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ORIGINAL ARTICLE

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Age-based inter-subject variability in platelet and white blood cell concentrations of platelet-rich plasma prepared using a new application to blood separation system

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Abstract

The benefit of autologous platelet-rich plasma (PRP) treatment is still under discussion. Variations in PRP products, consequence of the lack of a standardised protocol for the multitude of commercially available blood separation systems and the lack of knowledge of the optimal composition of PRP or its suitability for the proposed indication are some of the reasons behind clinical inconsistencies. The impact of inter-subject variability in PRP has received less attention in comparison. The purpose of this study was to determine the inter-subject variability, based on age, in the concentrates prepared by a new blood concentration system. Twenty-six healthy volunteers of both genders (29-93 years) were enrolled. Whole blood (WB) was collected from each participant to prepare PRP using the Easy PRP kit. Platelets and white blood cells (WBC) from WB and PRP were analysed after split population by age; patients younger than 65 years (n = 13) and patients ≥65 years old (n = 13). Among the demographic characteristics tested, only age was significantly different between the groups. Cell capture efficiency of the system was specific for each type of blood cell and identical for both age groups. Platelets and WBC in PRP were higher than in WB ($P < .001$). In WB, platelets and WBC concentrations were significantly lower in older group ($P \leq .035$). These differences persisted in the prepared PRP ($P \leq .004$). The ageing of population has a strong influence on the haematocrit and therefore on the composition of PRP. Because the efficiency of blood separator system seems to be constant across individuals, the inter-subject haematocrit variability based on age could be used as a predictor of resulting PRP. The clinical application of PRP should be restricted to the specific cell capture capacity of the different commercial devices.

KEYWORDS
blood, leukocytes, platelet-rich plasma

Key Messages

- we have analysed the inter-subject variability, based on age, on the concentrates prepared by a new blood concentration system
- ageing of population has a strong influence on the haematocrit and on the composition of platelet-rich plasma (PRP)
- clinical application of PRP should be restricted to the specific cell capture capacity of the different commercial devices

1 | INTRODUCTION

The use of platelet-rich plasma (PRP) as a therapy for orthopaedic injuries has gained popularity in the past few decades.[1-5] PRP is defined as an autologous preparation of platelets in a small volume of plasma.[6]

Platelet concentrations in PRP commonly range from a 3- to 5-fold increase over whole blood (WB) or a minimum concentration of 300×10^6 to 1000×10^6 platelets/mL.[7-10]

The benefits of PRP are hypothesised to derive from the regenerative effects of growth factors released by platelets as well as anti-inflammatory effects that help tissue healing and relieve pain.[11-13] In this line, autologous PRP has emerged recently as an alternative therapy in the treatment of diabetic ulcers by providing essential growth factors that promote healing.[14]

Recent studies have been reported a wide clinical outcome variability with PRP or autologous blood concentrates therapies, calling into question about the use of this treatment modality to resolve all the proposed indications.[6,15,16] A recent meta-analysis of 33 randomised trials and prospective cohort studies found little evidence to support the use of PRP in orthopaedic bone and soft-tissue injuries.[15] Others claim significant benefit from its use.[6] The dramatic lack of consistency in primary outcome measures across studies is important to understand a part of these discrepancies.[15,16]

However, the variations in the clinical outcomes are largely attributed to large number of methods and commercial blood separation devices available and the lack of standardisation of the blood fractionation and separation process, which result in great disparities in cellular and proteomic contents of PRP.[17-22]

Furthermore, intra-subject variations have been also suggested because a given system provides different yields and final products for the same individual when the preparations are repeated over time following a standardised working protocol.[17,23,24]

These inconsistencies in PRP efficacy are not altogether surprising given that the optimal content of PRP remains undefined and the lack of understanding regarding the true content of prepared final product, making not all PRP preparations equally effective depending on the intended clinical use.[24,25] For instance, density and composition of white blood cells (WBCs) in PRP preparations differ significantly[24] and the effects of WBCs in tissue regeneration following injury are controversial.[24,25]

Biological variability of platelet and WBC concentrations among individuals because of several causes may also contribute to the efficacy of PRP therapy but this correlation has been poorly explored.[10] Our hypothesis is that the ageing of population has a strong influence on the haematocrit and therefore on the composition of PRP.

The objective of this study was to determine the inter-subject variability based on the age, in the platelet and WBC content of the PRP prepared using a new commercial blood separation method.

