The effectiveness of Platelet-Rich Plasma as a biological treatment in the management of Gluteal Tendinopathy

Dr Jane Fitzpatrick

MB.BS, FACSEP

This thesis is presented for the degree of Doctor of Philosophy at the University of Western Australia

School of Surgery

Faculty of Medicine and Dentistry

2017
Statement of Contribution

I, Dr Mary Jane Fitzpatrick, certify that:

This thesis has been substantially accomplished during enrolment in the degree.

This thesis does not contain material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution.

No part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of The University of Western Australia and where applicable, any partner institution responsible for the joint-award of this degree.

This thesis does not contain any material previously published or written by another person, except where due reference has been made in the text.

The work(s) are not in any way a violation or infringement of any copyright, trademark, patent, or other rights whatsoever of any person.

The research involving human data reported in this thesis was assessed and approved by Epworth Healthcare Human Research Ethics Committee (57412 May 2013). The University of Western Australia accepted this ethics approval (RA/4/1/6242).

Written patient consent has been received and archived for the research involving patient data reported in this thesis.

The following approvals were obtained prior to commencing the relevant work described in this thesis: Australian New Zealand Clinical Trials Registry: ACTRN12613000677707.

This thesis contains published work and/or work prepared for publication, some of which has been co-authored.
Dr Jane Fitzpatrick (Candidate)

Signed: Winthrop Professor Ming Hao Zheng (Co-ordinating Supervisor)
# Table of Contents

Statement of Contribution ......................................................................................... 2
Abstract ..................................................................................................................... 11
  Background ........................................................................................................... 11
  Purpose .................................................................................................................. 12
  Methods ............................................................................................................... 13
  Results .................................................................................................................. 14
  Conclusion .......................................................................................................... 16
Acknowledgement .................................................................................................... 18
Authorship Declaration: Co-Authored Publications ............................................. 20
Prizes ......................................................................................................................... 28

Chapter 1. Introduction ......................................................................................... 30
  1.1 Preface ............................................................................................................ 30
  1.2 Background - Tendinopathy ....................................................................... 30
  1.3 Pathophysiology of tendinopathy ............................................................... 32
  1.4 Current treatments for gluteal tendinopathy ............................................. 33
    1.4.1 Activity modification, home training and Physiotherapy ................. 34
    1.4.2 Shock Wave therapy ............................................................................. 34
    1.4.3 Non-steroidal anti-inflammatory drugs ............................................ 34
    1.4.4 Glucocorticoid injections .................................................................... 35
    1.4.5 Other Injection therapies ..................................................................... 36
    1.4.6 Surgical Procedures .............................................................................. 36
  1.5 Platelet-Rich Plasma as a possible biological management for tendinopathy ................................................................. 37
  1.6 The aims of this thesis: .................................................................................. 39
  1.7 Outline of the thesis ...................................................................................... 40

Chapter 2. The Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy ................................................................. 42
  2.1 Preface: .......................................................................................................... 42
  2.2 Abstract: ........................................................................................................ 43
  2.3 Background .................................................................................................... 45
4.2 Abstract .................................................................................................................. 101
4.3 Background .......................................................................................................... 102
4.4 Methods............................................................................................................... 104
  4.4.1 Description of Common Commercial Kits ...................................................... 104
    Table 1. Commercially Available Kits for the Production of Platelet Products$^a$ .................................................................................................................. 106
  4.4.2 Sample Collection and Processing ................................................................. 107
    Table 2. Preparation of PRP Samples$^a$ ................................................................ 107
  4.4.3 Classification of the PRP Produced ............................................................... 108
  4.4.4 Statistical Analysis ....................................................................................... 108
4.5 Results .................................................................................................................. 108
  4.5.1 Comparison of Cellular Components ............................................................. 108
    Table 3. Cellular Data$^a$ .................................................................................. 110
    4.5.1.1 Platelets ............................................................................................... 111
      Figure 1. Platelet Counts by Kit Type ............................................................ 112
    4.5.1.2 Red Blood Cells ................................................................................ 112
      Figure 2. Red Blood Cells by Kit Type ............................................................ 112
    4.5.1.3 White Blood Cells ................................................................................ 113
      Figure 3. Total White Cell Count by Kit Type ................................................ 113
      Table 4. White Cell Differential Counts$^a$ ..................................................... 115
      Figure 4. Total White Cell Counts, Neutrophil and Lymphocyte Counts by kit type ........................................................................................................ 116
  4.5.2 Comparison of Chemical Composition .......................................................... 117
    Table 5. pH and Glucose Data .......................................................................... 117
    Figure 5. pH by kit type .................................................................................... 118
    Figure 6. Glucose concentration by kit type ..................................................... 119
4.6 Discussion .............................................................................................................. 119
    Figure 7. PRP in a GPS kit after centrifugation .................................................. 121
    Table 6. PRP kits by PAW and Mishra (Sports Medicine) Classifications .......... 125
4.7 Conclusion ............................................................................................................ 126

Chapter 5. The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Randomised, Double-Blind Controlled Trial comparing a single Platelet-Rich Plasma injection with a single corticosteroid injection. ........................................... 127
6.3 Background ........................................................................................................ 152
6.4 Methods .............................................................................................................. 155
   6.4.1 Objective .................................................................................................... 155
   6.4.2 Trial Design ............................................................................................... 155
      6.4.2.1 Subject selection .................................................................................. 156
      6.4.2.2 Intervention ......................................................................................... 156
      6.4.2.3 Outcomes ............................................................................................. 156
      6.4.2.4 Blinding ................................................................................................. 157
      6.4.2.5 Crossover ............................................................................................. 157
      6.4.2.6 Statistical Assessment .......................................................................... 157
6.5 Results ................................................................................................................ 157
6.6 Discussion .......................................................................................................... 166
6.7 Conclusion ......................................................................................................... 169

Chapter 7. Discussion, Conclusion and implications for future research .......... 170
7.1 Overview ........................................................................................................... 170
7.2 Discussion ......................................................................................................... 170
7.3 Implications for future research ...................................................................... 174
   7.3.1 Natural History of tendinopathy ................................................................. 174
   7.3.2 Pathological Grading of tendinopathy ......................................................... 174
   7.3.3 Develop a treatment algorithm for the management of tendinopathy .......... 175
7.4 Conclusion ......................................................................................................... 176
8 References ............................................................................................................ 177

Appendix A ............................................................................................................. 186

1 Protocol and ethics certificate The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Double-Blind Randomised, Controlled Trial (The PRP-HIP Trial) ......................... 186
   1.2 Synopsis ....................................................................................................... 187
   1.3 Table of Contents ......................................................................................... 188
   1.4 Abbreviations and Definitions .................................................................. 190
   1.5 Introduction ................................................................................................. 192
   1.6 Objectives .................................................................................................. 193
      1.6.1. Primary Objective ................................................................ ............... 193
1.6.2. Secondary Objectives ................................................................. 193
1.7. Investigational Plan ........................................................................ 193
  1.7.1. Summary of Trial Design ............................................................ 194
1.8. Trial Population ............................................................................... 194
  1.8.1. Inclusion Criteria ........................................................................ 194
  1.8.2. Exclusion Criteria ........................................................................ 195
  1.8.3. Discontinuations ........................................................................... 196
1.9. Treatment ........................................................................................ 197
  1.9.1. Treatments Administered .............................................................. 197
  1.9.2. Materials and Supplies ................................................................. 198
  1.9.3. Method of Assignment to Treatment ............................................. 198
  1.9.4. Rationale for Selection of Doses and Timing of Treatment in the Trial ........................................................................... 199
  1.9.5. Blinding ......................................................................................... 199
  1.9.6. Concomitant Therapy ................................................................. 199
  1.9.7. Treatment Compliance ............................................................... 199
1.10. Efficacy and Safety Evaluations, and the Appropriateness of Measurements ........................................................................... 200
  1.10.1. Efficacy Measures ....................................................................... 200
  1.10.2. Safety Evaluations ....................................................................... 200
  1.10.3. Appropriateness of Measurements ............................................... 202
1.11. Data Quality Assurance .................................................................. 203
  1.11.1. Data Capture System .................................................................. 203
1.12. Sample Size and Statistical Methods .............................................. 204
  1.12.1. Determination of Sample Size ..................................................... 204
  1.12.2. Statistical and Analytical Plans .................................................... 205
1.13. Informed Consent, Ethical Review, and Regulatory Considerations ........................................................................... 209
  1.13.1. Informed Consent ....................................................................... 209
  1.13.2. Ethical Review .............................................................................. 210
  1.13.3. Regulatory Considerations ......................................................... 210
1.14. References ....................................................................................... 211

2 HREC Certificate .................................................................................. 215

3 The Effectiveness of Platelet-Rich Plasma in the treatment of Tendinopathy ........................................................................... 217
4 Analysis of Platelet-Rich Plasma Extraction ......................................................... 225

Appendix B Conference Presentations related to this thesis................................. 233

2013 1st Melbourne International Hip Arthroscopy Meeting Jan
12-13 ......................................................................................................................... 233

2013 Raine Symposium University of Western Australia July 4 ............ 233

2013 ACSEP Registrar Teaching July ................................................................. 233

2013 New Zealand annual Sports Medicine Scientific Meeting
Wellington November 20-21............................................................ 233

2014 2nd Melbourne International Hip Arthroscopy Meeting Jan
16-17 ......................................................................................................................... 233

2015 Western Hospital Journal Club October 16 ............................................. 233

2015 Orthocell User Meeting Sydney August 8 ............................................. 233

2015 Continuing Orthopaedic Education – Australian
Orthopaedic Association, Melbourne July 24 ........................................ 233

2016 3rd Melbourne International Hip Arthroscopy Meeting
January 21-22 ....................................................................................................... 233

2017 SportsKongres European Sports Medicine Congress,
Copenhagen February 2-4 ............................................................................... 234

2017 ACSEP Annual Scientific Meeting Gold Coast February
10-14 ....................................................................................................................... 234

2017 Epworth Research Week June 5-9 ......................................................... 234
Abstract

Background

Tendinopathy can occur in all tendons in the human body but is most common in the gluteal tendons on the lateral aspect of the hip, the achilles tendon, the patellar tendon, the common extensor tendon and the rotator cuff. Tendinopathy is responsible for 30% of all primary presentations for musculoskeletal conditions and as the direct and indirect costs associated with tendinopathy are known to be high, the disease burden is significant. Tendinopathy of the gluteal tendons is the most prevalent of all lower limb tendinopathies causing high levels of dysfunction equal in severity to that of severe osteoarthritis of the hip.

The specific cause of gluteal tendinopathy is unknown but the clinical history of gluteal tendonitis suggests that there is a degenerative progression of disease associated with inflammation and bursitis in the early stage to partial thickness and later full thickness tears of the tendons.

There is a lack of high-quality research into treatment for gluteal tendinopathy. A myriad of treatments have been described including activity modification, home training, physiotherapy, shock wave therapy, non-steroidal anti-inflammatory drugs, corticosteroid, polidocanol and glucose injections, and surgical interventions. All of these have been reported to provide short term relief of symptoms but have not been shown to result in good long term outcomes.
Platelet-Rich Plasma (PRP) has emerged as a possible biological treatment in the management of tendinopathy. PRP is made from an autologous sample of whole blood which is centrifuged and separated to create a platelet-rich concentrate with greater than the baseline levels of platelets when compared to whole blood. It has been hypothesised that the effect of PRP is due to the growth factors: Platelet derived growth factor (PDGF), transforming growth factor beta (TGF-Beta), vascular endothelial growth factor (VEGF), insulin like growth factor 1 (IGF-1) and hepatocyte growth factor (HGF), which are released from the alpha granules of platelets during in vivo activation of the platelets.

However, there is currently uncertainty as to the method of preparation of the PRP and its effectiveness in treatment of gluteal tendinopathy. It remains unclear whether the efficacy of the PRP is affected by the inclusion of leucocytes or the concentration of platelets. Despite these issues, there have been randomised controlled trials using PRP in different sites including tennis elbow, achilles, patellar tendon and the rotator cuff with both positive and negative outcomes. Systematic reviews have therefore found variable outcomes and a Cochrane review suggested the reason for the variation may lie in the large variation in the types of PRP used and the variable technique for injections.

**Purpose**

This thesis aims to identify whether; there is evidence to support the use of PRP in tendinopathy from previously reported trials; the cellular and biochemical makeup of the PRP affect the outcome in the management of tendinopathy; there is variation in PRP cellularity (including red cells, platelets and full white cell differential) and biochemistry.
(particularly pH) in all commercially available preparation kits across Australia and whether PRP is effective in the treatment of gluteal tendinopathy over both the short and longer term.

**Methods**

A systematic review and meta-analysis of randomised controlled trials using autologous blood products, including PRP of all cellular variations used in tendinopathy with follow-up time of 3 months were assessed using the PRISMA guidelines. In contrast to all previous meta-analyses, the specific type of PRP and the technique for injection was defined and used in the meta-analysis in order to determine whether the cellular concentration or technique influenced the outcome. In addition, all controls were specified and analysed to determine whether the type of control influenced the outcome of the clinical trial comparator arm. All sites for tendinopathy were included and analysed together due to the limited number of trials in each site.

A controlled laboratory study was undertaken to assess all PRP separation kits commercially available in Australia. These were GPS III (Biomet Biologics), Smart-Prep2 (Harvest Terumo), Magellan (Arteriocyte Medical Systems), and ACP (Device Technologies). The resultant PRP was tested for platelet count, red cell count, and white cell count. In addition, measurements were obtained for white cell differential count, pH, potassium, sodium, chloride, glucose, and lactate: parameters which have not previously
been reported in the literature. The results were analysed to determine whether there was uniformity across PRP preparation kits.

The effectiveness of a single leucocyte-rich PRP (LR-PRP) injection in gluteal tendinopathy was assessed in a randomised double-blind controlled single centre clinical trial conducted between May 2013 and May 2015, with long term follow-up in June 2017. This trial was registered with the Australian New Zealand Clinical Trials Registry. 228 consecutive patients referred with gluteal tendinopathy were screened to enrol 80 participants, who were randomised (1:1, 40 in each group) to receive either a blinded glucocorticoid or platelet-rich plasma injection intra-tendinously under ultrasound guidance. A pain and functional assessment was performed using a Modified Harris Hip Sore (MHHS) questionnaire at 0, 2, 6, 12, 24, 52 and 104 weeks. Further analyses of the patient acceptable symptomatic state (PASS) and minimally important clinical difference (MICD) were performed at 12 weeks and 12 months. An open labelled extension allowed subjects to receive crossover treatment after three months.

**Results**

A total of 18 studies (1070 Subjects) were included in the systematic review and meta-analysis. 8 were deemed to be at low risk of bias. The most significant outcome in the PRP groups was those treated with highly cellular leucocyte-rich preparations: PRP-GPS (SMD 35.75 CI 28.4 – 43.10), Mycells-PRP (SMD 31.84 CI 17.56 – 46.13), Prosys-PRP
(SMD 42.99 CI 37.73-48.25), unspecified LR-PRP (SMD 34.62 CI 31.69-37.55). When the LR-PRP system types were grouped there was a strongly positive effect: LR-PRP (SMD 36.38 CI 34.0-38.77) when compared to leucocyte poor PRP (LP-PRP) SMD 26.77 CI 18.31-35.22. In assessing the control groups: there was no clear difference between different types of control injections saline (SMD 14.62 CI 10.74-18.5), Local anaesthetic (SMD 15.00 CI 7.66-22.34), Cortisone (SMD 23.82 CI 10.74-18.5) or Dry Needling (SMD 25.22 CI 21.27-29.16).

The laboratory study further demonstrated this variation. The three kits taking samples from the “buffy coat layer” were found to have much greater concentrations of platelets (3-6 times baseline) and an equally increased concentration of white cells (3-6 times baseline) including neutrophils, leucocytes, and monocytes. The kit taking samples from plasma was found to have low platelet concentrations of only 1.5 times baseline. A small drop in pH across several preparations was thought to relate to the citrate used in the sample preparation, as was the unexpected finding of an increase in glucose concentrations in all samples.

The Clinical trial was developed from the concept that the best results in the treatment of tendinopathy identified in the meta-analysis were found with the use of highly cellular LR-PRP. The laboratory study had validated the GPS III kit as one that produced LR-PRP and this kit was selected for use in the gluteal tendinopathy study. 228 patients were screened to enrol 80 subjects with a mean age of 60, a ratio of female to male of 9:1 and mean length of symptoms >14 months. Pain and function measured by the mean MHHS
showed no difference at 2 weeks CSI 66.95 (SD 15.14) vs PRP 65.23 (SD 11.60) or 6 weeks CSI 69.51 (SD 14.78) vs PRP 68.79 (SD 13.33). The mean MHHS was significantly improved at 12 weeks PRP 74.05 (SD 13.92) compared to the CSI group with a mean score of 67.13 (SD 16.04, p=0.048). The PRP group achieved a PASS score of 74 at 12 weeks, reflecting clinical recovery. The proportion of subjects who achieved the MICD of more than 8 points at 12 weeks was 21/37 (56.7%) in the CSI group and 32/39 (82%) in the PRP group (p=0.016).

At 24 weeks the LR-PRP group 77.60 (SD 11.88) had improved further compared to the CSI group 65.72 (SD 15.28) p=0.0003. 27 subjects were deemed to have failed the CSI treatment at 16-24 weeks with an exit score of 59.22 (SD 11.54) and then had treatment with LR-PRP. The crossover group improved with the LR-PRP: baseline 59.22 (SD 11.22) to 75.55 (SD 16.05) at 12 weeks, 77.69 (SD 15.30) at 24 weeks and 77.78 (SD 14.00) at 104 weeks. The LR-PRP group retained 38/39 subjects to 54 weeks and continued to improve. Their baseline scores of 53.77 (SD 12.08) improved to 81.8 (SD 9.97) at 104 weeks. Perhaps the most impressive difference between the groups was the number who had fully recovered at 2 years. When assessed on an intention to treat basis at 12 months 8/40 (20%) of the CSI had recovered compared to 31/40 (77.5%) of the LR-PRP group.

**Conclusion**

There is marked variation in PRP preparation in commercial kits. The systematic review and network meta-analysis showed that PRP improves the outcome in tendinopathy. The
trial results in gluteal tendinopathy confirm that PRP results in clinical recovery in the long term, whereas cortisone has only a short term effect. The results of this study have a significant impact on the future management of gluteal and other tendinopathies and for further study into the development of a treatment algorithm for gluteal tendinopathy.
Acknowledgement

This thesis began over a now memorable glass of champagne in Sydney with Dr Carolyn Broderick. I unwittingly unveiled my ideas relating to a clinical trial I postulated undertaking, to which Carolyn replied; “If you’re going to do all that work, you might as well do it properly and finish up with a doctorate!” I am indebted to Carolyn for triggering the concept and for agreeing to be a mentor in my doctoral process. Her mentorship and encouragement to ‘Celebrate every milestone’ within the process made this journey fun and allowed me to focus on my small achievements without losing sight of the task ahead!

To my Co-ordinating supervisor Winthrop Professor Ming Hao Zheng, my sincere thanks for your exacting scientific rigour and the enthusiasm you have had for this project. Your extraordinary ability to listen to my left field ideas which were often contradictory to the published scientific body of evidence in this area and guide me in defining how to test these hypotheses has been invaluable. For returning my short message service (SMS) enquiries from all continents at any hour of the day or night, for entire days set aside in Perth when I flew in for advice, for imparting true academic principles and ensuring some time for a deep appreciation of Margaret River wines was achieved, my most sincere thanks.

To my co-supervisors Professor Max Bulsara and Associate Professor Paul McCrory my most enduring thanks for your efforts particularly as we all reside in different locations. Max, without your support this thesis would simply not exist! Thanks for your patience
whilst I grappled with the demands of statistical analysis and for taking the time to listen to the clinical relevance of our issues so that we could design an appropriate analysis, even when that meant going beyond what the statistical package was capable of performing. For knowing where to find the best coffee in Freemantle and allowing me the moments of joy when graphs of our data made clinical sense!

To Associate Professor John O’Donnell for your career long association, honesty and genuine support I am deeply grateful. Your support in referring subjects for this trial personally and by promoting it within the orthopaedic community was instrumental in it’s success.

To Sally Boyd whose support as clinical trial co-ordinator was invaluable. For your level of organisation, patience and kindness to all of our subjects, my most sincere thanks. For accepting my moments of frustration and remaining calm throughout, my admiration.

To my family, Peter Snr, Peter Jnr and James McIntosh my most gracious thanks! It is difficult to keep a stable work-life balance during the writing of a thesis and perhaps the greatest toll during this time is on the family. For your encouragement, positivity and great certainty that I would make it to the end successfully, I applaud you.

This research was supported by an Australian Government Research Training Program (RTP) Scholarship.
Authorship Declaration: Co-Authored Publications
Authorship Declaration: Co-Authored Publications

This thesis contains work that has been published and prepared for publication.

Details of the work:

**The Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy: A Meta-analysis of Randomized Controlled Clinical Trials**

; Fitzpatrick, Jane ; Bulsara, Max ; Zheng, Ming H;


Location in thesis: Chapter 2

Student contribution to work:

The student undertook the planning, the literature reviews, the systematic review and meta-analysis, the data collection, statistical analysis, result interpretation, discussion and conclusion within this paper. The student was responsible for 100% of the drafting of this paper and 90% of the authorship of the included peer-reviewed publication.

Co-author signatures and dates:

Ming Hao Zheng Date: 18/09/17

Max Bulsara Date: 21/8/17
Details of the work:

**Effectiveness of PRP in the Treatment of Tendinopathy: Response**

Fitzpatrick, Jane; Bulsara, Max; Zheng, Ming Hao;


Location in thesis:

Chapter 3.2

Student contribution to work:

The student undertook the planning, discussion and conclusion within this paper. The student was responsible for 100% of the drafting of this paper and 90% of the authorship of the included peer-reviewed publication.

Co-author signatures and dates:

Ming Hao Zheng Date: 18/09/17

Max Bulsara Date: 22/09/17
Details of the work:

**Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy:**

**Response**

Fitpatrick, Jane; Bulsara, Max; Zheng, Ming Hao;


Location in thesis:

Chapter 3.5

Student contribution to work:

The student undertook the planning, discussion and conclusion within this paper. The student was responsible for 100% of the drafting of this paper and 90% of the authorship of the included peer-reviewed publication.

Co-author signatures and dates:

Ming Hao Zheng  Date: 18/09/17

Max Bulsara  Date: 22/09/17
Details of the work:

**Analysis of Platelet-Rich Plasma Extraction**

; Fitzpatrick, Jane ; Bulsara, Max K ; McCrory, Paul Robert ; Richardson, Martin D ; Zheng, Ming Hao;

; Orthopaedic Journal of Sports Medicine, 2017, Vol.5(1)

Location in thesis:

Chapter 4

Student contribution to work:

The student undertook the planning, arranged the ethics approval, set up and ran the laboratory study works, the literature review, the data collection, statistical analysis, result interpretation, discussion and conclusion within this paper. The student was responsible for 100% of the drafting of this paper and 90% of the authorship of the included peer-reviewed publication.

Co-author signatures and dates:

Ming Hao Zheng

Date: 18/09/17

Max Bulsara

Date: 23/8/17

Paul McCrory

Date: 22/08/2017

Martin Richardson

Date: 22nd August 2017
Details of the work: The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Randomised, Double-Blind Controlled Trial comparing a single Platelet-Rich Plasma injection with a single corticosteroid injection.

Location in thesis:
Chapter 5

Student contribution to work:
The student undertook the planning, arranged the ethics approvals, the clinical trial, the literature review, the data collection, statistical analysis, result interpretation, discussion and conclusion within this paper. The student was responsible for 100% of the drafting of this paper and 90% of the authorship of the included peer-reviewed publications appearing herein. Subjects for inclusion in the clinical trial were referred by medical colleagues in the course of normal specialist practice.

Co-author signatures and dates:

<table>
<thead>
<tr>
<th>Name</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ming Hao Zheng</td>
<td>18/09/17</td>
</tr>
<tr>
<td>Max Bulsara</td>
<td>22/08/17</td>
</tr>
<tr>
<td>Paul McCrory</td>
<td>22/06/2017</td>
</tr>
<tr>
<td>John O'Connell</td>
<td>21/06/2017</td>
</tr>
</tbody>
</table>
Details of the work: 2 year follow up of Leucocyte-rich Platelet-rich Plasma treatment of gluteal tendinopathy: A double blind Randomised Controlled Trial.

Location in thesis: Chapter 6

Student contribution to work:

The student undertook the planning, arranged the ethics approvals, the clinical trial, the literature review, the data collection, statistical analysis, result interpretation, discussion and conclusion within this paper. The student was responsible for 100% of the drafting of this paper and 90% of the authorship of the included peer-reviewed publications appearing herein. Subjects for inclusion in the clinical trial were referred by medical colleagues in the course of normal specialist practice.

Co-author signatures and dates:

Ming Hao Zheng  Date: 18/09/17
Max Bulsara  Date: 22/8/17
John O'Donnell  Date: 21/8/2017
Student signature: ____________________________

Date: 21 Aug 2017

I, [insert name of coordinating supervisor] certify that the student statements regarding their contribution to each of the works listed above are correct.

Coordinating supervisor signature: ____________________________

Date: 18/09/17
Prizes

The data from the randomised clinical trial presented in Chapter 5, The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Randomised, Double-Blind Controlled Trial comparing a single Platelet-Rich Plasma injection with a single corticosteroid injection, was presented at SportsKongres 2017, Copenhagen, Denmark in February. It was selected as a Finalist in the Open Papers Presentation Competition.

It was also presented as a poster at Epworth Research Week, June 5 2017 where it was awarded 2017 Experienced Researcher Poster Award.
Dr Jane Fitzpatrick

2017 Experienced Researcher Poster Award

Research Institute
Epsworth
Chapter 1. Introduction

1.1 Preface

This thesis is written as individual chapters which can be read separately. The University of Western Australia allows for a thesis to be presented as a series of published or unpublished papers. The full PDFs of the published papers appear in the Appendices but each paper has been reproduced in Word format for consistency of reading within each chapter.

1.2 Background - Tendinopathy

Tendinopathy is a term used to describe chronic overuse tendon disorders. This pathology can occur in all tendons in the human body but is most commonly discussed in the lower limb at the achilles at the ankle, the patellar tendon at the knee, the gluteal tendons on the lateral aspect of the hip and the hamstring posteriorly and, in the upper limb as De Quervains tendinopathy at the wrist, tennis and golfers elbow and as rotator cuff tendinopathy in the shoulder.

Tendinopathy is responsible for 30% of all primary presentations for musculoskeletal conditions. It has been reported that up to 5% of patients with tennis elbow are unable to work for an average of 29 days per year and that tennis elbow in the United Kingdom
alone is estimated to cost £27 million\textsuperscript{62}. In a recent report on the economic impact of tendinopathy, Hopkins et al demonstrated that the direct and indirect costs associated with tendinopathy are known to be high and with the emerging high prevalence of tendinopathy, the disease burden may be significantly greater than is currently recognised\textsuperscript{62}.

Tendinopathy of the gluteus medius or minimus tendons is a major cause of lateral hip pain. It has a reported incidence of between 1.8\textsuperscript{78} and 5.6\textsuperscript{80} per 1000 per year. It is more than four times more common in women and is the most prevalent of all lower limb tendinopathies\textsuperscript{36}. In 1961, Gordon identified that lateral hip pain was related to underlying tendinopathy with associated secondary trochanteric bursitis\textsuperscript{53,76}. Since the advent of imaging with ultrasound and MRI, tendinopathy as the underlying pathology has been confirmed and the term trochanteric bursitis is less commonly used, as bursitis is frequently absent on imaging\textsuperscript{71}. Tendinopathy of the gluteal tendons is incorporated into the term greater trochanteric pain syndrome (GTPS) but this is a less useful term as it includes other causes of lateral hip pain being somewhat of a vague umbrella-type terminology\textsuperscript{46}.

High levels of dysfunction have been found in people with gluteal tendinopathy who fail conservative treatment\textsuperscript{120} including less full time employment, higher pain levels and poorer quality of life\textsuperscript{45}. This has been equated with the disability of severe osteoarthritis of the hip\textsuperscript{45}. 

31
The diagnosis of gluteal tendinopathy can be confirmed by a clinical history of lateral hip pain localised to the greater trochanter, pain with activities such as walking and stair climbing and pain lying on the affected side at night. The diagnosis is further confirmed by the clinical signs of tenderness at the greater trochanter and localised lateral hip pain with rotational hip movements. Both ultrasound and MRI can reliably predict the presence of gluteal tendinopathy and tears. Patients with both clinical signs and symptoms and radiological appearance of gluteal tendinopathy can be regarded as having symptomatic disease involving the gluteal tendons.

1.3 Pathophysiology of tendinopathy

The cause of gluteal tendinopathy is currently unknown. The clinical history of gluteal tendonitis suggests there is a degenerative progression of disease. In early cases, tendinopathy of one or both tendons is present sometimes with associated trochanteric bursitis. Moderate cases show partial thickness tears either longitudinally or at the insertion of the tendons and more advanced cases demonstrate full thickness tears with progression to tendon retraction and fatty atrophic changes of the muscle belly seen on MRI.

The cellular mechanism for this progression of degenerative change in tendinopathy has been described as a cycle of micro-trauma and improper healing leading to tendinopathy and eventual structural failure in tendons. Four grades of tendinopathy are described. Grade 1 disease is characterised by a change in collagen fibre pattern with an increasing
wavy appearance and an increase in type 3 collagen. Grade 2 disease has angio-fibroblastic hyperplasia, rounding of tenocytes and further disorganisation of collagen fibres. In Grade 3 disease cell apoptosis leads to a depletion of tenocytes and a breakdown of collagen and extra cellular matrix. Finally in Grade 4 there is gross structural disruption and tendon breakdown seen as tearing.

This concept of the continuum model of tendinopathy was first described by Cook and Purdham\textsuperscript{25} and introduced the relationship between the clinical progression of disease with the underlying pathology. It has been suggested that this model be used to develop a treatment algorithm which recommends differing treatments depending on the underlying pathophysiological degree of severity of the tendinopathy\textsuperscript{14}.

A continuum model is ideal for researchers and clinicians alike as it allows us to more accurately identify the appropriate treatment for different stages of the tendinopathy as it progresses. However, there is still a discrepancy between the clinical history, the relationship of pain to the histopathology and radiological structural changes that do not allow this model to be used effectively at this stage.

1.4 Current treatments for gluteal tendinopathy

A recent review of the current treatments for gluteal tendinopathy suggested there is a lack of high-quality research into conservative treatments of gluteal tendinopathy\textsuperscript{11}.
1.4.1 Activity modification, home training and Physiotherapy

One study exists looking at the effectiveness of home based training in gluteal tendinopathy\textsuperscript{101}. This study used a program consisting of three stretches (piriformis and iliotibial band stretches and a straight leg raise) and two active strength exercises (wall squats and prone hip extensions). Based on pain as an outcome measure, home training was found to be inferior to corticosteroid injections at one month but better at 15 months and was superior to shock wave therapy. However, only 34\% of patients were able to return to previous activity levels.

1.4.2 Shock Wave therapy

Shock wave therapy has been used in two trials in gluteal tendinopathy\textsuperscript{51, 101}. Rompe showed that shock wave therapy was less effective at one month than corticosteroid injections and no more effective than home training at 15 months. Furia showed shock wave therapy was more effective at 12 months but the comparator group was allowed any other form of conservative treatment so this is not a strong conclusion.

1.4.3 Non-steroidal anti-inflammatory drugs

The use of non-steroidal anti-inflammatory drugs in tendinopathy generally has been discussed by Kaux\textsuperscript{65} as useful for pain modification but has not been specifically studied in gluteal tendinopathy.
1.4.4 Glucocorticoid injections

Most reviews of the treatments of gluteal tendinopathy identify that glucocorticoid injections are commonly used and effective as a short term treatment\textsuperscript{11, 36, 57, 80}

McEvoy\textsuperscript{84} reviewed 65 corticosteroid injections given for gluteal tendinopathy and concluded that injections given in to greater trochanteric bursa were more effective than those given into the sub-gluteal bursa.

Shbeeb\textsuperscript{110} found in a prospective case series of 75 patients that 77\% were improved at 1 week and 61\% at 26 weeks. Labrosse\textsuperscript{75} found 72\% were improved at 1 month in a similar prospective case series of 54 patients. Rassmussen\textsuperscript{97} similarly found 66\% of 36 patients improved but noted relapses after 10 months.

Two randomised controlled trials confirmed similar findings. Rompe\textsuperscript{101} reported on 229 patients randomised to three groups (CSI, Home training and Shock wave therapy) and found that corticosteroid injections were superior at 1 month but not at 4 or 15 months. Likewise, Brinks\textsuperscript{17} reported on 120 patients randomised to regular treatment or corticosteroid injections and found that corticosteroid injections were superior at 3 months but there was no difference at 12 months.

Thus across prospective case series and randomised controlled trials there is a similar finding of improvement from 1-4 weeks and then poorer outcomes over the longer term and a tendency towards recurrence of symptoms.
1.4.5 Other Injection therapies

Autologous tenocyte implantation has been used in one prospective case series of 12 patients with chronic recalcitrant gluteal tendinopathy with improvement from 12 to 24 months in 8 out of 12 patients\textsuperscript{19}.

Other injections therapies such as autologous blood, sclerosants such as Polidocanol, botulinum toxin and serine proteinase inhibitors such as Aprotinin have been discussed in tendinopathy generally\textsuperscript{65} but have not been used in gluteal tendinopathy.

1.4.6 Surgical Procedures

Surgical procedures have been described for recalcitrant end stage cases but there have been only small case series reported in the literature. Slawski\textsuperscript{111} retrospectively reviewed 5 cases where a release of the Iliotibial band (ITB) and bursectomy were performed showing an improvement in Harris Hip Scores. Barnthouse\textsuperscript{10} described the same operation could be performed endoscopically but did not report on any outcomes. Drummond\textsuperscript{40} retrospectively reviewed 49 patients using a similar surgical technique. There was large variability in the type of surgery performed as some patients had additional tendon repairs but overall they reported high satisfaction rates of 78% following the surgery. Craig\textsuperscript{28} described a different technique for iliotibial band lengthening but also good outcomes with 16 of 17 patients reporting good or excellent results. Walsh\textsuperscript{120} looked prospectively at the results of 72 surgical tendon repairs reporting that 90% had minimal or no pain at follow-up.
1.5 Platelet-Rich Plasma as a possible biological management for tendinopathy

Platelet-Rich Plasma (PRP) has emerged as a possible biological treatment in the management of tendinopathy. Platelet-Rich Plasma (PRP) is defined as a platelet-rich concentrate with greater than the baseline levels of platelets when compared to whole blood. This product is made by the collection of a venous sample of whole blood which is then spun by centrifugation and separated allowing cellular and non-cellular components to be collected. DeLong et al\textsuperscript{37}, described that PRP preparations can be divided into two types – one plasma-based and the other based on buffy coat preparations. Plasma-based preparations aim to capture platelets from the plasma after centrifugation and exclude red and white cells. Generally this produces smaller increases in platelets than the methods that take platelets from both the plasma and the more cellular ‘buffy coat’\textsuperscript{20, 22, 44}.

It has been hypothesised that the effect of PRP is due to the growth factors Platelet derived growth factor (PDGF), transforming growth factor beta (TGF-Beta), vascular endothelial growth factor (VEGF), insulin like growth factor 1 (IGF-1) and hepatocyte growth factor (HGF) which are released from the alpha granules of platelets during in vivo activation of the platelets\textsuperscript{5, 9, 16, 22, 29, 32, 82, 124} or subsequently produced by the cellular matrix of the tendon.
There remains a lot of uncertainty as to the method of preparation of the PRP and its cellular content. It remains uncertain whether the efficacy of the PRP is affected by the inclusion of leucocytes. Moojen et al, suggested that there may be positive effects from the white cells acting as anti-microbial agents. Other authors have suggested that the platelets themselves may already have this anti-microbial property. Questions have been raised as to whether there may also be negative effects from these white cells in causing further inflammation leading to fibrosis or from the release of catabolic cytokines. It is postulated that this effect may be more prevalent with neutrophils than other white cells.

