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Platelet Distribution Amplitude (PDW) and Mean Platelet Volume (MPV): two simple, practical and specific indicators of platelet activation in Liquid PRF

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Abstract

Background: platelet indices are potentially useful markers in diagnosing liquid PRF platelet activation and its quality. An increase both in mean platelet volume (MPV) and platelet distribution width (PDW) is due to platelet activation and comes from platelet enlargement and pseudopodia formation. Our hypothesis is that, using different vials and centrifuges to obtain liquid PRF, there might be different MPV and PDW values, which would point toward different type or level of activation.

Methods: Platelet indices (MPV, PDW, MPV/Plt, PDW/Plt) were measured in different Liquid PRF types/groups. Five different types of Liquid PRF were prepared: Liquid Fibrinogen 2700 rpm × 3' (820 g) (RCF clot=608 g; RCF max =816 g; RCF min =326 g); i-PRF 3300 rpm (1220 g) × 3'(RCF clot =765 g; RCF max = 1008 g; RCF min =403 g); i-PRF 700 rpm (55 g) × 5'(RCF clot =38 g; RCF max =55 g; RCF min =22 g); A-PRF 1300 rpm (189 g) × 5'(RCFclot=142 g; RCFmax=189 g; RCFmin=66 g); C-PRF 2500 rpm (700 g) × 8' and also PRP (Platelet rich Plasma) [2200 rpm, (1147 *g*) x 20 min]. The studied hematological parameters were: platelet count (Plt), mean platelet volume (MPV) and platelet distribution width (PDW), as well as MPV/Plt ratio and PDW/Plt ratio.

Results: the highest MPV found in Vacumed FL vials was of 9.428±0.69 fL, while in original S- PRF vials it was 9.56±0.77 fL p=0.49, whatever the type of centrifuge employed; highest PDW found in Vacumed FL vials was of 11.71±0.66 %, while with original S-PRF vials it was 11.7±1.95 % p=0.974, whatever the type of centrifuge employed. Hence no difference tied to the type of vial and centrifuge was found between obtained values, but only based on the type of Liquid PRF produced.

Conclusions: PDW and MPV are the most specific markers for platelet activation, as they do not increase in values during simple platelet enlargement and their value is not related to the type of vial and centrifuge used, but only to the type of PRF produced.

Keywords: Platelet distribution width; Platelet activation; Mean platelet volume; Anisocytosis; Liquid platelet rich Fibrin

Introduction

Topical use of Platelet-Rich Fibrin (PRF) can play an important role in initiating the repair process of chronic wounds [1]. Recently, a significant and extensive group of reliable markers for platelet activation has been studied upon, including β-thromboglobulin (β-TG) and *P*-selectin. Several researchers used a series of platelet indices, measured via hematological

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machine analysis, since platelet activity is linked to significant morphological changes in platelets themselves. Mean platelet volume (MPV) is probably the most studied marker of platelet activation. Recently, novel platelet indices, like mean platelet component (MPC) and platelet component distribution width (PCDW) have been studied, and our group, in this study, did so as potential markers of platelet activation in Liquid PRF (liquid Platelet rich fibrin) [2] as an index for production of growth factors by platelet contained in it. This effort to research and find simple and widely used indices was focused on the morphological changes platelet activation brings in platelet themselves, including both their spherical form and pseudopod formation (Figure1). Platelets with increased number and size of pseudopods are different in size, possibly influencing platelet distribution width. PDW is also a platelet anisocytosis marker, which occurs after platelet activation, increasing diameter and apparent volume of platelets themselves; hence PDW itself can be a simple and cheap marker of platelet activation [3]. Platelet distribution width (PDW) reflects with its values the variability in platelet dimension and is generally considered a proper index for platelet function and activation. Increased PDW values were noted in diabetic patient [4]. Hence, the possibility that platelet activation increases MPV and PDW was analyzed.

