

**Platelet rich plasma injection with arthroscopic acromioplasty for chronic rotator cuff
tendinopathy: a randomized controlled trial**

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Key words: tendinopathy, platelet rich plasma, acromioplasty, rotator cuff

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Abstract

Background Platelet Rich Plasma (PRP) has been proposed to augment tendon healing through improving tissue structure during the initial repair phase. We report the clinical outcomes and tissue effects of PRP with arthroscopic acromioplasty (AA) for the treatment of chronic rotator cuff tendinopathy.

Purpose To investigate both the clinical and tissue effects of the co-application of PRP injection with arthroscopic acromioplasty in patients with chronic rotator cuff tendinopathy.

Design Parallel two-arm superiority type randomised controlled trial.

Methods The study comprised 60 randomised patients diagnosed with rotator cuff tendinopathy (55% women) aged between 35-75 years. Patients were randomised to AA alone or in combination with an injection of autologous platelet rich plasma (PRP) into the sub-acromial bursa (AA+PRP). Efficacy of treatment was assessed by analysis of patient reported outcomes up to 2 years after treatment using the Oxford Shoulder Score (OSS) and by analysis of tendon biopsies taken 12 weeks post-treatment.

Results There was no significant difference in the OSS between AA alone and AA+PRP at any time point in the study. From 12 weeks onwards, there was a significant increase in the OSS for both groups compared to their baseline scores ($p < 0.001$). Bonar scoring determined no significant change in tissue structure with the co-application of PRP compared to surgery alone. The number of blood vessels and tendon cellularity were significantly decreased in tissue biopsies taken from PRP treated patients. The expression of p53 positive apoptotic cells increased after AA+PRP but decreased after AA alone.

Conclusions Arthroscopic acromioplasty significantly improves long-term clinical outcomes up to 2 years. The co-application of PRP did not affect clinical outcomes. PRP significantly alters the tissue characteristics in tendons post-surgery with reduced cellularity and vascularity and increased levels of apoptosis.

Clinical Relevance The co-application of PRP did not improve clinical outcomes and may have potential deleterious effects on healing tendons.

Keywords tendinopathy, platelet-rich plasma, acromioplasty, rotator cuff

What is known: PRP has been proposed to aid in early tendon healing through improving tissue structure during the initial repair phase. It contains a significantly higher concentration of platelets than is present in autologous blood.

What this study adds: Arthroscopic acromioplasty significantly improves long-term clinical outcomes up to two years. The co-application of PRP did not affect clinical outcomes. PRP significantly alters the tissue characteristics in tendons post-surgery with reduced cellularity and vascularity and increased levels of apoptosis, which could be detrimental to the long-term structural properties of the tendon.

Introduction

Shoulder pain is the third most common musculoskeletal problem to present to General Practitioners³². Rotator cuff tendinopathy frequently causes shoulder pain and loss of function⁹. Arthroscopic acromioplasty (AA) is often performed in patients with persistent symptoms that have failed to respond to conservative therapy including physiotherapy and glucocorticoid injections when the rotator cuff is either intact or impossible to repair. Although most patients improve after AA some fail to improve after surgery and many do not return to normal⁹. Some studies have questioned whether AA adds value to patients²⁵. Approximately 25,000 arthroscopic acromioplasties are performed each year in England and rates of surgery increased by over 700% between 2001 and 2010²³. The failure of these conservative and surgical strategies to resolve symptoms in some patients has resulted in the increasingly widespread use of platelet-rich plasma (PRP) injections in clinical practice with high expectations¹.

Platelet alpha granules are a rich source of growth factors that augment tissue healing, including TGF β , VEGF, PDGF, IGF and EGF^{3,34}. Utilising platelet concentrate provides the potential for an autologous and relatively inexpensive solution to facilitate tissue repair. PRP has been used for a wide variety of applications including periodontal, craniofacial and spinal surgery^{2, 18, 19, 34}. It has recently been used to treat patients with tendon pathology with conflicting evidence on clinical outcome^{1,5,8,12-14}. The mechanisms by which PRP works are not fully elucidated. In a surgical rat model of rotator cuff injury PRP treatment adversely influenced tendon mechanical properties, vascularity and healing, inducing a reactive fibroblastic response⁶. Studies investigating the use of PRP for rotator cuff tendinopathy have shown mixed results. Whilst positive outcomes have been reported for prospective and randomized controlled trials^{5, 17, 22, 35-37}, other randomized controlled trials have suggested the use of PRP did not significantly improve clinical outcome^{8, 24}. Furthermore, the composition of PRP is not standardised, varying widely between individuals and preparation methods^{34, 39} and there is a limited evidence basis to support the use of biological approaches to enhance rotator cuff healing after injury^{21,30}. We are aware that PRP is increasingly being used to treat patients with chronic tendinopathy but without a strong evidence base for its use³¹.

