Review of concentration yields in commercially available platelet-rich plasma (PRP) systems: a call for PRP standardization

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ABSTRACT

Platelet-rich plasma (PRP) has become increasingly popular in pain medicine with hopes of becoming a safe, effective alternative to routine treatments. However, given its autologous nature, PRP injectate may differ depending on the specific manufacturer and protocol. Currently, there is no standardization of reporting protocol. This systematic review compiles and standardizes values on PRP preparation and final product composition of platelets, white cell count, and growth factors for ease of comparison. On review of 876 studies, 13 studies were selected according to our inclusion criteria. Data from 33 PRP systems and protocols were extracted and standardized. Overall, PRP final product concentrations as well as PRP preparation protocols varied widely between systems. However, platelet concentration was directly correlated with both volume of blood collected and device centrifugal force. In conclusion, there is a large heterogeneity between PRP separation systems that must be resolved for proper study of this promising treatment.

INTRODUCTION

Platelet-rich plasma (PRP) is currently solely characterized by its absolute platelet concentration: it is a plasma volume with any platelet concentration above that of baseline whole blood, which is 150 000/µL[1] to 450 000/µL.[2] While PRP was first used in the 1970s for wound and bone healing in the field of oral and maxillofacial surgery,[3] there are now several high-quality systemic reviews, meta-analyses and randomized controlled trails supporting its applications in many fields, including dermatology,[4] cardiology,[5] plastic surgery,[6] orthopedic surgery,[7] pain management[8] and sports medicine.[9 10] As the popularity of this autologous derivative of whole blood grows and it is further studied, the definition of PRP is shifting to be having a minimum platelet concentration of 1 000 000/µL or approximately a fivefold increase in platelets from baseline.[11]

However, there are other important components aside from platelets in PRP that should be also characterized. Platelets are a rich source of growth factors and cytokines, including platelet-derived growth factor αβ (PDGF-αβ), transforming growth factor β1 (TGF-β1) and vascular endothelial growth factor (VEGF).[12] Once released on platelet activation, these factors play a key role in the healing process by directing cell proliferation, chemotaxis and angiogenesis.[13] While there is no single standardized PRP protocol, most products follow this basic recipe: whole blood is collected from a patient, anticoagulant is added and the sample is centrifuged within an hour of collection. This initial spin aims to separate red blood cells and platelet-poor plasma from the 'buffy coat', a layer rich in white blood cells (WBCs) and platelets. PRP can either be derived from the buffy coat or from the platelet-poor plasma. There remains to be a consensus on whether WBC inclusion in the final injectate is beneficial or merely causes unnecessary inflammation, so additional spins either remove or keep the WBCs and further processing is performed to isolate the platelet concentrate for use. Lastly, just prior to use, the PRP product may be activated with calcium chloride, thrombin or another agent—again there is no consensus which agent is optimal or even if activation is necessary.

Conventionally, PRP was created through a manual laboratory preparation where all materials were obtained individually and blood was prepared following one of the accepted protocols, such as Landesberg[14] or Anitua.[15] Now, there is an abundance of commercially available systems that provide kits with specially designed receptacles and preset, modified centrifuges. These PRP systems claim to yield consistent products and higher platelet counts than manual laboratory preparation.[16]

Interestingly, PRP falls into a category of products that does not require abiding to the US Food and Drug Administration (FDA) traditional regulatory pathway of animal and clinical trials.[17] While several PRP systems on the market do have FDA clearance, there are no clear regulations for PRP injectate composition, and currently PRP can vary greatly in platelet, WBC and growth factor concentration depending on the spinning protocol and other preparation protocol.[18 19]

Efforts to characterize PRP have been made via primary experiments by independent groups but are usually limited to an analysis of five or fewer commercial systems.[20] Other noteworthy efforts have been made to compile PRP composition information in the literature but sometimes include manufacturer data or are incomplete.[21]

The purpose of this study is to review all peer-reviewed published data on PRP preparation from commercially available PRP systems and compile and standardize values on PRP preparation and product composition of platelets, WBCs and growth factors for ease of comparison.