Despite these issues, there have been some randomised controlled trials using PRP in different sites including tennis elbow, achilles, patellar tendon and the rotator cuff with variable outcomes. Not surprisingly, systematic reviews have therefore found both positive and negative outcomes, without any good explanation as to the difference.

A recent Cochrane review suggested the reason for this may lie in the large variation in the types of PRP used and the technique for injections. It is possible that tendons may be different in their response, for example the achilles tendon has not shown improvement following the injection of PRP in trials to date. Part of the reason for this controversy may relate to the fact that PRP has a slow onset of action and it takes 3-6 months to see the effectiveness. It may also relate to the number of trials.
with a lack of statistical significance over times shorter than 3 months or with small numbers of participants\textsuperscript{39, 73, 98}.

This uncertainty is further compounded by concerns relating to the appropriate selection of a control or comparator for clinical trials. It has been argued that cortisone has a negative effect on tendinopathy and thus when used as a control it will make the mean difference greater than it would if it were compared to other types of injectable controls\textsuperscript{34}. Corticosteroid injections show an improvement up to 3 months and then a decline in effectiveness\textsuperscript{35}. Several groups have reported that it is difficult to get subjects in a control group to avoid drop out when they have no clinical effect from the control treatment or a short term benefit only\textsuperscript{73, 88, 98} thus making the use of a placebo control or inactive treatment difficult.

\textbf{1.6 The aims of this thesis:}

a. To identify whether there is evidence to support the use of PRP in tendinopathy from previously reported trials.

b. To determine whether the cellular and biochemical makeup of the PRP affect the outcome in the management of tendinopathy.

c. To determine whether there is variation in PRP cellularity (including red cells, platelets and full white cell differential) and biochemistry (particularly pH) in all commercially available preparation kits across Australia.
d. To determine whether PRP is effective in the treatment of gluteal tendinopathy.

1.7 Outline of the thesis

Chapter 2 looks at whether there is evidence to support the use of PRP in tendinopathy from previously reported trials. It presents the publication “The Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy, A Meta-analysis of randomized controlled clinical trials”. This meta-analysis describes the data from all randomised controlled clinical trials by PRP type and treatment technique.

Chapter 3 examines the letters to the editor relating to the meta-analysis and the author responses to these. It includes the continuing education module commissioned by the editor of the American Journal of Sports Medicine relating to the meta-analysis.

Chapter 4 looks at whether the cellular and biochemical makeup of the PRP affect the outcome in the management of tendinopathy. It presents the publication “Analysis of Platelet-Rich Plasma Extraction Variations in Platelet and Blood Components between 4 Common Commercial Kits” which describes a laboratory study analysis of the cellular and chemical composition of PRP made in currently available commercial kits in Australia.

Chapter 5 and 6 look at whether PRP is effective in the treatment of gluteal tendinopathy, a tendon which has not previously been investigated in the literature. The publication “The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Randomised, Double-Blind Controlled Trial comparing a single Platelet-Rich Plasma
injection with a single corticosteroid injection” is presented. This describes the outcome at three months of the clinical trial using leucocyte-rich platelet-rich plasma in the management of gluteal tendinopathy. Chapter 6 outlines the 2 year follow up of the subjects in the gluteal tendon clinical trial.

Chapter 7 provides a discussion and conclusion to the thesis.
Chapter 2. The Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy

2.1 Preface:

What is known about the subject: PRP is defined as platelet-rich plasma concentrate with platelet levels greater than baseline when compared to whole blood. There is widespread interest in the use of PRP in the treatment of tendinopathy and there are increasing numbers of randomised controlled trials studying the effectiveness of PRP in tendinopathy, particularly in tennis elbow. There is still no consensus as to whether PRP confers a beneficial effect as not all trials demonstrate a positive benefit. Six systematic reviews have been published between 2010 and 2014 assessing the effectiveness of PRP in tendinopathy. Despite analysing the same data, they reported contrasting conclusions from concluding that PRP is efficacious to finding that there is ‘strong evidence against Platelet-Rich Plasma’. Gosens concluded that ‘it would be better to break out the results by specific study design and PRP type’.

What this study adds to existing Knowledge: This meta-analysis is designed to assess the comparative effectiveness of PRP types and injection technique in tendinopathy. It shows a positive outcome in treating tendinopathy when leucocyte-rich PRP kits were used. All previous meta-analyses have grouped different PRP types together and have shown conflicting results.
2.2 Abstract:

**Background:** Tendinopathies are very common in the population. There are increasing numbers of clinical studies referring to Platelet-Rich and Platelet-poor (PRP and PPP) as a treatment for tendinopathy.

**Purpose:** To perform a meta-analysis of the outcome of the PRP groups by PRP preparation method and injection technique in tendinopathy. To determine clinical effectiveness of the preparations and to evaluate the effect of controls.

**Study Design:** Systematic review and meta-analysis

**Methods:**

**Data Sources:** Data bases of PubMed, EMBASE, CINAHL and Medline were searched in March 2012, April 2014 and August 2015.

**Study Selection:** Randomised controlled trials using autologous blood, PRP, PPP or autologous conditioned plasma in tendinopathy with outcome measures of pain and follow-up time of 3 months were included. Trials including surgery, tendon tears, muscle, ligament injuries were excluded.

**Data Extraction:** Study quality was assessed using the Cochrane collaboration risk of bias tool by two reviewers. Data was pooled using random effects meta-analysis.
**Main outcomes and Measures:** The primary outcome measure was a change in pain and function. Where more than one pain scale was included, we selected a functional score ahead of a visual analogue score.

**Results:** A total of 18 studies (1070 Subjects) were included. 8 were deemed to be at low risk of bias. The most significant outcome in the PRP groups was those treated with highly cellular leucocyte-rich preparations: PRP-GPS (SMD 35.75CI 28.4 – 43.10), Mycells-PRP (SMD 31.84 CI 17.56 – 46.13), Prosys-PRP (SMD 42.99 CI 37.73-48.25), unspecified LR-PRP (SMD 34.62 CI 31.69-37.55). When the LR-PRP system types are grouped there is a strongly positive effect: LR-PRP (SMD 36.38 CI 34.0-38.77) when compared to leucocyte poor PRP (LP-PRP) SMD 26.77 CI 18.31-35.22. In assessing the control groups: there was no clear difference between different types of control injections saline (SMD 14.62 CI 10.74-18.5), Local anaesthetic (SMD 15.00 CI 7.66-22.34), Cortisone (SMD 23.82 CI 10.74-18.5) or Dry Needling (SMD 25.22 CI 21.27-29.16).

**Conclusion and Clinical relevance:** There is good evidence to support the use of a single injection of leucocyte-rich PRP in tendinopathy. Both the preparation and intra-tendinous injection technique of the PRP appear to be of great clinical significance.
2.3 Background

Introduction

Tendinopathy is one of the most common reasons for presentation to a medical practitioner representing 30% of all presentations for musculoskeletal complaints. The most frequently discussed sites include the elbow (both tennis and golfers elbow), rotator cuff, achilles tendon, patellar tendon and the gluteal tendons. There are multiple treatments described in the literature including physiotherapy, shock wave therapy, the use of non-steroidal anti-inflammatory drugs, injections of glucocorticoid, prolotherapy, autologous blood, polidocanol, botulinum toxin and Platelet-rich Plasma (PRP).

Despite the pathophysiological role of inflammation being debated, the most commonly used treatment for chronic tendinopathy is glucocorticoid injections. These give a good short term improvement, less than 3 months, but do not confer a benefit in the longer term. PRP is one treatment that has been embraced in recent years as a potentially safe, effective treatment for tendinopathies.

PRP is defined as platelet-rich plasma concentrate with platelet levels greater than baseline when compared to whole blood. The potential uses of PRP extend from skin and wound healing to the treatment of tendinopathy and osteoarthritis. There is widespread interest in the use of PRP in the treatment of tendinopathy and there are increasing numbers of randomised controlled trials studying the effectiveness of PRP in tendinopathy, particularly in tennis elbow. There is still no
consensus as to whether PRP confers a beneficial effect as not all trials have failed to demonstrate a positive benefit.\textsuperscript{33, 73}

We found 6 systematic reviews published between 2010 and 2014 assessing the effectiveness of PRP in tendinopathy.\textsuperscript{2, 9, 32, 34, 72, 90} Despite analysing the same data, they reported contrasting conclusions from concluding that PRP is efficacious\textsuperscript{2} to finding that there is ‘\textit{strong evidence against Platelet-Rich Plasma}’\textsuperscript{34}. The majority of comments state that there is great difficulty reaching a conclusion due to the variance of the type of PRP produced. In the Cochrane review of PRP in soft tissue injuries, Moraes indicated ‘\textit{there is need for standardisation of PRP preparation methods}’\textsuperscript{90}. In his editorial review, Gosens commented on the systematic review done by De Vos\textsuperscript{34}, concluding that ‘\textit{it would be better to break out the results by specific study design and PRP type}’\textsuperscript{55}

Thus we conducted a meta-analysis to assess the comparative effectiveness of PRP types in tendinopathy. We assessed the effectiveness of different controls used in the RCTs.

### 2.4 Methods

Our review followed the PRISMA guidelines (Preferred reporting items for systematic reviews and meta-analysis) and the PRISMA-IPD statement\textsuperscript{77, 113}. Figure 4.

#### 2.4.1 Eligibility Criteria, Patients and Interventions

Randomised controlled trials (RCTs) using injections of PRP or autologous blood products in the treatment of tendinopathy (of any type) were included if they treated
adults (≥18 years of age). Trials which included subjects having surgery or treatment of non-tendon soft tissue injuries eg muscle, ligament or fascia, were not eligible. Eligible interventions included injections of any autologous blood product including whole blood, platelet-rich or poor plasma or autologous conditioned serum. We allowed any dosage, volume, number of injections and peri-tendinous or intra-tendinous injections. Controls were accepted as other active injections, placebo or conservative management.

2.4.2 Outcomes

We considered the most important primary outcome measure as change in pain intensity or function. Previous meta-analyses have demonstrated that the “benefit from PRP is most evident at longer time points”\(^2\) or “have a significant impact on improving pain and/or function over time”\(^3\). Therefore, a minimum acceptable follow up of 12 weeks for studies was included and data from 6 and 12 month follow up was included where available. In the event that more than one pain or function scale was included in the study, we selected the function score such as the Patient-Rated Tennis Elbow Evaluation (PRTEE) (or equivalent for other tendons) ahead of a visual analogue score (VAS) or verbal rating scales. Only one pain score measure was used for each study.

2.4.3 Data Sources and Search Strategy

A search strategy for RCTs investigating the treatment of tendinopathy with autologous blood products was carried out. The full search strategy is contained in Supplemental Figure 2, Search Strategies, but key search terms included platelet-rich plasma, autologous conditioned serum, autologous blood and tendonitis, tendinopathy and the
terms for all common tendinopathy such as tennis elbow, achilles tendonitis/opathy, patellar tendonitis, hamstring, rotator cuff and gluteal tendinopathy. The data bases of PubMed, EMBASE, CINAHL and Medline were searched for 5 years up to March 2012. A repeat search was performed in April 2014 and August 2015. The language was restricted to English.

2.4.4 Study Selection

The initial screening and study selection was performed by two authors (JF and MB). Any disagreement was discussed between these 2 authors and a third author MZ was available to determine a consensus.

For more information, visit www.prisma-statement.org.
Figure 1. ‘Flow of information through systematic review for PRP in tendinopathy’ shows 72 records were identified through database searching. An additional 3 papers were obtained from review articles. After duplicates were removed 65 records were screened. 21 records were excluded on review of the abstract as they were protocol registrations, not RCTs, related to surgical procedures or conditions other than tendinopathy. The number of full text articles assessed for eligibility was 44. Of these 22 studies were excluded; 5 related to cuff tears, 2 to muscle injury, 13 to surgical interventions and 2 non PRP studies. Of the 22 papers available for analysis, two sets of papers were combined after discussion as they related to the same data sets 31, 33, 56, 95. Two papers were excluded, Kazemi, had data only available to 8 weeks, which did not meet the minimum criteria for analysis 66 and, Mishra 2006, had no analysable data available in the published form and despite personal contact with the authors it was not possible to get data for analysis for this work 87. This meant there were 18 papers available for full analysis. (Table1).
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Tendon</th>
<th>No</th>
<th>Therapy</th>
<th>Outcome</th>
<th>Time Mths</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell</td>
<td>2013</td>
<td>Achilles</td>
<td>53</td>
<td>ABI/DN</td>
<td>VISA-A</td>
<td>6</td>
<td>Included</td>
</tr>
<tr>
<td>Pearson</td>
<td>2012</td>
<td>Achilles</td>
<td>28</td>
<td>ABI/Ecc</td>
<td>VISA-A</td>
<td>3</td>
<td>Included</td>
</tr>
<tr>
<td>Thanassos</td>
<td>2011</td>
<td>TE</td>
<td>27</td>
<td>GPS/ABI</td>
<td>VAS</td>
<td>3,6</td>
<td>Included</td>
</tr>
<tr>
<td>Creaney</td>
<td>2011</td>
<td>TE</td>
<td>130</td>
<td>LR-PRP/ABI</td>
<td>PRTEE</td>
<td>3,6</td>
<td>Included</td>
</tr>
<tr>
<td>Wolf</td>
<td>2011</td>
<td>TE</td>
<td>28</td>
<td>ABI/CSI/Saline</td>
<td>DASH</td>
<td>2,6</td>
<td>Included</td>
</tr>
<tr>
<td>DeVos</td>
<td>2010/2011</td>
<td>Achilles</td>
<td>54</td>
<td>GPS/Saline</td>
<td>VISA-A</td>
<td>3,12</td>
<td>Included</td>
</tr>
<tr>
<td>De Jonge</td>
<td>2010/2011</td>
<td>TE</td>
<td>100</td>
<td>GPS/CSI</td>
<td>DASH</td>
<td>3,6,12</td>
<td>Included</td>
</tr>
<tr>
<td>Gosens</td>
<td>2010/2011</td>
<td>TE</td>
<td>60</td>
<td>ABI/CSI</td>
<td>DASH</td>
<td>2</td>
<td>Not included</td>
</tr>
<tr>
<td>Peerbooms</td>
<td>2010</td>
<td>TE</td>
<td>60</td>
<td>ABI/CSI</td>
<td>DASH</td>
<td>2</td>
<td>Not included</td>
</tr>
<tr>
<td>Kazemi</td>
<td>2010</td>
<td>TE</td>
<td>57</td>
<td>ABI/CSI/SWT</td>
<td>VAS</td>
<td>3,6,12</td>
<td>Included</td>
</tr>
<tr>
<td>Ozturan</td>
<td>2013</td>
<td>TE</td>
<td>60/17</td>
<td>GPS-H</td>
<td>PRTEE</td>
<td>3,12</td>
<td>Included</td>
</tr>
<tr>
<td>Krogh</td>
<td>2013</td>
<td>TE</td>
<td>225</td>
<td>GPS/LA</td>
<td>PRTEE</td>
<td>3,6</td>
<td>Included</td>
</tr>
<tr>
<td>Behera</td>
<td>2015</td>
<td>TE</td>
<td>25</td>
<td>LP-PRP/LA</td>
<td>MMCPI</td>
<td>3,6,12</td>
<td>Included</td>
</tr>
<tr>
<td>Arik</td>
<td>2014</td>
<td>TE</td>
<td>80</td>
<td>ABI/CSI</td>
<td>PRTEE</td>
<td>3</td>
<td>Included</td>
</tr>
<tr>
<td>Dragoo</td>
<td>2014</td>
<td>PT</td>
<td>25</td>
<td>GPS/DN</td>
<td>VISA-P</td>
<td>3,6</td>
<td>Included</td>
</tr>
</tbody>
</table>
### 2.4.5 Data Collection Processes

Data from the included trials was extracted by one reviewer (JF) and checked by a second reviewer (MB). The extracted data was included in an excel spreadsheet and included the title of the paper and authors, the kit or product type and technique eg whole blood (ABI), GPS, MyCells, the region being treated eg achilles, tennis elbow, the number of participants in the trial enrolled and completed, whether the trial was an RCT, the type of pain score used and it’s maximum score, and the 2, 3, 6 and 12 month scores and their standard deviations. Where the standard deviations were not reported, they were calculated from the 95% confidence intervals. Where neither of these was available the authors were approached directly using the email address on their publication to obtain the raw data. One paper, Mishra, had no published standard deviations or 95% confidence intervals but these were provided following personal contact with the author. The technique used in all PRP groups was described as single/multiple injections, intratendinous (peppering), with or without local anaesthetic. One author was approached to confirm his technique as it was not clear from the publication whether local anaesthetic was used.
2.4.6 Assessment of risk of bias

Since it is accepted that the inclusion of trials with a high risk of bias may distort the results of a meta-analysis,\textsuperscript{60,77} the Cochrane Collaboration’s tool for assessing the risk of bias was used. The following were assessed: randomisation sequence generation, concealment, blinding of subjects, investigator and assessor, attrition rates and financial interest by companies. These were given a rating of low, unclear or high risk of bias. An RCT was ranked with low, medium or high risk overall based on the key areas of if it had a low risk of bias in the key areas of allocation concealment, reporting of attrition rates and patient and assessor blinding, low if all key areas low, medium if two or three factors high or uncertain and high if all four factors rated high.

2.4.7 Measures of treatment effect

Weighted mean difference (WMD) with 95% CI was calculated when continuous outcomes are measured on standard scales. Where continuous outcomes reported on non-standard scales, standardised mean difference (SMD) was calculated. All analysis was performed on the basis of intention-to-treat (ITT). As change from baseline scores were analysed, the Imputing a change-from-baseline standard deviation using a correlation coefficient was done based on the Cochrane guidelines\textsuperscript{61}. 
2.4.8 Assessment of heterogeneity

Heterogeneity among trials was assessed using the $I^2$ test statistic (> 50% is considered having substantial heterogeneity). We used a random-effects meta-analysis as an overall summary when appropriate.

2.4.9 Statistical Analysis

We used the scores for the pain intensity at baseline, 3, 6 and 12 months where available. These were standardised mean differences for each study and each control/treatment group. There was a variety of pain scales used across the studies which were based on 0-10 or 0-100. These were all converted to 0-100 by multiplication of a factor of 10. Given the variety of pain scores, the application of an individual arm-based approach to the meta-analysis was used so each blood product type and each control type was evaluated separately, within each study trial. Data appears as scores for each time period, baseline, 6, and 12 months with standard deviations and 95% CI for each time point. A fixed-effect model was used if no significant heterogeneity existed between studies.

All statistical analysis was performed using STATA version 13 (Stata Corp. 2013 Stata Statistical Software: Release 13. College Station, TX: Stata Corp LP.) Data was analysed in the network meta-analysis using STATA network meta-analysis functions and Forest plots were utilized to assess statistical heterogeneity.
2.5 Results

Of the 75 studies identified by the search, a total of 18 studies were included in the qualitative synthesis. As outlined in Figure 1, studies were excluded if they related to rotator cuff tears rather than tendinopathy, assessed muscle injury, were duplicates, related to ligament injury, had surgical intervention or did not use an autologous blood or PRP product.

Studies were analysed for type of control and type and technique of treatment. All treatments consisted of intra-tendinous injections with prior administration of 1-2 mls local anaesthetic, unless specified otherwise as follows: 1. autologous blood (ABI) 7 studies\(^7, 13, 29, 93, 94, 115, 123\). 2. Leucocyte-rich PRP produced from the buffy coat layer (a. GPS, Biomet Biologistics, USA 6 studies\(^33, 39, 68, 88, 95, 115\). b. MyCells, Kaylight Limited, Israel 1 study\(^118\). c. Pros, Tozaiholdings Inc., Seoul, Korea 1 study\(^98\) d. unspecified kit as LR-PRP) 2 studies\(^29, 52\). 3. Leucocyte-rich PRP produced from the buffy coat layer with 10-15 mls of local anaesthetic injected prior (GPS-HLA) 1 study\(^73\). 4. Leucocyte-poor PRP (LP-PRP) 1 study\(^12\) 5. Autologous conditioned plasma – Leucocyte-poor, platelet poor plasma (ACP) 1 study\(^112\).

Nine groups used a single injection\(^12, 33, 39, 52, 68, 73, 88, 95, 115\) and four groups used two injections\(^29, 98, 112, 118\). All groups used ultrasound guidance except two\(^88, 95\). All groups used 1-3 mls of local anaesthetic injected superficially except one group who injected the local with the PRP\(^95\) and one group who used 10-15 mls of local anaesthetic superficially\(^73\). Only one group activated the PRP prior to injection – Behera’s group who
also used LP-PRP\textsuperscript{12}. Four groups buffered the PRP prior to use with sodium bicarbonate\textsuperscript{33, 73, 88, 95}.

Controls were divided into 1. Injections a. Corticosteroid (CSI) 6 studies\textsuperscript{7, 52, 73, 93, 95, 123}, b. Saline 4 studies\textsuperscript{33, 68, 73, 123} c. Local Anaesthetic (LA) 2 studies\textsuperscript{12, 88} d. Dry Needling (DN) 4 studies\textsuperscript{39, 98, 112, 115} 2. Non-injection a. Eccentric Training (Ecc) 1 study\textsuperscript{94} b. Shock Wave Treatment (SWT) 2 studies\textsuperscript{93, 118}. Two studies used two control arms – Wolf used cortisone and saline as controls against autologous blood\textsuperscript{123}, and Krog used also cortisone and saline as controls against GPS – PRP\textsuperscript{73} No differentiation was made for differing tendon sites.

\textbf{2.5.1 Risk of bias assessment}

No studies were eliminated on bias risk alone. Table 2, shows eight studies were deemed to have a low risk of bias based on the four key areas of Allocation concealment, patient and assessor blinding and reporting of attrition.
<table>
<thead>
<tr>
<th>Trial</th>
<th>Rx</th>
<th>No</th>
<th>Company Interest</th>
<th>Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Patient Blinding</th>
<th>Doctor Blinding</th>
<th>Assessor Blinding</th>
<th>Attrition</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell</td>
<td>ABI</td>
<td>53</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Pearson</td>
<td>ABI</td>
<td>28</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>HRB</td>
<td>HRB</td>
<td>HRB</td>
<td>LRB</td>
<td>MRB</td>
</tr>
<tr>
<td>Thanassios</td>
<td>PR</td>
<td>27</td>
<td>LRB</td>
<td>LRB</td>
<td>HRB</td>
<td>HRB</td>
<td>HRB</td>
<td>LRB</td>
<td>LRB</td>
<td>MRB</td>
</tr>
<tr>
<td>Creamey</td>
<td>PR</td>
<td>13</td>
<td>LRB</td>
<td>URB</td>
<td>LRB</td>
<td>HRB</td>
<td>HRB</td>
<td>LRB</td>
<td>MRB</td>
<td></td>
</tr>
<tr>
<td>Wolf</td>
<td>ABI</td>
<td>28</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>HRB</td>
<td>HRB</td>
<td>HRB</td>
<td>MRB</td>
</tr>
<tr>
<td>DeVos</td>
<td>PR</td>
<td>54</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td></td>
</tr>
<tr>
<td>Gosens</td>
<td>PR</td>
<td>10</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td></td>
</tr>
<tr>
<td>Ozturan</td>
<td>ABI</td>
<td>57</td>
<td>LRB</td>
<td>URB</td>
<td>URB</td>
<td>HRB</td>
<td>HRB</td>
<td>HRB</td>
<td>LRB</td>
<td>MRB</td>
</tr>
<tr>
<td>Krogh</td>
<td>PR</td>
<td>60</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td></td>
</tr>
<tr>
<td>Vetrano</td>
<td>PR</td>
<td>46</td>
<td>LRB</td>
<td>LRB</td>
<td>HRB</td>
<td>HRB</td>
<td>LRB</td>
<td>LRB</td>
<td>MRB</td>
<td></td>
</tr>
<tr>
<td>Mishra</td>
<td>PR</td>
<td>22</td>
<td>HRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td></td>
</tr>
<tr>
<td>Behera</td>
<td>PR</td>
<td>25</td>
<td>LRB</td>
<td>URB</td>
<td>URB</td>
<td>URB</td>
<td>HRB</td>
<td>URB</td>
<td>LRB</td>
<td>MRB</td>
</tr>
</tbody>
</table>
1. LRB: Low risk Bias  
2. URB: Uncertain risk bias  
3. HRB: High risk bias  
4. MRB: Medium risk bias

### 2.5.2 Network meta-analysis

A total of 18 studies (1070 Subjects) were included. Seventeen studies were deemed to be at low or medium risk of bias. Figure 3, Forest plot by technique and PRP kit type, shows the changes in pain scores for treatments and controls presented by treatment type.

The most significant outcome in the PRP groups was those treated with highly cellular leucocyte-rich preparations: PRP-GPS (SMD 35.75CI 28.4 – 43.10), Mycells-PRP (SMD 31.84 CI 17.56 – 46.13), Prosys-PRP (SMD 42.99 CI 37.73-48.25), unspecified LR-PRP (SMD 34.62 CI 31.69-37.55).
The ACP group also had a positive response (SMD 32.67 (1.42, 63.93). The leucocyte-poor PRP did not appear to be as effective: LP-PRP(SMD 26.77 CI 18.31-35.22).

Since it appeared that leucocyte rich, platelet-rich preparations produce a more positive outcome than leucocyte poor preparations this was compared in Figure 2, forest plot grouped analysis. Once the LR-PRP system types are grouped, there is a strongly positive effect: LR-PRP (SMD 36.38 CI 34.0-38.77) when compared to leucocyte poor PRP (LP-PRP) SMD 26.77 CI 18.31-35.22.

One group using LR-PRP with administration of 10-15 mls of local anaesthesia did not get positive results\(^7\). (SMD 14.83 CI 11.11-18.55). Whilst there was no local anaesthetic administered at the time of the PRP injection, the volume injected prior was more than ten times the amount used by other groups. Given the potential negative effect of local anaesthetic on the PRP, this may be the reason this group performed poorly\(^21\).

In assessing the control groups: there was no clear difference between different types of control injections saline (SMD 14.62 CI 10.74-18.5), Local anaesthetic (SMD 15.00 CI 7.66-22.34), Cortisone (SMD 23.82 CI 10.74-18.5) or Dry Needling (SMD 25.22 CI 21.27-29.16). None of these controls is truly a placebo as all these injections produce a measurable effect on the outcome but they do produce effective controls for this type of clinical trial.
Figure 2 Forest Plot by Grouped Analysis

1 ABI: Autologous blood injection
CI: Control Injections grouped together – includes local anaesthetic, Cortisone, dry needling, saline injections
LR-PRP: Leucocyte-rich platelet-rich plasma, single (one study with 2) intra-tendinous peppered injection, with local anaesthetic prior
LR-PRP-HLA: Leucocyte-rich platelet-rich plasma, single intra-tendinous peppered injection, high volume local anaesthetic used
Control-SWT: Control – Shock wave therapy
Control-Ecc: Control – Eccentric strength training
LP-PRP: Leucocyte-poor platelet-rich plasma, single intra-tendinous injection with local anaesthetic
LP-PPP: Leucocyte-poor platelet-poor plasma, two intra-tendinous injections with local anaesthetic and dry needling
ES: 95% Confidence Intervals
% weight: % weight allocated to study
2 ABI: Autologous blood injection (technique not isolated in this protocol)
Control-DN: Control, dry needling
2.6 Discussion

Essentially there are two main types of PRP produced. The first is from the plasma layer. These aim to exclude red and white cells from the preparation and to collect as many platelets from the remaining ‘plasma’ layer as possible. The resultant product is low in red and white cells and has a relatively lower 1.5-3 times baseline level of platelets. The ACP kit works in this way and has been shown to have 1.3\(^2\) to 2.6\(^8\)\(^\text{81}\) times baseline platelet concentrations with low white cell counts. Thus the ACP kit was classified as PPP – being lower in platelet count but also low in white cell count. The second type of product is made from the ‘buffy coat’ layer. It aims to take platelets from both the plasma and the cellular layer and thus is generally much higher in platelet count yielding approximately 3-8 times baseline levels of platelets\(^2\)\(^\text{20},\text{38},\text{69}\). It does however, concentrate the white cells in equal amounts and is thus high in both leucocytes and platelets (LR-PRP). It is possible to produce leucocyte poor PRP (LP-PRP) by filtering out the white cells after preparation as was conducted by Behera’s group. A recent laboratory study by these authors (unpublished data) showed that the difference between PRP kit preparation

---

LR-PRP: Leucocyte-rich PRP, unspecified kit, single intra-tendinous peppered injection with local anaesthetic
Control-Saline: Control injection of saline
GPS-PRP-HL: Leucocyte-rich PRP (GPS Kit), single intra-tendinous injection with high volume local anaesthetic
Control-CSI: Control injection of cortisol
Control-LA: Control injection of local anaesthetic
GPS-PRP: Leucocyte-rich PRP (GPS Kit), single intra-tendinous peppered injection with local anaesthetic
Control-SWT: Control – Shock wave therapy
Control-Ecc: Control – Eccentric strength training
Mycells-PRP: Leucocyte-rich PRP (Mycells Kit), single intra-tendinous peppered injection with local anaesthetic
LP-PRP: Leucocyte-poor PRP, single intra-tendinous peppered injection with local anaesthetic
Prosys-PRP: Leucocyte-rich PRP (Prosys Kit), two intra-tendinous peppered injections with local anaesthetic
ACP-PRP: Leucocyte-poor platelet-poor plasma, (ACP Kit), two intra-tendinous injections with local anaesthetic and dry needling
ES: 95% Confidence Intervals
% weight: % weight allocated to study
is quite profound in terms of the total white cell count ranged from $35.8 \times 10^9/l$ in LR-PRP to $1.3 \times 10^9/l$ in LP-PRP.

This study shows that the outcome of PRP is different depending on the method of preparation of the PRP and the injection technique. There were four different types of PRP preparations and techniques studied. Highly cellular LR-PRP shows strongly positive outcomes in treating tendinopathy, when assessed in the network meta-analysis.

The type of PRP (LR-PRP) and the usually single injection technique using small volumes of superficial local anaesthetic administration with a 5-6 pass peppering technique for the LR-PRP, generally under ultrasound guidance, are consistent across the studies: Tendons included in this analysis included 5 studies on tennis elbow, 2 studies on rotator cuff, 2 studies on patellar tendon and one on the achilles tendon. Only one trial was included using LP-PRP, hence the data is too limited to draw conclusions at this stage. There is some evidence that the use of local anaesthetic reduces the effectiveness of PRP in-vitro\textsuperscript{21}. This meta-analysis demonstrates that LR-PRP is effective but it is important to note that all groups used local anaesthetic injected \textit{prior to} and \textit{superficial to} the tendon.

We have not presented the data in contrast to placebo/control in part, as many studies have active controls for example Creaney\textsuperscript{29}, who compared autologous blood injections with Platelet-Rich Plasma, and, because our secondary goal was to determine whether the choice of control made a difference to the outcome. Several reviewers have suggested that glucocorticoid injections should not be used as a control as they confer a negative
outcome and therefore make the difference in the active (PRP) treatment look greater. We would contest that *all injections* are clinically active treatments whether this is dry needling, saline or local anaesthetic administration\textsuperscript{63, 106, 112}. Thus the data have been presented as change in pain scores from baseline for all modalities, be they controls or active treatments.

We also wished to identify whether the type of control may affect the result of trials, particularly the use of cortisone. It has been argued by De Vos\textsuperscript{34} that cortisone has a negative effect on tendinopathy and thus when used as a control it will make the mean difference greater than it would if it were compared to other types of injectable controls. Corticosteroid injections show an improvement up to 3 months and then a decline in effectiveness, as shown in the most recent Cochrane review by Dean\textsuperscript{35}. Our network meta-analysis found that cortisone, dry needling and saline injections did not have a positive outcome in the treatment of tendinopathy. Saline (SMD 14.62 CI 10.74-18.5), Local anaesthetic (SMD 15.00 CI 7.66-22.34), Cortisone (SMD 23.82 CI 10.74-18.5) or Dry Needling (SMD 25.22 CI 21.27-29.16). In fact, Cortisone and dry needling both have a greater change from baseline than saline or local anaesthetic and would thus show a less positive outcome when compared to active treatment groups – the opposite effect to that postulated by De Vos\textsuperscript{34}. It is considered, therefore, that any of cortisone, dry needling or saline injections are good controls for clinical trials assessing tendinopathy and consequently trials using cortisone, saline, local anaesthetic or dry needling as a control would be valid when used in a meta-analysis. Taking into account the recommendations
of the World Medical Association’s Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects, which states “the benefits, risks, burdens and effectiveness of a new intervention must be tested against the best current proven intervention, except in the following circumstances: The use of a placebo, or no treatment, is acceptable in studies where no current proven treatment exists,” our network meta-analysis would support the inclusion of data where cortisone, local anaesthetic, saline or dry needling are used as a control in the treatment of tendinopathy.

The strength of this meta-analysis is that we have shown a difference in the outcome in treating tendinopathy directly related to the type of PRP produced. All previous meta-analyses have grouped PRP types together.

