Original Article

Substantial Variability in Platelet-Rich Plasma Composition Is Based on Patient Age and Baseline Platelet Count

Luciano Rossi, M.D., Ph.D., Maximiliano Ranalletta, M.D., Ph.D., Ignacio Pasqualini, M.D., Juan Pablo Zicaro, M.D., Matías Costa Paz, M.D., Pablo Camino, M.D., and Nicolas S. Piuzzi, M.D.

Purpose: To evaluate the effect of age, sex, body mass index (BMI), and baseline blood count on the final composition of platelet rich-plasma (PRP) and to evaluate the variability of PRP applied in the same patient at 2 different times. **Methods:** Potential subjects treated with PRP between January 2019 and December 2021 were identified in an institutional registry. Patient demographics and baseline blood count were prospectively recorded in a consecutive series of patients treated with PRP for a musculoskeletal condition in our Institution. The influence of sex, BMI, age, and baseline blood count on final platelet concentrations in PRP was evaluated. Finally, intrapersonal variability was evaluated. **Results:** A total of 403 PRP injections from 357 patients were analyzed from an institutional prospective registry of PRP between January 2019 and December 2021. A directly proportional variation in PRP platelet count of 3.8× was observed for each unit increase in baseline blood platelet count. For every decade increase, we observed an approximate decrease of 32,666 platelets. When the first dose of PRP platelet counts was compared with the second dose of PRP platelet counts between the same patients, significant differences were found. A mean of 890,018 platelets in the first PRP and a mean of 1,244,467 in the second PRP with a mean difference of 354,448 was found ($P = .008$). We did not find differences in the final concentration of platelets regarding sex, BMI, or PRP protocol. **Conclusions:** Overall the final composition of PRP (platelet count) was significantly influenced by patient's age and baseline platelet count. In contrast, BMI, sex and the rest of the components of the baseline blood count did not have a significant influence on final PRP. Furthermore, in patients who received 2 doses of PRP, the final concentration of platelets varied significantly between the 2 preparations. **Level of Evidence:** Level IV, prognostic case series.

The use of platelet-rich plasma (PRP) for the treatment of different orthopaedic pathologies has increased substantially in recent years.[1] PRP can be defined as an autologous blood preparation in which the platelets have been concentrated to levels exceeding that in the whole blood from the same patient. These therapies aim to deliver proregenerative growth factors (GFs) and cytokines, which are released from a concentrated pool of degranulating platelets, to the site of pathology. The other important component of PRP is white blood cells, which have a strong influence on the GFs and cytokines delivered to the target tissue.[2] PRP is usually described as poor or rich in leukocytes, depending on whether the final concentration of leukocytes in the PRP is above or below the baseline whole blood from the patient.[2] GFs released by platelets have been demonstrated to perform proregenerative functions in vitro, including promoting stem and progenitor cell proliferation and recruitment, modulating inflammatory responses, and stimulating angiogenesis.[3] However, clinical outcomes on the use of PRP in orthopaedics have not consistently matched the findings of basic science studies.[3-10] As major variability exists among reported clinical outcomes on the application of

From the Departments of Orthopaedic Surgery (L.R., M.R., J.P.Z., M.C.P.) and Hematology (P.C.), Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; and Departments of Orthopedic Surgery (I.P., N.S.P.) and Biomedical Engineering, Lerner Research Institute (N.S.P.), Cleveland Clinic, Cleveland, Ohio, U.S.A.

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Address correspondence to Luciano Andrés Rossi, Peron 4190 (C1199ABB) Buenos Aires, Argentina. E-mail: luciano.rossi@hospitalitaliano.org.ar

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PRP across different musculoskeletal pathologies, this has remained a topic of interest. Among the factors that could explain this variability are the lack of consensus regarding the classification of the PRP, the incomplete reporting of the PRP preparation protocols, the variability in the delivery methods, and the doses used, among others.[11,12]

Altogether, an additional challenge when assessing PRP is the oftentimes-unaddressed variability related to clinical characteristics of the patients, which drive intra- and interpersonal variability. Studies have shown that the final composition of PRP could vary in relation to clinical variables such as the sex, age, and the baseline blood count of the patient.[13-16] However, the specific literature on this topic is scarce and includes a limited number of patients.[13-16] In addition, as some PRP protocols advocate for the use of more than one PRP application, it is unknown how much PRP applied to the same patient at different times varies.[17,18] The purposes of this study were to evaluate the effect of age, sex, body mass index (BMI), and baseline blood count on the final composition of PRP and to evaluate the variability of PRP applied in the same patient at 2 different times. Our hypothesis was that there would be interpersonal and intrapersonal differences in the final PRP composition.

