarflexion, inversion, eversion)</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
</tr>
<tr>
<td>Progress to resistance band</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
</tr>
<tr>
<td>Balance on one leg</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
</tr>
<tr>
<td>Progress to one leg on wobble board</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
</tr>
<tr>
<td>Balance on one leg and play catch with partner</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
</tr>
<tr>
<td>Progress to one leg catch on wobble board</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
</tr>
<tr>
<td>One-leg mini squats with other leg extended in different directions</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
</tr>
<tr>
<td>Progress to single leg hoping to step</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
</tr>
<tr>
<td>Squats on stable surface</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
</tr>
<tr>
<td>Progress to Standing squat jumps: start in a squat position; jump and land softly</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
</tr>
<tr>
<td>Calf raises</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
</tr>
<tr>
<td>Progress to weighted calf raises</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
</tr>
<tr>
<td>Lunges on stable surface</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
</tr>
<tr>
<td>Progress to Scissor hops: start in a lunge position; jump and land with the other foot forward</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
</tr>
<tr>
<td>Walking</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
</tr>
<tr>
<td>Progress to Bounding: take large bounding steps at 50 percent of maximal running speed</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
<td>1.</td>
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<tr>
<td>Progress to running</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
<td>2.</td>
</tr>
<tr>
<td>Side step</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
<td>3.</td>
</tr>
<tr>
<td>Progress to shuffling</td>
<td></td>
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</tr>
</tbody>
</table>
Appendix C: American Orthopedic Foot and Ankle Society Scale

**AOFAS Ankle-Hindfoot Scale**

**I. Pain (40 points)**
- □ None +40
- □ Mild, occasional +30
- □ Moderate, daily +20
- □ Severe, almost always present +0

**II. Function (50 points)**

**Activity limitations, support requirements**
- □ No limitations, no support +10
- □ No limitation of daily activities, limitations of recreational activities, no support +7
- □ Limited daily and recreational activities, cane +4
- □ Severe limitation of daily and recreational activities, walker, crutches, wheelchair, brace +0

**Maximum walking distance, blocks**
- □ Greater than six +5
- □ Four-six +4
- □ One-three +2
- □ Less than one +0

**Walking surfaces**
- □ No difficulty on any surface +5
- □ Some difficulty on uneven terrain, stairs, inclines, ladders +3
- □ Severe difficulty on uneven terrain, stairs, inclines, ladders +0

**Gait abnormality**
- □ None, slight +8
- □ Obvious +4
- □ Marked +0

**Sagittal motion (flexion plus extension)**
- □ Normal or mild restriction (30° or more) +8
- □ Moderate restriction (15° - 29°) +4
- □ Severe restriction (less than 15°) +0

**Hindfoot motion (inversion plus eversion)**
- □ Normal or mild restriction (75% - 100% normal) +6
- □ Moderate restriction (25% - 74% normal) +3
- □ Marked restriction (less than 25% of normal) +0

**Ankle-hindfoot stability (anteroposterior, varus-valgus)**
- □ Stable +8
- □ Definitely unstable +0

**III. Alignment (10 points)**
- □ Good, plantigrade foot, ankle-hindfoot well aligned +10
- □ Fair, plantigrade foot, some degree of ankle-hindfoot malalignment observed, no symptoms +5
- □ Poor, nonplantigrade foot, severe malalignment, symptoms +0

**IV. Total Score (100 points):**

\[ \text{Pain Points} + \quad \text{Function Points} + \quad \text{Alignment Points} = \]

\[ \text{Total Points}/100 \text{ points} \]

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Appendix D: Visual Analog Scale

![Visual Analog Scale Diagram]
Appendix E: PAASS Framework

P: Pain severity
- During sport participation
- Over last 24 hours

A: Ankle impairments
- Ankle range of motion
- Ankle muscle strength, endurance and power

S: Athlete perception
- Perceived ankle confidence/reassurance
- Perceived ankle stability
- Psychological readiness

S: Sensorimotor control
- Proprioception
- Dynamic postural control/balance

S: Sport/functional performance
- Hopping and jumping
- Agility
- Sport-specific activities
- Ability to complete a full training session

Suggested evaluation
- VAS
- Pain on palpation
- Active and passive range of motion
- Heel raise test
- Single leg squat
- Injury Psychological Return to Sport scale (I-PRRS)
- Star Excursion Balance Test (SEBT)
- Foot and Ankle Disability Index Sport (FADI Sport)
- Shuttle runs
- T-test
Appendix F: Sample Size Calculation

Using the following parameters, the sample size was calculated:

Alpha = .05 (two tailed)

Beta = .20

Power= 80%

Effect size of 35% calculated from Laver et al. study

Standard deviation- 12 from Laver et al study.

Baseline for control group 42 days from Malliaropoulos et al. study

15% loss to follow up rate

28 participants total with 14 in the intervention and 14 in the control
### Appendix G: Baseline Characteristics/ Inclusion & Exclusion Survey

**Baseline Characteristics/ Inclusion & Exclusion Survey**

**FOR RESEARCH ASSISTANT TO FILL OUT**

<table>
<thead>
<tr>
<th>Participant Number</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Center</td>
<td></td>
</tr>
<tr>
<td>Research Assistant Name</td>
<td></td>
</tr>
<tr>
<td>Baseline VAS score</td>
<td></td>
</tr>
<tr>
<td>Baseline AOFAS score</td>
<td></td>
</tr>
</tbody>
</table>

**FOR PARTICIPANT TO FILL OUT**

<table>
<thead>
<tr>
<th>Age</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Weight (pounds)</td>
<td></td>
</tr>
<tr>
<td>Height (Inches)</td>
<td></td>
</tr>
<tr>
<td>Side of Injury (right or left)</td>
<td></td>
</tr>
<tr>
<td>Sport played</td>
<td></td>
</tr>
<tr>
<td>Level of Competition (professional or college)</td>
<td></td>
</tr>
<tr>
<td>Have you taken antiplatelet medication in last 10 days (aspirin, Plavix, Brilinta, Effient, etc)?</td>
<td></td>
</tr>
<tr>
<td>Have you taken NSAIDs in last 10 days (Advil, Motrin, Naprosyn, Celebrex, Indocin, Aleve, Etc.)?</td>
<td></td>
</tr>
<tr>
<td>Have you had injections into the injured ankle?</td>
<td></td>
</tr>
<tr>
<td>Have you had surgery to the injured ankle?</td>
<td></td>
</tr>
<tr>
<td>Have you had prior sprains to the injured ankle?</td>
<td></td>
</tr>
<tr>
<td>Do you have Diabetes?</td>
<td></td>
</tr>
<tr>
<td>Are you currently pregnant?</td>
<td></td>
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</tbody>
</table>
Bibliography


