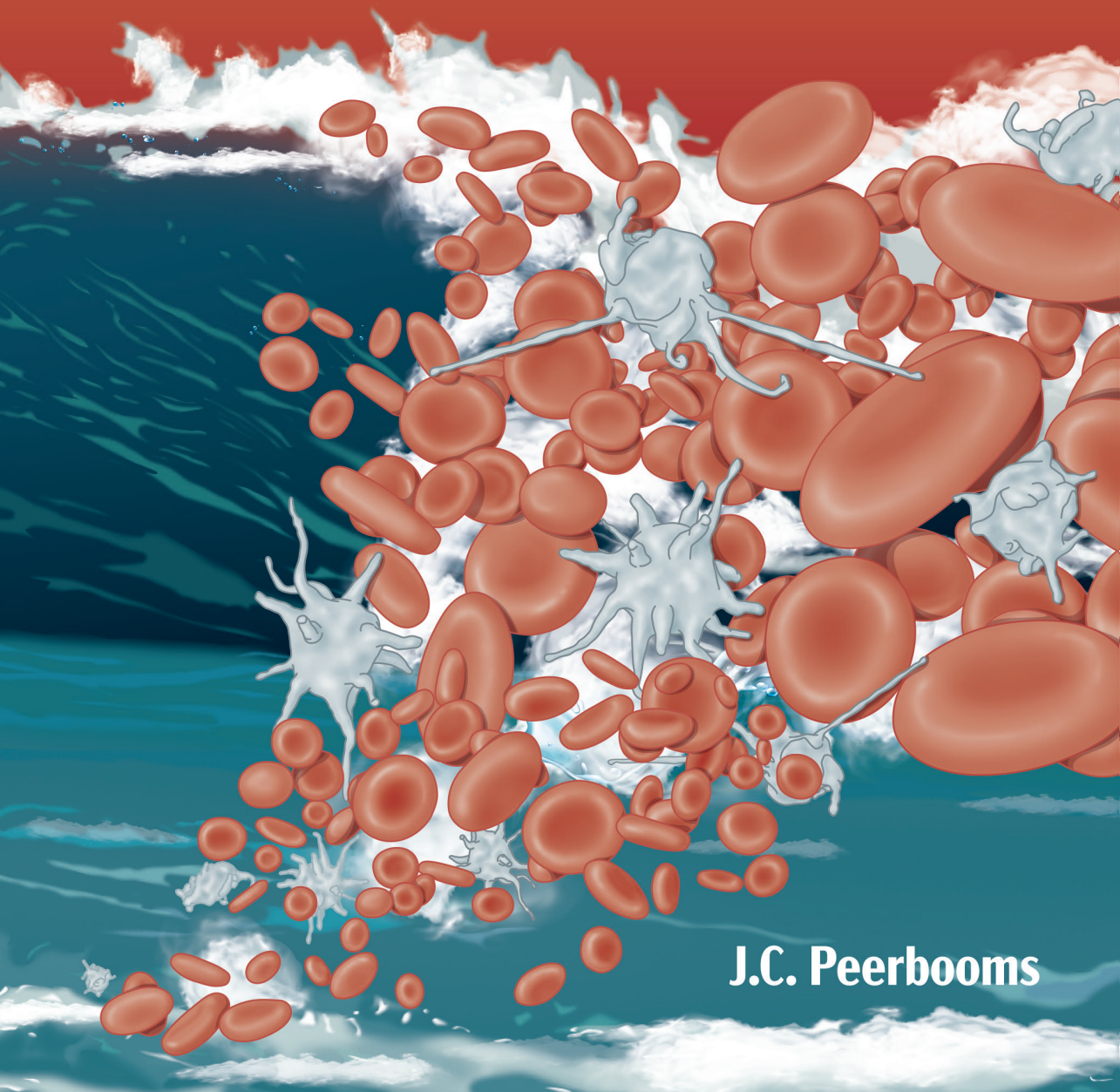
Platelet - Rich Plasma in Musculoskeletal Disorders



J.C. Peerbooms

Platelet-Rich Plasma in Musculoskeletal Disorders

Trombocytenrijk plasma bij musculoskeletale aandoeningen

Joost Christiaan Peerbooms

Colophon

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I know you would be proud.

Wishing you could join me here today……

For the memory of my father, Frans.

CHAPTER 1

General Introduction

PREFACE

Musculoskeletal disorders are a major cause of pain and disability affecting hundreds of millions of people globally. An ageing population and prolonged life expectancy are expected to result in an increase in the number of patients suffering from musculoskeletal disorders in the future, with significant social and economic impacts.[1] Over the years, surgical options for the treatment of musculoskeletal disorders have increased. More effective implants, surgical techniques, and post-operative rehabilitation programmes have led to an increase in the number of orthopaedic surgical procedures performed each year. However, many musculoskeletal conditions have a quite favourable natural history, with a reduction or complete disappearance of symptoms occurring over the course of weeks or months. Non-surgical treatment is important in reducing costs for patients and society as well as avoiding the risks of surgical interventions. The main tools for non-surgical treatments are pharmacological pain reduction with paracetamol or nonsteroidal anti-inflammatory drugs (NSAIDs), exercise therapy, and orthosis, among other techniques.[2] The effectiveness of these techniques has not been proven for all musculoskeletal conditions, and some may have no benefit over allowing the disorder to run its natural course.[3]

Over the years, researchers have developed and promoted several techniques that have been claimed to stimulate or augment the repair of tissues, which may result in the improvement of symptoms. The basis for these techniques is the local injection of biological substances that are naturally produced in the patient's body or cells (e.g., mesenchymal stem cells derived from bone marrow, adipose-derived mesenchymal cells, or growth factors).[4] The purpose of these techniques is to enhance biological repair processes. Among such 'orthobiologic' techniques, platelet-rich plasma (PRP) injection has received much attention and is the topic of this thesis.

PLATELET-RICH PLASMA (PRP)

What is PRP?

To create a PRP preparation, the patient's blood components (platelets, leukocytes, red blood cells, and plasma) must first be separated by centrifugation. The platelet-rich fraction must then be isolated. This yields PRP, which is a small volume of autologous blood plasma with a platelet concentration that is 5 to 10 times greater than normal.[5] The platelet granules contain hundreds of proteins called growth factors. The activation and subsequent degranulation of the platelets in PRP results in the release of these growth factors.[6,7] The basic growth factors that enhance the repair process include platelet-derived growth factor-AB (PDGF-AB), transforming growth factor ß1 (TGF-ß1), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF).[7,8] When injected, these growth factors are thought to play an important role in cell proliferation,

chemotaxis, cell differentiation, and angiogenesis. Therefore, PRP is used to promote new tissue growth, preserve remaining tissue, reduce pain, and improve function.[9]

History of PRP

The concept and description of PRP originate from haematology. The term PRP was first used by Kingsley in 1954 to earmark platelet concentrate during blood coagulation experiments in the field of transfusion medicine.[10] In the 1970s, the term PRP was used to describe plasma with a platelet count higher than that of peripheral blood. This product was also investigated in a rat model to improve skin tissue healing.[11] In the following years, researchers established the role of platelets in enhancing tissue healing during the treatment of skin ulcers in humans.[12] Approximately 10 years later, PRP was studied in bone repair by Marx et al.[5], who reported the beneficial effect of platelet-rich product on bone healing after maxillofacial surgery. Over the last decade, the popularity of PRP has increased, leading to much publicity about its use as a biological treatment for athletic injuries. Moreover, its application has grown exponentially in orthopaedics.

Different Types of PRP

When considering the use of PRP, health care practitioners should understand that the treatment has various forms. When whole blood is centrifuged, the different components of the blood are separated. Different protocols for the separation of blood components exist. On one end of the spectrum, hardly any separation of leukocytes and platelets occurs. On the other end of the spectrum, the platelets and leukocytes are (mostly) separated. If separation is limited, this technique is called buffy coat-based separation. If more separation between the cells is created, this is known as a plasma-based technique.[13-15] Because every commercially available product is made according to a different protocol (centrifugation time and speed, number of times the process is repeated, and G-forces used), a great deal of variation exists among the different products.

The plasma-based technique yields *leukocyte-poor* PRP (LP-PRP), while the buffy coat-based technique creates *leukocyte-rich* PRP (LR-PRP). The fluid that remains after centrifuging the platelets and removing the leukocytes and erythrocytes is called platelet-poor plasma (PPP).

For this thesis, we used a buffy coat-based preparation system and hence used LR-PRP in all studies. PPP was used alongside LR-PRP to investigate wound healing.

Furthermore, there are different techniques for initiating platelet degranulation and the subsequent release of growth factors.[16] One such technique is to rely on the patient's collagen to release thrombin in vivo and to start the degranulation of the platelets. Other available techniques use bovine thrombin or calcium chloride to antagonise the anticoagulant in donated blood.[17] The different PRP preparation methods could potentially affect the characteristics and therapeutic efficacy of the resulting product,

theoretically giving each form of PRP unique properties. Most published studies do not provide a complete characterisation of the PRP compositions used, so reliable comparison between studies remains a challenging problem.[18] Several PRP preparation classification systems have been developed to facilitate comparison between studies and promote the standardisation of the PRP preparation process. However, there is currently no world-wide consensus on which classification system is to be used.[19]

Clinical Effectiveness of PRP Injection

The scientific literature has shown that the activated growth factors released from the platelets in PRP injections have several potential effects in a laboratory setting.[18] De Mos et al. demonstrated that both platelet-rich clot release and platelet-poor clot release increased tenocyte cell count and collagen production in vitro.[20] Another in vitro study by Zhang and Wang showed that PRP activated by calcium chloride promotes the differentiation of tendon stem cells into active tenocytes.[21] However, the available clinical research on the use of PRP has produced inconsistent results.[22,23] Caution is advised when translating in vitro results to a clinical setting, and randomised controlled clinical studies of high methodological quality should be conducted to determine the appropriate indications for PRP.

TENDINOPATHY

The pathophysiology and origins of pain in chronic tendon pathologies are not yet entirely clear. However, tendinopathy is known to be a degenerative process for which inflammation is generally not observed. Recently, however, Millar et al.[24], reported an increased number of macrophages and the presence of specific interleukins, suggesting some inflammatory response. Diseased tendons are characterised by disorganisation of collagen fibres, often excessive production of extracellular matrix (ECM) proteins, an increase in micro-vascularisation and sensory nerve innervation, increased immune cells and inflammatory mediators, and improved cellular apoptosis.[24,25] Tendinopathy clinically presents as a combination of pain, swelling, and reduced function. A variety of tendons can be affected in humans. NSAIDs offer short-term pain relief but may have a negative effect on tendon structure.[3] In the past, corticosteroid injections were a frequently used treatment for chronic tendinopathy. These injections offer effective short-term pain relief but become less effective after three months; they may also cause tendon rupture by decreasing tendon cell proliferation and inducing degenerative changes in the tendon.[26-28] PRP injection has been suggested as a suitable replacement for NSAIDs and corticosteroid injections. Accordingly, there is a growing interest in the use of PRP for the treatment of tendinopathy, although high level clinical evidence for its effectiveness in this regard is limited.

WOUND HEALING

One of the main concerns in total joint arthroplasty is wound healing because of the risk of wound separation and surgical site infections.[29] Identification of methods to enhance or accelerate wound healing and reduce infection may be important, especially for high-risk patients (e.g., patients who have diabetes mellitus, use tobacco, or have a high body mass index). Outside the field of orthopaedics, the application of PRP for wound healing has been explored in both in vivo and in vitro studies.[30,31,32] Combining platelet gel and fibrin sealant derived from PRP could theoretically reduce blood loss and promote wound healing in a surgical wound.[30] Moreover, the presence of leukocytes in PRP may have an additional effect because the leukocytes may have a local antimicrobial effect, leading a reduction of infections.[33-34]

AIMS AND OUTLINE OF THIS THESIS

This thesis aims to study the effectiveness of PRP in the treatment of some musculoskeletal disorders. We performed several clinical trials to investigate the effectiveness of PRP injection in our orthopaedic practice, focusing on tendinopathy and wound healing. To analyse the available techniques, we reviewed the literature on the available systems of PRP for musculoskeletal disorders (**Chapter 2**). Wound healing is one of the major topics of interest regarding the use of PRP across several clinical fields. In **Chapter 3**, we analyse the use of PRP and PPP for function, pain, wound healing, and blood loss in total knee arthroplasty in a randomised controlled trial (RCT). In **Chapter 4**, we describe a literature review that we performed on the use of PRP in upper limb conditions, mainly focusing on tendinopathy. Based on this review, we conducted an RCT comparing the use of corticosteroids versus PRP in patients with chronic tennis elbow conditions and published the results after one year (**Chapter 5**); this was followed by a second publication examining the long-term results after two years (**Chapter 6**). Based on the positive findings in our elbow RCT and favourable results in a clinical setting with other upper limb conditions, we also studied the use of PRP in a lower limb condition. In **Chapter 7**, we discuss our analysis of the use of corticosteroids versus PRP in plantar fasciitis in a double-blind RCT.

We discuss the results of these studies and draw conclusions in **Chapter 8**. We then formulate suggestions for further research in the same chapter. **Chapters 9** and **10** provide summaries of this thesis in English and Dutch, respectively.

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CHAPTER 2

Concentrations of blood components in commercial platelet-rich plasma separation systems: A review of the literature

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ABSTRACT

Background: Platelet-rich plasma (PRP) has proven to be a very safe therapeutic option in the treatment of tendon, muscle, bone, and cartilage injuries. Currently, several commercial separation systems are available for the preparation of PRP. The concentrations of blood components in PRP among these separation systems vary substantially.

Purpose: To systematically review and evaluate the differences between the concentrations of blood components in PRP produced by various PRP separation systems.

Study Design: Systematic review.

Methods: MEDLINE/PubMed, the Cochrane Central Register of Controlled Trials (CENTRAL), and EMBASE were searched for studies that compared the concentrations of blood components and growth factors in PRP between various separation systems and studies that reported on the concentrations of blood components and growth factors of single separation systems. The primary outcomes were platelet count, leukocyte count, and concentration of growth factors (e.g., platelet-derived growth factor-AB [PDGF-AB], transforming growth factor-ß1 [TGF-ß1], and vascular endothelial growth factor [VEGF]). Furthermore, the preparation protocols and prices of the systems were compared.

Results: There were 1079 studies found, of which 19 studies were selected for inclusion in this review. The concentrations of platelets and leukocytes in PRP differed largely between, and to a lesser extent within, the studied PRP separation systems. Additionally, large differences both between and within the studied PRP separation systems were found for all the growth factors. Furthermore, preparation protocols and prices varied widely between systems.

Conclusion: There is a large heterogeneity between PRP separation systems regarding concentrations of platelets, leukocytes, and growth factors in PRP. The choice for the most appropriate type of PRP should be based on the specific clinical field of application. As the ideal concentrations of blood components and growth factors for the specific fields of application are yet to be determined for most of the fields, future research should focus on which type of PRP is most suitable for the specific field.

Keywords: platelet-rich plasma; systematic review; concentration; platelets; leukocytes; growth factors

INTRODUCTION

Platelet-rich plasma (PRP) is a small volume of autologous blood plasma that has been enriched with blood-derived platelets.[21] PRP is considered to have beneficial effects on many healing processes as a result of the growth factors contained in the platelet alpha-granules.[43] The use of PRP for clinical applications in periodontal and oral surgery, maxillofacial surgery, plastic surgery, and the treatment of chronic skin and soft tissue ulcers has been extensively investigated.[22,33,47,53] PRP has proven to be a very safe therapeutic option; complications are rarely reported, as PRP is derived from autologous blood.[42] In orthopaedic surgery and sports medicine, the use of PRP has been of increasing interest over the last decade. PRP has shown to have a beneficial effect on the healing of tendon, muscle, bone, and cartilage injuries.[15,58] Clinical studies on the efficacy of PRP in the treatment of symptomatic knee osteoarthritis [31,39,52] and chronic tendinopathy such as patellar tendinopathy [14,17] and lateral epicondylitis [19,23,40,41] have shown beneficial effects of PRP injections.

Currently, several commercial separation systems are available for the preparation of PRP.[15] The concentrations of blood components in PRP (platelets, leukocytes, and growth factors) among these separation systems vary substantially.[15] Studies comparing the differences in blood components in PRP from these separation systems report varying outcomes in terms of the concentrations of blood components and growth factors.[7,36,50] To gain more insight into the differences between the concentrations of blood components and growth factors in PRP produced by the different separation systems, we conducted a systematic review of the literature on studies investigating the blood components and growth factors in PRP.

METHODS

Inclusion Criteria

The literature search performed for this review was limited to studies that compared the concentrations of blood components and growth factors in PRP between different PRP separation systems and studies that reported on the concentrations of blood components and growth factors of single PRP separation systems. We only included studies investigating human blood taken from healthy adult (age > 18 years) volunteers. The literature search was limited to articles in the English, German, French, and Dutch languages. Only studies reporting on PRP separation systems that are currently commercially available were included.

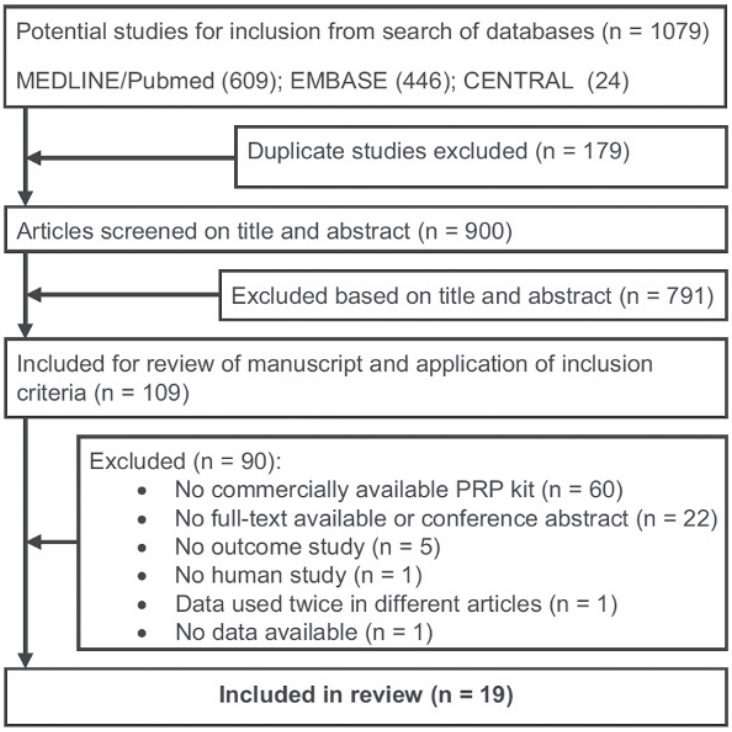

Figure 1. Flow diagram of the search process.

Outcome Measures

This review primarily focused on the platelet count, leukocyte count, platelet enrichment factor ([platelet concentration in PRP]/[platelet concentration in whole blood]), and growth factors (platelet-derived growth factor-AB [PDGF-AB], platelet-derived growth factor-BB [PDGF-BB], transforming growth factor-ß1 [TGF-ß1], vascular endothelial growth factor [VEGF], epidermal growth factor [EGF], fibroblast growth factor-2 [FGF-2], hepatocyte growth factor [HGF], and insulin-like growth factor [IGF]). Furthermore, the preparation protocols (amount of whole blood needed, number of centrifugations, time of centrifugation) and prices of the different PRP separation systems were compared.

Search Strategy

We searched MEDLINE/PubMed, the Cochrane Central Register of Controlled Trials (CENTRAL), and EMBASE up until March 2017 to identify relevant studies concerning the concentrations of blood components in PRP. There were no constraints based on the publication status. In MEDLINE, the following search strategy was used and modified for other databases:

1. Humans
2. Platelet-rich plasma
3. 1 AND 2
4. Blood platelets or platelet count
5. Leukocytes or leukocyte count
6. Platelet-derived growth factor
7. 3 AND 4 AND 5
8. 3 AND 6
9. 7 OR 8

The search was performed by one of the authors (B.W.O.). References of retrieved publications were also used to add studies potentially meeting the inclusion criteria that were missed by the electronic search. Abstracts from scientific meetings and review articles were excluded.

Review Process

To identify relevant articles for this review, the title and abstract of the articles found by the abovementioned search strategy were reviewed. After selection, the full articles were reviewed for definitive selection. All identified studies were independently reviewed by 2 reviewers (B.W.O. and J.C.P.) for inclusion using the abovementioned criteria. In case of disagreement, a third reviewer (A.J.H.V.) was consulted to resolve the disagreement.

Data Collection

The following data were extracted from the included trials: study design (comparative study or study describing one separation device), study characteristics (e.g., number of blood samples), concentration analysis methods, type of outcome, results of the study, and main conclusion(s) of the study. This information was extracted by one author (B.W.O.). If necessary, authors were contacted for additional information about their specific article.

The companies producing the PRP separation systems were contacted to gain information about the specific preparation protocols. In case a company did not respond to the request, the literature was searched for the preparation protocol.

STATISTICAL ANALYSIS

First, 95% CIs were calculated for each of the blood components studied in the included studies using the mean concentration, SD, and number of samples. The following formula was used: $x \pm \gamma \frac{\sigma}{\sqrt{n}}$ where x is the mean concentration, γ the critical value of the t distribution based on the sample size of the study, σ the standard deviation and n the number of samples studied. Forest plots were created using the mean and

95% CI. Differences in concentrations within and between the different PRP separation systems were explored informally by the eyeball test. Additional statistical analyses of differences within and between the different separation systems were not conducted. As a substantial part of the data in the included studies was presented in graphs, which led to missing quantitative data, descriptive results of the studies that compared 2 PRP preparation systems were summarized in a table. Analyses were conducted in SPSS (version 15.0; SPSS) and Excel (Microsoft).

RESULTS

Search Results

The search was performed on September 17, 2016, with a final search update to check for recently published relevant articles on April 11, 2017. The search of MEDLINE/ PubMed, the Cochrane Central Register of Controlled Trials (CENTRAL), and EMBASE databases provided 1079 citations, of which 179 were duplicates. After reviewing the titles and abstracts of the 900 remaining studies, 791 studies were excluded for not meeting the inclusion criteria. The articles of the remaining 109 studies were reviewed, after which 90 studies were excluded: 19 studies were selected for inclusion in this review (Figure 1). No additional studies were found by checking the references of the selected articles.

Characteristics of Included Studies

The characteristics of the included studies are summarized in Table 1. Fourteen studies compared the concentrations of blood components in PRP between different PRP separation systems. In 8 studies, commercially available separation systems were compared. Five studies reported the concentrations of blood components of single separation systems. The number of samples analysed varied between 3 and 102. Ten different commercially available separation systems were studied. The GPS III system (Zimmer Biomet) was studied the most, with 10 articles in total, followed by the ACP system (Arthrex), which was studied in 5 articles. The Endoret (BTI Biotechnology Institute), Magellan (Arteriocyte), and SmartPrep (Harvest Technologies) systems were all studied in 3 articles; the Cascade (Musculoskeletal Transplant Foundation) and RegenPRP (RegenLab) systems were studied in 2 articles; and the Prosys (Prodizen), KYOCERA (Kyocera Medical), and GLO (Glofinn Oy) systems were only studied in 1 article.

Outcome Measures

The platelet concentration was the most studied outcome measure, studied in 13 of 17 articles. Other outcome measures were the leukocyte concentration (12/17), red blood cell concentration (5/17), and platelet enrichment factor (7/17). With regard to growth factors, TGF-ß1 was studied the most (9/17), followed by PDGF-AB and VEGF (both 8/ 17). Other reported growth factors were IGF (4/17), PDGF-BB (3/17), EGF (3/17), HGF (2/17),

and FGF-2 (1/17). As TGF-ß1, PDGF-AB, and VEGF were by far the most studied growth factors, further statistical analyses were only performed for these 3 growth factors.

Table 1 Characteristics of the Included Studies*

	No. of Samples	No. of PRP Kits Studied	PRP Kits Studied	Outcome Measures
Anitua et al[3] (2013)	3	1	Endoret	PEF, WBCC, PDGF-AB, VEGF, HGF, IGF
Castillo et al[7] (2011)	5	3	GPS III, Cascade, Magellan	PC, WBCC, RBCC, PEF, PDGF-AB, PDGF-BB, TGF-B1, VEGF, PCE, FC
Dragoo et al[13] (2012)	40	1	GPS III	PDGF-BB, TGF-B1, VEGF, IGF
Evanson et al[16] (2014)	102	1	ACP	PC, WBCC, RBCC, PDGF-AB, PDGF-BB, TGF-B1, VEGF, EGF, FGF-2, HGF, IGF
Everts et al[18] (2008)	20	1	Magellan	PC, WBCC, PEF
Hamilton et al[24] (2015)	10	1	GPS III	PC, WBCC, PDGF-AB, HGF, IGF, VEGF
Howard et al[25] (2014)	4	2	Cascade, SmartPrep	PC, PEF, PDGF-AB, TGF-B1
Kaux et al[27] (2011)	6	1	GPS III	PC, WBCC, RBCC
Kaux et al[26] (2011)	5	1	GPS III	WBCC, RBCC, PEF
Kushida et al[29] (2014)	5	3	GLO, KYOCERA, Magellan	PC, PDGF-AB, TGF-B1, VEGF
Leitner et al[30] (2006)	3	1	SmartPrep	PC, WBCC, RBCC
Magalon et al[32] (2014)	10	3	ACP, GPS III, RegenPRP	PC, WBCC, PEF, PDGF-AB, TGF-B1, VEGF, EGF, PCE
Mazzocca et al[36] (2012)	8	2	ACP, GPS III	PC, WBCC, RBCC, PDGF-AB, TGF-B1, VEGF, EGF, FGF-2, HGF, IGF
Mazzucco et al[37] (2009)	Not provided	1	RegenPRP	PC, PEF, PDGF-BB, TGF-B1, VEGF, EGF, IGF
Oh et al[46] (2015)	14	3	ACP, GPS III, Prosys	PC, WBCC
Schar et al[51] (2015)	11	1	GPS III	TGF-B1, VEGF
Sundman et al[54] (2011)	11	2	ACP, GPS III	PC, WBCC, PEF
Weibrich et al[56] (2005)	51	1	Endoret	PC, WBCC, PDGF-AB, TGF-B1, PCE
Weibrich et al[57] (2012)	54	2	Endoret, SmartPrep	PC, WBCC, PDGF-AB, TGF-B1, IGF

*EGF, epidermal growth factor; FC, fibrinogen concentration; FGF-2, fibroblast growth factor-2; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; PC, platelet concentration; PCE, platelet capture efficiency; PDGF-AB, platelet-derived growth factor-AB; PDGF-BB, platelet-derived growth factor-BB; PEF, platelet enrichment factor; PRP, platelet-rich plasma; RBCC, red blood cell concentration; TGF-B1, transforming growth factor-B1; VEGF, vascular endothelial growth factor; WBCC, white blood cell concentration

PRP Separation Systems

The preparation protocols for the different PRP separation systems are summarized in Table 2. The majority of the systems use a dual spin method (6/10). Both the centrifugal force (range, 350-2008g) and the total centrifugation time (range, 5-21 minutes) differed largely between the systems. Also, a wide variation in price per kit (range, US$50-US$500) was found between the systems.

Table 2 Preparation Protocols and Costs for the Different PRP Separation Systems*

			Centrifugal Force, *g*		Centrifugation Time, min			
	Type of System	Whole Blood Volume, mL	First Spin	Second Spin	First Spin	Second Spin	Final Volume of PRP, mL	Cost/Kit, $
ACP	Plasma	11	350	—	5	—	2.0-5.0	150
GPS III	Buffy coat	54	1100	—	15	—	6.0	350
Cascade	Plasma	9	1100	1450	6	15	2	NP
Endoret	NP	9	580	—	8	—	2.0	NP
GLO	Buffy coat	9	1200	600	5	2	0.6	50-75
SmartPrep	Buffy coat	60	1250	1050	14	7.0-10.0	NP	NP
KYOCERA	NP	20	600	2000	7	5	2	60
Magellan	Buffy coat	60	610	1240	4	6	3	500
Prosys	NP	30	1660	2008	3	3	3	NP
RegenPRP	NP	8	1500	—	5	—	4	NP

* NP, not provided by manufacturer (unknown); PRP, platelet-rich plasma

Laboratory Results

Platelets, Leukocytes, and Platelet Enrichment Factors.