Furthermore, there are no studies reporting the tissue effects of PRP injection in humans. The aim of this randomized controlled trial was to investigate in patients with chronic rotator cuff tendinopathy both the clinical effect and, for the first time to our knowledge, the tissue effect of the co-application of PRP injection with arthroscopic acromioplasty. We hypothesized that PRP would improve both clinical outcome and tissue characteristics.

Methods

Study population

Inclusion criteria included the diagnosis by a shoulder specialist of sub-acromial impingement syndrome or a partial thickness rotator cuff tear in the outpatient clinic at the Nuffield Orthopaedic Centre, part of Oxford University Hospitals NHS Trust, in patients aged between 35-75 years. We performed a painful arc test and an external rotation resistance test, which were positive in all patients²⁰. High definition ultrasound examination was performed in all patients to determine if there was evidence of a rotator cuff tear. Eligible patients had failed conservative treatment, including a course of physiotherapy and / or glucocorticoid injection into the sub-acromial space and had been painful for a minimum of 6 months as previously described⁷. None had received glucocorticoid treatment for 12 weeks prior to surgery. Exclusion criteria included; presence of a full thickness rotator cuff tear; a history of trauma, previous surgery to the shoulder, osteoarthritis or other pathologies of the affected shoulder; absence of conservative treatment with physiotherapy and a minimum of one and no more than three glucocorticoid injections; unable to consent; or contraindications to PRP. The full trial protocol is published elsewhere (ISRCTN 10464365)⁷.

Study Design and Treatment

This trial was a parallel two-arm superiority type randomised controlled trial. The groups consisted of those undergoing arthroscopic acromioplasty alone (AA) and AA with the co-application of a platelet rich plasma injection (AA+PRP). Participants were randomised prior to surgery using sealed envelopes based on a computer generated simple random list. The allocation sequence was concealed from the patients and clinical staff directly involved in patient care and assessments. Each patient's treatment allocation was revealed to members

of the study team 3 days prior to surgery at the earliest to allow for organisational requirements in relation to the operating schedules and required equipment. These study team members did not undertake follow-up assessment. Participants remained unaware of their treatment allocation throughout the trial. A standard AA was performed using posterior and anterolateral arthroscopy portals with the patient under general anaesthetic and interscalene block in the beach chair position. The glenohumeral joint and bursa were inspected for evidence of other pathology and patients were withdrawn from the study if a full thickness rotator cuff tear, frozen shoulder or osteoarthritis was found because these were previously determined exclusions. The surgeon was not informed of the randomization until after the arthroscopic assessment. Autologous leucocyte rich PRP and thrombin were prepared using the Magellan® Autologous Platelet Separator System (Arteriocyte, Hopkinton, MA, USA). From 50mls of whole venous blood, approximately 5mls of PRP and 1ml of thrombin was collected. PRP was made intra operatively after an arthroscopic assessment had been performed and was only made for patients treated with PRP. The PRP injection needle was placed into the subacromial space (bursa) under arthroscopic guidance to confirm correct location. The PRP was injected into the sub-acromial space using dual syringes after the AA had been performed and arthroscopic portals had been closed to prevent escape of the PRP injection. Surplus PRP was used for platelet analysis. Assessments of the patients were carried out by blinded assessors on the day of surgery, 6 weeks and 3, 6, 12 and 24 months. At each time point patients underwent a clinical examination and were asked to complete the Oxford Shoulder Score (OSS) questionnaire, as well as information on patient satisfaction, pain and overall quality of life (EQ-5D-3L). The OSS is a validated and widely used clinical outcome measure validated for rotator cuff tendinopathy scoring from 0 (severe pathology) to 48 (normal function)¹¹.