Legend: Resting platelets are smooth and disc shaped (left). Activated platelets have an irregular shape with many protruding pseudopodia (right).

Figure 1: Platelets in their resting form and at the beginning of the activation.

Thanks to automatic analyzing machines, MPV has been extensively used in clinical practice even if highly susceptible to pre-test variables, including the type of anticoagulant employed, time for analysis and pre-test storage temperature. MPV largely depends on the platelet counting technique (impedance or optical) used in the automatic analyzer as well as the sample dilution conditions before counting: osmolality, temperature, type of detergent, which explains the high discrepancy between different counters. MPV values reported in healthy people vary widely in scientific literature: from 6,0 to 13,2 fL. Our laboratory reports ranges from 8,0 to 12,0 fL as normal MPV values, while PDW ranges between 8,0 and 18,0%.

The relevance of PDW as a possible general index of platelet activation remains, however, poorly defined. Currently we also wanted to study the platelet-dependent functional significance of PDW variability in the various types of Liquid PRF, clarifying its action in relation to platelet activation in vivo in the synthesis of platelet growth factors.

Materials and Methods

No ethical committee approval was required for this study since no human samples were identified, as previously described by Miron, Fujioka-Kobayashi et al. [5]. All subjects from which blood samples were drawn agreed to sign the informed consent.

The main objective is to investigate whether the *g*-force adaptation for the above modifications on liquid PRF (liquid C-PRF, liquid A-PRF and i-PRF), using an oscillating or a 41.3° fixed angle centrifuge, as well as different types of vials, has some influence on their characteristics in terms of morphology and platelet content. Low speed centrifugation (Crisci A., 2021) [6] of PRF in negative pressure plastic (PET) collection vials Vacumed FL (code 44909) and with the original green S-PRF vial (recommended by the manufacturing company Process, France), allows PRF to be prepared in liquid form and to be used in an injectable manner. Liquid C-PRF (RCFclot=525 g; RCFmax=700 g; RCFmin=280 g) according to Miron [7,8] or other types of liquid PRF (A-PRF, i-PRF, C- PRF, Liquid Fibrinogen) [9] were found after post-centrifugation sampling of the blood, with a Vacutainer system under vacuum and 18 gauge needle, and these can be injected or even solidified into clots and membranes.

Twenty subjects in apparent good health, 10 males and 10 females, aged between 18 and 60 years, were subjected to blood sampling in two phases for a total of 45 ml (5 Vacumed FL tubes × 9 ml) in a first phase and another 45 ml (5 S-PRF tubes × 9 ml) in a second phase. The baseline Plt, MPV and PDW values were evaluated. All the liquid samples for analyzing PRF Liquid were collected in tubes (Vacutest K2 EDTA 6 ml code: K135400) containing dipotassium ethylenediaminetetraacetate (EDTA) as an anticoagulant.

9 mL of whole blood was centrifuged in S-PRF plastic vials and Vacumed FL PET vials to obtain Liquid PRFs. The centrifuge (Duo centrifuge, Process for PRF™, Nice, France) used had a fixed angle, no brake and a rotor size of 110 mm according to protocol (rotor angle of 43.1°, radius of 75 mm in the center of the pipe, 100 mm maximum and 35 mm minimum). A comparison was carried out with a second centrifuge, of tilting type with a 90° angle (CenLee CTL420 Horizontal Centrifuge, Hunan, China). Samples were analyzed by a HECO 5 hematology analyzer (Seac Radim Company), and the platelet concentration and all platelet parameters were evaluated two hours after blood sampling (the mean values ± s.d. obtained for each type of liquid PRF produced have been reported) (Table 1,2). Five different types of Liquid PRF were produced: Liquid Fibrinogen 2700 rpm × 3' [8] (RCF clot=608 *g*; RCF max =816 *g*; RCF min

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=326 *g*); i-PRF 3300 rpm (1220 g) × 3'(RCF clot =765 *g*; RCF max = 1008 *g*; RCF min =403 *g)*; i-PRF 700 rpm (55 g) × 5'(RCF clot =38 *g*; RCF max =55 *g*; RCF min =22 *g*); A-PRF 1300 rpm (189 g) × 5'(RCFclot=142 *g*; RCFmax=189 *g*; RCFmin=66 *g*); C-PRF 2500 rpm (700 g) × 8' [6].