The concentrations of platelets and leukocytes found in the included studies are presented in Figure 2. The concentration of platelets in PRP differed largely between, and to a lesser extent within, the studied PRP separation systems. The highest concentration of platelets was produced by the Cascade system; the lowest concentration of platelets was produced by the ACP system. Regarding the concentration of leukocytes in PRP, large differences were found between, but not within, the separation systems. The highest concentration of leukocytes was found in PRP produced by the GPS III system; PRP produced by the ACP system contained the lowest number of leukocytes. Although only reported in 4 studies, large differences between PRP separation systems were found for the platelet enrichment factor. The highest platelet enrichment factors were found for the GPS III and SmartPrep systems (3.93 [32] and 3.79 [30], respectively) and the lowest for the ACP, RegenPRP, and Cascade systems (1.31 [32], 1.59 [32] and 1.62 [7], respectively).

Growth Factors.

The concentrations of the growth factors PDGF-AB, TGF-ß1, and VEGF found in the included studies are presented in Figure 3. Large differences both between and within the studied PRP separation systems were found for all the growth factors. Additionally, no differences in the concentrations of PDGF-AB and TGF-ß1 were found between the higher (GPS III, SmartPrep, and Magellan) and lower platelet-yielding devices (ACP, Cascade, Endoret, and RegenPRP) as for the higher (GPS III, SmartPrep, Magellan, and RegenPRP) and lower leukocyte-yielding devices (ACP and Cascade). However, the concentration of VEGF tended to be higher in PRP produced by systems that yield higher concentrations of platelets and leukocytes (GPS III and Magellan).

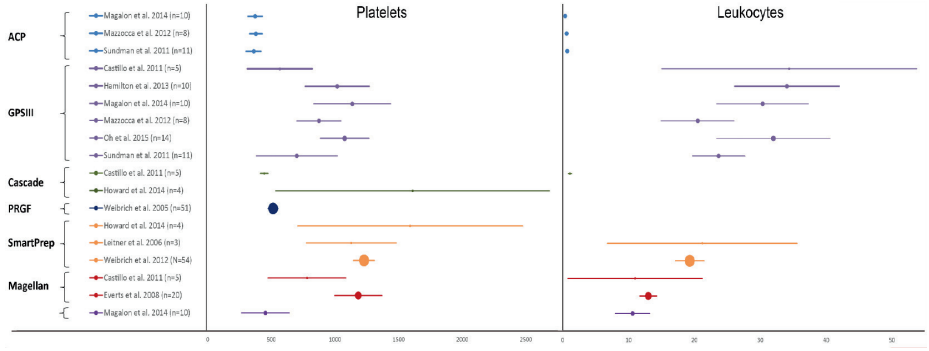

Figure 2. Concentrations of platelets (×10³ µL) and leukocytes (×10³ µL) found in the included studies.

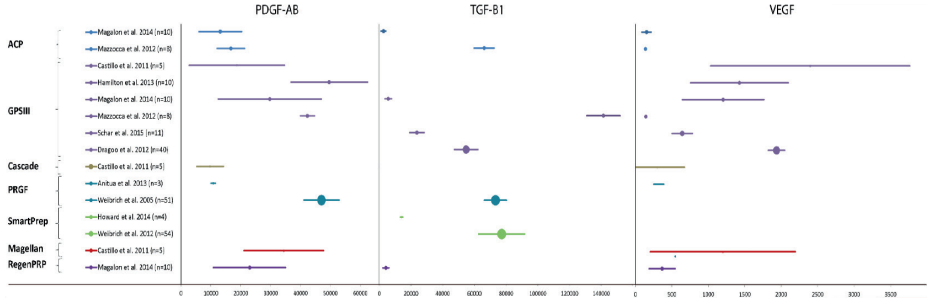

Figure 3. Concentrations of PDGF-AB (pg/mL), TGF-B1 (pg/mL), and VEGF (pg/mL) found in the included studies.

Comparative Studies

As not all selected studies provided exact data, descriptive results of the studies comparing 2 PRP separation systems were used.[7,25,29,32,36,46,54,57] The ACP and GPS III were the only systems that have been compared in more than 1 study: the concentrations of platelets, leukocytes, and growth factors were significantly higher in favour of the GPS III.[32,36,46,54] Overall, the ACP showed lower platelet and leukocyte concentrations in studies comparing the ACP with systems other than the GPS III; the concentrations of growth factors, however, were largely comparable.[32,46] The GPS III, on the other hand, showed a significantly higher concentration of leukocytes compared with other systems.[7,32,46] Furthermore, the GPS III produced a higher concentration of platelets than the RegenPRP and Prosys,[32,46] but no significant differences in the platelet concentration were found between the GPS III and the Cascade and Magellan.[7] The concentrations of growth factors did not significantly differ in most of the studies.

DISCUSSION

The objective of this review was to assess the differences between the concentrations of blood components and growth factors in PRP between the various PRP separation systems. The findings in this review demonstrate that there is a large heterogeneity among various systems regarding the concentrations of platelets and leukocytes. Regarding the concentrations of growth factors, there is a large heterogeneity both between and within the different systems. Furthermore, the concentration of VEGF tended to be higher in PRP produced by systems that produce higher concentrations of platelets and leukocytes.

Concentration of Platelets

There was a large difference in the concentration of platelets between the systems studied in this review. Roughly, the systems studied in this review can be divided into high- and low-yielding devices. This division into high- and low-yielding devices has been described before by Dhurat and Sukesh.[11] Dhurat and Sukesh [11] described that PRP devices can usually be divided into lower (2.5-3 times the baseline concentration) and higher (5-9 times the baseline concentration) systems. The low-yielding devices in this review produce PRP with a platelet concentration around 500 x 10^3 µL, whereas the high-yielding devices generally produce a platelet concentration over 750 x 10^3 µL. Among the high-yielding devices were the GPS III, SmartPrep, and Magellan systems; the lower concentration systems were the ACP, Cascade, Endoret, and RegenPRP. These findings correlate well with the findings in this review.

The concentration of platelets in PRP is of importance, as the mechanism of action of PRP is mainly based on the growth factors and cytokines found in the alpha-granules in the platelets. However, there is no consensus about the optimal concentration of platelets in PRP: some authors have reported platelet concentrations greater than 200 x 10^3 µL [37] to be therapeutic, whereas others have reported concentrations of 1000 x 10^3 µL.[34] In the present study, the platelet concentrations of all of the PRP separation systems exceeded a platelet concentration of > 200 x 10^3 µL, which implies that all the devices met the definition for therapeutic and effective PRP as defined by Mazzucco et al.[37]

Concentration of Leukocytes

Comparable with the concentration of platelets in PRP, the concentration of leukocytes differed largely between the systems studied in this review. Additionally, no large differences within the systems were found. PRP separation systems can be divided into systems producing a high and a low concentration of leukocytes. The concentration of leukocytes in PRP is a direct result of the preparation method that is used. Buffy coat-based systems, for example, produce PRP with a high concentration of leukocytes, as the buffy coat is rich in leukocytes. Plasma-based systems, in contrast, are designed to separate only the platelet and plasma portions of whole blood and therefore contain a

low concentration of leukocytes.[11,15,50] The majority of separation systems in the current literature yield leukocyte-rich PRP. As also shown in this review, the ACP, Cascade, and Endoret systems are known to produce leukocyte-poor PRP. Currently, the inclusion of leukocytes in PRP is subject to debate, as both beneficial and adverse effects of leukocyte inclusion have been suggested.[50] Potential beneficial effects of leukocyte inclusion include their role in tissue remodelling and their increased antibacterial and immunological resistance.[12,44] Furthermore, the presence of leukocytes in PRP is associated with an increased concentration of growth factors, especially VEGF.[9,10,28,64] On the other hand, the inclusion of leukocytes might have catabolic and inflammatory effects on the targeted tissue as a result of the release of proinflammatory cytokines by leukocytes, which is associated with decreased proliferation and increased apoptosis.[2,4,5,8,38,49,59-62] As the aim of this review was to evaluate the differences between the concentrations of blood components in PRP produced by the various PRP separation systems, no definitive answer can be provided on whether leukocyte-rich or leukocyte-poor PRP is best based on the results of this review. There is, however, increasing evidence that the type of PRP (leukocyte-rich or leukocyte-poor) should be matched to the specific clinical field of application. In the treatment of knee osteoarthritis, for example, the use of leukocyte-poor PRP seems to be more beneficial than leukocyte-rich PRP.[48] In the treatment of chronic tendinopathy, in contrast, the use of leukocyte-rich PRP is superior to leukocyte-poor PRP.[20] To gain more insight in the specific indications for the different types of PRP, future research should focus on which type of PRP is most suitable for the specific fields of application.

Concentrations of Growth Factors

A wide variation was found regarding the concentrations of growth factors both between different systems as well as within systems. These differences can partly be explained by the use of the specific enzyme-linked immunosorbent assay kits. The assays of growth factors contained in the platelets may be influenced by the incomplete removal of platelets and red blood cells and therefore give variable results.[36] Data within the studies are comparable, but a comparison between studies is less reliable, which limits the relevance of these findings. In this review, it seemed, however, that the concentration of VEGF tended to be higher in PRP produced by systems with higher concentrations of platelets and leukocytes. Higher amounts of growth factors have indeed been correlated with higher amounts of platelets and leukocytes.[55,63] Although evidence about the role of the specific growth factors is scarce, in vitro studies have suggested that PDGF and TGF-B are the 2 most important growth factors in PRP.[1,6,35,45] In contrast to the platelet and leukocyte concentrations, there is no evidence about ideal concentrations of growth factors in PRP for tissue regeneration. Therefore, future studies are necessary to reveal the exact mechanisms of growth factors in PRP and their role in tissue regeneration.

Preparation Protocols

Besides a large heterogeneity in the concentrations of platelets, leukocytes, and growth factors between systems, the preparation protocols for the different systems also differed largely. Wide ranges were found for both the centrifugal force (350-2008g) and the total centrifugation time (5-21 minutes). There are many ways of preparing PRP; the most common methods are the plasma-based and buffy coat-based methods.[29] Although not known for all systems in this review, most systems use the buffy coat-based method. As mentioned earlier, buffy coat-based systems produce PRP with a high concentration of leukocytes, as the buffy coat is rich in leukocytes.[11,15,50] Although the ideal concentrations of blood components and growth factors for the specific fields of application have yet to be determined, the field of application should play an important role in the choice for the most appropriate PRP separation system. Other factors such as the volume of whole blood needed, the final volume of PRP, and the usability and reliability of the separation system could also be taken into consideration. Finally, the price of the systems can be taken into consideration, as a wide variation in price per kit ($95-$500) was found.

Strengths and Limitations

This is the first systematic review that offers a comprehensive overview of the concentrations of blood components in PRP produced by all the commercially available PRP separation systems and that analyses the differences between the systems in terms of the concentrations of blood components and growth factors. Initially, this study was designed as a meta-analysis. Unfortunately, despite all the authors who were contacted, we had to deal with a lot of missing data, and no raw data were available for the majority of the studies. This limited the statistic options available for analysing the differences between systems, and therefore, a meta-analysis could not be conducted. To overcome the missing data, descriptive results of the studies that compared 2 PRP preparation systems were summarized. Furthermore, the number of samples studied in the included studies was rather small; only 5 of the 19 studies used 20 samples, and 10 of the 19 studies used 10 samples, which also limits a comparison between systems.

However, as this review of the literature showed, future research on the components of PRP should not focus on the concentrations of the components but rather on the optimal concentrations of platelets, leukocytes, and growth factors for the different fields of application. The use of leukocyte-rich PRP in chronic tendinopathy has been extensively investigated and been proven to be superior to leukocyte-poor PRP.[20] For other applications, osteoarthritis, for example, the evidence is limited, and well-designed clinical studies are necessary to gain more insight to which formulation of PRP is most suitable.

In conclusion, this review demonstrates that there is a large heterogeneity among different systems with regard to the concentrations of platelets, leukocytes, and growth

factors in PRP. Also, the preparation protocols for the different systems differ largely. The choice for the most appropriate type of PRP should be based on the specific clinical field of application. As the ideal concentrations of blood components and growth factors for the specific fields of application are yet to be determined for most of the fields, future research should focus on which type of PRP is most suitable for the specific field.

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CHAPTER 3

No positive effect of autologous platelet gel after total knee arthroplasty. A double-blind randomized controlled trial: 102 patients with a 3-month follow-up

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ABSTRACT

Background and purpose: Activated platelets release a cocktail of growth factors, some of which are thought to stimulate repair. We investigated whether the use of autologous platelet gel (PG) in total knee arthroplasty (TKA) would improve wound healing, knee function, and decrease blood loss and the use of analgesics.

Patients and methods: 102 patients undergoing TKA were randomly assigned in a PG group (n= 50) or in a control (C) group (n = 52). The primary analysis was based on 73 participants (PG = 32, C = 41) comparing the postoperative wound scores, VAS, WOMAC, Knee function, use of analgesics, and the pre- and postoperative haemoglobin values after a follow up of 3 months. 29 participants were excluded due to insufficient data.

Results: Characteristics of the protocol-compliant patients were similar to those who were excluded. Analysis was per protocol and focused on the remaining 73 patients. At baseline and after 3 months follow up, there were no differences between both groups for age, height, weight, sex, side of operation, platelet count, haemoglobin values, severity of complaints (WOMAC) and level of pain.

Interpretation: In our patients undergoing TKA the application of PG to the wound site did not promote wound healing. Also, no effect of PG was found on pain, knee function and haemoglobin values.

Trial registration: METC protocol number 04-17. Date of approval 27th October 2004.

INTRODUCTION

Identification of methods to enhance or accelerate wound healing may be important, especially in high-risk patients (e.g., with type 1 diabetes mellitus, tobacco use, or previously irradiated tissue). The requirement of growth factors within the wound healing cascade has been confirmed.[1, 15, 16] In a canine model, treatment with autologous blood platelet concentrate enhanced and accelerated early wound healing.[22] In humans autologous blood platelet concentrate was shown to increase bone formation in maxillofacial surgery.[18] Since this latter result was regarded as a general stimulation of repair rather than a specific increase in bone formation, we investigated in a double-blind randomized trial whether the application of a platelet concentrate (in spray form) could improve repair of wounds after TKA. The primary outcome parameter was wound healing, but we also studied the effects on knee function, use of analgesics, and haemoglobin values.

PATIENTS AND METHODS

This double-blind, randomized study included 102 consecutive patients scheduled for primary unilateral TKA for osteoarthritis between June 2005 and March 2007. All procedures took place in a training hospital using the same surgical procedure performed by an orthopaedic consultant or a supervised senior orthopaedic resident. There was no age limit for inclusion. Criteria for participation included pain and radiographic knee osteoarthritis. Exclusion criteria were: platelet count ≤ $150x10^9$/L, haemoglobin level ≤ 6.5 mmol/L, BMI > 33 and systemic disorders such as diabetes, rheumatoid arthritis and hepatitis.

In the current study we tested the hypothesis that the application of a platelet concentrate (in spray form) could improve repair of wounds after TKA. The primary endpoint was wound healing, but we also studied the effects on knee function, use of analgesics, and haemoglobin values.

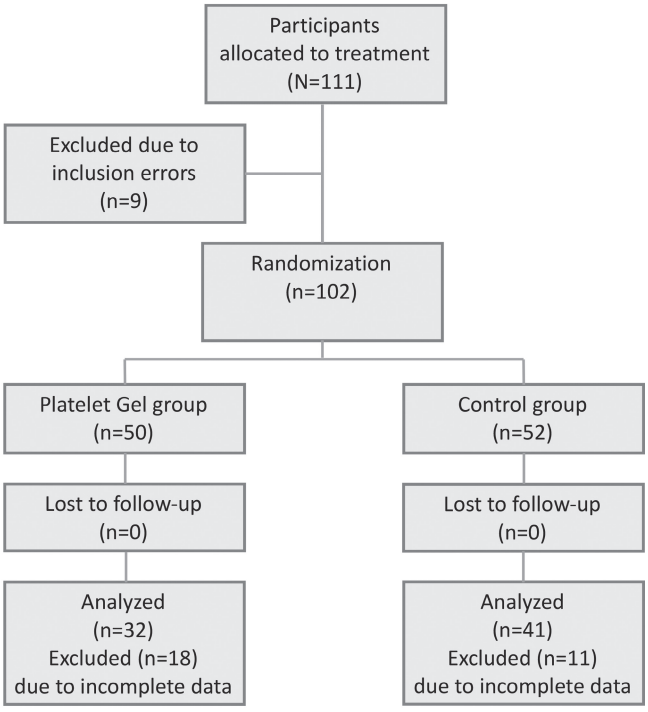

Figure 1. Flow chart of the patients.

Randomization

Block randomization of the patients was performed after they were deemed eligible and had provided informed consent. Patients were randomly allocated to the PG arm or to the C arm. Treatment assignments (placed in sequentially numbered opaque envelopes) were assigned by the trial managers, who also arranged the facilities needed for the procedure.

Surgical procedure

The medial para-patellar approach was used, averting the patella laterally. A tourniquet was used. In all cases a cemented posterior cruciate retaining prosthesis (AGC; Biomet Biologics, Warsaw, Indiana) was used. After implantation of the components, the tourniquet was deflated, and primary haemostasis was achieved. Before closure of the wound layers, the knee soft tissues and joint were rinsed with saline solution to remove all debris. After closure of the joint capsule the subcutaneous tissues of the patients randomized to receive PG were sprayed with the platelet-poor plasma (PPP) fraction (±10 mL), and the skin was closed with staplers. We did not use a deep or subcutaneous drain. In all patients the knee incision was dressed postoperatively with compression bandages and rehabilitation was started the day after surgery. In this fashion, patients, nurses and physical therapist were all blinded to which procedure was used.

Platelet-rich plasma preparation

In the group randomized to receive PRP the patient's own platelets were collected using the GPS System (Biomet Biologics, Warsaw, Indiana). This device uses a desktop-size centrifuge with disposable cylinders to isolate the platelet rich fraction from a small volume of the patient's anticoagulated blood drawn at the time of the procedure.

First a 60 mL syringe was filled with 7 mL of anticoagulant citrate phosphate dextrose formula A and 53 mL of whole blood was drawn via an intravenous catheter in the medial cubital vein using a 17 G needle. Proper mixing with the anticoagulant was done by 8 times inverting the syringe. The platelet rich fraction was prepared according to the instructions for the use for the GPS System. In brief, blood was drawn into a 60 mL bowl of the blood cell separator and centrifuged 15 min at a rate of 3200 RPM for sequestration. Approximately 6 mL PRP was obtained for each patient. The PRP was then buffered to physiologic pH using 8.4% sodium bicarbonate. Autologous thrombin was isolated from 4 mL PRP and 0.17 mL of 10% calcium chloride to antagonize the anticoagulant in the donated blood. Addition of calcified thrombin activated the platelets. The total time from blood draw to injection in the patients was about 90 min. No specialized equipment, other than the centrifuge to process the GPS disposable, was required. A person that is certified for blood management performed all the procedures under sterile conditions.

Injection technique

Using an aerosol spraying at a distance of 10-15 cm with the knee flexed in 90 degrees, which expose the knee cavity, 6 mL PRP was applied to the dried wound site (synovium and bony cutting edges of femur and tibia); thereafter, the wound was closed in layers. After closure of the joint capsule the subcutaneous tissues of the patients randomized to receive PG were sprayed with the platelet-poor plasma (PPP) fraction (±10 mL).

Rehabilitation

Postoperative pain relief was achieved using a standard protocol (paracetamol 3 g daily and diclofenac 50 mg 3 x daily, with pantoprazol 40 mg daily as ulcer protective). All patients received thrombosis prophylaxis via a subcutaneous injection of 0.3 ml low-molecular-weight heparin daily before operation and continued until sufficient effect of oral anticoagulants (acenocoumarol) was achieved. The oral anticoagulants were used up to 12 weeks postoperatively. Rehabilitation, which was started the day after surgery using crutches, was according to the Joint Care program (Biomet, Indiana, USA). The physiotherapist was also blinded to which procedure was used.

Wound score form

A wound score form was used for scoring wound healing (Table 1). A pilot study showed the wound score form to be sufficiently reliable (K = 0.8, unpublished data). The score ranges from 0 to 100, where 0 represents a dry wound without any sign of infection, and 100 represents wound leakage with signs of infection. Points of changes of wound

dressings (question 2) are multiplied by the type of wound dressing (question 1: x 1 or x 2). Questions 3 and 4 are indicators of the wound leakage, questions 5 and 7 are indicators of the type of leakage, and question 6 addresses wound infection parameters. The wound scores were measured by a trained orthopaedic resident (medical ward), who did not know whether PG had been used.

Statistics

There is little information if the effect of the application of a platelet concentrate could improve repair of wounds after TKA. The purpose of this study was to investigate whether the application of a platelet concentrate could reduce 25% of the wound leakage. Wound leakage was defined as a binary result (leakage or no leakage).

With a bilateral alpha of 0.05 and a power of 80%, the intention to treat was 43 patients in each group to show a significant difference ($\alpha = 0.05$, $\beta = 0.8$, $2n = 86$). This difference is based on a study of Gardener et al.[12]

Wound scores and function scores were measured on days 3 to 5, and at the regular control every 2 weeks. The function scores were also measured at 6- and 12-weeks post-surgery. For purpose of analysis the wound scores were dichotomized according to either “wound closure” (score of 0; no leakage or signs or infection) or “wound leakage” (scores > 0). Absolute difference in rate of wound closure with corresponding confidence intervals were computed according to Altman.[2] Wound closure was analysed with Chi-square between the two groups for each day.

Haemoglobin values were measured pre- and postoperatively. The haemoglobin drop was analysed using unpaired Student’s t-test.

VAS (Visual Analogue Scale) for pain at rest and pain during walking was measured at intake and 6 weeks and 12 weeks post-operative. The analogue scores 0-10 mm (negligible), 10-30 mm (mild), 30-50 mm (painful), 50-80 mm (moderate), and 80-100 (severe) were regarded as ordinal categories. Analysis was focused on the changes between measurement points in time, using Mann-Whitney U tests. Postoperative frequency use of analgesics was scored on a 5-point scale (never - always) pre-operative, 6 weeks and 3 months post-operative. Changes between measurement points in time were analysed using Mann-Whitney tests.

The range of motion of the operated knee was analysed on the second day, third day, fourth day, 2 weeks, 6 weeks, and 3 months post-operative and was analysed using ANOVA for repeated measurements. WOMAC function scores were measured pre-operative, 6- and 12-weeks post-operative. Analysis focused on changes between measurement points in time, using Mann-Whitney U tests. A trained medical person, who was blinded to treatment group, measured all scores.

Table 1 Wound Score Form

1. Dressing material:

• Cutiplast (sterile plaster)	X 1
• Absorbing bandage (sterile) + plaster (unsterile)	X 2
• Absorbing bandage (sterile) + Tegaderm (sterile)	X 2
• Gauze dressing + Tegaderm (sterile)	X 1

2. Changes of dressing material in 24 hours

	Points
• None	0
• 1	2
• 2	4
• 3	6
• 4	8

NB! Points of question 2 multiply by the sort of dressings in question 1 (X 1 or X 2)

3. Extent of leakage of the wound with stamp technique (sterile gauze with hand pressure)

	Points
• Wound is dry on stamping/no leakage in old dressing	0
• Wound is dry on stamping/leakage in old dressing	12
• Wound is leaking	24

4. Location of leakage

	Points
• None	0
• Hole of the drain	5
• Part of the wound	10
• Entire wound	15

5. Condition of leakage in stamp

	Points
• None/stamp is dry	0
• Clear leakage	2
• Sanguine leakage	4
• Blood	6
• Troubled leakage	8
• Pus	10

6. Condition of the wound

	Yes / No
• Redness	5 / 0
• Warmth	5 / 0
• Swelling	5 / 0
• Pain	5 / 0
• Temperature	5 / 0

7. Condition of leakage in old dressing

	Points
• None/dressing is dry	0
• Clear leakage	2
• Sanguine leakage	4
• Blood	6
• Troubled leakage	8
• Pus	10

All data analysis were intended to be carried out according to a pre-established analysis plan based on the principle of intention to treat. The significance level was set at $P = .05$. SPSS version 16.0 for Windows (SPSS Inc., Chicago, Illinois) was used.

Ethics

All patients had to be able to read and understand the protocol and the informed consent. The Medical-Ethical Committee (METC) and the National and Institutional Review Board approved the study. METC protocol number 04-17. Date of approval 27th October 2004. The trial was performed in compliance with the Helsinki Declaration 2000 and Good Clinical Practice 1997.

RESULTS

From June 2005 to March 2007 a total of 111 patients with an indication for TKA were included in the study. 9 patients had to be excluded due to inclusion errors. Analysis was per protocol and focused on the remaining 102 patients to a pre-established analysis plan based on the principle of intention to treat: 50 TKA were treated with PG and 52 without PG. Patient characteristics at baseline were similar between the two groups (Table 2).

However, due to a reorganization of the patient's ward, no or partial measurements were recorded in a number of the included patients during hospital stay. Missing data were imputed based on the "last known result carried forward" principle.

Characteristics of the patients who were fully recorded compared to the other patients were similar (Table 3). Eventually, full data were recorded for 32 patients in the PG group and 41 patients in the C group. Analysis for possible bias caused due to missing data showed no differences between resulting groups at baseline (Table 3) (Figure 1).

Table 2 Patient Characteristics at Inclusion

	PG group	Control group
No. of patients	50	52
No. of males	13 (26%)	11 (21%)
Age, mean (SD)	77 (4.4)	78 (5.1)
Length in cm, mean (SD)	168 (9.1)	168 (8.1)
Weight in kg, mean (SD)	83 (16)	79 (12)
Platelet count in 10^9/L, mean (SD)	253 (63)	273 (64)
Hb in mmol/L, mean (SD)	8.6 (0.9)	8.5 (0.7)
Right side	29 (58%)	30 (59%)
WOMAC score, median (range)	49 (8–78)	43 (14–74)
Pain at rest, median (range)	3 (1–5)	3 (1–5)
Pain during activity, median (range)	4 (1–5)	4 (1–5)

Primary endpoint (wound healing)

Both groups had normal wound healing (Table 4). No clinical or statistical differences in wound closure were notable between the two groups during hospital stay (third day, 95% CI: 11% to 25% difference; fourth day, 95% CI: 30% to 10% difference). At 2 weeks

more patients in the control group had total wound closure compared to the PG group (*P* = 0.02) (95% CI: -41% to -7% difference).