Tissue Biopsies and Histological Assessment

Tendon biopsies for histology were taken at baseline during the surgical procedure under general anaesthetic, and at their 3-month follow-up visit under local anaesthesia. The tissue biopsy was obtained in a standardized manner using a 14G trucut biopsy needle under ultrasound guidance. The specimen was taken approximately 5-10mm posterior to the

anterior edge of the supraspinatus tendon using a well established technique³³. Samples were immediately immersed in 10% buffered formalin and left for approximately 0.5mm/hr. Tissue processing, antibody staining and image analysis for immunostaining are described in online supplementary information.

Image Analysis

Two scientists experienced in analysis of histopathology independently performed quantitative analysis of stained tissue sections. All sections stained with H&E and alcian blue were analysed using an established Bonar scoring system that evaluates tissue structure¹⁰. This semi quantitative tendon scoring system was used to grade tenocyte morphology, ground substance, collagen and vascularity. Each parameter was graded 0-3 according to their microscopic appearance, where 0 indicated a normal appearance and 3 an abnormal appearance. Immunostained images were analysed using a validated ImageJ[®] algorithm to quantify the percentage (%) of DAB (3,3'-Diaminobenzidine) immunostaining present in each image and has previously been described in more detail¹⁶.

Statistical Analysis

Sample size calculation

The sample size for the trial was determined based on the study being able to detect at least the minimally clinically important difference of three points on the OSS. The common standard deviation was assumed to be 3 points on the OSS, giving an effect size of 1. Using 80% power and a significance level of 5%, at least 17 participants were required in each of the trial groups (i.e. 34 participants overall). Ethical approval was obtained to increase the sample size to 60 to allow for sufficient numbers for the translational biomarker research.

Patient reported assessments:

Differences in the OSS between the treatment arms at 6 weeks and 3, 6 and 12 months post randomization were tested using a two-sample t-test with a 5% significance level, or a non-parametric equivalent (Mann-Whitney U test) as appropriate, in accordance with the protocol. The robustness of the results was confirmed by the use of multiple imputation considering

missing at random and missing not at random mechanisms (for the primary outcome at six months), repeating the statistical analysis using differences from baseline and adjusting for patient characteristics (baseline OSS, age and gender), and analysing the data as a multi level mixed-effects linear regression model including all follow-up data (including 24 month follow-up data from a subset of patients) into the model and allowing for treatment and time interactions. This analysis was performed in StataIC, version 12. All other patient reported outcomes were analyzed descriptively.

Biomarker assessments:

Inter-observer and intra-observer reliability were tested one month apart using the Intra-class Correlation Coefficient (ICC). Distributions of immunopositive staining were assessed for normality and for non-parametric data Mann Whitney tests were used, with statistical significance set at a level of $p < 0.05$ unless otherwise stated.

Relationship between biomarkers and patient reported outcomes:

Spearman correlation coefficients were calculated to test the relationships between the tissue markers and the OSS scores. Statistical analysis was carried using GraphPad Prism 5 (Graphpad Software).

Results

Patients

Overall, 483 patients were assessed for eligibility to take part in the trial over a period of 12 months. Of these, 60 patients fitted all the inclusion criteria and were taken through to randomisation. Thirty patients were allocated AA alone and 30 patients received AA with PRP as outlined in the consort flow diagram (Figure 1). While waiting for surgery 4 patients withdrew (2 from the AA+PRP group and 2 from the AA group). A further 8 patients were withdrawn after arthroscopic assessment due to the discovery of a diagnosis excluding them from the trial that had not been revealed during pre-operative assessment (3 from the AA+PRP group and 5 from the AA group). As the same post-randomisation exclusion criteria

were applied to both trial arms and numbers of post-randomisation exclusions are similar in both treatment groups, this was not thought to introduce bias into the analysis and is in line with recommendations by Fergusson et al¹⁵. Therefore, the analysis includes 23 patients in the AA group and 25 patients in the AA+PRP group. At the time of clinical presentation, baseline characteristics were comparable in terms of gender, age, length of symptoms, OSS or pre-operative platelet count between the patient groups (Table 1).

