CHARACTERISTICS OF AUTOLOGOUS PLATELET-RICH PLASMA CONCENTRATES OBTAINED BY THE BUFFY COAT TECHNIQUE USING DIFFERENT PROTOCOLS

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ABSTRACT

INTRODUCTION: In the last decade, platelet-rich concentrates, including the so-called platelet-rich plasma (PRP), have been gaining extreme popularity and are widely used in variety of clinical fields of medicine—dermatology, orthopedics, traumatology, plastic and reconstructive surgery, maxillofacial surgery, and others.

AIM: The aim of the article was to analyze the quantitative characteristics of the blood components in autologous platelet-rich plasma concentrates obtained by the buffy coat technique using different protocols.

MATERIALS AND METHODS: Thirty participants (avg. age 42.8) were included in the present study. Venous blood (35 mL) was drawn from each participant—3 mL were separated for control group to determine average blood cell level. Four protocols for PRP obtaining (4x8 mL) were performed by using vacutainer tube with separating gel and monovette (S-Monovette) without a separating gel. The concentration factor (%) for each protocol was calculated relative to the average baseline blood values of the control group.

RESULTS: Protocol I showed increase in platelet concentrations by 91.55% and the leukocyte level was 107% higher compared to the control group. The second protocol (separating gel) demonstrated increase in platelet count by 337.06% and in leukocytes by 82.37%. The third protocol, based on double centrifugation, showed 352.08% increase in platelets and 40.54% decrease in white blood cell compared to controls. The fourth protocol demonstrated 389.84% increase in platelet level and 74.36% decrease in leukocyte number.

CONCLUSION: The use of separating gels into various techniques for obtaining PRP facilitates blood cell aspiration and makes the procedure faster and safer in ambulatory practice.

Keywords: *autologous platelet products, platelets, growth factors*

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INTRODUCTION

The administration of autologous platelet-rich plasma (PRP) products is defined as a revolutionary, alternative method in medicine. Increased proliferation and induction of human mesenchymal stem cell differentiation have been found to be possible explanations for the clinical success reported with the use of autologous platelet products. Platelets excrete sev-

eral growth factors and cytokines that are associated with the healing capacity and the regeneration process in human body. Many studies demonstrate the chondro-inductive and osteo-inductive potential of PRP. Platelet-rich plasma is a general term referring to any sample of autologous plasma product with values of platelet concentrations above the baseline in the peripheral blood (1-5).

Depending on the different classification systems, variations in PRP products can be divided into several groups: pure platelet-rich plasma, platelet-rich and leukocyte-rich plasma, platelet-rich and leukocyte-poor plasma, growth factor-rich plasma, autologous platelet-rich plasma gel, and others. The terms are often mistaken and many clinicians do not take into account the significant differences between each of these blood products (6,7). Whole blood drawn from the patient by conventional phlebotomy techniques is centrifuged to form a distinct middle layer that contains platelets and white blood cells (WBC). Thereafter, this layer is placed in the damage area, where growth factors and other cytokines are released to allow faster and more predictable regeneration (8,9,10).

The buffy coat (BC) technique for obtaining PRP includes the following steps (Fig. 1). Whole blood (8 mL) is drawn by venipuncture into vacutainers with trisodium citrate as anticoagulant. The blood is centrifuged at high speed—above 1000×g ("g" centrifugal force) for a certain time at 20°C to 24°C. Due to the different relative weight of the blood cells, three layers are formed: a lower layer consisting of erythrocytes, a middle one consisting of platelets and leukocytes, the so-called buffy coat, and a top layer containing platelet-poor plasma (PPP). The surface layer of the supernatant is removed and the lower 2/3 of PPP with the buffy coat layer is carefully aspirated and transferred to a new sterile tube without anticoagulant. In order to reduce part of the leukocyte count, the resulting plasma can be subjected to a second centrifugation with a lower centrifugal force—below 1000×g (11,12). There are many different protocols for obtaining autologous platelet-rich products that have been described in the literature. A lot of commercial available kits are used, but there is lack of standardization and consensus on the best approach for preparation (13).