Table 3 Data of Patients With and Without In-Hospital Recorded Data

	Complete recovery	Incomplete recovery	p-value	PG group	Control group	p-value
No. of patients	73	29		32	41	
No. of males	14 (19%)	10 (34%)	0.1	6 (19%)	8 (20%)	1
Age, mean (SD)	77 (4.8)	77 (4.8)	0.9	76 (4.1)	78 (5.2)	0.1
Length in cm, mean (SD)	167 (7.6)	169 (10.7)	0.3	166 (6.5)	168 (8.3)	0.4
Weight in kg, mean (SD)	80 (14)	81 (15)	0.8	81 (17)	80 (12)	0.6
Platelet count in 10^9/L, mean (SD)	269 (66)	249 (58)	0.2	261 (68)	275 (64)	0.4
Hb in mmol/L, mean (SD)	8.6 (0.8)	8.6 (0.9)	0.9	8.6 (0.8)	8.5 (0.7)	0.3
Right side	43 (59%)	16 (57%)	1	16 (50%)	27 (66%)	0.2
WOMAC score, median (range)	45 (8–76)	41 (11–76)	0.4	50 (8–76)	43 (14–74)	0.6
Pain at rest, median (range)	3 (1–5)	3 (1–5)	0.8	4 (1–5)	3 (1–5)	0.6
Pain during activity, median (range)	4 (1–5)	4 (2–5)	0.9	4 (1–5)	4 (1–5)	0.3

Table 4 Data on the Platelet Gel Group (PG) and the Control Group

	PG group	Control group	95% CI	p-value
Wound closure)	n = 32	n = 41		
Second day postoperatively	0	0		–
Third day postoperatively	7	6	7% (–11% to 25%)	0.5
Fourth day postoperatively	7	13	–9% (–30% to 10%)	0.4
Wound closure	n = 36	n = 46		
2 weeks postoperatively	4	16	–24% (–41% to 7%)	0.02
Drop in Hb, mean (SD)	n = 50	n = 52		
mmol/L	–1.58 (0.63)	–1.75 (0.58)	0.16 (–0.07 to 0.4)	0.2
Pain at rest, median (range)	n = 50	n = 52		
At inclusion	3 (1–5)	3 (1–5)		0.8
6 weeks	2 (1–5)	2 (1–5)		0.08
3 months	2 (1–5)	2 (1–5)		0.8
Pain during walking, median (range)	n = 50	n = 52		
At inclusion	4 (1–5)	4 (1–5)		0.4
6 weeks	2 (1–5)	2 (1–5)		0.07
3 months	2 (1–5)	2 (1–5)		0.9
Use of pain medication, median (range)	n = 50	n = 52		
At inclusion	2 (1–5)	2 (1–5)		0.5
6 weeks	2 (1–5)	2 (1–5)		0.9
3 months	2 (1–5)	2 (1–5)		0.1
WOMAC score, mean (range)	n = 50	n = 52		
At inclusion	45 (8–76)	44 (14–74)		
6 weeks	26 (3–76)	24 (0–65)	0 (–8 to 8)	0.7
3 months	25 (0–76)	21 (0–66)	–3 (–6 to 1)	0.4
ROM, mean (SD)	n = 32	n = 36		
Second day postoperatively	53 (14)	50 (17)		
Third day postoperatively	68 (13)	65 (16)		
Fourth day postoperatively	72 (13)	73 (14)		0.7
ROM, mean (SD)	n = 34	n = 45		
2 weeks postoperatively	91 (13)	89 (13)		
6 weeks postoperatively	99 (11)	100 (13)		
3 months postoperatively	102 (12)	101 (12)		0.9

Secondary endpoints

There was a difference in the postoperative drop in levels of haemoglobin between the 2 groups (between group difference 0.16 mmol/L, *P* = 0.2; 95% CI: -0.07 to 0.4 difference) (Table 4).

Reported pain was reduced from (moderately) painful to mild pain 6 weeks postoperatively. There was a trend to greater pain reduction (both at rest and while walking) at 6 weeks postoperative for the C group.

The median frequency of medication use was "sometimes", and this remained the median answer though out the study period. There was no difference in reduction of pain medication use between the PG group and C group neither at 6 weeks post-surgery ($P = 0.9$) or at 3 months ($P = 0.1$).

As expected, during hospitalization the range of motion of the operated knee increased from 50 degrees 2 days post-surgery to 75 degrees at discharge from hospital. During these days no benefit was seen for the PG group (2-way ANOVA, $P = 0.7$). From 2 weeks, 6 weeks up to 3 months follow-up, no differences were seen between the two groups ($p = 0.9$).

At 6 weeks post-operative the self-rated knee function (WOMAC score) had increased by 20 points, but the recovery rate between the two groups was similar (Mann-Whitney U test, $P = 0.7$; 95% CI: -8 to 8 difference); similarly, there were no differences between groups at 3-months follow-up (95% CI: -6 to 1 difference).

Complications

After discharge from hospital, superficial wound infections occurred in 2 patients (1 in each group; both coagulase-negative Staphylococcus); these infections were successfully treated with antibiotics. No deep infections were seen.

DISCUSSION

In this randomized study we found no effect of wound healing of platelet gel used after TKA.

PG is promoted as an ideal autologous biological blood-derived product, which can be exogenously applied to various tissues where it releases high concentrations of platelet growth factors that enhance wound healing. In addition, PG possesses antimicrobial properties that may contribute to the prevention of infections.[9] When platelets become activated, growth factors are released and initiate the body's natural healing response.

The actual quantity of platelets needed to achieve an improved outcome when PG is used is still questionable. Marx et al.[18] found in their study that a 3-4 times higher platelet count improved the mandibular continuity defects. The GPS system that we used produces a 6-8 times higher platelet count. Much higher concentrations might have an inhibitory effect.[25] The activator for the platelets we used was a mixture of thrombin and calcium chloride. After combining these substances, platelet-rich gel is formed, and numerous regulatory molecules and antimicrobial proteins are released to the injury site.[26] Thrombin derived from bovine plasma is used in the USA, despite

the fact that bovine thrombin has been associated years ago with the development of antibodies to thrombin and factor V, which had led to recurrent bleeding in patients who were exposed.[27] Alternatively, the platelets can be activated by autologous thrombin, produced with commercially available thrombin kits.[7, 8] Tsay et al.[23] showed the use of a synthetic peptide that mimics thrombin known as peptide-6 SFLLRN (TRAP).

Using the GPS system, the patient's own platelets (which travel through the bloodstream) can be collected into a highly concentrated formula.

We found a slight difference in the haemoglobin drop: 0.16 mmol/L. This is 10 % of the total drop. The mean haemoglobin before operation was 8.6 mmol/L. After operation the haemoglobin in the PG dropped to 7.1 mmol/L and in the control group the haemoglobin dropped to 7.0 mmol/L. This finding is in contrast to an earlier report and might be explained by differences in the technique and methodology. For example, Everts et al.[6] used a PG and fibrin sealant technique, a preparation that differs from our technique. Moreover, their trial included more patients, and the haemoglobin values were scored not only on the first postoperative day (as we did) but also on days 2 to 4 post-surgery and again on the day of hospital discharge. Everts et al.[6] only scored function during the first 5 days and on the day of discharge, whereas we scored function on the first 4 days, and at 2-, 6- and 12-weeks post-surgery.

Beneficial effects of concentrated growth factors are said to decrease wound leakage by 25%, minimizing the need for postoperative blood transfusion, decreasing the risk of postoperative infections, and promoting faster functional rehabilitation with less pain.[11, 12] Most reports on PG have discussed its use for healing chronic wounds.[5, 14, 15, 21, 22] To our knowledge, no blinded randomized study has previously been performed.

The type of wound dressing is also important. It has been shown that with the use of occlusive dressings both re-epithelialisation and subsequent collagen synthesis are 2-6 times faster than they are in wounds exposed to air. On a cellular level, dressings assist wound healing by creating a hypoxic wound environment wherein fibroblasts proliferate, and angiogenesis occurs more rapidly.[10] The proper timing of dressing removal remains a controversial topic. Studies on clean, and clean contaminated, wounds showed no difference in infections rates according to whether the dressing was removed on the first postoperative day or at the time of suture removal.[4, 20] In our patients, all wounds were dressed with sealed bandages directly after surgery and undressed the second day post-surgery; no beneficial effect of PG was seen. The use of PG has shown good results in difficult to heal wounds and in wounds compared to normal wound treatment.[13, 17, 19] But exogenous applied platelets have no haemostatic effect. Exogenous applied platelets have no haemostatic effect. They have a poor tensile strength to accomplish wound sealing. Altmeppen et al.[3] have shown that an autologous platelet-enriched plasma cannot be used as a glue in the common sense to seal stitches

or prosthesis. Platelet gels, however, have a high concentration of platelets that release the bioactive proteins and growth factors are necessary to initiate and accelerate tissue repair and enhance dermal and epidermal regeneration.

We used a specially designed wound study scoring system; to our knowledge no measure of wound leakage has previously been described for this type of study.

Several of the patients had incomplete data sets. This is explained by the integration of two hospitals into one during the second half of the trial; we underestimated the deleterious effect of this reorganization on the quality of data collection at ward level. There is no clear explanation for the difference between the rate at which patients from both groups were not recorded; we can only assume that this is coincidental. Between group analysis of patient characteristics at baseline did not show any statistically significant differences of the patients with or without complete data recoding. Due to this dropout of data, we had a severe loss of statistical power with regard to our primary outcome measure (wound closure). However, our results regarding to wound closure, follow a similar trend as all secondary outcome measures, and are compliant with our clinical observations and strengthening our conclusion. Another limitation of our study is that we did not investigate the effect of varying platelet and fibrinogen concentrations.

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CHAPTER 4

Platelet-rich plasma in upper limb conditions

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ABSTRACT

Platelet-rich plasma (PRP) is an autologous platelet concentrate. It has been used since 1990 in the dental and facial reconstructive surgery and its application in other areas is increasing rapidly as a result of the presumably positive effects on bone, muscle and tendon regeneration as well as wound healing. Recently we have learned more about growth factors that play an important role in the healing process. In vitro studies show that growth factors released by platelets have a positive effect on the healing of soft tissue. Despite the presence of minimal clinical evidence, the application of PRP for upper limb conditions has increased. The medical industry promotes the application of PRP significantly. Several randomized studies currently are implemented, although we have to be careful with the use of PRP for upper limb conditions until 'higher level' research is published.

INTRODUCTION

The literature contains many studies documenting the safe and efficacious use of platelet-rich plasma (PRP) for upper limb injuries. In many cases, the terms autogenous platelet concentrate, platelet gel and fibrin glue are used as synonyms for PRP. Many of these studies claim to have excellent outcomes, although they are limited to case series; consequently, it is difficult to draw conclusions from these case reports, which may or may not have controls, have small sample sizes, and do not define a standardized preparation of PRP. This makes it hard to interpret any of the results obtained.[1] Standardized dosing and composition of PRP is necessary in order to compare the data from different studies. Unfortunately, most of these human clinical studies lack statistical significance because of small sample sizes and a paucity of randomized controlled trials. Recently the first two randomized controlled trials using PRP were published. Both studies are of Dutch origin. One deals with chronic achilles tendinopathy and the other one deals with chronic lateral epicondylitis.[2,3] Because of the outcome of the former study, the *New York Times* reported that 'Popular Blood Therapy Might Not Work'. This in contrast to an earlier publication in the *New York Times* of "'New Blood Therapy Saves Superbowl'.[4] The latter study published a positive effect of PRP treating chronic lateral epicondylitis. Efforts are currently underway aiming to design clinical studies that will help further delineate the effects of PRP.

PLATELET-RICH PLASMA

In 1998, discussion started about the use of PRP.[5] PRP is a volume of autologous blood plasma with a platelet concentration above the reference value. The reference value for platelet count in blood is between 150,000/µL and 440,000/µL. PRP commercial application systems have shown that the platelet concentration in PRP can increase between 160% and 740%. To create a possible therapeutic effect, a 400% to 500% increase of platelets is needed to achieve a PRP platelet volume reaching 1,000,000 / µL in a volume of 5 mL.[6]

PRP can accelerate healing by releasing a variety of growth factors and cytokines from activated platelets. The basic cytokines released from platelets include: transforming growth factor (TGF), platelet-derived growth factor (PDGF), insulin-like growth factor-I and II (IGF-I, IGF-II) and vascular endothelial growth factor (VEGF). These cytokines play an important role in cell proliferation, chemotaxis, cell differentiation and angiogenesis (Table 1). [7] All these cytokines in the PRP are normally present in biological ratios.

Table 1 Function of Growth Factors Within Platelet-Rich Plasma

Growth factor	Target	Function
PDGF	Blood vessels, skin cells, fibroblasts, many other cells	Cel growth, recrutement, differentiation, cytokine secretion
PDGF A + B	Fibroblasts, smooth muscle cells, chondrocyts, osteoblasts, mesenchymal stem cells	Cell growth, recrutement, blood vessel growth, granulation, growth factor secretion
TGF-ß1	Blood vessels, skin cells, fibroblasts, osteoblasts	Blood vessel/collagen synthesis, inhibition of growth, apoptosis, differentation, activation
IGF-1, -2	Bone, blood vessels, skin cells, fibroblasts	Cell growth, differentation, recrutement, collagen synthesis together with PDGF
VEGF	Blood vessel cells	Cell growth, migration, blood vessel growth, anti-apoptosis

PDGF, platelet-derived growth factor; TGF, transforming growth factor;
IGF, insulin-like growth factor; VEGF, vascular endothelial growth factor

CELLULAR EVIDENCE FOR PRP

The three general stages of healing (wound, bone, tendon) are inflammation, proliferation and remodelling. The inflammatory phase begins with tissue injury. Platelets are then stimulated to release growth factors, cytokines and haemostatic factors which are required in the initial phase of the clotting process. Histamine and serotonin are issued by the platelets. Both increase capillary permeability, thus improving accessibility to the inflammatory cells to the wound surface and the activation of macrophages. Polymorphonucleaire leukocytes migrate into the tissue where the inflammation occurs. Soon, the cells begin to proliferate, and fibroblasts form a base substance. Activation of adenosine receptor promotes the inflammatory process during wound healing.[7]

Repetitive use of the tendon can lead to changes in the collagen fibre matrix predisposing the tendons to microtears and scarring, affecting functionality and increasing risk for re-injury. In chronic conditions, histologically, there is no further inflammatory response seen. It is a degenerative condition, affecting the metabolic and healing process in a negative manner, and prolonging their chronicities.[8,9]

Currently, injections with corticosteroids have been used as a nonsurgical method. However, this method only has a temporary effect.[10] Furthermore, concerns for atrophy and adverse structural changes, including tears, have limited the use of corticosteroids. PRP has been used in vivo as a potential alternative to corticosteroids. In vitro studies have found that the use of PRP in tendinopathy and human mesenchymal stromal stem cell proliferation can strengthen. The quality of the tendon.[11] The use of PRP has shown that the macrophage proliferation and IL-1 production within the first 72 hours after exposure suppressed.[12] This difference in induction of the cells has important implications for tendon and muscle healing. Initially, PRP is able to inhibit the inflammatory process, whereas it stimulates the proliferation and maturation process. This is particularly important in preventing the formation of fibrous scar tissue that occurs macrophage-mediated tendon to bone healing. Animal studies have shown that the PRP can strengthen tendons.[13,14]

A study by Hall et al.[15] showed that human tenocytes treated with PRP increased the collagen and endothelial expression for vascular repair. Moreover, an increased concentration of the growth factors described above was shown, creating a synergistic regulation of fibrosis, which promotes full muscle and tendon function.[15] Sanchez et al.[9] concluded that PRP facilitated the proliferation of human tendon cells, stimulating the release of multiple repair factors, especially an abundance of hepatocyte growth factor, which help reduce scar formation around tendon tissues. Other studies have also shown promise that local delivery of PRP shortened the recovery time after injury in small animal models, expediating myogenesis and tendinous repair.[16] Histologically, PRP appears to promote cellular repair that would otherwise be limited.

HOW TO PREPARE PRP

Several systems are available for the preparation of PRP in outpatients (Table 2). As an example, we describe the Biomet Biologics III GPS system (Biomet Biologics. LLC, Warsaw, IN, USA). This device uses a desktop-size centrifuge with disposable tubes for the various parts of blood separation. Thirty millilitres to 60 mL of whole blood is increased. An 18- or 19-gauge butterfly needle is recommended so as to avoid irritation and damage of the platelets, which are in a resting position. Thereafter, 3 mL to 6 ml of 8.4% of sodium bicarbonate is added to the blood to avoid clotting. The blood is then placed in a Food and Drug Administration approved device and centrifuged for 15 minutes at a speed of 3200 r.p.m. Subsequently, the blood is separated into platelet-poor plasma (PPP), red blood cells and PRP. Then, the PPP is removed through a special portal from the centrifuge tube. Finally, the PRP can be withdrawn. Depending on the blood, approximately 3 mL to 6 mL of PRP is available. After isolation, the PRP can be administered with or without an activating agent. Combination with calcium chloride and / or injection of trombine initiates platelet activation, clot formation and growth factor release at the injection site. Administration of PRP without an exogenous activator

is often performed and is supported by literature showing that platelets can be slowly activated by exposure to tendon derived collagen alone.[17] For surgical applications, PRP is often treated with calcium chloride before application of trombine. This forms a gel-like substance, which can be applied directly.[18] Several PRP systems are now available that allow an efficient preparation for outpatient use (Table 2). Differences such as the volume of autologous blood, spin rate, activating agent, leukocyte concentration, final PRP volume, and final platelet concentration and growth factor, distinguish the systems which are available. Haematological variation between patients (e.g., the number of leukocytes, platelets) may also affect the final PRP preparation.

The optimal amount of platelets and growth factors necessary for the healing of musculoskeletal injuries is still unclear and remains a matter of discussion. PRP has a clinical effect only if used at a concentration of at least four times the normal concentration of platelets.[6] However, the efficacy of PRP is shown in less concentrated amounts.[18] Given the nature of the autologous PRP, there are concerns about safety. Each injection should be prepared and given by an aseptic technique. Relative contra-indications apply to patients with a history of trombocytopenia, use of anticoagulants, active infection, tumour, metastatic disease or pregnancy. There is no documentation of carcinogenesis, hyperplasia or tumour growth associated with the use of PRP.[19]

PRP activation and the pH of the PRP represents other parameters that are discussed in the literature. Trombine and calcium are traditionally used to activate platelets. This combination results in the formation of a gel that can be used in open surgery but cannot be injected. Trombine and calcium activation results in a rapid release of the contents of the platelets. This requires an immediate use of the PRP. Platelets can also be slowly activated by exposure to collagen derived from the tendon. Variations in partial activation of calcium are also examined.[20] The release of growth factors from PRP is pH-dependent.[21]

Table 2 Platelet-Rich Plasma Preparation Systems

System	Blood volume (mL)	Centrifuge (min/r.p.m.)	PRP volume (mL)	Platelet count	Activator	Growth factors
Autologous Conditioned Plasma (Arthrex Naples, FL, USA)	9	5 min/1500 r.p.m.	3–5	2–3×	None	PDGF (25×) EGF (5×) VEGF (11×) TGF-ß1 (4×) IGF-1 (1×)
Cascade (Musculoskeletal Tissue Foundation Edison, NJ, USA)	9 or 18	First: 6 min/1850 r.p.m. Second: 15 min/ 2450 r.p.m.	2–4	Unknown	Calcium	PDGF (?) EGF (5–10×), VEGF (5–10×) TGF-ß1 (5–10×) IGF-1 (5–10×)
GPS III (Biomet, Warsaw, IN, USA)	27 or 54	15 min/3200 r.p.m.	3–6	4–8×	Calciumchloride or trombine	PDGF (?) EGF (3.9×) VEGF (6.2×) TGF-ß1 (3.6×) IGF-1 (1×)
SmartPReP (Harvest Technologies Plymouth, MA, USA)	20 or 60	14 min/1700 r.p.m.	3–7	4.4–7.6×	Trombine	PDGF (4.4×) EGF (4.4×) VEGF (4.4×) TGF-ß1 (4.4×) IGF-1 (?)

PDGF, platelet-derived growth factor; TGF, transforming growth factor; IGF, insulin-like growth factor; EGF, epithelial growth factor VEGF, vascular endothelial growth factor; (?), concentration of the growth factor is not known

LATERAL EPICONDYLITIS

A PRP injection can be used for patients with refractory lateral epicondylitis of the elbow who have failed conservative treatment, including physical therapy, a counter-force brace, and corticosteroid injections. It is recommended that imaging studies including either magnetic resonance imaging or ultrasound should confirm extensor carpi radialis brevis tendinopathy. The post-injection protocol includes standard rehabilitation for eccentric strengthening and functional progressions, with a gradual return to activities over 6 weeks to 8 weeks. It is not necessary to immobilize the elbow after the injection. The criteria for return to sports may include painless full range of motion with no localized pain or tenderness. Mishra and Pavelko [17] evaluated a series of 140 patients with chronic lateral epicondylar elbow pain. Of those patients, 20 met the inclusion criteria and were offered PRP injection as an alternative to surgery. Fifteen patients underwent PRP injection, and five patients served as controls by undergoing local anaesthetic injection only. The patients undergoing PRP therapy were noted to have 60% improvement at 8 weeks, 81% at 6 months, and 93% at final follow-up (range, 12 months to 38 months). At 8 weeks, three of the five patients in the control group sought treatment outside the study or formally withdrew from the study, limiting the possible comparisons. Therefore, the final outcome data reflects only the patients who were treated with PRP. At the final follow-up (range 12 months to 38 months), 93% of patients were completely satisfied with the treatment, 94% (range 90% to 100%) were able to return to work and sports, and 99% were able to return to activities of daily living. No adverse events or complications were reported. This study has significant design flaws; the sample size is small, and the attrition rate approaches 60%. However, it is one of the few studies performed in a prospective fashion and includes a control group.

In 2003, Edwards and Calandruccio [22] reported a 79% success rate in treating a group of patients with respect to treating a group of patients with refractory chronic epicondylitis. Twenty-two of the 28 patients were reported to be pain-free after autologous blood injection therapy. They injected whole blood that had not been centrifuged, which is different than the preparation for PRP. No adverse events and no recurrences were reported. However, the authors do not comment on the discomfort level at the site of injection in a large portion of their patients in the immediate period following the autologous blood injection. This is a Level 4 study with a small sample size and no control group; consequently, it is difficult to draw definitive conclusions.

The authors of this review have demonstrated in a recent double blind randomized study that the use of PRP after 26 weeks and 1 year follow up has a significant difference in decrease of pain and disability of function in favour of the platelet application measured by the Visual Analogue Scale and Disabilities of the Arm, Shoulder and Hand score compared with the use of corticosteroids for patients with chronic symptoms of tennis elbow (alpha = 0.05 and power 0.9).[23]

SUBACROMIAL IMPIGMENT

Open subacromial decompression (OSD) treatment for chronic impingement syndrome of the shoulder has been well documented.[23-25] The space between the acromion and humeral head is normally narrow and decreases with abduction of the arm. Overuse leads to the development of tendinosis and the formation of granulation tissue in an attempt to repair the damaged tendon. This creates the 'impingement'. Most common symptoms of shoulder impingement are pain, weakness and limitation in the range of motion. During and after OSD surgery, the patients' own defence mechanism is activated to reduce bleeding and initiate wound healing. Platelets play a pivotal role in this process through the formation of a platelet plug and activation of the blood coagulation cascade. Activated platelets at the wound site release several platelet growth factors, which initiate connective tissue healing and increase mitogenesis, angiogenesis and macrophage migration.[26,27] Treatment with PRP provides a source of concentrated platelets, with granules that contain PDGF and TGF. These growth factors augment the wound healing process.[28,29] Everts et al.[30] report the results of a randomized controlled trial that evaluated PRP application in patients undergoing open subacromial decompression. The purpose of their study was to evaluate the effect of PRP on surgical wound healing with emphasis on the restoration of range of motion, activities of daily living, pain and pain medication, as part of the treatment of the impingement syndrome (Neer grade II) using the American Shoulder and Elbow Surgeons shoulder assessment method to evaluate the study objectives. [31] In the PRP-treated group, patients had a statistically faster recovery with less pain medication requirement, greater range of motion, and greater ability to perform activities of daily living.

The high concentration of non-activated leukocytes, present in the PRP, promotes antimicrobial activity at the wound site through destruction of bacteria and foreign materials and removal of damaged tissue. [32,33] The success rate of an OSD depends on a rapid recovery of the shoulder function. Time to recovery can be improved through good wound healing and less post-operative pain.

ROTATOR CUFF TEAR

The literature reveals that, despite the technical expertise of the surgeon, a significant failure rate can be expected after rotator cuff repair. The biological milieu at the rotator cuff footprint and the inherent poor healing potential of the distal rotator cuff tendon create an environment that is not optimal for healing of the tendon to bone. Augmentation of the rotator cuff repair with PRP could hypothetically optimize the biologic environment at the repair site and allow for a more robust healing response at the osseous-tendon interface. The intra-operative use of PRP augmentation of the rotator cuff repair has been gaining in popularity among shoulder surgeons. Gamradt et al.[34] have reviewed the basic science regarding the use of autogenous platelets and growth factors used to enhance the healing of the repaired rotator cuff. PRP augmentation of rotator cuff repair at the tendon-bone interface is described in this review. This same group of researchers is currently conducting a randomized clinical trial assessing the efficacy of their described technique.

In addition, the use of stem cells for the augmentation of rotator cuff repair is under investigation. Stem cells can give rise to specialized cells. When unspecialized stem cells give rise to specialized cells, the process is called differentiation. Scientists are just beginning to understand the signals inside and outside cells that trigger stem cell differentiation. The internal signals are controlled by a cell's genes, which are interspersed across long strands of DNA, and carry coded instructions for all the structures and functions of a cell.

Randelli et al.[35] conducted an uncontrolled pilot study of PRP augmented arthroscopic rotator cuff repair to evaluate safety and outcome. In 14 patients, PRP was injected with thrombin (GPS II Platelet Concentrate System, Biomet Biologics, LLC) into the footprint after the repair was performed and the irrigation ceased.[35] There were no complications, and, at a mean of 2 years, all patients had statistically significant improvements in VAS, Constant, and University of California Los Angeles shoulder scores compared with pre-operative values. The authors are currently conducting a randomized controlled trial to ascertain the efficacy of PRP in arthroscopic rotator cuff repair.

SHOULDER OSTEOARTHRITIS

The use of PRP for total shoulder arthroplasty (TSA) has not been well documented. The only publication so far know is by Zavadil et al.[39] They described that the recovery of patients undergoing TSA can be adversely affected by a number of complications. Their study examines the effect of autologous platelet gel (APG) and PPP treatment on TSA patients post-operatively. Forty patients underwent TSA. They were enrolled in either a study group (n = 20) or a control group (n = 20), with the study group receiving APG and PPP. The patients and all affected parties in the post-operative care were blinded. Pre-operative demographic data, pre- and post-operative laboratory data, pain scores, pain medication, pre- and post-operative range of motion scores, complications and post-operative length of stay was recorded for each patient in each group. The treatment group had significantly lower pain and medication scores compared with the control group. The internal rotation index improvement factor was significantly higher in the treatment group.

Zavadil et al.[39] showed that the use of APG in combination with PPP has a beneficial outcome regarding pain control and range of motion after TSA.

CONCLUSION

Acceleration of tendon healing with PRP appears to be promising, although there is currently little clinical evidence available to support its use. Well-designed, controlled clinical trials are under way and are necessary to determine the therapeutic value of PRP. More substantiated, clinical data are needed to determine its efficacy; standardized preparation and composition will be necessary to compare results. Post-procedure rehabilitation protocols must also be established to determine optimal tendon healing.

Further laboratory research must be performed to determine the optimal activation in addition to growth factor, platelet, and leukocyte concentrations. Several PRP preparation systems are now available, and orthopaedic surgeons and sports medicine physicians must be aware of their differences. Given its excellent safety profile and ease of preparation, the use of PRP in orthopaedic surgery will likely continue to grow; however, clinical use should proceed cautiously because there is little, if any, high-level clinical evidence supporting the efficacy of this therapeutic modality.

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CHAPTER 5

Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial: Platelet-rich plasma versus corticosteroid injection with a 1-year follow-up

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ABSTRACT

Background: Platelet-rich plasma (PRP) has shown to be a general stimulation for repair.

Purpose: To determine the effectiveness of PRP compared with corticosteroid injections in patients with chronic lateral epicondylitis.

Study Design: Randomized controlled trial; Level of evidence, 1.

Patients: The trial was conducted in 2 teaching hospitals in the Netherlands. One hundred patients with chronic lateral epicondylitis were randomly assigned in the PRP group (n = 51) or the corticosteroid group (n = 49). A central computer system carried out randomization and allocation to the trial group. Patients were randomized to receive either a corticosteroid injection or an autologous platelet concentrate injection through a peppering technique. The primary analysis included visual analogue score and DASH Outcome Measure scores (DASH: Disabilities of the Arm, Shoulder, and Hand).