The weakness of this meta-analysis is that it has not been possible to separate the results into grouping by tendon as there are insufficient trials in each area at present. However, as the number of trials increases, it will be possible to determine whether there are differences across tendon locations with different PRP preparations. Nevertheless, the pathology of tendinopathy is similar and conclusions can be drawn for tendinopathy as a group.\textsuperscript{79, 100, 107}

\section*{2.7 Conclusion:}

This network meta-analysis has identified that the type of PRP and the techniques used affect the outcome and should always be included in any meta-analysis in the future. As predicted by Moraes\textsuperscript{90} and recommended by Gosens when an analysis is done \textit{it would}
be better to break out the results by specific study design and PRP type. Our systematic review and network meta-analysis found strong evidence that leucocyte rich PRP (LR-PRP) improves the outcome in tendinopathy and confirms the results published by Baksh. The technique for injection of the LR-PRP includes the use of 1-2 mls of local anaesthetic injected prior to the LR-PRP superficial to the tendon. The single LR-PRP is injected using a peppering technique intra-tendinously into the affected area, generally under ultrasound guidance.
2.8 Search Strategy and Prisma Checklist
### Databases (searched 6.8.13)

<table>
<thead>
<tr>
<th>Database</th>
<th>Search Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medline MESH headings</td>
<td>search term 1 = (Platelet-Rich Plasma) search term 2 = tendinopathy or (tennis</td>
</tr>
<tr>
<td>Medline keyword</td>
<td>search term 1 = (Platelet-Rich Plasma) search term 2 = tendinopathy or (tennis</td>
</tr>
<tr>
<td>Embase MESH headings</td>
<td>search term 1 = (thrombocyte rich plasma) search term 2 = tendinopathy or (tennis</td>
</tr>
<tr>
<td>Embase keyword</td>
<td>search term 1 = (thrombocyte rich plasma) search term 2 = tendinopathy or (tennis</td>
</tr>
<tr>
<td>Pubmed MESH headings</td>
<td>search term 1 = (Platelet-Rich Plasma) search term 2 = tendinopathy or (tennis</td>
</tr>
<tr>
<td>PubMed keyword</td>
<td>search term 1 = (thrombocyte rich plasma) search term 2 = tendinopathy or (tennis</td>
</tr>
<tr>
<td>CINAHL: CINAHL headings</td>
<td>search term 1 = (Platelet-Rich Plasma) search term 2 = tendinopathy or (tennis</td>
</tr>
<tr>
<td>CINAHL keyword</td>
<td>search term 1 = (thrombocyte rich plasma) search term 2 = tendinopathy or (tennis</td>
</tr>
<tr>
<td>WoS</td>
<td>search term 1 = (Platelet-Rich Plasma) search term 2 = tendinopathy or (tennis</td>
</tr>
<tr>
<td>Scopus</td>
<td>search term 1 = (thrombocyte rich plasma) search term 2 = tendinopathy or (tennis</td>
</tr>
</tbody>
</table>

**Searching Notes:**
- Number in brackets (x) = number of records.
- red = search term not listed in your database.
search term 1
Platelet-Rich Plasma (1,351), Intercellular Signaling Peptides and Proteins (20,387), autologous blood transfusions (6,615), plasma (14,2430), autologous conditioned plasma (9).
platelet poor plasma (1,088), autologous conditioned serum (22), autologous blood (4,559)

thrombocyte rich plasma (4,289), thrombocyte poor plasma (717), growth factor (55,604), blood autotransfusion (7,830), autologous conditioned plasma (10).
platelet rich plasma (6,734), autologous conditioned serum (54), autologous blood transfusion (975), autologous blood plasma (13).
platelet rich plasma (1,250), growth factors (735, 697), autologous conditioned serum (119), autologous blood transfusion (6,482)
platelet poor plasma (17,95), autologous blood (44,522), autologous conditioned plasma (58), autologous blood plasma (5,796)
platelet rich plasma (40), growth substances (7,185), blood transfusion, autologous (795), blood component transfusion (1,335)
platelet poor plasma (46), growth factors (6,792), autologous conditioned serum (9), autologous conditioned plasma (3), autologous blood plasma (9)
epworth has no access but you may want to check your search strat
epworth has no access but you may want to check your search strat

cords for that search term.
mail 5.8.13; ic new synonyms.
elected terms and the results were tabulated. if the selected term did not appear in the thes

h 2, limited to RCT or prospective study, limit to human, limit to last 5 years = 182 records.
) or (intercellular signaling peptides and proteins) or (autologous blood transfusions) or plasma (elbow) or (tendon injuries) or (achilles tendon) or (rotator cuff) or (patellar ligament) or bi

h 2, limited to RCT or prospective study, limit to human, limit to last 5 years = 85 records.
isms) or (thrombocyte poor plasma) or (growth factor) or (blood autotransfusion) or (autologous) or hamstring or (achilles tendinitis) or tendinitis or tendinopathy or tendinosis or ten

h 2, limited to RCT or prospective study, limit to human, limit to last 5 years = 37 records.
) or (growth factors) or (autologous conditioned serum) or (autologous blood transfusion) or (elbow) or (lateral epicondylitis) or achilles or (rotator cuff) or (jumper’s knee) or (patellar tende

2, limited to RCT or prospective studies, limit to human, limit to last 5 years = 16 records.
) or (growth substances) or (blood transfusion) or autologous or (blood component transfusions) or (elbow) or (achilles tendinopathy) or (patellar tendinopathy) or (hamstring muscles) or (rot
<table>
<thead>
<tr>
<th>search term 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>tendinopathy (4,125), tennis elbow (1,238), tendinosis (10,274), achilles tendon (6,076), rotator cuff (4810), patellar ligament (15,32), buttocks (4,388),</td>
</tr>
<tr>
<td>tendinitis (676), lateral epicondylitis (507), patellar tendinitis (23), jumper's knee (131), hamstring (3085), gluteal (3,440)</td>
</tr>
<tr>
<td>tendinitis (6,318), tennis elbow (1821), hamstring (3,619), achilles tendinitis (618),</td>
</tr>
<tr>
<td>tendinosis (448), tendinopathy (1,834), tendinosis (24), tendinosis (783), tenosynovitis (10), tenosynovitis (2), lateral epicondylitis (736), patellar tendinosis (38), jumper's knee (202), gluteal (4,688), rotator cuff (3,833)</td>
</tr>
<tr>
<td>tendinosis (4,437), tennis elbow (1,187), lateral epicondylitis (1,187), achilles (5,653), rotator cuff (4,541)</td>
</tr>
<tr>
<td>jumper's knee (145), patellar tendinopathy (471), hamstring tendinopathy (28), hamstring* (4,490), gluteal (3,695)</td>
</tr>
<tr>
<td>tendinopathy (1,390), tennis elbow (799), achilles tendinopathy (383), patellar tendinopathy (118), hamstring muscles (1,343), rotator cuff injuries (1,606)</td>
</tr>
<tr>
<td>tendinitis (246), lateral epicondylitis (238), buttocks (907),</td>
</tr>
</tbody>
</table>

surs, then it was searched as a key word.

s.

pra or (autologous conditioned plasma) or (platelet poor plasma) or (autologous conditioned serum) or (lateral epicondylitis or (tendinitis or (tennis elbow) or hamstring or (achilles tendinitis)

ous conditioned plasma) or (platelet rich plasma) or (autologous conditioned serum) or (autologous blood or (tenosynovitis or tenosynovitis or (lateral epicondylitis) or (patellar tendinosis) or (jumper's knee) or gluteal or (platelet poor plasma) or (autologous blood or (autologous conditioned plasma) or (autologous blood or (lateral epicondylitis) or hamstring* or gluteal

n) or (platelet poor plasma) or (growth factors) or (autologous conditioned serum) or (autologous conditioned plasma) or (tenon) or (lateral epicondylitis) or buttocks
autologous blood

blood transfusion or (autologous blood plasma) or (rotator cuff)

plasma

filtrated plasma or (autologous blood plasma)
# PRISMA 2009 Checklist

<table>
<thead>
<tr>
<th>Section/topic</th>
<th>#</th>
<th>Checklist item</th>
<th>Reported on page #</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TITLE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Title</td>
<td>1</td>
<td>Identify the report as a systematic review, meta-analysis, or both.</td>
<td>1</td>
</tr>
<tr>
<td><strong>ABSTRACT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structured summary</td>
<td>2</td>
<td>Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.</td>
<td>2-3</td>
</tr>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rationale</td>
<td>3</td>
<td>Describe the rationale for the review in the context of what is already known.</td>
<td>4</td>
</tr>
<tr>
<td>Objectives</td>
<td>4</td>
<td>Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICO-S).</td>
<td>4-5</td>
</tr>
<tr>
<td><strong>METHODS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protocol and registration</td>
<td>5</td>
<td>Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.</td>
<td></td>
</tr>
<tr>
<td>Eligibility criteria</td>
<td>6</td>
<td>Specify study characteristics (e.g., PICO-S, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.</td>
<td>5</td>
</tr>
<tr>
<td>Information sources</td>
<td>7</td>
<td>Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.</td>
<td>5</td>
</tr>
<tr>
<td>Search</td>
<td>8</td>
<td>Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.</td>
<td>5 + Attached</td>
</tr>
<tr>
<td>Study selection</td>
<td>9</td>
<td>State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).</td>
<td>5</td>
</tr>
<tr>
<td>Data collection process</td>
<td>10</td>
<td>Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.</td>
<td>6</td>
</tr>
<tr>
<td>Data items</td>
<td>11</td>
<td>List and define all variables for which data were sought (e.g., PICO-S, funding sources) and any assumptions and simplifications made.</td>
<td>6</td>
</tr>
<tr>
<td>Risk of bias in individual studies</td>
<td>12</td>
<td>Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.</td>
<td>6</td>
</tr>
<tr>
<td>Summary measures</td>
<td>13</td>
<td>State the principal summary measures (e.g., risk ratio, difference in means).</td>
<td>6</td>
</tr>
<tr>
<td>Synthesis of results</td>
<td>14</td>
<td>Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I²) for each meta-analysis.</td>
<td>6</td>
</tr>
</tbody>
</table>
# PRISMA 2009 Checklist

<table>
<thead>
<tr>
<th>Section/topic</th>
<th>#</th>
<th>Checklist Item</th>
<th>Reported on page #</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk of bias across studies</strong></td>
<td>15</td>
<td>Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).</td>
<td>6</td>
</tr>
<tr>
<td><strong>Additional analyses</strong></td>
<td>16</td>
<td>Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.</td>
<td>6-7</td>
</tr>
<tr>
<td><strong>RESULTS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Study selection</strong></td>
<td>17</td>
<td>Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.</td>
<td>7</td>
</tr>
<tr>
<td><strong>Study characteristics</strong></td>
<td>18</td>
<td>For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.</td>
<td>7</td>
</tr>
<tr>
<td><strong>Risk of bias within studies</strong></td>
<td>19</td>
<td>Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).</td>
<td>8+ Table</td>
</tr>
<tr>
<td><strong>Results of individual studies</strong></td>
<td>20</td>
<td>For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group; (b) effect estimates and confidence intervals, ideally with a forest plot.</td>
<td>8 + Forest plot</td>
</tr>
<tr>
<td><strong>Synthesis of results</strong></td>
<td>21</td>
<td>Present results of each meta-analysis done, including confidence intervals and measures of consistency.</td>
<td>8</td>
</tr>
<tr>
<td><strong>Risk of bias across studies</strong></td>
<td>22</td>
<td>Present results of any assessment of risk of bias across studies (see Item 15).</td>
<td>7 + Table</td>
</tr>
<tr>
<td><strong>Additional analysis</strong></td>
<td>23</td>
<td>Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).</td>
<td>8</td>
</tr>
<tr>
<td><strong>DISCUSSION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Summary of evidence</strong></td>
<td>24</td>
<td>Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).</td>
<td>8-9</td>
</tr>
<tr>
<td><strong>Limitations</strong></td>
<td>25</td>
<td>Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).</td>
<td>10</td>
</tr>
<tr>
<td><strong>Conclusions</strong></td>
<td>26</td>
<td>Provide a general interpretation of the results in the context of other evidence, and implications for future research.</td>
<td>10</td>
</tr>
<tr>
<td><strong>FUNDING</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Funding</strong></td>
<td>27</td>
<td>Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.</td>
<td>10</td>
</tr>
</tbody>
</table>


For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org)
Chapter 3. Editorial Responses to The Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy

3.1 Preface

The aim of the meta-analysis was to look at the highest level of evidence available in the scientific literature relating to the treatment of tendinopathy using autologous blood products. The Cochrane guidelines for Systematic reviews state that the highest level of evidence is provided by the inclusion of randomised controlled trials. Other non-randomised trials, case series or case reports were not included as they have a lower level of evidence. The meta-analysis was based on the highest level of evidence possible in line with the Cochrane guidelines.59

Three different systems have been used to assess the quality of report, methodology and evidence in systematic reviews: The PRISMA guidelines are used to support a meta-analysis113, where the AMSTAR96 and GRADE105 systems are used to assess multiple meta-analyses or as tools to develop policy strategies in public health platforms. Most journals have specific guidelines relating to which reporting guidelines are required for publication and in line with the American Journal of Sports Medicine recommendations, the PRISMA guidelines were used. However, the GRADE system similarly says that the level of quality in a meta-analysis is the highest where only RCT studies are included in the analysis, as was done in the paper.
‘The Cochrane Collaboration's tool for assessing risk of bias in randomised trials’ was used in this study. It was not necessary to use assessment tools for non-randomised studies of interventions, having included only randomised controlled trials.

There were two letters to the editor of the American Journal of Sports Medicine following the e-publication in June 2016, ahead of print. The editor commissioned responses to these letters which were published in January 2017 at the time of print publication of the original article.

3.2 Letter to the editor – Russo

Effectiveness of PRP in the Treatment of Tendinopathy: Letter to the Editor


© 2016 The Author(s)

Dear Editor:

We read with great interest the network meta-analysis of Fitzpatrick et al7 and regard it as a timely addition to the literature to help inform clinicians of the effectiveness ranking of treatments for tendinopathy pain and impaired function. Using a standardized mean difference (SMD) cut-off of 26, there appeared to be inadequate benefit from saline, local anesthetic, corticosteroid, and dry needling and a positive benefit with leukocyte-
rich platelet-rich plasma (PRP) (SMD, 36.4), leukocyte-poor PRP (SMD, 26.8), and autologous conditioned plasma (SMD, 32.7).

The authors made a qualified statement that leukocyte-rich PRP appears to produce a greater response than leukocyte-poor PRP, but as the authors acknowledged, this is based on 10 studies for leukocyte-rich PRP and only 1 study for leukocyte-poor PRP. In fact, the leukocyte-poor study2 consisted of only 15 patients in the active arm who were treated with a noncommercial preparation of centrifuged blood, which was then passed through a white cell filter (Imugard III PL; Terumo Penpol). This preparation approach should probably not stand proxy for the range of commercial leukocyte-poor preparation systems that currently exist. The Imugard leukocyte filter leaves a residual leukocyte cell count of less than 0.2 $\times 10^5$,14 whereas a typical leukocyte-poor commercial preparation of PRP leaves 0.6 $\times 10^6$ to 1.65 $\times 10^6$ leukocytes (.85% reduction) (RegenKit BCT; RegenLab USA).12 The study by Behera et al2 should properly be characterized as leukocyte absent and thus may explain the reduced efficacy seen compared to leukocyte-rich PRP results.

We therefore propose that, currently, there is inadequate evidence to formulate an opinion as to whether leukocyte-rich or leukocyte-poor PRP may produce the optimal response in treating symptomatic tendinopathy. Indeed, we believe that the search for a binary outcome by commercial interests or investigators is a flawed approach compared to the ideal goal of having a PRP preparation that is uniquely contoured to the requirements
of tendon cell stimulation, tendon strengthening, and analgesic responses rather than “rich” or “poor” in leukocyte number.4,8,11

There is evidence that tendon cells need a certain amount of leukocytes present in PRP to stimulate tendon cell production, although an excessive amount of leukocytes can be detrimental and may produce apoptosis and tendon cell death.11,15,16 Therefore, the approach that should be sought is “leukocyte–optimally enhanced PRP,” and we encourage a collaboration between basic science researchers and clinicians to achieve this outcome.1,3-6,8,10,11,13,16

We also note the use of multiple time points contained within studies being used as separate data entries in the network meta-analysis and that this was conducted with a random-effects process. We suggest that a random-effects process is not the optimal way to address the issue of multiple time points because there is the potential for bias towards the results of a study with a small sample size but with a large number of time points sampled, as each time point contributes equally in the overall network meta-analysis. We recommend the use of fractional polynomials for longitudinal data as per the recommendations of Jansen et al.9 which overcome this bias predisposition without sacrificing the usefulness of multiple time point data. We are most interested as to whether the authors would be able to re-present their results using this statistical approach and believe the conclusions would be more robust for doing so.

Like most good research, the meta-analysis by Fitzpatrick et al7 adds seminal knowledge (efficacy of PRP and inefficacy of saline, local anesthetic, corticosteroid, and dry
needling) and points the way to further research on optimizing the cellular matrix of PRP for tendinopathy treatment. Until further studies are conducted, we believe that current commercial PRP preparations, whether leukocyte rich or poor, are suitable for clinical use.

Marc Russo, MBBS, DA(UK), FANZCA, FFPMANZCA
Willem Volschenk, MBChB, FCA(SA), FANZCA
Danielle Santarelli, PhD

Broadmeadow, New South Wales, Australia

Address correspondence to Marc Russo, MBBS, DA(UK), FANZCA, FFPMANZCA (email: algoguy@gmail.com).

The authors declared that they have no conflicts of interest in the authorship and publication of this contribution.
REFERENCES


3.3 Response to letter to the editor – Russo

Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy - A Meta-analysis of Randomized Controlled Clinical Trials, Fitzpatrick et al -Author's Response

The authors thank the respondents for their comments that this paper is ‘a timely addition to the literature to help inform clinicians of the effectiveness ranking of treatments for tendinopathy pain and impaired function.’

The authors accept that there are more RCTs included for analysis that have used LR-PRP. We have included all papers available in the literature at the dates searched. It is possible as further evidence becomes available we will be able to make stronger statements relating to the difference between PRP preparations. However, with the data available at present we have concluded that with reference to LP-PRP (or leucocyte deficient PRP as Russo et al prefer): ‘Only 1 trial was included using LP-PRP; hence, the data are too limited to draw conclusions at this stage.’ However as there was sufficient data relating to LR-PRP we can more confidently say that ‘This meta-analysis demonstrates that LR-PRP is effective.’

Further we agree with the comments by Russo et al that presence of leucocytes may be an important part of the PRP and may confer a more positive outcome. We support the comment that further research to determine the optimal number of white cells in PRP for
clinical efficacy in the management of tendinopathy will be of great assistance in the future.

With respect to the use of fractional polynomials as a statistical analysis, we have performed the more conventional random effects process of statistical analysis due to the limitation of interpreting the parameters of the treatment effect using the fractional polynomials models. Although there are several time points assessed in each trial, the number of time points analysed by this meta-analysis in each trial is consistent and thus the use of the random effects statistical analysis is valid for the studies assessed and would not lead to bias towards studies with more time point assessments.

We thank you for your positive comments and suggest that the data available in the scientific literature to date would support the statement that there is good evidence that LR-PRP improves outcomes in tendinopathy. Further, the technique for the injection of LR-PRP includes the use of 1 to 2 mL of local anesthetic injected prior to LR-PRP superficial to the tendon and that a single LR-PRP is injected using a peppering technique intra-tendinously into the affected area, generally under ultrasound guidance.

3.4 Letter to the editor – Scott

Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy: Letter to the Editor

DOI: 10.1177/0363546516669321 The American Journal of Sports Medicine, Vol. 44, No. 10 © 2016 The Author(s)
Dear Editor:

I was surprised to read the recent meta-analysis, “The Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy: A Meta-analysis of Randomized Controlled Clinical Trials,”1 published in the AJSM. The authors of this trial reported in their abstract that “there is good evidence to support the use of a single injection of LR-PRP [leukocyte-rich platelet-rich plasma] under ultrasound guidance in tendinopathy,” and in the conclusion of the article itself, the authors further stated that there is “strong evidence that LR-PRP improves outcomes in tendinopathy” (emphases added). Unfortunately, this conclusion is simply not justified by the results of the article.

To decide whether the evidence for or against a given treatment is “good,” “strong,” or something else entirely, there is consensus among over 20 organizations (including the World Health Organization, American College of Physicians, and Cochrane Collaboration) that the evidence should be ranked using a systematic approach (the GRADE [Grading of Recommendations Assessment, Development and Evaluation] system), which should ideally be applied by those with training in clinical trial design and evaluation. The Cochrane Collaboration conducted its own systematic review of PRP for soft tissue musculoskeletal injuries and concluded (as of April 2014) that there was only “very weak (very low quality) evidence” regarding the clinical efficacy of PRP.2 Considering that the AJSM review concludes the evidence to be “strong,” whereas the Cochrane Collaboration concluded it to be “very weak,” prompts a closer look.
The systematic review by Fitzpatrick et al actually did not report any formal assessment of the quality of evidence (other than an assessment of the risk of bias, which is just 1 component of the GRADE scheme). Several other aspects of this study are also somewhat unconventional and potentially misleading. Typically, forest plots of standardized mean differences are used to graphically represent the difference between a treatment and the control, using a similar outcome measure at a similar point in time. However, in this case, the forest plots depicted the differences between baseline measures (taken at the start of each clinical trial) and the clinical outcomes. Thus, the conclusion that “LR-PRP improves outcomes in tendinopathy” is incorrect. For any given sample of people with tendinopathy, the average clinical outcomes of the whole group will improve over time, even in a “wait-and-see” group. Therefore, evidence of efficacy requires more than a documentation of changes from baseline.

Fitzpatrick et al went a step further and argued that the use of placebos/controls was a bad idea: “We have not presented the data in contrast to placebos/controls in part as many studies have active controls.” Admittedly, it is now accepted that the placebo effect is a real biological phenomenon that can synergize with an active treatment to improve clinical efficacy. Nevertheless, I and many others believe that an important medical goal is to achieve treatments that exceed the placebo response, or a simple reaction to the needle, which is typically a very short-lived response.

The authors also reported that they have conducted a network meta-analysis; however, there is not enough specific information of how this was performed to evaluate or
replicate their results. Their analysis led them to conclude that there was ‘‘no clear difference between different types of control injections.’’ Needless to say, this conclusion is at odds with a large body of evidence demonstrating different clinical responses between saline and corticosteroids. Therefore, it leads to the question of whether the network meta-analysis was conducted properly.

The authors’ use of the I2 statistic is also unconventional.

The I2 statistic is, by convention, a measure of the heterogeneity of treatment effect sizes among different clinical trials that have assessed a common outcome. Here, it is applied to trials using vastly different outcome measures (visual analog scale, Disabilities of the Arm, Shoulder and Hand [DASH], Patient-Rated Tennis Elbow Evaluation [PRTEE], Victorian Institute of Sport Assessment–Achilles [VISA-A], etc) and even at different time points. In some cases, the I2 value is calculated for a single trial with more than 1 time point, grouped together for the purposes of assessing heterogeneity.

In their conclusion, the authors stated that ‘‘the most significant outcomes in the PRP groups were seen in those treated with LR-PRP preparations.’’ It is not always clear to a reader what is meant by a ‘‘significant outcome,’’ so this should have been defined more clearly from the outset. A ‘‘clinically significant outcome’’ for a patient with tendinopathy might be, for example, a 50% reduction in pain with tendon loading, an increased participation level in sport, or a feeling that his or her condition has improved substantially. What the authors are apparently referring to is the size of the standardized
mean difference from baseline. If that is the case, then the most likely reason that there
were more studies reporting significant changes from baseline with LR-PRP is because
the majority of studies in the review used this preparation.

In summary, after reading the results of this AJSM article, I believe that it is still the case
that patients should be cautioned that the evidence that has shown support for PRP in the
treatment of soft tissue injuries is of low quality, as concluded by the Cochrane
Collaboration.

Alex Scott, PhD

Vancouver, British Columbia, Canada

Address correspondence to Alex Scott, PhD (email: ascott@interchange.ubc.ca).

The author declared that he has no conflicts of interest in the authorship and publication
of this contribution.

REFERENCES

3.5 Response to letter to the editor – Scott

Letter to the editor regarding Fitzpatrick et al, "The effectiveness of platelet rich plasma in the treatment of tendinopathy - A Meta-analysis of Randomized Controlled Clinical Trials” - Response.

We reported ‘strong evidence that LR-PRP improves outcomes in tendinopathy.’\textsuperscript{47} This meta-analysis was based on the highest level of evidence available in the scientific literature. The Cochrane guidelines for Systematic reviews state that the highest level of evidence is provided by the inclusion of randomised controlled trials. We have not included any papers with a lower level of evidence and thus the meta-analysis is based on the highest level of evidence possible in line with the Cochrane guidelines\textsuperscript{59}.

Three different systems have been used to assess the quality of report, methodology and evidence in systematic reviews: The PRISMA\textsuperscript{113}, AMSTAR and GRADE systems. The GRADE system is used to ensure the level of evidence is high, however we have used the PRISMA reporting guidelines for meta-analyses, in line with the accepted standards of the AJSM, JAMA and BMJ. With the GRADE system the level of quality is the highest where only RCT studies are included in the analysis, as we have done. We have also used ‘The Cochrane Collaboration's tool for assessing risk of bias in randomised trials”\textsuperscript{60}. We believe our study does fulfil the criteria for assessing quality as defined by the accepted PRISMA guidelines.
The Cochrane study’ Platelet-rich therapies for musculoskeletal soft tissue injuries concluded there was positive although ‘very low quality evidence from a subset of these trials for a marginal short-term benefit in pain from PRP’. The authors claim that part of the reason for this was the ‘major concern particular to PRP research is the methodology for its preparation’ and point out in addition, ‘classification proposals of platelet-rich products are available and have demonstrated from the clinical science perspective that the effectiveness of these products may be strongly linked to three key items: 1) the absolute number of platelets, 2) the manner in which platelet activation occurs, and 3) the presence or absence of white cells.’

This study was designed to address these issues by analysing the outcome based on the particular PRP preparation (initially analysing by exact kit type for preparation), method of injection technique and including only studies relating to tendinopathy. The Cochrane study included all soft tissues including ligamentous and muscle injury as well as surgical interventions such as ACL reconstruction. The benefit of our study protocol is that it has been able to limit the injury only to tendinopathy. Additionally we have been able to differentiate between specific PRP treatment types and answer the question posed by Mishra – ‘it would be better to break out the results by specific study design and PRP type.’

With reference to the forest plots, we have included all the data for standard mean differences so that the reader can see the ‘natural history of the condition’ as the change from baseline for the controls. They can see the conventionally expressed difference
between the treatment and the control as the difference between the scores of the controls and the active treatment groups. Whether this is plotted as both or one group – the delta value is the same. Thus the outcome that PRP improves the outcome in tendinopathy is clearly demonstrated correctly by this forest plot but allows the reader to see all of the data leading to this analysis.

The authors agree that the ‘placebo effect is a real biological phenomenon which can synergize with an active treatment to improve clinical efficacy’. Further we have demonstrated that both active and placebo controls are shown by these RCTs to demonstrate a change from baseline. Whether this is due to the placebo effect or accounted for by the natural history of the condition is unclear but nevertheless we have shown that the type of control (active or placebo) does not negatively influence the outcome of the trial and thus, we accept that all trials with either form of control may be critically analysed in the meta-analysis. This differs from the Cochrane report which did not assess all trials if their control was active, i.e corticosteroid.

Further, the Declaration of Helsinki would support the use of active controls rather than placebo controls as stated in our paper: ‘the benefits, risks, burdens and effectiveness of a new intervention must be tested against the best current proven intervention, except in the following circumstances: The use of a placebo, or no treatment, is acceptable in studies where no current proven treatment exists’1. Whilst Dr Scott may not agree that the type of control does not influence the outcome of the trial, our statement that the type of control does not influence the outcome is based on the data from the RCTs and does not reflect
an opinion. Given the opinion of de Vos$^{34}$ that the use of cortisone as a control may bias the outcome of the trial, we stated our finding that ‘corticosteroid and dry needling both have a greater change from baseline than saline or local anesthetic and would thus show a less positive outcome when compared with active treatment groups, the opposite effect to that postulated by de Vos et al’. In this sense the data do not support the theory held by Dr Scott to the contrary.

This systematic review was conducted according to the PRISMA guidelines and the full supporting documentation is available in the Supplementary information for this article. This article has been conducted to the highest standards for systematic review and meta-analysis.

The final criticism that the use of the I-squared statistical analysis was unconventional is incorrect. The I-squared statistical analysis is used to assess heterogeneity and this has been correctly assessed here. This paper cannot assess individually each tendinopathy type and outcome measure separately as is done in a Cochrane assessment (which has unlimited publication space).

In conclusion the aim of this paper was to perform a meta-analysis of the outcomes of the PRP groups by preparation method and injection technique in tendinopathy. Our systematic review and network meta-analysis found evidence that LR-PRP improves outcomes in tendinopathy.
3.6 CME Quiz questions American Journal of Sports Medicine

3.6.1 Invitation

The editor of the American Journal of Sports Medicine commissioned a series of
Continuing Medical Education (CME) questions in conjunction with the publication of
the article The Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy.

The link to the CME questions appears as a part of the article as follows:

An online CME course associated with this article is available for 1 AMA PRA Category
1 Credit™ at
Continuing Medical Education (ACCME), it is the policy of The American Orthopaedic
Society for Sports Medicine that authors, editors, and planners disclose to the learners all
financial relationships during the past 12 months with any commercial interest (A
‘commercial interest’ is any entity producing, marketing, re-selling, or distributing
health care goods or services consumed by, or used on, patients). Any and all disclosures
are provided in the online journal CME area which is provided to all participants before
they actually take the CME activity. In accordance with AOSSM policy, authors, editors,
and planners’ participation in this educational activity will be predicated upon timely
submission and review of AOSSM disclosure. Noncompliance will result in an
author/editor or planner to be stricken from participating in this CME activity.
3.6.2 CME Questions


1. Platelet-rich Plasma is defined as:
   a. Platelet-rich concentrate with platelet levels > 3 times baseline
   b. Platelet-rich concentrate with platelet levels > baseline
   c. Platelet-rich concentrate with red blood cell levels > baseline
   d. Platelet-rich concentrate with negligible levels of leucocytes

2. The overall risk of bias in this study:
   a. Was based on allocation concealment, funding and blinding
   b. Found High risk of bias in approximately half of the studies
   c. Found a low risk of bias in approximately half of the studies
   d. All of the above

3. In assessing the controls used in each RCT:
   a. Corticosteroid injection produces the most negative outcome
   b. Saline and local anaesthetic are comparable in outcome
   c. Dry needling and Saline are comparable in outcome
   d. Controls are used because they produce no change from baseline

4. Regarding the method of PRP preparation:
   a. All PRP is derived from the buffy coat layer after centrifugation
   b. LP-PRP is derived from the buffy coat layer after centrifugation
   c. PPP is derived from the buffy coat layer after centrifugation
   d. LR-PRP is derived from the buffy coat layer after centrifugation
5. Which of the following statements are true regarding the treatment for tendinopathy?
   a. Due to the inflammatory nature of tendinopathy corticosteroids are used commonly
   b. Despite the non-inflammatory nature of tendinopathy corticosteroids are used commonly
   c. Corticosteroids are commonly used because they have the best long term result in tendinopathy
   d. None of the above

6. This systematic review and meta-analysis:
   a. Included only RCTs in soft tissue injuries
   b. Included all trials in tendinopathy
   c. Included only RCTs in tendinopathy
   d. Included RCTs in tendinopathy with surgical intervention

7. The data analysed in this meta-analysis:
   a. Included standard means of pain scores at 2, 3, 6 and 12 months
   b. Included the technique used as a single or multiple injections
   c. Included the technique relating to local anaesthetic administration
   d. All of the above

8. Regarding the results of the meta-analysis:
   a. Only the GPS, Mycells and ACP kits produce a positive outcome in this meta-analysis
   b. Results for pooled data for LR-PRP showed positive outcomes in this meta-analysis
   c. All kits that produce LR-PRP showed similar results irrespective of technique or tendon
   d. The authors were able to break down the results by geographical tendon type eg achilles
9. Regarding the analysis of controls used in this meta-analysis:
   a. The authors identified that none of the controls showed an change from baseline
   b. The authors identified corticosteroid showed a similar improvement to dry needling
   c. The authors identified that saline and local anaesthetic had a greater change from baseline than corticosteroid
   d. None of the above
Chapter 4. Analysis of Platelet-Rich-Plasma Extraction.

Variations in Platelet and Blood Components between Four Common Commercial Kits.

4.1 Preface

It is clear from the results of the meta-analysis (Chapter 2) that the cellular composition of PRP is critical to the clinical efficacy in the management of tendinopathy. Therefore the preparation of PRP needs to be standardised and the components understood. There are many commercially available systems and spin techniques for the production of PRP in the clinical setting. This Chapter looks at the variation in cellular composition of these methods more critically as well as expanding this to include the biochemical composition of PRP preparations.

This laboratory study is designed to explore the standardization of platelet-rich plasma extraction for clinical use. It compares the variations in composition of the PRP sample produced including platelet, red cell, white cell, pH and glucose. The clinical significance of this is to evaluate which variations in product must be taken into account when assessing the results of clinical trials and in the choice of preparation by practitioners. From this, we can determine whether the difference between the kits is clinically relevant by undertaking high level controlled trials with validated kits.
4.2 Abstract

**Background:** Platelet-rich plasma (PRP) has been extensively used as a treatment in tissue healing in tendinopathy, muscle injury, and osteoarthritis. However, there is variation in methods of extraction, and this produces different types of PRP.

**Purpose:** To determine the composition of PRP obtained from 4 commercial separation kits, which would allow assessment of current classification systems used in cross-study comparisons.

**Study Design:** Controlled laboratory study.

**Methods:** Three normal adults each donated 181 mL of whole blood, which served as a control and was then processed through 4 PRP separation kits: GPS III (Biomet Biologics), Smart-Prep2 (Harvest Terumo), Magellan (Arteriocyte Medical Systems), and ACP (Device Technologies). The resultant PRP was tested for platelet count, red cell count, and white cell count, including differential in a commercial pathology laboratory. Glucose and pH measurements were obtained from a blood gas autoanalyzer machine.

**Results:** Three kits taking samples from the “buffy coat layer” were found to have greater concentrations of platelets (3-6 times baseline) while 1 taking samples from plasma was found to have platelet concentrations of only 1.5 times baseline. The same 3 kits produced an increased concentration of white cells (3-6 times baseline). These comprised neutrophils, leucocytes, and monocytes. This represents high concentrations of platelets and white cells. A small drop in pH was thought to relate to the citrate used in
the sample preparation. Interestingly, an unexpected increase in glucose concentrations, with 3 to 6 times greater than baseline levels, were found in all samples.

**Conclusion:** This study reveals the variation of blood components including platelets, red blood cells, leukocytes, pH, and glucose in PRP extractions. The high concentrations of cells are important as the white cell count in PRP samples has frequently been ignored, being considered insignificant. The lack of standardization of platelet-rich plasma preparation for clinical use has at least in part contributed to the varying clinical efficacy in PRP use.

**Clinical Relevance:** The variation of platelet and other blood component concentrations between commercial PRP kits may impact clinical treatment outcomes. There is a need for standardization of PRP for clinical use.

### 4.3 Background

Platelet-rich plasma (PRP) is defined as a platelet-rich concentrate with greater than the baseline levels of platelets when compared with whole blood. PRP is increasingly used in prospective clinical studies to improve tissue healing, particularly with regarding to tendonitis. There are small numbers of randomized controlled trials emerging showing the positive benefit of PRP in tendinopathy. It has been hypothesized that this is due to the growth factors platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-beta), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), and hepatocyte growth factor (HGF), which are
released from the alpha granules during in vivo activation of platelets, or subsequently produced by the cellular matrix of the tendon.

DeLong et al considered that PRP preparations can be divided into 2 forms: 1 plasma-based and the other based on buffy coat preparations. Plasma-based preparations aim to capture platelets from the plasma after centrifugation and exclude red and white cells. Generally these kits produce smaller increases in platelets than the kits that take platelets from both the plasma and the more cellular “buffy coat.”

There has been some discussion about whether the efficacy of the PRP is affected by the inclusion of the white cells. Moojen et al considered that there may be positive effects from the white cells acting as antimicrobial agents. Other authors have suggested that the platelets themselves may already have this property. There may also be negative effects from these white cells in causing further inflammation, leading to fibrosis, or from the release of catabolic cytokines. This effect may be more prevalent with neutrophils than other white cells. Recent meta-analyses of PRP in tendinopathy identified that leucocyte-rich PRP had a strongly positive outcome in the treatment of tendinopathies.

There has also been discussion about whether the pH of the resultant PRP will affect platelet function, and thus, whether the PRP produced should be “buffered.” Since it is likely to be important in the management of different conditions to have certain types of PRP used, all commercial kits should be validated for cell and PRP
type, but this has not always been the case. The purpose of this study was to validate all kits available in Australia for their composition of platelet, red and white cell counts, pH, and glucose levels using a single-donor model. A recommendation could then be made as to which PRP kits/types are associated with the best results in the treatment of different musculoskeletal conditions such as tendinopathy and osteoarthritis.

4.4 Methods

Three healthy adult human subjects were recruited and consented for this trial (2 women, 1 man; age range, 25-35 years).

4.4.1 Description of Common Commercial Kits

A review of all kits was undertaken as shown in Table 1 based on the International Olympic Committee (IOC) consensus paper on the use of platelet-rich plasma in sports medicine. It was decided that only kits producing platelet-rich plasma (PRP), autologous conditioned plasma, or pure platelets would be assessed. Only kits producing PRP from whole blood for use in musculoskeletal conditions such as tendonitis, muscle injuries, or osteoarthritis were selected. Kits were excluded if they produced platelet-rich fibrin or bone marrow samples. Thus, 8 potential kits were available for testing.

