

Immature Platelet Fraction as a Thrombopoietic Index in Donor's Plateletpheresis

This article was published in the following Scient Open Access Journal:

Journal of Blood Disorders, Symptoms & Treatments

Received June 25, 2018; Accepted July 05, 2018; Published July 13, 2018

Valery M. Pogorelov*

Department of industrial and clinical transfusion
Russia, AI Evdokimov Moscow State Medical and
Dental University, Russia

Abstract

The purpose of this study was to examine the activity of thrombocytopoiesis in repeat donating platelets for the preparation of platelet concentrates (PC). The work was based on a retrospective analysis of databases. Donors undergoing plateletpheresis by differential centrifugation for the first time (Group 2, n= 24) and repeat donors donating both by differential centrifugation (Group 3, n= 44) and by apheresis (Group 4, n= 121) every other week for 2–5 years were compared to healthy volunteers (Group 1, n= 87). The platelet count (PLTs), percentage of immature platelets fraction (IPF%) and their absolute values (A-IPF) was performed with the automated analyzer Sysmex XE-2100 (Sysmex, Cobe, Japan). The parameter IPF was chosen by us as a thrombopoietic index. There was not a significant difference in the PLTs for donors before the procedures and the control. Using the IPF obtained in Group 1 we found a significantly higher level in the donor's blood before the collection of the second buffy coat (BC) bag both in Group 2 and in Group 3. Moreover, in Groups 3 and 4 the IPFs shown have elevated levels before the start of procedures. In donors of Group 4, the A-IPF after apheresis decreased. Both in Group 2 and in Group 3, thrombocytopenia (TCP) occurred before the collection of the second BC bag and increase at the end of the procedure. In Group 4 a TCP was more pronounced. By day 14, donor's PLTs recovered to baseline. Thus, thrombocytopoiesis reacts quickly to depletion of platelets. However, apheresis platelet donation might lead to "a tension of thrombopoiesis", as evidenced by the low levels of A-IPF. Further investigation of IPF may be of critical clinical importance for the practice of platelet transfusion.

Keywords: Thrombocytopenia, Thrombocytopoiesis, Reticulated platelets, Thrombogenicity, Platelet concentrates

Introduction

There are findings that platelets may be able to adjust their phenotypic composition in response to environmental factors [1]. The key question is: how does this process occur in vivo?

Ingram & Coopersmith (1969) first described a previously unreported type of platelet released into the peripheral blood of dogs following acute blood loss [2]. These cells display coarse, punctate condensations (reticulum) when stained supravivally with new methylene blue and are probably analogous to reticulated erythrocytes. Both types of reticulated cells occur in increased numbers following acute blood loss.

According Harrison, et al. (1997) young or reticulated platelets (RPs) contain detectable mRNA and are thus analogous to red cell reticulocytes [3]. They can be easily detected either with supravital dye staining on blood films, or more commonly with fluorescent dyes and flow cytometry. Using the latter technique many different groups have demonstrated that the measurement of RPs has much clinical potential. Platelet reticulocyte analysis should thus become part of accepted haematological practice and provide useful clinical information for the investigation and monitoring of platelet production in various thrombocytopenic conditions. In particular, measurement of RPs will provide an excellent and simple means for monitoring the response of chemotherapy and transplant patients to growth factors (e.g. thrombopoietin) resulting in a decrease in the demand for platelets transfusion.

Kickler et al. [4] concluded that in consumptive thrombocytopenic disorders, thrombopoietin (TPO) production is increased, leading to an increased platelet production by megakaryocytes. These authors found that a high fluorescent platelet fraction percentage had a predictive value in the thrombocytopenia assessment.

*Corresponding Author: Valery M. Pogorelov,
Department of industrial and clinical transfusion
Russia, AI Evdokimov Moscow State Medical and
Dental University, Russia,
Email: pogorelov.valery@gmail.com

A new automated method to reliably quantify RPs, expressed as the immature platelet fraction (IPF), has been developed utilizing the XE- 2100 blood cell counter with upgraded software (Sysmex, Kobe, Japan), IPF as a percentage of total platelet count has been examined as a diagnostic tool to differentiate aplastic and consumptive thrombocytopenic states [5].

The biological essence of the formation of the progeny of platelets can be used clinically, for example, in resuscitation measures and requires further study [1]. It is interesting that Stohlawetz et al. (1998) first concluded that the repeat platelet donation might lead to "a relative exhaustion of thrombopoiesis" and the RPs count could be used to monitor the thrombopoietic capacity of long-term platelet donors [6].

