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THE ROLE OF PLATELET-DERIVED MICROPARTICLES IN THE FORMATION OF FUNCTIONAL AND PROCOAGULANT ACTIVITY OF PLATELET CONCENTRATES

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Introduction. Platelet activation and vesiculation processes negatively affect the functional state of platelets and can accelerate cell apoptosis in platelet concentrates (PCs). Platelet-derived microparticles (PMPs) are biologically active vesicles formed as a result of platelet activation or apoptosis. PMPs can be considered as a marker for predicting the functional properties of PCs.

Objective. Assessment of the role of PMPs to the formation of functional and procoagulant activity of PCs.

Materials and methods. The study included 20 regular platelet donors. The PMP level in peripheral blood and PCs was determined by flow cytometry. Aggregation activity in PCs was assessed using adenosine diphosphate (ADP), collagen, and ristocetin as inducers. During thromboelastography, the following parameters were analyzed: clot formation time (R), maximum amplitude, and clot elasticity index. Statistical analysis was performed using the Mann–Whitney *U*-test, Wilcoxon test, and Pearson correlation.

Results. The PMP level in donor blood was not significantly associated with sex, age, or donor tenure. During storage, the PMP level in PCs increased 3.5-fold ($p < 0.0001$). Platelet aggregation activity decreased by day 5, especially in the presence of ADP and collagen. Thromboelastography parameters also decreased, which may indicate a reduction in hemostatic potential. An inverse correlation was found between PMP levels and the increase in clot formation time ($r = -0.490$; $p = 0.028$).

Conclusions. PMPs can serve as a marker of platelet activation and the PCs quality. No statistically significant correlations were found between individual donor characteristics and PMP levels, suggesting that technological factors of blood component collection and processing have a dominant influence on the microparticle formation process. The patterns of PMP level in peripheral blood are of substantial scientific interest in the context of developing new quality control parameters for platelet-containing components.

Keywords: platelet-derived microparticles; platelet concentrate; aggregation; transfusion; flow cytometry; platelet activation

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ВКЛАД ТРОМБОЦИТАРНЫХ МИКРОЧАСТИЦ В ФОРМИРОВАНИЕ ФУНКЦИОНАЛЬНОЙ И ПРОКОАГУЛЯНТНОЙ АКТИВНОСТИ КОНЦЕНТРАТОВ ТРОМБОЦИТОВ

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Введение. Активация тромбоцитов и процессы везикуляции негативно влияют на функциональное состояние тромбоцитов и способны ускорять процессы апоптоза клеток в концентрате тромбоцитов. Тромбоцитарные микрочастицы (ТМЧ) — биологически активные везикулы, образующиеся в результате активации или апоптоза тромбоцитов. ТМЧ могут быть рассмотрены в качестве маркера для прогнозирования функциональных свойств концентратов тромбоцитов.

Цель. Оценка вклада тромбоцитарных микрочастиц в формирование функциональной и прокоагулянтной активности концентратов тромбоцитов.

Материалы и методы. В исследование включены 20 кадровых доноров тромбоцитов. Уровень ТМЧ в периферической крови и концентратах тромбоцитов определяли методом проточной цитометрии. Агрегационную активность в концентратах тромбоцитов оценивали с использованием индукторов аденозиндифосфата, коллагена и ристомicina. При проведении тромбоэластографии анализировали: время образования сгустка (R), максимальную амплитуду, показатель упругости сгустка. Статистическую обработку результатов проводили с использованием критериев Манна – Уитни, Вилкоксона, корреляции Пирсона.

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Результаты. Содержание тромбоцитарных микрочастиц в крови доноров не имело значимой связи с полом, возрастом и донорским стажем. В процессе хранения концентрация ТМЧ в концентратах тромбоцитов увеличивалась в 3,5 раза ($p < 0,0001$). Агрегационная активность тромбоцитов уменьшалась к пятым суткам, особенно в присутствии аденозиндифосфата и коллагена. Показатели тромбоэластограммы также снижались, что может указывать на уменьшение гемостатического потенциала. Обнаружена обратная связь между уровнем ТМЧ и нарастанием времени образования сгустка ($r = -0,490$; $p = 0,028$).