PRP (Platelet Rich Plasma) was prepared from samples drawn from 20 patients [2200 rpm, (1147 g) × 20 min] with 15ml BioReb Base vial (Biodevice & Advanced Materials S.r.l. Naples, Italy) with separating and anticoagulant gel (ACD-A: Anticoagulant Citrate Dextrose Solution, Solution A, USP) using a HETTICH EBA200 centrifuge with fixed 33° angle, following protocol indicated by the manufacturing company. Liquid PRF cell separation was carried out immediately (no later than 2 minutes) through centrifugation. All centrifugation was performed at room temperature (22–25°C) and all vials before venipuncture were preheated to 37°C in an incubator, to simulate body temperature as much as possible [10].

In general, after centrifugation, an average of approximately 1.5 ml of liquid PRF was obtained from vials containing 9 ml of blood, equal to 9.1 g. The average weight of the liquid PRF obtained is 1.3 g.

Data analysis

All liquid samples for Liquid PRF analysis were collected in vials containing ethylenediaminetetraacetate dipotassium (EDTA) as anticoagulant. The following hematological parameters were studied in all extracted Liquid PRF samples: platelet count (Plt), mean platelet volume (MPV) and platelet distribution width (PDW), as well as MPV/Plt and PDW/Plt Ratios. All continuous data following normal distribution were calculated as Mean±Standard Deviation (SD) and evaluated for significant differences at each time point with Student's independent parametric test for paired data, while unpaired data was analyzed with Mann-Whitney rank test, using the Statistics for Biomedical Disciplines software by Santon A. Glatz Ed. 2007 Version 6.0. Differences were considered significant with p values less than 0.05 (*), And highly significant for p values less than 0.01 (**) and 0.001 (***). Furthermore, to investigate the apparent distribution relationships between PDW and other platelet indices, we calculated the Spearman correlation coefficient (r) to evaluate the PDW correlations with Mean Platelet Volume (MPV) and Platelet Count (Plt) [11]. We also proceeded to calculate the significance with Wilcoxon and Friedman tests when Spearman test resulted in Low Correlation. The platelet concentration (Plt) is expressed in K/mL, the Platelet count (Pct) in %, the MPV (mean platelet volume) in fL, the PDW (platelet distribution width) in %, the MPV/Plt ratio in fL/10^9 L and the PDW/Plt ratio in %/10^9 L.