Gyongyossy-Issa, et al. has shown that the median levels of RPs were significantly lower in the repeat donors than in the new ones [7]. The RPs are regarded by Gyongyossy-Issa, et al. to be present in circulation of plateletpheresis donors until Day 7. The authors also concluded that repeat PLTs donation might lead to "a relative exhaustion of thrombopoiesis", as evidenced by the low levels of RPs exhibited by repeat donors. According Lazarus et al. regular plateletpheresis donors develop sustained decreases in platelet count, however, clinically significant thrombocytopenia is unusual when rigorous ongoing review and prudent deferral policies are established and followed [8]. The frequency of donation correlated directly with decrease in PLTs count [9]. In our previous study, we also demonstrated a significant decrease in the number of platelets, but repeated donors showed a higher level of significance than the first donor's plateletpheresis [10]. Using IPF% samples from 21 healthy men we found a significantly higher level of IPF% in the samples with relative thrombocytopenia in terms of PLTs $\times 10^3/\mu\text{L}$ after plateletpheresis.

Process-induced platelet activation occurs with all production methods, including apheresis. Recent studies have highlighted the range and consistence of interindividual variation in the PLT response [11], but little is known about the effect of plateletpheresis on donors' PLTs. Today, PCs are generally produced from blood by differential centrifugation (pooled buffy coat-derived platelet concentrates - BCs) or whole by apheresis (apheresis derived platelet concentrates - APCs). Some data suggests that APCs are characterized by a superior hemostatic capacity over BCs in vitro [12]. It has already been shown, for example, that newly formed RPs are inherently more reactive and more prone to recruiting into clots, they stimulate the formation of thrombi even in standard therapy with a double antiplatelet [13].

Today is considered that evaluating of the percentage of RPs may be helpful in assessing the efficacy of thrombopoiesis and circulating immature platelet fraction reflects real-time thrombopoiesis and correlates with platelet recovery from thrombocytopenic presentations [14,15].

The purpose of this study was to examine the activity of donor' thrombocytopenia in response to plateletpheresis in donating platelets for the preparation of PC. The parameter IPF was chosen by us as a thrombopoietic index. The work was based on a retrospective analysis of databases.

Materials and Methods

Peripheral blood PLTs were examined on 276 people, 87

volunteers and 189 donors, including the first-time donors with one donation whole blood in the history and the repeat donors who underwent of preparing PCs every other week for 2-5 years. All subjects were male, aged 18-55 years (median 25 years), with no clinically significant physical findings or abnormalities in laboratory result sat screening.

The control group consisted of volunteers without donation (Group 1, n= 87). Two methods of preparing PCs from donor's blood was in use. The first-time donors undergoing intermittent-flow centrifugation plateletpheresis for BCs on the Sorvall RS 3C, USA pheresis platform (Group 2, n= 24). In the repeated donors were prepared both BCs (Group 3, n= 44), or APCs by platelet apheresis procedure on MCS+ Hemonetics, USA (Group 4, n= 121). In the second and third groups, the production of PCs lasted 80 minutes on average, 2052 ml of blood was treated, and the volume of the concentrate ranged from 150 to 250 ml (4 doses). In the fourth group, the duration of apheresis was 90-100 minutes, 3100-3900 ml of blood was treated, and the concentrate volume was 400 ml (8 doses).

Peripheral blood samples (7 mL) was collected in tube (BD Vacutainer, UK) with dipotassium ethylene-diaminetetraacetate (EDTA KE) Flow cytometry determination immature PLTs from the samples was performed with the automated analyzer Sysmex XE-2100 (Sysmex, Kobe, Japan) in the RET channel. The XE-2100 incorporates two state-of-the-art platelet methodologies, impedance (PLT-I) and fluorescent optical (PLT-O), to assure accurate and reliable platelet counts; a switching algorithm is incorporated within the analyzer software to report the most 'correct' platelet count, either optical or impedance [16]. The percentage of immature platelets (IPF%), their absolute number (A-IPF $\times 10^3/\mu\text{L}$), as well as the platelet count (PLTs $\times 10^3/\mu\text{L}$) in donors during the BCs were calculated before filling on each from three platelet pooling stages (BCs bag), and in the case of APCs at the beginning and at the end of apheresis. Finally, all the values of each point of the study were compared with the parameters of volunteers. Blood was examined only once at the time of PCs preparation for each observation. Signed informed consent was obtained from each subject and the study was conducted in compliance with the Declaration of Helsinki.

Statistical Analyses

All data were given as median, 95% confidence interval (95% CI) and coefficients of variation (CV%). Significance of differences between donor groups and healthy volunteers were evaluated using the two-sample t-test with different variances. Data was calculated using SPSS Version 10.0.5 for Windows (SPSS, Chicago, IL). The level of $p < 0.05$ was considered significant for all tests.

Results

The percentage IPF, A-IPF, and platelet count for control (Group 1), the first-time donors (Group 2) and repeat donors undergoing both the three platelet pooling stages (Group 3) and apheresis (Group 4) who donate PLTs every two weeks for a period of 2 to 5 years separately in the dynamics of procedures are included in Tables 1-3 and Figures 1-3.

