Rotator cuff calcific tendinitis: another entity of rotator cuff problems

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Chapter 5

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 injury. Currently, several commercial PRP 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 concentration of blood components in PRP produced by various PRP separation systems.

Methods

MEDLINE/Pubmed, the Cochrane Central Register of Controlled Trials (CENTRAL) and EMBASE were searched for studies that compared the concentration of blood components and growth factors in PRP between various separation systems and studies that reported on the concentration of blood components and growth factors of single separation systems. The primary outcomes were platelet count, leukocyte count, and concentration of growth factors (Platelet Derived Growth Factor-AB (PDGF-AB), Transforming Growth Factor-B1 (TGF-B1), Vascular Endothelial Growth Factor (VEGF). Furthermore, the preparation protocols and prices of the systems were compared.

Results

1079 studies were found of which 19 studies were selected for inclusion in this review. The concentration of platelets and leukocytes in PRP differed largely between, and to a lesser extend within, the studied PRP separation systems. Additionally, large difference 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 concentration of blood components and growth factors for the specific fields of application are yet to be determined for most of the fields of application, future research should focus on which type of PRP is most suitable for the specific fields of application.

Introduction

Platelet-rich plasma (PRP) is 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 platelets alphagranules[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; complication are rarely reported as PRP is derived from autologous blood[42]. In orthopaedic surgery and sports medicine, the use of PRP is of increasing interest over the last decade. PRP has shown to have a beneficial effect on the healing of tendon, muscle, bone and cartilage injury[15,58]. Clinical studies on the efficacy of platelet-rich plasma 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 PRP separation systems are available for the preparation of PRP[15]. The concentrations of blood components in PRP (platelets, leukocytes and growth factors e.g. PDGF, TGF-B1 and VEGF) 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 concentration of blood components and growth factors[7,36,50]. To gain more insight into the differences between the concentration of blood components and growth factors in PRP produced by the different separation systems, we conducted a systematic review of literature on studies investigating the blood components and growth factors in PRP.

Materials and methods

Inclusion criteria

Studies

The literature search performed for this review was limited to studies that compared the concentration of blood components and growth factors in PRP between different PRP separation systems and studies that reported on the concentration of blood components and growth factors of single PRP separation systems. We only included studies investigating human blood taken from healthy adult (>18 years) volunteers. The literature search was limited to papers in the English, German, French and Dutch language.

PRP separation systems

Only studies reporting on PRP separation systems that are currently commercially available were included.

Outcome measures

This review primarily focused on the platelet count, the leukocyte count, the 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-B1 (TGF-B1), 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 concentration of blood components in PRP. There were no constraints based on publication status. In MEDLINE, the following search strategy was used and was modified for uses in 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.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 above-mentioned search strategy were reviewed. After the selection, the full manuscripts were reviewed for definitive selection. All identified studies were independently reviewed by two reviewers (B.O. and J.P.) for inclusion using the above-mentioned criteria. In case of disagreement, a third reviewer (A.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 the main conclusion(s) of the study. This information was extracted by one author (B.O.). If necessary, authors were contacted for additional information about their specific paper.

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, literature was searched for the preparation protocol.

Statistical analysis

First, the 95% confidence intervals (CI) were calculated for each of the blood components studied in the included studies using the mean concentration, the standard deviation and number of samples. The following formula was used: $x \pm \times \frac{\sigma}{\sqrt{n}}$ 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 the 95% CI. Differences in concentrations within and between the different PRP separation systems were explored informally by eye-ball 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 two or more PRP preparation systems were summarized in a table. Analyses were conducted in SPSS (version 15.0; SPSS, Chicago, Illinois) and Microsoft Excel, Microsoft Office 365 (Microsoft Corporation, Redmond, WA, USA).

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 manuscripts 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.