Cell saver–based pure platelet systems requiring a minimum sample of 200 mL of whole blood for processing were not deemed appropriate to study as this large sample was
regarded as impractical for office use. The Caption pure platelet kit was not commercially available at the time of testing, and therefore, 6 potential kits were available for study. Of these, only 4 were commercially available in Australia at the time of testing. These included: GPS III (Biomet Biologics), Smart-Prep2 (Terumo Harvest), Magellan (Arteriocyte Medical Systems), and ACP (Device Technologies, Arthrex). All companies agreed for their kits to be used in the trial and provided the kits.
Table 1. Commercially Available Kits for the Production of Platelet Products

<table>
<thead>
<tr>
<th>Device name</th>
<th>Company</th>
<th>Name of Product</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPS III</td>
<td>Biomet</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>SmartPrep2</td>
<td>Harvest</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>Magellan</td>
<td>Arteriocyte Medical</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>Angel</td>
<td>Sorin</td>
<td>Platelet-rich plasma</td>
<td>Not available for testing</td>
</tr>
<tr>
<td>CS</td>
<td>Genesis</td>
<td>Platelet-rich plasma</td>
<td>Not available for testing</td>
</tr>
<tr>
<td>ACP</td>
<td>Arthrex</td>
<td>Autologous conditioned plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>PRFM Fibrinet System</td>
<td>Cascade</td>
<td>Platelet-rich fibrin</td>
<td>Not tested, fibrin membrane</td>
</tr>
<tr>
<td>PRF and Vivostat</td>
<td>Choukroun’s</td>
<td>Platelet-rich fibrin</td>
<td>Not tested, fibrin membrane</td>
</tr>
<tr>
<td>BMAC</td>
<td>Depuy</td>
<td>Platelet-rich plasma and stem cells</td>
<td>Not tested, bone marrow</td>
</tr>
<tr>
<td>Cell saver–based systems</td>
<td>Several</td>
<td>Pure platelets</td>
<td>Not tested, volume required &gt;200 mL</td>
</tr>
<tr>
<td>Haemonetics, CATS, BRAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caption</td>
<td>Not yet marketed</td>
<td>Pure platelets</td>
<td>Not tested, not available</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>Pure platelets</td>
<td>4</td>
</tr>
</tbody>
</table>

*Table derived from Engebretson et al.42*
4.4.2 Sample Collection and Processing

All samples were collected from the subjects by the senior author and processed immediately. One hundred eighty-one milliliters of blood was drawn from each subject: 5 mL was used for the control sample, 52 mL for each of the PRP-based kits (GPS III, SmartPrep2, and Magellan), and 15 mL for the ACP kit. The samples were processed according to the manufacturers’ instructions to produce 6 to 7 mL of finished product, as shown in Table 2.

Table 2. Preparation of PRP Samples

<table>
<thead>
<tr>
<th>System</th>
<th>Blood Volume, mL</th>
<th>Anticoagulant Volume, mL</th>
<th>Centrifugal Force, G Force</th>
<th>Centrifuge Time, min</th>
<th>Volume Produced, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPS III</td>
<td>52</td>
<td>ACD-A 8</td>
<td>1100</td>
<td>15</td>
<td>6 to 7</td>
</tr>
<tr>
<td>SmartPrep2</td>
<td>52</td>
<td>ACD-A 8</td>
<td>1250/1050</td>
<td>14</td>
<td>6 to 7</td>
</tr>
<tr>
<td>Magellan</td>
<td>52</td>
<td>ACD-A 8</td>
<td>1200</td>
<td>17</td>
<td>6 to 7</td>
</tr>
<tr>
<td>ACP</td>
<td>15</td>
<td>ACD-A 2</td>
<td>1500</td>
<td>5</td>
<td>6 to 7</td>
</tr>
</tbody>
</table>

*ACD-A, anticoagulant citrate dextrose solution A; PRP, platelet-rich plasma.

The samples were then processed: 1.5 mL from the PRP samples and the control blood were put into a tube for analysis on the Radiometer ABL800 Flex blood gas testing machine generating results for pH, K+, Na+, Cl-, glucose, and lactate. The remaining
control blood and PRP samples were placed into a collection tube for analysis on a Coulter LH 250 automated analyzer within 30 minutes of collection to measure full blood count and white cell count with differential.

4.4.3 Classification of the PRP Produced

The results from the analysis were assessed based on the PAW (Platelet, Activation, White cells)\textsuperscript{38} and the sports medicine platelet-rich plasma classification\textsuperscript{86} systems. The PAW system classifies PRP based on platelet numbers, the manner in which activation occurs and the presence or absence of white cells. The Mishra Sports Medicine PRP classification system is based on platelet concentration, the presence or absence of white blood cells, and whether the PRP has been activated with exogenous thrombin or calcium chloride.

4.4.4 Statistical Analysis

All statistical analyses were performed using STATA version 13 (Stata Corp). All variables had a calculated mean and standard deviation. Each subject was used as their own control, and thus, change from mean was relative to their own control result.

4.5 Results

4.5.1 Comparison of Cellular Components

We first compared the cellular components of platelets, leukocytes, and red blood cells between these 4 kits using standard methods on 3 human subjects. A summary of data is
presented in Table 3. The values for total platelet count as well as red and white cell counts are presented compared with controls.
Table 3. Cellular Data

<table>
<thead>
<tr>
<th>Kit</th>
<th>Cell Type</th>
<th>Mean x 10⁶/L</th>
<th>SD x 10⁶/L</th>
<th>Median x 10⁶/L</th>
<th>Min x 10⁶/L</th>
<th>Max x 10⁶/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Platelets</td>
<td>269</td>
<td>106</td>
<td>290</td>
<td>154</td>
<td>362</td>
</tr>
<tr>
<td></td>
<td>WCC</td>
<td>8.73</td>
<td>3.75</td>
<td>8.9</td>
<td>4.9</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>RBC</td>
<td>4.7</td>
<td>0.436</td>
<td>4.5</td>
<td>4.4</td>
<td>5.2</td>
</tr>
<tr>
<td>ACP</td>
<td>Platelets</td>
<td>412</td>
<td>140</td>
<td>424</td>
<td>266</td>
<td>546</td>
</tr>
<tr>
<td></td>
<td>WCC</td>
<td>1.3</td>
<td>0.781</td>
<td>7.7</td>
<td>0.4</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>RBC</td>
<td>0.0333</td>
<td>0.0577</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>GPS</td>
<td>Platelets</td>
<td>964</td>
<td>551</td>
<td>760</td>
<td>544</td>
<td>1588</td>
</tr>
<tr>
<td></td>
<td>WCC</td>
<td>35.8</td>
<td>10.8</td>
<td>41.8</td>
<td>23.3</td>
<td>42.3</td>
</tr>
<tr>
<td></td>
<td>RBC</td>
<td>1.03</td>
<td>0.289</td>
<td>1.2</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Smart Prep</td>
<td>Platelets</td>
<td>1224</td>
<td>560</td>
<td>1262</td>
<td>646</td>
<td>1764</td>
</tr>
<tr>
<td></td>
<td>WCC</td>
<td>24.7</td>
<td>8.69</td>
<td>26.1</td>
<td>15.4</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>RBC</td>
<td>1.43</td>
<td>0.306</td>
<td>1.5</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Magellan</td>
<td>Platelets</td>
<td>1266</td>
<td>831</td>
<td>1153</td>
<td>497</td>
<td>2148</td>
</tr>
<tr>
<td></td>
<td>WCC</td>
<td>31.4</td>
<td>9.4</td>
<td>35.2</td>
<td>20.7</td>
<td>38.3</td>
</tr>
<tr>
<td></td>
<td>RBC</td>
<td>1.03</td>
<td>0.153</td>
<td>1.0</td>
<td>0.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*RBC,: red blood cell count; WCC, total White cell count.*
4.5.1.1 Platelets

An increase in platelet production was demonstrated compared with baseline in all kits (Figure 1). The ACP kit produced a 1 to 1.7 times baseline level of platelets (412 x10^9/L), which is consistent with the literature for this kit and open-tube single- or double-spin systems. The Magellan (1266 x 10^9/L), GPS (964 x 10^9/L), and SmartPrep (1224 x 10^9/L) kits produce 3 and 6 times baseline platelet concentrations, consistent with previous data.
4.5.1.2 Red Blood Cells

All kits significantly reduced red cell counts compared with controls, as seen in Figure 2. The ACP kit virtually eliminated red cells. The GPS, SmartPrep, and Magellan kits reduced the red cells by 3 to 6 times baseline levels.

Figure 1. Platelet Counts by Kit Type

Figure 2. Red Blood Cells by Kit Type
4.5.1.3 White Blood Cells

White cell counts are of great importance. Compared with controls (white cell count, $8.73 \times 10^9$/L), the only kit to reduce the white cell count was the plasma system (ACP) ($1.3 \times 10^9$/L), which reduced the white cell count by 5 to 22 times, almost eliminating the white cells. The GPS III ($35.8 \times 10^9$/L), SmartPrep2 ($24.7 \times 10^9$/L), and Magellan ($31.4 \times 10^9$/L) kits actively concentrated white cells 3 to 5 times baseline levels (Figure 3). This is consistent with the results found by Carmona\textsuperscript{20}. Similar increases across all 3 kits were demonstrated. Our results show much higher levels of white cell concentration than have been indicated by others\textsuperscript{20,26}.

![Total White Cell Count](image)

**Figure 3. Total White Cell Count by Kit Type**
When the white cell count is broken into a differential white cell count, the majority of cells are neutrophils and lymphocytes (Table 4, Figure 4).
### Table 4. White Cell Differential Counts\(^a\)

<table>
<thead>
<tr>
<th>Kit</th>
<th>Cell Type</th>
<th>Mean (10^9/L)</th>
<th>SD (10^9/L)</th>
<th>Median (10^9/L)</th>
<th>Min (10^9/L)</th>
<th>Max (10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>WCC</td>
<td>8.73</td>
<td>3.75</td>
<td>8.9</td>
<td>4.9</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td>5.5</td>
<td>2.91</td>
<td>5.3</td>
<td>2.7</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>2.37</td>
<td>0.85</td>
<td>2.4</td>
<td>1.5</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Monocytes</td>
<td>0.6</td>
<td>0.173</td>
<td>0.5</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>ACP</td>
<td>WCC</td>
<td>1.3</td>
<td>0.781</td>
<td>1.7</td>
<td>0.4</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td>0.4</td>
<td>0.265</td>
<td>0.5</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>0.7</td>
<td>0.436</td>
<td>0.9</td>
<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Monocytes</td>
<td>0.167</td>
<td>0.115</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>GPS</td>
<td>WCC</td>
<td>35.8</td>
<td>10.8</td>
<td>41.8</td>
<td>23.3</td>
<td>42.3</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td>15.4</td>
<td>5.05</td>
<td>14</td>
<td>11.2</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>15.9</td>
<td>7.73</td>
<td>14.6</td>
<td>8.9</td>
<td>24.2</td>
</tr>
<tr>
<td></td>
<td>Monocytes</td>
<td>3.8</td>
<td>1.1</td>
<td>3.8</td>
<td>2.7</td>
<td>4.9</td>
</tr>
<tr>
<td>Smart Prep</td>
<td>WCC</td>
<td>24.7</td>
<td>8.69</td>
<td>26.1</td>
<td>15.4</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td>6.47</td>
<td>1.86</td>
<td>7</td>
<td>4.4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>14</td>
<td>6.36</td>
<td>13</td>
<td>8.2</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>Monocytes</td>
<td>3.57</td>
<td>1</td>
<td>3.5</td>
<td>2.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Magellan</td>
<td>WCC</td>
<td>31.4</td>
<td>9.4</td>
<td>35.2</td>
<td>20.7</td>
<td>38.3</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td>15.1</td>
<td>3.93</td>
<td>16.6</td>
<td>10.6</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td>12.5</td>
<td>5.72</td>
<td>12</td>
<td>7.1</td>
<td>18.5</td>
</tr>
</tbody>
</table>
WCC, total white cell count.

Compared with controls (5.5 x 10^9/L), the GPS and Magellan kits contain greater mean neutrophil counts (15.4 and 15.1 x 10^9/L, respectively). The SmartPrep kit has a lower mean neutrophil count (6.47 x 10^9/L), and the ACP kit had a negligible mean neutrophil count (0.4 x 10^9/L).

Compared with controls (2.37 x 10^9/L), the mean lymphocyte counts of the GPS (15.9 x 10^9/L), SmartPrep (14.0 x 10^9/L), and Magellan (12.5 x 10^9/L) were higher but similar.
across kits. The ACP kit had negligible lymphocytes (0.7 x 10⁹/L). The increase in total white cell count was similar across the three buffy coat layer kits (GPS, SmartPrep, and Magellan). However, the relative increase in neutrophils was much greater for the GPS and the Magellan kits.

4.5.2 Comparison of Chemical Composition

PRP from the kits was assessed for glucose and pH using the Radiometer ABL800 Flex. The data for pH and glucose are shown in Table 5 and Figures 5 and 6.

Table 5. pH and Glucose Data

<table>
<thead>
<tr>
<th>Kit</th>
<th>Chemical</th>
<th>Mean, mmol/L</th>
<th>SD, mmol/L</th>
<th>Median, mmol/L</th>
<th>Min, mmol/L</th>
<th>Max, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>pH</td>
<td>7.1</td>
<td>0.28</td>
<td>7.12</td>
<td>7.07</td>
<td>7.12</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>4.2</td>
<td>0.529</td>
<td>4.4</td>
<td>3.6</td>
<td>4.6</td>
</tr>
<tr>
<td>ACP</td>
<td>pH</td>
<td>6.87</td>
<td>0.256</td>
<td>6.99</td>
<td>6.57</td>
<td>7.04</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>18.5</td>
<td>2.73</td>
<td>17.7</td>
<td>16.2</td>
<td>21.5</td>
</tr>
<tr>
<td>GPS</td>
<td>pH</td>
<td>7.05</td>
<td>0.02</td>
<td>7.05</td>
<td>7.03</td>
<td>7.07</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>15.8</td>
<td>1.07</td>
<td>15.2</td>
<td>15.1</td>
<td>17.0</td>
</tr>
<tr>
<td>Smart Prep</td>
<td>pH</td>
<td>6.59</td>
<td>0.329</td>
<td>6.61</td>
<td>6.55</td>
<td>6.61</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>23.6</td>
<td>1.36</td>
<td>23.8</td>
<td>22.2</td>
<td>24.9</td>
</tr>
<tr>
<td>Magellan</td>
<td>pH</td>
<td>6.66</td>
<td>0.102</td>
<td>6.69</td>
<td>6.54</td>
<td>6.74</td>
</tr>
</tbody>
</table>
The mean pH of the controls was 7.1. The mean pH of the PRP produced ranged from 6.59 (SmartPrep) to 7.05 (GPS). The lower pH in the kit samples is related to the use of the anticoagulant citrate dextrose solution-formula A (ACD-A) anticoagulant. The amount of ACD-A used was the same ratio for all kits by volume.

Figure 5. pH by kit type

Compared with the glucose control of 4.2 mmol/L, all PRP produced contained a high level of glucose ranging from 15.8 to 23.6 mmol/L. This reflects an increase in glucose of 4 to 6 times baseline.
4.6 Discussion

There are various kits available for the separation of PRP in clinical practice. As a specific “dose” of platelets may be required to achieve a clinical effect, it is important to identify the kits that produce different doses. Using a single-donor protocol, we have analyzed the blood components of 4 of the most common commercial kits available to medical practitioners in Australia. We have shown an increase in platelets from baseline in all of the kits with large variations. This was found to be 1 to 1.5 for plasma-type kits (ACP) and between 3 and 6 times for buffy coat layer kits (GPS III, Magellan,
SmartPrep2). This confirms the work of Castillo et al, who similarly compared 3 kits (Magellan, Cascade and GPS). In addition, there is variation in the numbers of neutrophils, leucocytes, and monocytes between the kits. The plasma system (ACP) reduced the white cell count by 5 to 22 times, almost eliminating the white cells. The buffy coat kits (GPS III, SmartPrep2, and Magellan) actively concentrated white cells 3 to 5 times baseline. The increase in total white cell count was similar across the 3 buffy coat layer kits (GPS, SmartPrep, and Magellan). However, the relative increase in neutrophils was much greater for the GPS and Magellan kits.

A small reduction in pH was thought to relate to the citrate used in the sample preparation. This reduction is not thought to be of clinical significance. Based on the small drop in pH, it does not seem necessary to buffer the PRP unless this change in pH can be shown to negatively impact the production of growth factors.

No studies have reported the level of glucose in PRP produced previously. One of the surprising findings in this study was the significant increases in glucose concentration of 4 to 6 times baseline in all kits. This has not been previously reported as a significant variable, and the clinical significance of this factor is unknown. It is of interest that glucose solutions at concentrations between 12% and 20% have been used in prolotherapy injections with varying results. It is likely this is derived from the use of the ACD-A and is thus related to the preparation technique consistent to all kits. If one takes 5 mL of a 10% glucose solution (2.8 mmol solution) for injection, this would contain 0.5 g of glucose. If we take 5 mL of PRP produced in any of the studied kits at 20
mmol/L (70% glucose solution), this would give us 3 g of glucose. In simple terms, our PRP samples are producing a 6-times glucose concentration compared with glucose solutions used in prolotherapy. This may be important as part of the factors producing a clinical response.

Essentially there are 2 main types of PRP preparation methods. After centrifugation, there are 3 key layers, as shown in Figure 7.

![Figure 7. PRP in a GPS kit after centrifugation](image)

Plasma-based systems take product from the yellow relatively acellular plasma layer. These systems aim to exclude red and white cells from the preparation and to collect as many platelets from the remaining “plasma” layer as possible. As many of the platelets are in the Buffy coat layer, the resultant product is low in red and white cells and has only a 1.5 to 1.7 times baseline level of platelets. This is well demonstrated by the results from
the ACP kit in our study. The second type of PRP product is made from the buffy coat floating above the red cell layer. Levels of platelets at 3 to 6 times baseline levels are expected as the product is coming from a more platelet-dense environment.\textsuperscript{16, 22} Again, this is confirmed by our testing, with the GPS III, Magellan, and SmartPrep2 kits having much higher platelet concentrations.

In producing PRP, all kits aim to reduce the red cell count\textsuperscript{16, 22, 32} and increase the collection of platelets. Some white cells are captured at the same time.\textsuperscript{16, 22} Due to the addition of citrate to the blood being collected, there is likely to be a drop in pH of the sample produced. This is the first paper to identify how other variables like lactate or glucose are changed by this process. Generally, in most literature review papers, the white cell count is ignored or regarded as negligible. These authors feel that these aspects of PRP systems should be more highly noted in future literature reports as the concentration of white cells is as great as that of the platelets and there is glucose present in the end product.

Studies by others have also shown variation of platelet levels, growth factor and cytokine levels,\textsuperscript{92} and total white cell counts across PRP preparation methods.\textsuperscript{20, 22, 43, 44, 69, 83, 114, 121, 124} However, the most important finding in our study is that the white cell counts are significantly more concentrated than previously thought. The ACP kit was the only one in our series that reduced the white cell count by a factor of about 9. This may be an important point of difference if the white cells are not beneficial. The other 3 kits (GPS III, Magellan, SmartPrep2) concentrated the white cell count by 3 to 5 times, a similar
increase to platelet concentration. Thus, these white cells are not contaminants as their levels are as high as the primary ingredient: platelets. Their levels may be regarded as potentially clinically significant. Further, we assessed the white cell differential count and found that the cellular concentration of white cells was up to 40% neutrophils and lymphocytes each and a further 10% made up of monocytes. The remainder of the cells were basophils and eosinophils in small quantities. An increase in the growth factor VEGF would be expected as the number of lymphocytes increases. These lymphocytes may play an important role in further enhancing the tissue repair processes, but they may also lead to increased local inflammation.

White cells may contribute to the modulation of inflammatory and platelet activation, thereby acting to potentiate the tissue repair mechanism. It is possible that the white cells may confer an advantage to the patient in reducing the chance of infection or modulating the inflammatory response. This may be an important consideration in those clinical settings where the patient is at greater risk, such as with intra-articular procedures or at the time of surgery. Furthermore, Zimmermann et al found that the increased white cell count was responsible for between one-third and one-half of the variation on growth factors found in his samples. They found a positive correlation between the white cell count and VEGF (known to come from the white cells) and PDGF.

On the other hand, others have shown that white cells appear to have a deleterious effect on the tissue resulting in increased inflammation and further scarring. These negative effects are largely due to neutrophils and include the release of oxygen-free radicals,
catabolic cytokines, matrix mettaloproteinases (MMPs), and interleukin B, which degrade tissue.\textsuperscript{37}

A recent meta-analysis of the effectiveness of PRP in tendinopathy has shown that leucocyte-rich PRP (LR-PRP) is the most effective in the treatment of tendinopathy.\textsuperscript{47} This study allows us to recommend PRP produced by the GPS III, SmartPrep2, and Magellan kits in the treatment of tendinopathy. By contrast, 2 recent reviews of the effectiveness of PRP in osteoarthritis have shown that leucocyte-poor PRP (LP-PRP) may be more effective.\textsuperscript{85,99}

One of the surprising new findings in this study was the significant increases in glucose concentration of 4 to 6 times baseline in all kits. This has not been previously reported as a significant variable, and the clinical significance of this factor is unknown. It is of interest that glucose solutions at concentrations of between 12\% and 20\% have been used in prolotherapy injections with varying results.\textsuperscript{3,30,50} The clinical significance of this remains uncertain. This was thought to be important as prolotherapy with glucose has been used in the treatment of musculoskeletal injuries.

Kit validation studies help to classify kits into those deemed similar enough to allow results from papers using kits to be compared. Some kit classification systems have been proposed in the past.\textsuperscript{38,86} Table 6 shows the PRP kits classified according to both the PAW and Mishra classification systems.
Table 6. PRP kits by PAW and Mishra (Sports Medicine) Classifications

<table>
<thead>
<tr>
<th>Kit</th>
<th>PAW Platelets</th>
<th>PAW WCC</th>
<th>PAW Neutrophils</th>
<th>Activation</th>
<th>PAW PAW/Mishra Result</th>
<th>Mishra WCC</th>
<th>Mishra Platelets</th>
<th>Mishra Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACP</td>
<td>P2</td>
<td>B</td>
<td>b</td>
<td>N/A</td>
<td>P2Bb</td>
<td>Minimal</td>
<td>&lt;5</td>
<td>3B</td>
</tr>
<tr>
<td>Magellan</td>
<td>P4</td>
<td>A</td>
<td>a</td>
<td>N/A</td>
<td>P4Aa</td>
<td>Increased</td>
<td>&lt;5</td>
<td>1B</td>
</tr>
<tr>
<td>GPS III</td>
<td>P3</td>
<td>A</td>
<td>a</td>
<td>N/A</td>
<td>P3Aa</td>
<td>Increased</td>
<td>&lt;5</td>
<td>1B</td>
</tr>
<tr>
<td>SmartPrep2</td>
<td>P3</td>
<td>A</td>
<td>a</td>
<td>N/A</td>
<td>P3Aa</td>
<td>Increased</td>
<td>&lt;5</td>
<td>1B</td>
</tr>
</tbody>
</table>

*N/A, not applicable; WCC, total white cell count.

PAW classification: platelet counts: P2 = baseline to 750,000, P3 = 750,000-1250,000, P4 = >1250,000; total WCC: A = above baseline, B = below or equal to baseline; neutrophil count: a = above baseline, b = below baseline.

Mishra classification: Type 1B = increased WCC, no activation, platelet count <5 times baseline, Type 3B = minimal WCC, no activation, platelet count <5 times baseline.

The classification proposed by Mishra allows for platelet concentrations >5 or <5 times baseline. We found 3 to 6 times baseline values in the buffy coat kits and 1.5 in the plasma kits. This classification system with a cut-off of greater or less than 5 times concentration does not fit for either buffy coat or plasma system results from our study. The white cells are recorded only as present or absent, and there is no accounting for the level of white cells. The PAW classification system proposed by DeLong et al is appropriate for the classification of platelets, classifying our tested kits into 3 different groups, but it simply classifies the white cells as absent or present. Given the high concentration of white cells found in our laboratory analysis, this classification system...
does not adequately account for the high numbers of white cells, including both neutrophils and lymphocytes found in the PRP preparations studied. If these cells are found to be significant for efficacy, a further breakdown of types of PRP to adequately include white cells will be needed. Kits classified in the same class would then be able to have their results compared as a group when meta-analysis or comparison studies are being undertaken.

4.7 Conclusion

This study identifies the large variations in composition and concentration of platelets, white cell counts, and the differential count of neutrophils and lymphocytes as well as the presence of high levels of glucose between 4 commercial PRP kits. The clinical significance of this is that these variations must be taken into account when assessing the results of clinical trials and in the choice of preparation by practitioners. This study highlights the need for standardization of platelet-rich plasma extraction for clinical use.
Chapter 5. The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Randomised, Double-Blind Controlled Trial comparing a single Platelet-Rich Plasma injection with a single corticosteroid injection.

5.1 Preface

**What is known about the subject:** Tendinopathy of the gluteus medius or minimus is the second most common cause of hip pain presenting in primary care and a common cause of lateral hip pain or greater trochanteric pain syndrome (GTPS). The diagnosis of gluteal tendinopathy is based on *both* clinical presentation and imaging, as imaging alone has not been shown to be diagnostic. Early cases of tendinopathy have been shown to benefit from physiotherapy interventions, analgesics and NSAIDs. The use of corticosteroids may provide short term benefits from twelve to twenty six weeks. Once the tendinopathy has progressed to a full thickness tear, conservative measures are generally ineffective and these ‘refractory patients’ are often managed surgically. A more effective treatment for chronic tendinopathy, without full thickness tears of the gluteal tendons would be of great benefit to clinical practice. The objective of this clinical trial was to examine the effectiveness of a single intra-tendinous PRP injection compared to a standard corticosteroid injection in the treatment of gluteal tendinopathy.
What this study adds to existing knowledge: This is the first published double blinded block-randomised controlled trial performed on the clinical use of PRP in gluteal tendinopathy. The result identifies that PRP has a role in the clinical management of this condition.

5.2 Abstract

Background: Gluteus medius/minimus tendinopathy is a common cause of lateral hip pain or greater trochanteric pain syndrome.

Hypothesis: There would be no difference in the modified Harris Hip score between a single Platelet-Rich-Plasma (PRP) injection compared to a corticosteroid injection (CSI) in the treatment of gluteal tendinopathy.

Study Design: Randomised Double-blind Controlled Single Centre Clinical Trial; Level of evidence 1, recruitment 29 May 2013 to May 2015, open labelled close out September 2016. Australian New Zealand Clinical Trials Registry: ACTRN12613000677707

Methods: 228 consecutive patients referred with gluteal tendinopathy were screened to enrol 80 participants. 148 excluded (refusal 42, previous surgery/sciatica 50, osteoarthritis 17, full thickness tears tendons 17, other 22). Subjects were randomised (1:1) to receive either a blinded glucocorticoid or platelet-rich plasma injection intratendinously under ultrasound guidance. A pain and functional assessment score was performed using a Modified Harris Hip Sore (MHHS) questionnaire at 0, 2, 6 and 12
weeks and patient acceptable symptomatic state (PASS) and minimally important clinical
difference (MICD) were derived from the MHHS at 12 weeks.

**Results:** Subjects had a mean age of 60, a ratio of female to male of 9:1 and mean length
of symptoms >14 months. Pain and function measured by the mean MHHS showed no
difference at 2 weeks CSI 66.95 (SD 15.14) vs PRP 65.23 (SD 11.60) or 6 weeks CSI
69.51 (SD 14.78) vs PRP 68.79 (SD 13.33). The mean MHHS was significantly
improved at 12 weeks PRP 74.05 (SD 13.92) compared to the CSI group with a mean
score of 67.13 (SD 16.04, p=0.048). Only the PRP group achieved a PASS score of 74 at
12 weeks, reflecting clinical recovery (CSI 67.13). The proportion of subjects who
achieved the MICD of more than 8 points at 12 weeks was 21/37 (56.7%) in the CSI
group and 32/39 (82%) in the PRP group (p=0.016).

**Conclusion:** Patients with chronic gluteal tendinopathy >14 months, diagnosed with both
clinical and radiological examinations, achieved greater clinical improvement at 12
weeks when treated with a single PRP injection than those treated with a single
corticosteroid injection.

**Clinical Relevance:** This is the first published double blinded block-randomised
controlled trial performed on the clinical use of PRP in gluteal tendinopathy.

**Key Terms:** Platelet-rich Plasma, Gluteal Tendinopathy, Leucocyte
5.3 Background

Tendinopathies constitute the most common reason for consultation with a primary care physician and make up thirty percent of all musculoskeletal consultations. Tendinopathy of the gluteus medius or minimus tendons is a major cause of lateral hip pain or greater trochanteric pain syndrome (GTPS). It is more than four times more common in women and is the most prevalent of all lower limb tendinopathies. High levels of dysfunction have been found in people with gluteal tendinopathy who fail conservative treatment, including less full time employment, higher pain levels and poorer quality of life. This has been equated with the disability of severe osteoarthritis of the hip where the economic impact has been estimated at 4400 Euros per patient (USD $4707) in indirect costs, having a major economic impact.

Fearon et al. identified that the diagnosis of GTPS can be confirmed by a clinical history of lateral hip pain localised to the greater trochanter, pain with activities such as walking and stair climbing and pain lying on the affected side at night. Positive clinical signs include tenderness at the greater trochanter and localised lateral hip pain with FABER testing. Both ultrasound and MRI can reliably predict the presence of gluteal tendinopathy and tears. Patients with both clinical signs and symptoms and radiological appearance of gluteal tendinopathy can be regarded as having symptomatic disease involving the gluteal tendons.

Although physiotherapy may be considered a first line treatment for tendinopathy, two recent reviews of treatment modalities for gluteal tendinopathy have found there is little evidence to support physiotherapy or an exercise program for gluteal tendinopathy.
Other interventions including analgesics and non-steroidal anti-inflammatories have also failed to provide a long term benefit\textsuperscript{80, 101, 122}. Today glucocorticoid injections are still considered to be one of most popular injection therapies for pain relief in many clinics, despite controversies regarding the use of glucocorticoid injection for treatment of tendinopathy. Several studies have shown that glucocorticoid injections provide short term benefits from twelve to twenty six weeks\textsuperscript{97, 101}, but no long term benefit\textsuperscript{26}.

Another commonly used injection modality in tendinopathy is platelet rich plasma (PRP) but inconsistent outcomes of PRP are reported. There have been numerous studies attempting to determine the best injection treatment, with varied, contradictory and inconclusive results\textsuperscript{33, 39, 52, 68, 73, 88, 98, 115, 118}.

Considering the lack of high level clinical evidence on injection modalities for treatment gluteal tendinopathy, we have designed a double blind, randomised controlled study to compare the effectiveness of glucocorticoid injection and PRP.

5.4 Methods

5.4.1 Objective

Our hypothesis was that there would be no difference in the modified Harris Hip score (MHHS) between a single intra-tendinous Platelet-Rich-Plasma (PRP) injection compared to a corticosteroid injection (CSI) in the treatment of gluteal tendinopathy.
5.4.2 Trial Design

This trial is a single site double blind prospective parallel group randomised controlled clinical trial, submitted August 2012 at Australian New Zealand Clinical Trials Registry (ACTRN12613000677707) and approved by the Epworth Healthcare Human Research Ethics Committee (57412). The subjects, Clinical Investigator (treating Physician) and investigators examining the data were blinded to the treatment allocation and results until the end of the study following statistical analysis. Informed consent was obtained and CONSORT guidelines followed. No changes were made to the trial design after commencement.

5.4.2.1 Subject Selection

Eligible subjects were aged 18 to 80 years, male or female with a history of gluteal tendinopathy of greater than 4 months and having lateral hip pain, pain with activity such as walking and stair climbing, and pain lying on the affected side at night. The clinical signs on examination included tenderness over the greater trochanter. Radiological confirmation of the diagnosis of Grade 2-3 tendinopathy (no tear) was made using ultrasound and MRI imaging.

Subjects were excluded if they had full thickness tears (Grade 4) demonstrated radiologically, had previous hip or tendon surgery, had a history of breast cancer, were taking warfarin (blood thinners) at the time of the procedure, had back surgery within the last 12 months, a history of recent sciatica or had a cortisone injection within the previous 6 weeks.
5.4.2.2 Randomisation

Assignment to a treatment group was determined by an independent statistician using a computer generated fixed-block randomisation scheme allowing for 80 subjects after screening and informed consent. This was electronically locked and accessible only by the single allocator. A unique trial patient identification number was allocated simultaneously with treatment allocation.

Allocation concealment was ensured as the allocation remained electronically locked after allocation and only the code given to the laboratory preparation technician at the time of trial substance preparation.

5.4.2.3 Interventions

All subjects had approximately 55 mls of blood withdrawn from the cubital fossa to ensure blinding. The PRP was prepared using the GPS III kit, Biomet Biologics USA according to the manufacturer’s instructions and the CSI by mixing Celestone Chronodose with saline to the same volume. No buffering or activating agents were added and the resultant syringe covered with tape to blind the injector and the subject to the contents.

Local anaesthetic was administered and then 6-7 mls of trial substance (PRP or CSI) was injected into the affected area of tendon in 5-6 passes using ultrasound guidance.

Both groups had the same 12 week unsupervised rehabilitation program with directed activity modification post treatment without engagement of clinical physiotherapists. In
the first 4 weeks subjects were instructed to avoid all aggravating activities including walking for exercise, stairs, squats, lunges and abduction exercises. At 6 weeks they were instructed to begin a progressive walking program which also included the use of stairs, return to gym and other sports. At 12 weeks there were no limitations on activity.

5.4.2.4 Outcomes

The primary outcome measure was a pain and functional assessment - the modified Harris Hip score (MHHS). The MHHS used as our primary outcome measure was completed by the subjects at baseline, two, six and twelve weeks. The MHHS is the Harris Hip Score (HHS) without the physician reported range of motion component. This retains the pain and function components including daily activities (stairs, use of public transport, sitting, and managing shoes and socks) and gait (limp, support needed, and walking distance)\textsuperscript{91}. There has been shown to be no meaningful difference between the HHS and the MHHS\textsuperscript{41}. This score has been widely used in other hip pathology such as replacement surgery and hip arthroscopy in patients of the same age and has been found to reflect patient satisfaction\textsuperscript{6}.

The patient acceptable symptomatic state (PASS) reflects the point at which patients feel well\textsuperscript{74}. The PASS score at which patients considered their status to be satisfactory at 12 months has been found to be 74 for the MHHS\textsuperscript{23}. Since there is no validated PASS score for gluteal tendinopathy, we used the validated score from patients with a similar hip condition and defined a PASS score of 74 to reflect an improvement representative of
clinical recovery. The minimal important clinical difference (MICD) for the MHHS has been shown to be 8 points\textsuperscript{67}.

Clinical assessment was performed at six and twelve weeks.

5.4.2.5 Sample Size

A power analysis determined the sample size at 72 was based on the hypothesis; there would be no difference in the modified Harris Hip score (MHHS) between a single intra-tendinous Platelet-Rich-Plasma (PRP) injection compared to a corticosteroid injection (CSI) in the treatment of gluteal tendinopathy. To account for a 10\% drop out rate at twelve weeks 80 subjects were recruited to the study.

5.4.2.6 Blinding

The treating /assessing clinicians and subjects were blinded to the treatment. The results were entered on a locked excel spread sheet, coded and analysed blinded.