The control's percentage IPF for this study was generally congruent with those at least the one previously reported study [17]. There was not a significant different in the PLTs count

Group			Median, 95% CI and CV, %		
			IPF		PLT x 10 ³ /μl
			IPF%	A-IPF x 10 ³ /μL	
The intermittent-flow centrifugation plateletpheresis on the Sorvall RS 3C pheresis platform (USA)	The first-time donors	First BC bag	1.3 1.2 – 2.5 (83.3)	3.7 3.2 – 5.04 (56.07)	228.5 219 – 259 (19.7)
		Second BC bag	2.0 ^a 1.7 – 3.0 (63.4)	4.8 ^d 3.72 – 6.16 (61.74)	201 ^h 192 – 234 (23.3)
		Third BC bag	2.65 ^d 2.4 – 4.3 (64.7)	5.1 ^d 4.17 – 6.99 (63.08)	183.5 ^b 167 – 211 (27.7)
Control			1.4 1.41 – 1.79 (57.14)	3.3 3.23 – 4.09 (56.28)	227 226 – 242 (16.17)

^ap = 0.0001; ^bp = 0.0002; ^cp = 0.0005; ^dp < 0.001; ^ep = 0.009; ^fp = 0.01; ^gp < 0.02; ^hp = 0.03

Abbreviation: IPF%, immature platelet fraction; n, number of observations; A-IPF x 10³/μL, immature platelet fraction expressed as the absolute-IPF (IPF% x the platelet count); PLT, platelets; 95% CI, 95% confidence interval; CV%, coefficient's variation; BC bag, the buffy coat; p-Values are group comparison values with control calculated using the two-sample t-test with different variances.

Table 1: The IPF values and PLT x 10³/μL results in healthy controls (Group 1, n = 87) and the first-time donors with one donations in history (Group 2, n = 24).

Group			Median 95% CI and CV, %		
			IPF		PLT x 10 ³ /μl
			IPF%	A-IPF x 10 ³ /μL	
The plateletpheresis on the Sorvall RS 3C pheresis platform (USA)	The repeat donors	First BC bag	1.9 ^f 1.83 – 2.49 (52.78)	4.6 ^f 4.06 – 5.51 (51.1)	232.5 224.8 – 248.8 (17.1)
		Second BC bag	2.1 ^c 2.17 – 3.0 (53.9%)	5.0 ^b 4.3 – 5.6 (44.22)	209 ^d 196.2 – 220.34 (19.5)
		Third BC bag	2.65 ^a 2.38 – 3.22 (51.07)	5.35 ^a 4.58 – 5.84 (40.31)	190.5 ^e 175.6 – 202 (23.5)
Control			1.4 1.41 – 1.79 (57.14)	3.3 4.09 – 3.23 (56.28)	227 226 – 242 (16.17)

^ap = 0.0001; ^bp = 0.0002; ^cp = 0.0005; ^dp < 0.001; ^ep = 0.009; ^fp = 0.01; ^gp < 0.02; ^hp = 0.03

Abbreviation: IPF%, immature platelet fraction; n, number of observations; A-IPF x 10³/μL, immature platelet fraction expressed as the absolute-IPF (IPF% x the platelet count); PLT, platelets; 95% CI, 95% confidence interval; CV %, coefficients variation; BC bag, the buffy coat; p-Values are group comparison values with control calculated using the two-sample t-test with different variances.

Table 2: The IPF values and PLT x 10³/μL results in healthy controls (Group 1, n = 87) and the repeat donors undergoing intermittent-flow centrifugation plateletpheresis for buffy coat-derived platelet concentrates on the Sorvall RS 3C pheresis platform for 2–5 years (Group 3, n = 44).

Group			Median 95% CI and CV, %		
			IPF		PLT x 10 ³ /μl
			IPF%	A-IPF x 10 ³ /μL	
The apheresis for 2–3 years on the Hemonetics platform (USA)	The repeat donors	The pre- plateletpheresis period	1.9 ^f 2.06 – 2.54 (59.56)	4.6 ^f 4.77 – 5.65 (47.79)	234.5 234.2 – 246.1 (18.3)
		The post- plateletpheresis period	2.2 ^d 2.32 – 2.96 (68.94)	3.7 3.72 – 4.46 (51.34)	159 ^d 160.35 – 171.21 (18.38)
Control			1.4 1.41 – 1.79 (57.14)	3.3 4.09 – 3.23 (56.28)	227 226 – 242 (16.17)

^ap = 0.0001; ^bp = 0.0002; ^cp = 0.0005; ^dp < 0.001; ^ep = 0.009; ^fp = 0.01; ^gp < 0.02; ^hp = 0.03

Abbreviation: IPF%, immature platelet fraction; n, number of observations; A-IPF x 10³/μL, immature platelet fraction expressed as the absolute-IPF (IPF% x the platelet count); PLT, platelets; 95% CI, 95% confidence interval; CV%, coefficients variation; p-Values are group comparison values with control calculated using the two-sample t-test with different variances.