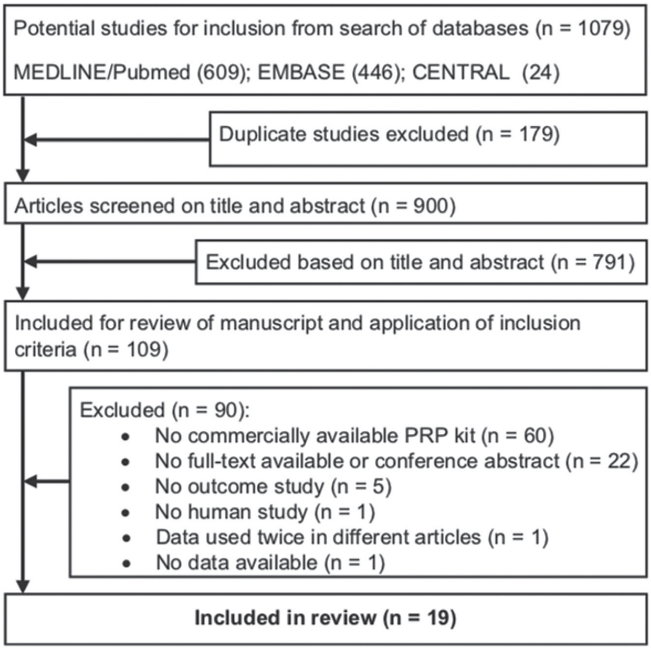

Figure 1: flow diagram of the search process.

Characteristics of the included studies

The characteristics of the included studies are summarized in table 1. 14 studies compared the concentration of blood components in PRP between different PRP

separation systems. In eight studies commercially available separation systems were compared. Five studies reported the concentration of blood components of single separation systems. The number of samples analyzed varied between 3 and 102. Ten different commercially available separation systems were studied. The GPSIII system was studied the most: ten times in total followed by the ACP system which was studied in five studies. The PRGF, Magellan and SmartPrep systems were all studied three times; the Cascade and RegenPRP were studied in two studies and the Prosys, KYOCERA and GLO systems were only studied in one study.

Outcome measures

The platelet concentration was the most studied outcome measure, studied in thirteen of seventeen studies. Other outcome measures were the leukocyte concentration (12/17), red blood cell concentration (5/17) and the platelet enrichment factor (7/17). With regard to growth factors, TGF-B1 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-B1, PDGF-A and VEGF were by far the most studied growth factors, further statistical analyses were only performed for these three growth factors.

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) as the total centrifugation time (range 5-21 minutes) differed largely between systems. Also, a wide variation in price per kit (range 95-500$) was found between the systems.