Methods

Patients who underwent a PRP procedure due to musculoskeletal conditions between January 2019 and December 2021 were identified in our institutional PRP Registry. The PRP Registry was developed in conjunction with the hemotherapy service and consists of an electronic database documenting for each patient who received a specific treatment with PRP including (1) clinical data of the patient; (2) baseline blood count; (3) PRP preparation protocol; and (4) final PRP composition analysis. The protocol of the following study was approved by the ethics committee of our institution (institutional review board: 00010193), and all patients gave their consent to participate in it.

Patient Clinical Data

Clinical data collected and recorded in the PRP Registry included age, height, weight, history of previous medication, and orthopaedic pathology (diagnosis, location, evolution time).

Baseline Blood Count Analysis

A 25-mL whole blood sample was obtained by a licensed clinical phlebotomist using a standard venipuncture technique between 7 and 9 AM before drawing the remainder of the blood for the PRP preparation. This was done with a holder system and blood collection needles. Three samples were taken, one in a tube for serum without an accelerator and 2 in a tube with K2 EDTA for plasma. With one of these plasma samples, a complete blood count was performed in all patients with a Sysmex model XT 2000i hematology counter. This study included the erythrocyte count, hematocrit, hemoglobin, mean corpuscular volume, leukocyte formula, and baseline platelet count.

PRP Preparation Protocol

After this stage and after patients signed the informed consent to participate in the study, 150 mL of blood was extracted in an extraction bag specially designed for this purpose. All this was done aseptically, with double cleaning with alcohol and povidone iodine. Samples were taken from the discarded bag to carry out the serology of transfusion-transmitted infections and immunohematology of the donor/patient. The extracted unit was subjected to double centrifugation in a Thermo Fisher Scientific Sorvall BP-16 Refrigerated Centrifuge for Blood Bank (Thermo Fisher Scientific, Waltham, MA). The first consists of a slight centrifugation for 4 minutes at 1400 rpm without braking for the production of PRP poor in leukocytes and with medium braking for the production of PRP rich in leukocytes. The PRP was separated from the globular mass by taking part of the buffy coat for the PRP rich in leukocytes and without touching the buffy coat for the PRP poor in leukocytes. The product obtained was separated into satellite bags without opening the circuit, guaranteeing the sterility of the process. Then, the PRP was centrifuged for 6 minutes at 3000 rpm to achieve a greater concentration of the product.

Final PRP Composition Analysis

At the end of the process, quality control was carried out on the product through an XT ROCHE hematology counter (Roche, Basel, Switzerland) before the infusion of PRP to the donor/patient. The control consisted of final volume, platelet count, white blood cell count, and product concentration calculation.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation or median and interquartile range according to distribution, and categorical variables are presented as absolute and relative frequencies. The *t*-test and Mann–Whitney *U* test were used to compare the means or medians of the quantitative variables between the 2 groups. One-way analysis of variance or Kruskal–Wallis tests were used to compare the differences between the means or medians between more than 2 groups based on their assumptions. According to their assumptions, a paired *t*-test or a Wilcoxon signed-rank test was used to test the hypothesis of similarity in

Table 1. Patient Baseline Demographic Characteristics (n = 403)

Age, y, % (n)	
<40	14% (57)
40-60	34% (136)
>61	52% (210)
Sex, % (n)	
Female	55% (221)
Male	45% (182)
BMI, % (n)	
<24.9	31% (123)
25-29.9	45% (183)
>30	24% (97)
PRP indication, % (n)	
Knee	46% (186)
Osteoarthritis	91% (170)
Focal cartilage lesions	5% (10)
Meniscal injuries	4% (6)
Shoulder	43% (174)
Rotator cuff tendinopathy	92% (160)
Rotator cuff tears	8% (14)
Elbow	8% (30)
Epicondylitis	100% (30)
Hip	2% (10)
Hip osteoarthritis	100% (10)
Foot and ankle	1% (3)
Diabetic foot ulcers	100% (3)
PRP protocol, % (n)	
PRP-LP	92% (371)
PRP-LR	8% (32)
PRP injections per subject, % (n)	
1	89% (318)
2	9% (34)
3	1% (3)
4	1% (2)

BMI, body mass index; PRP, platelet-rich plasma; LP, leukocyte poor; LR, leukocyte rich.

the mean or median platelet count between the first PRP and the second PRP injections. Given that the distribution of platelet count in the PRP was frankly asymmetric, we conducted a quantile regression, modeling the median of this variable based on 4 potential predictor variables (age in decades, BMI, basal number of platelets, and sex), considering each person (n = 357) as a cluster to whom between 1 and 2 interventions were performed (n = 403). The statistical analysis was performed using Stata, version 13 (Stata Corp., College Station, TX), *P* values less than .05 were considered statistically significant.