Table 3: The IPF and PLT x 10³/μL values results in healthy controls (Group 1, n = 87) and the repeat donors who underwent platelet apheresis for 2–3 years for apheresis-derived platelet concentrates on the Hemonetics platform (Group 4, n = 121).

(p>0.05) both first-time and repeat donors before the procedures in comparison with the control values.

When PC was obtained from first-time donors (Group 2), relative TCP occurred before the collection of the second BC bag and the PLTs statistically significant decreased with respect to

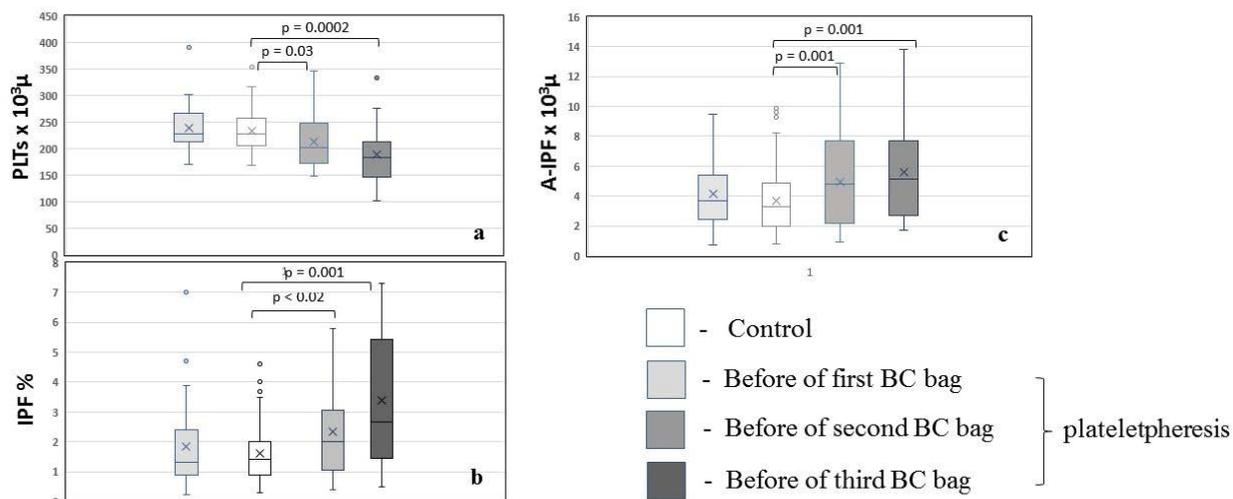


Figure 1: Results of count platelets (PLTs x 10³/μL), a proportional value (IPF%) and the absolute-IPF (A-IPF x 10³/μL) in control (n= 87), and first-time donors undergo differential centrifugation on the Sorvall RS 3C pheresis platform (Group 2, n= 24).
 Abbreviations: PLT – platelets, IPF -Immature Platelet Fraction, P-Values are group comparison values calculated using the two-sample t-test with different variances; BC bag, the huffy coat.