The Oxford Shoulder Score

There was no evidence of significant differences in the OSS between AA alone and AA+PRP at any time point in the study ($p > 0.05$). The median (IQR) of the OSS were 41 (33-46) in the AA group and 43 (38-45) in the AA+PRP group at 6 months, 44 (36-47) in the AA group and 45 (38-48) in the AA+PRP group at 12 months and 48 (46-48) in the AA group and 46 (42-48) in the AA+PRP group at 24 months. The results are robust to varying assumptions about the underlying missing data mechanism. A repeated measures model confirmed that there was insufficient evidence to suggest a difference in OSS between the treatment arms. From 12 weeks onwards, there was an increase in the OSS for both groups compared to their baseline scores. Box plots representing the median OSS with interquartile ranges over time are shown in Figure 2.

Secondary patient reported outcomes

No clinically relevant differences were observed in terms of the secondary outcomes. 95% of participants in both trial arms are reported to be pleased with their shoulder surgery, and at least 80% would choose to have the same procedure again at 12 months. Overall quality of life as measured by the EQ-5D-3L index score increased slightly from an overall median of 0.656 at baseline to 0.760 at 12 months, with similar outcomes in both groups.

Some variation can be observed in the pain visual analogue scale (VAS) over the first 12 weeks of the trial, but over the 12 months of follow-up, pain as measured on a 10 point VAS decreased from a median (IQR) score of 6.8 (4.1, 7.6) and 5.1 (2.2, 8.8) to 0.6 (0.3, 3.6) and 0.5 (0, 2.0) in the AA and AA + PRP arms respectively.

Metrics: Reliability and Validity

The ICCs relating to inter-rater measurement were between 0.722 and 0.909 for three full patient sets. The intra-observer ICC was 0.830 after two months.

Tissue parameters

Three main histological changes were observed between treatment groups including cell number, vascularity and apoptosis (Figure 3). The tissue changes brought on by AA surgery included increased cellularity, an increase in CD34 positive blood vessels, and a decrease in a marker of apoptosis, p53. Compared to AA alone, AA+PRP significantly reduced the number of cells in the tendon biopsy after 12 weeks ($p=0.03$). PRP also significantly decreased the amount of CD34 positive blood vessels post surgery ($p=0.04$). In contrast, p53 protein was significantly increased after PRP treatment ($p=0.02$) compared to AA alone. The expression of other histology markers studied in this trial was not altered (Supplementary Table 2). There was no significant difference in the Bonar score between the two groups at 12 weeks. There was also no change in the Bonar score compared to baseline. In all biopsies, tenocytes had a slightly irregular shape with a few regions containing healthy looking tenocytes with an elongated appearance, there were patches of disorganised collagen with a large amount of ground substance. There were no statistically meaningful observations when comparing tissue biomarkers to the OSS.

Discussion

PRP is widely used to treat a variety of clinical conditions, yet there is a limited evidence base to support its use. Studies of the use of PRP for rotator cuff tendinopathy have been of variable quality and shown mixed results^{24, 36, 37}. Furthermore, the mechanisms of action and biological effects of PRP are not fully elucidated. In this randomised controlled clinical trial, we sought to investigate the effects of the co-administration of PRP into the sub-acromial bursa with arthroscopic acromioplasty in patients with chronic rotator cuff tendinopathy compared to arthroscopic acromioplasty alone. The effect of treatment was determined by evaluation of clinical outcome and by analysing tendon tissue 3 months after treatment, facilitating an insight into the local biological effects of PRP. The 3-month time interval was chosen to try

and capture the maximal effect of PRP but avoid any immediate changes associated with surgery.