METHODS

This study was conducted in accordance with the 2009 Preferred Reporting Items for Systematic

Reviews and Meta-Analyses (PRISMA) statement.[22] Studies were searched electronically on the PubMed/MEDLINE database in March 2017 with combinations of the following: "platelet-rich plasma" AND "systems" OR "concentration" OR "commercial" OR "comparison". All studies with quantification of platelet concentration or platelet factor increase in PRP and preparation from a commercial system were considered. Studies or values directly from manufacturers were excluded to reduce bias. When multiple studies of the same commercial device existed, up to three were selected that provided the most complete analysis of the final PRP product (ie, WCC concentration, growth factor concentration and preparation). Additional preferences included publication in the English language and original experiments in peer-reviewed articles. The references of review articles and included studies were also scanned to identify other potentially eligible studies that fit the aforementioned criteria. Attempts to contact the authors for clarification or additional information were not made, but previously published works were cross-referenced to obtain additional data points. Two investigators independently reviewed the selected articles, and a third investigator was consulted to resolve any disagreements.

Data from the selected studies were extracted and compiled into online supplementary table 1.[23] Data were collected regarding protocol for PRP preparation, processing machine, spinning parameters, final platelet count, final WBC count and growth factor analysis. To standardize the values, units were converted. The standard deviations (SD) are listed if given in the study. Values from the studies were used whenever possible. In efforts to have a complete table, values not provided were calculated by the following:

Platelet capture efficiency %=(PRP volume × PRP platelet concentration) / (whole blood volume × whole blood platelet concentration)

Platelet factor increase=PRP platelet concentration / whole blood platelet concentration

When the whole blood platelet concentration was not given in the study, the average platelet count 200 000 platelet/μL[11] was used for calculations. Calculated values are noted in online supplementary table 1.

Statistical analyses were performed with JMP Pro software, Version 10.0.0 (SAS Institute Inc, Cary, North Carolina, USA) and Microsoft Excel 2016. Linear correlations between PRP platelet concentration PRP volume, blood volume, centrifuge spin time and force as well as between PRP, WBC and growth factor concentration were analyzed by Pearson correlation coefficient *r*. Means and SD were calculated for each of the variables of interest.

RESULTS

We reviewed 876 studies and were able to extract information from 13 studies that met the selection criteria detailed above (figure 1). From these studies, we compiled data points from 33 different commercially available PRP systems in online supplementary table 1.

Preparation protocol

There was no standardization in PRP preparation for the 33 PRP systems selected and not all studies reported their preparation protocols. Based on the variables most commonly reported throughout all selected studies, we chose blood volume collected, centrifugal force and centrifuge time as our parameters for comparison.

Blood volume collected

The mean blood volume collected for centrifugation was 30.90±26.35 mL and ranged from 6 mL with Plateltex (Bratislava, Slovakia)[20] and Regenlab RegenKit (Lausanne, Switzerland)[20] to 120 mL for Vivostat (Allerød, Denmark).[24] A Pearson correlation coefficient was computed to assess the relationship between the amount of blood processed and PRP platelet concentration. There was a moderate positive correlation between the two variables, r=0.42, n=39 (figure 2A).

Centrifugal force

Relative centrifugal force provided in gravitational force (*g*) was used since rotor radius was not provided or readily available for conversion from revolutions per minute. With systems using double spins, the higher spin force was used. The mean centrifugal force used was 1240±830 g and ranged from 180 g with Liège University Hospital Protocol (Jouan BR4i centrifuge),[20] Biomet GPSII (Warsaw, Indiana, USA)[25] and Plateltex[24] to 3200 g with Biomet GPSIII Mini.[26] There was a significant positive correlation between maximum centrifugal spin force and PRP platelet concentration, r=0.63, n=22 (figure 2B).

Centrifuge time

For systems with double spins, the spin times were combined. The mean total centrifuge spin time was 12.07±5.69 min and ranged from 3 min for DePuy PEAK[27] to 25 min for Curasan AG[20] and the Friadent-Schütze Protocol.[28] There was no significant correlation between length of spin and PRP platelet concentration, r=0.13, n=31 (figure 2C).