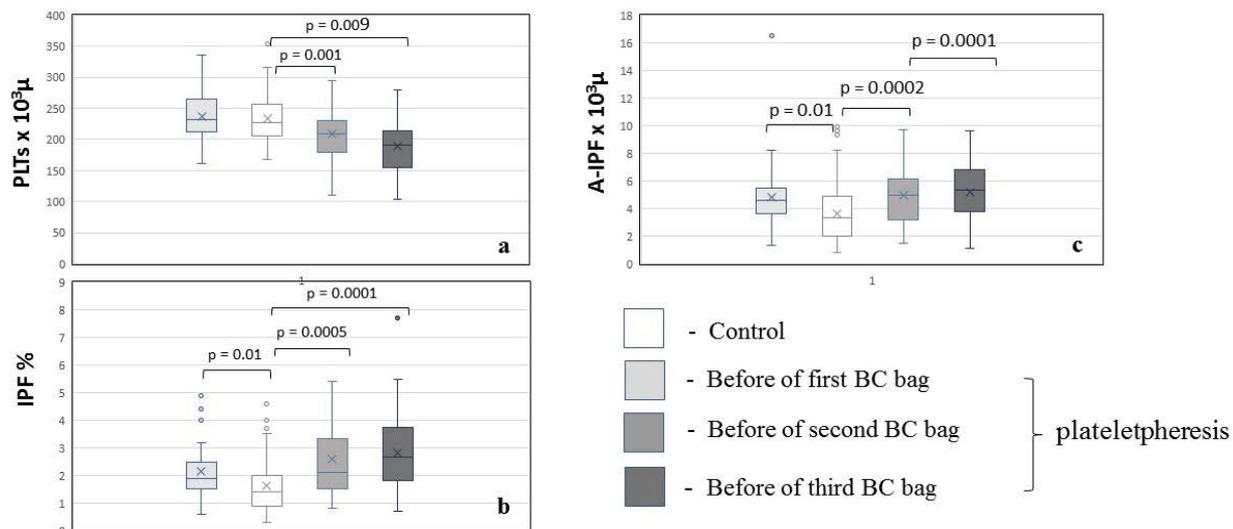


Figure 2: Results of count platelets (PLTs x10³/ μL), a proportional value (IPF %) and the absolute-IPF (A-IPF x 10³/μL) in control (Group 1, n= 87), and repeat donors undergoing differential centrifugation on the Sorvall RS 3C pheresis platform for 2-5 years (Group 3, n= 44).
 Abbreviations: PLT – platelets, IPF -Immature Platelet Fraction, P-Values are group comparison values calculated using the two-sample t-test with different variances; BC bag, the huffy coat.

the control (Group 1) until the end of the procedure. In the same time interval, the rise of the IPF% was observed, and the A-IPF x 10³/μL value here increased reliably both before the second and before the third BC (Table 1).

When a buffy coat-derived PCs was obtained from repeat donors (Group 3) number of circulating PLTs also decreased before the second BC bag was collected and statistically significant fell at the end of the procedure (Table 2). Unlike the Group 2 percentage of RPs here were high even before the procedure began and significantly increased at its end and. The A-IPF x 10³/

μL value at the same time was increased also and significantly increased at its end and for each BC bags respectively.

In the repeat donors undergoing apheresis (Group 4), as in Group 3, the values of IPF (both types) before the procedure was statistically significantly higher than control ones (Table 3). Whereas the IPF% index showed a small but statistically significant increase in Group 4 after apheresis, a A-IPF% x10³ / μL showed a tendency to decrease. The number of circulating PLTs decreased after apheresis.

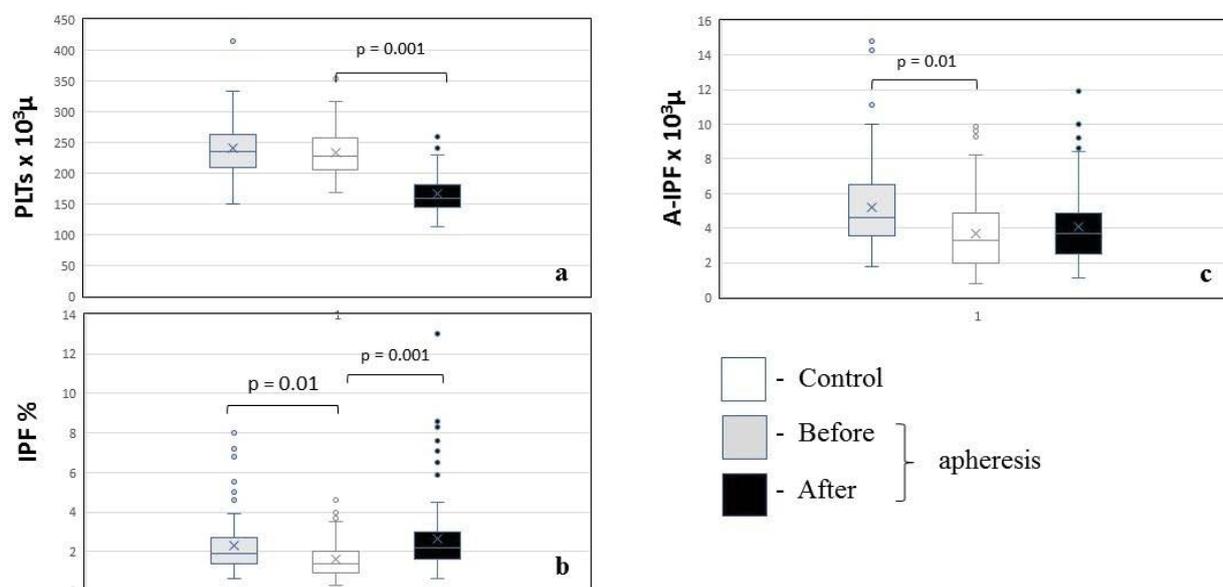


Figure 3: Results of count platelets (PLTs x 10³/μL), a proportional value (IPF %) and the absolute-IPF (A-IPF x 10³/μL) in control (Group 1, n = 87), and repeat donors, who underwent platelet apheresis for 2-5 years for APCs on the Hemonetics platform (Group 4, n = 121).
Abbreviations: PLT - Platelets, IPF - Immature Platelet Fraction, apheresis derived platelet concentrates. APCs - apheresis derived platelet concentrates. P-Values are group comparison values calculated using the two-sample t-test with different variances.