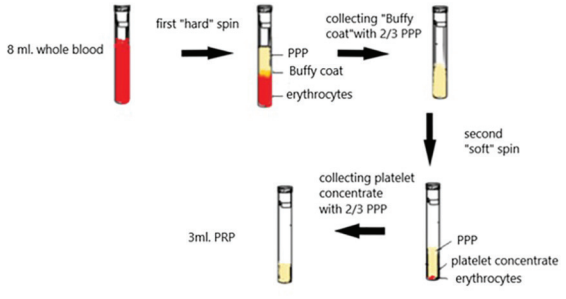

Fig. 1

AIM

The aim of the present study was to analyze the quantitative characteristics of the blood components in autologous PRP concentrates obtained by the buffy coat technique using different protocols.

MATERIALS AND METHODS

Materials

The present study had permission from the Ethic Committee at Medical University of Varna (61/30.03.2017). Thirty healthy participants (16 men and 14 women) at an average age of 42.8 years (min. 26; max. 64; SD±11.840) were included in the present study after their informed knowledge and consent. In sterile manner, 35 mL of venous blood was drawn from each participant. Two types of laboratory consumables were used. The first was vacutainer tubes (8 mL) with separating biocompatible gel (cycloaliphatic polymer inert gel) and the second type was a monovette (S-Monovette, 8 mL) without a separating gel. Each tube had 3.2% trisodium citrate as anticoagulant. The venipuncture was performed with a 22G needle in order to avoid premature activation of platelets. Three milliliters of whole blood of each patient were used as a control group to determine the baseline levels. The average values of blood cells (baseline levels) from the control group were compared to the average values of blood cells of protocols I, II, III, and IV. Each blood sample was processed within 1 hour after its withdrawal. OHAUS Frontier 5000 Series Multi laboratory centrifuges were used for the needs of the research. Symmetrical position of the tested tubes and calibration of the weight were mandatory before centrifugation.

Methods

Four protocols for PRP obtaining based on the buffy coat technique were utilized and blood cell counts were analyzed. The first protocol involved single-spin centrifugation at room temperature of a monovette without gel for 10 minutes at 1500×g (Fig. 2). The second protocol was under the same conditions of single-spin centrifugation with relative centrifugal force (RCF) of 1500×g for 10 minutes but using a vacutainer tube with a separating gel (Fig. 3). The third protocol included a process of double centrifugation of the monovette without gel. The first spin (separation) had a centrifugal force of 1150×g for 10 minutes (Fig. 4a) and the second spin (concentration) had a centrifugal force of 350×g for 5 minutes (Fig. 4b). The fourth protocol used 8 mL of whole blood in a vacutainer tube with a separating gel in conditions as in protocol three, namely first spin at RCF of 1150×g for 10 minutes (Fig. 5a) and second concentration spin with a centrifugal force of 350×g for 5 minutes (Fig. 5b) (Table 1).