PRP platelet concentration

The mean PRP platelet concentration was 742.78±463.39×10^3/μL and ranged from 88×10^3/μL with Selphyl[29] to 1643±421×10^3/μL for Emcyte GenesisCS (Fort Myers, Florida, USA),[30] n=47. There was wide variability in the platelet concentration factor increase among systems, which ranged from a 0.52 to 9.7 fold increase from baseline whole blood platelet concentration. Additionally, the mean PRP volume produced was 5.20±2.92 mL and ranged from 0.34 mL with Plateltex[20] to 10.6±2.4 mL for the Landesberg Protocol (Mistral 3000i centrifuge).[20 24 31] There was no correlation between PRP volume and PRP platelet concentration, r=−0.22, n=45 (figure 2D).

PRP WBC concentration

The mean PRP WBC concentration was 41.66±95.16 × 10^3/μL and ranged from 0.0003×10^3/μL with Selphyl[29] to 320×10^3/μL for Curasan AG (Kleinostheim, Germany).[20] There was no significant relationship between the PRP platelet concentration and PRP WBC concentration, r=0.34, n=27 (figure 3A).

PRP growth factor concentration

The mean PRP PDGF-αβ concentration was 72.40±60.86 ng/mL and ranged from 9.47±5.83 ng/mL with Selphyl (Bethlehem, Pennsylvania, USA)[26] to 251.6±115.4 ng/mL with the Friadent-Schütze Protocol.[28] The mean PRP TGF-β1 concentration was 373.17±594.46 ng/mL and ranged from 0.1±0.08 ng/mL with Biomet GPSIII[32] and MTF Cascade PRP (Cleveland, Ohio, USA)[32] to 1719 ng/mL with Arteriocyte MagellanPRP.[29] The mean PRP VEGF concentration was 0.42±0.63 ng/mL and ranged from 0.029 ng/mL with Selphyl[29] to 2.4±1.1 ng/mL with Biomet GPSIII.[32] There was a significant positive correlation between PRP platelet concentration and PDGF-αβ concentration, r=0.63, n=34, a moderate positive correlation between