Discussion

The purpose of this study was to examine the activity of thrombocytopoiesis in response to plateletpheresis in donating platelets for the preparation of PC. The work was based on a retrospective analysis of databases.

In the present paper we compare the values of IPF (both types) and PLT count in the control (Group 1, n = 87), in the first-time donors undergoing differential centrifugation (Group 2, n = 24) and in the repeat donors which undergo both differential centrifugation (Group 3, n = 44) and platelet apheresis (Group 4, n = 121) every two weeks for a period of 2 to 5 years when obtaining PCs from blood. The parameter IPF was chosen by us as a thrombopoietic index.

The facts obtained in this paper can be interpreted as meaning that thrombocytopoiesis reacts quickly to plateletpheresis. A few hours after the onset of procedure, bone marrow increases the production of functionally and metabolically active RPs that provide effective hemostasis.

First, there was not a significant difference (p>0.05) in the platelet count both for the first-time and the repeat donors before the procedures in comparison with the control values (Figures 1a, 2a, 3a).

Second, using the IPF% and the A-IPF x 10³/μL values obtained in Group 1, we found a significantly higher level of RPs in the donor's whole blood samples before the collection of the second BC bag both in the first-time donors of Group 2 (Figure 1b, p<0.02 and c, p=0.001) and in the repeat donors of Group 3 (Figure 2b, p=0.0005 and c, p=0.0002). However, IPF shown have elevated levels of RPs in repeat donors in compared with control already before the start of procedures (p=0.01), i.e. after 14 days of rest (Figure 2b, 2c, 3b, 3c). These changes in the IPF

during plateletpheresis in our donors, in analogy with Albanyan et al., is most likely to be a limitation of the method used rather than a reflection of a biological process [18]. However, the one-pointedness of changes in IPF% in our donors and changes of TPO from the blood of donors according to the data of other authors makes it possible to the thrombopoietic index of IPF with confidence [19].

In contrast to Group 3, in the repeated donors of Group 4, the A-IPF at the end of apheresis decreased, although it remained non-significantly above control (Figure 3c, p>0.05). A high IPF percentage is indicative of consumptive disorders in contrast to a low IPF percentage seen in aplastic states; absolute number of immature platelets (A-IPF) specifically reflects the number of RPs in circulation, i.e., platelet production [20]. In addition, Bat, et al. clearly demonstrated that A-IPF reflects platelet production by the bone marrow in various clinical settings [21].

Third, when the PCs was obtained both from the first-time donors (Group 2) and from the repeat donors of Group 3, relative TCP occurred before the collection of the second BC bag (Figure 1a, p=0.03, Figure 2a, p=0.001) and statistically significant increase at the end of the procedure (Figure 1a, p=0.0002, Figure 2a, p=0.009). There was also relative TCP, but more pronounced, in the repeat donors undergoing apheresis (Figure 3a, p=0.001). In our previous study, we demonstrate a significant decrease in the platelet count, and the repeat donors showed also higher level of significance than the first-time donors [10]. By day 14, donor's platelet count recovered to baseline in majority of the donors. At the same time, the combination of A-IPF reduction and relative TCP after apheresis in our repeated donors it is may to speculate an indication of "tension of thrombocytopoiesis". In fact, this term is also conditional as "a relative exhaustion of thrombopoiesis" described by Stohlawetz, et al. [6] or Gyongtyossy-Issa et al. [7].

Aim of study Thokala et al. was to analyze the recovery of platelet count to baseline among apheresis platelet donors [22]. According to these authors, by day 7, donor's platelet count recovered to baseline in majority of the donors. Allowing enough recovery periods for donor PLTs count, the minimum interval between two apheresis donations can be 7 days till more prospective studies conclude on the frequency and minimum interval between plateletpheresis donations. That author did not investigate of the immature platelets fraction.

It is known that these young or RPs are functionally and metabolically more active than the resting ones. This capacity of platelets may have significant consequences for normal and pathologic thrombopoiesis in humans in addition to having clinical implications.

The mobilization of thrombocytopoiesis reserves may release an increased number of immature platelets in the peripheral blood, left adaptation shift [23]. Circulating PLTs consist of subpopulations with different age, maturation state and size. Understanding of the features that characterize platelet subpopulations is very relevant for the methods of PCs production, which may enrich or deplete platelet subpopulations [24]. Under normal conditions the lipid membranes of PLTs are studded with glycoproteins with multiple functionalities; the platelet membrane glycoprotein is the most abundant among these, and includes the GP Ib-IX-V, GP Ia/IIa, GP VI, GP IIb/IIIa, and P-selectin [25-26]. They play a role in platelet activation, adhesion, and aggregation, and participate in hemostasis and thrombosis. Early studies suggested that young platelets have more receptors and shown also that one's are stainable by thiazole orange (TO), suggesting that they may be are functionally more active than mature thrombocytes by 3 independent criteria, namely P-selectin levels, annexin V binding, and release of calcium after adenosine diphosphate (ADP) activation. According to Fager et al. the ability of activated PLTs to support thrombin generation is defined by a subpopulation of platelets expressing a nondissociable pool of platelet-derived factors Va (FVa) and increased adhesive receptor density [25]. This subpopulation is hypothesized to play a significant role in regulating both normal hemostasis and pathological thrombus formation because the adherent properties of PLTs and their ability to mount and sustain a procoagulant response are crucial steps in both these processes: increases in receptor density were determined as the ratio of mean fluorescence intensity in the young (TO-positive) platelets ($p < 0.001$) compared with mature platelets.