Table 1: Study characteristics of the studies included in this review

Study	Number of samples	Number of PRP kits studied	PRP kits studied	Outcome measures
Anitua 2013[2]	3	1	Endoret	PEF, WBCC, PDGF-AB, VEGF, HGF, IGF-I
Castillo 2011[7]	5	3	Biomet GPS III, Cascade, Magellan	PC, WBCC, RBC, PEF, PDGF-AB, PDGF-BB, TGF-B1, VEGF, PCE, FC
Dragoo 2012[13]	40	1	Biomet GPS III	PDGF-BB, TGF-B1, VEGF, IGF
Evanson 2014[16]	102	1	Arthrex ACP	PC, WBCC, RBC, PDGF-AB, PDGF-BB, TGF-B1, VEGF, EGF, FGF, HGF, IGF-1
Everts 2008[18]	20	1	Magellan	PC, WBCC, PEF
Hamilton 2013[24]	10	1	Biomet GPS III	PC, WBCC, PDGF-AB, HGF, IGF-1 and VEGF
Howard 2014[25]	4	2	Cascade, Harvest SmartPRep	PC, PEF, PDGF-AB, TGF-B1
Kaux 2011(1)[27]	6	1	Biomet GPS III	PC, WBCC, RBCC
Kaux 2011 (2)[26]	5	1	Biomet GPS III	WBCC, RBCC, PEF
Kushida 2014[29]	5	3	GLO, Kyocera, Magellan	PC, PDGF-AB, TGF-B1, VEGF
Leitner 2006[30]	3	1	Harvest SmartPReP	PC, WBCC, RBCC
Magalon 2014[32]	10	3	Arthrex ACP, Biomet GPS III, RegenPRP	PC, WBCC, PEF, PDGF-AB, TGF-B1, VEGF, EGF, PCE
Mazzocca 2012[36]	8	2	Arthrex ACP, Biomet GPS III	PC, WBCC, RBCC, PDGF-AB, TGF-B1, VEGF, EGF, FGF-2, HGF, IGF
Mazzucco 2009[37]	Not provided	1	RegenPRP	PC, PEF, PDGF-BB, TGF-B1, VEGF, EGF and IGF-I
Oh 2015[46]	14	3	Arthrex ACP, Biomet GPS III, Prosys PRP	PC, WBCC
Schar 2015[51]	11	1	Biomet GPS III	TGF-B1, VEGF
Sundman 2011[54]	11	2	Arthrex ACP, Biomet GPS III	PC, WBCC, PEF
Weibrich 2005[56]	51	1	Endoret	PC, WBCC, PDGF-AB, TGF-B1, PCE
Weibrich 2012[57]	54	2	Endoret, Harvest SmartPReP	PC, WBCC, PDGF-AB, TGF-B1, IGF

PC: platelet concentration; WBCC: white bloodcell concentration; RBCC: red bloodcell concentration; PEF: platelet enrichmentfactor; PDGF-AB: platelet-derived growth factor-AB; PDGF-BB: platelet-derived growth factor-BB; TGF-B1: transforming growth factor-B1; VEGF: vascular endothelial growth factor; EGF: epidermal growth factor; FGF-2: fibroblast growth factor-2; HGF: hepatocyte growth factor; IGF: insuline-like growth factor; PCE: platelet capture efficiency; FC: fibrinogen concentration.

Table 2: Preparation protocols and costs for the different PRP separation systems

System	Type of system	Whole blood volume (mL)	Centrifugal force (g) first spin	Centrifugal force (g) second spin	Centrifugation time (min) first spin	Centrifugation time (min) second spin	Final volume of PRP (mL)	Cost/kit ($)
ACP	Plasma-based	11	350	-	5	-	2.0-5.0	150
GPS III	Buffy coat	54	1100	-	15	-	6.0	350
Cascade	Plasma-based	9	1100	1450	6	15	2	*
PRGF	*	9	580	-	8	-	2.0	*
GLO	Buffy coat	9	1200	600	5	2	0.6	50-75
SmartPrep	Buffy coat	60	1250	1050	14	7.0-10.0	*	
Kyocera	*	20	600	2000	7	5	2	60
Magellan	Buffy coat	60	610	1240	4	6	3	500
Prosys	*	30	1660	2008	3	3	3	*
RegenPRP	*	8	1500	-	5	-	4	*

*= unknown/not provided by producer

Laboratory results

Concentration platelets, leukocytes and platelet enrichment factor

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 extend 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 the PRP produced by the GPS III system, the PRP produced by the ACP system contained the lowest number of leukocytes. Although only reported in four studies, large difference between PRP separation systems were found for the platelet enrichment factor. The highest platelet enrichment factors were found for the GPS III and SmartPrep system (respectively 3.93[32] and 3.79[30]); the lowest for the ACP, RegenPRP and Cascade systems (respectively 1.31[32], 1.59[32] and 1.62[7]).

Concentration growth factors

The concentrations of the growth factors PDGF-AB, TGF-B1 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 concentration of PDGF-AB and TGF-B1 were found between the higher (GPSIII, SmartPrep and Magellan) and lower platelet yielding devices (ACP, Cascade, PRGF and RegenPRP) as for the higher (GPSIII, 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, Magellan).