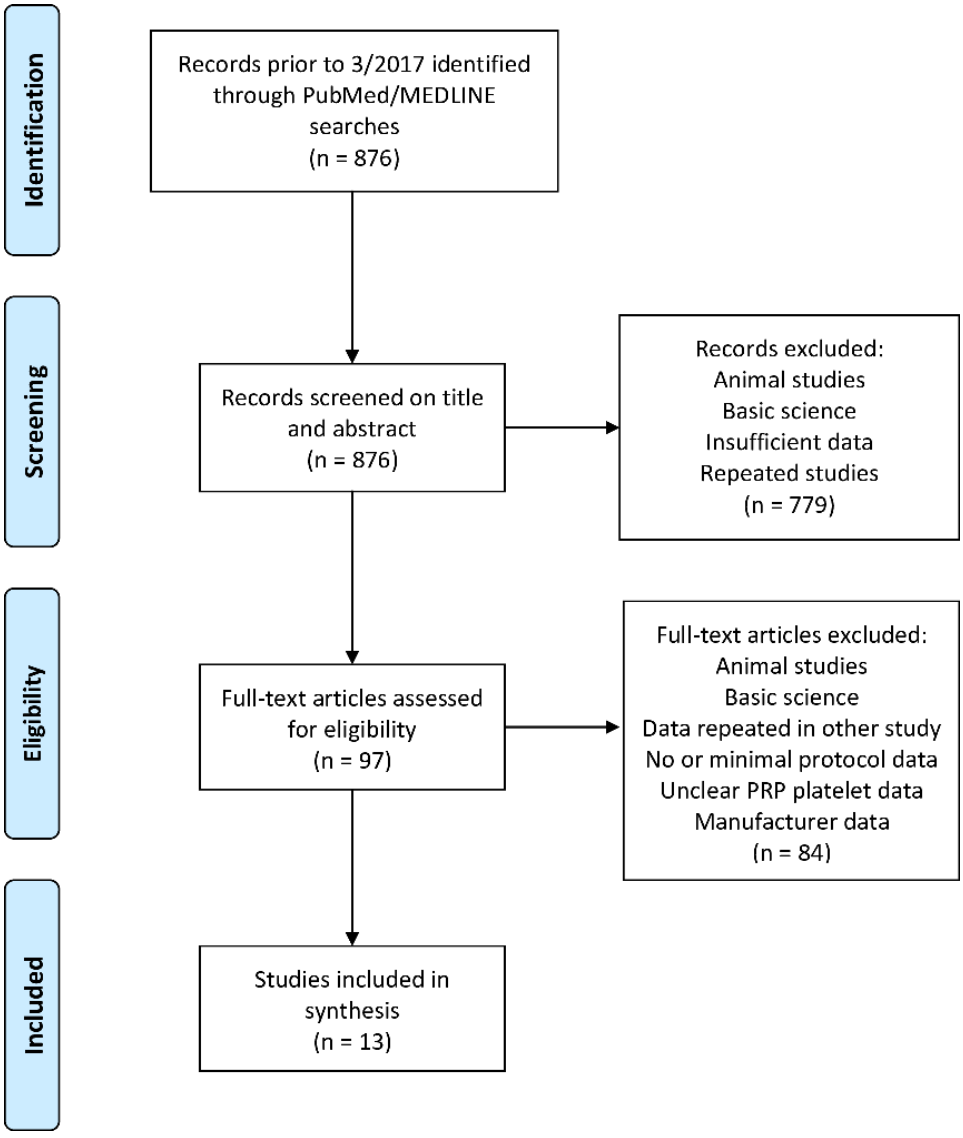

Figure 1 PRISMA 2009 flow diagram depicting the systematic review process used in this study. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PRP, platelet-rich plasma.

PRP platelet concentration and TGF-β1 concentration, r=0.45, n=26, and no correlation between PRP platelet concentration and VEGF concentration, r=0.005, n=17 (figure 3). PRP WBC concentration had a moderate positive correlation with PDGF-αβ concentration, r=0.47, n=19, a moderate negative correlation with TGF-β1 concentration, r=−0.50, n=21, and a significant positive correlation with VEGF concentration, r=0.66, n=17 (figure 4).

DISCUSSION

PRP platelet concentration

Of the 33 systems and protocols we analyzed, only 11 met the definition of PRP as defined by Marx *et al*[11] as having a minimum platelet concentration of 1 000 000 platelets/μL. Additionally, Marx *et al* and others have stated that platelets in PRP should be concentrated to at least five times that of baseline to be efficacious[2]—only 10 of the 33 systems and protocols reviewed met this definition. Lastly, 3 of the 33 systems and protocols reviewed even resulted in a final PRP product with a platelet count less than that of whole blood, with a PRP platelet factor increase of 0.737 with Biotechnology Institute PRGF-Endoret,[24] 0.65 with Curasan AG[24] and 0.52 with Selphyl.[29] Of the systems analyzed, Emcyte GenesisCS had the highest platelet concentration tested at $1643\pm421\times10^3$/μL.[30] The lowest platelet concentration was 88×10^3/μL with Selphyl.[29] This variable range highlights the importance of system selection and protocol adherence.

However, an optimal platelet concentration for PRP has yet to be identified. More is not necessarily better as too high of a platelet concentration can be detrimental to the healing process.[33] Yamaguchi *et al*[34] showed PRP concentration had a dose-dependent effect on intestinal anastomotic healing in rats, where a lower PRP platelet concentration (2 000 000/μL) exerted positive effects, while the higher PRP platelet count (5 000 000/μL) was harmful to healing. A second study by Giusti *et al* demonstrated positive effects on human tenocyte proliferation using a concentration of 500 000 and 1 000 000/μL while again 5 000 000/μL induced cell death.[35] There currently is not a consensus for an optimal PRP dose, but further clinical studies will hopefully identify therapeutic dose ranges for particular indications. For example, for knee osteoarthritis[36] and tendinopathies,[37] platelet concentrations between 3 and 4 fold higher than baseline are recommended.