5.4.2.7 Statistical Methods

Statistical analysis was conducted on an intention-to-treat basis (ITT) using STATA version 13 (Stata Corp. 2013 Stata Statistical Software: Release 13. College Station, TX: Stata Corp LP.) Treatment comparisons were based on the MHHS at 12 weeks with significance of p < 0.05. Standard t tests with equal variance were done at 12 weeks.
5.5 Results

5.5.1 Patient Study and Follow Up

During the recruitment period (May 2013 to May 2015), 228 patients were assessed. Figure 1 Flow Diagram PRP Trial Gluteal tendinopathy, shows the flow of participants through the study. 148 subjects were excluded due to ineligibility criteria. A standardised physiotherapy program had been ineffective prior to enrolment in all of these subjects. The enrolment period was extended for 1 month to meet the target recruitment of 80 subjects. Subjects were randomly assigned to the PRP treatment group (n=40) or the corticosteroid injection group (n=40). One subject in each group was not treated as assigned: one due to difficult venesection and one withdrew. 37 subjects in the corticosteroid group and 39 in the PRP group were available for analysis at 12 weeks.
Number assessed for eligibility: 228

Number excluded: 148
No. refused to participate: 42
No. not meeting inclusion exclusion criteria:
- Previous hip/back surgery and/or sciatica: 50
- OA hip (no tendinopathy): 17
- Full thickness tendon tears: 17
- Sent for Physio: 10
- Other: 12

Number randomised: 80

Number assigned to receive CSI: 40
Number treated as assigned: 39
Number not treated as assigned: 1
Reasons: 1 - difficult venesection

Number lost to follow-up: 1
Reasons: no follow-up after 2 weeks
Number discontinued: 1
Reasons: developed breast Cancer and withdrew

Number included in analysis: 37
Number excluded from analysis: 0

Number assigned to receive PRP: 40
Number treated as assigned: 39
Number not treated as assigned: 1
Reasons: 1 - withdrew from study

Number lost to follow-up: 0
Number discontinued: 0

Number included in analysis: 39
Number excluded from analysis: 0
The baseline demographic and initial MHHS data is shown in Table 1. The groups showed similar baseline data relative to MHHS, sex, BMI, age, tendon grading, length of symptoms and previous cortisone injections. In accordance with the prevalence of this condition there were more females than males recruited.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CSI Group (n=40)</th>
<th>LR-PRP Group (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean, (range), y</td>
<td>59.7 (23-78)</td>
<td>60.3 (23-76)</td>
</tr>
<tr>
<td>Sex n, (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2 (5)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Female</td>
<td>38 (95)</td>
<td>34 (85)</td>
</tr>
<tr>
<td>BMI kg/m^2 Mean, (SD)</td>
<td>26.96 (4.33)</td>
<td>28.42 (4.58)</td>
</tr>
<tr>
<td>Range</td>
<td>18.8-39.5</td>
<td>20 – 43.9</td>
</tr>
<tr>
<td>Number of previous CSI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0, n, (%)</td>
<td>21 (52.5)</td>
<td>13 (32.5)</td>
</tr>
<tr>
<td>1, n, (%)</td>
<td>14 (35)</td>
<td>19 (47.5)</td>
</tr>
<tr>
<td>2, n, (%)</td>
<td>3 (7.5)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>3 or more, n, (%)</td>
<td>2 (5)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Mean group (SD)</td>
<td>0.65 (0.83)</td>
<td>0.975 (0.97)</td>
</tr>
<tr>
<td>Trial of Physiotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n, (%)</td>
<td>39 (100)</td>
<td>39 (100)</td>
</tr>
<tr>
<td>Grade of tendinopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, n, (%)</td>
<td>24 (60)</td>
<td>20 (50)</td>
</tr>
<tr>
<td>II, n (%)</td>
<td>9 (22.5)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>III, n (%)</td>
<td>7 (17.5)</td>
<td>14 (35)</td>
</tr>
<tr>
<td>IV, n, (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mean Grade (SD)</td>
<td>1.6 (0.7)</td>
<td>1.8 (0.9)</td>
</tr>
<tr>
<td>Initial MHHS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, (SD)</td>
<td>54.15 (10.88)</td>
<td>53.77 (12.88)</td>
</tr>
<tr>
<td>Range</td>
<td>32-71</td>
<td>23-77</td>
</tr>
</tbody>
</table>
5.5.2 Primary Outcome

Table 2 shows the mean for MHHS at baseline, 0, 2, 6 and 12 weeks for the groups and
the 12 week figures for MICD and the PASS scores. The end of the follow up period was
September 2015. The mean MHHS at 2 weeks was CSI 66.95 (SD 15.14) vs PRP 65.23
(SD 11.60) and 6 weeks CSI 69.51 (SD 14.78) vs PRP 68.79 (SD 13.33). The mean
MHHS improved significantly at 12 weeks with the PRP group with a mean score of
74.05 (SD 13.92) compared to CSI group with a mean score of 67.13 (SD 16.04). This
was statistically significant with a p value of 0.048. This data is shown graphically in
Figure 2.

The proportion of subjects who achieved the MICD of a change in score from baseline of
more than 8 points at 12 weeks was 21/37 (56.7%) in the CSI group and 32/39 (82%) in
the PRP group (p=0.016).
### Table 2. Main outcome measures at 0, 2, 6 and 12 weeks

<table>
<thead>
<tr>
<th>Time</th>
<th>Baseline</th>
<th>2 weeks</th>
<th>6 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MHHS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSI Group</td>
<td>54.15 (10.88)</td>
<td>66.95 (15.14)</td>
<td>69.51 (14.78)</td>
<td>67.13 (16.04)</td>
</tr>
<tr>
<td>PRP Group</td>
<td>53.77 (12.08)</td>
<td>65.23 (11.60)</td>
<td>68.79 (13.33)</td>
<td>74.05 (13.92)</td>
</tr>
<tr>
<td><strong>p=0.048</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PASS Score &gt; 74</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSI Group, n/total n, (%)</td>
<td>17/37 (45.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRP Group, n/total n, (%)</td>
<td>25/39 (64.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MICD &gt; 8 points difference on MHHS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSI Group, n/total n, (%)</td>
<td>21/37 (56.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRP Group, n/total n, (%)</td>
<td>32/39 (82.0)</td>
<td></td>
<td></td>
<td>p=0.016</td>
</tr>
</tbody>
</table>

**Legend:**

- MHHS: Modified Harris Hip Score
- SD: Standard Deviation
- CSI: Corticosteroid injection
- PRP: Platelet-rich plasma
- PASS: Patient acceptable symptomatic state
- n: Number in group
- %: Percentage
5.5.3 Secondary Outcome

The subjects’ ability to return to normal activities can be measured by the PASS score. This reflects the point at which the MHHS improvement correlates with clinical recovery. At this point, the subjects have resumed normal activity and are unlikely to require further treatment. The proportion of subjects who achieved an outcome score of greater
than or equal to 74 at 12 weeks was 17/37 (45.9%) in the CSI group and 25/39 (64.1%) in the PRP group. There was no correlation between the outcome and the BMI, length of symptoms or the number of previous corticosteroid injections.

5.5.4 Compliance with Rehabilitation

All subjects were compliant with the 12 week unsupervised rehabilitation program. This was reviewed at 6 weeks where further instructions relating to the progressive walking program and return to other activity were outlined. No protocol deviations were recorded relating to non-compliance of the rehabilitation program.

5.5.5 Adverse Events

There were no treatment related significant adverse events in either group. Treatment related minor adverse events occurred in both groups and generally related to post treatment localised soreness within 48 hours.

5.6 Discussion

5.6.1 General

This study compared the change in pain and function measured by a MHHS in subjects treated with a single PRP injection compared to a corticosteroid injection in gluteal tendinopathy. The results showed a statistically significant improvement in patients treated with PRP over 12 weeks. The CSI group showed good improvement to 6 weeks but their subsequent scores declined as compared to the PRP treatment group.
The use of PRP has been controversial in the management of tendinopathy as trials have shown variable results\(^3\)\(^3\), \(^3\)\(^9\), \(^5\)\(^2\), \(^5\)\(^6\), \(^6\)\(^8\), \(^8\)\(^8\), \(^9\)\(^8\), \(^1\)\(^1\)\(^5\), \(^1\)\(^1\)\(^8\). In a recent meta-analysis, which included 18 studies (1066 participants), significant positive outcomes were seen in those treated with highly cellular leukocyte-rich PRP (LR-PRP) preparations. There was good evidence to support the use of a single injection of LR-PRP under ultrasound guidance in tendinopathy\(^4\)\(^7\). This study provides further evidence for the use of LR-PRP in gluteal tendinopathy using LR-PRP produced by the GPS III Kit\(^4\)\(^8\). Treatment with PRP provides a successful non-surgical management option which is more effective than corticosteroid injections and is less invasive than surgical treatment.

The demographic data shown in Table 1 shows the groups to be well randomised with no significant differences between groups when measured by t-tests. A mean age of 60 and a 9:1 ratio of females to males is consistent with previous findings of a higher ratio of females\(^1\)\(^1\). The BMI was similar in both groups (Mean CSI 29.96 (SD 4.33), Mean PRP 28.42(SD 4.58) contrary to our expectation that patients with a higher BMI may be represented in this group. To ensure the group had chronic tendinopathy, the minimum length of symptoms (LOS) was 4 months. The mean LOS was 15.25 (SD12.52) for the CSI group and 14.78 (SD 12.33) for the PRP group. Almost half (47.5%) of the subjects had symptoms longer than 12 months suggesting the natural history of chronic gluteal tendinopathy is not to resolve over 12 months.

In line with the World Medical Association’s Declaration of Helsinki\(^1\) that the benefits, risks, burdens and effectiveness of a new intervention must be tested against the best
current proven intervention, the currently accepted treatment for gluteal tendinopathy, a corticosteroid injection was used as a control in this study\textsuperscript{11,80}. 34 subjects (42.5\%) had not had a previous CSI and only 13 (16.2\%) had 2 or more injections prior to entry in the study. A sub group analysis found there was no difference in the outcome for patients who had more previous injections. Although this would suggest there is no long term detrimental effect on the tendon from CSI, we had few subjects who had more than 2 injections and the outcome may be different with larger number of injections.

From a statistical perspective, several additional tests were run to ensure accuracy of the data. Since there were 37 in group 1 and 39 in Group 2 at the 12 weeks – in order to ensure that these 2 subjects did not make the difference, we re-tested the data to exclude all data from these individuals. Since T-tests rely on the fact that the underlying distribution of scores has a normal distribution we have also run a non-parametric test to ensure the data is accurately assessed. A two-sample Kolmogorov-Smirnov test (Statistics-Exact statistics-Two-sample K-S test in STATA using scores as the variable and against group) has been run. This shows that the t=12 is the only time point where there is significance. Thus, we have significance with both a t-test and nonparametric tests.

Physiotherapy has been used as a first line therapy for gluteal tendinopathy despite no evidence for it’s efficacy\textsuperscript{11}. All the subjects in this study had failed previous physiotherapy interventions and further physiotherapy was avoided not being considered
an evidence based treatment for this study. The results of this study can be attributed directly to the response to the injection treatment received by the subject. The results shown in Table 2 show the CSI group improved up to 6 weeks but then began to decline. The MHHS of 54.15(SD 10.88) at baseline improved to 69.51 (SD 14.78) at 6 weeks and then dropped to 67.13(SD 16.04) at 12 weeks. However, as the increase in standard deviation shows, there was a lot of variation in the outcome in this group. Our study confirms previous studies findings that cortisone injections are effective for less than 3 months\textsuperscript{75, 80, 97, 101}. By contrast, the PRP group showed a consistent progressive improvement with scores of 53.77 (SD12.08) at baseline progressively improving to 74.05(SD 13.92) at 12 weeks (p=0.048).

5.6.2 Strengths of the Study
The strength of this study is that we are able to recruit only subjects with chronic tendinopathy of greater than 4 months. Some previous studies have included subjects with acute reactive pathology thus including subjects who may recover with physiotherapy alone or with no additional treatment\textsuperscript{18, 33, 39}. The power calculation of sample size has been adequate to provide statistical significance and there were only 2 subjects lost to follow up at 12 weeks.

5.6.3 Limitations of the Study
The limitations of this study are the short duration of follow up, the use of corticosteroid as a control and the inability to determine the economic impact of the treatment.
While the use of corticosteroid as a control is controversial in longer term studies\textsuperscript{34} the use of corticosteroid as a control compared to placebo, local anaesthetic or saline injections did not affect the outcome of similar trials\textsuperscript{47}. The 3 month follow up was chosen based on a meta-analysis of previous studies showing the effectiveness of CSI was maximal at 2-6 weeks and that the effect of PRP was emerging at 12 weeks and continues to show a trend of improvement out to 12 months\textsuperscript{47}. The 12 week point would therefore be the first point at which the groups would be likely to diverge, as has been found in our study. A longer duration than 12 weeks would be likely to suffer from dropout in the control group. An open labelled follow up has demonstrated that the result from the PRP is sustained at 1-2 years as anticipated and that the CSI group continues to decline. (unpublished data)

5.6.4 Econometrics

Our study did not specifically aim to look at econometrics. However, using data from Coombes et al\textsuperscript{27}, it is possible to assess the direct costs of the economic impact of this new treatment. Based on Coombes Australian figures a standard physio treatment is AUD$75 and a CSI AUD$159. Our demographics show that 58% of subjects have both CSI and physiotherapy. The cost of a 12 week physiotherapy program is AUD$900, CSI AUD $159 and the combined treatment AUD$1059. The cost of a single LR-PRP injection using a GPS III kit and a home based rehabilitation program is AUD$500. Whilst this is more expensive than a CSI, it is more effective and it represents a
considerable saving compared to physiotherapy alone or in combination with a corticosteroid injection.

5.7 Conclusion

Patients with chronic gluteal tendinopathy of greater than 4 months, diagnosed with both clinical and radiological examinations, achieved greater clinical improvement at 12 weeks when treated with a single PRP injection than those treated with a single corticosteroid injection.

5.8 Supplementary Documentation

5.8.1 Trial Protocol

The full Trial Protocol is available in Appendix A.

5.8.2 Ethics Certificate

The Ethics Certificate is available in Appendix A.
Chapter 6. 2 year follow-up of Platelet-Rich Plasma treatment of Chronic Gluteal Tendinopathy: A Randomised double-blind controlled trial with open labelled extension

6.1 Preface

Clinical Relevance

This is the first published double blinded randomised controlled trial performed on the clinical use of PRP in gluteal tendinopathy with long term follow up of 2 years.

What is known about this subject

Tendinopathy of the gluteus medius or minimus is the second most common cause of hip pain presenting in primary care and a common cause of lateral hip pain or greater trochanteric pain syndrome (GTPS). Early cases of tendinopathy have been shown to benefit from physiotherapy interventions, analgesics and NSAIDs. The use of corticosteroids may provide short term benefits from twelve to twenty six weeks. Once the tendinopathy has progressed to a full thickness tear, conservative measures are generally ineffective and these ‘refractory patients’ are often managed surgically. A new treatment for more chronic tendinopathy, without full thickness tears of the gluteal tendons has been sought by these authors as an alternative to surgical intervention. Results of the study published after 12 weeks follow up showed that patients with chronic gluteal tendinopathy >4 months, diagnosed with both clinical and radiological
examinations, achieved greater clinical improvement at 12 weeks when treated with a single PRP injection than those treated with a single corticosteroid injection. The objective of this trial was to determine whether there would be a sustained long term difference in the Modified Harris Hip Score (MHHS) at 2 years for the LR-PRP injection in the treatment of chronic gluteal tendinopathy.

What this study adds to existing knowledge

This is the first published double blinded block-randomised controlled trial performed on the clinical use of PRP in gluteal tendinopathy with 2 year follow up. The results show the mean MHHS improved significantly at 12 weeks in the PRP group with a mean score of 74.05 (SD 13.92) compared to CSI group with a mean score of 67.13 (SD 16.04) p=0.048. At 24 weeks the LR-PRP group 77.60 (SD 11.88) had improved further compared to the CSI group 65.72 (SD 15.28) p=0.0003. The LR-PRP group retained 38/39 subjects to 54 weeks and continued to improve. Their baseline scores of 53.77 (SD 12.08) improved to 78.18 (SD 14.53) at 52 weeks and 82.59 (SD 9.71) at 104 weeks. The results of the LR-PRP were sustained at 2 years. This study provides evidence for the use of PRP in gluteal tendinopathy with sustained results to 2 years.

6.2 Abstract

Background
A previously published trial has shown patients with chronic gluteal tendinopathy achieved greater clinical improvement at 12 weeks when treated with a single PRP injection than those treated with a single corticosteroid injection.

**Purpose**

This 2 year follow-up study was to determine whether there would be a sustained long term difference in the Modified Harris Hip Score (MHHS) at 2 years for LR-PRP injection in the treatment of chronic gluteal tendinopathy.

**Study Design**

Randomised Double-blind Controlled Single Centre Clinical Trial with open labelled crossover; Level of evidence 1, recruitment 29 May 2013 to May 2015, follow-up June 2017. Australian New Zealand Clinical Trials Registry: ACTRN12613000677707

**Methods**

This trial included 80 patients randomised 1:1 to receive either leucocyte-rich Platelet-Rich Plasma (LR-PRP) or corticosteroid (CSI) injected intra-tendinously under ultrasound guidance. Subjects had a mean age of 60, 9:1 ratio of females, a mean BMI of 29 and a mean length of symptoms > 14 months. No subjects had full thickness tears of the gluteal tendons. An open labelled extension allowed subjects to receive crossover treatment after three months. The main outcome measure was the modified Harris Hip Score (MHHS).
Results

The mean MHHS improved significantly at 12 weeks in the PRP group with a mean score of 74.05 (SD 13.92) compared to CSI group with a mean score of 67.13 (SD 16.04) \( p=0.048 \). At 24 weeks the LR-PRP group 77.60 (SD 11.88) had improved further compared to the CSI group 65.72 (SD 15.28) \( p=0.0003 \). 27 subjects were deemed to have failed the CSI treatment at 16-24 weeks with an exit score of 59.22 (SD 11.54) and then had treatment with LR-PRP. The crossover group improved with the LR-PRP: baseline 59.22 (SD 11.22) to 75.55 (SD 16.05) at 12 weeks, 77.69 (SD 15.30) at 24 weeks and 77.53 (SD 14.54) at 104 weeks. The LR-PRP group retained 38/39 subjects to 52 weeks and continued to improve. Their baseline scores of 53.77 (SD 12.08) improved to 82.59 (SD 9.71) at 104 weeks.

Conclusion

In patients with chronic gluteal tendinopathy, length of symptoms >14 months, a single intra-tendinous LR-PRP injection performed under ultrasound guidance, results in a greater improvement in pain and function than a single corticosteroid injection. The improvement following LR-PRP injection is sustained at 2 years whereas the improvement from a CSI is maximal at 6 weeks and returns to baseline by 2 years.

6.3 Background

Gluteal tendinopathy of the gluteus medius or minimus tendons is acknowledged as one of the primary causes of lateral hip pain or greater trochanteric pain syndrome (GTPS). It
is more than four times more prevalent in middle aged women than men and is the most prevalent of all lower limb tendinopathies. Significant levels of dysfunction have been noted in people with gluteal tendinopathy who fail conservative treatment which has been equated to the level of disability of severe osteoarthritis of the hip. Thus long term effective strategies for the treatment of gluteal tendinopathy are important for the wellbeing of this group.

The cause of tendinopathy of the gluteal tendons is unknown. The clinical history of gluteal tendonitis suggests a degenerative progression of disease. In early cases, tendinopathy of one or both tendons is present sometimes with trochanteric bursitis. Moderate cases show partial thickness tears either longitudinally or at the insertion of the tendons. More advanced cases demonstrate full thickness tears with progression to tendon retraction and fatty atrophic changes of the muscle belly seen on MRI.

Bhabra et al described the cellular mechanism for this progression of degenerative change in tendinopathy where a cycle of microtrauma and improper healing leads to tendinopathy and eventual structural failure in tendons. Four grades of tendinopathy are described. In grade 1 disease, the collagen fiber pattern becomes increasingly wavy. Although cellular and vascular changes are minimal, there is an increase in the proportion of type 3 collagen. In grade 2, there is tendinosis and angio-fibroblastic hyperplasia, with further disorganization and fragmentation of the collagen fibers, cellular hyperplasia, rounding of tenocytes, and neovascular hyperplasia. In grade 3 tendinopathy, programmed cell death leads to the depletion of functional tendon cells and breakdown of
collagen and extracellular matrix. Finally, grade 4 presents with gross structural
disruption and mechanical failure.”

Each stage of the tendinopathy responds differently to different treatment modalities. Early cases of tendinopathy have been shown to benefit from physiotherapy interventions, analgesics and non-steroidal anti-inflammatories. The use of corticosteroid injections may provide short term benefits from twelve to twenty-six weeks, but the recurrence rate is high. Once the tendinopathy has progressed to a full thickness tear, conservative measures are generally ineffective and these ‘refractory patients’ are often managed surgically.

Given the paucity of treatments for higher grade tendinopathy, there is interest as to whether biological treatments might provide a better long term outcome for patients. A recent meta-analysis on the use of platelet-rich plasma (PRP) in tendinopathy found that a single leucocyte-rich platelet-rich plasma (LR-PRP) injection showed positive outcomes in the management of tendinopathy. Further laboratory study has shown that different PRP kits and preparation produce varying levels of both platelets and white cells, even when PRP is made using the buffy coat layer.

These authors performed a double blind randomised controlled trial assessing the effectiveness of a single LR-PRP injection compared to a single corticosteroid injection in the management of gluteal tendinopathy. 80 subjects were recruited with a mean age of 60, 9:1 ratio of females, a mean BMI of 27 and a mean length of symptoms of 15 months. They were randomised to receive either a LR-PRP or a CSI intra-tendinously.
under ultrasound guidance. The mean MHHS improved significantly at 12 weeks in the PRP group with a mean score of 74.05 (SD 13.92) compared to CSI group with a mean score of 67.13 (SD 16.04) (p=0.048). The proportion of subjects who achieved the minimally important clinical difference (MICD) of a change in score from baseline of more than 8 points at 12 weeks was 21/37 (56.7%) in the CSI group and 32/39 (82%) in the PRP group (p=0.016). Whilst the 3 month follow up showed the effectiveness of CSI was maximal at 2-6 weeks and that the effect of PRP was emerging at 12 weeks the clinical results need to be sustained beyond 12 months for this to be an effective long term treatment. This study presents the longer term follow up of these patients out to two years.

6.4 Methods

6.4.1 Objective

Our hypothesis was that there would be a sustained long term difference in the Modified Harris Hip Score (MHHS) at 2 years for the LR-PRP injection in the treatment of chronic gluteal tendinopathy.

6.4.2 Trial Design

This double blind randomised controlled trial with open labelled extension to 2 years included 80 patients randomised 1:1 to receive either LR-PRP or CSI injected intra-tendinously under ultrasound guidance between May 2013 and May 2015. 2 year follow up was completed in June 2017. This trial was registered at Australian New Zealand
Clinical Trials Registry (ACTRN12613000677707) and undertaken with ethics approval from the Epworth Healthcare Human Research Ethics Committee (57412). The full trial design is outlined in the 3 month follow up report.

6.4.2.1 Subject selection
Subjects had a mean age of 60 (range 23=78), 9:1 ratio of females, a mean BMI of 27 (SD 4.48) and a mean length of symptoms of 15 months (SD 12.35). No subjects were included with full thickness tears of the gluteal tendons.

6.4.2.2 Intervention
The PRP was prepared using the GPS III kit, Biomet Biologics USA which has been demonstrated to produce leucocyte-rich PRP\textsuperscript{48}. There were no limitations on the patient’s activity after 12 weeks.

6.4.2.3 Outcomes
The primary outcome measure was the modified Harris Hip score (MHHS) - a pain and functional assessment. The MHHS was completed by the subjects at baseline, two, six and twelve weeks and then at 6, 12 and 24 months.

The patient acceptable symptomatic state (PASS) was used to determine when clinical recovery had been achieved\textsuperscript{74} and determined by a PASS score of 74 as defined by Chahal\textsuperscript{23}.
6.4.2.4 Blinding

Subjects and investigators were blinded for a minimum of 3 months. After this, subjects were un-blinded if they determined they had not made a recovery and requested further treatment. Subjects remained blinded until June 2017, if they had not had any further intervention.

6.4.2.5 Crossover

Subjects were offered further treatment in the form of a cortisone injection, a LR-PRP injection or surgical intervention if they failed treatment beyond 3 months. This was un-blinded.

6.4.2.6 Statistical Assessment

Statistical analysis was conducted on an as treated basis using STATA version 14 (Stata Corp. 2016 Stata Statistical Software: Release 14. College Station, TX: Stata Corp LP.) Treatment comparisons were based on the MHHS at 3, 6, 12 and 24 months with significance of p < 0.05. Standard t tests with equal variance were done at 24 months.

6.5 Results

Patient Study and Follow up
Table 1: MHHS at all time points for each group.

<table>
<thead>
<tr>
<th>TimePoint</th>
<th>Group 1 = CSI n, Mean MHHS, (SD)</th>
<th>Group 2 = LR-PRP n, Mean MHHS, (SD)</th>
<th>Group 3 = CSI+LR-PRP n, Mean MHHS, (SD)¹</th>
<th>Group 4 = Surgery N, Mean MHHS (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40, 54.15 (10.88)</td>
<td>40, 53.77 (12.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>39, 66.95 (15.14)</td>
<td>39, 65.23 (11.59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>37, 69.51 (14.78)</td>
<td>39, 68.79 (13.32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>37, 67.13 (16.04)</td>
<td>39, 74.05 (13.92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 (T2=0 group 3)</td>
<td>37, 65.72&quot; (15.28)</td>
<td>38, 77.60 (11.88)</td>
<td>27, 59.22 (11.54)</td>
<td></td>
</tr>
<tr>
<td>26 (T2 + 2 weeks)</td>
<td></td>
<td></td>
<td>27, 67.48 (13.97)</td>
<td></td>
</tr>
<tr>
<td>30 (T2 + 6 weeks)</td>
<td></td>
<td></td>
<td>27, 70.37 (15.21)</td>
<td></td>
</tr>
<tr>
<td>36 (T2 + 12 weeks)</td>
<td></td>
<td></td>
<td>27, 75.55 (16.05)</td>
<td></td>
</tr>
<tr>
<td>48 (T2 + 26 weeks)</td>
<td></td>
<td></td>
<td>26, 77.69 (15.30)</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>15, 70.53 (23.80)</td>
<td>38, 78.18 (14.53)</td>
<td>3, 57.66 (10.96)</td>
<td></td>
</tr>
<tr>
<td>76 (T2 + 52 weeks)</td>
<td></td>
<td></td>
<td>21, 79.04 (14.28)</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>11, 71.27 (25.78)</td>
<td>35, 82.59 (9.71)</td>
<td>5, 67.80 (13.33)</td>
<td></td>
</tr>
<tr>
<td>128 (T2 + 104 weeks)</td>
<td></td>
<td></td>
<td>13, 77.53 (14.54)</td>
<td></td>
</tr>
</tbody>
</table>

Legend:

- TimePoint = Time in weeks
- n = Number subjects
- mean MHHS = mean Modified Harris Hip Score
SD = Standard Deviation

" = Scores marked with " represent scores at approximately 24 weeks. The 24 week score is recorded once for each subject but represents either the last score taken before crossover or the continuing score if no crossover occurred.

Scores for this group are recorded from a new baseline where TimePoint = 0 is the new starting point following crossover from Group 1 CSI after 24 weeks
Number assessed for eligibility: 228

Number excluded: 148
No. refused to participate: 42
No. not meeting inclusion exclusion criteria:
- Previous hip/back surgery or sciatica: 50
- OA hip (no tendinopathy): 17
- Full thickness tendon tears: 17
- Sent for Physio: 10
- Other: 12

Number randomised: 80
Number assigned to receive CSI: 40
Number treated as assigned: 39
Number not treated as assigned: 1
Reasons: 1 - difficult venesection

Number assigned to receive PRP: 40
Number treated as assigned: 39
Number not treated as assigned: 1
Reasons: 1 - withdrew from study

Number lost to follow-up at 12 weeks: 1
Reasons: no follow-up after 2 weeks
Number discontinued: 1
Reason - developed breast cancer and withdrew
Number included in analysis: 37

2 years analysed in CSI group: 11
Crossover to Group 3 LR-PRP: 26
Crossover from Group 3 - Group 4 Surgery: 3
Lost to follow-up: 0
Total Number analysed: 37

Number lost to follow-up at 12 weeks: 0
Number discontinued: 0
Number included in analysis: 39

2 years analysed in LR-PRP Group: 35
Crossover to Group 4 Surgery: 2
Lost to follow-up: 2
Total number analysed: 37
Figure 1 shows the flow of participants in the study. 40 subjects were randomised to each group. At 12 weeks, 37 subjects in the CSI and 39 in the LR-PRP groups were analysed. The mean MHHS improved significantly at 12 weeks in the PRP group with a mean score of 74.05 (SD 13.92) compared to CSI group with a mean score of 67.13 (SD 16.04). This was found to be significant \( p=0.048 \).

At 24 weeks the mean MHHS in the LR-PRP group 77.60 (SD 11.88) had improved further compared to the CSI group 65.72 (SD 15.28). This was found to be highly statistically significant \( p=0.0003 \). This is shown graphically in Figure 2. The LR-PRP group retained 38 subjects to 52 weeks and continued to improve; from a baseline score of 53.77 (SD 12.08) the scores improved to 78.18 (SD 14.53) at 52 weeks and 82.59 (SD 9.71) at 104 weeks.

Figure 2. Graph of the MHHS in groups 1 CSI and 2 LR-PRP at 0, 2, 6, 12 and 24 weeks.
Between 16 and 24 weeks, subjects from either group who requested further treatment continued to be followed but form new groups. The largest number was from Group 1 CSI who was then followed up as Group 3 CSI + LR-PRP. The subjects recorded an exit score which is recorded with the remaining 24 month scores and can be seen as the time 0 score for group 3 – CSI + LR-PRP. 27 subjects were deemed to have failed the CSI treatment at 16-24 weeks with an exit score of 59.22 (SD 11.54) having almost returned to their baseline scores of 54.15 (SD 10.88). There were only 11 subjects remaining in the CSI group at 104 weeks, the rest having failed and had further treatment. The remaining
15 subjects retained a score of 70.53 (SD23.80) at 52 weeks but by 104 weeks only 11 remained with scores of 71.27 (SD 25.78).

The crossover group 3 CSI + LR-PRP had baseline scores equivalent to the other 2 groups; they had returned to their baseline before having the crossover treatment. They improved progressively from baseline 59.22 (SD11.22) to 75.55 (SD16.05) at 12 weeks, 77.69 (SD15.30) at 24 weeks and 77.53 (SD 14.54 at 104 weeks. This has been shown graphically in Figure 3 by comparison with group 2 LR-PRP.

Figure 3. Graph of MHHS in groups 2 LR-PRP and 3 CSI + LR-PRP at 0,2,6,12, 24, 52 and 104 weeks

Legend:
Scores: MHHS

2: Group 2 – LR-PRP
3: Group 3 – CSI + LR-PRP

Timepoints recorded as 0, 2, 6, 12, 24, 52 and 104 weeks

Overall, 5 patients failed their treatment and progressed to surgery, two from Group 2 LR-PRP and 3 from the CSI group. All 3 of the patients from the CSI group had LR-PRP and then surgery.

PASS scores > 74 were used to determine the number of subjects who had fully recovered following their treatment and the outcomes are seen in Table 2.
Table 2. PASS Scores > 74 at 12, 24, 52 and 104 weeks

<table>
<thead>
<tr>
<th>TimePoint</th>
<th>Group 1 CSI</th>
<th>Group 2 LR-PRP</th>
<th>Group 3 CSI + LR-PRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>17/37 (45.9%)</td>
<td>25/39 (64.1%)</td>
<td>18/27 (66.6%)</td>
</tr>
<tr>
<td>24</td>
<td>10/37 (27%)</td>
<td>25/38 (65.8%)</td>
<td>18/26 (69.2%)</td>
</tr>
<tr>
<td>52</td>
<td>9/15 (60%)</td>
<td>29/38 (76.34%)</td>
<td>15/21 (71.4%)</td>
</tr>
<tr>
<td>104</td>
<td>8/11 (72.2%)</td>
<td>31/35 (88.6%)</td>
<td>*9/13 (69.2%)</td>
</tr>
</tbody>
</table>

Legend:

- **TimePoint = Time in weeks**
- n= Number subjects
- total n = total number subjects in group at that time point
- % = percentage
- *= Note due to this group commencing the crossover 16-24 weeks, not all of the subjects have reached the 104 weeks at the assessment time.

Only 4 patients were lost to follow up at 2 years. Overall, at 24 weeks only 10/37 (27%) subjects in the CSI group had achieved the PASS score compared to 25/38 (65.8%) of the LR-PRP group. By 2 years only 8 patients remained in the CSI group with a PASS score greater than 74. By contrast in the LR-PRP group 31/35 (88.6%) had reached the PASS score of 74 or greater.
6.6 Discussion

This study compared long term pain and function outcomes as measured by a MHHS in subjects with natural history resistant tendinopathy receiving either a CSI or a LR-PRP injection for gluteal tendinopathy. The blinded results of the study were reported at 12 weeks follow up showing a statistically significant improvement in the LR-PRP group compared to the CSI group. The results at 24 weeks further demonstrate that the effect of the CSI 65.72 (SD 15.28) has declined as has been shown by previous authors, and, the LR-PRP group has had a sustained improvement 77.69 (SD 15.30) (p=0.0003). This effect is also sustained at 12 months 78.18 (SD 14.53) and 2 years 82.59 (SD 9.71).

The use of PRP in the management of tendinopathy has been controversial. Some tendons may be different in their response, for example the achilles tendon. Part of the reason for this controversy lies in the fact that the PRP has a slow onset of action and it takes 3-6 months to see the effectiveness. It has been difficult to get statistical significance over times shorter than 3 months or with small numbers of participants. This study shows a slow onset of action in the first 6 weeks. The mean MHHS changed 15.02 points (from 53.77 (12.08) to 68.79 (13.32)) by 6 weeks. 12 weeks marks the first time point where subjects reach the PASS score showing recovery and then there is continued improvement out to 1 year where the change is much greater at 24.41 points (from 53.77 (12.08) to 78.18 (14.53)). Interestingly, and perhaps unexpectedly, there is a further improvement from 1 year to 2 years where the mean MHHS reaches 82.59 (SD 9.71). This may represent the ongoing cellular and structural
adaptations occurring in the tendon. This will be explored in a subsequent study looking at the radiological findings beyond 24 months.

The second reason for the controversy is that it is difficult to get subjects in a control group to avoid drop out when they have no clinical effect from the control treatment or a short term benefit only. This has been reported by several groups. The use of placebo or inactive controls makes this more difficult due to the long timeframe required to reach the full effect of the PRP. This study aimed to avoid this by ending the blind at 3 months and allowing a crossover treatment so that subjects were not lost to follow up. Only 1 subject from the control group (CSI) was lost to follow up at 12 months and 1 from the LR-PRP group.

The crossover (failed treatment) rate in the CSI group after 24 weeks made statically comparison between groups difficult at 52 and 104 weeks with only 15 subjects remaining in the CSI group at 1 year and 11 at 2 years. This reflects the failure rate of the corticosteroid and is anticipated.

Having a crossover group allows us to assess whether having a corticosteroid injection prior may negatively or positively influence the outcome of the LR-PRP injection. Using general linear model analysis, there was no relationship to previous number of corticosteroid injections and outcome scores in Groups 1 CSI and 2 LR-PRP. The crossover group 3 (CSI + LR-PRP), who had a corticosteroid initially and then a LR-PRP injection at approximately 16-24 weeks followed a very similar pathway with baseline scores of 59.22 (11.54) compared to the LR-PRP baseline of 53.77 (12.08). The scores at
24 weeks showed no statistical difference CSI + LR-PRP 77.69 (15.30) and LR-PRP 77.60 (11.88) or at 52 weeks CSI + LR-PRP 79.04 (14.28) and LR-PRP 78.18 (14.53).

This is reassuring for the clinician who wishes to know if a previous corticosteroid injection may confer a less positive outcome for a patient.