Results: Successful treatment was defined as more than a 25% reduction in visual analogue score or DASH score without a reintervention after 1 year. The results showed that, according to the visual analogue scores, 24 of the 49 patients (49%) in the corticosteroid group and 37 of the 51 patients (73%) in the PRP group were successful, which was significantly different (P <.001). Furthermore, according to the DASH scores, 25 of the 49 patients (51%) in the corticosteroid group and 37 of the 51 patients (73%) in the PRP group were successful, which was also significantly different (P = .005). The corticosteroid group was better initially and then declined, whereas the PRP group progressively improved.

Conclusion: Treatment of patients with chronic lateral epicondylitis with PRP reduces pain and significantly increases function, exceeding the effect of corticosteroid injection. Future decisions for application of the PRP for lateral epicondylitis should be con- firmed by further follow-up from this trial and should take into account possible costs and harms as well as benefits.

Keywords: lateral epicondylitis; platelet rich plasma; corticosteroids; pian; function

INTRODUCTION

Lateral epicondylitis is the most commonly diagnosed condition of the elbow, affecting approximately 1% to 3% of the population. The condition mostly occurs in patients whose activities require strong gripping or repetitive wrist movements. Individuals between the ages of 35 and 50 years are at high risk. The dominant arm is most frequently affected.[7,8,13]

The cause of lateral epicondylitis is unknown. It is thought that lesions occur in the common origin of the wrist and finger extensors on the lateral epicondyle owing to a combination of mechanical overloading and abnormal microvascular responses.[12,19,24]

Numerous methods have been advocated for treating elbow tendinosis, including rest, nonsteroidal anti-inflammatory medication, bracing, physical therapy, extracorporeal shock wave therapy, and botulism toxin injection. Injection of corticosteroids (once considered the gold standard but now controversial), whole blood injections, and various types of surgical procedures have also been recommended.[2,3,16,18,25]

In an animal model, the addition of growth factors to the ruptured tendon has been shown to increase the healing of the tendon.[1,11] In humans, it has been shown that the injection of whole blood into the tendon decreases the pain.[3]

Platelet-rich plasma (PRP) is promoted as an ideal autologous biological blood-derived product that can be exogenously applied to various tissues, where it releases high concentrations of platelet-derived growth factors that enhance wound healing, bone healing, and tendon healing.[14] In addition, PRP possesses antimicrobial properties that may contribute to the prevention of infections.[5] When platelets become activated, growth factors are released and initiate the body's natural healing response. In a double-blind randomized trial, we investigated whether injection of concentrated autologous platelets improves the outcome of patients with lateral epicondylitis more so than corticosteroid injection. The primary outcome parameters were pain and daily use of the elbow.

METHODS

This double-blinded randomized trial included 100 consecutive patients with lateral epicondylitis scheduled for injection therapy in 2 Dutch training hospitals between May 2006 and January 2008.

All procedures used the same injection procedure, performed by an orthopaedic consultant or a supervised orthopaedic resident. Criteria for participation included lateral epicondylitis for longer than 6 months and pain of at least 50 on a visual analogue

score (VAS) for pain (0, no pain; 100 maximum pain possible). Lateral epicondylitis was defined as pain over the lateral epicondyle on direct palpation and pain in that area during resisted wrist extension. All affected elbows were screened with radiography and all proved to be normal, except for some calcifications of the common extensor origin. Sonography and magnetic resonance imaging were not standardly used. Patients had a clinical diagnosis of lateral epicondylitis, or lateral elbow pain increased by pressure on the lateral epicondyle and during resisted extension of the wrist. All patients suffered for more than 6 months. Before 6 months of the trial, they were treated with cast immobilization, injections with corticosteroids, or physiotherapy.

Exclusion criteria were as follows: age less than 18 years, pregnancy, history of carpal tunnel syndrome or cervical radiculopathy, and systemic disorders such as diabetes, rheumatoid arthritis, and hepatitis. Also, patients were excluded if they had been treated for lateral epicondylitis with surgical intervention or with a corticosteroid injection in the past 6 months.

The primary endpoint was a 25% reduction in the VAS score or DASH Outcome Measure score (DASH: Disabilities of the Arm, Shoulder, and Hand) without a reintervention after 1 year. In the current study, we tested the hypothesis that the injection of concentrated autologous platelets increases the healing of patients with tendinitis compared with those treated with a steroid injection.

Statistical data were collected to determine the power of both groups. Successful treatment in the PRP group was determined by using the results of Mishra and Pavelko.[10] In this study, 93% of the patients with chronic lateral epicondylitis that received PRP were considered successful-that is, with more than a 25% decrease in pain. Successful treatment in the control group was determined by using the results of Hay and colleagues,[6] who studied the effect of corticosteroid injections for chronic lateral epicondylitis. Full recovery or decrease in complaints without complications was seen in 65% of the patients in the corticosteroid group. With a bilateral alpha of .05 and a power of 90% (p1 = .93 and p2 = .65), 42 patients per group are necessary to measure the difference with the Chi-square test. To correct for the patients who were lost to follow-up, we included a minimum of 50 patients in each group. The Medical Ethical Committee and the National and Institutional Review Board approved the study.

Randomization

Randomization was performed after patients were deemed eligible and had provided informed consent. Patients were randomly allocated to the concentrated autologous platelet group (PRP group) or the corticosteroid group (control group). A computer using block randomization of 10 patients was used to create a randomization schedule. Treatment assignments (placed in sequentially numbered opaque envelopes) were assigned by the trial managers, who also arranged the facilities needed for the procedure.

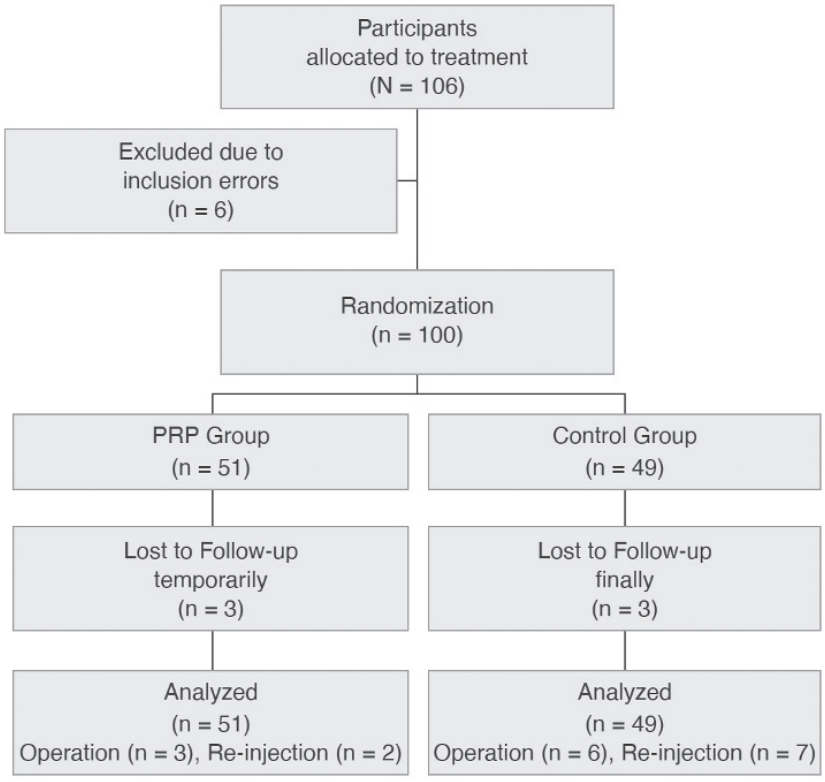


Figure 1. Flow diagram of a trial of injection therapy for chronic lateral epicondylitis. The diagram includes the number of patients actively followed up at different times during the trial.

PRP Preparation

In the group randomized to receive PRP, the patient's own platelets were collected with the Recover System (Biomet Biologics, Warsaw, Indiana). This device uses a desktop-size centrifuge with disposable cylinders to isolate the platelet-rich fraction from a small volume of the patient's anticoagulated blood, drawn at the time of the procedure. As part of the double-blind procedure, blood was also collected from the patients in the control group. In sum, 27 mL of whole blood was collected from the uninvolved arm into a 30-mL syringe that contained 3 mL sodium citrate. The platelet-rich fraction was prepared according to the instructions of the Recover System. Approximately 3 mL PRP was obtained for each patient. The PRP was then buffered to physiologic pH using 8.4% sodium bicarbonate, and bupivacaine hydrochloride 0.5% with epinephrine (1:200000) was added. No activating agent was used. After masking the tubes with opaque tape, the investigator returned and injected 3 mL of this PRP into the patient. The total time from blood draw to injection in the patients was about 30 minutes. No specialized equipment was required, other than the centrifuge to process the Recover disposable cylinders. All the procedures were performed in the same office setting by

an independent person certified for blood management, without the investigator or the patient present.

Injection Technique

Approximately 1 mL of PRP or corticosteroids (kenacort 40 mg/mL triamcinolone acetonide) with bupivacaine hydrochloride 0.5% with epinephrine (1:200000) was injected directly into the area of maximum tenderness. Then, using a 22-gauge needle and a peppering technique, the investigator injected the remaining PRP or corticosteroids with bupivacaine hydrochloride 0.5% with epinephrine (1:200000, ± 4 mL) into the common extensor tendon. This technique involved a single skin portal and 5 penetrations of the tendon.

Postprocedure Protocol

Immediately after the injection, the patient was kept in a supine position without moving the arm for 15 minutes. Patients were sent home with instructions to rest the arm for approximately 24 hours. If necessary, patients were allowed to use acetaminophen, but the use of nonsteroidal anti-inflammatory medication was prohibited. After 24 hours, patients were given a standardized stretching protocol to follow for 2 weeks under the supervision of a physiotherapist. A formal eccentric muscle- and tendon-strengthening program was initiated after this stretching. At 4 weeks after the procedure, patients were allowed to proceed with normal sporting or recreational activities as tolerated. The VAS and DASH function scores were measured before injection and at 4, 8, 12, 26, and 52 weeks after injection. The DASH score is a validated upper limb functional score.[22]

STATISTICAL ANALYSIS

All data analysis was carried out according to a preestablished analysis plan, on a last-observation-carried-forward basis. The categorical values are compared with the Pearson Chi-square test. The preoperative continuous variables are compared with the *t* test. The VAS and DASH scores are compared with an analysis of variance with repeated measurements test. The significance level was set at $P = .05$ for all tests, and SPSS 16.0 was used.

RESULTS

From May 2006 to January 2008, a total of 100 eligible patients with lateral epicondylitis were randomized into groups. Eight patients were lost to follow-up or had incomplete data sets; however, they needed no reintervention (Figure 1). Their data are included in the analysis until their last visit. Analysis of the demographics (sex, side, and centre) between the protocol-compliant patients and those lost to follow-up showed no significant differences (Table 1).

Table 1 Patient Demographics

	Corticosteroid	Platelet-Rich Plasma	*P*
Number	51	49	
Age, y	47.3 ± 7.6	46.9 ± 8.4	.797[a]
Sex: male, female	25, 26	23, 26	.840[b]
Side: right, left	32, 19	31, 18	.957[b]
Lost to follow-up	1	3	.700[b]
Reinterventions	13	5	.970[b]

[a] *t* test. [b] Chi-square test.

The mean patient age was 47 years. There were 48 men and 52 women. The study included 63 patients with lateral epicondylitis on the right elbow and 37 patients with symptoms on the left elbow. The ratio between dominant and nondominant side was according to the literature: 65%. In most cases, the dominant side was involved.[23] The ratio was equally distributed. The activity level of the patients, preintervention and postintervention, has been noted in the DASH score.

Eighteen patients needed a reintervention. The patients who needed a reintervention were all scored as nonsuccessful. Between the 2 hospitals, there were no significant differences between the protocol-compliant patients and the reintervention patients (*P* = .168). The primary analysis was conducted on a carried-forward principle and involved 100 patients.

In total, 18 reinterventions or operations were needed after an average of 5 months (range, 2-6 months). In the PRP group, 3 patients obtained an operation and 2 patients a reinjection with corticosteroids. In the corticosteroid group, 6 patients required an operation, 1 a reinjection with corticosteroid, and 6 a reinjection with PRP after 6 months of follow-up (Table 2). The percentages of reintervention did not depend on age, gender, side, treatment, or preoperative VAS or DASH score.

Table 2 Flow Chart of Patients

	Corticosteroid Group	Platelet Group	Total
Free of complications	35	43	78
Temporarily lost to follow-up	0	3	3
Operation	6	3	9
Corticosteroid injection (second injection)	1	2	3
Platelet concentrate injection (second injection)	6	0	6
Lost to follow-up	1	0	1
Total	49	51	100

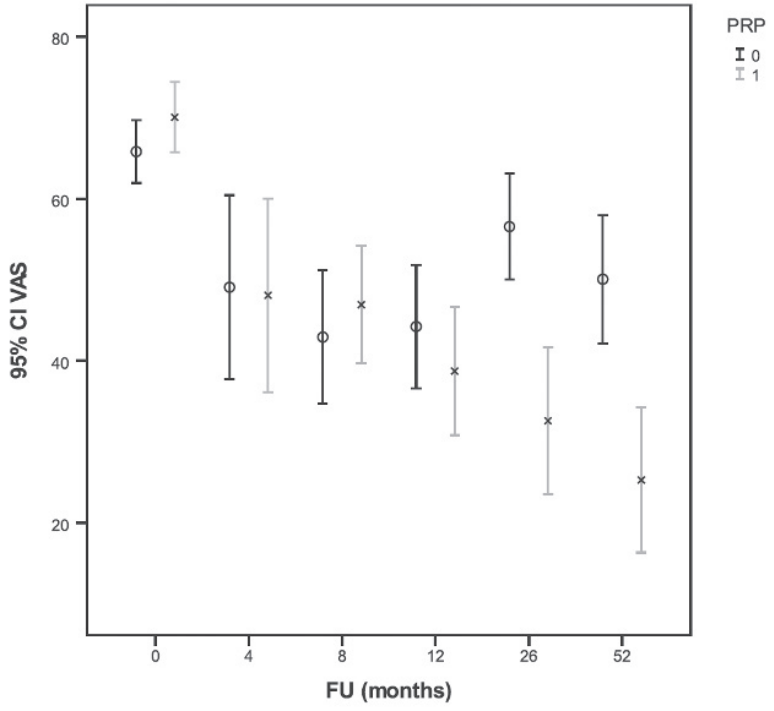
Six months after the initial treatment, the patients who were operated had VAS and DASH scores (respectively, 54.3 ± 26 and 112.2 ± 75.2) that were significantly worse than those of the nonoperated patients (P = .04). The patients needing a second injection had comparable VAS and DASH scores (60.6 ± 29 and 94.6 ± 62.2; P = .0196) as the patients who did not have a second injection.

Initially, the PRP-treated patients had a mean VAS score of 70.1 ± 15.1 and a mean DASH score of 161.3 ± 62.3. The control patients had a mean VAS score of 65.8 ± 13.8 and a mean DASH score of 131.2 ± 58.2. Four weeks after the procedure, PRP-treated patients reported a mean improvement of 21% in their VAS scores (70.1 to 55.4) compared with the initial values, whereas the corticosteroid-treated patients reported a 32.8% improvement (65.8 to 44.2; P = .077) (Figure 2). Also, after 4 weeks, DASH scores had improved 15.7% (161.3 to 135.9) in PRP patients versus a 25.8% improvement (131.3 to 97.4) in corticosteroid-treated patients (P = .469) (Figure 3).

Eight weeks after the procedure, PRP-treated patients reported a mean improvement of 33.1% (70.1 to 46.9) in their VAS scores compared with the initial values, whereas the corticosteroid-treated patients reported a 34.8% improvement (65.8 to 42.9; P = .818) (Figure 2). After 8 weeks, DASH scores improved 29.7% (161.3 to 113.4) in PRP patients versus a 35.5% improvement (131.26 to 84.7) in corticosteroid-treated patients (P = .999) (Figure 3).

Twelve weeks after the procedure, PRP-treated patients reported a mean improvement of 44.8% (70.1 to 38.7) in their VAS scores compared with the initial values, whereas the corticosteroid-treated patients reported a 32.8% improvement (65.8 to 44.2; P = .206) (Figure 2). Also, after 12 weeks, DASH scores had improved 43.0% (161.3 to 92.0) in PRP

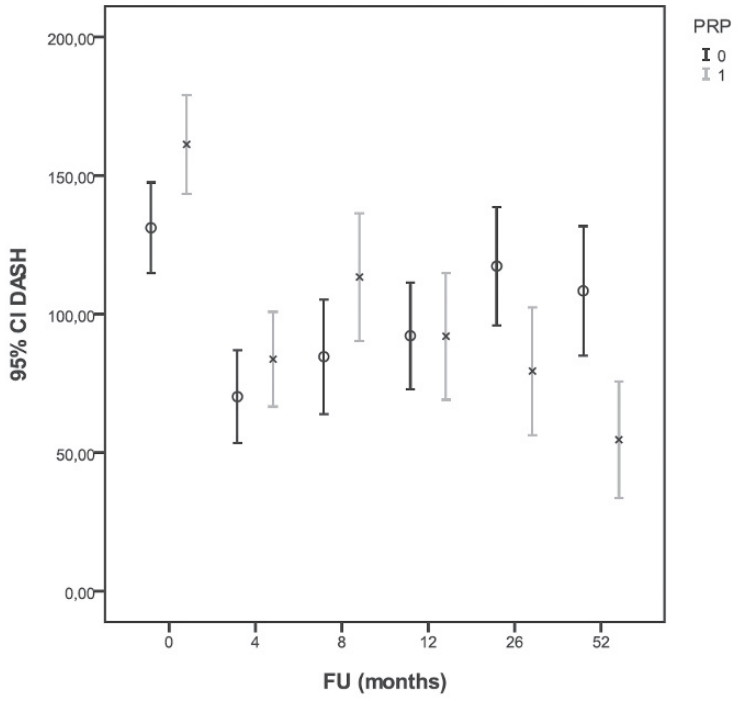
patients versus a 29.8% improvement (131.3 to 92.2) in corticosteroid-treated patients (*P* = .060) (Figure 3).



	t (wks)	CS			PRP		
		average	±	St.dev.	average	±	St.dev.
VAS	0	65,8	±	13,8	70,1	±	15,1
	4	44,2	±	26,4	55,4	±	24,2
	8	42,9	±	29,2	46,9	±	24,9
	12	44,2	±	27,1	38,7	±	27,2
	26	56,6	±	23,2	32,6	±	31,5
	52	50,1	±	28,1	25,3	±	31,2

Figure 2. Twenty-four of the 49 patients (49%) in the corticosteroid (CS) group and 37 of the 51 patients (73%) in the platelet-rich plasma (PRP) group were defined as successful with the visual analogue score (VAS), a significant difference (P < .001). CI, confidence interval.

Six months after the procedure, PRP-treated patients reported a mean improvement of 53.5% (70.1 to 32.6) in their VAS scores compared with the initial values, whereas the corticosteroid-treated patients reported a 14.0% improvement (65.8 to 56.6; *P* < .001) (Figure 2). Also, after 6 months, DASH scores had improved 50.7% (161.3 to 79.5) in PRP patients versus a 10.7% improvement (131.3 to 117.3) in corticosteroid-treated patients (*P* = .003) (Figure 3).



	t (wks)	CS			PRP		
		average	±	St.dev.	average	±	St.dev.
DASH	0	131,2	±	58,2	161,3	±	62,4
	4	97,4	±	69,0	135,9	±	78,0
	8	84,7	±	73,4	113,4	±	79,6
	12	92,2	±	68,7	92,0	±	78,8
	26	117,3	±	75,6	79,5	±	80,3
	52	108,4	±	82,2	54,7	±	73,2

Figure 3. Twenty-five of the 49 patients (51%) in the corticosteroid (CS) group and 37 of the 51 patients (73%) patients in the platelet-rich plasma (PRP) group were defined as successful with the DASH Outcome Measure, a significant difference (P = .005). CI, confidence interval.

One year after the procedure, PRP-treated patients reported a mean improvement of 63.9% (70.1 to 25.3) in their VAS scores compared with the initial values, whereas the corticosteroid-treated patients reported a 24.0% improvement (65.8 to 50.1; $P < .001$) (Figure 2). Also, after 1 year, DASH scores improved 66% (161.3 to 54.7) in PRP patients versus a 17.4% improvement (131.3 to 108.4) in corticosteroid-treated patients ($P = .001$) (Figure 3).

Regarding the patients who failed their treatment, those who crossed over to the PRP group and those who received surgery did finally benefit. The patients who received a

reinjection with corticosteroids did not see a resolution of pain and disability, according to the mentioned criteria.

Successful treatment was defined as more than a 25% reduction in VAS or DASH score without a reintervention after 1 year. The results showed that 24 of the 49 patients (49%) in the corticosteroid group and 37 of the 51 patients (73%) in the PRP group were successful with the VAS score, which was significant ($P < .001$). Twenty-five of the 49 patients (51%) in the corticosteroid group and 37 of the 51 patients (73%) in the PRP group were successful with the DASH score, which was also significant ($P = .005$).

No fevers or rashes were reported. Apart from the local inflammation causing increased pain 3 to 4 weeks after the injection, no systemic or other local reactions were seen. The effect can be characterized as a local mechanism, without systemic side effects.

If we set the criteria for success at 50% or 75% improvement of both scores (instead of 25% improvement), the results still show significant differences between both groups, as shown in Tables 3 and 4.

Table 3 Pain Resolution for the Corticosteroid (CS) and Platelet-Rich Plasma (PRP) Groups

			Pain Reduction		Percentage Pain Reduction (%)[a]				
Time, wks	Group	n	Average ± SE	*t*	>75%	50%-75%	25%-50%	<25%	x^2
4	CS	51	–21.6 ± 3.5		17.6	15.7	19.6	47.1	
	PRP	48	–15.8 ± 3.6	.14	4.2	10.4	29.2	56.3	.12
8	CS	51	–22.9 ± 4.0		23.5	11.8	17.6	47.1	
	PRP	48	–22.9 ± 3.5	.99	12.5	16.7	31.3	39.6	.22
12	CS	51	–21.6 ± 3.6		15.7	21.6	15.7	47.1	
	PRP	48	–31.1 ± 4.2	.09	18.8	33.3	14.6	33.3	.46
26	CS	51	–9.3 ± 3.1		3.9	7.8	27.5	60.8	
	PRP	49	–37.4 ± 4.6	< .001	40.8	18.4	10.2	30.6	< .001
52	CS	50	–15.7 ± 3.5		13.7	9.8	23.5	52.9	
	PRP	49	–44.8 ± 4.4	< .001	57.1	8.2	10.2	24.5	<.001

[a] For all patients, the pain reduction score is classified as > 75%, 50%-75%, 25%-50%, or < 25% pain reduction. For all time points, the percentage of patients in each category is calculated. The 2 groups are compared with the Chi-square test.

Table 4 Disability Resolution for the Corticosteroid (CS) and Platelet-Rich Plasma (PRP) Groups

			Disability Reduction		Percentage Disability Reduction (%)[α]				
Time, wks	Group	n	Average ± SE	*t*	>75%	50%-75%	25%-50%	<25%	x^2
4	CS	51	–33.8 ± 5.2		17.6	15.7	17.6	49.0	
	PRP	48	–24.6 ± 6.4	.42	4.2	12.5	29.2	54.2	.12
8	CS	51	–46.5 ± 6.7		29.4	9.8	17.6	43.1	
	PRP	48	–57.1 ± 8.7	.96	16.7	14.6	20.8	47.9	.48
12	CS	51	–39.0 ± 6.5		23.5	5.9	13.7	56.9	
	PRP	48	–68.5 ± 9.7	.03	27.1	20.8	18.8	33.3	.05
26	CS	51	–13.8 ± 7.7		13.7	7.8	11.8	66.7	
	PRP	49	–79.4 ± 11.8	< .001	38.8	14.3	14.3	32.7	.01
52	CS	50	–22.4 ± 8.6		24.0	12.0	14.0	50.0	
	PRP	49	–106.6 ± 8.7	<.001	57.1	4.1	14.3	24.5	.01

[α] For all patients, the disability reduction score is classified as > 75%, 50%-75%, 25%-50%, or < 25% disability reduction. For all time points, the percentage of patients in each category is calculated. The 2 groups are compared with the Chi-square test.

Regarding the cost, PRP is not cost-effective when compared with corticosteroid on a short-term basis. A PRP treatment costs around €200 (current US$300, as of November 2009). The DBC price for injection treatment is €360 (US$540; DBC stands for Diagnose Behandeling Combinatie, or Diagnosis Treatment Combination). A DBC is an administrative code that combines diagnosis, treatment, and all the related costs; a DBC therefore includes all treatments per diagnosis, from the first visit to the last check-up. So, the overall cost for a PRP injection will be around €560 (US$840) compared with the corticosteroid injection of around €200 (US$300). But this does not include all socioeconomic costs.

DISCUSSION

This randomized study was designed to test the use of concentrated autologous platelets in patients with lateral epicondylitis; its application proved to be both safe and easy. The corticosteroid group was actually better initially and then declined, whereas the PRP group progressively improved. There was a significant difference in decrease of pain and disability of function following the platelet application after 26 weeks and 1 year.

Lateral epicondylitis is a common problem with many available treatment methods. The most commonly recommended treatment is physiotherapy and bracing. Approximately 87% of the patients benefit from this combination of treatment methods.[20]

Now controversial, corticosteroid injection was once considered the gold standard in the treatment of lateral epicondylitis. However, studies show that it is merely the best treatment option in the short-term, when compared with physiotherapy and wait-and-see policy. Poor results are often seen after the 12-week follow-up.[18] Treatment with corticosteroids has a high frequency of relapse and recurrence, probably because intratendinous injection may lead to permanent adverse changes within the structure of the tendon and because patients tend to overuse the arm after injection as a result of direct pain relief.[18]

In a meta-analysis, Smidt and colleagues [17] showed that the effects of steroid injections as compared with placebo injection, injection with local anaesthetics, injection with another steroid, or another conservative treatment are not significantly different in the intermediate and long-term. However, the patients who were examined all had short-term lateral epicondylitis.

There are various types of surgical procedures for patients with chronic lateral epicondylitis. Verhaar and colleagues noted an improvement in 60% to 70% of the patients after surgical treatment, although higher success rates (80% to 90%) have more recently been reported.[21,23] Patients remain, however, interested in an alternative to surgical intervention.

Platelet-rich plasma is promoted as an ideal autologous biological blood-derived product that can be exogenously applied to various tissues where, after being activated, it releases high concentrations of platelet-derived growth factors that enhance tissue healing.[5,26] With the Recover System, the patient's own platelets can be collected into a highly concentrated formula. No activation agent was used during our procedure. The activation of the platelets will occur through the exposure of platelets to the thrombin, which is released from the tendon tissue during the peppering technique.

During the first 2 days of tendon healing, an inflammatory process is initiated by migration of neutrophils and, subsequently, macrophages to the degenerative tissue site. In turn, activated macrophages release multiple growth factors, including platelet-derived growth factor, transforming growth factors alpha and beta, interleukin-1, and fibroblast growth factor.[4] Angiogenesis and fibroplasia start shortly after day 3, followed by collagen synthesis on days 3 to 5. This process leads to an early increase in tendon breaking strength, which is the most important tendon healing parameter, followed by epithelization and, ultimately, the remodelling process. This was confirmed in an animal study.[1]

The treatment of tendinosis with an injection of concentrated autologous platelets may be a nonoperative alternative. Injection of autologous platelets has been shown to improve repair in tendinosis in several animal and in vitro models.[9,15] A possible

explanation for the long-lasting effect of platelets could be that platelets improve the early neotendon properties so that the cells are able to perceive and respond to mechanical loading at an early time point.[1] The results of the present study confirm the suggested positive effect in vivo as described by Mishra and Pavelko.[10] They reported a significant improvement of symptoms after 8 weeks in 60% of the patients treated with PRP versus 16% of the patients treated with a local anaesthetic. After 6 months the improvement in patients treated with PRP was 81%. They compared PRP with a local anaesthetic, which is not an accepted treatment for lateral epicondylitis in the Netherlands. Furthermore, they injected only 15 patients with PRP and compared them with 5 patients treated with a local anaesthetic. The study was underpowered, and the patients were not randomized.