During the course of the study, arthroscopic acromioplasty with PRP did not significantly improve clinical outcome compared with surgery alone, with similar Oxford Shoulder Scores in both treatment groups. These findings are supported by other studies investigating the clinical outcome of PRP treatment in Achilles and rotator cuff tendinopathy^{8, 12, 13}. Tendon biopsies from AA+PRP treated patients showed anti-proliferative and anti-neovascular effects compared to patients undergoing AA alone. Our study is the first human trial to evaluate the local tissue effects of PRP treatment in tendons so comparison of these tissue findings to other human studies is not possible. Animal models of surgically induced tendinopathy have been used to investigate the effects of PRP treatment. In a surgical rat model of rotator cuff injury, PRP treatment adversely influenced tendon mechanical properties, vascularity and healing, inducing a reactive fibroblastic response⁶. Other animal *in vivo* studies report increased vascularity and tensile strength of PRP treated tendons, however these inconsistencies in the literature may be attributed to the type of surgical model used and methods of PRP preparation⁴. Furthermore, surgically induced animal models of tendinopathy may not necessarily recapitulate human tendinopathies that develop as a consequence of ageing and repetitive overload.

Increased levels of p53 have been previously reported in partial and full thickness rotator cuff tendon tears^{28, 29}. Importantly, the current study showed a further potentially harmful effect of PRP. Increased levels of p53 protein were present in PRP treated tendons compared to surgery alone, suggesting PRP treatment induces apoptosis. Collectively, these anti-proliferative, anti-neovascular, and pro-apoptotic effects of PRP treatment on rotator cuff tendons in this study suggest significant deviation in the microstructure of PRP treated tendons compared to surgery alone 3 months after treatment. Inflammation and vascularity are known to be critical factors involved in tissue remodelling after injury^{27, 40}. Furthermore, an accumulation of apoptotic cells in PRP treated tendons implies failure of restoration of tissue homeostasis after injury^{26, 38}. The findings from this study demonstrate that PRP does

not improve clinical outcome at 2 years and the anti-proliferative, anti-neovascular, and pro-apoptotic tissue changes observed in PRP treated tendons are likely to have a deleterious effect on tendon healing which may ultimately culminate in structural failure of the tissue.

Limitations of this study

Our study has several limitations. Firstly, our cohort was derived from a single tertiary orthopaedic centre and from a relatively small sample size. All patients had chronic symptoms and had previously received physiotherapy and glucocorticoid treatment, although none had received glucocorticoid treatment for 12 weeks prior to surgery. There was no difference between the groups in this regard. Secondly, this study assessed the co-application of PRP with AA and may not reflect the clinical effect of PRP used alone. Thirdly, we evaluated the effects of AA and AA+PRP without including a placebo control. However, the practicalities and ethical concerns associated with obtaining repeat tissue biopsies in sham surgery treated patients were felt to be insuperable at the time. Fourthly, concentrations of PRP are known to vary between individuals and methods of production resulting in an inconsistent therapeutic effect^{34, 39}. A single dose and lack of knowledge about an optimal PRP dosing regime may be a limiting factor in this study. Fifthly the time interval of 12 weeks after surgery was chosen for the tendon biopsy to best capture the effect of the PRP injection; it is not known from this study how persistent the tissue changes might be. There were insufficient numbers of patients in the study to evaluate clinical outcome 24 months after treatment, however the study was adequately powered for evaluation of the primary endpoint, which was clinical outcome at 12 months post-treatment. Although there is a potential selection bias due to the need for an ultrasound-guided biopsy, no patients refused to participate at screening due to this component of the study.

Conclusions

Our findings show that AA with and without PRP injection significantly improves clinical outcomes up to 2 years. However, there was no evidence that the co-application of PRP has any additional effect on clinical outcomes. Importantly the tissue effects of PRP included reduced cellularity and vascularity and an increase in a marker of apoptosis. These tissue

findings have not previously been reported and reveal a potentially detrimental effect of PRP to the long-term structural properties of the tendon, which may increase the likelihood of tendon tears. More trials are needed to determine the longer-term consequences of PRP injection.