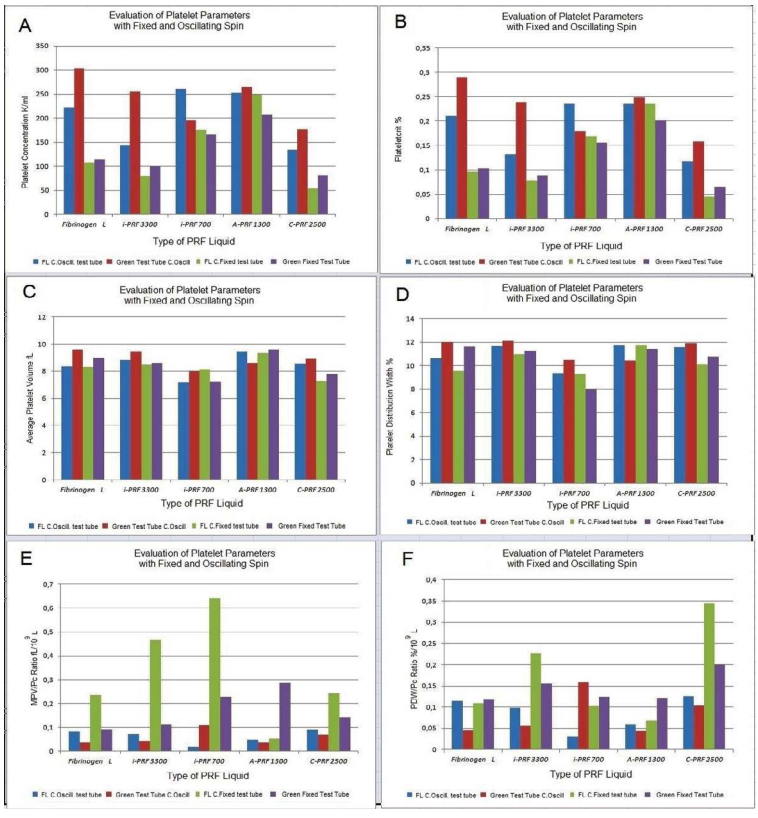

Figure 2: Evaluation of platelet parameters with Horizontal (H) and Fixed Centrifuge (F) in various types of Liquid PRF with Vacumed FL vial (code 44909) and original Green S-PRF vial.

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Table 1: Platelet parameters evaluation with Horizontal (H) and Fixed Centrifuge (F) in various types of Liquid PRF with Vacumed FL vials (code 44909).

TypePRF	Fibr.L.H.	Fibr.L.F.	Significativity		i-PRF H.	i-PRF F.	Significativity		i-PRF H.	i-PRF F.	Signif.
	2700 rpm x 3'/816 g		t-test	t-M/W	3300 rpm x 3'/1220 g		t-test	t-M/W	700 rpm x 5'/55 g		t-M/W
Plt	222.7±138.3	107.8±73.5	0.009**		144.3±68.1	79.5±62.8	0.017*		260.6±154.4	175.0±161.3	
MPV	8.34±3.05	8.32±2.21		>0.05	8.84±0.99	8.48±1.07		>0.05	7.22±3.84	8.15±3.63	>0.05
Pct	0.21±0.13	0.097±0.07	0.007**		0.132±0.08	0.079±0.06	0.057		0.235±0.14	0.169±0.15	
PDW	10.6±3.76	9.53±4.6		>0.05	11.65±0.85	10.97±2.83		>0.05	9.35±4.95	9.28±5.57	>0.05
MPV/Plt	0.082±0.15	0.236±0.63		0.033*	0.072±0.03	0.466±0.87	0.048*	0.006**	0.02±0.02	0.64±2.08	0.053
PDW/Plt	0.114±0.23	0.109±0.13		>0.05	0.098±0.05	0.226±0.19		0.044*	0.03±0.02	0.102±0.22	>0.05

TypePRF	A-PRF H.	A-PRF F.	Signif.	C-PRF H.	C-PRF F.	Significativity		PRP
	1300 rpm x 5'/189 g		t-M/W	2500 rpm x 8'/700 g		t-test	t-M/W	2200 rpm x 20'
Plt	252.3±120.6	249.0±97.9		135.1±66.2	54.1±47.2	0.000**		32.3±57.5
MPV	9.47±0.72	9.34±0.7	>0.05	8.57±0.65	7.29±2.64		0.035*	6.52±2.68
Pct	0.23±0.11	0.235±0.09		0.118±0.06	0.046±0.05	0.000**		0.027±0.05
PDW	11.7±0.8	11.77±0.6	>0.05	11.58±0.46	10.12±3.57		>0.05	7.93±5.32
MPV/Plt	0.047±0.03	0.052±0.05	>0.05	0.089±0.07	0.243±0.31		0.037*	0.986±1.46
PDW/Plt	0.059±0.03	0.068±0.08	>0.05	0.125±0.11	0.344±0.45		0.041*	0.596±0.77

with Vacumed FL tube (code 44909); p<0.05* (significant difference); p<0.01**; p<0.001***(highly significant difference);

Table 2: Platelet parameters evaluation with Horizontal (H) and Fixed Centrifuge (F) in various types of Liquid PRF with original Green S-PRF vials.