Our results confirm the results of Edwards and Calandruccio.[3] They injected whole blood into patients with lateral epicondylitis. Treatment success was seen in 79% of patients; however, multiple injections were necessary in 32% of patients. The limitation of this study is that all patients had failed previous nonsurgical treatments, including prior steroid injections. Furthermore, some patients had a beneficial effect after receiving more than 1 injection. In our study, a single percutaneous injection of PRP or corticosteroid was used with a peppering technique. Repeated injections might be beneficial in patients who had suboptimal results after the initial injection, although no evidence for a beneficial effect of more than one injection exists.

Twenty-six weeks (6 months) was chosen as the cut-off point to consider whether the therapy was successful or not; however, we achieved significant results after only 26 weeks. We know that the natural history of lateral epicondylitis predominantly results in healed patients (80%) within 1 year, but all patients in the present study had complaints for at least 6 months, thereby putting their improvement past the 1-year mark. In both the corticosteroid group and the PRP group, each patient has a natural history; as such and because the population was randomized, we can expect natural history to have the same influence on both groups.

In conclusion, this report describes the first comparison of an autologous platelet concentrate with the gold standard, corticosteroid injection, as a treatment for lateral epicondylitis in patients who have failed nonoperative treatment. It demonstrates that a single injection of concentrated autologous platelets improves pain and function more so than corticosteroid injection. These improvements were sustained over time with no reported complications. Perhaps for athletes it is less optimal, but all depends on the demands of the patient. We had no elite athletes in our population.

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CHAPTER 6

Ongoing positive effect of platelet-rich plasma in lateral epicondylitis. A double-blind randomized controlled trial: PRP versus corticosteroid injection with a 2-year follow-up

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ABSTRACT

Background: Platelet-rich plasma (PRP) has been shown to be a general stimulation for repair and 1-year results showed promising success percentages.

Purpose: This trial was undertaken to determine the effectiveness of PRP compared with corticosteroid injections in patients with chronic lateral epicondylitis with a 2-year follow-up.

Study Design: Randomized controlled trial; Level of evidence, 1.

Methods: The trial was conducted in 2 Dutch teaching hospitals. One hundred patients with chronic lateral epicondylitis were randomly assigned to a leukocyte enriched PRP group (n = 51) or the corticosteroid group (n = 49). Randomization and allocation to the trial group were carried out by a central computer system. Patients received either a corticosteroid injection or an autologous platelet concentrate injection through a peppering needling technique. The primary analysis included visual analogue scale (VAS) pain scores and Disabilities of the Arm, Shoulder and Hand (DASH) outcome scores.

Results: The PRP group was more often successfully treated than the corticosteroid group ($P < .0001$). Success was defined as a reduction of 25% on VAS or DASH scores without a reintervention after 2 years. When baseline VAS and DASH scores were compared with the scores at 2-year follow-up, both groups significantly improved across time (intention-to-treat principle). However, the DASH scores of the corticosteroid group returned to baseline levels, while those of the PRP group significantly improved (as-treated principle). There were no complications related to the use of PRP.

Conclusion: Treatment of patients with chronic lateral epicondylitis with PRP reduces pain and increases function significantly, exceeding the effect of corticosteroid injection even after a follow-up of 2 years. Future decisions for application of PRP for lateral epicondylitis should be confirmed by further follow-up from this trial and should take into account possible costs and harms as well as benefits.

Keywords: lateral epicondylitis; platelet-rich plasma; corticosteroids; pain; disability

INTRODUCTION

Lateral epicondylitis is the most commonly diagnosed condition of the elbow and affects approximately 1% to 3% of the population. The condition mostly occurs in patients whose activities require strong gripping or repetitive wrist movements. Individuals between the ages of 35 and 50 years are at high risk. The dominant arm is most frequently affected.[11,12,19]

The cause of lateral epicondylitis is unknown. It is thought that lesions occur in the common origin of the wrist and finger extensors on the lateral epicondyle because of a combination of mechanical overloading and abnormal microvascular responses.[18,29,34]

Numerous methods have been advocated for treating elbow tendinosis, including rest, nonsteroidal anti-inflammatory medication, bracing, physical therapy, extracorporeal shockwave therapy, and botulinum toxin injection. Injection of corticosteroids, which was considered to be the gold standard before but is actually currently controversial, or whole-blood injections and various types of surgical procedures have also been recommended.[2,6,25,28,35]

In an animal model, the addition of growth factors to the ruptured tendon has been shown to increase the healing of the tendon.[1,16] In humans, the injection of whole blood into the tendon at least decreases pain.[6]

Platelet-rich plasma (PRP) is promoted as an ideal biologic autologous blood-derived product. It can be exogenously applied to various tissues where, upon platelet activation, a release of high concentrations of platelet-derived growth factors occurs. Platelet-rich plasma applications enhance wound healing, bone healing, and also tendon healing.[22] In addition, PRP also possesses antimicrobial properties that may contribute to the prevention of infections.[8] As nowadays various different ways to produce PRP are available, it is of eminent importance to discriminate between leukocyte-enriched or leukocyte-deleted PRP. Accordingly, platelet concentrates have been categorized in either pure PRP (P-PRP), in which leukocytes are purposely eliminated from the PRP, or leukocyte and PRP (L-PRP), containing a high concentration of leukocytes.[5]

We recently published the 1-year results of a double-blind randomized trial showing the improved outcome of patients with epicondylitis after an injection of concentrated autologous leukocytes and platelets compared with corticosteroid injection.[20] Few studies have examined the effectiveness of PRP against corticosteroids. The primary outcome parameters were pain and daily use of the elbow. However, as data on a longer follow-up regarding the effectiveness of PRP are currently lacking, we now present the 2-year follow-up of this trial using the same outcome parameters.

METHODS

This double-blind randomized trial included 100 consecutive patients with lateral epicondylitis for injection therapy in 2 Dutch training hospitals (St Elisabeth Hospital and Haga Hospital) between May 2006 and January 2008. The PRP preparation was done using the Recover system (Biomet Biologics, Warsaw, Indiana). This device uses a desktop-size centrifuge with disposable cylinders to isolate the platelet and leukocyte-rich fraction from a small volume of the patient's anticoagulated blood drawn at the time of the procedure. Both PRP and corticosteroids were injected into the common extensor tendon using a 22-gauge needle and a peppering technique. Further information on the study design, power analysis, enrolment criteria, and treatment methods can be found in the 1-year follow-up report.[20] The Medical Ethical Committee and the National and Institutional Review Board approved the study. This trial is registered with identifier number 2007-004947-31 at http:// www.clinicaltrials.gov.

Instruments

Patients completed 2 self-report instruments at every time point: The Disabilities of the Arm, Shoulder and Hand (DASH) outcome measure and a visual analogue scale (VAS) for pain.

The DASH is a 30-item, self-report questionnaire designed to assess physical function and symptoms in persons with any of several musculoskeletal disorders of the upper limbs.[3,32] The items assess the degree of difficulty in performing various physical activities because of an arm, shoulder, or hand problem (21 items), the severity of each of the symptoms of pain, activity-related pain, tingling, weakness, and stiffness (5 items), and the problem's effect on social activities, work, and sleep, and its psychological effect (4 items). The DASH also contains 2 optional 4-item scales concerning the ability to perform sports and/or to play a musical instrument (sport/music scale), and the ability to work (work scale). In this study, the 2 optional scales were not used in the analyses. A 5-point Likert scale ranging from 1 (no difficulty or no symptom) to 5 (unable to perform activity or very severe symptom) is used. The scores for all items are then used to calculate a total scale score ranging from 0 (no disability) to 100 (severest disability). The psychometric properties of the DASH outcome measure are adequate to good.[3,32]

A VAS is a measurement instrument to quantify the amount of pain reported by the patient. Scores can range from 0 (no pain) to 100 (severest pain).

Data concerning type of treatment (corticosteroids or PRP), type of reintervention, complications, side, sex, and age were retrieved from medical files.

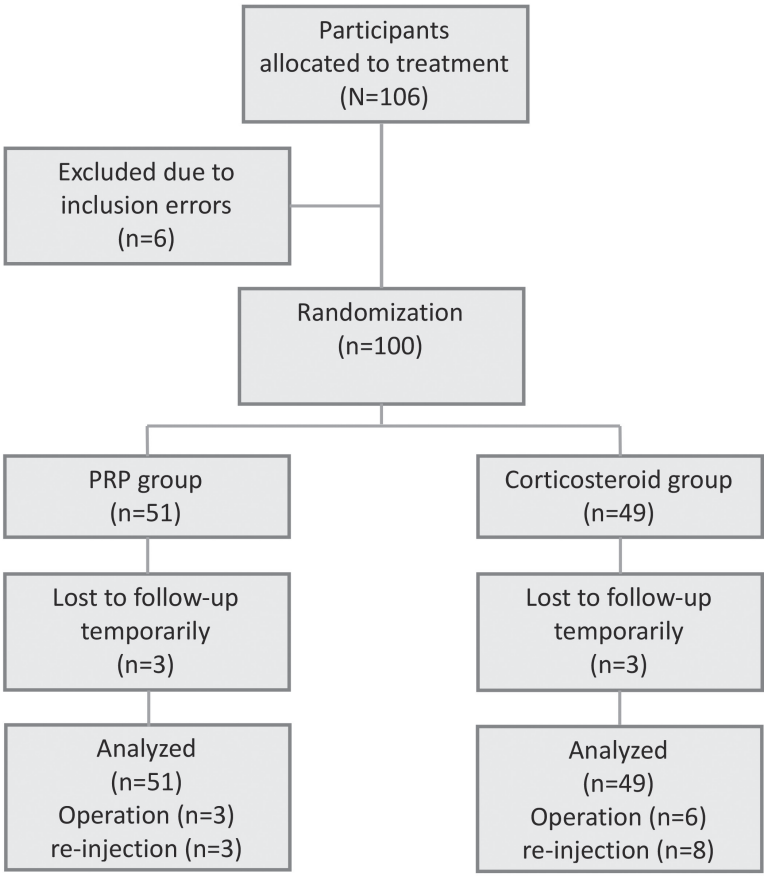

Figure 1. Flow diagram of a trial of injection therapy for chronic lateral epicondylitis. The diagram includes the number of patients actively followed up at different times during the trial.

Statistical Analyses

Frequencies were used to present the available sociodemographic and clinical data. Student *t* tests (continuous data) and x^2 tests (categorical data) were used to examine differences between (1) the protocol-compliant group and the reintervention patients, (2) the corticosteroid group and the PRP group, and (3) the successfully treated group and the nonsuccessfully treated group. The protocol-compliant group was defined as the group of patients who did not need a reintervention (i.e., reinjection, crossover, or surgery). Successful treatment was defined as more than 25% reduction on the VAS pain score and the DASH total scores without a reintervention after 2 years compared with the pre-injection scores. This 25% reduction closely resembles the MCID (minimum clinically important difference), which is 10.2 points for the DASH score.[21] To examine differences in VAS pain scores and DASH total scores between the PRP group and the corticosteroid group before and after the intervention, multivariate analyses of variance

for repeated measures were used. Multiple post hoc comparisons were corrected with the Bonferroni method. To determine whether the corticosteroid group and PRP group scored significantly different at a specific time point, Student *t* tests were used. The VAS pain score and DASH total scores were analysed according to the intention-to-treat principle (based on the allocated intervention) and according to the as-treated principle (based on the received treatment). Missing values are replaced by the last observed value of that variable for each individual (last observation carried forward).

All statistical analyses were performed using the Statistical Package for the Social Sciences (version 17.0, SPSS, Chicago, Illinois).

RESULTS

From May 2006 to January 2008, a total of 100 eligible patients with epicondylitis were randomized into either a PRP injection group or a corticosteroid injection group. Six patients were lost to follow-up because of wrongful inclusion (Figure 1). Analysis of the baseline characteristics (age, sex, side, hand dominance, VAS score, DASH score) between the protocol-compliant patients and those lost to follow-up showed no significant differences ($P > .05$). Sociodemographic and clinical characteristics of the participants are shown in Table 1. The PRP group and the corticosteroid group did not differ on demographic or clinical characteristics ($P > .05$). However, at baseline, the PRP group did score significantly higher on the DASH total score compared with the corticosteroid group ($P < .0001$).

Table 1 Baseline Characteristics of the Corticosteroid and Platelet-Rich Plasma Groups α

	Corticosteroid	Platelet-Rich Plasma	*P* value
	(n = 49)	(n = 51)	
Age, mean ± SD	47.3 ± 7.8	46.8 ± 8.5	.780
Male sex, no. (%)	23 (44.2)	23 (47.9)	.712
Right side, no. (%)	32 (61.5)	30 (62.5)	.921
Dominant hand involved, no. (%)	37 (75.5)	38 (74.5)	.908
VAS, mean ± SD	67.1 ± 13.5	70.2 ± 15.2	.285
DASH, mean ± SD	44.1 ± 16.2	56.3 ± 17.7	<.0001

[α] SD, standard deviation; VAS, visual analogue scale; DASH, Disabilities of the Arm, Shoulder and Hand outcome measure

Course of VAS Pain Scores (Intention-to-Treat Principle)

As shown in Figure 2A, the course of the VAS scores across assessment points is different for the group treated with corticosteroids and the PRP group ($P < .0001$). Table 2 shows the mean scores and standard deviations. The base-line scores of the corticosteroid group were significantly higher compared with all subsequent time points ($P < .0001$), except for 26 weeks ($P = .029$). Between 8 weeks and 26 weeks, pain scores temporarily got worse ($P = .007$). In contrast, compared with baseline scores, the scores of the PRP group significantly improved during the entire duration of the study ($P < .002$). Overall, the average VAS scores differed significantly between the 2 groups ($F_{1,98} = 6.3$, $P = .014$).

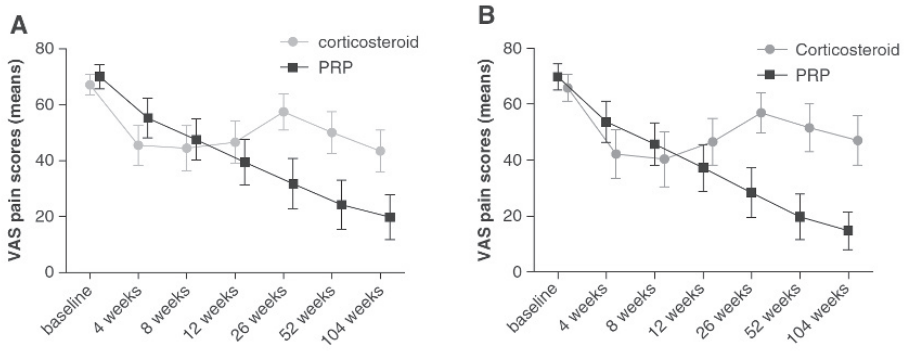

Figure 2. The course of visual analogue scale (VAS) pain scores across assessment points. Bars present 95% confidence intervals. Patients with chronic lateral epicondylitis were randomly assigned to the platelet-rich plasma (PRP) group or the corticosteroid group. A, intention-to-treat; B, reintervention excluded.

When VAS scores were compared at each assessment point separately, the PRP group scored significantly worse at 4 weeks after the injection ($P < .023$), while the opposite was found at 26 weeks ($P < .0001$), 52 weeks ($P < .0001$), and 104 weeks ($P < .0001$) after treatment. No differences between the PRP group and the corticosteroid group were found at baseline, 8 weeks, and 12 weeks. In general, the results of the intention-to-treat analysis and the as-treated analysis were comparable (Figure 2B).

Table 2 DASH and VAS scores for the Corticosteroid Group and the PRP Group at the Various Time Points (Intention-to-Treat Analyses) α

		DASH		VAS	
Time	Intervention	Mean ± SD	*P* Value	Mean ± SD	*P* Value
Baseline	Corticosteroid	43.3 ± 16.1	.002	66.2 ± 14.0	.340
	PRP	54.3 ± 19.5		69.0 ± 15.9	
4 weeks	Corticosteroid	31.2 ±6 20.8	.005	44.3 ± 26.3	.023
	PRP	43.1 ± 21.6		55.7 ± 24.1	
8 weeks	Corticosteroid	28.3 ± 22.2	.060	43.4 ±28.9	.411
	PRP	37.2 ± 24.7		47.7 ± 25.0	
12 weeks	Corticosteroid	32.3 ± 21.7	.813	45.5 ± 27.1	.319
	PRP	21.3 ± 22.0		40.2 ± 27.5	
26 weeks	Corticosteroid	37.6 ± 23.1	.037	55.8 ± 24.1	<.0001
	PRP	27.8 ± 24.7		32.9 ± 30.8	
52 weeks	Corticosteroid	36.8 ± 24.0	<.0001	48.8 ± 27.0	<.0001
	PRP	20.0 ± 23.5		25.9 ± 30.6	
104 weeks	Corticosteroid	36.5 ± 23.8	<.0001	42.4 ± 26.8	<.0001
	PRP	17.6 ± 24.0		21.3 ± 28.1	

[α] DASH, Disabilities of the Arm, Shoulder and Hand outcome measure; VAS, visual analogue scale; PRP, platelet-rich plasma; SD, standard deviation

Successful Treatment (VAS Score)

In total, 60 of 100 patients were successfully treated, which was defined as a reduction of 25% on the VAS pain score without a reintervention after 2 years. Table 3 shows that the PRP group was more often treated successfully (n = 39) than the corticosteroid group (n = 21; $P < .0001$). However, compared with baseline VAS pain scores, a number of patients (n = 11) had deteriorated in VAS pain scores at 2-year follow-up. Of these 11 patients, the majority received a corticosteroid injection (n = 9), while 2 patients received a PRP injection ($P = .017$). Eventually, 1 patient had received a reinjection with corticosteroids, 1 patient crossed over to the PRP group, and 2 patients received surgery.

Table 3 Baseline Characteristics of the Successful and Nonsuccessful Groups α

	Successful (n = 60)	Nonsuccessful (n = 40)	*P* Value
Age, mean ± SD	45.9 ± 8.7	48.8 ± 7.0	.084
Sex, male/female, no. (%)	29 (48.3)/31 (51.7)	17 (42.5)/23 (57.5)	.566
Side, right/left, no. (%)	35 (58.3)/25 (41.7)	27 (67.5)/13 (32.5)	.355
Treatment, PRP/ corticosteroid, no. (%)	39 (65.0)/21 (35.0)	9 (22.5)/31 (77.5)	<.0001
VAS, mean ± SD	67.6 ± 14.4	70.2 ± 14.4	.382
DASH, mean ± SD	52.9 ± 17.9	45.5 ± 17.2	.044

[α] SD, standard deviation; PRP, platelet-rich plasma; VAS, visual analogue scale; DASH, Disabilities of the Arm, Shoulder and Hand outcome measure

Course of DASH Disability Symptom Scores (Intention-to-Treat Principle)

As shown in Figure 3A, the course of the DASH disability symptom scores showed an overall improvement ($F_{6,93}$ = 18.4, *P* < .0001). The baseline DASH scores of the corticosteroid group were significantly higher compared with the scores at 8 weeks (*P* < .0001) and 12 weeks after injection (*P* < .006). Between baseline and 4 weeks, DASH scores significantly deteriorated (*P* < .0001).

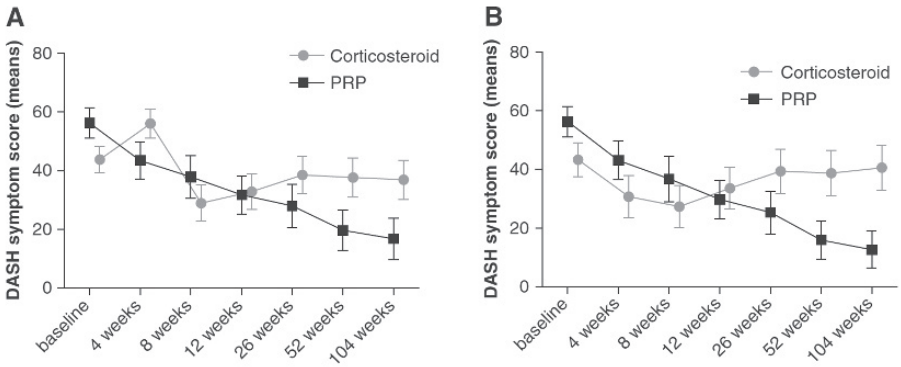

Figure 3. The course of Disabilities of the Arm, Shoulder and Hand (DASH) disability scores across assessment points. Bars present 95% confidence intervals. Patients with chronic lateral epicondylitis were randomly assigned to the PRP group or the corticosteroid group. A, intention-to-treat; B, reintervention excluded.

Although differences were not significant, after 12 weeks, DASH scores deteriorated. In contrast, compared with baseline scores, the scores of the PRP group significantly improved during the entire duration of the study (*P* < .002). Overall, the average DASH disability symptom scores did not differ significantly between the intervention groups

(*P* = .455). However, when DASH scores were compared at each assessment point separately, the PRP group scored significantly worse at baseline and at 4 weeks after the injection (*P* < .005), while the opposite was found at 26 weeks (*P* =.037), 52 weeks (*P* < .0001), and 104 weeks (*P* < .0001) after treatment. In general, the results of the intention-to-treat analysis and the as-treated analysis were comparable (Figure 3B). However, when the baseline scores of the corticosteroid group were compared with the 2-year results in the as-treated analysis, no significant difference was found (*P* = .438), indicating that the corticosteroid group returned back to baseline levels. In addition, the deterioration in the corticosteroid group between baseline and 4 weeks disappeared.

Successful Treatment (DASH Symptom Score)

In total, 56 of 100 patients (56.0%) were successfully treated, which was defined as a reduction of 25% on the DASH score without a reintervention after 2 years. Patients in the PRP group were more often treated successfully (n = 37; *P* < .0001) compared with the corticosteroid group (n = 19). However, compared with baseline DASH scores, a number of patients (n = 30) had deteriorated at 2-year follow-up. The majority of patients in this group received a corticosteroid injection (n = 23), while 7 patients received a PRP injection (*P* = .001). Eventually, 1 patient received a reinjection, 1 patient crossed over to the PRP group, and 4 patients received surgery.

Failures (Reinterventions)

Table 4 shows the characteristics of the 20 reinterventions. On average, reinterventions or operations were needed after an average of 6 months (range, 2-14 months). At baseline, 14 patients were allocated to corticosteroids and 6 patients received an injection with PRP (*P* =. 036). The protocol-compliant group and the reintervention group differed significantly regarding sex (*P* = .015) and side (*P* = .027).

There were 6 reinterventions in the PRP group: 3 patients who required an operation and 3 patients who required a reinjection with corticosteroids. Except for 1 reinjection, all reinterventions were performed in the first year after the initial treatment; 2 operations and 1 reinjection with corticosteroids occurred within 3 months after the PRP injection. There were 14 reinterventions in the corticosteroid group: 6 patients required an operation, 1 patient required a reinjection with corticosteroids every 3 months and declined surgery, and 7 patients crossed over to have a PRP injection.

In the corticosteroid group, all reinterventions were performed in the first year of follow-up except for 1 crossover patient receiving a PRP injection. Regarding the patients who failed their initial treatment, those who crossed over to the PRP group significantly improved on both VAS pain scores (*P* < .001) and DASH disability symptom scores (*P* = .019). However, patients who received surgery or a reinjection with corticosteroids did not benefit when their VAS and DASH scores at 2 years were compared with their baseline scores. No complications were seen concerning the use of PRP, except for the

initial worsening of pain because of the activation of the inflammation cycle, which usually lasted for 1 to 2 weeks.

Table 4 Baseline Characteristics of the Protocol-Compliant and the Reintervention Group α

	Protocol-Compliant (n = 80)	Reintervention (n = 20)	*P* Value
Age, mean ± SD	46.5 ± 8.2	49.2 ± 7.6	.206
Sex, male/female, no. (%)	41 (51.2)/39 (48.8)	5 (25.0)/15 (75.0)	.015
Side, right/left, no. (%)	46 (57.5)/34 (43.5)	16 (80.0)/4 (20.0)	.027
Treatment, PRP/corticosteroid, no. (%)	45 (56.3)/35 (44.7)	6 (30.0)/14 (70.0)	.036
VAS, mean ± SD	68.1 ± 14.9	70.8 ± 11.9	.464
DASH, mean ± SD	50.4 ± 18.2	48.2 ± 17.2	.663

[α] SD, standard deviation; PRP, platelet-rich plasma; VAS, visual analogue scale; DASH, Disabilities of the Arm, Shoulder and Hand outcome measure

DISCUSSION

This randomized, double-blind study was designed to compare the use of concentrated autologous platelets to corticosteroid in patients with lateral epicondylitis; its application proved to be both safe and easy. The corticosteroid group was actually better initially and then declined, returning to baseline level concerning functional impairment, while the PRP group progressively improved. There was a significant difference in decrease of pain and disability of function after the platelet application even after 2 years. Comparing the results presented here with the results of the 1-year follow up, the effect in the corticosteroid group declined, whereas the result in the PRP group was maintained. A remarkable finding was that the PRP group had worse DASH scores before treatment and better ones after 26 weeks of the initial treatment. This adds to the power of our conclusion that that PRP was helpful.

Lateral epicondylitis is a common problem with many available treatment methods. The most commonly recommended treatment is physiotherapy and bracing. Approximately 87% of the patients benefit from this combination of treatment methods.[30] Corticosteroid injection, nowadays seen as controversial, was considered the gold standard in the treatment of lateral epicondylitis. However, studies show it is merely the best treatment option in the short term, when compared with physiotherapy and a wait-and-see policy. Often, poor results are seen after 12 weeks of follow-up.[28] Treatment with corticosteroids has a high frequency of relapse and recurrence, probably because intratendinous injection may lead to permanent adverse changes within the structure of the tendon and because patients tend to overuse the arm after injection as a result of direct pain relief.[28] In our study, the recurrence rate and need for repeated

injection or surgery was also larger in the corticosteroid group than in the PRP group. Actually, of the 11% getting worse after the injection, the vast majority was found in the corticosteroid group. Smidt et al.[26] showed in a meta-analysis that the effects of steroid injections compared with placebo injection, injection with local anaesthetics, injection with another steroid, or another non-operative treatment are not significantly different in the intermediate and long term. However, the studies acknowledging the relatively good results of a wait-and-see policy, physiotherapy, and even corticosteroid injections are studies that included patients who all had nonchronic lateral epicondylitis (i.e., patients with complaints of less than 6 months' duration). The current study included patients with a duration of symptoms of > 6 months. Smidt et al.[27] showed most patients recover from lateral epicondylitis within 1 year but that beyond 6 months, not much natural recovery is seen.

Our original power analysis in the 1-year follow-up paper [20] with an alpha of .05 and a beta of .9 was based on the 93% success in the Mishra and Pavelko [15] study for PRP and the 65% success in the Hay et al.[10] study for corticosteroid injection, both obtained after 6 months. Our study presents the results after 2 years so possibly the power at 6 months is correct, but the power after 2 years of follow-up does not need to be, rendering this study underpowered at the 2-year follow-up. However, a beta of .9 is higher than in most studies. More important, there is no additional improvement in symptoms from a wait-and-see policy or a steroid injection beyond 1 year (actually, there seems to be no additional gain in recovery percentages in waiting beyond 6 months).[27] Although we do not know what the success percentages will be at 2 years of natural history or after 1 steroid injection, there is no evidence to suggest it would be very different from what we used for the 6-month power analysis.

For those who do not recover, there are various types of surgical procedures for patients with chronic lateral epicondylitis. Verhaar et al.[33] noted an improvement in 60% to 70% of the patients after surgical treatment, although more recently higher success rates (80%-90%) have been reported.[31] Patients remain, however, interested in an alternative to surgical intervention.