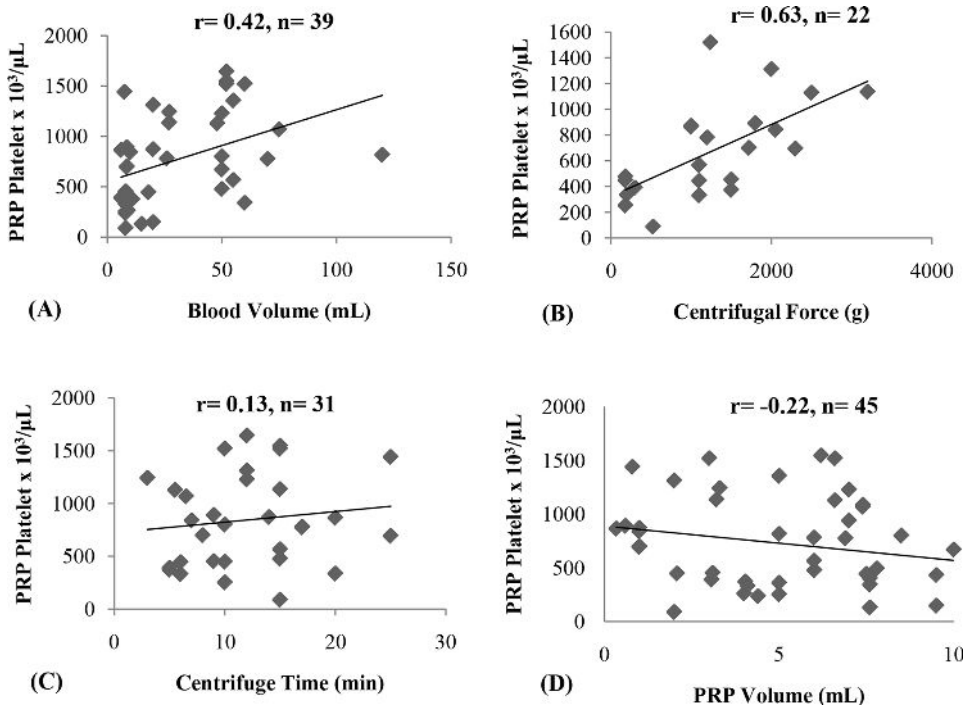

Figure 2 Correlation between PRP platelet concentration and (A) blood volume drawn, (B) centrifugal force, (C) centrifuge time and (D) PRP volume produced. n, sample size; PRP, platelet-rich plasma; *r*, Pearson correlation coefficient.

PRP WBC concentration

Similar to platelet concentration, optimal WBC concentrations have also been debated, and the PRP systems studied reflected this by their wide range in WCC concentration. Whether to include the WBCs in the injected concentrate has been a rather controversial topic. Proponents of the inclusion of WBCs claim that there is a decreased likelihood of infection due to the antimicrobial properties of WBCs, which may be of benefit when used intraoperatively.[38–40] Additionally, Zimmermann *et al* reports that the leukocytes in leukocyte-rich PRP (LR-PRP) account for up to half of the increased variance of growth factors PDGF-αβ, PDGF-β1 and VEGF compared with leukocyte-poor PRP (LP-PRP). Therefore, WCC concentration can be used to optimize growth factor concentrations.[41] Interestingly, our study