Выводы. Тромбоцитарные микрочастицы могут служить маркером активации тромбоцитов и качества концентратов тромбоцитов. Статистически значимых корреляций между индивидуальными характеристиками доноров и уровнем ТМЧ не зафиксировано, что позволяет предположить доминирующее влияние технологических факторов заготовки и обработки компонентов крови на процесс формирования микрочастиц. Закономерности содержания ТМЧ в периферической крови представляют существенный научный интерес в контексте разработки новых параметров контроля качества тромбоцитсодержащих компонентов.

Ключевые слова: тромбоцитарные микрочастицы; концентрат тромбоцитов; агрегация; трансфузия; проточная цитометрия; активация тромбоцитов

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INTRODUCTION

Platelet-derived microparticles (PMPs) are vesicles ranging in size 100–1000 nm that are formed as a result of platelet activation or apoptosis [1]. PMPs contain various biologically active molecules, including membrane receptors, cytokines, and signaling proteins, and play an important role in hemostasis, inflammation, angiogenesis, and other physiological processes [2].

In recent years, increasing attention has been paid to PMPs as markers of platelet activation, particularly in the context of the collection, storage, and use of platelet concentrates (PCs) [3, 4]. Possessing pro-aggregant activity, they are able to enhance hemostatic properties through the exposure of phosphatidylserine, and are involved in modulating the immune response and inflammatory reactions [5]. When using PCs in transfusion therapy, the possibility of an increased risk of post-transfusion reactions in recipients with high PMP level should be taken into account [6–9]. Conversely, the transfusion of non-activated platelets with low PMP level in hematological and oncological patients may reduce the risk of developing refractoriness [10, 11].

Due to the constantly growing demand and the improvement of blood component collection technologies, special attention is being paid to new methods for assessing the quality and safety of blood components [12, 13]. The functional and procoagulant properties of PCs can be assessed by the PMP level in the PCs, and

their concentration in the donor's peripheral blood may reflect baseline platelet activation [14, 15]. The baseline platelet activation status of the donor directly affects the formation of the platelet microparticle pool in the final component. Determining this status prior to donation may be key to ensuring the quality and safety of transfusion therapy [16].

The study purpose was to assess the PMPs role to the formation of functional and procoagulant activity of PCs.

MATERIALS AND METHODS

The study included 20 regular platelet donors, of whom 12 were male and 8 were female. The inclusion criteria for donors in the study were: age 18 years or older; weight at least 50 kg; hemoglobin level at least 130 g/L for men, at least 120 g/L for women; platelet count at least $200 \times 10^3/\mu\text{L}$; informed consent of the donor to participate in the study; for women — platelet donation not earlier than 10 days after the end of menstruation; absence of contraindications for blood donation¹. Information on the age structure and donor tenure is presented in Table.

Before donation, venous blood from the donor was collected into S-Monovette 4.3 mL vacuum tubes containing 3.2% sodium citrate (Sarstedt AG & Co., Germany). Platelet collection from donors was performed using automatic apheresis with cell separators. After completion of the apheresis procedure, a

¹ Order of the Ministry of Health of the Russian Federation dated 28.10.2020 No. 1166n "On approval of the procedure for medical examination of donors and the list of medical contraindications (temporary and permanent) for blood and/or its components donation and the deferral period applicable to an individual in the presence of temporary medical indications from blood and/or its components donation".

Table. Age structure and donor tenure of donors (M_e (min–max))

	Males	Females
Age, years	35 (25–54)	42 (29–48)
Donor tenure, number of donations	14 (2–32)	17 (0–44)

The table was compiled by the authors based on their own data

Note. M_e — median; min — minimum value; max — maximum value.

sample of at least 5 mL was taken from the PC container for laboratory testing. The samples were stored at 22 ± 2 °C under constant agitation (Vuce-Vortex shaker, Labotek, Latvia). Laboratory parameters in the components were assessed on the day of collection and on the fifth day of storage. A comparison of peripheral blood parameters, including platelet count, at different storage time points was performed to assess the overall dynamics of changes.