PLTs contribute to hemostasis by forming the platelet plug and then contributing to coagulation by providing a catalytic surface where thrombin generation occurs efficiently. This catalytic activity, known as the platelet procoagulant response, is being recognized as a nuanced response. Mazepa et al. examined platelets' response to strong stimuli, which results in the formation of a platelet subpopulation (superactivated platelets) with several unique properties, including enhanced procoagulant activity [26]. These PLTs contribute uniquely to thrombus architecture and seem to have thrombus regulatory activity. Superactivated platelets' role in diseases of thrombosis and hemostasis, as either potentiating or mitigating factors, is not currently known, but may be an important pharmacological target. These agents could potentially increase superactivated platelet potential by 2 mechanisms: (1) directly inducing of

phosphatidylserine exposure and enhanced procoagulant activity; and (2) selectively affecting older platelets (reticulated platelets are relatively resistant to the drugs' effect), leading to a larger proportion of circulating young platelets.

Baaten et al. have shown that the gradual increase in thrombogenicity after cessation of prasugrel treatment, prasugrel is a more potent the ADP purine receptor P2Y₁₂ antagonist, in patients with acute coronary syndromes is due to the increased activity of juvenile PLTs [27]. The compromised reactivity of juvenile PLTs during the initial days of offset can contribute to a risk of bleeding upon surgery: when urgent surgery is required, or when bleeding must be controlled, PLT transfusions have shown to be effective in restoring hemostasis at 6 h after a loading dose of prasugrel.

Thus, the value of our study is also to obtain new data that is important for understanding the mechanisms of the response of thrombocytopoiesis to the influence of factors of the internal and external environment in which the process is the selection of a sufficient number of functionally active platelets and preparing the organism for life in conditions of increased requirements for hemostasis. Besides, further investigation of IPF with blood loss may be of critical clinical importance for the practice of PCs transfusion.

Conclusions

The mobilization of thrombocytopoiesis reserves may release an increased number of reticulated (RPs) or immature platelets in the peripheral blood, left adaptation shift. Understanding of the features that characterize platelet subpopulations is very relevant for the methods of platelet concentrates (PCs) production, which may enrich or deplete platelet subpopulation. Effects of plateletpheresis on the regenerative capacity of thrombocytopoiesis have not been studied enough. Besides, the biological essence of the formation of the progeny of platelets can be used clinically, for example, in blood loss resuscitation measures and it requires further study. Therefore, the purpose of this study was to examine the activity of thrombocytopoiesis in response to apheresis in repeat donating platelets for the preparation of PC. The parameter IPF both the percentage of immature platelets (IPF%), and their absolute number ($A\text{-IPF} \times 10^3/\mu\text{L}$) was chosen by us as a thrombopoietic index. The facts obtained in this paper can be interpreted as meaning that thrombocytopoiesis reacts quickly to plateletpheresis. A few hours after the onset of procedure, bone marrow increases the production of functionally and metabolically active RPs that provide effective hemostasis. However, the combination of a decrease in the A-IPF and relative thrombocytopenia after apheresis in our repeated donors may possibly be indicative of "a tension of thrombocytopoiesis". The RPs count can be used to monitor the thrombopoietic capacity of long-term platelet donors. Further investigation of IPF may be of critical clinical importance for the practice of platelet transfusion.

Competing Interests

No external funding and no competing interests declared.

Acknowledgments

I am grateful to the staff of the National Hematology Research Center in Moscow (Russia), as well as my graduate student Victoria Ufimtseva for working with blood donors. I thank of the

staff of the Department of industrial and clinical transfusion AI Evdokimov Moscow State Medical and Dental University Prof. Becsoltam M. Urtaev and Roman A. Simanin, without whom the very idea of this article would not have appeared. I thank my family for their patience.