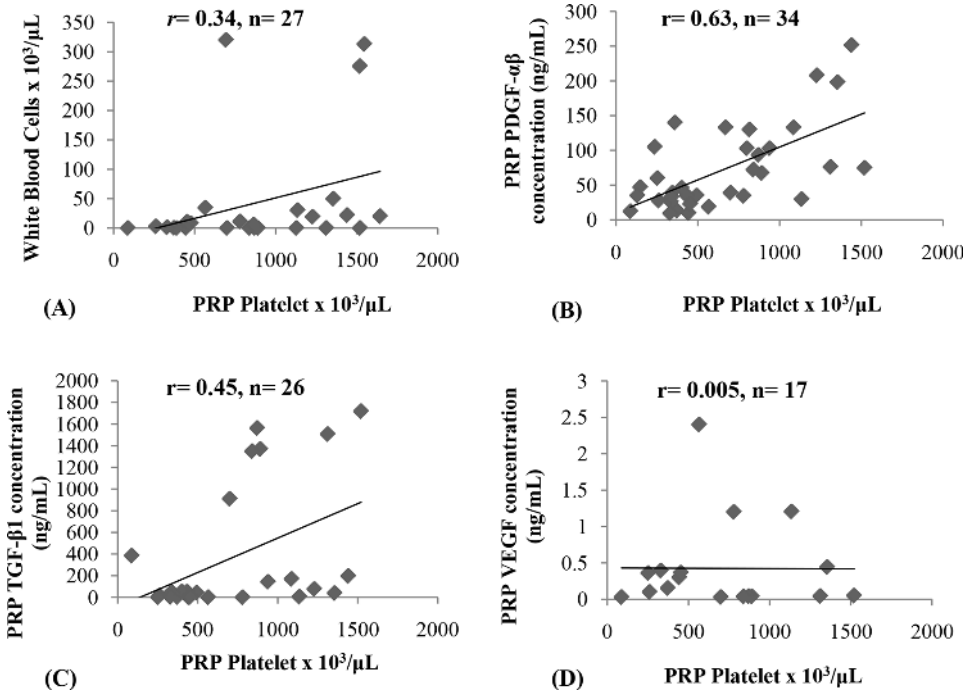

Figure 3 Correlation between PRP platelet concentration and (A) white cell count in PRP, (B) PRP PDGF-αβ concentration, (C) PRP TGF-β1 concentration and (D) PRP VEGF concentration. n, sample size; PDGF-αβ, platelet-derived growth factor αβ; PRP, platelet-rich plasma; *r*, Pearson correlation coefficient; TGF-β1, transforming growth factor β1; VEGF, vascular endothelial growth factor.

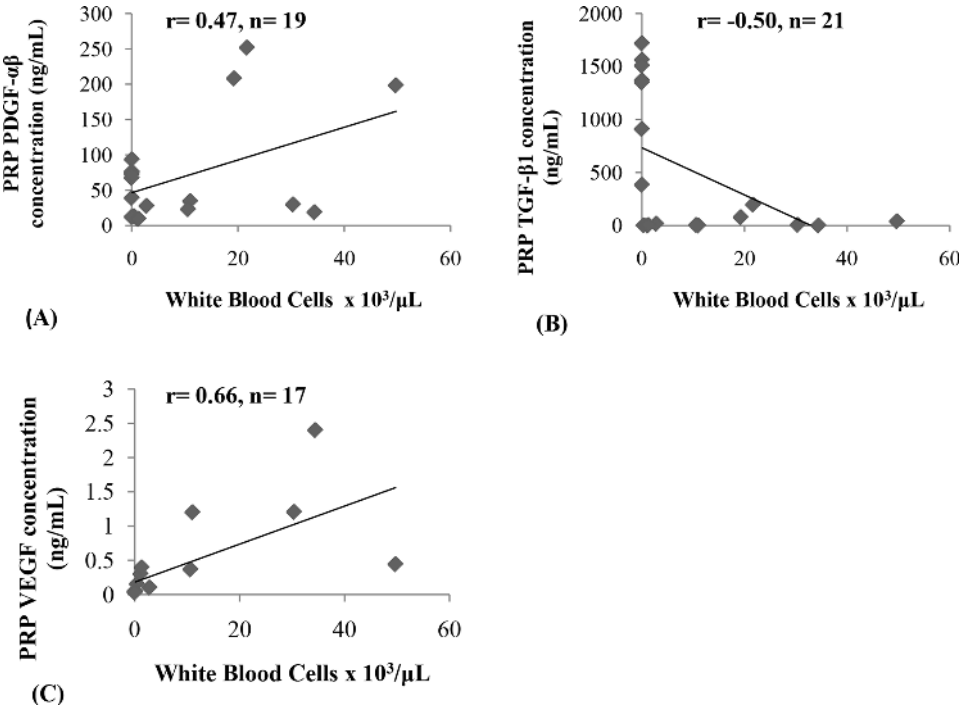

Figure 4 Correlation between PRP white cell count concentration and (A) PRP PDGF-αβ concentration, (B) PrP TGF-β1 concentration and (C) PRP VEGF concentration. n, sample size; PDGF-αβ, platelet-derived growth factor αβ; PRP, platelet-rich plasma; *r*, Pearson correlation coefficient; TGF-β1, transforming growth factor β1; VEGF, vascular endothelial growth factor.

found that increasing WBCs correlated with increased PDGF-αβ and VEGF, while there was an inverse relationship with TGF-β1. Opponents of WBC inclusion claim that WBCs increase acute pain and discomfort after the injection and their catabolic and proinflammatory effect have been found to have detrimental effects on articular cartilage healing.[42] Separate clinical studies validate that while there may be more acute swelling and pain after intra-articular LR-PRP injection, both LR-PRP and LP-PRP yielded similarly statistically significant improvement.[43 44] However, the majority of current evidence guide WBC by the injection site: LP-PRP for intra-articular areas[45] and LR-PRP for tendons.[46] Additional clinical studies are necessary for further indication-specific determination of WBC inclusion.