TypePRF	Fibr.L.H.	Fibr.L.F.	Significatività		i-PRF H.	i-PRF F.	Signif.	i-PRF H.	i-PRF F.	Signif.
	2700 rpm x 3'/816 g		t-test	t-M/W	3300 rpm x 3'/1220 g		t-test	700 rpm x 5'/55 g		t-M/W
Plt	303.5±97.37	114.4±51.8	0.000***	0.000***	254.6±108.3	100.2±52.4	0.000***	195.6±163.9	166.1±172.1	
MPV	9.58±0.55	8.98±0.63		>0.05	9.48±0.65	8.61±0.99	0.027*	8.01±2.99	7.25±3.94	>0.05
Pct	0.29±0.09	0.104±0.05	0.000***	0.000***	0.239±0.09	0.088±0.05	0.000***	0.18±0.15	0.156±0.16	
PDW	12.01±0.58	11.61±0.71		>0.05	12.12±0.69	11.29±1.03	0.042*	10.46±3.73	7.95±5.9	>0.05
MPV/Plt	0.036±0.02	0.091±0.03	0.000***	0.000***	0.044±0.02	0.112±0.07	0.008*	0.11±0.18	0.228±0.55	>0.05
PDW/Plt	0.045±0.02	0.119±0.05	0.000***	0.000***	0.056±0.02	0.155±0.12	0.018*	0.158±0.27	0.124±0.33	>0.05

TypePRF	A-PRF H.	A-PRF F.	Signif.	C-PRF H.	C-PRF F.	Significativity	
	1300 rpm x 5'/189 g		t-M/W	2500 rpm x 8'/700 g		t-test	t-M/W
Plt	264.8±152.7	207.1±132.8		176.9±75.4	82.0±52.1	0.002**	0.005**
MPV	8.6±3.11	9.61±1.01	>0.05	8.91±0.37	7.78±0.7	0.000**	0.001**
Pct	0.249±0.14	0.202±0.13		0.158±0.07	0.066±0.05	0.002**	0.001**
PDW	10.4±3.72	11.43±3.7	>0.05	11.9±1.40	10.75±0.77	0.024*	0.019*
MPV/Plt	0.037±0.03	0.028±0.71	>0.05	0.071±0.07	0.142±0.09		0.015*
PDW/Plt	0.044±0.03	0.121±0.2	>0.05	0.104±0.12	0.20±0.13		0.013*

with original Green S-PRF vial; p<0.05* (significant difference); p<0.01**; p<0.001***(highly significant difference);

MPV Max= PDW Max=

Table 3: Spearman Correlation Coefficient between platelet parameters with Horizontal (H) and Fixed Centrifuge (F) in various types of Liquid PRF with Vacumed FL vial (code 44909).

TypePRF	Fibr.L.H.	Fibr.L.F.	i-PRF H.	i-PRF F.	i-PRF H.	i-PRF F.	A-PRF H.	A-PRF F.	C-PRF H.	C-PRF F.
	2700 rpm x 3'/816 g		3300 rpm x 3'/1220 g		700 rpm x 5'/55 g		1300 rpm x 5'/189 g		2500 rpm x 8'/700 g	
Plt/MPV	r=0.388 p=0.255	r=0.542 p=0.026*	r=0.394 p=0.247	r=0.570 p=0.015*	r=0.542 p=0.102	r=0.474 p=0.036*	r=-0.548 p=0.098	r=0.128 p=0.629	r=0.264 p=0.443	r=0.759 p=0.001**
Plt/PDW	r=0.979 p=0.811	r=0.111 p=0.665	r=0.318 p=0.354	r=0.400 p=0.099	r=0.285 p=0.407	r=0.253 p=0.277	r=-0.773 p=0.011*	r=-0.121 p=0.646	r=-0.152 p=0.657	r=-0.627 p=0.004**
PDW/MPV	r=-0.785 p=0.009**	r=0.520 p=0.033*	r=-0.864 p=0.002**	r=0.534 p=0.024*	r=0.885 p=0.001**	r=0.585 p=0.008*	r=-0.885 p=0.001**	r=0.690 p=0.004**	r=0.803 p=0.007**	r=0.778 p=0.001**