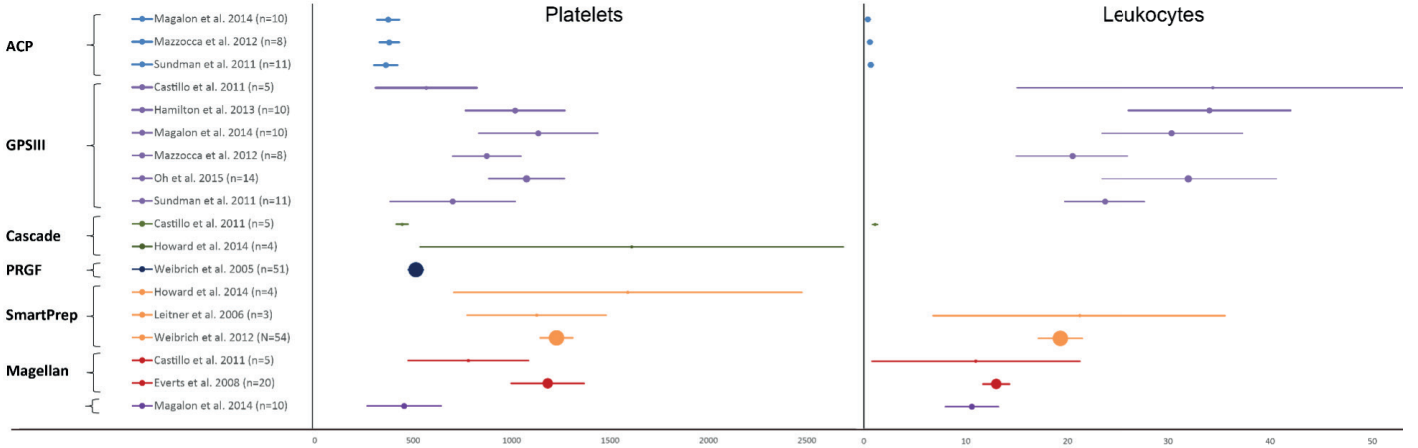

Figure 2: the concentrations of platelets (x10^3µL) and leukocytes (x10^3µL) found in the included studies

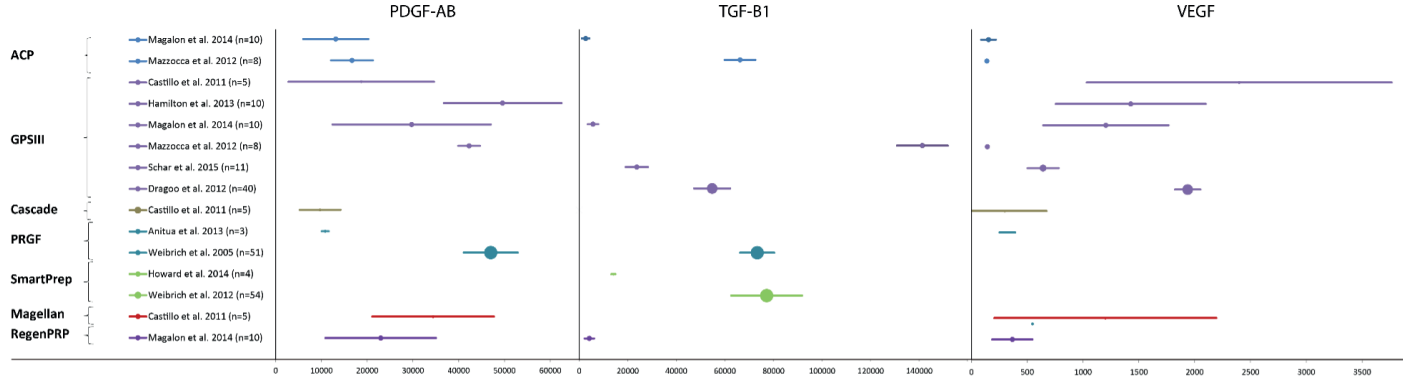

Figure 3: the concentration of PDGF-AB (pg/mL), TGF-B1 (pg/mL) and VEGF (pg/mL) found in the included studies.