PRP growth factor concentration

Additionally, the relationship between platelet and growth factor concentration in PRP remains ambiguous, although their functions appear to be beneficial to the healing process. PDGF has been demonstrated to stimulate the chemotaxis of macrophages and neutrophils and to promote the secretion of TGF-β1 from macrophages.[47] In turn, TGF-β1 promotes an anabolic environment by decreasing type I collagen gene expression, which leads to a regulatory increase in type II collagen.[48] Leitner *et al*[49] found a strong positive correlation between platelet count and amount of PDGF-αβ, which is the only significant correlation our analysis showed between platelet concentration with PDGF-αβ, TGF-β1 or VEGF concentration. While we only found a moderately positive correlation with platelet concentration and VEGF concentration, however, significant positive correlations between the two have been identified in other studies.[50] Literature shows that there is patient variation in growth factor counts and even differences in product storage, such as time from preparation and temperature, can affect PRP growth factor concentration.[41]

Preparation protocol

Furthermore, it has become clear that PRP product preparation makes a significant difference in regard to its final composition and potentially even its efficacy. We found that processing a larger initial blood volume and using a higher spin force was correlated with increased platelet counts. A longer spin time did not indicate a higher platelet yield. Kececi *et al* suggests that adjusting the centrifugal force of the second spin may be used to create a final PRP product with the desired range of platelets. We agree with the authors that regardless of the baseline platelet count of the individual or the desired platelet injectate concentration, an adjustable centrifugal force should allow for the production of a precise range of platelet concentrations.[51] Once the optimal dosages of certain injections are determined, individual protocols may be developed to produce that ideal PRP consistency for a given treatment.

Few studies completely reported details of the protocol they followed when preparing PRP. One may assume that the authors precisely followed the manufacturer's protocol, however, at times there may have been adjustments or modifications based on patient need or provider preference. Regardless, the protocol followed should be readily accessible for interprovider reproducibility. Different provider protocol was found to affect final PRP product composition. This was particularly evident when multiple separate studies analyzing the same manufacturer's system had significant differing results. For a few systems, the outcomes were relatively consistent across different studies such as for Emcyte Pure PRP, which averaged a PRP platelet factor increase of 6.7 ± 0.3[52] and 6.9 ± 0.7[53] by Mandle *et al* and Mandle *et al*, respectively, and Emcyte GenesisCS, which averaged a PRP platelet factor increase of 9.13[30] and 9.7[52] by Kevy *et al* and Mandle *et al*, respectively. However, the results were inconsistent among multiple studies for other systems, the most striking being a 3.23 times PRP platelet factor increase difference with

Curasan AG: Mazzucco *et al* report a 0.65[24] times PRP platelet factor increase compared with Kaux *et al* that had a 2.75[20] times PRP platelet factor increase. Hence, there may be great individual provider variability in the concentration of PRP factors even given the same manufacturer and assumed protocol.

STRENGTHS AND LIMITATIONS

As aforementioned, not all publications shared detailed descriptions of the protocol being used. When the details for the preparation were described, studies were not consistent about which details of the protocol they provided. For instance, we did not compile information about activators and anticoagulants used because it was reported in less than half of the studies we analyzed. However, specific activator and anticoagulant use has been shown to affect both platelet count[54] and growth factor release,[55] thus it is important to report such values in future studies.

Another limitation resulted from several studies lacking documentation of the baseline whole blood platelet concentration. When not specifically stated, we standardized the average platelet count to the reference value of 200 000 platelet/μL[11] to calculate the platelet factor increase and/or PRP platelet concentration. Our primary reason for study exclusion was when only a PRP platelet concentration or factor increase was provided, without any details on preparation or other cell or growth factor analysis—this may have skewed which studies were included in our review. Furthermore, despite careful study selection, several unit discrepancies were found within studies, and at times, exact values were not stated and had to be estimated from a figure as detailed in the figure legend of online supplementary table 1. Additionally, while an increasing number of studies are beginning to report WBC count, studies rarely characterize WBCs into neutrophils, lymphocytes and monocytes.[18] The deleterious effects of WBCs in PRP are largely attributed to neutrophils. Neutrophils can cause inflammation by degrading tissue through releasing oxygen-free radicals and matrix metalloproteinases.[56 57] Knowledge of the WBC differential ratios may further fine-tune formulations for certain indications. The authors regret not being able to evaluate the WBC differential as an outcome in this review since it was not widely available in the literature.