2 | METHODS

2.1 | Study design and sample collection

This study was approved by the research ethics committee of the Rey Juan Carlos University of Madrid (internal registration number 190420164416). All methods were performed in accordance with the relevant guidelines and regulations. The study was conducted following Helsinki declaration, as well as all national and international ethical standards for human experimentation.[26] All participants were informed about the procedures used in the study and signed in the informed consent form.

Healthy volunteers of both genders (n = 26), split into two groups based on age, were enrolled in the observational study, which was conducted using the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[27] The control group (n = 13) consisted of individuals younger than 65 years, and the

older adult group (n = 13) with individuals ≥65 years old.[25] All participants who agreed to be included as volunteers in the studio met the inclusion criteria: non-anaemic, chronic disease-free individuals who were not on aspirin or anti-inflammatory drugs at the start of the trial.

A 15 mL WB sample was obtained from each donor by venipuncture from the cephalic, basilic or median vein, using standard aseptic technique.

2.2 | Preparation of PRP

The PRP was harvested using the Easy PRP commercial system (Mesotech, Napoli, Italy, https://www.mesotech.it/products/) according to the instructions of the manufacturer. After loading 1 mL of anticoagulant into the syringe, 11 mL of WB was slowly collected from each patient. The syringe was gently balanced 10 times to evenly mix the blood with the anticoagulant. The upper part of the kit was punctured with a needle to extract the vacuum from the device. Then, with a long 19 G 90 mm needle, the blood was slowly injected into the device, filling the lower compartment and the lower half of the central body of the system.

The device was centrifuged (Nahita, Ibor Médica, Spain) at 3500 RPM for 5 minutes. Later, the buffy coat and the fraction of the PRP were extracted using a 5 mL syringe and a 19 G and 90 mm needle. A total of 2 mL of the concentrated final product was transferred to an EDTA tube to determine the count of platelets and leukocytes (WBC), including neutrophils, lymphocytes and monocytes (Echevarne laboratory, HM San Francisco Hospital, León). In addition, 2.5 mL of WB from each donor was collected into an EDTA tube in order to carry out the baseline analysis.

The platelet and WBC concentration in WB and in the prepared PRP were expressed per millilitre of the sample. The increase in the platelet or WBC concentration factor (the mean cell fold change from WB basal concentration) was calculated as the ratio of each cell concentration in final product and WB (PRP:WB ratio).[22]

2.3 | Statistical analysis

A descriptive analysis of the characteristics of the participants of both groups was performed. Continuous variables were reported using the mean, SD and range, as well as median and interquartile range. The paired sample *t*-test or the Wilcoxon test was performed depending on the distribution of data based on the Shapiro-Wilk test, for comparisons between WB and Easy PRP kit concentrates. Independent Student's *t*-test or the Mann–Whitney *U*-test was used for comparisons between groups. For all analyses, a value of $P < .05$ was considered statistically significant. The data obtained were analysed using SPSS software for Mac (Version 22; IBM Corp, Armonk, New York).

3 | RESULTS

Descriptive data of the sample population are displayed in Table 1. All variables, except age, followed a Gaussian distribution ($P > .05$; Shapiro-Wilk test). There were no significant differences in weight, height or the body mass index after dividing the population of volunteers into young and older adult groups.

As can be seen in the Table 2, the platelet and WBC count per ml in WB were significantly different between both age groups ($P \leq .035$), with 25% to 30% higher counts in the group of younger volunteers. Within the white cell series, the neutrophil and monocyte count were approximately 29% ($P \leq .032$) and the lymphocyte count was 49% higher in the group of younger adults ($P < .001$).

Despite these differences, the efficiency for cell capture of the Easy PRP kit system was identical for both age groups (Figure 1), with a mean platelet fold change of 4.05 ± 0.98 and 3.74 ± 1.20 and a mean WBC fold change of 2.03 ± 0.69 and 1.93 ± 0.85 for the control and the older age group, respectively ($P > .05$). Mean platelet and WBC fold change for all volunteers were 3.89 ± 1.09 and 1.98 ± 0.76. The increase in lymphocyte factor was higher than that of monocytes and neutrophils in both age groups.

The platelet and WBC density in the final product were significantly higher than that obtained in WB ($P < .001$) in both aged groups or in all volunteers as a whole (Table 3).