Results comparative studies

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

There was a large difference in concentration of platelets between the systems studied in this review. Roughly, the systems studied in this review can be divided in high- and low-yielding devices. This division in high- and low-yielding devices has been described before by Dhurat et al.[11]. Dhurat et al. described that PRP devices can usually be divided into lower (2.5-3 times baseline concentration) and higher (5-9 times baseline concentration) systems. The low-yielding devices in this review produce PRP with a platelet concentration around 500x10^3 µL, whereas the high-yielding devices generally produce platelet concentrations over 750x10^3 µL. Among the high-yielding devices were the GPSIII, SmartPrep and the Magellan systems; the lower concentration systems concerned the ACP, Cascade, PRGF and RegenPRP systems. 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 α-granules in the platelets. However, there is no consensus about the optimal concentration of platelets in PRP: some authors report platelet concentrations of >200x10^3 uL[37] to be a therapeutic concentration, whereas others report concentrations of 1000x10^3 uL[35]. In the present study, the platelet concentrations of all of the PRP separation systems exceeded a platelet concentration of >200*103 uL which implies that all the devices met the definition for therapeutic effective PRP as defined by Mazzucco[37].

Concentration leukocytes

Comparable to 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 high and systems producing 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 high concentrations of leucocytes 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 low concentrations of leukocytes[11,15,50]. The majority of separation systems in current literature yield leukocyte-rich PRP. As also shown in this review, the ACP, Cascade and PRGF 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 remodeling 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 pro-inflammatory cytokines by leukocytes which is associated with decreased proliferation and with increased apoptosis[1,4,5,8,38,49,59,60,61,62]. As the aim of this review was to evaluate the differences between the concentration 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 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.

Concentration growth factors

A wide variation was found regarding the concentrations of growth factors both between different systems as within systems. These differences can partly be explained by the use of the specific ELISA 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 studies are comparable but 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 PRP kits that produce 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 suggest that that PDGF and TGF-b are the two most important growth factors in PRP[3,6,34,45]. In contrast to the platelet and leukocyte concentration, there is no evidence about an ideal concentration 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 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) as the total centrifugation time (5-21 minutes). There are many ways of preparing PRP, the most common methods are the plasma-based and the buffy coat- based method[29]. Although not known for all systems in this review, most systems used the buffy coat-based method. As mentioned earlier, buffy coat-based systems produce PRP with high concentrations of leucocytes as the buffy coat is rich in leukocytes[11,15,50]. Although the ideal concentration of blood components and growth factors for the specific field of application has yet to be determined by future research, the field of application should play an important role in the choice for the most appropriate PRP separation system. Other factors like 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 (range 95-500$) was found.

Strength and limitations

This is the first systematic review that offers a comprehensive overview of the concentration of blood components in PRP produced by all the commercially available

PRP separation systems and that analyzes the differences between the systems in terms of concentration of blood components and growth factors. Initially this study was designed as a meta-analysis. Unfortunately, despite all authors were contacted, we had to deal with a lot of missing data and no raw data was available for the majority of the studies. This limited the statistic options available for analyzing 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 two or more PRP preparation devices were summarized. Furthermore, the number of samples studied in the included studies was rather small; only five of the 19 studies used 20 or more samples and ten of the 19 studies used even 10 or less samples, which also limits the comparison between systems.

However, as the review of literature provided in the discussion showed, future research on the components of PRP should not focus on the concentration of the components, but rather on the optimal concentration 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 into which formulation of PRP is most suitable.

In conclusion, this review demonstrates that there is a large heterogeneity between 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 concentration of blood components and growth factors for the specific fields of application are yet to be determined for most of the fields of application, future research should focus on which type of PRP is most suitable for the specific fields of application.

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