with Vacumed FL vial (code 44909); p<0.05* (significant difference); p<0.01**; p<0.001***(highly significant difference);

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Results

In general, PDW values at the time of the study were directly and positively correlated to MPV values (r=0.370 vs 0.945, p≤0.001) and inversely correlated to platelet counts (r=-0.627 vs 0.945, p≤0.001). The Mann-Whitney rank test was not performed in case of paired data and/or significance was established with the *t* Student's test (Table 1-4).

Highest obtained MPV values, indicating platelet activation, were detected using original S- PRF vials using the oscillating centrifuge to produce Liquid Fibrinogen at 2700 rpm × 3' (816 g) (9.58 ± 0.55 fL) and i-PRF at 3300 rpm × 3 ' (1220 g)(9.48±0.65 fL) , as well as with fixed centrifuge to produce A-PRF (9.61±1.01 fL) (Table 2). Even using Vacumed FL vials (code 44909) with both Fixed and Oscillating centrifuge, high MPV values were found in producing A-PRF (9.34±0.7 fL Fixed Centr.)(9.47±0.72 fL Centr.H) (Table 1) (Figure 2C). The highest PDW values, which also could be surmised as activation index, were detected using original S-PRF vials using the oscillating centrifuge to produce Liquid Fibrinogen (12.01±0.58%), i-PRF at 3300 rpm × 3' (1220 g)(12.12 ±0.69 fL) and C-PRF at 2500 rpm × 8' (700 g)(11.9±1.4 %) and with fixed centrifuge to produce Liquid Fibrinogen (11.61±0.71 %) and A-PRF (11.43±3.7 %) (Table 2). Even using Vacumed FL vials (code 44909) with both Fixed and Oscillating centrifuges, high PDW values were found in producing A-PRF (11.7±0.8% Centr.H)(11.77±0.6% Fixed Centr.) (Table 1) and i-PRF at 3300 rpm × 3' (1220 g)(11.65±0.85 % Centr.H) and also C-PRF at 2500 rpm × 8' (700 g)(11.58±0.46 % Centr.H) (Table 1). The maximum MPV found with the Vacumed FL vials was 9.428±0.69 fL, with the original S-PRF vials it was 9.56±0.77 p=0.49 fL whatever the type of centrifuge used; the maximum PDW found with the Vacumed FL vials was 11.71±0.66%, with the original S-PRF vials it was 11.7±1.95 p=0.974% whatever the type of centrifuge used. Therefore, there was no significant difference in values relating to both the type of test tube and centrifuge used. PDW was significantly and negatively correlated with Platelet Count (Plt) (r=- 0.773, p<0.05) in the production of A-PRF H and in C-PRF with Fixed centrifuge (r=-0.627, p<0.01) with Vacumed FL vials (Table 3), and also with MPV (r=-0.885, p<0.01) in the production of A-PRF H (Figure 2D). An inverse but not significant correlation was also observed between MPV and Plt (r=-0.548, p=0.09) with Vacumed FL vials in the production of A-PRF H. Examining the production of C-PRF with Fixed centrifuge and Vacumed FL vials, a positive and significant correlation was found between MPV and Plt (r=0.759, p<0.01) and between PDW and MPV (r=0.778, p<0.01) and negative but significant correlation between PDW and Plt (r=-0.627, p<0.01) (Table 3). The correlation between PDW and MPV with Vacumed FL vials was also strongly positive in all liquid PRF formulations except for Liquid Fibrinogen, i-PRF (3300 rpm × 3') and A-PRF produced with a horizontal centrifuge, where we found a strongly negative correlation. The correlation between PDW and MPV with S-PRF vials was strongly positive in all liquid PRF formulations except in Liquid Fibrinogen, i-PRF (3300 rpm × 3') and A-PRF produced with horizontal centrifuge (H) where a strongly negative correlation was observed. In summary we found no differences between the use of Vacumed FL and/or S-PRF vials in the PDW/MPV correlation. PDW was significantly and negatively correlated with MPV (r=-0.815, p<0.01) in the production of Fibrinogen L H and A-PRF H (r=-0.945, p<0.01) with the use of an S-PRF tube. PDW was significantly and positively correlated with MPV (r=0.917, p<0.01) in Fixed centrifuge production of i-PRF 700 rpm × 5' and between MPV and Plt (r=0.730, p<0.01) and in C- PRF (r=0.853, p<0.01) (Table 4). The values obtained on the PRP samples of the 21 patients are as follows: Plt: 32.3±57.51 K/mL; MPV: 6.52±2.68 fL; Pct: 0.027±0.051%; PDW: 7.93±5.32%; MPV/plt: 0.986±1.46 fL/109 L; PDW/Plt: 0.596±0.77 %/109 L, all values were significantly lower than those obtained in all types of Liquid PRFs.