However, in consistence with pre-existing differences in basal WB concentrations, the cell density in the prepared PRP from each group was markedly different. With a slightly more favourable PRP:WB ratio for individuals in the control group, the system concentrated 32% to 40% more platelets ($P = .004$) and WBC ($P < .001$) for this group than for the older adults group. Neutrophils, lymphocytes and monocytes PRP counts were also 34%, 52% and 37% higher, respectively.

4 | DISCUSSION

The results of this study show that the ageing of population has a strong impact on the recovery rate of platelets and WBC in the PRP preparations. In our study cohort, we found dramatic differences in the platelet and WBC count obtained in the PRP product recovered from the

TABLE 1 Demographic and descriptive data of the sample population according to the control group and older adult group

	Total group N = 26		Control group n = 13		Older adult group n = 13		
	Mean ± SD (range)	Median (interquartile range [IQR])	Mean ± SD (range)	Median (IQR)	Mean ± SD (range)	Median (IQR)	*P* value
Age (yr)	62.32 ± 23.43 (29.00–93.00)	70.00 (48.00)	40.75 ± 11.69 (29.00–64.00)	36.50 (11.75)	82.23 ± 8.82 (72.00–93.00)	83.00 (17.50)	<.001[a]
Weight (kg)	71.92 ± 16.27 (51.00–113.00)	72.00 (26.00)	78.42 ± 16.56 (53.00–113.00)	74.50 (16.00)	65.92 ± 14.02 (51.00–93.00)	64.00 (22.50)	.053[b]
Height (cm)	169.40 ± 10.01 (152.00–193.00)	168.00 (11.00)	173.08 ± 8.05 (160.00–190.00)	171.50 (11.00)	166.1 ± 10.75 (152.00–193.00)	165.00 (10.50)	.080[b]
Body mass index (kg/m^2)	25.04 ± 5.13 (17.75–34.58)	24.68 (7.56)	26.17 ± 5.10 (18.34–34.11)	25.24 (9.16)	23.99 ± 5.12 (17.75–34.58)	22.22 (8.03)	.298[b]

Note: A *P* value <.05 (with a 95% confidence interval) was considered statistically significant. Range (min–max).
[a]Mann-Whitney *U*-test.
[b]Independent *t* test.

TABLE 2 Platelets and white blood cells (WBC) count (cells × 10^6/mL) in whole blood in the control group and older adult group

	Control group n = 13			Older adult group n = 13			
Variables	Mean ± DS (range)	Median (interquartile range [IQR])	*P**	Mean ± DS (range)	Median (IQR)	*P**	*P* value
Platelets	256.60 ± 76.37 (153.00–382.00)	237.00 (124.50)	.483	190.30 ± 71.38 (56.0–347.00)	181.00 (58.00)	.234	.035[b]
WBC	8.44 ± 2.64 (5.80–14.80)	7.56 (3.26)	.036	5.70 ± 12 933 (1.02–10.30)	5.70 (2.90)	.840	.001[a]
Neutrophils	4.84 ± 1.87 (2.26–8.70)	4.25 (3.13)	.440	3.46 ± 1.69 (1.81–7.38)	2.72 (2.25)	.019	.032[a]
Lynfocytes	2.75 ± 0.95 (1.81–5.45)	2.60 (0.78)	.003	1.46 ± 0.62 (0.72–2.78)	1.32 (0.88)	.128	<.001[a]
Monocytes	0.60 ± 0.15 (0.31–0.84)	0.59 (0.20)	.644	0.42 ± 0.14 (0.20–0.73)	0.42 (0.15)	.645	.006[b]

Note: WBC, Leukocytes. Range (min–max). A *P* value <.05 (with a 95% confidence interval) was considered statistically significant.
*Shapiro-Wilk test Statistical: [a]Mann-Whitney *U*-test; [b]Independent *t* test.