Results

Study Population

A total of 403 PRP injections were administered in a total of 357 patients. Patient baseline demographic, blood, and administered PRP characteristics are reported in Tables 1, 2, and 3, respectively.

Table 2. Baseline Blood Characteristics (n = 403)

Hematocrit	42% (40-44)
Erythrocytes	4,730,000 × mm^3 (4,380,00-5,004,00)
Platelets	230,000 × mm^3 (200,000-267,000)
Leukocytes	6,370 × mm^3 (5,400-7,430)
Neutrophils	3,560 × mm^3 (2,910-4,400)
Basophils	30 × mm^3 (20-40)
Eosinophils	160 × mm^3 (100-260)

NOTE. Values are shown as median (interquartile range).

PRP Interpersonal Variability

Overall, age was the only demographic factor to have a significant effect on PRP composition (*P* = .005). Patients >60 years old had the lowest amount of PRP platelet counts whereas patients <40 had the highest (Table 4). There was no difference in PRP based on sex, BMI, or PRP protocol.

Variables Predicting PRP Platelet Counts

For every decade increase, we observed an approximate decrease of 32,666 platelets. A directly proportional variation in PRP platelet count of 3.8 was observed for each unit increase in baseline platelet count. Although we did not find significant differences, men showed a lower platelet count in PRP compared with women (a median of −64,000). No statistical differences were observed between BMI and PRP platelet count (Table 5).

PRP Intrapersonal Variability

Thirty-four patients received 2 doses of PRP separated by 15 days from each other. When comparing the first dose of PRP with the second dose for the same patients, platelet counts PRP showed significant differences. Overall, there was a mean of 890,018 platelets in the first PRP, and a mean of 1,244,467 in the second PRP with a mean difference of 354,448 (*P* = .008).

Discussion

Overall, the main finding of this study was that significant variation in the final composition of PRP was seen in relationship to the number of baseline platelets and patient's age. For every decade increase, there was a decrease of 32,666 platelets. And, as expected, a directly proportional variation in PRP platelet count of 3.8 was observed for each unit increase in baseline platelet count. Moreover, regarding interpersonal variability, when we evaluated patients who received 2 PRP treatments at different time, we found that the PRP of the second application contained an average of 354,448 more platelets.

Despite the fact that in the last decade numerous clinical trials have been published evaluating the effectiveness of PRP, reports have been inconsistent, even for the same indication.[18,19] Several factors can

Table 3. PRP Characteristics (n = 403)

Volume	30 mL (25-30)
Hematocrit	0.2% (0.1-0.3)
Erythrocytes	40,000 × mm^3 (20,000-70,000)
Platelets	996,000 × mm^3 (807,000-1,162,000) 4.33× (4-4.35)
Leukocytes	760 × mm^3 (460-1,990)
Neutrophils	60 × mm^3 (30-120)
Lymphocytes	400 × mm^3 (190-780)
Basophils	10 × mm^3 (0-10)
Eosinophils	1 × mm^3 (0-10)

NOTE. Values are shown as median (interquartile range).
PRP, platelet-rich plasma.

explain the variability that exists in terms of reported clinical outcomes. First, the preparation protocols and the report of the final composition of the PRP used across the different studies is oftentimes missing or highly variable.[6,12,20] In a recent systematic review of clinical trials on PRP for the treatment of musculoskeletal diseases reported that PRP preparation protocols are only reported in 10% of the studies. Furthermore, only 16% provided quantitative metrics on the composition of the final PRP product.[12] As PRP preparation protocols vary, there is an additional intrinsic variability associated with the several commercial density separation systems available in the market as well as the unstandardized nomenclature, and the ambiguous classifications available.[11,21,22]