Platelet-rich plasma is promoted as an ideal biologic autologous blood-derived product. It can be exogenously applied to various tissues, where after platelet activation, high concentrations of platelet-derived growth factors that enhance tissue healing are released.[8,36] Utilizing the Recover system, the patient's own platelets can be collected into a highly concentrated formula. No activation agent was used during our procedure. The activation of the platelets will occur through the exposure of platelets to the thrombin. The thrombin is produced as a reaction to the injection of the platelets into the tendon tissue using a peppering technique. The exposed collagen may also serve as an activator. Several negative side effects are known when using bovine thrombin as an exogenous activator, limiting its clinical use: undesirable immune responses in humans,[13]

and inhibition of cell proliferation and viability in vitro.[17] This may be overcome when using an autologous-derived thrombin. Collagen is an attractive alternative to bovine thrombin as it is naturally involved in the intrinsic clotting cascade. Fufa et al.[9] measured clinically relevant levels of transforming growth factor beta (TGF-ß1), platelet-derived growth factor (PDGF-AB), and vascular endothelial growth factor (VEGF) from both type I collagen–activated as well as bovine thrombin-activated PRP.

During the first 2 days of tendon healing, an inflammatory process is initiated by migration of neutrophils and subsequently macrophages to the degenerative tissue site. In turn, activated macrophages release multiple growth factors, including PDGF, TGF-*α* and TGF-ß, interleukin-1, and fibroblast growth factor.[7] Angiogenesis and fibroplasia start shortly after day 3, followed by collagen synthesis on days 3 to 5. This process leads to an early increase in tendon breaking strength, which is the most important tendon healing parameter, followed by epithelialization and the ultimately the remodelling process. This course of repair was confirmed in a previous animal study.[1]

The presence of an elevated concentration of leukocytes in the PRP is a topic of discussion nowadays. Companies that concentrate white blood cells argue that leukocytes are useful in creating an antibacterial response and have the ability to debride dead tendon tissue and jump-start healing (because they also contain growth factors). A basic study in horses showed no lengthening of the inflammation phase when L-PRP was used to treat an acute lesion of the bow tendon when compared to the control group.[4] Companies that purposely eliminate white blood cells from PRP argue that leukocytes have detrimental effects on healing tissue, because of the enzymes from the matrix metalloproteinase family that are released by neutrophils.[24] This is, however, not proven in prospective randomized controlled studies. The treatment of tendinosis with an injection of concentrated autologous platelets may be a nonoperative alternative. Injection of autologous platelets has been shown to improve repair in tendinosis in several animal and in vitro models.[14,23] The effect of 1-injection PRP is shown to last longer than 1 year, while the percentage of success after a single corticosteroid injection drops from 51% at 1 year to 40% after 2 years of follow-up. This figure resembles the number for an invasive placebo treatment. A possible explanation for the long-lasting effect of platelets could be that platelets improve the very early neotendon properties so that the cells are able to perceive and respond to mechanical loading at an early time point.[1]

In our study, a single percutaneous injection of PRP or corticosteroid was performed, using a peppering technique in both groups. Repeated injections might be beneficial in patients who had suboptimal results after the initial injection, although no evidence for a beneficial effect of more than 1 injection exists. On theoretical grounds, by studying the inflammation cascade in tendon repair, a reinjection after 3 to 4 weeks seems logical because at this stage the cell proliferation and matrix deposition activity will

have peaked and can be expected to subsequently decline. However, at this time no true indication of what the result of second injection would be can be determined. Routinely injecting a second time would be unnecessary in 73% of the cases, as they were already successful after 1 injection. Regarding the patients who failed their initial treatment, those who crossed over to the PRP group significantly improved on both VAS pain scores and DASH disability symptom scores. The decision to proceed to further treatment was based on patient preference. However, patients who received surgery or a reinjection with corticosteroids did not benefit when their VAS and DASH scores at 2 years were compared with their baseline scores. When interpreting these results, strong conclusions regarding these findings are not possible, because the numbers of patients in these reintervention groups were relatively small.

We know that the natural history of nonchronic lateral epicondylitis is benign, resulting in normalization of complaints in the vast majority of patients within 1 year with little gain in recovery between 6 and 12 months.[27] All patients included in this study had complaints for at least 6 months. Patients receiving a corticosteroid injection also have a natural history and because the population was randomized, we can expect that the natural history will have the same influence in both groups. In the current study, 70% of the patients were already injected with corticosteroids at least 6 months before inclusion into this study. The PRP group should have experienced this negative effect also. Whether the positive effect of PRP is in fact the natural course of lateral epicondylitis cannot be determined from the current work. Still, the inclusion of patients with a minimum complaint history of 6 months indicates a chronic patient population was enrolled in the study. The positive effect of PRP compared with a corticosteroid injection on the course of lateral epicondylitis thus seems not be caused by natural history or a negative effect of the corticosteroid injection (which is not present in this study [Figures 2 and 3]). A critique of the original study was that the corticosteroid treatment is not the same as a placebo and might be worse than a placebo. In the Netherlands, the Institutional Review Board would not allow a placebo, and therefore this is a limitation of this study as the corticosteroid injection (and those before inclusion in the study) may have adversely affected the long-term results compared with a true placebo injection or dry needling.

In the Netherlands, a PRP treatment costs approximately twice as much as a corticosteroid treatment and surgery for lateral epicondylitis is twice the cost of a PRP treatment and thus 4 times as much as a corticosteroid injection. The PRP treatment therefore costs 2 units; a steroid injection costs 1 unit and surgery, 4 units. Thus, in the PRP group 51 patients were treated with PRP, costing 51 times 2 units of money, and in the corticosteroid group, 49 patients were treated, costing 49 times 1 unit. In this study we had 20 reinterventions: 3 surgeries (12 units) and 3 reinjections with corticosteroids (3 units), making a total of 6 reinterventions, costing 15 extra units in the PRP group; and 6 surgeries (24 units), 1 reinjection with corticosteroids (1 unit),

and 7 reinjections with PRP (14 units), making a total of 14 reinterventions, costing 39 extra units in the corticosteroid group. Regarding cost, PRP is not cost effective compared with corticosteroid on a short-term basis, but if the costs of those patients failing on the corticosteroid injection who proceed to surgery are taken into account, the differences in cost effectiveness will level out (102 + 15 = 117 units in the PRP group versus 49 + 39 = 88 units in the corticosteroid group), especially if the costs for those who failed on corticosteroids were turned into a success by a consecutive PRP injection. This cost analysis does not include all socioeconomic costs of a recurrence, time off work, and the extra efforts reinterventions required from the patient and doctor. Moreover, although it is difficult to draw conclusions from small numbers, those patients who were reinjected with corticosteroids or those who had surgery did not improve compared with baseline, with those who were reinjected with PRP (those who crossed over) showing significant improvement. The crossover patients actually were patients who were offered either an operation or to try the experimental PRP injection; without this offer, an additional 7 patients in the corticosteroid group would have been operated on. Actually, the number of operations in the PRP group might have been less if we had realized that an initial flare-up of inflammation signs (i.e., pain) is to be expected when using PRP. Two operations and 1 reinjection with corticosteroids were carried out within 3 months after the PRP injection, whereas in fact these patients still might have been in their inflammation and healing phase. Taking all these incidents into account, the PRP procedure might actually be a cheaper method in the long run, but a formal cost analysis should be performed.

In conclusion, this report demonstrates that a single injection of concentrated autologous platelets improves pain and function more effectively than corticosteroid injection in chronic lateral epicondylitis. These improvements were sustained over a 2-year follow-up time with no reported complications.

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CHAPTER 7

Positive effect of platelet-rich plasma on pain in plantar fasciitis. A double-blind multi centre randomized controlled trial: Platelet-rich plasma versus corticosteroid injectionwith a 1-year follow-up

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ABSTRACT

Background: When conservative treatment for chronic plantar fasciitis fails, often a corticosteroid injection is given. Corticosteroid injection gives temporary pain reduction, but no healing. Platelet-rich plasma (PRP) has proven to be a safe therapeutic option in the treatment of tendon, muscle, bone, and cartilage injuries.

Purpose: To determine the effectiveness of PRP compared with corticosteroid injections for chronic plantar fasciitis.

Study design: Randomized controlled double-blind clinical trial.

Methods: Patients with chronic plantar fasciitis were allocated to have steroid injection or PRP. The primary outcome measure was the Foot Function Index (FFI) pain score. Secondary outcome measures were function scored by the FFI Activity, FFI Disability and the American Orthopaedic Foot and Ankle Score (AOFAS) and quality of life scored with the WHOQOL-BREF. All outcomes were measured at baseline and at 4, 12, 26 weeks, and 1 year after the procedure.

Results: Of the 115 patients, 63 were allocated to PRP group (of which 46 patients (73%) completed the study) and 52 were allocated to the control group (of which 36 patients (69%) completed the study). In the control group, FFI Pain scores decreased quickly and then remained stable during follow-up. In the PRP group, FFI Pain reduction was more modest, but reached a lower point after 12 months than the control group. After adjusting for baseline differences, the PRP group showed significantly lower pain scores at the 1-year follow-up than the control group (mean difference, 14.4; 95% CI, 3.2-25.6). The number of patients with at least 25% improvement (FFI Pain score) between baseline and 12-month follow-up differed significantly between the groups. Of the 46 patients in the PRP group, 39 (84.4%) improved at least 25%, while only 20 (55.6%) of the 36 patients in the control group showed such an improvement ($p = 0.003$). The PRP group showed significantly lower FFI Disability scores than the control group (mean difference, 12.0; 95% CI, 2.3-21.6).

Conclusion: Treatment of patients with chronic plantar fasciitis with PRP seems to reduce pain and increase function more, compared to the effect of corticosteroid injection.

Registration: NCT00758642 (ClinicalTrials.gov identifier).

Key Words: Plantar fasciitis; Platelet Rich Plasma; Corticosteroids; Pain; Function.

BACKGROUND

Chronic plantar fasciitis is the most common cause of foot complaints in the United States. Up to 11 to 15% of these complaints require professional care among adults.[6,21] The incidence of plantar fasciitis peaks in persons between the ages of 40 to 60 years with no bias towards either sex.[30]

The underlying condition that causes plantar fasciopathy is a degenerative tissue condition that occurs near the site of origin of the plantar fascia at the medial tuberosity of the calcaneous.[3] In acute cases, plantar fasciitis is characterized by classical signs of inflammation including pain, swelling, and loss of function. For more chronic conditions, however, inflammation is not the underlying tissue disruption. In fact, histology of chronic cases has shown no signs of inflammatory cell invasion into the affected area.[16] Instead, the tissue is characterized histologically by infiltration with macrophages, lymphocytes, and plasma cells; tissue destruction; and repair involving immature vascularization and fibrosis.[16] The normal fascia tissue is replaced by an angiofibroblastic hyperplastic tissue, which spreads itself throughout the surrounding tissue creating a self-perpetuating cycle of degeneration.[16]

Numerous methods have been advocated for treating plantar fasciitis, including rest, nonsteroidal anti-inflammatory medication, night splints, foot orthosis, stretching protocols, and extracorporeal shock wave therapy.[6,7,25,33] Corticosteroid injections are a popular method of treating the condition as well, but only seem to have small and short-term effects.[7] Other various types of surgical procedures have also been recommended.[1,6,8,19,29,33] The use of corticosteroids is particularly troubling since several studies have linked plantar fascia rupture to repeated local injections of a corticosteroid.[1,6,15,27] When neither rest and neither activity restriction nor conservative treatments result in a satisfactory outcome, the patient is often interested in treatment options other than surgery.

Platelet-rich plasma (PRP) is promoted as an ideal autologous biological blood-derived product, which can be exogenously applied to various tissues where it releases high concentrations of platelet derived growth factors that enhance wound healing, bone healing, and tendon healing.[9,32]

When platelets become activated, growth factors are released and initiate the body's natural healing response.[34] In animals, the addition of growth factors to the ruptured tendon has been shown to increase the healing of the tendon.[8] In humans, it has been shown that the injection of platelets into the tendon decreases pain.[6]

In a double-blind randomized trial, we investigated whether an injection of PRP improves the outcome of patients with chronic plantar fasciitis more so than corticosteroid

injections. The primary outcome parameter was pain. Secondary parameters were function and quality of life.

METHODS

Study design

Peerbooms et al.[24] published the study design in the journal of BMC Musculoskeletal Disorders in 2010. Double-blind randomization was performed after patients were deemed eligible and provided informed consent. Patients were randomly allocated to the concentrated autologous platelet group (PRP group) or to the corticosteroid group (control group). A computer using block randomization of 10 patients was used to create a randomization schedule. Treatment assignments (placed in sequentially numbered opaque envelopes) were assigned by the trial managers who also arranged the facilities needed for the procedure. The investigator who assessed the outcomes was blinded to the treatment that the patient received. The treatment was given by another investigator who also prepared the two injections (J.C.P., H.M.S., T.G.).

For both groups blood was drawn to make the groups as equal as possible.

All patients with a plantar fasciitis who were admitted to one of the participating hospitals and met the inclusion criteria were asked to join the study. Plantar fasciitis was defined as pain at the point of the fascia plantaris origin at direct palpation.[6] All patients with plantar fasciitis were screened with X-ray of the calcaneus for bony abnormalities and to differentiate for subtalar arthritis. Sonography and magnetic resonance imaging were not used standardly.

The Medical Ethical Committee of The Netherlands approved the study design, procedures, and informed consent.

Study population

The study was conducted at the Orthopaedic Departments of the HAGA Ziekenhuis Den Haag, Alrijne Ziekenhuis Leiden, Albert Schweitzer Ziekenhuis Dordrecht, Maastricht University Medical Centre and St. Elisabeth Ziekenhuis Tilburg (The Netherlands) between November 2008 and January 2015. J.C.P and T.G. were responsible for the data and safety monitoring. Inclusion criteria: patients aged > 18 years, with plantar fasciitis (at least 6-months duration), who failed conservative treatment are included. Patients were able to understand the informed consent. The FFI pain score in the morning should be higher than 5 (0-10 scale).

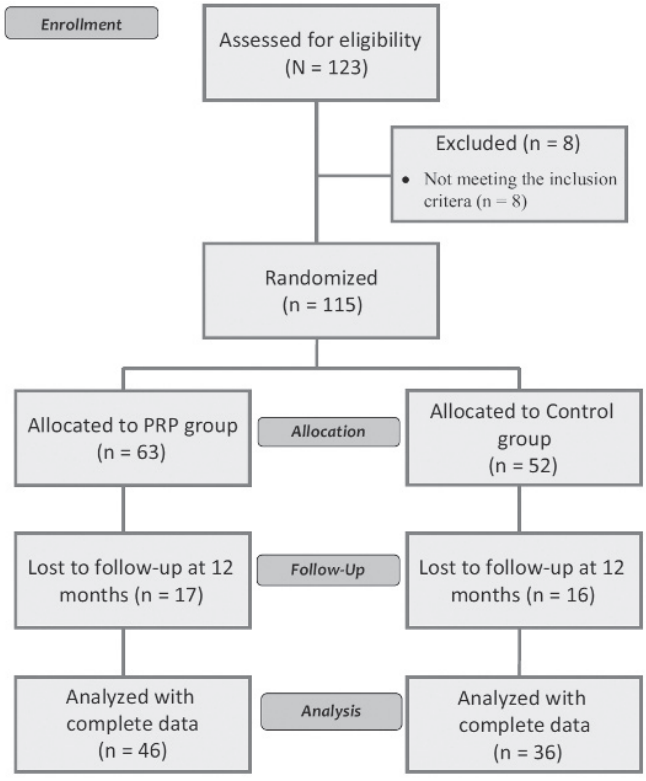

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flowchart.
PRP, platelet-rich plasma.

Patients were excluded from the study when they received local steroid injections within 6 months, physical/occupational therapies within 4 weeks, or non-steroidal anti-inflammatory within 1 week prior to randomization. In addition, patients were excluded with the inability to fulfil follow-up criteria; significant cardiovascular; renal or hepatic disease; pregnancy, (local) malignancy; history of anaemia (haemoglobin < 5.0); previous surgery for plantar fasciitis; active bilateral plantar fasciitis; diagnosis of vascular insufficiency or neuropathy related to heel pain; hypothyroidism; and diabetics.

Interventions

Platelet Concentrate Preparation

Fifty-five millilitres whole blood was collected from the uninvolved arm into a 60-mL syringe that contained 5 mL sodium citrate. A peripheral complete blood count was also collected at the time of the initial blood draw. The blood was then prepared according to the Gravitational Platelet System (GPS) instructions (Zimmer Biomet). This device is a desktop-size centrifuge with disposable cylinders for the blood approximately 5

mL platelet concentrate is obtained for each patient. Autologous platelet concentrate contains concentrated white blood cells and platelets that are suspended in plasma. Since an acidic anticoagulant is introduced to the whole blood used to produce the platelet concentrate, the platelet concentrate must be buffered to increase the pH to normal physiologic levels. This was accomplished with 8.4% sodium bicarbonate solution added at a ratio 0.05 mL of sodium bicarbonate solution to 1 mL of platelet concentrate. The resulting buffered platelet concentrate contains approximately a 6 to 8 times concentration of platelets compared to baseline whole blood. No activating agent was used. The total time from blood draw to injection in the patients was about 30 minutes. No specialized equipment, other than the GPS machine, was required.

Corticosteroid
The type of steroid that is used during the study is kenacort 40 mg/mL triamcinolone acetonide.

Injection Technique
Initially, bupivacaine was infiltrated into the skin and the subcutaneous tissue of both groups as a local field block. Approximately 0.5 mL was also injected directly into the area of maximum tenderness. Then, either 5 to 6 mL platelet concentrate, or 1 mL corticosteroid was injected using a 22-gauge needle into the plantar fasciitis using a peppering technique. This technique involved a single skin portal and then 5 penetrations of the fascia.

Postprocedure Protocol
Immediately after injection, patients in both groups were kept in sitting position without moving the foot for 15 minutes. Patients were referred to the physiotherapist to obtain stretching exercises. Patients were sent home with instructions to limit their use of the feet for approximately 48 hours and they used hydrocodone or acetaminophen for pain. The use of nonsteroidal medication was prohibited. After 48 hours, patients were given a standardized stretching protocol to follow for 2 weeks. A formal strengthening program was initiated after this stretching. At 4 weeks after the procedure, patients were allowed to proceed with normal sporting or recreational activities as tolerated. Any type of foot orthoses was not allowed.

Study Endpoints

Pain
The primary outcome measure pain was measured using a visual analogue scale score of the FFI at all time points.[4,14] The FFI pain score records the patient's reported pain using a scale of 0 (pain-free) to 10 (worst pain imaginable). The scale is a 10-cm line, and the score is marked at the point on the line corresponding with the patient response.

Treatment was considered being a success if patients showed a FFI pain score reduction of 25% between baseline and 12-month follow-up. In addition, patients should not have required escape therapy and pain medication beyond the protocol defined allowable period. Patients that obtained a different treatment were classified as unsuccessful. To determine the percent of change, first the baseline pain score was subtracted from the endpoint pain score. Subsequently, this difference score was divided by the baseline pain score and multiplied by 100. If a patient was lost to follow-up, the last available measurement was used to determine the treatment success.

Function and Quality of Life

The secondary outcome measures of this study were the FFI Disability, FFI Activity [2,4,14] and American Orthopaedic Foot & Ankle Society (AOFAS) score.[13,26] Lastly, patients' quality of life was assessed using the World Health Organization Quality of Life (WHOQOL-BREF). [20, 31] This is the short version of the WHOQOL-100. The WHOQOL-BREF consists of 4 domains (Physical Health, Psychological Health, Social Relationships, and Environment) and 2 items assessing overall quality of life and general health. The response scale consists of 5-point Likert scales. Higher scores indicate better quality of life. All outcomes in this study were measured at baseline, 1, 3, 6 months and at 1 year after the procedure.

Determination of Sample Size

Our main hypotheses will be tested by investigating the interaction effect between treatment and measurement occasion, indicating whether the treatments differ in their change in the outcome over time. We are not aware of earlier research comparing PRP with corticosteroid treatments for chronic plantar fasciitis on pain, function and quality of life with a follow up of at least 1 year. Therefore, we take a conservative stance by assuming a small partial eta-squared effect size of 0.02 and a correlation between the repeated measurements of 0.3. To detect such effect sizes with a power of 0.80 and a significance level of .05, at least 84 participants are required (42 in each group).

Statistical Analysis

For dichotomous baseline characteristics, frequencies and percentages were reported. Means and standard deviations were calculated for continuous and normally distributed baseline characteristics. For nonnormally distributed continuous characteristics, the median and interquartile range were reported.

To test the null hypothesis that the treatment groups do not differ in their change on the outcome measures over time, linear mixed modelling analyses were used, focusing on the interaction effect between treatment group and time. The influence of dosage on this treatment effect was assessed by inspecting the 3-way interaction effect between treatment group, time and injection dosage. For all outcome measures,

individual differences in growth trajectories were taken into account by allowing both the intercept and slope to vary across all patients. Time was modelled continuously and both linear as well as quadratic and cubic time effects were investigated. Any differences between the treatment groups on the baseline scores of the outcome measures were handled with a constrained longitudinal data analysis that constrains the baseline means of the treatment groups to be equal by omitting the main effect for treatment from the statistical model.[5] Inferences regarding the difference between treatments are based on the interaction effect between treatment group and time. In the linear mixed model analysis, parameters were estimated using restricted maximum likelihood estimation.

Analysis of covariance was used to test the null hypothesis of equal outcome means at the 12-month follow-up, adjusted for baseline differences. The effects of all abovementioned analyses were adjusted for the potential confounders sex, smoking, and duration of symptoms before treatment. Differences between groups in the number of patients showing at least 25% improvement in pain symptoms was assessed using a Chi-square test.

P-values < .05 were considered statistically significant for the primary outcome measure (FFI Pain), while a Bonferroni correction was applied to adjust for multiple testing of the treatment effects for the secondary and remaining outcome measures. In order to retain sufficient statistical power, the Bonferroni correction was applied separately to the secondary outcomes (FFI Disability and FFI Activity, AOFAS; significance level = .05 / 3 = .0167) and the remaining outcome measures (WHOQOL-BREF; significance level = .05 / 5 = .01). Confidence intervals were calculated at the 95% level. All data were analysed by a blinded researcher (P.L.) using SPSS (v 23; IBM).

RESULTS

The flowchart in Figure 1 indicates that of all 115 randomized patients, 63 were allocated to the PRP group and 52 to the control group. Of the 63 patients in the PRP group, 46 completed the study and 17 patients were lost during the 12-month follow-up. For logistic reasons, 16 patients were treated with an injection made of the 30-mL PRP kit instead of the 60-mL PRP kit. The influence of dosage on the treatment effect was assessed by inspecting the 3-way interaction effect between treatment group, time, and injection dosage. No differences were seen between the 30- and 60-mL doses. In the control group, 36 patients completed the study, and 16 patients were lost to follow-up.

Table 1 presents the baseline characteristics for patients allocated to the PRP and control group separately.

Appendix Table A1 indicates that for all outcome measures, the Little MCAR test (missing completely at random) failed to reach significance, suggesting that the missing values on those outcome measures are likely missing completely at random. This result allowed

for handling missing data on the outcome measures by means of maximum likelihood estimation in the mixed model analysis, as this method assumes the missing values to be either missing at random or missing completely at random.

Table 1 Baseline Characteristics for Patients in the PRP and Control Groups α

	PRP group (n=63)	Control group (n=52)
Baseline characteristics	M (SD) / n (%)	M (SD) / n (%)
Female sex	48 (76.2)	34 (65.4)
Age, y	50.73 ± 11.33	47.5 ± 11.19
Length, cm	170.33 ± 10.16	174.65 ± 10.11
Weight, kg	84.27 ± 15.62	91.92 ± 19.39
Body Mass Index	29.12 ± 5.17	30.16 ± 6.29
Duration of Symptoms, weeks[b]	70 (40-130)	52 (35- 90)
Smoking, yes	9 (14.3)	10 (19.2)
Previous Foot Surgery: yes	2 (3.2)	3 (5.8)
Codisease: yes	15 (23.8)	11 (21.2)
Comedication: yes	20 (31.7)	13 (25.0)
Baseline scores of outcome measures	M (SD)	M (SD)
FFI Pain	64.7 ± 16.95	56.96 ± 20.13
FFI Disability	51.56 ± 20.6	41.15 ± 21.71
FFI Activity	31.64 ± 16.64	25.2 ± 16.57
AOFAS	49.44 ± 15.29	57.71 ± 16.61
WHOQOL-BREF		
Overall QOL and General Health	7.17 ± 1.55	7.51 ± 1.29
Physical Health	12.26 ± 2.74	12.99 ± 2.58
Psychological Health	14.98 ± 2.29	15.37 ± 2.44
Social Relationships	15.44 ± 2.79	16.42 ± 1.89
Environment	16.2 ± 4.32	16.1 ± 2

[a] Data are provided as n (%) or mean ± SD. AOFAS, American Orthopaedic Foot & Ankle Society; FFI, Foot Function Index; PRP, platelet-rich plasma; WHOQOL-BREF, World Health Organization Quality of Life.
[b] Median (interquartile range) reported because this variable is not normally distributed.

Table 2 presents the results of the linear mixed modelling analysis for all outcome measures. For each outcome, the treatment × time interaction effects in the second column pertain to a model including only a linear time effect, while the similar tests in the third column are derived from a model including both linear as well as quadratic

and cubic time effects. The linear time models assume that patients change linearly over time on the outcome measures. For these models, the FFI Pain, FFI Disability, FFI Activity, and AOFAS outcomes showed significant interaction effects between treatment and time, suggesting that the treatment groups differ in their change in these outcomes over time. However, when inspecting the results of the models that also include quadratic and cubic time effects, it turns out that the treatment groups now only show significant differences in their change on the FFI pain scores over time and no longer on the FFI Disability, FFI activity, and AOFAS outcomes.

Table 2 ***F*** **Test on the Interaction Between Treatment and Time α**

Outcome measure	Time modeled linearly		Time modeled linearly, quadratically and cubically	
	F-test	*P* value	*F*-test	*P* value
FFI Pain[b,c]	*F*(1,96.634) = 8.140	0.005[d]	*F*(1,178.118) = 6.884	0.009[d]
FFI Disability[b]	*F*(1,117.727) = 11.227	0.001[d]	*F*(1,210.506) = 2.856	0.092
FFI Activity[b]	*F*(1,122.090) = 7.176	0.008[d]	*F*(1,202.982) = 1.393	0.239
AOFAS[b]	*F*(1,126.818) = 12.955	<0.001[d]	*F*(1,211.989) = 2.922	0.089
WHOQOL-BREF				
Overall QOL and General Health	*F*(1,116.178) = 0.579	0.448	*F*(1,213.492) = 1.967	0.162
Physical Health[b]	*F*(1,122.849) = 6.201	0.014	*F*(1,175.289) = 0.360	0.549
Psychological Health	*F*(1,113.228) = 0.168	0.683	*F*(1,167.993) = 0.376	0.541
Social Relationships	*F*(1,104.092) = 0.252	0.617	*F*(1,187.356) = 2.393	0.124
Environment	*F*(1,126.934) = 0.940	0.334	*F*(1,192.926) = 0.037	0.847

[α] For all outcome measures, the results of the *F* test on the interaction between treatment and time for models with and without adjustment for quadratic and cubic time effects. AOFAS, American Orthopaedic Foot & Ankle Society; FFI, Foot Function Index; WHOQOL-BREF, World Health Organization Quality of Life.
[b] These models showed significant quadratic and cubic time effects.
[c] Primary outcome measure.
[d] Statistically significant after the Bonferroni correction (see the Statistical Analysis section).