The Spearman correlation coefficient for PRP between Plt and MPV is r=0.635, p=0.001 and between Plt and PDW is r=0.628 p=0.001, hence correlation between values is considered strongly positive. We proceeded to verify the significance through the Wilcoxon and Friedman tests of the values with poor correlation reported in Table 3 and 4 between Plt, MPV and PDW. Between Plt and MPV in the production

Table 4: Spearman Correlation Coefficient between platelet parameters with Horizontal (H) and Fixed Centrifuge (F) in various types of Liquid PRF with original Green S-PRF Vial.

TypePRF	Fibr.L.H.	Fibr.L.F.	i-PRF H.	i-PRF F.	i-PRF H.	i-PRF F.	A-PRF H.	A-PRF F.	C-PRF H.	C-PRF F.
	2700 rpm x 3'/816 g		3300 rpm x 3'/1220 g		700 rpm x 5'/55 g		1300 rpm x 5'/189 g		2500 rpm x 8'/700 g	
Plt/MPV	r=-0.188 p=0.584	r=0.467 p=0.166	r=-0.118 p=0.726	r=0.584 p=0.060	r=0.624 p=0.054	r=0.730 p=0.003**	r=-0.103 p=0.759	r=0.187 p=0.529	r=0.321 p=0.349	r=0.853 p=0.001**
Plt/PDW	r=-0.552 p=0.096	r=0.167 p=0.626	r=-0.358 p=0.296	r=0.195 p=0.548	r=0.370 p=0.279	r=0.654 p=0.009**	r=-0.079 p=0.811	r=-0.021 p=0.939	r=-0.385 p=0.259	r=-0.413 p=0.177
PDW/MPV	r=-0.815 p=0.006**	r=0.633 p=0.05	r=-0.515 p=0.123	r=0.655 p=0.031*	r=0.745 p=0.016*	r=0.917 p=0.001**	r=-0.945 p=0.001**	r=0.598 p=0.033*	r=0.370 p=0.279	r=0.563 p=0.057

with original Green S-PRF vials; p<0.05* (significant difference); p<0.01**; p<0.001***(highly significant difference);