It is important to assess the economic impact of any new treatment. This study has shown that a single LR-PRP injection with a home based exercise program is effective out to 2 years. The base cost of this treatment is AUD $500 (Consumables AUD$250 and injection AUD$250). The currently accepted treatment for this condition is a corticosteroid injection followed by a 12 week physiotherapy program or when this fails, surgical intervention. Using data from Coombes et al27 who studied the econometrics of similar injections for tennis elbow, the cost of a corticosteroid injection is AUD $159 and the cost of a 12 week physiotherapy program is AUD$900 making the combined treatment cost AUD $1059. This group of patients had already had a course of physiotherapy and frequently one or more corticosteroid injections prior to entry in the trial. Without the new treatment this group who had failed treatment were likely to undergo surgical treatment for which the cost is much greater at AUD $5478 (Surgeon AUD$978, Anaesthetist AUD$950, Assistant Surgeon AUD$250, Theatre fee and one night admission in private hospital AUD$3300). Thus treatment with a single LR-PRP injection followed by a home based exercise program is considerably more cost effective than the current treatment of Physiotherapy and CSI or surgical intervention.
The strength of this study is the follow up period of 2 years allows us to see that the positive effect of the PRP has been sustained. The study has controlled for the natural history of lower grades of tendinopathy by inclusion of subjects with an average length of symptoms greater than 14 months. The crossover allows us to be confident of this as there was not a significant dropout rate (as the failed subjects are captured by the crossover arm).

The limitations of this study are that comparisons between groups after 24 weeks are difficult due to the failure in the CSI group. Conclusions relating to the sustained outcome in the LR-PRP group however, are not affected by this. The finding that there was progressive improvement up to 2 years suggesting a somewhat prolonged recovery has not been checked with radiological studies. Other authors have looked at radiological changes earlier\textsuperscript{31, 52, 73} but this finding suggests these changes may be delayed, if they are present. Further study of imaging in these patients after 2 years may help to resolve whether there are longer term structural changes.

\section*{6.7 Conclusion}

In patients with chronic gluteal tendinopathy, length of symptoms greater than 14 months, a single intra-tendinous LR-PRP injection performed under ultrasound guidance, results in a greater improvement in pain and function than a single corticosteroid injection. These results continue to improve out to 2 years.
Chapter 7. Discussion, Conclusion and implications for future research

7.1 Overview

The aim of this thesis is to identify whether there is evidence to support the use of PRP in tendinopathy, the ideal cellular and biochemical makeup of this product and to determine whether PRP is an effective treatment for gluteal tendinopathy. The effectiveness of PRP in gluteal tendinopathy has not previously been studied. The data from this thesis show that there is evidence to support the use of a leucocyte-rich platelet-rich plasma injection as a new treatment for chronic gluteal tendinopathy.

7.2 Discussion

The most recent Cochrane report on the use of PRP in soft tissue injuries found weak evidence to support PRP improved pain levels but no change in functional outcomes. This study was not limited to tendinopathy, including surgical and non-surgical treatments as well as tendon, ligament and muscle injuries. It did highlight the need for good quality meta-analysis in each pathological condition, such as tendinopathy or
muscle injury. It also raised the question as to whether the PRP preparation or the technique for injection might be themselves important in the outcome.

The systematic review and network meta-analysis presented in Chapter 2 provides evidence that PRP improves the outcome in tendinopathy and that the technique of injection and the preparation of the PRP are important.

This data answered the questions raised as to the efficacy in tendinopathy of different types of PRP preparation. Having pooled the data from PPP, LP-PRP and LR-PRP this analysis found that there was evidence for the use of LR-PRP in tendinopathy. As to the technique of injection, the pooled data supported the technique of a single intra-tendinous injection.

However, it was not possible to determine whether other types of PRP such as leucocyte-poor PRP or platelet poor plasma were effective as there was insufficient data to make a conclusion relating to this. If further trials are conducted in tendinopathy using PPP or LP-PRP these can be included in future meta-analyses and may answer the question that is now raised specifically as to whether it is only LR-PRP that is efficacious in the treatment of tendinopathy.

In addition, the meta-analysis was conducted across tendinopathy from all tendons in the human body. It was not possible to separate the results into grouping by individual tendon as there are insufficient trials in each tendon location at present. Future research is
needed with trials using well described formulations of PRP in specific geographical tendinopathy locations to allow for this to be determined.

There has been much discussion about the composition of PRP and its relative efficacy. Little is known about the biochemistry and the cellular content of PRP made with different methods. It has previously been assumed that all PRP is similar.

The results from the laboratory analysis of PRP preparation kits in Chapter 3 identify large variations in cellular and chemical composition, particularly platelets, white cell counts, and the differential count of neutrophils and lymphocytes in PRP preparations. Further, new scientific findings of high levels of glucose in the preparations was documented and the pH of the PRP was described.

The clinical significance of this is that these variations must be taken into account when assessing the results of clinical trials and in the choice of preparation by practitioners. Since leucocyte-rich PRP has been demonstrated to be effective in tendinopathy, clinicians need to be cognisant of which kits and preparation techniques produce standardised leucocyte-rich platelet-rich plasma for clinical use in tendinopathy.

Further research is needed to identify the role of specific types of PRP preparation in other clinical conditions such as osteoarthritis. All new preparation kits and methods in the future will need to be validated prior to use in a clinical setting.

The surprising finding of the high glucose content in all PRP preparations has implications for future research. It is unknown whether the glucose plays any role in the
efficacy of the PRP. Future studies using only glucose in high concentrations or PRP made without the addition of ACD-A are required to determine how significant this factor is and may also have implications for certain patient populations such as those with diabetes.

No previously reported conservative treatment has been demonstrated to be effective beyond three to six months for gluteal tendinopathy. There is a need for a new effective treatment for patients with chronic gluteal tendinopathy of longer duration than 6 months.

The results from the clinical trial presented in Chapters 5 and 6 show that in patients with chronic gluteal tendinopathy, a single intra-tendinous LR-PRP injection performed under ultrasound guidance, results in a significant improvement in pain and function and that this is sustained beyond two years. This is achieved with a home based exercise program removing the additional cost of rehabilitation.

This study has highlighted the slow onset of action of LR-PRP. Twelve weeks marks the first time point where subjects reach an improvement level consistent with resolution of the tendinopathy clinically and then there is continued improvement out to two years.

This has implications for future research using PRP in tendinopathy as clinical trials will need to continue for up to two or more years to demonstrate the long term result. This is made more difficult by the issue of the choice of control used in the clinical trials. Since placebo, inactive treatments and interventions known to have short term benefits are the only currently available comparators and this generally leads to high dropout rates, consideration for the type of protocol design need to be determined that will allow for
extended follow up in one arm of a trial when the other arm has insufficient numbers to
determine statistical significance. This has been achieved in the clinical trial described in
Chapters 5 and 6 by allowing an open labelled crossover extension with data captured for
2 years.

7.3 Implications for future research

7.3.1 Natural History of tendinopathy

It is not known whether all tendinopathy results in healing over time or whether this is
specific to younger tendons. It is unknown whether the aging tendon when injured
behaves in a similar way or may become more pathological with time. Further research
into the natural history of tendinopathy is needed to determine whether the natural history
of tendinopathy in all demographics such as age and sex, is to recover and whether there
is a specific group who go on to develop ‘natural history resistant’ tendinopathy.

7.3.2 Pathological Grading of tendinopathy

This clinical trial controlled for the natural history of lower grades of tendinopathy by
inclusion of subjects with an average length of symptoms greater than 14 months. At
present, the grade of tendinopathy or its chronicity are not well documented in clinical
trials in tendinopathy. This makes it difficult in assessing the outcome of interventions
particularly where trials have allowed the inclusion of many different stages of
tendinopathy.
Further study developing the grading of tendinopathy would be helpful to correlate the pathophysiology with the clinical presentation. Further study needs to be done to develop correlation between the clinical presentation of length of symptoms, clinical signs and the pathological grading. Further research developing MRI or ultrasound classification tools for the grading of tendinopathy will help in this correlation.

Tools such as this would allow further research to look for structural changes in tendinopathy following treatment with PRP (or other treatments) and provide additional outcome measures for clinical trials in this area.

7.3.3 Develop a treatment algorithm for the management of tendinopathy

First, our understanding of the natural history of tendinopathy with different demographics (such as age or sex) will allow us to focus on the patients who need treatment. We could thus identify which patients have different grades of tendinopathy determined by the correlation between clinical, histopathological and radiological findings.

Second, future research could identify which treatments are effective in different grades of tendinopathy. More research is needed to determine whether specific rehabilitation or physiotherapy has a role to play in lower grades of tendinopathy for example. Further
research into the role of glucose and the cellular composition of PRP therapies is needed to determine the best composition for efficacy in tendinopathy.

Third, further research needs to aim to develop a treatment algorithm that allows practitioners to understand the correlation between the grading (or severity) of the tendinopathy and the correct treatment for the tendon at that grading-point. For example, that Grade I tendinopathy may be managed with activity modification and active physiotherapy, Grade II-III tendinopathy with cellular injections such as LR-PRP and Grade IV with surgical intervention. This would allow for an ideal treatment scenario where the health resources are targeted to the patients according to their needs based on pathological grading and clinical outcomes.

7.4 Conclusion

There is marked variation in PRP preparation in commercial kits. The systematic review and network meta-analysis showed that PRP improves the outcome in tendinopathy. The randomised controlled clinical trial confirms that PRP results in clinical recovery in the long term, whereas cortisone has only a short term effect in gluteal tendinopathy. The mean MHHS baseline scores of 53.77 (SD 12.08) improved to 82.59 (SD 9.71) at 104 weeks in the LR-PRP group whereas the CSI group had returned to their baseline scores at 26 weeks. The results of this study have a significant impact on the future management of gluteal and other tendinopathies and for further study into the development of a treatment algorithm for gluteal tendinopathy.
8 References

8. Arthrex. Arthrex ACP Double Syringe [Company Literature]. Available at: http://secure.cdn.arthrex.com/pdfs/sj/jf_jEEeCRTQBQVoRHOw/sj/jf_jEEeCRTQBQVoRHOw.pdf?Expires=1381108223&Signature=ImPOQ2BluH8QtQF5pfepxMQQIniyc5WUIt2h0uf4ZUm5p3GcnvBxmn4cVhfs3u0HKpCCQdpptom6-Rn2XKWCYiwX70jpbyYrErOc%7EtikCwCj9Lw5YLqG8DePVGWlw8tIjxBrlflagY %7EJISawcouMv1Xs0vXS202SdxNk&Key-Pair-Id=APKAIXYFKLX6GHMYCAUQ. Accessed 2 Dec, 2015.


104. Schnabel LJ, MS; Miller, BJ; McDermott, WG; Fortier, LA. PLATELET RICH PLASMA (PRP) TREATMENT FOR TENDINITIS.


Appendix A

1 Protocol and ethics certificate

The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Double-Blind Randomised, Controlled Trial (The PRP-HIP Trial)

Confidential Information

The information contained in this protocol is confidential and is intended for the use of clinical investigators. It should not be copied by or distributed to people not involved in the clinical trial, unless they are bound by a confidentiality agreement.

Protocol Approved by Dr Jane Fitzpatrick (dd/mm/yyyy) ____________
Clinical Trial Protocol Synopsis: The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Double-Blind Randomised, Controlled Trial (The PRP Trial)

**Name of Investigational Product:** Platelet-rich plasma (PRP) injections

**Title of Trial:** The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Double-Blind Randomised, Controlled Trial (The PRP Trial)

**Number of Planned Patients/Subjects:**
- Enrolled/Randomised: 80
- Completed: 72

**Phase of Development:** III

**Length of Trial:** 18 months
- Planned first patient visit: June 2013
- Planned last patient visit: May 2015

**Objectives:**
- The primary objective of this trial is to test the effectiveness of platelet-rich plasma (PRP) injections in patients who have painful gluteal tendonopathies. The trial aims to see if PRP use in patients who have gluteal tendonopathies reduces pain and improves functional levels at 12 weeks follow-up, compared to cortisone injection.
- The secondary objectives of the trial are:
  - treatment group comparisons in terms of MHHS at other follow-up times
  - safety information will be collected over the course of the trial.

**Trial Design:**
- This is a randomised, double-blinded, parallel group trial comparing a single PRP injection with a single cortisone injection. The trial population is adult patients with painful gluteal tendonopathies, treated on an out-patient basis.

**Diagnosis and Main Criteria for Inclusion and Exclusion:**
- Trial patients can be men or women, aged between 18 and 80 years inclusive, with gluteus medius and/or minimus tendonopathy and without tears of the tendons on MRI and ultrasound, a history of breast cancer, previous hip surgery, current warfarin treatment, recent back surgery or sciatic pain, or cortisone treatment in the previous six weeks.

**Test Product, Dosage and Mode of Administration:**
- A single platelet-rich plasma (PRP) injection (Biomet Biologics), injected intra-tendonously.

**Planned Duration of Treatment:**
- treatment period: single treatment
- observation period: 12 months

**Reference Therapy, Dose & Mode of Administration:**
- A single injection of Celestone Chronodose (cortisone)

**Criteria for Evaluation:**
- **Efficacy:** Modified Harris Hip Score, Womac Score
- **Safety:** Proportions of patients experiencing adverse events.

**Statistical Methods:**
- Statistical: The sample size requirement is 36 completed patients per treatment arm, for an unpaired t test with 90% power. Allowing for 10% loss to follow-up at 12 weeks brings the total number of randomised patients needed to 80. The primary analysis will be ANCOVA with baseline adjustment.
### 1.3 Table of Contents

The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Randomised, Controlled Trial (The PRP Trial)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Protocol The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Randomised, Double-Blinded Controlled Trial (The PRP Trial)</td>
<td>186</td>
</tr>
<tr>
<td>2. Synopsis</td>
<td>187</td>
</tr>
<tr>
<td>3. Table of Contents</td>
<td>188</td>
</tr>
<tr>
<td>4. Abbreviations and Definitions</td>
<td>190</td>
</tr>
<tr>
<td>5. Introduction</td>
<td>192</td>
</tr>
<tr>
<td>6. Objectives</td>
<td>193</td>
</tr>
<tr>
<td>6.1. Primary Objective</td>
<td>193</td>
</tr>
<tr>
<td>6.2. Secondary Objectives</td>
<td>193</td>
</tr>
<tr>
<td>7. Investigational Plan</td>
<td>193</td>
</tr>
<tr>
<td>7.1. Summary of Trial Design</td>
<td>194</td>
</tr>
<tr>
<td>8. Trial Population</td>
<td>194</td>
</tr>
<tr>
<td>8.1. Inclusion Criteria</td>
<td>194</td>
</tr>
<tr>
<td>8.2. Exclusion Criteria</td>
<td>195</td>
</tr>
<tr>
<td>8.3. Discontinuations</td>
<td>196</td>
</tr>
<tr>
<td>8.3.1. Discontinuation of Trial Sites</td>
<td>196</td>
</tr>
<tr>
<td>8.3.2. Discontinuation of the Trial</td>
<td>196</td>
</tr>
<tr>
<td>9. Treatment</td>
<td>197</td>
</tr>
<tr>
<td>9.1. Treatments Administered</td>
<td>197</td>
</tr>
<tr>
<td>9.2. Materials and Supplies</td>
<td>198</td>
</tr>
<tr>
<td>9.3. Method of Assignment to Treatment</td>
<td>198</td>
</tr>
<tr>
<td>9.4. Rationale for Selection of Doses and Timing of Treatment in the Trial</td>
<td>199</td>
</tr>
<tr>
<td>9.4.1. Continued Access to Trial Treatment</td>
<td>199</td>
</tr>
<tr>
<td>9.5. Blinding</td>
<td>199</td>
</tr>
</tbody>
</table>
14. References ...................................................................................................................... 211

Table of Contents (concluded)

List of Protocol Attachments
Protocol Attachment 1. Trial Schedule

1.4 Abbreviations and Definitions

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>event (AE)</td>
<td>Any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the product.</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of Covariance.</td>
</tr>
<tr>
<td>case report form (CRF)</td>
<td>Sometimes referred to as clinical report form; a printed or electronic form for recording trial participants’ data during a clinical trial, as required by the protocol.</td>
</tr>
<tr>
<td>consent</td>
<td>The act of obtaining informed consent for participation in a clinical trial from patients deemed eligible or potentially eligible to participate in the trial. Patients entered into a trial are those who sign the informed consent document directly or through their legally acceptable representatives.</td>
</tr>
<tr>
<td>end of trial</td>
<td>End of trial is the date of the last visit or last scheduled procedure shown in the Trial Schedule for the last active subject in the trial.</td>
</tr>
<tr>
<td>Ethics/Ethical review committee</td>
<td>A committee (institutional, regional, or national) composed of medical and non-medical members whose responsibility is to verify that the safety, welfare and human rights of the patients participating in a clinical trial are protected.</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice.</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonisation.</td>
</tr>
<tr>
<td>intention to treat (ITT)</td>
<td>The principle asserting that the effect of a treatment policy is best assessed by evaluating on the basis of the intention to treat a patient (i.e. the planned treatment regimen) rather than on the basis of the actual treatment given. Thus, patients allocated to a treatment group should be followed up, assessed and analysed as members of that group irrespective of their compliance with the planned treatment.</td>
</tr>
<tr>
<td>investigator</td>
<td>A person responsible for the conduct of the clinical trial at a trial site.</td>
</tr>
<tr>
<td>MHHS</td>
<td>Modified Harris Hip Score.</td>
</tr>
<tr>
<td>ml/mls</td>
<td>millilitres.</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging.</td>
</tr>
<tr>
<td>patient</td>
<td>A trial participant who has the disease or condition for which the investigational product is targeted.</td>
</tr>
<tr>
<td><strong>per protocol set (PPS)</strong></td>
<td>The set of data generated by the subset of patients whose compliance with the protocol was sufficient to ensure that these data would likely exhibit the effects of treatment.</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>PRP</strong></td>
<td>Platelet-rich plasma.</td>
</tr>
<tr>
<td><strong>randomise</strong></td>
<td>The act of assigning a patient to a treatment.</td>
</tr>
<tr>
<td><strong>SAE</strong></td>
<td>Serious Adverse Event.</td>
</tr>
<tr>
<td><strong>screen</strong></td>
<td>The act of determining whether an individual meets minimum eligibility requirements for participation in a clinical trial. (In this trial, screening involves diagnostic procedures. Separate informed consent for these will not be obtained.)</td>
</tr>
<tr>
<td><strong>treatment-emergent adverse event (TEAE)</strong></td>
<td>Any untoward medical occurrence that either occurs or worsens at any time after treatment is given. A TEAE does not necessarily have to have a causal relationship with the treatment.</td>
</tr>
</tbody>
</table>
The Effectiveness of Platelet-Rich Plasma Injections in Gluteal Tendonopathy – A Double-Blind Randomised, Controlled Trial (The PRP-HIP Trial)

1.5 Introduction

The incidence of gluteal tendonopathies is high\(^2\). It occurs in around 15\% of women and 7\% of men in middle-aged and elderly populations with an annual incidence of 1.8 patients per 1000\(^3\).

Although it is often seen in association with degenerative hip disease, it is not caused by osteoarthritis and frequently occurs separately. Many patients are severely limited by this condition for years with lateral hip pain, weakness and pain on walking, pain on sitting and inability to lie on the affected side, with disturbances to their sleep pattern.

The currently accepted treatments for this condition are conservative measures such as rest from walking, physiotherapy and non-steroidal anti-inflammatory drugs. Cortisone injections into the trochanteric bursa are also common and have been demonstrated to give temporary relief from pain for a variable time but generally only for 3 to 12 weeks. Most patients receive multiple cortisone injections, with limited success.

Tendonopathy has been researched widely and the currently accepted scientific position is that it is not an inflammatory condition\(^4\). The absence of inflammatory cells in the biopsies of patients with chronic tendonopathies offers little or no evidence to support the use of cortisone.

Over the last five years, there has been increasing evidence from randomised control trials\(^1,5,6,7\) that platelet-rich plasma (PRP) has significantly better short- and long-term results in the treatment of tendonopathies compared to other treatments. Tennis elbow has been researched
with long-term follow up extending beyond two years, where it has proved far superior to cortisone injections.

No data exist on the use of PRP in gluteal tendonopathies. A small, uncontrolled study carried out by the investigators has shown promising results. Therefore we wish to study the use of PRP injections in patients with gluteal tendonopathy prospectively, via a randomised, controlled trial.

1.6 Objectives

1.6.1. Primary Objective
The primary objective of this randomised, double-blind, clinical trial is to assess the efficacy of a single PRP injection, compared to a single cortisone injection, on the change in Modified Harris Hip Score (MHHS) from baseline to 12 weeks, in patients with gluteus medius or minimus tendonitis. The two treatment groups will be compared in terms of the MHHS change via analysis of covariance (ANCOVA) with a baseline MHHS adjustment.

1.6.2. Secondary Objectives
Secondary objectives are to compare the treatment groups in terms of MHHS at other follow-up times. The two treatment groups will be compared in terms of the MHHS change between baseline and follow-up time via ANCOVA with a baseline MHHS adjustment.

Safety information will be collected over the course of the trial and summarised.

1.7. Investigational Plan
1.7.1. Summary of Trial Design

This is a single centre, randomised, double-blind, parallel group trial. In the event that recruitment is slow, further centres may be added, in which case extensions to the randomisation scheme will be produced.

Patients will be randomised to either a single PRP injection or a single cortisone injection. The patient population will be adults with gluteus medius and/or minimus tendonopathy.

A total of 72 completed trial patients is required; patients will be randomised to one of the two treatment arms on a 1:1 basis.

1.8. Trial Population

The participants will be patients of either gender, between the ages of 18 and 80 years inclusive, referred to either Dr Jane Fitzpatrick or Mr John O’Donnell with gluteus medius and/or minimus tendonopathy. Participants will be excluded if they have demonstrated tears of the tendons on magnetic resonance imaging (MRI) and ultrasound; if they have had breast cancer; previous hip surgery; are on warfarin; had recent back surgery or sciatic pain; had cortisone treatment within the previous six weeks. Entered patients who meet all of the inclusion criteria and are not excluded by any of the exclusion criteria may be enrolled into the trial. Participation will be voluntary and written informed consent will be obtained by the investigator or his/her designee from all patients prior to the performance of any trial procedures.

1.8.1. Inclusion Criteria

Patients are eligible for inclusion in the trial only if they meet both of the following criteria:

[1] Present with gluteus medius and/or minimus tendonopathy.
[2] Are aged between 18 and 80 years (inclusive).
1.8.2. Exclusion Criteria

Patients will be excluded from the trial if they meet any of the following criteria:

[1] Are investigator site personnel directly affiliated with this trial and/or their immediate families. Immediate family is defined as a spouse, parent, child or sibling, whether biological or legally adopted.

[2] Have demonstrated full thickness tears (not partial thickness tears) of the tendons on MRI and ultrasound.


[4] Have had previous hip surgery or previous repair to gluteal tendons.


[6] Have had back surgery in the last 18 months.


[8] Have had cortisone treatment within the previous six weeks.

[9] Are currently enrolled in, or discontinued within the last 30 days from, any type of medical research judged by the investigator not to be scientifically or medically compatible with this trial.

[10] Have previously completed or withdrawn from this trial or any other trial investigating PRP or cortisone injection for the current trial’s indications.

[11] Are, in the opinion of the investigator, unwilling or unable to comply with trial data collection.
1.8.3. Discontinuations

Participants will be offered surgery to the tendons if, in the opinion of the investigator, they do not respond to their allocated trial treatment within six months. Note: This would involve discontinuation from the trial, but not discontinuation of trial treatment, the latter being a single treatment at the beginning of the trial.

If any of Exclusion Criteria [2] to [7] are observed, or develop after randomisation, the patient should be discontinued from the trial as soon as possible.

The investigator may decide that a patient should be withdrawn. If this decision is made because of a serious adverse event, appropriate measures are to be taken.

If the patient, for any reason, requires treatment with another therapeutic agent that has been demonstrated to be effective for treatment of the trial indication, the patient can be discontinued. In this case, discontinuation from the trial should occur prior to introduction of the new treatment.

The investigator may stop the trial or stop a patient’s participation in the trial for medical, safety, regulatory or other reasons consistent with applicable laws, regulations or good clinical practice.

Patients who discontinue the trial early will have end-of-trial procedures performed as per the Trial Schedule (Protocol Attachment 1).

1.8.3.1. Discontinuation of Trial Sites

Trial site participation may be discontinued if the investigator, or the ethics review committee of the trial site judges it necessary for medical, safety, regulatory or other reasons consistent with applicable laws, regulations or good clinical practice.

1.8.3.2. Discontinuation of the Trial

The trial will be discontinued if the investigator judges it necessary for medical, safety, regulatory or other reasons consistent with applicable laws, regulations or good clinical practice.
1.9. Treatment

1.9.1. Treatments Administered

Patients will receive either a single PRP injection or a single cortisone injection.

All Patients will have 55 mls of blood taken. Those randomised to the PRP group will have their blood processed according to the directions supplied with the kit from Biomet. The kit is fully sealed and supplied sterile. The blood is placed in a balanced centrifuge and spun for 15 minutes at the requisite speed.

The patient is placed on their side and the site and extent of tenderness is marked and visualised under ultrasound to ensure that the abnormality corresponds to the site of tenderness. Two mls of lignocaine 2% with adrenaline and eight mls of Naropin 0.75% are injected into the area.

The cells are then separated and loaded into a syringe. Using the ultrasound to guide the injection the cells are injected in a “peppering” technique into the abnormal area of tendon (intratendonously). There will be 4 to 6 passes as the cells are injected. The area is then covered with a waterproof dressing. The patient will be given a script for analgesia and cautioned against the use of non-steroidal anti-inflammatory medicines. A review appointment will be made for six weeks hence, and the patient is instructed to alert the physician to any concerns or questions they have.

They will not use crutches and will be allowed to do normal activities as comfortable. No high load activities are recommended in the post-procedure period. No physiotherapy will be given in the first six weeks following treatment. After the six-week review, the patient will be progressively returned to high-load activities. Patients will be reviewed at three months and at 12 months.
For patients randomised to cortisone treatment, the patient is placed on their side and the site and extent of tenderness is marked and visualised under ultrasound to ensure that the abnormality corresponds to the site of tenderness. Two mls of lignocaine 2% with adrenaline and eight mls of Naropin 0.75% are injected into the area.

Then an injection of Celestone Chronodose (one ampoule) will be given under ultrasound guidance in the same technique as the PRP as the Investigator will be blinded to the treatment. The syringe will be given to the Investigator in a covered syringe. The injection site will be covered with a dressing. The patient will be given the same instructions as the PRP group and will follow the same course until the six-week review.

Patients will be instructed to contact the investigator as soon as possible if they have a complaint or problem with the trial treatment so that the situation can be assessed.

1.9.2. Materials and Supplies

GPS® III PRP kits (Biomet, USA) will be used to treat patients allocated to the PRP arm. The kits are fully sealed and supplied sterile.

Patients allocated to the cortisone treatment group will each receive a single injection of Celestone Chronodose (one ampoule).

Clinical trial materials will be labeled according to local regulatory requirements.

1.9.3. Method of Assignment to Treatment

Patients who meet all criteria for enrollment will be randomised to open-label treatment at the initial patient visit. Assignment to treatment group will be determined by a computer-generated,
fixed block randomisation scheme. A unique patient trial identification number will be allocated simultaneously with treatment allocation.

To achieve between-group comparability for the site factor, the randomisation will be stratified by site, if indeed more than one site is used.

1.9.4. Rationale for Selection of Doses and Timing of Treatment in the Trial

The treatment doses and treatment timing used in this trial were chosen to be the same as those used in current clinical practice.

1.9.4.1. Continued Access to Trial Treatment

Not applicable. Only a single treatment is given.

1.9.5. Blinding

This is a double-blind trial. Both the participants and the investigator will be blinded. The sample to inject will be given to the Investigator covered so it is not visible to either.

1.9.6. Concomitant Therapy

Not applicable. There are no concomitant therapies in this trial.

Patients will be instructed to avoid the use of non-steroidal anti-inflammatory medicines.

1.9.7. Treatment Compliance

Not applicable.
1.10. Efficacy and Safety Evaluations, and the Appropriateness of Measurements

Trial procedures and their timing (including any tolerance limits for timing) are summarised in the Trial Schedule.

1.10.1. Efficacy Measures

1.10.1.1. Primary Efficacy Measure

The primary efficacy measure for this trial is the change in Modified Harris Hip Score (MHHS). This score will be completed by the patient. The MMHS is an ordinal scale variable with scores ranging from zero (no problems) to 100 (worst problems).

1.10.2. Safety Evaluations

Investigators are responsible for monitoring the safety of patients who have entered this trial and for alerting the sponsor or its designee to any event that seems unusual, even if the event may be considered an unanticipated benefit to the patients.

The investigator is responsible for the appropriate medical care of patients during the trial.

The investigator remains responsible for following, through an appropriate health care option, adverse events that are serious or that caused the patient to discontinue before completing the trial. The patient should be followed until the event is resolved or explained. Frequency of follow-up evaluation is at the discretion of the investigator.

The following safety parameters will be evaluated during the trial:

- Discontinuations
• Adverse events.

1.10.2.1. **Adverse Events**

Lack of treatment effect is not an adverse event (AE) in clinical trials, as the purpose of the trial is to establish a treatment effect, if one exists.

Cases of trial patient pregnancy that occur during maternal or paternal exposures to trial treatment should be reported.

Trial personnel will record the occurrence and nature of each patient’s pre-existing conditions, including clinically significant signs and symptoms of the disease under treatment in the trial.

After Patient Informed Consent is given, trial personnel will record any change in patient condition and the occurrence and nature of any AEs. All AEs related to protocol procedures are reported to the ethics committee.

Investigators will record their assessment of the potential relatedness of each AE to trial treatment and/or treatment delivery system.

1.10.2.1.1. **Serious Adverse Events**

Serious adverse event (SAE) collection begins after the patient has given informed consent and has received a trial treatment. If a patient experiences an SAE after signing informed consent, but prior to receiving trial treatment, the event will NOT be collected unless the investigator feels the event may have been caused by a protocol procedure.

Trial site personnel must report any SAEs to the ethics committee according to local ethics committee procedures. An SAE is any adverse event from this trial that results in one of the following outcomes:
• death

• initial or prolonged inpatient hospitalisation

• a life-threatening experience (i.e. immediate risk of dying)

• persistent or significant disability/incapacity

• congenital anomaly/birth defect

• considered significant by the investigator for any other reason.

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

When a condition related to the trial treatment delivery system requires medical or surgical intervention to preclude either permanent impairment of a body function or permanent damage to a body structure, the serious outcome of “required intervention” will be assigned.

1.10.2.2. Safety Monitoring

The investigators will monitor patient safety throughout the trial.

1.10.3. Appropriateness of Measurements

The efficacy score used in this trial (the Modified Harris Hip Score⁹) is a standard assessment that, in the opinion of the trial team, is widely used and generally regarded as reliable, accurate and relevant. The safety measures used in this trial are also standard.
1.11. Data Quality Assurance

To ensure accurate, complete, and reliable data, the trial Statistician will:

- provide instructional material to the trial sites, as appropriate.
- provide training to investigators and trial personnel. This training will give instruction on the completion of the case report forms (CRFs) and data capture procedures.
- be available for consultation with trial site personnel by telephone and email.
- review and evaluate CRF data to detect errors in data collection.
- conduct a quality review of the database.

To ensure the safety of trial participants and to ensure accurate, complete and reliable data, the investigators will keep records of clinical notes and patient medical records as original source documents for the trial. If requested, the investigator will provide the ethics committee with access to original source documents.

1.11.1. Data Capture System

An electronic data capture system will be used in this trial, in the form of an EpiData\textsuperscript{10} data entry system and database, constructed by the trial Statistician.

In most cases, trial data will be recorded initially on hard-copy case report forms (CRF) for later entry into the trial database.

Any data, for which the CRF or paper documentation provided by the patient will serve as a source document, will be identified and documented by each site in that site’s trial file.
1.12. Sample Size and Statistical Methods

1.12.1. Determination of Sample Size

The sample size is based on the primary objective - to assess the efficacy of a single treatment with PRP, compared with cortisone, on the change in Modified Harris Hip Score from baseline to 12 weeks, in patients with gluteus medius and/or minimus tendonopathy, in an open-label, randomised trial.

For an unpaired $t$ test with 90% power and a 2-sided alpha level of 5%, the number of evaluable patients needed per group ($N_{pg}$) is given by:

$$N_{pg} = 21\sigma^2/\Delta^2$$

Where $\sigma^2$ is the variance of change from baseline in the MHHS (Modified Harris Hip Score) after 12 weeks treatment and $\Delta$ is the change in MHHS, specified here as 20 points on the MHHS.

The variance was estimated from eight patient records (John O’Donnell’s practice) as 182.05.

Given the uncertainty in this estimate, the upper 95% confidence limit (CL) for the variance was used in place of the point estimate, to err on the side of caution.

The upper 95% CL was calculated as: $(n-1)s^2/\chi^2_{0.975,n-1} = 8 \times 182.05/2.1797 = 688.16 (= 25.85^2)$.

Thus, $N_{pg} = 21 \times 182.05 / (20)^2 = 21 \times 688.16 / 400 = 36$ evaluable patients per treatment group.

Allowing for 10% loss to follow-up at the 12 week main evaluation, 36/0.9 = 40 randomised patients per treatment group are required.
1.12.2. Statistical and Analytical Plans

1.12.2.1. General Considerations

Statistical analysis of this trial will be the responsibility of Dr Jane Fitzpatrick and Max Bulsara (Biostatistician).

Analyses will be conducted on an intention-to-treat (ITT) basis, unless otherwise specified. An ITT analysis is one of data by the groups to which patients were assigned by random allocation, even if the subject did not receive the assigned treatment, did not receive the correct treatment, or otherwise did not follow the protocol (e.g. was wrongly enrolled). All randomised patients who received any trial treatment will be included in the safety data summaries.

If more than one investigative sites site is used, those with fewer than five patients with post-baseline data will be pooled for any statistical analysis of treatment × site interaction. Unless otherwise specified, treatment comparisons will be based on contrasts between the PRP and cortisone treatment groups. Any significance tests will be conducted at a two-sided alpha level of 0.05. Any tests of interaction effects will be conducted at a two-sided alpha level of 0.10. Unless otherwise specified, when a total score is calculated from individual items, it will be considered missing if any of the individual items are missing.

Also, unless otherwise specified, if an average score is calculated it will be based on non-missing values.

All statistical analysis will be conducted using SAS® Version 9.2 or later and run in a PC environment.

In general, descriptive summary statistics will include:
for categorical variables: number missing, number and percentage (with the percentage excluding the number missing in the denominator); and

for continuous variables: number missing, number, mean, median, standard deviation, 95% confidence interval (CI), inter-quartile range, minimum and maximum.

Percentages will be reported to one decimal place; mean and median to one decimal place more than the raw data; standard deviation and standard error to two decimal places more than the raw data; and minimum and maximum to the same decimal place as the raw data.

Any change to the data analysis methods described in the protocol will require an amendment only if it changes a principal feature of the protocol. Any other change to the data analysis methods described in the protocol, and the justification for making the change, will be described in the clinical trial report. Additional exploratory analyses of the data will be conducted as deemed appropriate.

1.12.2.2. Patient Disposition

The number of randomised patients who complete the trial or discontinue early will be tabulated for each treatment group. The statistical significance of the differences between treatment groups for each discontinuation reason will be assessed using Fisher’s exact test, if the data are sufficient for such a comparison to be meaningful.
1.12.2.3. **Patient Characteristics**

Patient demographics and illness characteristics will be summarised along with baseline efficacy measurements (MHHS). Treatment group means will be tabulated for continuous variables, and percentages will be presented for categorical variables.

1.12.2.4. **Treatment Compliance**

Not applicable.

1.12.2.5. **Primary Outcome and Methodology**

The primary analysis of mean change from baseline to 12 weeks in the Modified Harris Hip Score will be analysis of covariance (ANCOVA). The model will include the fixed effect of treatment and the continuous, fixed covariate of baseline MHHS score. Significance tests will be based on least-squares means and Type III sum-of-squares, using a two-sided $\alpha = 0.05$ (two-sided 95% confidence intervals).

Analyses will use SAS® PROC MIXED. The primary comparison is the contrast between treatment groups of the change in MHHS between baseline and week 12.

Patients who discontinue without a week 12 follow-up MHHS will still have their baseline information included in the primary endpoint efficacy analysis.