Note that in the models including linear, quadratic and cubic time effects, we report in Table 2 only the interaction effects between treatment and the linear time. The interactions between treatment and quadratic and cubic time were included in the model, although their significance was similar to those of the linear time × treatment interaction. We decided to report the results of the linear time × treatment interaction to test the null hypothesis that the 2 groups were equal in their change on the outcome over time, after accounting for nonlinear change in the outcome measure. This is exactly

what we were interested in; therefore, we did not report the interactions of treatment with the quadratic and cubic time effects.

A possible explanation for this discrepancy is that the assumption of linear change over time is implausible. If change in an outcome over time is not linear yet time is modelled only linearly, then spurious interaction effects between time and other variables may arise.[17] Indeed, the FFI Pain, FFI Disability, FFI Activity, AOFAS, and WHOQOL Physical Health models showed linear as well as significant quadratic and cubic time effects, suggesting that the change in these outcome measures cannot be considered linear. This finding is corroborated by visual inspection of the growth curves of both treatment groups (Figure 2). The treatment groups did not show differences with respect to their change in quality of life over time, as indicated by the nonsignificant treatment × time interaction effects for all models involving the WHOQOL-BREF outcomes.

Appendix Table A2 is similar to Table 2, yet it presents the results of the 3-way interaction among treatment group, time and dosage. This test indicates whether the differences between treatment groups in the change in the outcome measures over time depend on the used injection dosage. For all outcome measures, this interaction effect failed to reach significance, both for models with linear time only and for models with linear, quadratic and cubic time effects. These results suggest that the injection dosage did not affect the differences between the treatment groups in their change on the outcomes over time.

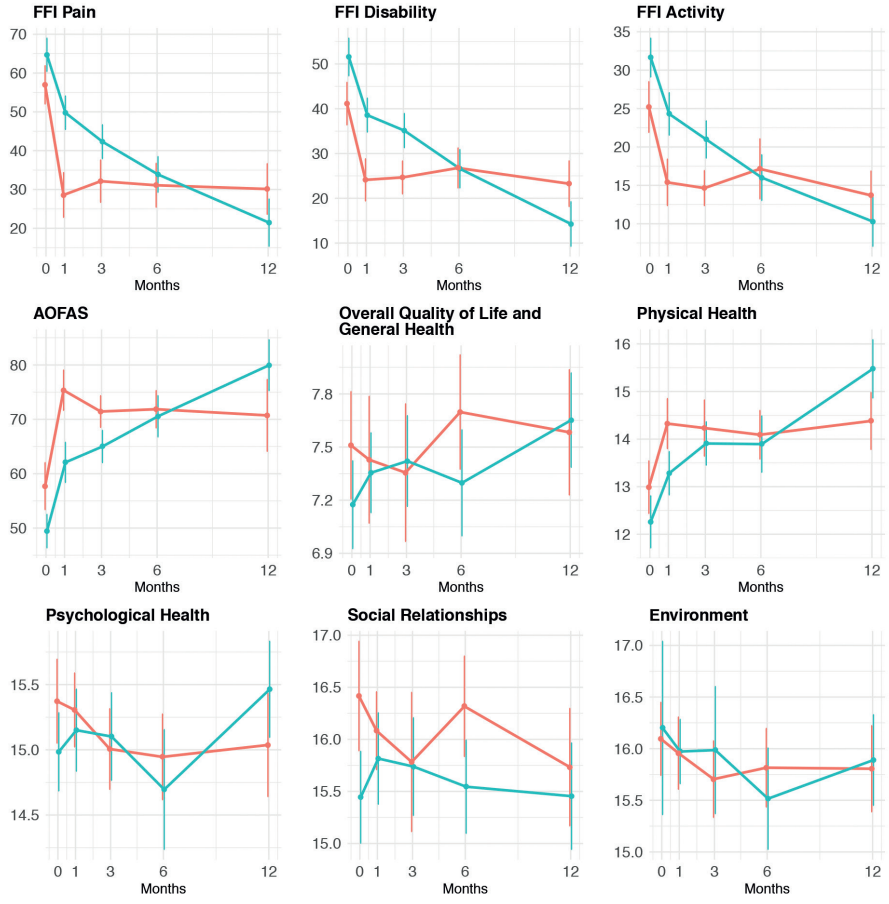

Figure 2. For the platelet-rich plasma group (blue) and the control group (red), change in outcome over time. Values are presented as mean ± SD. AOFAS, American Orthopaedic Foot & Society; FFI, Foot Function Index.

Based on the mixed model analysis only the change in FFI Pain scores differed significantly between the treatment groups. Inspection of Figure 2 indicates that both treatment groups show decreased pain over time. In the control group the pain scores decrease quickly after the treatment and then remain stable during the follow-up. In the PRP group the pain reduction is more modest yet reaches a lower point at the 12-month follow-up than the control group. This finding is confirmed by the analysis of covariance reported in Table 3. After adjusting for baseline differences in FFI Pain scores, the patients in the PRP group showed significantly lower pain scores than patients in the control group (mean difference, 14.4; 95% CI, 3.2-25.6). Although the mixed model analysis did not indicate a significant treatment effect for the FFI disability outcome, the analysis of covariance suggests that after adjusting for baseline differences, patients

in the PRP group also showed significantly lower FFI Disability scores than patients in the control group (mean difference, 12.0; 95% CI, 2.3-21.6). For all WHOQOL-BREF outcomes the differences between the treatment groups at the 12-month follow-up failed to reach significance.

Table 3 Difference Between Treatment Groups at the 12-Month Follow-up for all Outcome Measures α

Outcome measure	Treatment Effect *F*-test	*P* value	Partial Eta Squared	Mean difference (95% CI)[b]
FFI Pain[c]	*F*(1,76) = 6.602	0.012[d]	0.080	14.40 (3.24, 25.57)
FFI Disability	*F*(1,76) = 6.064	0.016[d]	0.074	11.97 (2.29, 21.64)
FFI Activity	*F*(1,76) = 3.288	0.074	0.041	5.93 (-0.58, 12.44)
AOFAS	*F*(1,75) = 5.674	0.020	0.070	-11.27 (-20.69, -1.84)
WHOQOL-BREF				
Overall QOL and General Health	*F*(1,76) = 0.001	0.975	0.000	-0.01 (-0.54, 0.52)
Physical Health	*F*(1,76) = 3.187	0.078	0.040	-1.18 (-2.50, 0.14)
Psychological Health	*F*(1,76) = 0.471	0.494	0.006	-0.25 (-0.96, 0.47)
Social Relationships	*F*(1,70) = 0.071	0.790	0.001	-0.15 (-1.24, 0.95)
Environment	*F*(1,76) = 0.029	0.866	0.000	0.08 (-0.82, 0.97)

[a] Adjusted for between-group differences in baseline scores. AOFAS, American Orthopaedic Foot & Ankle Society; FFI, Foot Function Index; WHOQOL-BREF, World Health Organization Quality of Life.
[b] A positive mean difference indicates a higher mean score in the control group than in platelet-rich plasma at 12-month follow-up.
[c] Primary outcome measure.
[d] Statistically significant after the Bonferroni correction (see the Statistical Analysis section).

Last, Table 4 shows for both treatment groups the number of patients with at least 25% improvement in their FFI Pain score between baseline and the 12-month follow-up. It turns out that of the 46 patients in the PRP group, 39 (84.4%) improved at least 25%, while only 20 (55.6%) of the 36 patients in the control group showed such an improvement. This difference was statistically significant (χ^2[1], 8.6; *P* = .003; odds ratio, 4.5; 95% CI, 1.6-12.7).

Table 4 Improvement in the FFI Pain Scores by 25% α

		25% Improvement in FFI pain, [b] n (%)		
		Yes	No	Total
Treatment group	PRP	39 (84.8)	7 (15.2)	46
	Control	20 (55.6)	16 (44.4)	36
	Total	59	23	82

[α] For both the PRP group and the control group, the number (percentages) of patients showing a 25% improvement in their FFI Pain scores between baseline and the 12-month follow-up. FFI, Foot Function Index; PRP, platelet-rich plasma.
[b] Patients in the PRP group showed a 25% improvement in the FFI Pain score significantly more often than patients in the control group (χ^2[1], 8.55; *P* = .003; odds ratio, 4.46; 95% CI, 1.58-12.66).

DISCUSSION

This randomized study was designed to test the effectiveness of PRP compared with corticosteroid injections for chronic plantar fasciitis.

There is no standard of care management for chronic recalcitrant plantar fasciitis that is nonresponsive to nonoperative treatment. Many researchers believe that, since plantar fasciitis is a degenerative disease, regenerative potential of platelet rich plasma could help. The treatment of a degenerative tendon disease with an injection of concentrated autologous platelets may be a nonoperative alternative. By utilizing the GPS system, the patient's own platelets can be collected into a highly concentrated formula. We postulate that the concentrated growth factors work in a synergetic manner to initiate a tendon healing response. This hypothesis is supported by in vitro research in the literature. Transforming growth factor β1 is shown to significantly increase type I collagen production by tendon sheath fibroblasts. This same mechanism is likely to be active in chronic plantar fasciitis.[34]

In this study, we followed the patients for 1 year after intervention; pain at the end of 1 year was our primary end point, as assessed with the FFI Pain scale. Function and quality of life were the secondary outcome measures. Our results show that the 2 treatments differed in their change in pain score over time. Patients in the PRP group showed significantly lower pain and disability scores than patients in the control group after adjusting for baseline differences. Differences between the treatment groups at 1-year follow-up were not found with respect to function (FFI Activity and AOFAS) and quality of life (WHOQOL-BREF). A larger percentage of patients showed at least a 25% improvement in pain score between baseline and the 1-year follow-up in the PRP group (84.8%) than in the control group (55.6%). Our findings in this study with a decrease in the pain and disability after a PRP injection compared well with other published studies on treatment of plantar fasciitis.[29] It also showed similar outcomes

if compared to a previous study, where the same GPS and injections techniques were used, for patients with chronic lateral epicondylitis.[23] Here the authors also concluded that the corticosteroid group initially was better and then declined, whereas the PRP group progressively improved.

According to Gonnade et al.[10] in their recent article, previous observational studies and a few randomized clinical trials on plantar fasciitis have concluded that PRP is an effective therapy in chronic cases, but still there is controversy due to lack of Level 1 evidence. In a single-blinded prospective randomized longitudinal case series of 40 patients, Monto [21] concluded that PRP injection is more efficacious and long-lasting than cortisone injection in the long-term management of severe chronic plantar fasciitis. One trial by Shetty et al.[28] also compared PRP with cortisone, but they found no difference between the two. The drawback of Shetty et al.'s study is the short follow-up of only 3 months. The most recent study by Mahindra et al.[18] found that PRP and cortisone are better than placebo, but at 3 months of follow-up, PRP injection was significantly better than corticosteroid injection.

To our knowledge, this is the first randomized that compared PRP with corticosteroids in > 100 patients with plantar fasciitis. Treatment of patients with chronic plantar fasciitis with PRP seems to reduce pain and increase function as compared with the effect of corticosteroid injection. Our findings are comparable with other studies, but this study had a 1-year follow up. All other randomized studies had a maximum follow-up of 3 months.[10]

There are some limitations of our study. First, we have to address the violation of protocol. Sixteen patients were treated with a 30-mL PRP kit instead off the 60-mL PRP kit as described in the protocol. This was due to logistic reasons and occurred in only 1 of the treating centres. Because the results suggest that the injection dosage did not affect the differences between the treatment groups in their change on the outcomes over time, we did not exclude them from this study. Our statistical analyses were also been adjusted to accommodate for this protocol violation. Furthermore, there is a large heterogeneity among different systems with regard to the concentrations of platelets, leukocytes, and growth factors in PRP. The choice for the most appropriate type of PRP should be based on the specific clinical field of application,[22] but there is no significant difference between the concentrations of PRP obtained with the GPS II (30-mL blood) and GPS III (60-mL blood).[12] Second, we did not use ultrasound-guided injections for both groups. There is always a debate about the fact that injections would not been given at the exact spot where they are needed. Ultrasound-guided technique is advocated in previous studies.[10] Kane et al.[11] showed no advantages of ultrasound guidance over direct palpation of the most tender area for guidance for the injections. A final limitation is that we have no data on the patient's characteristics, between the study group and the 8 patients who were not suitable for further allocation. Potentially, this could lead to a bias.

In conclusion, this report describes the first comparison of an autologous platelet concentrate with corticosteroids as a treatment for chronic plantar fasciitis in patients who have undergone failed nonoperative treatment, with a follow up of 1-year. It demonstrates that a single injection of concentrated autologous platelets improves pain and function more so than corticosteroid injection. These improvements were sustained over time with no reported complications. Future decisions for application of the PRP for plantar fasciitis should be confirmed by further follow-up from this trial and should take into account possible costs and harms as well as benefits.

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Appendix Table A1

For all outcome measures the results of Little's MCAR test

Outcome measure	Chi-Square test	*P*-value
FFI Pain	$\chi^2(30) = 16.330$	0.980
FFI Disability	$\chi^2(27) = 21.993$	0.738
FFI Activity	$\chi^2(27) = 24.285$	0.614
AOFAS	$\chi^2(28) = 17.190$	0.945
WHOQOL Overall QoL and General Health	$\chi^2(24) = 19.058$	0.749
WHOQOL Physical Health	$\chi^2(27) = 25.478$	0.548
WHOQOL Psychological Health	$\chi^2(27) = 32.715$	0.207
WHOQOL Social Relationships	$\chi^2(24) = 47.296$	0.265
WHOQOL Environment	$\chi^2(27) = 27.631$	0.430

Abbreviations: QoL = Quality of Life, FFI = Foot Function Index, AOFAS = American Orthopaedic Function & Ankle Society; WHOQOL = World Health Organization Quality of Life instrument

Appendix Table A2

For all outcome measures the results of the *F*-test on the three-way interaction between treatment, dosage and time, both for models with- and without adjustment for quadratic and cubic time effects

Outcome measure	**Time modelled linearly**		**Time modelled linearly, quadratically and cubically**	
	F-test	*P*-value	*F*-test	*P*-value
FFI Pain†∇	F(1,96.636) = 3.157	0.079	F(1,178.136) = 0.237	0.627
FFI Disability†	F(1,117.726) = 0.434	0.511	F(1,210.527) = 0.602	0.439
FFI Activity†	F(1,122.073) = 0.001	0.977	F(1,203.000) = 0.235	0.628
AOFAS†	F(1,126.834) = 1.919	0.168	F(1,212.003) = 1.249	0.265
WHOQOL				
Overall QOL and General Health	F(1,116.196) = 2.687	0.104	F(1,213.489) = 0.475	0.491
Physical Health†	F(1,122.828) = 3.645	0.059	F(1,175.282) = 1.549	0.215
Psychological Health	F(1,113.226) = 0.747	0.389	F(1,167.989) = 1.320	0.252
Social Relationships	F(1,104.084) = 3.447	0.066	F(1,187.336) = 0.085	0.771
Environment	F(1,126.952) = 0.631	0.429	F(1,192.948) = 2.174	0.142

* Statistically significant based on the criteria reported in our statistical analysis paragraph
† These models showed significant quadratic- and cubic time effects
∇ Primary outcome measure

CHAPTER 8

Discussion

Platelet-rich plasma (PRP) is derived from a patient's own blood and is injected locally for several musculoskeletal disorders. In the laboratory it has been shown that PRP contains a high concentration of growth factors, and by injecting PRP, local repair reactions might be stimulated. The aim of injecting PRP would be to enhance biological repair processes, leading to reduction of patient complaints. Therefore, PRP has a place in the field of orthobiologics. Due to the limited results of conventional non-surgical treatment for some musculoskeletal disorders, there is great interest in the use of orthobiologics. Application of PRP has become popular, but there is limited evidence of its effectiveness.[1]

The overall aim of this thesis was to study the place of PRP in the treatment of two frequently occurring tendon disorders, tennis elbow and plantar fasciitis, and for wound healing in total knee arthroplasty (TKA). We concluded that PRP injections outperforms corticosteroid injections in tennis elbow and plantar fasciitis. We did not find added value in using PRP in wound healing after TKA.

In this chapter, we discuss the main findings of our studies and their significance for clinical practice. We will also discuss the complex comparison between different systems of PRP application and opportunities for further research.

SECTION I: TENDINOPATHY

In our studies we concluded that the group patients who received a PRP injection for their tennis elbow (**Chapter 5 and 6**) or for plantar fasciitis (**Chapter 7**) had a better outcome than patients who received a corticosteroid injection. This suggests that PRP reduces the patient complaints, but it may also demonstrate negative long-term effects of corticosteroid injections. The treatment of tendinopathy is challenging because the precise pathophysiology of tendinopathy is not known, and the origin of pain is not clear yet. Thus, this treatment is not based on hard science but on theoretical considerations related to the condition.

Corticosteroid Injections

Corticosteroid injections have been used to treat tendinopathy since the 1950s.[2] These injections are given directly into the area around the tendon.[3] Corticosteroid injections aim to modulate inflammatory cells and mediators, such as lymphocytes, macrophages and mast cells.[3] Essentially, corticosteroid injections reduce the pain caused by inflammation. However, injections of corticosteroids also increase protein catabolism, reduce the synthesis of type I collagen and therefore slow down repair reactions.[3] Given the rather limited inflammation in prolonged tendinopathy and the inhibition of collagen repair after corticosteroid injection, the usefulness of corticosteroid injections for chronic symptoms in tendinopathy has been subject to much debate.[4-6] Corticosteroid injections have shown to be effective in relieving pain in the short term. However, after longer periods, there tends to be a recurrence of symptoms.[4,5] In addition, several

complications have been reported with injections of corticosteroids, such as fad pad atrophy and tendon rupture.[4,5,7]

Lateral Epicondylitis

Lateral epicondyle tendinopathy, also known as 'tennis elbow', is a common cause of pain and disability. It has been suggested that repetitive microtrauma of the extensors of wrist and hand can induce an angiofibroblastic reaction, leading to pain.[8] Our study shows that corticosteroid injections are inferior to PRP injections in the treatment of tendinopathy.

Our results are supported by several meta-analyses.[9-11] Li et al. conducted a meta-analysis to compare PRP injections with corticosteroid injections in patients with lateral epicondylitis.[9] They used seven medium to high quality randomised controlled trials (RCTs) for inclusion in their study.[12-18] The meta-analysis revealed that corticosteroid injections result in better outcomes in the Disability Arm, Shoulder, and Hand (DASH) scores compared to PRP treatments for lateral elbow epicondylitis during the first few weeks (four to eight weeks). PRP injections resulted in reduced pain and improved function in the visual analogue scale (VAS) and DASH scores and were more effective than corticosteroid injections after a longer follow-up period (24 weeks after the injection).

Tang et al. published a meta-analysis to compare PRP, autologous blood (AB) injections and corticosteroid injections in patients with lateral epicondylitis.[10] They included RCTs that compared any two forms of these injections. PRP yielded a better outcome in pain (VAS) and function (DASH) in the long term than AB or corticosteroid injections. However, in the short term, corticosteroids ranked first. In another meta-analysis, Xu et al. came to the same conclusion. PRP injections showed a superior improvement in reducing pain and improving elbow function compared with corticosteroids injections in the long term.[11]

However, studies comparing PRP with a normal saline solution did not show that PRP was more effective.[14,19,20] The same was seen for autologous blood compared to PRP.[21-23]

Comparing our studies (**Chapter 5 and 6**) with the main conclusions of reviews and meta-analyses, we conclude that our own studies are in line with the literature. We conclude that PRP injections have a better and longer-lasting effect than corticosteroid injections do. Other studies, however, found no benefit of PRP injections over other substances, including saline solutions. Therefore, final conclusions about the effectiveness of PRP cannot be drawn yet.[24]

Plantar Fasciitis

Plantar fasciitis is a syndrome characterised by pain at the attachment site of the plantar fascia to the calcaneus. It is one of the most common causes of heel pain. Plantar fasciitis is more common in runners and people who are overweight. Small tears in the fascia at the attachment site are believed to cause that pain. The condition of more than 90% of

patients with plantar fasciitis improves within 10 months of starting simple treatment methods. Among these methods is rest, ice, nonsteroidal anti-inflammatory medication, exercise, supportive shoes and orthotics, soft heel pads, night splints, physical therapy, extracorporeal shockwave therapy (ESWT) and corticosteroid injection.[25]

In our plantar fasciitis study (**Chapter 7**), patients in the PRP group showed significantly lower pain and disability scores than patients in the corticosteroid group. A larger percentage of patients showed an improvement of at least 25% between the baseline pain score and the one-year follow-up score in the PRP group (85%) than in the corticosteroid group (56%).

In systematic reviews and meta-analyses which compare injections of PRP to corticosteroid injections, the use of PRP is supported mainly because of its superiority over corticosteroids, especially in providing long-term pain relief.[26-29] Ling and Wang concluded that PRP injections offered better effects than corticosteroids in American Orthopaedic Foot and Ankle Score (AOFAS), and their effects were sustained in the long term.[29] As described in lateral epicondylitis, PRP outperformed corticosteroid injections, but further studies are necessary to determine if the effect of PRP is better than a placebo injection. Therefore, there is no scientific basis yet to recommend PRP as a standard treatment option for plantar fasciitis.

SECTION II: TOTAL KNEE ARTHROPLASTY

Biological components used to enhance haemostasis and wound healing following total knee arthroplasty have been the subject of research. In 2000 Mooar et al. showed that using PRP for TKA resulted in a positive outcome.[30] In **Chapter 3** we tested the hypothesis that the application of PRP would improve repair of wounds after total knee arthroplasty (TKA). In patients undergoing TKA, application of PRP to the wound site did not promote wound healing. Additionally, we found that it had no effect on pain, knee function or haemoglobin drop (as indicator of blood loss).

In their two-year follow-up study, Guerreiro et al. showed that the PRP group, the tranexamic acid group and a combination of these two found better pain control post-operation.[31] Their study was in line with our study. Our own results are also supported by a meta-analysis by Li et al.[32] and by Ma et al.[33] Other than observing an improvement of the range of motion (ROM), there was no difference with respect to pain and the infection rate. Almost all of the reported studies had a short follow up. For TKA, in particular, a longer follow-up would be necessary to show the effects on knee function and periprosthetic infections.

No studies were found focusing on the possible antimicrobial effect of PRP leading to reduction of infection rate after TKA. Especially in high-risk cases (obesity, smoking, diabetics) studying the reduction of the infection risk by using PRP seems to be valuable.

So far, a consensus on using PRP as a routine treatment for wound healing after TKA has not been reached. Wound healing is a complex process, and many factors, including surgical techniques and approaches may contribute to the process. Other patient conditions, including diabetes, obesity and smoking, may also impact wound healing. Therefore, a robust study with a large number of patients would be necessary to show the place of PRP in enhancing wound healing and infection prevention in TKA.

SECTION III: PRP SYSTEMS

Blood Components

In **Chapter 2** we reviewed literature about the concentrations of blood components in commercially available PRP separation systems. The literature revealed a significant amount of heterogeneity between the PRP separation systems available on the market, especially involving concentrations of platelets, leukocytes and growth factors in PRP. In our review we found a wide variety of growth factors, not only between the different separation systems, but also when different studies compared the same separation systems. We also found a strong correlation between the concentration of vascular endothelial growth factor (VEGF) and the concentrations of platelets and leukocytes. This correlation is also supported by other studies.[29,34]

The concentration of platelets in PRP is important because the mechanism of action of PRP is mainly based on growth factors. Growth factors released by the platelets, platelet-derived growth factor (PDGF), transforming growth factor ß1 (TGF-ß1), VEGF and epidermal growth factor (EGF) are the most important factors involved in tissue repair.[35] The concentration of these growth factors is generally considered a marker of the quality of the PRP preparation.[35,36] However, there is no consensus about the optimal concentration of growth factors.[35,36]

Currently, physicians can choose from over 30 PRP processing systems.[37-39] A lack of a consensus on standardising PRP has contributed to the significant variation in PRP products. Although the ideal concentrations of blood components and growth factors for specific fields of application have yet to be determined, the field of application should play an important role in the choice for the most appropriate PRP separation system. Other factors such as the volume of whole blood needed, the final volume of PRP and the usability and reliability of the separation system should also be taken into consideration.

PRP Preparation

There are a number of standard steps for preparing PRP. The key steps in the preparation process are collecting peripheral blood from the patients by vena puncture, conducting blood centrifugation to retrieve platelet-enriched fraction and activating platelets to release growth factors.[40,41] In each of these phases, there is variability based on the volume of blood collected, the type of anticoagulant, centrifugation protocols and the type of platelet activators.[36,42]

The concentration of leukocytes also differed extensively between the systems studied (**Chapter 2**). PRP separation systems are typically divided into systems producing high and low concentrations of leukocytes.[44,45] Plasma-based methods minimise leukocyte fractions. Buffy coat-based methods actively concentrate leukocytes.[42,46,47] This type of PRP preparation is generally referred to as *leukocyte-rich* PR*P* (LR-PRP).

Currently, the presence of leukocytes in PRP is under debate, as both beneficial and adverse effects of leukocytes have been suggested.[48] Potential benefits of the presence of leukocytes include their role in tissue remodelling and increased antibacterial and immunological resistance.[50,51] Furthermore, the presence of leukocytes in PRP is associated with an increased concentration of growth factors, especially VEGF.[41,51,52] On the other hand, leukocytes may have catabolic and inflammatory effects due to their release of pro-inflammatory cytokines, which is associated with decreased proliferation and increased apoptosis.[53-58]

In the studies we performed, LR-PRP was used. (**Chapter 3, 5, 6, 7**). This leukocyte-rich type of PRP is also most commonly used for treatment of tendinopathy in other studies. Hardly any study, however, provides a detailed characterisation of the compositions of the PRP used, which makes comparing different studies a challenge.[59] The characterisation of the cell types in PRP used is important, but many other parameters need to be considered (e.g., the activation of the growth factors, the presence of red blood cells).[60-62] All of these factors make it intrinsically difficult to compare the effectiveness of PRP in different studies. Furthermore, there is no proof or consensus on the optimal preparation method and composition of PRP for different musculoskeletal conditions.[63]

STRENGTHS and LIMITATIONS

All of our RCTs (**Chapter 3, 5, 6, 7**) were double blind multicentre RCTs. They all provide a thorough description of the preparation of the PRP and the system used. Both of our RCTs on tendinopathy (**Chapter 5, 6, 7**) involved an above average enrolment of patients. On the other hand, in the plantar fasciitis study, the number of patients with which we were unable to follow up after a year was quite high (**Chapter 7**). 27% (17 out of 63) of the patients in the PRP group and 31% (16 out of 52) in the control group

were lost in follow-up. The same can be concluded with regard to follow-up in the arthroplasty study. In this part of the study, we were unable to follow up with 36% (18 out of 50) in the PRP group and 21% (11 out of 52) in the control group due to incomplete primary outcome (wound healing) datasets.

In both studies on tendinopathy (**Chapter 5, 6, 7**), we did not use ultrasound-guided injections for either group. Ultrasound-guided techniques were used in some other studies.[64] However, Kane et al. did not find advantages in using ultrasound guidance over direct palpation of the most tender area in terms of guidance of the injections.[65] All injections were given via the same technique for both corticosteroids and PRP.

In the plantar fasciitis study (**Chapter 7**), 16 patients were treated with a 30 ml PRP kit instead of the 60 ml PRP kit described in the protocol. This was due to logistical reasons in one of the treatment centres. This was discussed with the Medical Ethics Committee (METC), and we analysed it statistically. The influence of dosage on the effect of treatment was assessed by inspecting the three-way interaction effect among treatment group, time and injection dosage.

However, this conclusion is based on a small sample size (only 16 patients). Several studies have demonstrated that the reaction of cells depends on the dosage of the growth factors, but that very high concentrations are not essential for optimal stimulation of cell processes and may have an opposite effect.[34,66,67] Because the results suggest that the injection dosage did not affect the differences between the treatment groups in the changes in their outcomes over time, we did not exclude them from this study.