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Figure legends

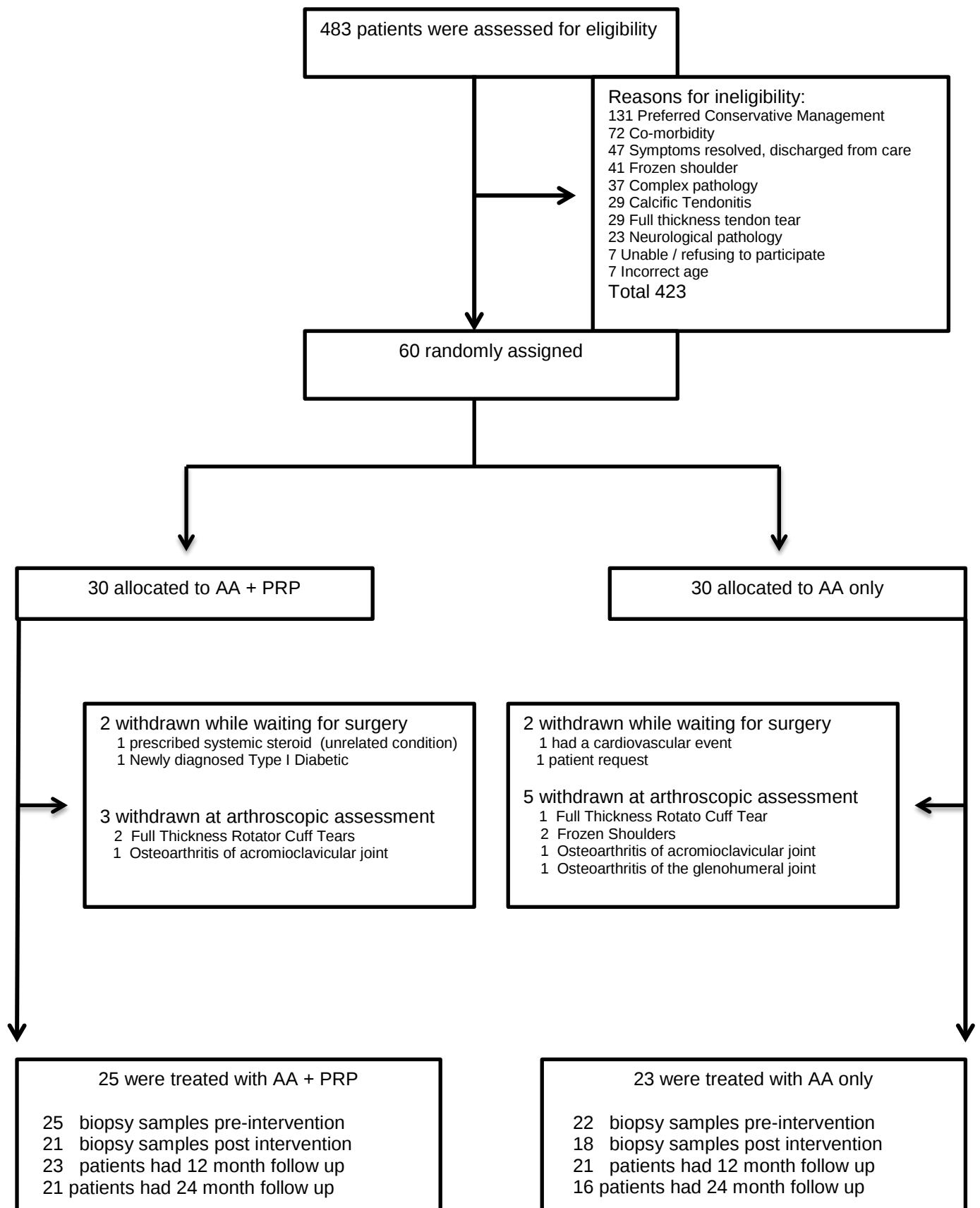
Figure 1. Consort flow diagram illustrating the research pathway of patients with rotator cuff tendinopathy in the study. AA denotes arthroscopic acromioplasty, PRP denotes platelet rich plasma.

Figure 2. Clinical outcomes of patients in the study over 24 months. The Oxford Shoulder Score (OSS) was used as a clinical outcome measure scoring from 0 (severe pathology) to 48 (normal function) during the 24 month post-operative period. There was no significant difference in the OSS between arthroscopic acromioplasty (AA) alone and arthroscopic acromioplasty plus platelet rich plasma (AA + PRP) at any time point in the study. From 12 weeks onwards, there was a significant increase in the OSS for both groups compared to their baseline scores at clinical presentation ($p < 0.001$). Box plots represent the median OSS with interquartile ranges over time.