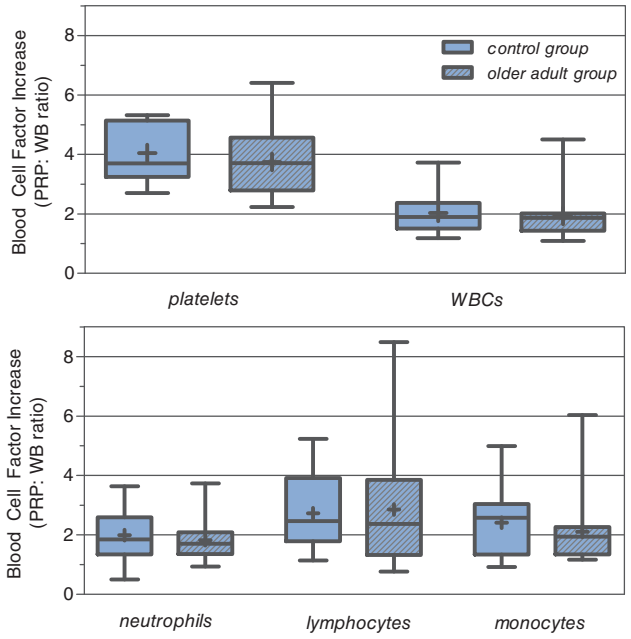

FIGURE 1 Platelet-rich plasma (PRP): whole blood (WB) ratio on control and older adult groups by using the Easy PRP kit. Top panel; mean platelets and white blood cells fold increase from WB. Bottom panel; mean neutrophils, lymphocytes and monocytes fold increase from WB (median; line in the middle of the IQR box, mean;+, whiskers; minimum to maximum)

WB of the older adult group, participants over 65 years old, using a new WB fractionation-separation system. In the older age group, the mean platelet and WBC count were between 30% and 40% lower than the cell count in the PRP of the control group; participants were younger than 65 years according to the WHO's definition of elderly person.[28]

Some previous studies have shown differences in the platelet concentration from PRP prepared using different WB separation methods,[18,20,21,29] largely related to the lack of a standardised PRP protocol.[22]

The number of centrifugation steps, the acceleration or the time used during centrifugation seem to play a determining role in the performance of each method of blood separation[22] as does the volume of blood incorporated or the particular geometry of the device.[30] Other reports have established important differences in the constituents of the final product of PRP, as a result of the wide range of extraction methods and protocols available.[17,19,22] Beyond platelets, PRP shows a variable composition of erythrocytes, WBC and other constituents such as electrolytes, soluble plasma hormones and other soluble proteins with bioactive functions, which together determine whether the clinical use of a PRP preparation is matched with the treated pathology.[17,19,22]

TABLE 3 Recovery of platelet and white blood cells (WBC) (cells × 10^6/mL) in the final platelet-rich plasma (PRP) product by using the Easy PRP kit in the control group and in the older adult group

	Control group n = 13			Older adult group n = 13			
Variables	**Mean ± SD (range)**	**Median (interquartile range [IQR])**	***P****	**Mean ± SD (range)**	**Median (IQR)**	***P****	***P* value**[a]
Platelets	999.2 ± 241.6 (564.50–1317.12)	1029.00 (418.80)	.766	676.50 ± 256.70 (359.00–1116.00)	643.20 (478.30)	.315	.004
WBC	16.80 ± 5.37 (8.88–24.00)	16.80 (10.16)	.492	9.89 ± 2.69 (4.59–14.25)	9.48 (4.09)	.968	.001
Neutrophils	8.71 ± 3.84 (3.27–16.99)	8.74 (5.35)	.818	5.71 ± 1.86 (2.36–8.76)	6.38 (2.69)	.813	.020
Lynfocytes	6.85 ± 2.29 (3.88–11.98)	6.49 (3.26)	.561	3.31 ± 1.38 (1.72–6.11)	3.67 (2.20)	.150	<.001
Monocytes	1.34 ± 0.52 (0.67–2.24)	1.28 (0.97)	.516	0.84 ± 0.40 (0.41–1.69)	0.76 (0.57)	.1967	.013

Note: WBC, Leukocytes. Range (min–max). A *P* value <.05 (with a 95% confidence interval) was considered statistically significant.
*Shapiro-Wilk test statistical; [a]Independent *t* test.

The platelet density in the final products of the control group, using the PRP protocol proposed by the manufacturer, was close to the maximum limit of the concentration range, which is assumed to determine the clinical effectiveness of PRP.[9,10] Because both groups were subjected to the same protocol to obtain PRP, only diluted fractions of platelet and WBC in WB of the older age group (30% lower) could be involved in the differences found between PRPs of both age groups. Recently, it has been shown that WB dilution increases the recovery rate of platelets and WBC in PRP preparations.[30] However, our study population exclusively included non-anaemic volunteers (data not shown) and thus it is feasible to think that (a) the improvement in the performance after blood dilution occurs by simple dilution of the red blood cells, avoiding their interference with the capture process of platelets or WBC by the system and (b) that the pre-existence of reduced values of platelets or WBC in the haematocrit would inexorably drive to a poor concentration of these cell lines in the PRP, regardless of whether the blood dilution is performed and the system used. Importantly, in our study, the cell capture efficiency of the Easy PRP system was the same in both age groups. Thus, the differences observed in the PRP preparations after dividing the study population according to age were exclusively because of the pre-existing significant differences in haematocrit of both population groups.