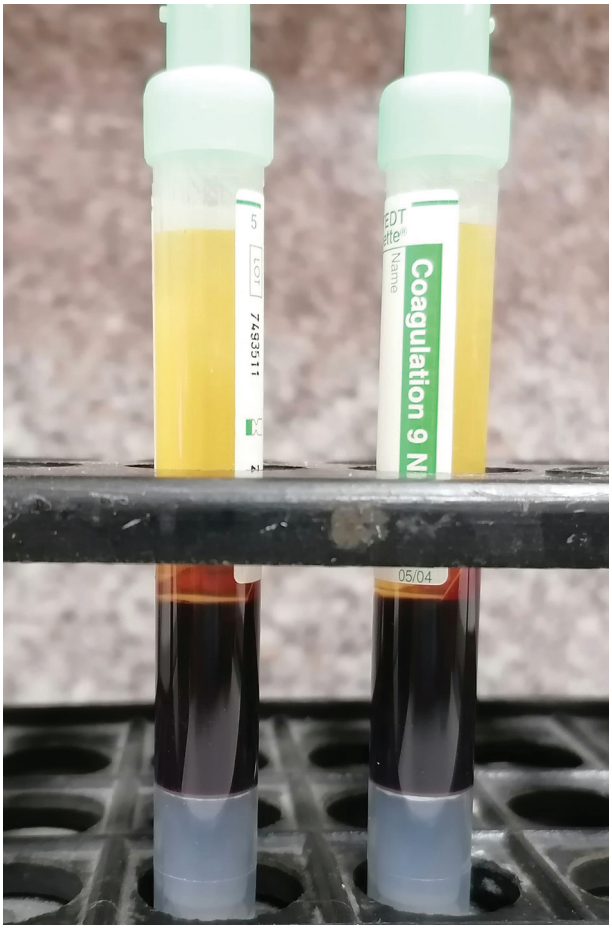

Fig. 2

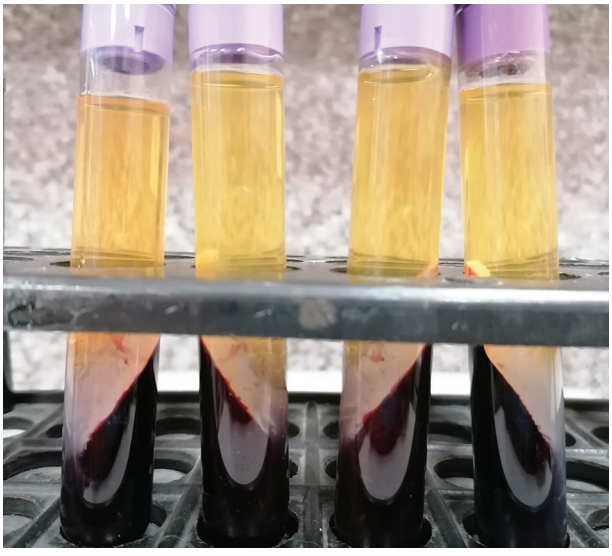

Fig. 3

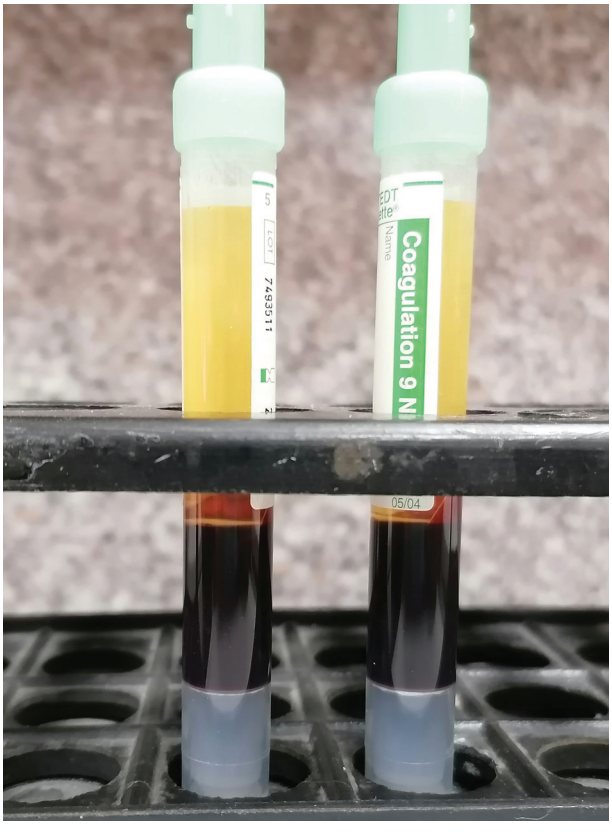

Fig. 4a

In all four protocols, after the first spin, there were three differentiated layers that were clearly visible. The lower layer included red blood cells, the mid-

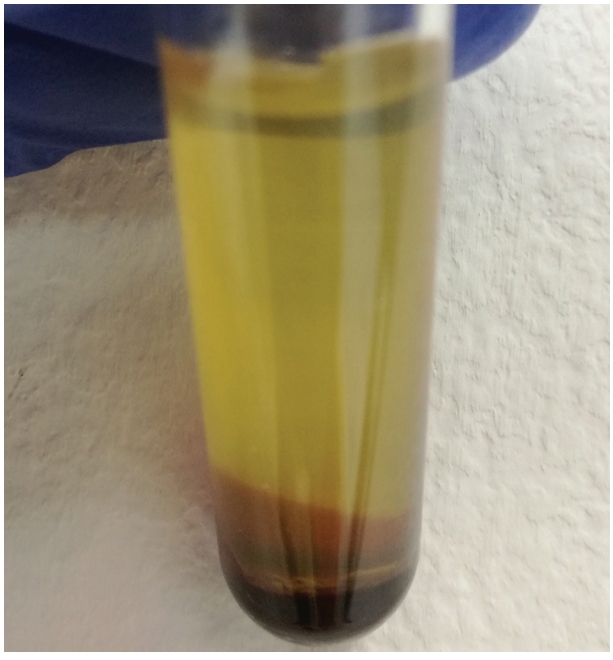

Fig. 4b

dle one consisted of concentrated platelets and leukocytes, and the upper layer was platelet-poor plasma (PPP). The superficial PPP layer was aspirated in order to gain a final product of 3 mL that was analyzed. An automatic Swelab Alfa Basic hemoanalyzer (Boule Medical AB, Sweden) was used for measurement of the platelet number, red blood cells, neutrophils, lymph cells, and monocytes in the control group and in the PRP products obtained by different protocols. The concentration factor (%) relative to the baseline blood cells values was also calculated.