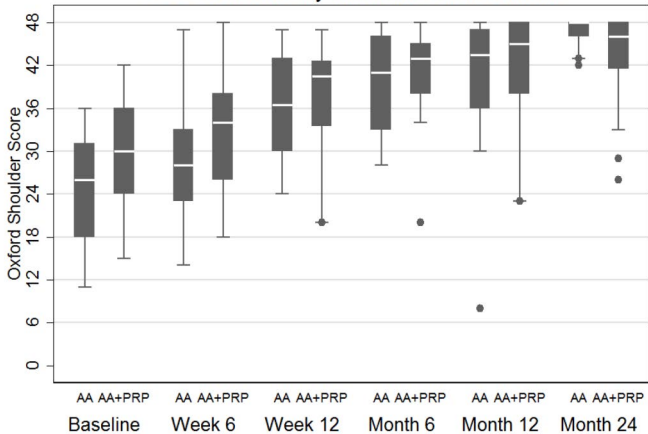
Figure 3. Tissue parameters of rotator cuff tendon biopsies 12 weeks after treatment. (A) Panel shows representative images of haematoxylin staining (blue) for quantification of cell number in stained sections. There was a significant decrease in the number of haematoxylin positive cells in tendon biopsies taken from the arthroscopic acromioplasty and platelet rich plasma (AA + PRP) treated patients compared to patients treated with arthroscopy alone (AA) ($p = 0.03$). Representative images of DAB immunostaining (brown) for CD34 and p53, blue represents haematoxylin nuclear counterstain. (B) The number of blood vessels determined by CD34 immunostaining significantly decreased in the AA + PRP group compared to AA alone ($p = 0.04$). (C) Expression of p53 positive apoptotic cells increased in the AA + PRP group but decreased in the AA group ($p = 0.02$). Data show mean with SEM. $*p < 0.05$. Scale bar = 10 μm .

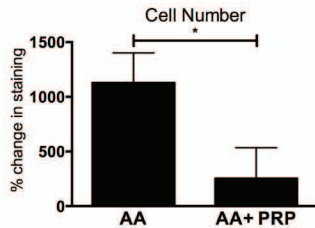
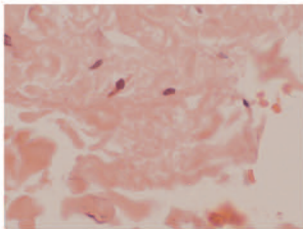
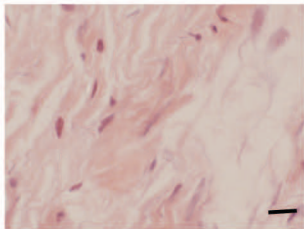
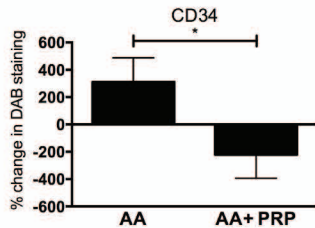
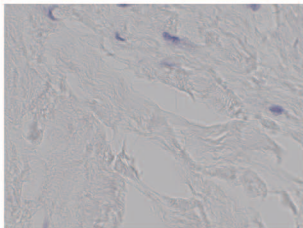
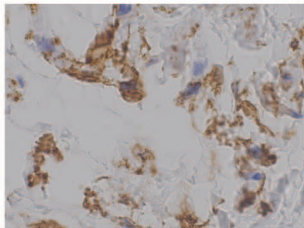
Table 1. Patient demographics for the study group. There were no significant differences between the two treatment groups. OSS = Oxford Shoulder Score.

Patient Parameter	Arthroscopic Acromioplasty alone n=23	Arthroscopic Acromioplasty + Platelet Rich Plasma Injection n=25
Gender	13 female; 10 male	13 female; 12 male
Age (years) (mean) (95% of CI)	54.87 (50.36-59.38)	52.2 (48.26-56.14)
Length of Symptoms (median with interquartile range)	24 (18-32)	19 (12-36)
Baseline OSS (median with interquartile range)	26.0 (18.0-31.0)	30.0 (24.0-36.0)
Pre-op Platelet Count (mean) (95% of CI)	258.8 (233.6-284.1)	243.8 (220.8-266.9)



OSS over time by randomised treatment



AA**AA+PRP****A****B****C**