Among the multiple clinical uses of PRP, the use of leukocyte-rich PRP is being explored as a strategy to promote the healing of infected ulcers.[31] Platelet concentrate, through the release of growth factors and proinflammatory cytokines or the modulation of chemotaxis, has been shown to be beneficial in the reduction of diabetic foot ulcers by contributing to angiogenesis and cell proliferation.[32] On the other hand, the presence of neutrophils and to a lesser extent monocytes as the main microbicidal effectors may aid in preventing or controlling infection at the site of injury.[22,25,33] It has been demonstrated that WBCs also enhance PRP growth factor concentrations through their own release of growth factors or by stimulating the release of platelet growth factors.[25]

In this regard, it must be considered the over-representation of lymphocyte fraction is in WBC concentrates, if the use of leukocytes for therapeutic purposes is among our applications. This occurs as a consequence of the greater concentration efficiency, method-independent, of the smaller to the detriment of the larger white cell lines.[24] As we have shown in the present study, lymphocytes within leukocyte concentrate (41% and 34% of WBCs for control and older adult group respectively) is appropriately 10% higher than that of the basal haematocrit (33% and 25% of WBCs respectively), whereas neutrophils and monocytes appear to maintain the proportionality in WB and in final products.

Despite the efforts to establish a standardised protocol that improves the performance of the different separation blood methods,[22,30] and the greater knowledge of the final products obtained and their suitability for the target clinical applications, little attention has been paid to the inter-subjects variability in the PRP preparations and the factors that trigger it.[10] As we have described, the age is, among potential subject natural variables that can alter the performance of commercial blood separation systems, a factor that would modify our clinical efficacy prediction of the final products. In the absence of underlying diseases in the studied population, the other demographic variables characterised were not statistically different between groups assuming that the variation in haematocrit is mainly a consequence of ageing. In this way, the patient's age, baseline haematocrit and the cell-capturing properties of the system could be useful variables to predict the composition of the prepared PRP. It is essential to confirm that the efficiency of a particular concentrator system remains intact in groups of subjects affected by other demographic variables or underlying pathologies as frailty,[34,35] which could affect the cell count in the haematocrit, as a basis to predict the effectiveness of the resulting PRP.

The impact of the inter-subject variability on the proteomic content or platelet competition or the potential for platelet activation remains unknown.

In summary, this study shows an inter-subject variability in the prepared PRP by considering the natural ageing of the population, which should be considered together with the potential variability in the concentration and composition of the final products achieved by different systems and working protocols.

There are exceptionally high number of intrinsic variables that may influence the final product and it would not be feasible include all potential factors for evaluation. Thus, it would be important for a consensus panel of experts to develop guidelines based on the best current available literature. Platelet and WBC count from the PRP prepared for administration, as well as factors considered relevant to the disease to be treated, may be appropriate reference points.

Our results do not intend to promote a standard or reference for purely commercial purpose. This variability should be considered regardless of commercial system or protocol used. From this study, we emphasise the need to use a personalised approach to the patient based on demographic or proteomic markers, which allow us to anticipate potential responders and non-responders to treatment with PRP.

5 | CONCLUSIONS

The ageing of population has a strong influence on the haematocrit and therefore on the composition of PRP. Because the efficiency of blood separator system seems to be constant across individuals, the inter-subject haematocrit variability based on age could be used as a predictor of resulting PRP. The clinical application of PRP should be restricted to the specific cell capture capacity of different commercial devices.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Bibiana Trevissón, Ricardo Becerro-de-Bengoa-Vallejo, David Sevillano, Marta Losa-Iglesias, Daniel López-López conceived and designed the analysis; Bibiana Trevissón, David Sevillano, Natalia González, Luis Alou, collect the data; David Sevillano, Ricardo Becerro-de-Bengoa-Vallejo, Natalia González, Luis Alou, Daniel López-López performed the analysis, David Sevillano, Ricardo Becerro-de-Bengoa-Vallejo, Marta Losa-Iglesias wrote the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonablerequest.

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