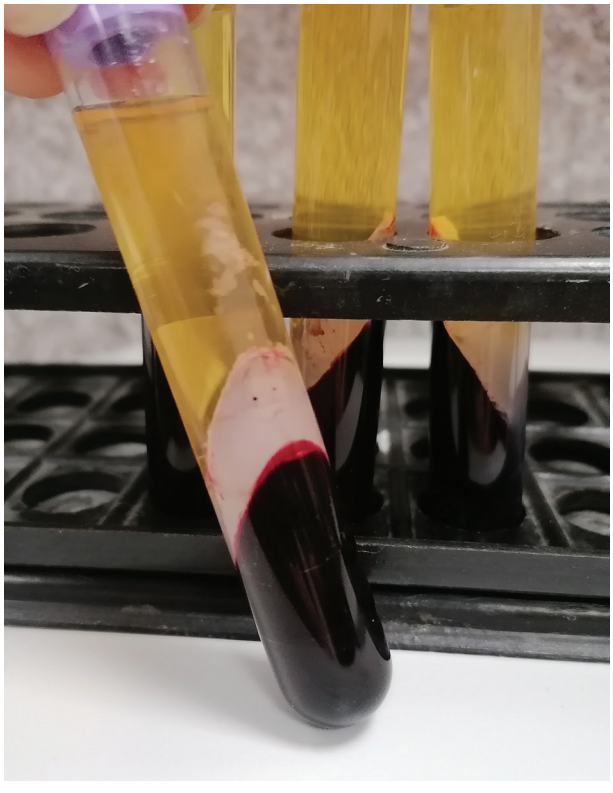

Fig. 5a

Statistical Analyses

The data were processed with statistical software IBM SPSS v. 20.0 for Windows, using descriptive indicators for quantitative variables and presented in graphics and tables. Statistically significant difference was assessed at a critical level of significance $p<0.05$.

RESULTS

The variable conditions proposed in these experimental protocols were: centrifugation of blood cells with and without a separating gel; single and double spin; change in relative centrifugal force (RCF) and time. The quantity of different blood cells (mean; SD) from different PRP products and the reference values from control group are presented in Table 2.

Results from protocol 1 showed a decrease by 51.9% in the mean value of erythrocytes compared to the values of the control group, increase in platelet concentration by 91.55% and an increase in leu-

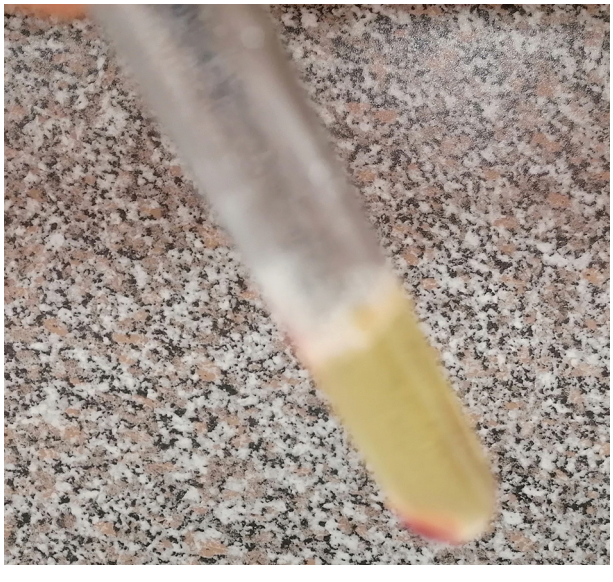

Fig. 5b

Tabl. 1. Protocols for obtaining PRP with different methods

Protocols centrifugal force (RCF)		First spin		Second spin	
		centrifugal force (RCF)	time (min.)	centrifugal force (RCF)	time (min.)
1	without gel	1500	10	N/A	
2	with gel	1500	10	N/A	
3	without gel	1150	10	350	5
4	with gel	1150	10	350	5

kocyte number by 107.21%. The second protocol included a separating gel and demonstrated 91.42% decrease in the mean value of erythrocytes compared to the values of the control group, an increase in platelet count by 337.06% and increase in leukocytes by 82.37%. The third protocol was based on a double centrifugation technique and showed 96.18% decrease in mean erythrocyte number compared to controls, 352.08% increase in platelet concentration and 40.54% decrease in white blood cells. The fourth protocol demonstrated 99.84% decrease in the mean erythrocyte count compared to the control group, 389.84% increase in platelet counts and 74.36% decrease in the white blood cell number.