1.12.2.6. **Secondary Analyses**

The primary analysis described above in Section 12.2.5 will be repeated for the MHHS treatment group comparisons at the other follow-up times, namely: 2 weeks; 6 weeks; 6 months; and 12 months. The latter two comparisons will not form part of any initial trial report due to the time needed for these follow-ups to become due.
Various analyses will be carried out to test the sensitivity of the primary outcome analysis to any missing data and to analysis technique.

First, any missing baseline MHHS data will be imputed using mean imputation. The procedures outlined in White & Thompson\textsuperscript{11} will be followed. An analysis identical to that described for the primary outcome will then be carried out on the resulting data set. If there are few missing baseline or 12-week MHHS data, these imputations may not be done.

Second, a REML-based, repeated measures mixed model (MMRM) will be fitted to the original data and treatment group differences estimated at the week 12 follow-up, using an adjustment for baseline MHHS, including the (unequally time-spaced) MHHS measurements at 2 weeks and 6 weeks post-treatment, but excluding the 6-month and 12-month follow-up MHHS measurements. The MMRM approach controls Type I error rates at a nominal level in the presence of missing completely at random (MCAR) or missing at random (MAR) and – in some cases – missing not at random (MNAR) data. This approach is superior to techniques like LOCF\textsuperscript{12}. The choice of covariance matrix structure will be made thus: each of Spatial Power (SP-POW), compound symmetry (CS) and unstructured (UN) covariance matrix structures will be fitted, and a choice made between them on the basis of the AICc criterion. In the event of similar AICcs for two or more structures, the simplest will be chosen. The Kenward- Roger approximation will be used to estimate denominator degrees of freedom. Significance tests will be based on least-squares means and Type III sum-of-squares, using a two-sided $\alpha$ of 5% and two-sided 95% CIs for all differences will be calculated.

A similar MMRM model may later be fitted, including the 6-month and 12-month follow-up measurements, if it is judged that missingness at these follow-ups is not too great a problem.
1.12.2.7. Pharmacokinetic/Pharmacodynamic Analyses

Not applicable.

1.12.2.8. Quality of Life Analyses

Not applicable.

1.12.2.9. Safety Analyses

Adverse events will be listed individually and summarised by treatment group. No significance testing will be carried out. (It is anticipated that adverse events will be rare and minor.)

1.12.2.10. Subgroup Analyses

Not applicable.

1.12.2.11. Interim Analyses

No interim analyses are planned for this trial. If an unplanned interim analysis is deemed necessary, the trial team will determine whether it is necessary to amend the protocol.

1.13. Informed Consent, Ethical Review, and Regulatory Considerations

1.13.1. Informed Consent

The investigator is responsible for ensuring that the patient understands the potential risks and benefits of participating in the trial, including answering any questions the patient may have throughout and sharing in a timely manner any new information that may be relevant to the patient’s willingness to continue his or her participation in the trial.
The informed consent document (ICD) will be used to explain the potential risks and benefits of trial participation to the patient in simple terms before the patient is entered into the trial, and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the trial and desires to participate in the trial.

The investigator is responsible for ensuring that informed consent is given by each patient or their legal representative. This includes obtaining the appropriate signatures and dates on the ICD prior to the performance of any protocol procedures and prior to the administration of trial treatment.

As used in this protocol, the term “informed consent” includes all consent and assent given by patients or their legal representatives.

1.13.2. Ethical Review

Ethical approval for the trial has been granted by the Epworth Healthcare Human Research Ethics Committee (57412).

All informed consent documents used in the trial must be compliant with the International Conference on Harmonisation (ICH) guideline on good clinical practice (GCP).

1.13.3. Regulatory Considerations

This trial will be conducted in accordance with applicable laws and regulations, GCP, and the Declaration of Helsinki. The investigator will, if required, submit the trial protocol to the ethical review committee.

1.13.3.1. Investigator Information
• Dr Jane Fitzpatrick, Specialist Sports Physician, Epworth HealthCare, Richmond.
  Phone No: 94296444
  Email Address: janefitz@ozemail.com.au

• Mr John O’Donnell, Orthopaedic Surgeon, Epworth HealthCare, Richmond.

1.13.3.2. **Protocol Signatures**

After reading the protocol, the principal investigator will sign the protocol signature page.

1.13.3.3. **Final Report Signature**

The principal investigator will sign the final clinical trial report, indicating their agreement with the analyses, results and conclusions of the report.

### 1.14. References


Perform procedures as indicated below, unless the cell relating to a time or time interval is shaded.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Baseline (Day -1 Pre-treatment)</th>
<th>During Therapy (Day 0)</th>
<th>During Therapy (Day 14 ± 2 days)</th>
<th>During Therapy (Day 42 ± 7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRF Visit (or follow-up) number</td>
<td>V1</td>
<td>V2</td>
<td>V3</td>
<td>V4</td>
</tr>
<tr>
<td>Informed consent (before trial procedures or tests)</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial history/pre-existing conditions</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHHS measurement</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Trial treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safety monitoring</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>

Abbreviations: CRF = case report form, V = visit (or follow-up), MHHS = Modified Harris Hip Score.
Human Research & Ethics Committee

Certificate of Approval

<table>
<thead>
<tr>
<th>Project Title:</th>
<th>A Phase III, randomised, parallel group trial, comparing PRP treatment with cortisone treatment.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal Investigator:</td>
<td>Dr Jane Fitzpatrick</td>
</tr>
<tr>
<td>Epworth study no:</td>
<td>57412</td>
</tr>
<tr>
<td>HREC Meeting date:</td>
<td>01 May 2013</td>
</tr>
<tr>
<td>Board of Management approval:</td>
<td>29 May 2013</td>
</tr>
<tr>
<td>Duration of Project:</td>
<td>19 August 2012 to 01 February 2015</td>
</tr>
</tbody>
</table>

Terms and conditions of approval:

Alan R. Kinkade
Group Chief Executive
The Principal Investigator is required to notify the Human Research Ethics Committee of the following;

**All Projects:**

2. Any proposed changes to the protocol or approved documentation or the addition of documents (including flyers, brochures, advertising materials etc) must be submitted to the Human Research Ethics Committee for approval prior to implementation.
3. The Principal Investigator must notify HREC of:
   a. Any serious adverse effects of the study on participants and steps taken to deal with them
   b. Any unforeseen events (e.g. protocol violations or complaints)
   c. Investigators withdrawing from or joining the project
4. A Progress Report must be submitted annually and at the conclusion of the project.
5. Epworth HealthCare HREC approval must remain current for the entire duration of the project. If the project is not completed in the allocated time a renewal request must be submitted to the HREC. Investigators undertaking projects without current HREC approval risk their indemnity, funding and publication rights.

**Clinical Trials:**

7. Must report all internal (occurring at Epworth HealthCare) Serious Adverse Events (SAE) to the sponsor and the HREC within 72 hours of occurrence.
8. Must report all Suspected Unexpected Serious Adverse Reactions (SUSARS) to the Therapeutic Goods Administration (TGA). For sponsored studies, the sponsor may take this responsibility.

I, ………………………………………………, accept the terms and conditions set out above.

Signature of Researcher: …………………………………….. Date: …………………..
The Effectiveness of Platelet-Rich Plasma in the Treatment of Tendinopathy

A Meta-analysis of Randomized Controlled Clinical Trials

Jane Fitzpatrick, MBBS, FACSP, Max Bulsara, BSc(Hons), MSc, PhD, and Ming H. Zheng, MD, PhD, FRCPA, FRCPath, FRCPA

Background: Tendinopathy is very common in the general population. There are increasing numbers of clinical studies referring to platelet-rich plasma (PRP) and platelet-poor plasma (PPP) as treatments for tendinopathy.

Purpose: To perform a meta-analysis of the outcomes of the PRP groups by preparation method and injection technique in tendinopathy. To determine the clinical effectiveness of the preparations and to evaluate the effect of controls used in the studies reviewed.

Study Design: Systematic review and meta-analysis.

Methods: The PubMed, EMBASE, CNHIL, and Medline databases were searched in March 2012, April 2014, and August 2015, and randomized controlled trials using autologous blood, PRP, PPP, or autologous conditioned plasma in tendinopathy with outcome measures of pain and follow-up time of 3 months were included in this review. Trials including surgery, tendon tears, and muscle or ligament injuries were excluded. Study quality was assessed using the Cochrane Collaboration risk-of-bias tool by 2 reviewers. Data were pooled using random-effects meta-analysis. The primary outcome measure was a change in pain intensity. Where more than 1 pain scale was included, a functional score was selected ahead of a visual analog scale score.

Results: A total of 18 studies (1068 participants) were included. Eight studies were deemed to be at low risk of bias. The most significant outcomes in the PRP groups were seen in those treated with highly cellular leukocyte-rich PRP (LR-PRP) preparations: GPR kit (standardized mean difference [SMD], 3.75; 95% CI, 2.60-6.30), MyCita kit (SMD, 1.44; 95% CI, 0.22-2.65), Prostaph kit (SMD, 0.39; 95% CI, 0.27-4.86), and unspecified LR-PRP (SMD, 0.42; 95% CI, 0.19-0.66). When the LR-PRP system type was compared, there was a strongly positive effect (SMD, 0.34; 95% CI, 0.00-0.68) when compared with leukocyte-poor PRP (SMD, 2.67; 95% CI, 1.83-3.52). In assessing the control groups, there was no clear difference between different types of control injections: saline (SMD, 1.42; 95% CI, 0.74-1.59), local anesthetic (SMD, 1.60; 95% CI, 0.76-0.18), and corticosteroid (SMD, 2.82; 95% CI, 1.04-18.56), or dry needling (SMD, 2.62; 95% CI, 2.12-29.42).

Conclusion: There is good evidence to support the use of a single injection of LR-PRP under ultrasound guidance in tendinopathy. Both the preparation and intra-tendinous injection technique of PRP appear to be of great clinical significance.

Keywords: platelet-rich plasma; tendinits; tendinopathy; platelet separation system; meta-analysis; injection therapy

Tendinopathy is one of the most common reasons for presentation to a medical practitioner, representing 30% of all presentations for musculoskeletal complaints. The most frequently discussed sites include the elbow (both tennis and golfer's elbow), rotator cuff, Achilles tendon, patellar tendon, and gluteal tendons. There are multiple treatments described in the literature including physical therapy; shock wave treatment; nonsteroidal anti-inflammatory drugs; and injections of glucocorticoids, prolotherapy, autologous blood, platelet-rich plasma, and/or platelet-rich plasma (PRP). Despite the pathophysiologic role of inflammation being debated, the most commonly used treatment for chronic tendinopathy is glucocorticoid injections. These offer good short-term improvement, less than 3 months, but do not confer a benefit in the longer term. PRP is one treatment that has been embraced in recent years as a potentially safe, effective treatment for tendinopathy. PRP is defined as platelet-rich concentrate with platelet levels greater than baseline when compared with whole blood. The potential uses of PRP extend from skin and wound healing to the treatment of tendinopathy and

The American Journal of Sports Medicine, Vol. 43, No. 1
DOI: 10.1177/0007123415564378 © 2016 The Author(s)
osteoarthritis. There is widespread interest in the use of PRP in the treatment of tendinopathy, as well as an increasing number of randomized controlled trials (RCTs) studying the effectiveness of PRP in tendinopathy, particularly in tennis elbow.\textsuperscript{11,14,23,27,29,30,40,45,46} There is still no consensus as to whether PRP confers a beneficial effect, as not all trials have failed to demonstrate a positive benefit.\textsuperscript{14,21}

We found 6 systematic reviews published between 2010 and 2014 assessing the effectiveness of PRP in tendinopathy,\textsuperscript{12,13,20,21,48,49} Despite analyzing the same data, they reported contrasting conclusions, from concluding that PRP is effective\textsuperscript{2} to finding that there is strong evidence against platelet-rich plasma.\textsuperscript{50} The majority of comments stated that there is great difficulty reaching a conclusion because of the variance of the type of PRP produced. In a Cochrane review of PRP in soft tissue injuries, Monroe et al.\textsuperscript{47} indicated that “there is need for standardization of PRP preparation methods.” In their editorial review, Goema and Mihm\textsuperscript{51} commented on the systematic review performed by de Vos et al.,\textsuperscript{18} concluding that “it would be better to break out the results by specific study design and PRP type.”

Thus, we conducted a meta-analysis to assess the comparative effectiveness of PRP types in tendinopathy. We also assessed the effectiveness of different controls used in RCTs.

METHODS

Our review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines and the PRISMA-PD Statement\textsuperscript{52} (see Appendix 1, available online at http://jssm.sagepub.com/supplemental).

Eligibility Criteria, Patients, and Interventions

RCTs using injections of PRP or autologous blood products in the treatment of tendinopathy (of any type) were included if they treated adults (aged >18 years). Trials that included patients undergoing surgery or treatment of non-tendinous soft tissue injuries (e.g., muscle, ligament, or fascia) were not eligible. Eligible interventions included injections of any autologous blood product including whole blood, PRP or platelet-poor plasma (PPP), or autologous conditioned plasma (ACP). We allowed any dosage, volume, number of injections, and periprosthetic or intratendinous injections. Controls were accepted as other active injections, placebo, or conservative management.

Outcomes

We considered the most important primary outcome measure as a change in pain intensity or function. Previous meta-analyses have demonstrated that the “benefit from PRP is most evident at longer time points” or “have a significant impact on improving pain and/or function over time.”\textsuperscript{12}

Therefore, a minimum acceptable follow-up of 12 weeks for studies was included, and data from 6- and 12-month follow-up were included where available. In the event that more than 1 pain scale was included in the study, we selected the Patient-Rated Tennis Elbow Evaluation (or equivalent for other tendons) ahead of a visual analog scale or verbal rating scales. Only 1 pain score measure was used for each study.

Data Sources and Search Strategy

A search strategy for RCTs investigating the treatment of tendinopathy with autologous blood products was carried out. The full search strategy is contained in Appendix 2 (available online), key search terms included “platelet-rich plasma,” “autologous conditioned serum,” “autologous blood and tendinitis,” “tendinopathy,” “and the terms for all common tendinopathy such as “tennis elbow,” “Achilles tendinosis/tendinopathy,” “patellar tendinitis,” “hamstring,” “rotator cuff,” and “gastrocnemius tendinopathy.” The PubMed, EMBASE, CINAHL, and Medline databases were searched for 5 years up to March 2012. A repeat search was performed in April 2014 and August 2015. The language was restricted to English.

Study Selection

Initial screening and study selection were performed by 3 authors (J.P. and M.B.). Any disagreement was discussed between these 2 authors, and a third author (M.Z.) was available to determine a consensus. A total of 723 records were identified through database searching (Figure 1). An additional 8 studies were obtained from reviews and conference proceedings. After duplicates were removed, 65 records were screened. Twenty-one records were excluded on review of the abstract, as they were protocol registrations, not RCTs, related to surgical procedures or conditions other than tendinopathy. The number of full-text articles assessed for eligibility was 44. Of these, 22 studies were excluded: 5 related to rotator cuff tears, 2 related to muscle injuries, 13 related to surgical interventions, and 2 were non-PRP studies. Of the 22 articles available for analysis, 2 sets of articles were combined after discussion, as they related to the same data sets.\textsuperscript{11,14,25,26} Two articles were excluded: Kazazi et al.\textsuperscript{27} had data only available to 8 weeks, which did not meet the minimum criteria for analysis, and Miwa and Pavelko\textsuperscript{28} had no analyzable data available in the published form, and despite personal contact with the authors, it was not possible to obtain data for analysis for this work. This meant that there were 18 articles available for full analysis (Table 1).

Data Collection Process

Data from the included trials were extracted by one reviewer (J.P.) and checked by a second reviewer (M.B.). The extracted data were included in an Excel spreadsheet (Microsoft Corp) and included the title of the article and authors; the kit or product type and technique; the region being treated; the number of participants in the trial enrolled and completed; whether the trial was an RCT; the type of pain score used and its maximum score; and
TABLE 1
Articles Available for Quantitative Analysis

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Tendinopathy</th>
<th>No. of Patients</th>
<th>Therapy</th>
<th>Outcome</th>
<th>Time, mo</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell et al(2013)</td>
<td>Achilles</td>
<td>53</td>
<td>ABLEIN</td>
<td>VISA-A</td>
<td>6</td>
<td>Included</td>
</tr>
<tr>
<td>Thanassas et al(2013)</td>
<td>TE</td>
<td>27</td>
<td>GIPS/ABE</td>
<td>VAS</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Crenney et al(2011)</td>
<td>TE</td>
<td>130</td>
<td>LR-PRP/PABI</td>
<td>PRST</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Wolf et al(2011)</td>
<td>TE</td>
<td>28</td>
<td>ABLEIS/EXS</td>
<td>DASH</td>
<td>2.6</td>
<td>Included</td>
</tr>
<tr>
<td>Peelee et al(2010, Gosens et al(2011)</td>
<td>TE</td>
<td>100</td>
<td>GIPS/CSI</td>
<td>DASH</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Kamami et al(2010)</td>
<td>TE</td>
<td>60</td>
<td>ABLEIS</td>
<td>VAS</td>
<td>2</td>
<td>Not included</td>
</tr>
<tr>
<td>Vanzen et al(2010)</td>
<td>TE</td>
<td>57</td>
<td>ABLEIS/EXS</td>
<td>VAS</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Kreh et al(2013)</td>
<td>TE</td>
<td>60</td>
<td>GIPS/EXS/ABEIS</td>
<td>DASH</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Mishra and Parvekar(2006)</td>
<td>TE</td>
<td>50</td>
<td>GIPS/LA</td>
<td>VAS</td>
<td>2.6</td>
<td>Included</td>
</tr>
<tr>
<td>Vatne et al(2013)</td>
<td>PT</td>
<td>46</td>
<td>MURATT</td>
<td>VISA-P</td>
<td>5.12</td>
<td>Included</td>
</tr>
<tr>
<td>Mishra et al(2014)</td>
<td>TE</td>
<td>25</td>
<td>GIPS/LA</td>
<td>PRST</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Rohrra et al(2015)</td>
<td>TE</td>
<td>25</td>
<td>LP-PFSSA</td>
<td>MMMP</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Birk et al(2014)</td>
<td>TE</td>
<td>80</td>
<td>ABLEIS</td>
<td>PRST</td>
<td>3</td>
<td>Included</td>
</tr>
<tr>
<td>Drugo et al(2014)</td>
<td>PT</td>
<td>25</td>
<td>GIPS/EN</td>
<td>VISA-F</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Rasa et al(2013)</td>
<td>RC</td>
<td>30</td>
<td>Posts/IN</td>
<td>VAS</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Stonehouse et al(2013)</td>
<td>TE</td>
<td>25</td>
<td>AC/PP</td>
<td>Nirsal</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Gersten et al(2015)</td>
<td>TE</td>
<td>30</td>
<td>LR-P/P</td>
<td>DASH</td>
<td>3.6</td>
<td>Included</td>
</tr>
<tr>
<td>Reisskram et al(2013)</td>
<td>RC</td>
<td>40</td>
<td>GIPS/Exs</td>
<td>WORK</td>
<td>3.6</td>
<td>Included</td>
</tr>
</tbody>
</table>

*ABE, autogenous blood injection; ACP, autogenous conditioned plasma; CSL, corticosteroid injection; DASH, Disabilities of the Arm, Shoulder and Hand; DNI, dry needling; Erc, electroacupuncture; GIPS, GIPS/ABE, GIPS kit and 16.5 mL of local anesthetic LA; local anesthetic injection; LP-PRP, leukocyte-poor platelet-rich plasma, no kit applied; LR-PRP, leukocyte-rich platelet-rich plasma, no kit applied; MC, Methasol; MURATT, modified Mayo Clinic Performance Index for the Elbow; Nirsal, Nirsal Score for elbow; Posts, Posts kit; PRST, Patient-Related Tennis Elbow Rehabilitation; PT, patellar tendonitis ( jumper's knee); RC, rocker cuff; Saline, saline injection; SFIP, Shoulder Pain and Disability Index; SWT, shock wave treatment; TE, tango elbow (lateral epicondyle); VAS, visual analog scale for pain; VISA-A, Victorian Institute of Sports Assessment-Achilles; VISA-P, Victorian Institute of Sports Assessment-Patellar; WORC, Western Ontario Rotator Cuff Index.

There were 60 patients at the beginning of the study; the final number of study patients was 17.

3. PRP-P produced from the buffy coat layer with 10 to 15 mL injected prior to the high volume (10 mL) local anesthetic injection.
4. Leukocyte-poor PRP (LP-PRP): 1 study
5. ACP (leukocyte-poor PPP): 1 study

Nine studies used a single injection, and 4 used 2 injections. All except for 2 studies used ultrasound guidance. All studies used 1 to 3 mL of local anesthetic injection, except for 1 study that injected the local anesthetic with PRP and 1 study that used 16.5 mL of local anesthetic. Only 1 study activated PRP before injection: Behera et al, who also used LP-PRP. Four studies buffered PRP before use with sodium bicarbonate.

Controls were divided into:
1. Injections:
   a. Corticosteroid: 6 studies
   b. Saline: 4 studies
   c. Local anesthetic: 2 studies
   d. Dry needle: 4 studies
2. Noninjections:
   a. Excenteric injection: 1 study
   b. Shock wave treatment: 2 studies

Two studies used 2 control arms: Wolf et al used corticosteroid and saline as controls against autologous blood, and Krehl et al also used corticosteroid and saline as controls against the GIPS kit. No differentiation was made for differing tendon sites.

Risk-of-Bias Assessment
No studies were eliminated on bias risk alone. Table 2 shows the 8 studies deemed to have a low risk of bias based on the 4 key areas of allocation concealment, patient and assessor blinding, and attrition.

Network Meta-analysis
A total of 18 studies (1068 participants) were included. Seventeen studies were deemed to be at low or medium risk of bias. The changes in pain scores for treatments and controls presented by treatment type are shown in Appendix Figure A1 (available online).

The most significant outcome in the PRP groups was observed in those treated with highly cellular LR-PRP preparations: GIPS kit (SMD, 30.75; 95% CI, 28.40-33.10), Methasol kit (SMD, 31.84; 95% CI, 17.56-46.13), Proxs kit (SMD, 42.99; 95% CI, 37.73-48.25), and unspecified LR-PRP (SMD, 54.62; 95% CI, 51.69-57.55).

References 1, 14, 15, 16, 20, 21, 22, 23, 24, 25.
the 2-, 3-, 6-, and 12-month scores and their SDs. Where the SDs were not reported, they were calculated from the 95% CIs. Where neither of these was available, the authors were approached directly using the email address on their publication to obtain the raw data. One study, Misbin et al., had no published SDs or 95% CIs, but these were provided after personal contact with the authors. The technique used in all PRP groups was described as single/multiple injections, intratendinous (peppered), with or without local anesthetic. One study's authors were approached to confirm their technique, as it was not clear from the publication whether local anesthetic was used.

Assessment of Risk of Bias

Because it is accepted that the inclusion of trials with a high risk of bias may distort the results of a meta-analysis, the Cochrane Collaboration tool for assessing the risk of bias was used. The following features were assessed: randomization sequence generation; allocation concealment; blinding of patients, investigator, and assessor; attrition rates; and financial interest by companies. These were given a rating of low, unclear, or high risk of bias. An RCT was ranked as having low, medium, or high risk overall based on the key areas of allocation concealment, reporting of attrition rates, and patient and assessor blinding (low = all key areas rated low, medium = 2 or 3 factors rated high or uncertain, and high = all 4 factors rated high).

Measures of Treatment Effect

The weighted mean difference with the 95% CI was calculated when continuous outcomes were measured on standard scales. Where continuous outcomes were reported on nonstandard scales, the standardized mean difference (SMD) was calculated. All analyses were performed on an intention-to-treat basis. As changes from baseline scores were analysed, we imputed a change-from-baseline SD using a correlation coefficient based on the Cochrane guidelines.

Assessment of Heterogeneity

Heterogeneity among trials was assessed using the I² test statistic (>50% is considered as having substantial heterogeneity). We used a random-effects meta-analysis as an overall summary when appropriate.

Statistical Analysis

We used the scores for the change in pain intensity at baseline and at 3, 6, and 12 months where available. These were SMDs for each study and each control/treatment group. There were a variety of pain scales used across the studies. Thus, the application of an individual arm-based approach to the meta-analysis was used so each blood product type and each control type were evaluated separately within each study trial. Data appear as the change in pain from baseline with SDs and 95% CIs for each time point. A fixed-effects model was used if no significant heterogeneity existed between studies.

All statistical analyses were performed using STATA version 13 (StatCorp LP). Forest plots were utilized to assess statistical heterogeneity.

RESULTS

Of the 75 studies identified by the search, a total of 18 studies were included in the qualitative synthesis. As outlined in Figure 1, studies were excluded if they related to rotator cuff tears rather than tendinopathy, assessed muscle injuries, were duplicates, related to ligament injuries, had surgical interventions, or did not use an autologous blood or PRP product.

Studies were analyzed for type of control and type and technique of treatment. All treatments consisted of intratendinous injections with a prior administration of 1 to 2 mL of local anesthetic unless specified otherwise, as follows:

1. Autologous blood injection (ABI): 7 studies
2. Platelet-rich plasma (PRP) produced from buffy coat layer:
   a. GPS kit (Biomet Biologicals): 6 studies
   b. MyCells kit (Stryker Ltd): 1 study
   c. Presys kit (Tomed Holdings Inc): 1 study
   d. Unspecified kit as LPR-PRP: 2 studies

Figure 1. Flow of information through a systematic review for platelet-rich plasma in tendinopathy.
TABLE 2
Risk-of-Bias Assessment for the Included Studies*

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>No. of Patients</th>
<th>Company</th>
<th>Sequence</th>
<th>Doctor</th>
<th>Allocation</th>
<th>Patient</th>
<th>Assessor</th>
<th>Attrition</th>
<th>Overall Risk of Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hell et al.²² (1993)</td>
<td>53</td>
<td>LRB</td>
<td>LRB</td>
<td>HBB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Pearson et al.³⁶ (2012)</td>
<td>28</td>
<td>LRB</td>
<td>LRB</td>
<td>HBB</td>
<td>HBB</td>
<td>HBB</td>
<td>HBB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Thounas et al.³⁵ (2013)</td>
<td>27</td>
<td>LRB</td>
<td>LRB</td>
<td>HBB</td>
<td>HBB</td>
<td>HBB</td>
<td>HBB</td>
<td>HBB</td>
<td>HBB</td>
</tr>
<tr>
<td>Crenney et al.³³ (2013)</td>
<td>130</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Wolf et al.¹⁴ (2011)</td>
<td>28</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>de la Fuente et al.³³ (2011)</td>
<td>54</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Goesse et al.³³ (2011)</td>
<td>100</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Ozturan et al.³³ (2013)</td>
<td>57</td>
<td>LRB</td>
<td>URB</td>
<td>URB</td>
<td>URB</td>
<td>URB</td>
<td>URB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Krogh et al.³³ (2013)</td>
<td>60</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Verrone et al.³³ (2014)</td>
<td>46</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Mihalis et al.³³ (2014)</td>
<td>225</td>
<td>LRB</td>
<td>URB</td>
<td>URB</td>
<td>URB</td>
<td>URB</td>
<td>URB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Bohra et al.³³ (2015)</td>
<td>25</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Ark et al.³³ (2016)</td>
<td>80</td>
<td>LRB</td>
<td>LRB</td>
<td>HBB</td>
<td>HBB</td>
<td>HBB</td>
<td>HBB</td>
<td>HBB</td>
<td>HBB</td>
</tr>
<tr>
<td>Dragos et al.³³ (2014)</td>
<td>36</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Rha et al.³³ (2013)</td>
<td>30</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Staniloumas et al.³³ (2013)</td>
<td>30</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Guzzanti et al.³³ (2013)</td>
<td>30</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
<tr>
<td>Kesikburun et al.³³ (2013)</td>
<td>40</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
<td>LRB</td>
</tr>
</tbody>
</table>

*The 8 studies were assessed as having a low risk of bias based on the key areas (allocation concealment, patient and assessor blinding, and attrition). LRB, a low risk bias; HBB, a high risk bias; MBB, a medium risk bias; PRP, platelet-rich plasma; URB, uncertain risk bias.

The ACP group also had a positive response (SMD, 2.57; 95% CI, 1.42-3.90). PRP did not appear to be as effective (SMD, 26.37; 95% CI, 18.31-35.22).

Because it appeared that LP-PRP preparations produced a more positive outcome than LP-PRP preparations, this was compared in a forest plot grouped analysis (see Appendix Figure A2, online only). Results showed a strong positive effect of LP-PRP (SMD, 46.38; 95% CI, 34.00-58.77) when compared with LP-PRP (SMD, 28.77; 95% CI, 18.31-35.22).

One study using LP-PRP with the administration of 10 to 15 mL of local anesthetic did not obtain positive results (SMD, 14.82; 95% CI, 11.13-18.53). While there was no local anesthetic administered at the time of the PRP injection, the volume injected prior was more than 10 times the amount used by other studies. Given the potential negative effect of local anesthetic on PRP, this may be the reason that this group performed poorly.⁷

In assessing the control groups, there was no clear difference between different types of control injections: saline (SMD, 14.62; 95% CI, 10.74-18.50), local anesthetic (SMD, 15.00; 95% CI, 7.66-22.34), corticosteroid (SMD, 23.82; 95% CI, 19.11-28.53), or dry needling (SMD, 20.22; 95% CI, 21.27-39.10). None of these controls was truly a placebo, as all these injections produce a measurable effect on the outcome, but they did produce effective controls for this type of clinical trial.

**DISCUSSION**

Essentially, there are 2 main types of PRP produced. The first is from the plasma layer. It aims to exclude red and white cells from the preparation and to collect as many platelets from the remaining "plasma" layer as possible. The resultant product is low in red and white cells and has a low level of platelets (1.5 to 3 times baseline levels). The ACP kit works in this way and has been shown to have LRB³ to LRB⁶ times the baseline platelet concentrations with low white cell counts. Thus, the ACP kit was classified as LRB, being lower in platelet count but also low in white cell count. The second type of product is made from the buffy coat layer. It aims to take platelets from both the plasma and the cellular layer and is thus generally much higher in platelet count, yielding approximately to 6 times the baseline level of platelets.⁸,¹²,¹⁹ It does, however, concentrate the white cells in equal amounts and is thus high in both leukocytes and platelets (LRP-PRP). It is possible to produce LP-PRP by filtering out the white cells after preparation, as was conducted by Behra et al.⁴ A recent laboratory study by these authors (unpublished data) showed that the difference between both preparations is quite profound in terms of the total white cell count, ranging from 35.8 × 10⁹/l in LP-PRP to 1.3 × 10⁷/l in LP-PRP.

This study shows that the outcome of PRP is different depending on the method of preparation of PRP and the injection technique. There were 4 different types of PRP preparations and techniques studied. Highly cellular LP-PRP shows strong positive outcomes in treating tendinopathy when assessed in the network meta-analysis.

For LP-PRP, the type of PRP and the usually single-injection technique using small volumes of superficial local anesthetic with a 5- to 10-gauge needle technique, generally under ultrasound guidance, are consistent across the studies. Tendons included in this analysis included 5
studies on tennis elbow, 2 studies on the rotator cuff, 2
studies on the patellar tendon, and 1 on the Achilles
tendon. Only 1 trial was included using LP-PRP; hence, the
data are too limited to draw conclusions at this stage.

There is some evidence that the use of local anesthetic
reduces the effectiveness of PRP in vitro.\textsuperscript{7} This meta-
analysis demonstrates that LP-PRP is effective, but it is
important to note that all groups used local anesthetic
injected prior to and superficial to the tendon.

We have not presented the data in contrast to placebo/controls in part as many studies have active controls, for
example, Crea and et al.,\textsuperscript{2} who compared ARIs with PRP, and
because our secondary goal was to determine whether the
choice of control made a difference to the outcomes. Several
reviewers have suggested that platelet-rich plasma injections
should not be used as a control as they confer a negative outcome
and therefore make the difference in the active (PRP) treatment
look greater. We would contest that all injections are clinically
active treatments whether this is dry needling, saline, or local
anesthetic administration.\textsuperscript{44,54} Thus, the data have been
presented as changes in pain scores from baseline for all
modalities, be they controls or active treatments.

We also wished to identify whether the type of control
could affect the results of trials, particularly the use of corti-
costeroid. It has been argued by de Vos et al.\textsuperscript{15} that cortico-
steroid has a negative effect on tendinopathy, and thus
when used as a control, it will make the mean difference
greater than it would if it were compared with other types
of injectable controls. Corticosteroid injections show an
improvement up to 3 months and then a decline in effective-
ness, as shown in the most recent Cochrane review by Deyn
et al.\textsuperscript{16} Our network meta-analysis found that cortico-
steroid, dry needling, and saline injections did not have a posi-
tive outcome in the treatment of tendinopathy; saline (SMD, 1.46; 95% CI, 10.74-18.50), local anesthetic (SMD, 1.05; 95%
CI, 7.66-22.34), corticosteroid (SMD, 2.12; 95% CI, 10.74-18.50), and dry needling (SMD, 25.22; 95% CI, 21.27-29.16). In fact, corticosteroid and dry needling both have a greater change from baseline than saline or local
anesthetic and would thus show a less positive outcome
when compared with active treatment groups, the opposite
effect to that postulated by de Vos et al.\textsuperscript{15} It is therefore
considered that any corticosteroid, dry needling, or saline injec-
tions are good controls for clinical trials assessing tendinopathy, and consequently, trials using corticosteroid,
saline, local anesthetic, or dry needling as a control would
be valid when used in a meta-analysis. Taking into account
the recommendations of the World Medical Association,\textsuperscript{68}
Declaration of Helsinki Ethical Principles for Medical
Research Involving Human Subjects, which states, "the
benefit, risks, burdens, and effectiveness of a new interven-
tion must be tested against the best current proven inter-
vension, except in the following circumstances: The use of
a placebo, or no treatment, is acceptable in studies where
no current proven treatment exists," our network meta-
analyses would support the inclusion of data where cortico-
steroid, local anesthetic, saline, or dry needling are used as
a control in the treatment of tendinopathy.

The strength of this meta-analysis is that we have shown
a difference in outcomes in treating tendinopathy directly
related to the type of PRP produced. All previous meta-
analyses have grouped PRP types together. The weakness
of this meta-analysis is that it has not been possible to sep-
arate the results into groups by tendon, as there are insuf-
ficient trials in each area at present. However, as the
number of trials increases, it will be possible to determine
whether there are differences across tendon locations with
different PRP preparations. Nevertheless, the causes of tend-
inopathy are similar, and conclusions can be drawn for tendinopathy as a group.\textsuperscript{64,65}

CONCLUSION

This network meta-analysis has identified that the type of
PRP and the techniques used affect the outcomes and
should always be included in any meta-analysis in the future, as predicted by Moreau et al.\textsuperscript{75} and recommended by Gosens and Mathias.\textsuperscript{8} Our systematic review and net-
work meta-analysis found strong evidence that LP-PRP
improves outcomes in tendinopathy and confirms the
results published by Fukih et al.\textsuperscript{17} The technique for the
injection of LP-PRP includes the use of 1 to 2 mL of local
anesthetic injected prior to LP-PRP superficial to the ten-
don. A single LP-PRP is injected using a peppering tech-
nique intratendinously into the affected area, generally
under ultrasound guidance.

ACKNOWLEDGMENT

The authors acknowledge Susie Morton of the Epworth
Healthcare Library, Richmond, Australia.

An online CME course associated with this article is available
for 1 AMA PRA Category 1 Credit™ at http://www
.amed重要因素/MemberEducation/AJSM%20Current%20Concepts%20Store.aspx. In accordance with
the standards of the Accreditation Council for Continuing
Medical Education (ACCME), it is the policy of The Amer-
ican Orthopaedic Society for Sports Medicine that
authors, editors, and planners disclose to the learners all
financial relationships during the past 12 months with
any commercial interest (A 'commercial interest' is any
entity producing, marketing, re-selling, or distributing
health care goods or services consumed by, or used on,
patients). Any and all disclosures are provided in the
online journal CME area which is provided to all partici-
pants before they actually take the CME activity. In accor-
dance with ACCME policy, authors, editors, and planners
participation in this educational activity will be predicated
upon timely submission and review of ACCME disclosure.
Noncompliance will result in an author, editor or planner
to be stricken from participating in this CME activity.