A second major challenge in the PRP field is the biological variability related to clinical characteristics of the patients that can influence the final composition of the PRP. A number of studies have shown that some clinical patients' demographics could significantly influence the final composition of the PRP.[13-16] More specifically, a significant correlation has been found between the final concentration of GFs and cytokines and sex and age. Xiong et al.[15] reported on the influence of sex on the final composition of PRP in 39 healthy patients, concluding that male patients had significantly greater levels of inflammatory cytokines (interleukein-1β and tumor necrosis factor-α) and GFs (fibroblast growth factor-basic and transforming growth factor-α b1) in PRP compared with female patients. In a similar study, Evanson et al.[16] analyzed the variability of different GFs in the final composition of PRP in relation to sex and age in 102 healthy patients. Of the GFs tested, 4 of 7 were significantly higher in women (epidermal growth factor, hepatocyte growth factor, insulin-like growth factor 1, platelet-derived growth factor [PDGF]-BB), and 5 of 7 GFs were significantly higher in people <25 years old (epidermal growth factor, insulin-like growth factor 1, PDGP-AB, PDGF-BB, and transforming growth factor P-1) than in those >25 years of age. Taniguchi et al.[13] analyzed 39 healthy Japanese subjects and found that age was negatively correlated with PDGF-BB and insulin-like growth factor-1 and sex had no influence on GFs. In contrast, other authors did not find a significant correlation of sex and age with final PRP GF concentrations. Weibrich et al.[14] analyzed 115 samples (stratified by donor age and sex) for GF concentrations and platelet counts. The authors did not observe a significant correlation between sex, age, or prior platelet count and final PRP GF concentrations. A strength of our study compared with previous literature is the large number of patients evaluated (n = 357). In our study, we found that the final concentration of platelets varied significantly according to the baseline blood count. Specifically, a directly proportional variation in PRP platelet count of 3.8 was observed for each unit increase in baseline blood platelet count. This is to be expected as density separation (centrifuges) may only concentrate between 2 to 6× the platelets that are already present in the baseline blood sample. This challenge raises questions regarding the required quality release criteria when using autologous blood derived products such as PRP. In a hypothetical situation in which patient A has a basal concentration of 200,000 platelets × μL and patient B 400,000 platelets × μL, using the same centrifuge, patient A will have 760,000 platelets × μL in the final PRP and patient B 1,520,000 platelets × μL, respectively. The clinical consequences of this variability in the final composition of the PRP are unknown due to the fact that most clinical trials on the use of PRP in orthopaedics do not report on the final composition of the PRP used.[12] Moreover, age also had a significant effect on platelet counts, as for every decade increase, there was a decrease of 32,666 platelets in the final PRP. From a clinical perspective, this is a relevant finding since the mechanism of action of PRP is believed to be based on the GFs and cytokines found in the

Table 4. Interpatient Variables Affecting PRP Platelet Count

Patient Characteristics (n = 403)	PRP Platelet Count*	*P* Value
Age, y		.005
20-40	1,056,000 (887,000-1,272,000)	
41-60	1,016,500 (824,000-1,201,500)	
>60	964,000 (761,000-1,129,000)	
Sex		.079
Female	1,018,000 (824,000-1,216,000)	
Male	954,000 (788,000-1,117,000)	
BMI (n)		.981
<24.9	982,000 (823,000-1,159,000)	
25-29.9	1,000,000 (788,000-1,156,000)	
>30	1,018,000 (826,000-1,172,000)	
PRP protocol		.683
PRP-LP	999,000 (811,000-1,162,000)	
PRP-LR	944,000 (789,500-1,159,000)	

BMI, body mass index; PRP, platelet-rich plasma; LP, leukocyte poor; LR, leukocyte rich
*Median (interquartile range).

Table 5. Patient Characteristics Predicting Final PRP Platelet Number

Predictable Variables	Change in Median Platelet Count of PRP (CI 95 %)	*P* Value
Basal blood platelet count	3.8× (3.4× to 4.1×)	<.001
Age in decades	−32,666 (−59,144 to −6,189)	.016
Male	−64,000 (−136,820 to 8,820)	.085
BMI	2,133 (−6,919 to 11,185)	.643

BMI, body mass index; CI, confidence interval; PRP, platelet-rich plasma.

alpha-granules in the platelets. Therefore, it is expected that, if the final platelet concentration is altered by factors such as the basal platelet count or the age of the patient, the biological effect on the target tissue will also be modified.

Finally, the intrapersonal variability of the final composition of PRP represents a major finding. When evaluating patients who received 2 PRP infiltrations, we found that the PRP of the second application contained an average of 354,448 more platelets. Consequently, even in the same patient, treated at the same center, by the same medical team and with the same centrifuge and PRP protocol, the mean final concentration of platelets varied significantly with only 2 weeks of difference between one preparation and the other. This highlights one of the greatest challenges of biological therapies today, which is being able to determine the ideal doses for the treatment of each pathology.

Limitations

This study has some limitations that should be considered. First, limited numbers of clinical variables were evaluated, and other not reported clinical factors could also influence the final composition of the PRP. Second, patient medications were not assessed, and their effect on the final platelet concentration was not reported. Although in our institution we do not indicate PRP therapy to antiplatelet or anticoagulated patients, other medications could have influenced the final concentration of platelets and these were not specifically analyzed. Finally, the concentration of specific GFs and cytokines was not assessed.

Conclusions

Overall, the final composition of PRP (platelet count) was significantly influenced by patient's age and baseline blood platelet count. In contrast, BMI, sex, and the rest of the components of the baseline blood count did not have a significant influence on final PRP. Furthermore, in patients who received 2 doses of PRP, the final concentration of platelets varied significantly between the 2 preparations.

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