Control group

In our tennis elbow studies (**Chapter 5, 6**), the recurrence rate and need for repeated injection or surgery was greater in the corticosteroid group than in the PRP group. This may suggest that corticosteroid injections may have a detrimental effect on tendons. This is supported by two high-quality reviews on treatment effects in chronic tendinopathies.[68,69] These studies showed that corticosteroids provided inferior clinical outcomes compared to a wait-and-see policy after a six-month follow-up. These results were also found in a high-quality double-blind RCT.[70]

This raises the question of whether the outcome seen in favour of PRP in our studies is due to the beneficial effect of PRP or due to the detrimental effect of corticosteroids. The difference between our studies and those of Coombes et al. (2013) and Smith et al. (2002) is that in our study the duration of complaints was chronic (more than six months), while in the Coombes et al. and the Smith et al. studies, there was a much shorter duration of complaints (six weeks or more).[69,70] Therefore, it would be valuable to have RCTs with normal saline as a control group because of the suggested placebo effect of normal saline. In a systematic review and meta-analysis, normal saline injections

proved to have a positive effect on pain relief and function in patients with lateral epicondylitis either due to their mechanical effect or because of a placebo effect.[19,21]

Outcome bias

PRP may have all the requirements of an ideal placebo. Filardo and Kon demonstrated that apart from expectations about outcome, marketing, a new sort of treatment and the way PRP is given to the patient are all parts of the placebo effect when patients receive PRP injections.[71] Needling in itself is a powerful tool, especially when patients are allocated for repeated injections.[72]

It is important to remember that the psychological effects of this innovative treatment may be in the financial interests of physicians in some countries and may bias the outcome of studies.

Additionally, industry-sponsored studies may bias final outcomes. Nessello et al. noted that industry-sponsored studies were more likely to show positive results, as did articles with a lower quality of evidence.[73]

In addition to the influences of the industry, another possible influence is that both the physician and the patient might be aware of emerging technologies, such as PRP.[74] The PRP products are often linked with keywords such as orthobiologics, growth factors, regenerative medicine, stem cells and others, which may be attractive to patients. In short, PRP is popular among physicians and patients for reasons beyond scientific evidence, such as competition and anecdotal evidence of efficacy.[1]

Regarding scientific evidence several systematic reviews and meta-analysis have been published in the last decade. It is important to keep in mind that a majority of studies regarding PRP and tendinopathy use a control group with active clinical treatments (i.e., corticosteroids, autologous blood injections).[75] This may also influence the final conclusions found in systematic reviews because of the (negative) effect of the treatments in the control groups.

Rehabilitation

Many studies do not provide sufficient information about PRP products, but many studies also fail in describing the rehabilitation protocols used after PRP treatment for tendinopathies. This makes it also difficult to determine which part of the treatment is effective. It is not clear whether the PRP injection, the exercise program after the injection or a combination of the two causes the outcome. It may also be possible that no treatment might enhance the self-limiting nature of these conditions.

The effect of rehabilitation itself is a compelling topic on its own, but this goes beyond of the scope of this thesis.

CONCLUSION and FUTURE PERSPECTIVES

From the studies presented in this thesis, we conclude that PRP outperformed corticosteroid injection for tennis elbow and plantar fasciitis. This is in line with other studies, reviews and meta-analyses of these topics.

However, this in itself does not constitute proof of a positive effect of PRP injections. Negative effects of corticosteroid injections in tendinopathy have been found; therefore, the negative effect of corticosteroid injections may bias perceptions of the effectiveness of PRP. We would recommend future studies with a better control group. We did not find added value in using PRP in wound healing after TKA, but studies in high-risk groups may be valuable.

PRP has become prominent in orthopaedic surgery and sports medicine over the last decades. However, studies that have been conducted on PRP up to now have been rather limited in quality. Investigation into PRP's therapeutic applications, optimal cellular compositions and treatment protocols lag far behind its widespread clinical use.

We recommend that each future study provide detailed, precise, and stepwise description of PRP preparation protocols. Then, a standardised rehabilitation protocol should be given to all patients to facilitate post-injection uniformization. Both of these steps will lead to more effective comparisons of future studies and will provide clearer information of the true potential of PRP. Future studies should also focus on measurements of adverse effects, patient satisfaction, cost effectiveness and quality of life among their primary outcomes.

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CHAPTER 9

Summary

In this thesis, the use of platelet-rich plasma (PRP) in the treatment of some musculoskeletal disorders is evaluated. Several studies are described to improve knowledge about the basic principles of PRP and to demonstrate its advantages and disadvantages in various applications.

Chapter 1 is a general introduction to the use of PRP in musculoskeletal disorders, with a focus on its use in tendinopathy and wound healing. A brief discussion of the history of PRP is provided. In addition, techniques for preparing PRP and releasing growth factors from the platelets are examined; this is followed by a discussion of the effectiveness of PRP. The last section of this chapter gives an overview of the thesis.

Chapter 2 is a review of the literature on commercial PRP separation systems and the resulting concentrations of blood components. There is a large heterogeneity among PRP separation systems regarding the concentrations of platelets, leukocytes, and growth factors in the resulting PRP. The type of PRP to be used in treatment should be chosen based on the specific clinical field of application. As the ideal concentrations of blood components and growth factors for any given application are yet to be established, future research should focus on determining the type of PRP most suitable for each specific field.

Chapter 3 describes a randomised clinical trial in patients undergoing total knee arthroplasty (TKA). We investigated whether the use of PRP in TKA would improve wound healing and knee function while reducing blood loss and the need for analgesics. A total of 102 patients undergoing TKA were randomly assigned to a PRP-group (n = 50) or to a control-group (C) (n = 52). The primary analysis included 73 participants (PRP = 32 and C = 41) and compared postoperative wound scores, VAS scores, WOMAC scores, knee function, use of analgesics, and pre- and post-operative levels of haemoglobin between the two groups. Twenty-nine participants were lost to follow-up. The characteristics of the protocol-compliant patients were similar to those of the excluded patients. Analysis was per protocol and focused on the remaining 73 patients. At baseline and after 3 months of follow-up, there were no statistically significant differences between the PRP group and the control group regarding age, height, weight, sex, side of operation, platelet count, haemoglobin values, severity of complaints, and level of pain. In patients undergoing TKA, the application of PRP to the wound site did not promote wound healing. Moreover, we also found that the use of PRP had no effect on pain, knee function, or haemoglobin values.

Chapter 4 reviews the use of PRP in upper extremity disorders. PRP has been used since 1990 in the dental and facial reconstructive surgery. The application in other areas has been increased rapidly as a result of the reported positive effects on bone, muscle and tendon regeneration as well as wound healing. In vitro studies show that growth factors released by platelets have a positive effect on the repair of soft tissue.

The application of PRP for upper limb conditions has also increased. In this chapter the literature is reviewed. We came to the conclusion that the use of PRP for upper limb conditions should be studied in more detail.

Chapter 5 describes a randomised clinical trial in patients with lateral epicondylitis. The trial was conducted in two teaching hospitals in the Netherlands. A total of 100 patients with chronic lateral epicondylitis were randomly assigned to a PRP-group (*n* = 51) or a corticosteroid-group (*n* = 49). Patients received either a corticosteroid injection or a PRP injection. The primary analysis included VAS and DASH scores. Successful treatment was defined as a reduction of at least 25% in VAS or DASH score after 1 year without reintervention. According to the VAS scores, 24 of the 49 patients (49%) in the corticosteroid-group and 37 of the 51 patients (73%) in the PRP group had a successful treatment outcome; this was a significant difference ($P < .001$). A significant difference ($P = .005$) was also observed between the DASH scores of the two groups, with 25 of the 49 patients (51%) in the corticosteroid-group and 37 of the 51 patients (73%) in the PRP-group displaying a successful treatment outcome. The decrease in the scores of the corticosteroid-group was greater initially but then declined, whereas the scores of the PRP group decreased more progressively. Treatment of chronic lateral epicondylitis with PRP reduces pain and significantly improves function, exceeding the effect of corticosteroid injection. When deciding to use PRP for lateral epicondylitis in the future, clinicians should take into account further follow-up from this trial and consider possible costs and harms as well as benefits.

In **Chapter 6** we describe the long-term effect of PRP injection after a follow-up of 2 years. The PRP-group was more often successfully treated than the corticosteroid-group ($P < .0001$). Success was defined as a 25% reduction in VAS or DASH score after 2 years without reintervention. When the baseline VAS and DASH scores were compared with the scores from the 2-year follow-up, both groups significantly improved over time. However, the DASH scores of the corticosteroid-group returned to baseline levels, while those of the PRP-group significantly improved. There were no complications related to the use of PRP.

The use of PRP in the treatment of patients with chronic lateral epicondylitis reduces pain and increases function significantly, exceeding the effect of corticosteroid injection even after a follow-up of 2 years.

Chapter 7 describes a randomised clinical trial in patients with plantar fasciitis. A total of 115 patients with plantar fasciitis were randomised to a PRP-group (*n* = 63) or a corticosteroid-group (*n* = 52). The primary analysis included the FFI Pain score. Secondary outcomes were the FFI Activity, FFI Disability, AOFAS score and the WHOQOL BREF. Seventy-three % of the patients in the PRP-group and 69% of patients in the corticosteroid-group completed the study. The PRP-group showed significantly lower

pain scores at 1 year follow-up than the corticosteroid- group (mean difference, 14.4; 95% CI, 3.2-25.6). At 1 year follow-up, 39 of the 46 patients in the PRP-group (84.4%) had an improved pain score of at least 25%. In the corticosteroid-group 20 out of the 36 patients (55.6%) had an improvement of their pain scores by at least 25% (*P* = .003). The PRP-group also showed significantly lower FFI Disability scores than the corticosteroid-group (mean difference, 12.0; 95% CI, 2.3-21.6). Treatment of chronic plantar fasciitis with PRP appears to have a better effect than corticosteroid treatment on pain and function.

In **Chapter 8** the outcomes of our PRP work are reviewed to evaluate the performance of PRP in the treatment of some musculoskeletal disorders. Based on the studies presented in this thesis, our conclusion is that PRP injection outperforms corticosteroid injection when used for tennis elbow and plantar fasciitis. However, this does not definitively prove the positive effect of PRP injections. Negative effects of corticosteroid injections have been found, creating a bias towards the effectiveness of PRP. Future studies should form a real control group. Over the last few decades, PRP has made a place for itself in orthopaedic surgery and sports medicine. However, until now, studies regarding PRP have often been of limited quality. We recommend that future studies provide a detailed, precise, and stepwise description of the PRP preparation protocol used. Moreover, a standardised rehabilitation protocol should be used with all patients to promote post-injection uniformisation. These steps will yield unambiguous comparisons between future studies and more accurate information on the true potential of PRP. Future studies should also include adverse effects, patient satisfaction, cost effectiveness, and quality of life measures among the primary outcomes.

CHAPTER 10

Summary in Dutch

Nederlandse Samenvatting

In dit proefschrift wordt het gebruik van trombocytenrijk plasma (PRP) bij de behandeling van een aantal musculoskeletale aandoeningen geëvalueerd. Verschillende onderzoeken worden beschreven om de kennis van de basisprincipes van PRP te verbeteren en de voor- en nadelen ervan in verschillende toepassingen aan te tonen.

Hoofdstuk 1 is een algemene introductie betreffende het gebruik van PRP bij musculoskeletale aandoeningen, waarbij met name het gebruik ervan bij tendinopathie en wondgenezing wordt besproken. Er wordt een korte uiteenzetting van de geschiedenis van PRP gegeven. Daarnaast wordt de techniek van het bereiden van PRP en het vrijkomen van groeifactoren uit de bloedplaatjes uitgelegd. Hierna volgt een bespreking van de effectiviteit van PRP. Het laatste deel van dit hoofdstuk geeft een overzicht van het proefschrift.

Hoofdstuk 2 is een overzicht van de literatuur betreffende de commerciële PRP-scheidingssystemen en de resulterende concentraties van de bloedbestanddelen. Er bestaat een grote heterogeniteit tussen de PRP-scheidingssystemen met betrekking tot de concentraties van bloedplaatjes, leukocyten en groeifactoren in het PRP. Het type PRP dat bij de behandeling moet worden gebruikt, moet worden gekozen op basis van het specifieke klinische toepassingsgebied. Aangezien de ideale concentraties van bloedbestanddelen en groeifactoren voor een bepaalde toepassing nog moeten worden vastgesteld, dient toekomstig onderzoek zich richten op het bepalen van het type PRP dat het meest geschikt is voor ieder afzonderlijk gebied.

Hoofdstuk 3 beschrijft een gerandomiseerde klinische studie bij patiënten die een totale knieprothese (TKP) hebben ondergaan. Wij hebben onderzocht of het gebruik van PRP bij TKP de wondgenezing en de kniefunctie zou verbeteren en het bloedverlies en het gebruik van pijnstillers zou verminderen. In totaal werden 102 patiënten die een TKP ondergingen, gerandomiseerd in een PRP-groep (n = 50) of in een controle-groep (C) (n = 52). De primaire analyse omvatte 73 deelnemers (PRP = 32 en C = 41) en vergeleek de postoperatieve wondscores, de VAS-scores, de WOMAC-scores, de kniefunctie, het gebruik van analgetica en de pre- en postoperatieve hemoglobinewaarden tussen de twee groepen. 29 deelnemers werden geëxcludeerd vanwege onvoldoende gegevens. De kenmerken van de geïncludeerde studie patiënten waren vergelijkbaar met die van de patiënten die werden geëxcludeerd. De analyse richtte zich op de overige 73 patiënten. Bij aanvang en na 3 maanden follow-up waren er geen statistisch significante verschillen tussen de PRP-groep en de controle-groep wat betreft leeftijd, lengte, gewicht, geslacht, kant van de operatie, aantal bloedplaatjes, hemoglobinewaarden, ernst van de klachten (WOMAC) en het pijn niveau. Bij de patiënten die een TKP kregen, bevorderde het aanbrengen van PRP op het wondgebied de wondgenezing niet. Tevens zagen wij dat PRP geen effect had op de pijn, de kniefunctie of de hemoglobinewaarden.

Hoofdstuk 4 beschrijft het gebruik van PRP bij aandoeningen van de bovenste extremiteit. PRP wordt sinds 1990 gebruikt in de tandheelkunde en bij reconstructieve chirurgie van het aangezicht. De toepassing van PRP in andere vakgebieden neemt snel toe, als gevolg van de beschreven positieve effecten op de genezing van botten, spieren en pezen, alsmede bij wondgenezing. In vitro studies tonen aan dat groeifactoren die vrijkomen uit de bloedplaatjes een positief effect hebben op de genezing van weke delen. De toepassing van PRP voor aandoeningen van de bovenste extremiteit neemt eveneens snel toe. In dit hoofdstuk wordt de literatuur besproken. Wij kwamen tot de conclusie dat het gebruik van PRP bij aandoeningen van de bovenste extremiteit meer in detail bestudeerd zal moeten worden.

Hoofdstuk 5 beschrijft een gerandomiseerde klinische studie bij patiënten met chronische epicondylitis lateralis (tenniselleboog). De studie werd uitgevoerd in 2 perifere ziekenhuizen in Nederland. In totaal werden 100 patiënten met chronische tenniselleboog klachten gerandomiseerd in een PRP-groep (n = 51) of in een corticosteroïd-groep (n = 49). De primaire analyse omvatte de VAS- en DASH-scores. Succesvolle behandeling werd gedefinieerd als een vermindering van ten minste 25% in de VAS- of DASH-score na 1 jaar zonder re-interventie. De resultaten toonden aan dat, volgens de VAS-score, 24 van de 49 patiënten (49%) in de corticosteroïd-groep en 37 van de 51 patiënten (73%) in de PRP-groep succesvol waren, hetgeen significant verschillend was (P < .001). Bovendien waren volgens de DASH-score 25 van de 49 patiënten (51%) in de corticosteroïd-groep en 37 van de 51 patiënten (73%) in de PRP-groep succesvol, hetgeen eveneens significant verschilde (P = .005). De corticosteroïd-groep was aanvankelijk beter en nam daarna af, terwijl de PRP-groep geleidelijk verbeterde. Behandeling van patiënten met chronische tenniselleboog klachten met PRP vermindert pijn en verbetert de functie significant ten opzichte van een injectie met corticosteroïden. Bij de beslissing om in de toekomst voor tenniselleboog klachten PRP te gebruiken, dienen clinici rekening houden met de verdere follow-up van dit onderzoek en met mogelijke kosten, complicaties en baten.

In **hoofdstuk 6** beschrijven we het lange termijn effect van de PRP-injectie na een follow-up van 2 jaar. De behandeling van de patiënten in de PRP-groep was succesvoller dan die in de corticosteroïd-groep (P < .0001). Succes werd gedefinieerd als een vermindering van 25% in de VAS- of DASH-scores zonder re-interventie na 2 jaar. Wanneer de baseline VAS- en DASH-scores werden vergeleken met de scores na 2 jaar, verbeterden beide groepen significant in de loop van de tijd. De DASH-scores van de corticosteroïd-groep keerden echter terug naar de uitgangswaarden, terwijl die van de PRP-groep significant verbeterden. Er waren geen complicaties gerelateerd aan het gebruik van PRP.

Behandeling van patiënten met chronische tenniselleboog klachten met PRP, vermindert de pijn en verbetert de functie significant ten opzichte van het effect van een injectie met corticosteroïden na een follow-up van 2 jaar.

Hoofdstuk 7 beschrijft een gerandomiseerde klinische studie bij patiënten met fasciitis plantaris (hielspoor). In totaal werden 115 patiënten met fasciitis plantaris gerandomiseerd in een PRP-groep (*n* = 63) of een corticosteroïd-groep (*n* = 52). De primaire analyse omvatte de FFI Pain score. Secundaire uitkomstmaten waren de FFI Activity, FFI Disability, AOFAS-score en de WHOQOL-BREF. 73% patiënten in de PRP-groep en 69% van de patiënten in de corticosteroïd-groep voltooide het onderzoek. Na 1 jaar follow-up liet de PRP-groep een significant lagere pijnscore zien dan de corticosteroïd-groep (gemiddeld verschil, 14,4; 95% CI, 3,2-25,6). Het aantal patiënten met een verbetering van ten minste 25% in hun FFI Pain score tussen aanvang en de follow-up na 1 jaar, verschilde significant tussen de groepen. Van de 46 patiënten in de PRP-groep verbeterden 39 patiënten (84,4%) hun pijnscore met minstens 25%, terwijl slechts 20 patiënten (55,6%) in de corticosteroïd-groep een dergelijke verbetering vertoonden (*P* = .003). De PRP-groep vertoonde ook significant lagere FFI-Disability score dan de corticosteroïd-groep (gemiddeld verschil, 12,0; 95% CI, 2,3-21,6). Behandeling van chronische fasciitis plantaris met PRP lijkt een beter effect te hebben dan de behandeling met corticosteroïden ten aanzien van de pijn en de functie.

In **hoofdstuk 8** worden de uitkomsten van ons PRP-onderzoek uiteengezet om zodoende de positie van PRP-toepassingen bij de behandeling van een aantal musculoskeletale aandoeningen te evalueren. Op basis van de studies die in dit proefschrift worden gepresenteerd, is onze conclusie dat een PRP-injectie een beter effect heeft dan een corticosteroïden-injectie bij chronische tenniselleboog en fasciitis plantaris. Dit is echter geen bewijs voor een positief effect van PRP-injecties. Negatieve effecten van injecties met corticosteroïden zijn bekend. Derhalve vertekent het negatieve effect van injecties met corticosteroïden de effectiviteit van een PRP-injectie. In toekomstige studies bevelen wij daarom een echte controle-groep aan. De afgelopen decennia heeft PRP een plaats veroverd in de orthopedische chirurgie en sportgeneeskunde. Tot nu toe waren onderzoeken met betrekking tot PRP echter vaak van beperkte kwaliteit. Wij raden aan dat elke toekomstige studie een gedetailleerde, nauwkeurige en stapsgewijze beschrijving geeft van het PRP-bereidingsprotocol. Bovendien moet bij alle patiënten een gestandaardiseerd revalidatieprotocol worden gebruikt om uniformiteit na injectie te creëren. Beide stappen zullen leiden tot een betere vergelijking van toekomstige studies en zullen betere informatie geven over het werkelijke potentieel van PRP. Toekomstige studies dienen ook bijwerkingen, patiënttevredenheid, kosteneffectiviteit en kwaliteit van leven als primaire uitkomsten te beschouwen.

APPENDICES

A

PhD PORTFOLIO JC Peerbooms

Summary PhD training and teaching

Erasmus MC Department of Orthopaedics and Sports Medicine
Promotor: prof. dr. JAN Verhaar
Copromotor: dr. T Gosens

General courses

Good Clinical Practice (GCP-WMO examen) 2020.
Cursus Wetenschappelijke Integriteit. Erasmus Universiteit Rotterdam 2020.
Basic Course SPSS. Erasmus Universiteit Rotterdam 2020.

Podium presentations

Peerbooms J, Gosens T, Sluimer J, Bruijn D. Prospective randomised study on the effect of autologous platelet injection in lateral epicondylitis compared with corticosteroid injection. Trends in Tendinopathy Symposium. Tilburg, The Netherlands. 9 June 2008.

Peerbooms J, Gosens T, Sluimer J, Bruijn D. Positive effect of an autologous platelet concentrate in lateral epicondylitis: a double blind randomized controlled trial: PRP versus corticosteroid injection.
Southend University Hospital, Clinical Orthopaedic Audit Meeting. Southend-on-Sea, United Kingdom. November 2009.

Peerbooms J, van Laar W, den Oudsten B, Gosens T. Ongoing positive effect of platelet rich plasma versus corticosteroid injection in lateral epicondylitis – a double blind randomized controlled trial: PRP versus corticosteroid injection with a 2-year follow up. Flinders Medical Center, Adelaide, Australia. September 2011.

Peerbooms J, van Laar W, den Oudsten B, Gosens T. Ongoing positive effect of platelet rich plasma versus corticosteroid injection in lateral epicondylitis – a double blind randomized controlled trial: PRP versus corticosteroid injection with a 2-year follow up. Combined annual scientific meeting of the AOA and the NZOA. Rotarua, New-Zealand. 9-14 October 2011.

Poster presentations

Peerbooms J, de Wolf G, Bruijn D.
Toepassing van Autoloog Trombocyten Concentraat bij TKA.
Stafdag Leyenburg ziekenhuis. The Hague, The Netherlands. 2006.

Peerbooms J, de Wolf G, Colaris J, Bruijn D, Verhaar J. No positive effects of autologous platelet gel after total knee arthroplasty: a blinded randomized controlled study. Stafdag HAGA ziekenhuis. The Hague, Dordrecht, The Netherlands. 2008.

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The positive effect of autologous platelets versus corticosteroid injections in chronic lateral epicondylitis: a blinded randomized controlled study
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ISPOR 15th Annual European Congress. Berlin, Germany. 3-7 November 2012.

Peerbooms J. Positive Effect of Platelet-rich Plasma on Pain in Plantar Fasciitis. International Webinar on Physical Health, Nursing and COVID-19 Management. Virtual meeting. 13 November 2020

Reviewer

2020 Nederlands Tijdschrift voor Geneeskunde
2020 Military Medical Research
2020 Nederlands Tijdschrift voor Orthopedie

LIST of PUBLICATIONS

Peerbooms J. Positive Effect of Platelet-Rich Plasma on Pain in Plantar Fasciitis: Response. Am J Sports Med. 2020; 48(2): 28-29.

Joosse P, Loggers S, Van de Ree C, Van Balen R, Steens J, Zuurmond R, Gosens T, Van Helden S, Polinder S, Willems H, Van Lieshout M. FRAIL-HIP study group (Peerbooms J). The value of nonoperative versus operative treatment of frail institutionalized elderly patients with a proximal femoral fracture in the shade of life (FRAIL-HIP); protocol for a multicenter observational cohort study. BMC Geriatr. 2019; 19(1): 301.

Peerbooms J, Lodder P, den Oudsten B, Doorgeest K, Schuller H, Gosens T. Positive Effect of Platelet-Rich Plasma on Pain in Plantar Fasciitis: Adouble-Blind Multicenter Randomized Controlled Trial. Am J Sports Med. 2019; 47(13): 3238-46.

Oudelaar B, Peerbooms J, Huis In ‘t Veld R, Vochteloo A. Concentrations of Blood Components in Commercial Platelet-Rich Plasma Separation Systems: A review of the Literature. Am J Sports Med. 2019; 47(2): 479-87.

Peerbooms J, Colaris J, Hakkert A, Van Appeldorn M, Bruijn D, Den Oudsten B, Gosens T. No Positive bone healing after using platelet rich plasma in a skeletal defect. An observational prospective cohort study. Int Orthop. 2012; 36(10): 2113-19.

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Gosens T, Peerbooms J, den Oudsten B. Ongoing positive effect of platelet-rich plasma in lateral epicondylitis. Shoulder and Elbow 2011; 3: 256-60.

Peerbooms J, Gosens T. Platelet-rich plasma in upper limb conditions. Shoulder and Elbow. 2011; 3: 8-12.

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Gosens T, Peerbooms J, Bruijn D, Sluimer J. Positive effect of an autologous platelet concentrate in lateral epicondylitis. Ned Tijdschrift voor Orthopedie 2009; 16(3): 136.

Peerbooms J, de Wolf G, Colaris J, Bruijn D, Verhaar J. No positive effect of autologous platelet gel after total knee arthroplasty. Acta Orthop. 2009; 80(5): 557-62.

Peerbooms J, Simons J, Tetteroo G, De Graaf E. Curative resection of rectal carcinoid tumors with transanal endoscopic microsurgery. J Laparoendosc Adv Surg Tech A. 2006; 16(5): 435-38.

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CURRICULUM VITAE

Joost Christiaan Peerbooms was born in Eersel, the Netherlands on May 16, 1975. He grew up in Enschede with his older brother and graduated from high school in 1994 (VWO, Kottenpark College, Enschede). After a year of studying Civil Engineering at the Delft Technical University, he started medical school at the Leiden University Medical Centre. At the end of his medical studies in 1999, he joined a research project at the Albert Schweitzer Hospital in Lambarene, Gabon.

In 2002, Joost fulfilled a medical elective placement in Orthopaedic Surgery at the Orthopaedic Department of Groote Schuur Hospital, Cape Town, Republic of South Africa, and at the Leyenburg Hospital, The Hague, the Netherlands. Later that year, he received his qualification as a medical doctor and started working as a resident in the Orthopaedic Department of the Leyenburg Hospital in The Hague.

At the end of 2002, Joost started his general surgical training at the Erasmus Medical Centre, Rotterdam (Prof. dr. H.J. Bonjer) and the IJsselland Hospital, Capelle aan den IJssel (Dr. I. Dawson) as part of his specialist training in orthopaedic surgery. In 2005, he continued his orthopaedic surgery training at the Leyenburg Hospital (Dr. L.N.J.E.M. Coene) and the Erasmus Medical Centre (Prof. dr. J.A.N. Verhaar). At that time, Joost initiated his first clinical study with platelet-rich plasma (PRP) under the supervision of Dr. D.J. de Bruijn and Prof. dr. J.A.N. Verhaar. In the following years, Dr. T. Gosens and Joost shared the same interest in the applications of PRP and further clinical study projects followed. After finishing his training as an Orthopaedic Surgeon in 2008, Joost fulfilled a one-year fellowship in hip and knee arthroplasty at the Southend University Hospital in Southend-on-Sea, the United Kingdom (Mr. A. White), a one-year fellowship in traumatology and arthroplasty at the Flinders Medical Centre in Adelaide, Australia (Prof. dr. J. Krishnan), and a one-month fellowship in traumatology at the Ganga Hospital in Coimbatore, India (Prof. dr. S. Rajasekaran).

Since 2012, Joost has been a staff member of the Orthopaedic Department at the Albert Schweitzer Hospital in Dordrecht and Zwijndrecht in the Netherlands. He lives in Rotterdam with Maayke Hunfeld and their two children, Boris (2013) and Okke (2015).