DISCUSSION

Platelet-rich plasma products have been reported to play an important role in regenerative processes. Platelets aggregate at the damaged area and release a variety of growth factors and cytokines that are associated with wound healing, thereby accelerating the process of reparation of soft tissues and bone (14, 15). Examples of growth factors that are secreted from α-particles by the activation of platelets include platelet-derived growth factor (PDGF), vas-

Tabl. 2. Blood cell characteristics in various protocols and control group

	Control group		Protocol 1		Protocol 2		Protocol 3		Protocol 4	
Blood cells analysis	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
RBC $\times 10^{12}$/L	5,01	±0,642	2,41	±0,547	0,43	±0,195	0,193	±0,041	0,008	±0,006
PLT $\times 10^{9}$/L	287,03	±63,214	549,80	±118,856	1254,50	±274,141	1297,60	±283,272	1406,00	±313,091
LEU $\times 10^{9}$/L	6,24	±1,283	12,93	±1,978	11,38	±2,199	3,71	±0,992	1,60	±0,313
NEU $\times 10^{9}$/L	3,60	±0,801	6,43	±1,389	5,82	±1,361	0,01	±0,009	0,005	±0,005
Mo $\times 10^{9}$/L	0,78	±0,185	2,98	±0,855	2,97	±0,775	0,48	±0,163	0,007	±0,007
Ly $\times 10^{9}$/L	2,01	±0,458	8,88	±2,055	8,80	±2,022	2,23	±0,451	1,05	±0,138

cular-endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), insulin-like growth factor (IGF) (16,17,18,19).

Factors that can affect the quality of the PRP product regarding different level of blood cells are: number of centrifugations, temperature, RCF, and time (20). In the present study, the analysis and comparison of the level of platelets showed that the platelet level from protocol I was 1.91 times higher than baseline values in control group; in protocol II—platelet concentration was 4.37 times higher; in protocol III (two spins)—4.52 times higher values; protocol IV (two spins/gel)—4.89 higher than the reference values. Regarding the leukocyte level, protocol I and II showed higher values in comparison to the control group—2.01 times and 1.75 times, respectively. Protocols that had two spins showed significant decrease in leukocyte number compared to the initial values: for method III—1.73 lower, method IV—4 times lower than the reference values.

For clinical application based on these data, surgeons should consider the individual necessities of the patient and the technical requirements for PRP preparation. It can be a time-consuming and expensive procedure. Protocols II, III, and IV showed significant enrichment of platelets in the PRP products. If a leukocyte-poor product is needed, then double centrifugation methods are recommended (protocol III or IV), if leukocyte-rich product is desired for clinical application, protocols with one centrifugation should be used (protocol II). We should emphasize that regarding tissue healing, there is no consensus about positive or negative effects of leukocytes in PRP. Some studies proposed that leukocytes stimulate the healing process in damaged tissue and simultaneously suppress the growth of some bacteria (21,22). On the other hand, a lot of studies showed positive correlation between the total number of leukocytes in PRP and increased levels of pro-inflammatory cytokines, indicating that leukocytes in PRP may inhibit the healing process (23,24).

Future perspectives should focus on analyzing the quantity of different growth factors in PRP products and its correlation to platelet and leukocyte levels (25,26). In contrast to commercial devices, an advantage of a standard clinical centrifuge is the ability to change force and time parameters and thus the final product can be affected depending on the desired clinical application

CONCLUSION

Platelet-rich plasma is an autologous product that facilitates the healing of soft tissues and bone after surgical treatment and procedures. Platelet-rich plasma is a general term, these products have specific variations in their structure and as a result—different use for clinical application is recommended. The use of separating gels facilitates blood cell aspiration and makes the procedure faster in ambulatory practice. The optimal concentration of platelets, leukocytes, and other plasma components remains to be clarified and the researcher should be aware that the PRP effect is not only based on thrombocyte concentration.

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