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of A-PRF with Fixed centrifuge and Vacumed FL vials, the $p<0.022$ and 0.000 were found respectively in the Wilcoxon and Friedman tests; between Plt and PDW in the production of A-PRF and Liquid Fibrinogen with Fixed centrifuge and Vacumed FL tube the $p<0.022$ and 0.000, of C-PRF the $p<0.020$ and 0.002 with oscillating centrifuge, respectively in the Wilcoxon and Friedman tests. Between Plt and PDW in the production of Liquid Fibrinogen and i- PRF (3300 × 3') with Fixed centrifuge and S-PRF vials the $p<0.020$ and 0.002; $p<0.018$ and 0.000 respectively for the Wilcoxon and Friedman tests; between Plt and MPV in the production of A-PRF and Liquid Fibrinogen with oscillating centrifuge and Sticky S-PRF vials, $p<0.020$ and 0.003; $p<0.020$ and 0.002; and i-PRF (3300 × 3') $p<0.020$ and 0.002 with oscillating centrifuge, respectively in the Wilcoxon and Friedman tests.

Discussion

Concentrated platelets present in Liquid-PRF are responsible, after their activation, for active secretion of growth factors and for the induction of proliferation and differentiation of various cells involved in tissue regeneration processes. Our recent study demonstrated that the reduction of relative centrifugal force leads to significant increases in platelets and leukocytes total number and growth factors amounts, hence signifying that low-speed centrifugation leads to increases in the potential for PRF regeneration (Crisci et al.2024)[2]. MPV and PDW are easily measurable platelet indices, increasing during platelet activation. In fact, to obtain a larger surface, platelets change shape during activation, morphing from discoidal to spherical. Pseudopodia are also formed. Hematology analyzers based on impedance technology measure platelet volume by means of deformation of the electric field, depending on platelets vertical diameter. Analyzers with laser optical technology determine cell volume based on their transversal diameter. Therefore activated platelets appear larger regardless of measurement method. PDW is an indicator of heterogeneity in platelet size. Higher PDW values reflect a wider platelet size range, which may result from increased platelet activation, destruction, and consumption. Another possible pathophysiological mechanism responsible for greater variability in platelet size is increased coagulation. We also observed that PDW was different in all five study groups (Tables 2,3) and hypothetically leading to different and various platelet activation. We attributed the increase in PDW to platelet anisocytosis, resulting from the formation of pseudopodia. Platelet indices should not be used individually as direct indicators of platelet activation, however simultaneous increases in MPV and PDW could indicate platelet activation, as illustrated in our observations. PDW and MPV both derive from the same platelet volume distribution histogram; theoretically, they measure platelet size distribution and average size, respectively. Our current analysis, however, shows that PDW and MPV are also largely coincident in their relationship with platelet concentration levels. The MPV was shown to be a valid platelet function test in the present study. This interpretation of our results is supported by the presence of significant correlations between PDW and MPV, whatever type of vial used and centrifuge adopted. Our study represents a first attempt to clarify the possible platelet functional significance of this indices, based on in-laboratory observational approach.

Conclusions

In conclusion, MPV and especially PDW both increase during platelet activation, as illustrated by automated hematology analyzers. Although our study sample was sufficient to reveal significant differences, smaller and less evident differences might have been omitted. PDW appears to be a more specific indicator of platelet activation than MPV, as it did not increase following single platelet enlargement caused by platelet swelling. The combined and simultaneous use of MPV and PDW might predict platelet activation more efficiently. Therefore, the most useful type of liquid PRF for platelet activation, ranked by analyzing the MPV and PDW values, is our study was found to be A-PRF, followed equally by Liquid Fibrinogen and i-PRF (3300 × 3'), then by C-PRF and i- PRF (700 × 5'), and at last PRP.

In particular, considering both MPV and PDW, with the use of Green S-PRF tubes used in a Fixed Angle centrifuge, the indices of greater platelet activation with the production of Liquid A-PRF are found, despite a recent study by our group on horses has demonstrated poor correlation between MPV, MPV/Pc ratio and A-PRF+ membrane dimensions [12].

However, it is believed that their contribution can be better demonstrated by further and larger clinical studies.

Disclosures

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