Factors that seem inconsequential for final PRP product composition can also affect platelet and growth factor concentration as well as PRP efficacy: smaller needles can prematurely cause platelet activation[58] and collection test tube shape and material can influence growth factor production.[59] While such details may seem tedious to report in every study, it creates further variability in PRP preparation and obstructs standardization.

Nonetheless, the main strength of this study was consolidating current studies about PRP system concentrate composition and standardizing the values for ease of comparison. From our study, it is evident that a wide variety of PRP platelet, WBC and growth factor concentrations are produced by current commercial systems and protocols. The current literature agrees that the lack of standardization in PRP preparation protocol as well as the under-reporting of key values impedes proper comparisons across different studies.[19 60] While the use and efficacy of PRP have been shown throughout several fields, when there are conflicting results about the efficacy of PRP, it is difficult to parse out if the treatment was not successful due to the inherent physiology of the PRP or due to a 'non-optimal' PRP formulation. The formulation is crucial as the biological properties of a given PRP injectate are altered by variations in the different platelet, WBC and growth factor concentrations as well as preparation protocols.[61–63]

Table 1 Recommended study reporting parameters, separated into minimal parameters that we believe all studies using PRP should report and comprehensive parameters that we believe cover most variables that affect the final PRP product

		Minimal parameters	Comprehensive parameters
PRP final product	PRP platelet concentration	X	X
	PRP platelet factor increase from whole blood	X	X
	PRP platelet concentration	X	X
	PRP WCC concentration	X	X
	PRP WBC differential (neutrophils vs lymphocytes vs monocytes)	X	X
	Growth factors: PDGF-αβ, TGF-β1, VEGF	X	X
Protocol	Baseline whole blood platelet count	X	X
	Centrifugal force (g)	X	X
	Centrifuge time	X	X
	Activators used	X	X
	Anticoagulant used		X
	Buffer used		X
	Cost		X
	Needle size		X
	Temperature of sample		X
	Time from preparation to use		X

PDGF-αβ, platelet-derived growth factor αβ; PRP, platelet-rich plasma; TGF-β1, transforming growth factor β1; VEGF, vascular endothelial growth factor; WBC, white blood cell.

DeLong *et al*[64] recommends a classification system to systematically identify the characteristics of PRP injectate used in each study. The platelets, activation, white cells (PAW) classification system identifies the number of platelets, the manner in which platelet activation occurs and the presence or absence of WBCs. However, considering cost, ease of access and value, we recommend that additional parameters must be reported to maximize reproducibility of results (table 1). In addition to the parameters in the PAW classification system, we also recommend that at minimum characterization of a WBC differential, growth factor concentration, centrifugal force and centrifuge time is necessary for all studies using PRP. Additionally, we suggest including anticoagulant use, buffer use, cost, needle sizes, temperature and time from preparation to use if studies are to be fully comprehensive in their reporting. Other variables such as number of applications of PRP, timings and activity postapplication[63] could also need to be recorded to determine clinical relevance. Perhaps with detailed reporting parameters we will be able to optimize PRP dosage for certain indications; hence, systems will be designed with a targeted indication in mind to streamline use, safety, cost and efficacy—but first, a strong call for standardization of PRP is required in order to do so.

CONCLUSIONS

With the overall premise of PRP being to deliver appropriate concentrations and ratios of cells, platelets and growth factors to a given area of interest, our study outlines which processing methods and commercially available platforms are in keeping with that philosophy, thus allowing users to make informed decisions when choosing among the available options for their patients. Our study suggests PRP platelet concentrations

positively correlate with increasing volume of blood collected and centrifugal force. Only 11 out of the 33 systems and protocols we reviewed fit the traditional definition of PRP. As needle size, spinning parameters, additives as well as other factors can all impact overall efficacy, future studies should attempt to meticulously describe and document their protocols in hopes of eventually gathering enough data to concur on a gold standard protocol for evidenced-based medicine.

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