REFERENCES

Analysis of Platelet-Rich Plasma Extraction

Variations in Platelet-Rich Plasma Components Between 4 Common Commercial Kits

Jane Fitzpatrick,*† FACSP, MBBS, Max K. Bulsara,* PhD, MSc, BSc(Hons), Paul Robert McCrorry,*† PhD, FFSEM, FACSP, FRACP, MBBS, Martin D. Richardson,*‡ FRACS, MBBS, MS, and Ming Hao Zheng,*‡ PhD, DM, FRCPA

Investigation performed at the University of Western Australia, Crawley, Western Australia, Australia

Background: Platelet-rich plasma (PRP) has been extensively used as a treatment in tissue healing in tendinopathy, muscle injury, and osteoarthritis. However, there is variation in methods of extraction, and this produces different types of PRP.

Purposes: To determine the composition of PRP obtained from 4 commercial separation kits, which would allow assessment of current classification systems used in cross-study comparisons.

Study Design: Controlled laboratory study.

Methods: Four normal adults each donated 181 mL of whole blood, some of which was served as a control and the remainder of which was processed through 4 PRP separation kits: GPS III (BioMetic Biologics), Smart-Prep2 (Harvest Tenure), Magellan (Anteriority Medical Systems), and ACP (Devios Technologies). The resultant PRP was tested for platelet count, red blood cell count, and white blood cell count, including differential, in a commercial pathology laboratory. Glucose and pH measurements were obtained from a blood gas analyzer machine.

Results: Three kits taking samples from the "buffy coat layer" were found to have greater concentrations of platelets (3-4 times baseline), while 1 kit taking samples from plasma was found to have platelet concentrations of only 1.5 times baseline. The same 3 kits produced an increased concentration of white blood cells (3-4 times baseline); the one separated the neutrophils, leukocytes, and monocytes. This represents high concentrations of platelets and white blood cells. A small dip in pH was thought to relate to the citrate used in the sample preparation. Interestingly, an unexpected increase in glucose concentrations, with 3 to 6 times greater than baseline levels, was found in all samples.

Conclusion: This study reveals the variation of blood components, including platelets, red blood cells, leukocytes, pH, and glucose in PRP extractions. The high concentrations of cells are important, as the white blood cell count in PRP samples has frequently been ignored, being considered insignificant. The lack of standardization of PRP preparation for clinical use has contributed at least in part to the varying clinical efficacy in PRP use.

Clinical Relevance: The variation of platelet and other blood component concentrations between commercial PRP kits may affect clinical treatment outcomes. There is a need for standardization of PRP for clinical use.

Keywords: platelet-rich plasma; PRP; leukocyte; osteoarthritis; tendinopathy

Platelet-rich plasma (PRP) is defined as a platelet-rich concentrate with higher-than-baseline levels of platelets when compared with whole blood. PRP is increasingly used in prospective clinical studies to improve tissue healing, particularly with regard to tendinopathy. A small number of randomized controlled trials have shown the positive effects of PRP in tendinopathy. It has been hypothesized that this is due to platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), and hepatocyte growth factor (HGF), which are released from the alpha granules during in vivo activation of platelet β3(αIIb) and subsequently produced by the cellular matrix of the tendon.

DeLong et al. considered that PRP preparations can be divided into 2 forms: 1 plasma based, the other based on the buffy coat preparations. Plasma-based preparations aim to capture platelets from the plasma after centrifugation and

The Orthopaedic Journal of Sports Medicine, 9(9), 23255671 16675572 DOI: 10.1177/2325567116675572 © The Authors 2017

This open-access article is published and distributed under the Creative Commons Attribution - NonCommercial - No Derivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits the noncommercial use, distribution, and reproduction of the article in any medium, provided the original author(s) and source are credited. You may not alter, transform, or build upon this article without the permission of the Author(s). For reprint and permission queries, please visit SAGE's Web site at http://www.sagepub.com/journalsPermissions.nav.
exclude red and white blood cells. Generally, these kits produce smaller increases in platelets than the kits that take platelets from both the plasma and the more cellular "buffy coat."18,19

There has been some discussion about whether the efficacy of PRP is affected by the inclusion of the white blood cells.20,21 Moneen et al.21 considered that there may be positive effects from the white blood cells acting as antimicrobial agents. Other authors have suggested that the platelets themselves may already have this property.20 There may also be negative effects from these white blood cells in causing further inflammation, leading to fibrosis, or from the release of cytokines.22,23 This effect may be more prevalent with neutrophils than other white blood cells.24 Recent meta-analyses of PRP in tendinopathy identified that leukocyte-rich PRP had a stronger positive outcome in the treatment of tendinopathies.16,18

There has also been discussion about whether the pH of the resultant PRP will affect platelet function,25 and thus whether the PRP produced should be "buffered." Because it is likely to be important in the management of different conditions to have certain types of PRP used,18 all commercial kits should be validated for cell and PRP type, but this has not always been the case. The purpose of this study was to validate all kits available in Australia for their composition of platelets, red and white blood cell counts, pH, and glucose levels using a single-donor model. A recommendation could then be made as to which PRP kit types are associated with the best results in the treatment of different musculoskeletal conditions such as tendinopathy and osteoarthritis.

METHODS

Three healthy adult human subjects were recruited and consented for this trial (2 women, 1 man; age range 28-35 years).

Description of Common Commercial Kits

A review of all kits was undertaken, as shown in Table 1 based on the International Olympic Committee (IOC) consensus paper on the use of PRP in sports medicine.18 It was decided that only kits producing PRP, autologous conditioned plasma, or pure platelets would be assessed. Only kits producing PRP from whole blood for use in musculoskeletal conditions such as tendinopathy, muscle injuries, or osteoarthritis were selected. Kites were excluded if they produced platelet-rich fibrin or bone marrow samples. Thus, 8 potential kits were available for testing.

Cell-saver-based pure platelet systems requiring a minimum sample of 200 mL of whole blood for processing25 were not deemed appropriate to study, as this large sample was regarded as impractical for office use. The Captive pure platelet kit was not commercially available at the time of testing, and therefore, 6 potential kits were available for

1 Address correspondence to Jane Fitzpatrick, FACS, MBBS, School of Surgery, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia (email: jane.fitzpatrick@research.uwa.edu.au).
2 School of Surgery, The University of Western Australia, Crawley, Western Australia, Australia.

3 QEII Medical Centre, Nedlands, Western Australia, Australia.
4 Chair in Biostatistics, Institute for Health Research, University of Notre Dame, Fremantle, Western Australia, Australia.
5 Monash University, Melbourne, Melbourne Brain Centre, Heidelberg, Victoria, Australia.
6 Department of Surgery, Orthopaedics and Trauma, University of Melbourne, Melbourne, Victoria, Australia.

Table 1

<table>
<thead>
<tr>
<th>Device Name</th>
<th>Company</th>
<th>Name of Product</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPR III</td>
<td>Bionet</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>SmartPrep2</td>
<td>Harvest</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>Magellan</td>
<td>ArterioMedica</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>Angel</td>
<td>Sinion</td>
<td>Platelet-rich plasma</td>
<td>Not available for testing</td>
</tr>
<tr>
<td>CS/ACP</td>
<td>Genosys</td>
<td>Platelet-rich plasma</td>
<td>Not available for testing</td>
</tr>
<tr>
<td>PRF and Vetrock</td>
<td>Arthrex</td>
<td>Autologous conditioned plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>PRM Fibrin/PRF System</td>
<td>CaseMed</td>
<td>Platelet-PRF fibrin</td>
<td>Not tested, fibrin membrane</td>
</tr>
<tr>
<td>BiMAC</td>
<td>Denmark</td>
<td>Platelet-PRF fibrin</td>
<td>Not tested, fibrin membrane</td>
</tr>
<tr>
<td>Cell saver-based systems</td>
<td>Kappa, Lambda, Gamma</td>
<td>Platelet-rich plasma and stem cells</td>
<td>Not tested, volume required &gt;200 mL</td>
</tr>
<tr>
<td>Captive</td>
<td>Not yet marketed</td>
<td>Pure platelets</td>
<td>Not tested, not available</td>
</tr>
<tr>
<td>Total</td>
<td>12 companies</td>
<td></td>
<td>4 tested</td>
</tr>
</tbody>
</table>

*Table derived from Engelschoten et al.*

---

25 Three healthy adult human subjects were recruited and consented for this trial (2 women, 1 man; age range 28-35 years).

26 Description of Common Commercial Kits

A review of all kits was undertaken, as shown in Table 1 based on the International Olympic Committee (IOC) consensus paper on the use of PRP in sports medicine.18 It was decided that only kits producing PRP, autologous conditioned plasma, or pure platelets would be assessed. Only kits producing PRP from whole blood for use in musculoskeletal conditions such as tendinopathy, muscle injuries, or osteoarthritis were selected. Kites were excluded if they produced platelet-rich fibrin or bone marrow samples. Thus, 8 potential kits were available for testing.

Cell-saver-based pure platelet systems requiring a minimum sample of 200 mL of whole blood for processing25 were not deemed appropriate to study, as this large sample was regarded as impractical for office use. The Captive pure platelet kit was not commercially available at the time of testing, and therefore, 6 potential kits were available for testing. The table is a summary of the kits available for testing. A recommendation could then be made as to which PRP kit types are associated with the best results in the treatment of different musculoskeletal conditions such as tendinopathy and osteoarthritis.

METHODS

Three healthy adult human subjects were recruited and consented for this trial (2 women, 1 man; age range 28-35 years).

Table 1

<table>
<thead>
<tr>
<th>Device Name</th>
<th>Company</th>
<th>Name of Product</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPR III</td>
<td>Bionet</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>SmartPrep2</td>
<td>Harvest</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>Magellan</td>
<td>ArterioMedica</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>Angel</td>
<td>Sinion</td>
<td>Platelet-rich plasma</td>
<td>Not available for testing</td>
</tr>
<tr>
<td>CS/ACP</td>
<td>Genosys</td>
<td>Platelet-rich plasma</td>
<td>Not available for testing</td>
</tr>
<tr>
<td>PRF and Vetrock</td>
<td>Arthrex</td>
<td>Autologous conditioned plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>PRM Fibrin/PRF System</td>
<td>CaseMed</td>
<td>Platelet-PRF fibrin</td>
<td>Not tested, fibrin membrane</td>
</tr>
<tr>
<td>BiMAC</td>
<td>Denmark</td>
<td>Platelet-PRF fibrin</td>
<td>Not tested, fibrin membrane</td>
</tr>
<tr>
<td>Cell saver-based systems</td>
<td>Kappa, Lambda, Gamma</td>
<td>Platelet-rich plasma and stem cells</td>
<td>Not tested, volume required &gt;200 mL</td>
</tr>
<tr>
<td>Captive</td>
<td>Not yet marketed</td>
<td>Pure platelets</td>
<td>Not tested, not available</td>
</tr>
<tr>
<td>Total</td>
<td>12 companies</td>
<td></td>
<td>4 tested</td>
</tr>
</tbody>
</table>

*Table derived from Engelschoten et al.*

---

25 Three healthy adult human subjects were recruited and consented for this trial (2 women, 1 man; age range 28-35 years).

26 Description of Common Commercial Kits

A review of all kits was undertaken, as shown in Table 1 based on the International Olympic Committee (IOC) consensus paper on the use of PRP in sports medicine.18 It was decided that only kits producing PRP, autologous conditioned plasma, or pure platelets would be assessed. Only kits producing PRP from whole blood for use in musculoskeletal conditions such as tendinopathy, muscle injuries, or osteoarthritis were selected. Kites were excluded if they produced platelet-rich fibrin or bone marrow samples. Thus, 8 potential kits were available for testing.

Cell-saver-based pure platelet systems requiring a minimum sample of 200 mL of whole blood for processing25 were not deemed appropriate to study, as this large sample was regarded as impractical for office use. The Captive pure platelet kit was not commercially available at the time of testing, and therefore, 6 potential kits were available for testing. The table is a summary of the kits available for testing. A recommendation could then be made as to which PRP kit types are associated with the best results in the treatment of different musculoskeletal conditions such as tendinopathy and osteoarthritis.

METHODS

Three healthy adult human subjects were recruited and consented for this trial (2 women, 1 man; age range 28-35 years).

Table 1

<table>
<thead>
<tr>
<th>Device Name</th>
<th>Company</th>
<th>Name of Product</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPR III</td>
<td>Bionet</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>SmartPrep2</td>
<td>Harvest</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>Magellan</td>
<td>ArterioMedica</td>
<td>Platelet-rich plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>Angel</td>
<td>Sinion</td>
<td>Platelet-rich plasma</td>
<td>Not available for testing</td>
</tr>
<tr>
<td>CS/ACP</td>
<td>Genosys</td>
<td>Platelet-rich plasma</td>
<td>Not available for testing</td>
</tr>
<tr>
<td>PRF and Vetrock</td>
<td>Arthrex</td>
<td>Autologous conditioned plasma</td>
<td>Tested</td>
</tr>
<tr>
<td>PRM Fibrin/PRF System</td>
<td>CaseMed</td>
<td>Platelet-PRF fibrin</td>
<td>Not tested, fibrin membrane</td>
</tr>
<tr>
<td>BiMAC</td>
<td>Denmark</td>
<td>Platelet-PRF fibrin</td>
<td>Not tested, fibrin membrane</td>
</tr>
<tr>
<td>Cell saver-based systems</td>
<td>Kappa, Lambda, Gamma</td>
<td>Platelet-rich plasma and stem cells</td>
<td>Not tested, volume required &gt;200 mL</td>
</tr>
<tr>
<td>Captive</td>
<td>Not yet marketed</td>
<td>Pure platelets</td>
<td>Not tested, not available</td>
</tr>
<tr>
<td>Total</td>
<td>12 companies</td>
<td></td>
<td>4 tested</td>
</tr>
</tbody>
</table>

*Table derived from Engelschoten et al.*

---

25 Three healthy adult human subjects were recruited and consented for this trial (2 women, 1 man; age range 28-35 years).

26 Description of Common Commercial Kits

A review of all kits was undertaken, as shown in Table 1 based on the International Olympic Committee (IOC) consensus paper on the use of PRP in sports medicine.18 It was decided that only kits producing PRP, autologous conditioned plasma, or pure platelets would be assessed. Only kits producing PRP from whole blood for use in musculoskeletal conditions such as tendinopathy, muscle injuries, or osteoarthritis were selected. Kites were excluded if they produced platelet-rich fibrin or bone marrow samples. Thus, 8 potential kits were available for testing.

Cell-saver-based pure platelet systems requiring a minimum sample of 200 mL of whole blood for processing25 were not deemed appropriate to study, as this large sample was regarded as impractical for office use. The Captive pure platelet kit was not commercially available at the time of testing, and therefore, 6 potential kits were available for testing. The table is a summary of the kits available for testing. A recommendation could then be made as to which PRP kit types are associated with the best results in the treatment of different musculoskeletal conditions such as tendinopathy and osteoarthritis.
TABLE 2
Preparation of PRP Samples

<table>
<thead>
<tr>
<th>System</th>
<th>Blood Volume, mL</th>
<th>Anticoagulant Volume, mL</th>
<th>Centrifugal Force, g-force</th>
<th>Centrifuge Time, min</th>
<th>Volume Produced, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPS III</td>
<td>52</td>
<td>ACD-A 8</td>
<td>1100</td>
<td>15</td>
<td>6.7</td>
</tr>
<tr>
<td>SmartPrep2</td>
<td>52</td>
<td>ACD-A 8</td>
<td>1250/1650</td>
<td>14</td>
<td>6.7</td>
</tr>
<tr>
<td>Magellan</td>
<td>52</td>
<td>ACD-A 8</td>
<td>1300</td>
<td>17</td>
<td>6.7</td>
</tr>
<tr>
<td>ACP</td>
<td>15</td>
<td>ACD-A 8</td>
<td>1000</td>
<td>5</td>
<td>6.7</td>
</tr>
</tbody>
</table>

*ACD-A, anticoagulant citrate-dextrose solution; PRP, platelet-rich plasma.

TABLE 3
Cellular Data

<table>
<thead>
<tr>
<th>Kit</th>
<th>Cell Type</th>
<th>Mean, × 10^6/L</th>
<th>SD, × 10^6/L</th>
<th>Median, × 10^6/L</th>
<th>Min, × 10^6/L</th>
<th>Max, × 10^6/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Platelets</td>
<td>299</td>
<td>106</td>
<td>290</td>
<td>154</td>
<td>362</td>
</tr>
<tr>
<td></td>
<td>WBC</td>
<td>8.73</td>
<td>3.75</td>
<td>8.9</td>
<td>4.9</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>RBC</td>
<td>4.7</td>
<td>0.416</td>
<td>4.5</td>
<td>4.4</td>
<td>5.2</td>
</tr>
<tr>
<td>ACP</td>
<td>Platelets</td>
<td>412</td>
<td>140</td>
<td>434</td>
<td>266</td>
<td>566</td>
</tr>
<tr>
<td></td>
<td>WBC</td>
<td>1.3</td>
<td>0.764</td>
<td>7.7</td>
<td>0.4</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>RBC</td>
<td>0.0033</td>
<td>0.0577</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>GPS</td>
<td>Platelets</td>
<td>964</td>
<td>551</td>
<td>769</td>
<td>544</td>
<td>1888</td>
</tr>
<tr>
<td></td>
<td>WBC</td>
<td>35.8</td>
<td>10.8</td>
<td>41.8</td>
<td>23.3</td>
<td>42.3</td>
</tr>
<tr>
<td></td>
<td>RBC</td>
<td>1.03</td>
<td>0.289</td>
<td>1.2</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>SmartPrep</td>
<td>Platelets</td>
<td>1224</td>
<td>550</td>
<td>1282</td>
<td>646</td>
<td>1764</td>
</tr>
<tr>
<td></td>
<td>WBC</td>
<td>28.7</td>
<td>8.69</td>
<td>28.1</td>
<td>15.4</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>RBC</td>
<td>1.43</td>
<td>0.306</td>
<td>1.5</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Magellan</td>
<td>Platelets</td>
<td>1266</td>
<td>831</td>
<td>1158</td>
<td>497</td>
<td>2148</td>
</tr>
<tr>
<td></td>
<td>WBC</td>
<td>31.4</td>
<td>9.4</td>
<td>35.2</td>
<td>20.7</td>
<td>35.3</td>
</tr>
<tr>
<td></td>
<td>RBC</td>
<td>1.9</td>
<td>0.153</td>
<td>1.9</td>
<td>0.9</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*RBC, red blood cell count; WBC, total white blood cell count.

study. Of these, only 4 were commercially available in Australia at the time of testing: GPS III (Bomet Biologics), SmartPrep2 (Terumo Harvest), Magellan (Artery md Medical Systems), and ACP (Device Technologies, Artherx). All companies agreed for their kits to be used in the trial and provided the kits.

Sample Collection and Processing

All samples were collected from the subjects by the senior author (J.P.) and were processed immediately. A total of 181 mL of blood was drawn from each subject: 5 mL was used for the control sample, 52 mL for each of the PRP-based kits (GPS III, SmartPrep2, and Magellan), and 35 mL for the ACP kit. The samples were processed according to the manufacturers' instructions to produce 6 to 7 mL of finished product, as shown in Table 3.

The samples were then processed: 1.5 mL from the PRP samples and the control blood were put into a tube for analysis on a blood gas testing machine (AE1800 Flex; Radiometer), generating results for pH, K+, Na⁺, Cl⁻, glucose, and lactate. The remaining control blood and PRP samples were placed into a collection tube for analysis on a Coulter LHI 350 automated analyzer (Beckman Coulter Inc) within 30 minutes of collection to measure full blood count and white blood cell count with differential.

Classification of the PRP Produced

The results from the analysis were assessed based on the PAW (platelet activation) and yellow blood cell (YBC) and the Mishra sports medicine PRP classification systems. The PAW system classifies PRP based on platelet numbers, the manner in which activation occurs, and the presence or absence of white blood cells. The Mishra sports medicine PRP classification system is based on platelet concentration, the presence or absence of white blood cells, and whether the PRP has been activated with exogenous thrombin or calciium chloride.

Statistical Analysis

All statistical analyses were performed using STATA version 13 (StataCorp). All variables had a calculated mean and standard deviation. Each subject was used as their own control, and thus, change from mean was relative to their own control result.

RESULTS

Comparison of Cellular Components

We first compared the cellular components of platelets, leukocytes, and red blood cells between these 4 kits using...
standard methods on 3 human subjects. A summary of data is presented in Table 3. The values for total platelet count as well as red and white blood cell counts are presented compared with controls.

**Platelets.** An increase in platelet production was demonstrated compared with baseline in all kits (Figure 1). The ACP kit produced a 1 to 1.7 times baseline level of platelets (412 x 10^9/L), which is consistent with the literature for this kit and open-tube single- or double-spin systems.41,42 The Magellan (1200 x 10^9/L), GFS (961 x 10^9/L), and SmartPrep (1234 x 10^9/L) kits produce 3 and 5 times baseline platelet concentrations, consistent with previous data.2,8,9,11,31,34

**Red Blood Cells.** All kits significantly reduced red blood cell counts compared with controls, as seen in Figure 2. The ACP kit virtually eliminated red blood cells. The GFS, SmartPrep, and Magellan kits reduced the red blood cells by 3 to 6 times baseline levels.

**White Blood Cells.** White blood cell counts are of great importance. Compared with controls (white blood cell count, 8.73 x 10^9/L), the only kit to reduce the white blood cell count was the plasma system (ACP) (1.3 x 10^9/L), which reduced the white blood cell count by 5 to 22 times, almost eliminating the white blood cells. The GFS III (8.8 x 10^9/L), SmartPrep (9.4 x 10^9/L), and Magellan (31.4 x 10^9/L) kits actively concentrated white blood cells 3 to 5 times baseline levels (Figure 3). This is consistent with the results found by Carmone et al.7 Similar increases across all 8 kits were demonstrated. Our results showed much higher levels of white blood cell concentration than have been indicated by others.14

When the white blood cell count is broken into a differential white blood cell count, the majority of cells are neutrophils and lymphocytes (Table 4 and Figure 4). Compared with controls (5.5 x 10^9/L), the GFS and Magellan kits contained greater mean neutrophil counts (15.4 and 15.1 x 10^9/L, respectively). The SmartPrep kit had a lower mean neutrophil count (6.47 x 10^9/L), and the ACP kit had a negligible mean neutrophil count (0.4 x 10^9/L). Compared with controls (2.37 x 10^9/L), the mean lymphocyte counts of the GFS (15.9 x 10^9/L), SmartPrep (14.0 x 10^9/L), and Magellan (12.5 x 10^9/L) were higher but similar across kits. The ACP kit had negligible lymphocytes (0.7 x 10^9/L). The increase in total white blood cell count was similar across the 3 buffy coat layer kits (GFS, SmartPrep, and Magellan). However, the relative increase in neutrophils was much greater for the GFS and the Magellan kit.

**Comparison of Chemical Composition**

PRP from the kits was assessed for glucose and pH using the Radiometer ABL800 Flex. The data for pH and glucose are shown in Table 5 and Figures 5 and 6.

Compared with the glucose control of 4.2 mmol/L, all PRP produced contained a high level of glucose ranging from 15.8 to 23.6 mmol/L. This reflects an increase in glucose of 4 to 6 times baseline.

The mean pH of the controls was 7.1. The mean pH of the PRP produced ranged from 6.69 (SmartPrep) to 7.06 (GFS). The lower pH in the kit samples is related to the use of the anticoagulant citrate dextrose solution—formula A (ACD-A) anticoagulant. The amount of ACD-A used was the same ratio for all kits by volume.
TABLE 4
White Blood Cell Differential Counts

<table>
<thead>
<tr>
<th>Kit</th>
<th>Mean ± SD, x10^9/L</th>
<th>Median (Range), x10^9/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>8.3 ± 2.75</td>
<td>8.9 (4.9-12.0)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>5.5 ± 2.92</td>
<td>5.3 (2.7-6.5)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2.9 ± 0.86</td>
<td>2.4 (1.8-3.3)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.6 ± 0.173</td>
<td>0.5 (0.5-0.8)</td>
</tr>
<tr>
<td>ACP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>1.3 ± 0.781</td>
<td>1.7 (0.4-1.8)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.4 ± 0.655</td>
<td>0.6 (0.1-0.6)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.7 ± 0.486</td>
<td>0.9 (0.2-1.2)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.167 ± 0.115</td>
<td>0.1 (0.1-0.4)</td>
</tr>
<tr>
<td>GPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>35.8 ± 10.8</td>
<td>41.8 (23.3-42.3)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>15.4 ± 5.05</td>
<td>14 (11-21)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>15.9 ± 7.73</td>
<td>14.6 (10.9-24.3)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3.8 ± 1.1</td>
<td>3.8 (2.7-4.9)</td>
</tr>
<tr>
<td>Smart Prep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>24.7 ± 8.69</td>
<td>26.1 (15.4-32.6)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>6.47 ± 1.68</td>
<td>7.4 (4.4-6)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>14 ± 6.36</td>
<td>13 (6.2-20.9)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3.57 ± 1</td>
<td>3.5 (2.6-6.6)</td>
</tr>
<tr>
<td>Magellan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>33.4 ± 9.4</td>
<td>35.2 (20.7-38.3)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>15.1 ± 3.09</td>
<td>16.6 (10.6-20)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>12.5 ± 5.72</td>
<td>12 (7.1-18.5)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3.27 ± 1.03</td>
<td>3.2 (2.4-4.4)</td>
</tr>
</tbody>
</table>

*WBC, total white blood cell count.

DISCUSSION

There are various kits available for the separation of PRP in clinical practice. As a specific "dose" of platelets may be required to achieve a clinical effect, it is important to identify the kits that produce different doses. Using a single-donor protocol, we have analyzed the blood components of 4 of the most common commercial kits available to medical practitioners in Australia. We have shown an increase in platelets from baseline in all the kits with large variations. This was found to be 1 to 1.5 for plasma-type kits (ACP) and between 3 and 6 times for buffy coat layer kits (GPS III, Magellan, SmartPrep). This confirms the work of Castillo et al. who similarly compared 3 kits (Magellan, Cascade, and GPS). In addition, there is variation in the numbers of neutrophils, leukocytes, and monocytes between the kits. The plasma system (ACP) reduced the white blood cell count by 5 to 22 times, almost eliminating the white blood cells. The buffy coat kits (GPS III, SmartPrep, and Magellan) actively concentrated white blood cells 5 to 5 times baseline. The increase in total white blood cell count was similar across the 3 buffy coat layer kits (GPS, SmartPrep, and Magellan). However, the relative increase in neutrophils was much greater for the GPS and Magellan kits.

A small reduction in pH was thought to relate to the citrate used in the sample preparation. This reduction is not thought to be of clinical significance. Based on the small drop in pH, it does not seem necessary to buffer the PRP unless this change in pH can be shown to negatively impact the production of growth factors.

No studies have reported the level of glucose in PRP produced previously. One of the surprising findings in this study was the significant increases in glucose concentration of 4 to 6 times baseline in all kits. This has not been previously reported as a significant variable, and the clinical significance of this factor is unknown. It is of interest that glucose solutions at concentrations between 15% and 20% have been used in prolotherapy injections with...
White blood cells may contribute to the modulation of inflammatory and platelet activation, thereby acting to potentiate the tissue repair mechanism. It is possible that the white blood cells may confer an advantage to the patient in reducing the chance of infection or modulating the inflammatory response.\textsuperscript{11-13} This may be an important consideration in those clinical settings where the patient is at greater risk, such as with intra-articular procedures or at the time of surgery. Furthermore, Zimmermann et al\textsuperscript{44} found that the increased white blood cell count was responsible for between one-third and one-half of the variation on growth factors found in their samples. They found a positive correlation between the white blood cell count and VEGF (known to come from the white blood cells) and PDGF.

On the other hand, others have shown that white blood cells appear to have a deleterious effect on the tissue,\textsuperscript{14} resulting in increased inflammation and further scarring. These negative effects are largely due to neutrophils and include the release of oxygen-free radicals, catabolic cytokines, matrix metalloproteinases (MMPs), and interleukin B, which degrade tissue.\textsuperscript{14}

A recent meta-analysis of the effectiveness of PRP in tendinopathy has shown that leukocyte-rich PRP (LR-PRP) is the most effective in the treatment of tendinopathy.\textsuperscript{15} This study allows us to recommend PRP produced by the GPS III, SmartPrep, and Magellan kits in the treatment of tendinopathy. By contrast, 2 recent reviews of the effectiveness of PRP in osteoarthritis have shown that leukocyte-poor PRP (LP-PRP) may be more effective.\textsuperscript{16,17}

One of the surprising new findings in this study was the significant increase in platelet concentration of 4 to 6 times baseline in all kits. This has not been previously reported as a significant variable, and the clinical significance of this factor is unknown. It is of interest that platelet solutions at concentrations of between 12% and 20% have been used in prolotherapy injections with varying results.\textsuperscript{18,19} The clinical significance of this remains uncertain. This was thought to be important as platelet-rich PRP is useful in the treatment of musculoskeletal injuries.

Kit validation studies help to classify kits into those deemed similar enough to allow results from papers using kits to be compared. Some kit classification systems have been proposed in the past.\textsuperscript{20,21} Table 6 shows the PRP kits classified according to both the PAW and Mishra classifications. The classification proposed by Mishra et al\textsuperscript{25} allows for platelet concentrations >3 or <6 times baseline. We found 3 to 6 times baseline values in the buffy coat kits and 1.5 in the plasma kits. This classification system with a cutoff of 3 times concentration does not fit for either buffy coat or plasma system results from our study. The white blood cells are recorded only as present or absent, and there is no accounting for the level of white blood cells. The PAW classification system proposed by DeLong et al\textsuperscript{23} is appropriate for the classification of platelets, classifying our tested kits into 3 different groups, but it simply classifies the white blood cells as absent or present. Given the high concentration of white blood cells found in our laboratory analysis, this classification system does not adequately account for the high numbers of white blood cells, including both neutrophils and lymphocytes found in the PRP preparations studied. If these cells are found to be significant for efficacy, a further breakdown of types of PRP to adequately include white blood cells will be needed. Kits classified in the same class would then be able to have their results compared as a group when meta-analysis or comparison studies are being undertaken.

**CONCLUSION**

This study identifies the large variations in composition and concentration of platelets, white blood cell counts, and the differential count of neutrophils and lymphocytes as well as the presence of high levels of glucose between 4 commercial PRP kits. The clinical significance of this is that these variations must be taken into account when assessing the results of clinical trials used in the choice of preparation by practitioners. This study highlights the need for standardization of platelet-rich plasma extraction for clinical use.

**ACKNOWLEDGMENT**

The authors extend thanks to Dr Ellen Maxwell, Melbourne Pathology, for advice and the pathology services. The authors acknowledge the Werow Hospital,
varying results. It is likely this is derived from the use of the ACD-A and is thus related to the preparation technique consistent in all kits. If one takes 5 ml of a 10% glucose solution (2.8 mmol/L) for injection, this would contain 0.5 g of glucose. If we take 5 ml of PRP produced in any of the studied kits at 20 mmol/L (70% glucose solution), this would give us 3 g of glucose. In simple terms, our PRP samples are producing a 6 times glucose concentration compared with glucose solutions used in prolotherapy. This may be important as part of the factors producing a clinical response.

Essentially there are 2 main types of PRP preparation methods. After centrifugation, there are 3 key layers, as shown in Figure 7. Plasma-based systems take product from the yellow relatively acellular plasma layer. These systems aim to exclude red and white blood cells from the preparation and to collect as many platelets from the remaining "plasma" layer as possible. As many of the platelets are in the buffy coat layer, the resultant product is low in red and white blood cells and has only a 1.5 to 1.7 times baseline level of platelets. This is well demonstrated by the results from the ACP kit in our study. The second type of PRP product is made from the buffy coat floating above the red blood cell layer. Levels of platelets at 3 to 6 times baseline levels are expected as the product is coming from a more platelet-dense environment. Again, this is confirmed by our testing, with the GPS III, Magellan, and SmartPrep kits having much higher platelet concentrations.

In producing PRP, all kits aim to reduce the red blood cell count and increase the collection of platelets. Some white blood cells are captured at the same time. Due to the addition of citrate to the blood being collected, there is likely to be a drop in pH of the sample produced. This is the first paper to identify how other variables like lactate or glucose are changed by this process. Generally, in most literature review papers, the white blood cell count is ignored or regarded as negligible. We feel that these aspects of PRP systems should be more highly noted in future literature reports as the concentration of white blood cells is as great as that of the platelets and there is glucose present in the end product.

Studies by others have also shown variation of platelet levels, growth factor and cytokine levels, and total white blood cell counts across PRP preparation methods. However, the most important finding in our study is that the white blood cell counts are significantly more concentrated than previously thought. The ACP kit was the only one in our series that reduced the white blood cell count by a factor of about 9. This may be an important point of difference if the white blood cells are not beneficial. The other 3 kits (GPS III, Magellan, SmartPrep) concentrated the white blood cell count by 3 to 5 times, a similar increase to platelet concentration. Thus, these white blood cells are not contaminants as their levels are as high as the primary ingredient: platelets. Their levels may be regarded as potentially clinically significant. Furthermore, we assessed the white blood cell differential count and found that the cellular concentration of white blood cells was up to 40% neutrophils and lymphocytes each and a further 10% made up of monocytes. The remainder of the cells were basophils and eosinophils in small quantities.

An increase in the growth factor VEGF would be expected as the number of lymphocytes increases. These lymphocytes may play an important role in further enhancing the tissue repair processes, but they may also lead to increased local inflammation.
REFERENCES


Appendix B Conference Presentations related to this thesis.

2013 1st Melbourne International Hip Arthroscopy Meeting Jan 12-13
Understanding Tendinopathies – A problem looking for a solution – by invitation

2013 Raine Symposium University of Western Australia July 4
Understanding tendinopathies – PRP – An Analysis

2013 ACSEP Registrar Teaching July
Hip and Groin case studies

2013 New Zealand annual Sports Medicine Scientific Meeting
Wellington November 20-21
PRP – A kit Validation Study

2014 2nd Melbourne International Hip Arthroscopy Meeting Jan 16-17
Management of gluteal tendinopathy using PRP

2015 Western Hospital Journal Club October 16
Tendinitis – New Frontiers

2015 Orthocell User Meeting Sydney August 8
Tendonitis – New Frontiers – Biologics for tendinopathy

2015 Continuing Orthopaedic Education – Australian Orthopaedic Association, Melbourne July 24
How I do it – Biologics for Tennis Elbow

2016 3rd Melbourne International Hip Arthroscopy Meeting January 21-22
Breakfast meeting: Conservative Management of Gluteal Tendinopathy
Conference Session: Conservative Management of Gluteal Tendinopathy – are all PRPs the same?

2017 SportsKongres European Sports Medicine Congress,
Copenhagen February 2-4
Finalist Open Presentations Prize Section

The effectiveness of Platelet-Rich Plasma Injections in Gluteal tendinopathy – A Double-Blind Randomised Controlled Trial comparing a single leucocyte-rich PRP injection to a single corticosteroid injection

2017 ACSEP Annual Scientific Meeting Gold Coast February 10-14
The effectiveness of Platelet-Rich Plasma Injections in Gluteal tendinopathy – A Double-Blind Randomised Controlled Trial comparing a single leucocyte-rich PRP injection to a single corticosteroid injection

2017 Epworth Research Week June 5-9
Winner : 2017 Experienced Researcher Poster Award

The effectiveness of Platelet-Rich Plasma Injections in Gluteal tendinopathy – A Double-Blind Randomised Controlled Trial comparing a single leucocyte-rich PRP injection to a single corticosteroid injection