References

- Schwartz H, Koster S, Kahr WHA, et al. A nucleate platelets generate progeny. *Blood*. 2010;115(8):3801-3809.
- Ingram M, Coopersmith A. Reticulated platelets following acute blood loss. *British J Haematol*. 1969;17:225-229.
- Harrison P, Robinson MSC, Mackie I, Machin S. Reticulated platelets. *Platelets*. 1997;8(6):379-383.
- Kickler TS, Oguni S, Borowitz MJ. A clinical evaluation of high fluorescent platelet fraction percentage in thrombocytopenia. *Am J Clin Pathol*. 2006;125:282-287.
- Briggs C, Kunka S, Hart D, et al. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. *Br J Haematol*. 2004;126(1):93-99.
- Stohlawetz P, Stiegler G, Jilma B, et al. Measurement of the levels of reticulated platelets after plateletpheresis to monitor activity of thrombopoiesis. *Transfusion*. 1998;38(5):454-458.
- Gyongyossy-Issa MIC, Miranda J, Devine DV. Generation of reticulated platelets in response to whole blood donation or plateletpheresis. *Transfusion*. 2001;41(10):1234-1240.
- Lazarus EF, Browning J, Norman J, et al. Sustained decreases in platelet count associated with multiple, regular plateletpheresis donations. *Transfusion*. 2001;41(6):756-761.
- Schrezenmeier H, Seifried E. Buffy-coat-derived pooled platelet concentrates and apheresis platelet concentrates: Which product type should be preferred? *Vox Sang*. 2010;99(1):1-15.
- Pogorelov VM, Beskorovainova V Ju, Chanieva MI, et al. Periodic acid-Schiff (PAS) staining of immature platelets in donors. *Platelets*. 2012;23(1):51-59.
- Garner SF, Jones CI, Stephens J, et al. Apheresis donors and platelet function: Inherent platelet responsiveness influences platelet quality. *Transfusion*. 2008;48(4):673-680.
- Böck M, Rahrig S, Kunz D, et al. Platelet concentrates derived from buffy coat and apheresis: Biochemical and functional differences. *Transfus Med*. 2002;12(5):317-324.
- Armstrong PC, Hoefer T, Knowles RB et al. Newly formed reticulated platelets undermine pharmacokinetically short-lived antiplatelet therapies. *Arterioscler Thromb Vasc Biol*. 2017;37(5):949-956.
- Żmigrodzka M. Evaluation of reticulated platelets in dogs with breed-related thrombocytopenia. *Polish J Veterinary Scient*. 2014;17(1):137-142.
- Hong H, Xiao W, Maitta RW. Steady increment of immature platelet fraction is suppressed by irradiation in single-donor platelet components during storage. *PLoS One*. 2014;9(1):854-865.
- Rowan RM, Linssen J. A picture is worth a thousand words. *Sysmex J Inter*. 2005;15:27-32.
- Jung H, Jeon H-K, Kim H-J, Kim S-H. Immature platelet fraction: establishment of a reference interval and diagnostic measure for thrombocytopenia. *Korean J Lab Med*. 2010;30(5):451-459.
- Albanyan A, Murphy MF, Wilcock M, Harrison P. Changes in the immature platelet fraction within ageing platelet concentrates. *J Thromb Haemost*. 2008;6(12):2213-2215.
- Dettke M, Hlousek M, Kurz M, et al. Increase in endogenous thrombopoietin in healthy donors after automated plateletpheresis. *Transfusion*. 1998;38(5):449-53.
- Barsam SJ, Psaila B, Forestier M, et al. Platelet production and platelet destruction: assessing mechanisms of treatment effect in immune thrombocytopenia. *Blood*. 2011;117(21):5723-5732.
- Bat T, Leitman SF, Calvo KR, et al. Measurement of the absolute immature platelet number reflects marrow production and is not impacted by platelet transfusion. *Transfusion*. 2013;53(6):1201-1204.
- Thokala RP, Radhakrishnan K, Anandan A, Panicker V. Recovery of platelet count among apheresis platelet donors. *J Clin Diagnos Res*. 2016;10(12):1-4.
- Pogorelov VM. Platelet hemostasis or the boundary between health and disease. *Archives Blood Transfus. & Discord*. 2018;1:1-2.
- Handtke S, Steil L, Greinacher A, Thiele T. Toward the relevance of platelet subpopulations for transfusion medicine. *Frontiers in Medicine*. 2018;5:1-8.
- Fager AM, Wood JP, Bouchard BA, et al. Properties of procoagulant platelets defining and characterizing the subpopulation binding a functional prothrombinase. *Arterioscler Thromb Vasc Biol*. 2010;30(12):2400-2407.
- Mazepa M, Hoffman M, Monroe D. Superactivated platelets thrombus regulators, thrombin generators, and potential clinical targets. *Arterioscler Thromb Vasc Biol*. 2013;33(8):1747-1752.
- Baaten CCFMJ, Veenstra LF, Wetzels R, et al. Gradual increase in thrombogenicity of juvenile platelets formed upon offset of prasugrel medication. *Haematologica*. 2015;100(9):1131-1138.