The platelet count in the obtained donor blood samples and platelet count was determined using a SMART 5 hematology analyzer (URIT Medical Electronic Group Co., China). The PMP level was determined in a suspension obtained by stepwise centrifugation using a Microspin 12 high-speed mini-centrifuge (SIA Biosan, Latvia). In the first step, the biomaterial samples were centrifuged at 2500g for 30 min, then a 200 μ L sample was taken and further centrifuged at 14,000g for 30 min. The resulting pellet was resuspended in annexin binding buffer (Annexin V, Elabscience, China). After obtaining the sample, staining was performed with annexin V antibodies and anti-Hu CD41APC monoclonal antibodies (Exbio Diagnostics, Czech Republic) against platelet surface markers [17]. The samples were then incubated for 20 min at room temperature in the dark, after which they were diluted with phosphate-buffered saline (1:300, Rosmedbio LLC). Flow cytometry was used for detection with a CytoFLEX cytofluorimeter (Beckman Coulter, USA). Analysis was performed according to a protocol previously described by us [18], according to which 10^6 events were collected per sample, and the PMP level was determined as the absolute number of PMPs per 10^6 events.

Platelet aggregation activity was assessed using the Born method on an AT-2 4-channel platelet aggregation analyzer (MedTech, Russia) [19]. CRONO-PAR reagents were used as aggregation inducers: ADP at a final concentration of 5 μ M, collagen at a final concentration of 2 μ g/mL, and ristocetin at a final concentration of 1.2 mg/mL (CRONO-LOG, USA). Since the optical aggregometry method is validated for platelet-rich plasma samples, the results were interpreted as relative parameters of the dynamics of platelet functional activity. Thromboelastogram parameters were also determined: R (clot formation time), MA (maximum amplitude), and

G (clot elasticity index) using a TEG® 5000 thromboelastograph (Haemoscope Corporation, USA).

Statistical processing of the results was performed in the Google Colab environment using the Python language with open-source libraries: Pandas (for data manipulation), NumPy (for vector operations), and SciPy (for scientific calculations). Quantitative data are presented as median (M_e), first and third quartiles [Q_1 ; Q_3]. The Mann–Whitney U -test was used to assess differences between two independent groups, and the Wilcoxon test was used to compare quantitative parameters in two dependent groups. Pearson correlation analysis was performed to study relationships between features. Differences were considered statistically significant at a significance level of $p < 0.05$.

RESULTS

The platelet count in the study group was $238 [207; 274] \times 10^3/\mu$ L, and the median PMP level in peripheral blood was 2244 [1580; 3998] per 10^6 events (Fig. 1). It was found that sex did not significantly affect the level of platelets or platelet-derived microparticles, although their levels in men were higher ($249 \times 10^3/\mu$ L and 2663 per 10^6 events, respectively, $p > 0.05$) than in women ($211 \times 10^3/\mu$ L and 1612 per 10^6 events, respectively, $p > 0.05$). The analysis revealed no significant association between the PMP level in donor blood and

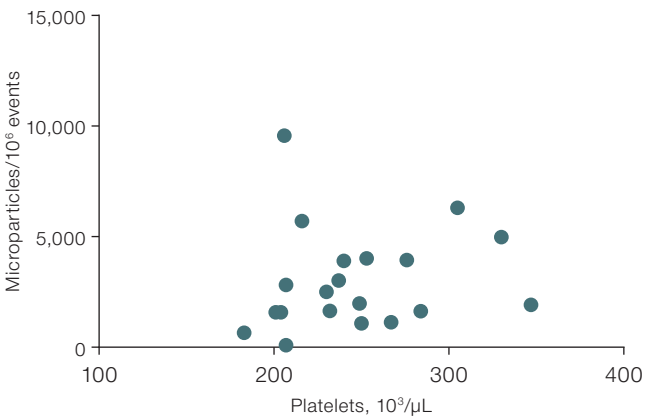


Figure prepared by the authors based on their own data

Fig. 1. Platelet and microparticle level in donor blood

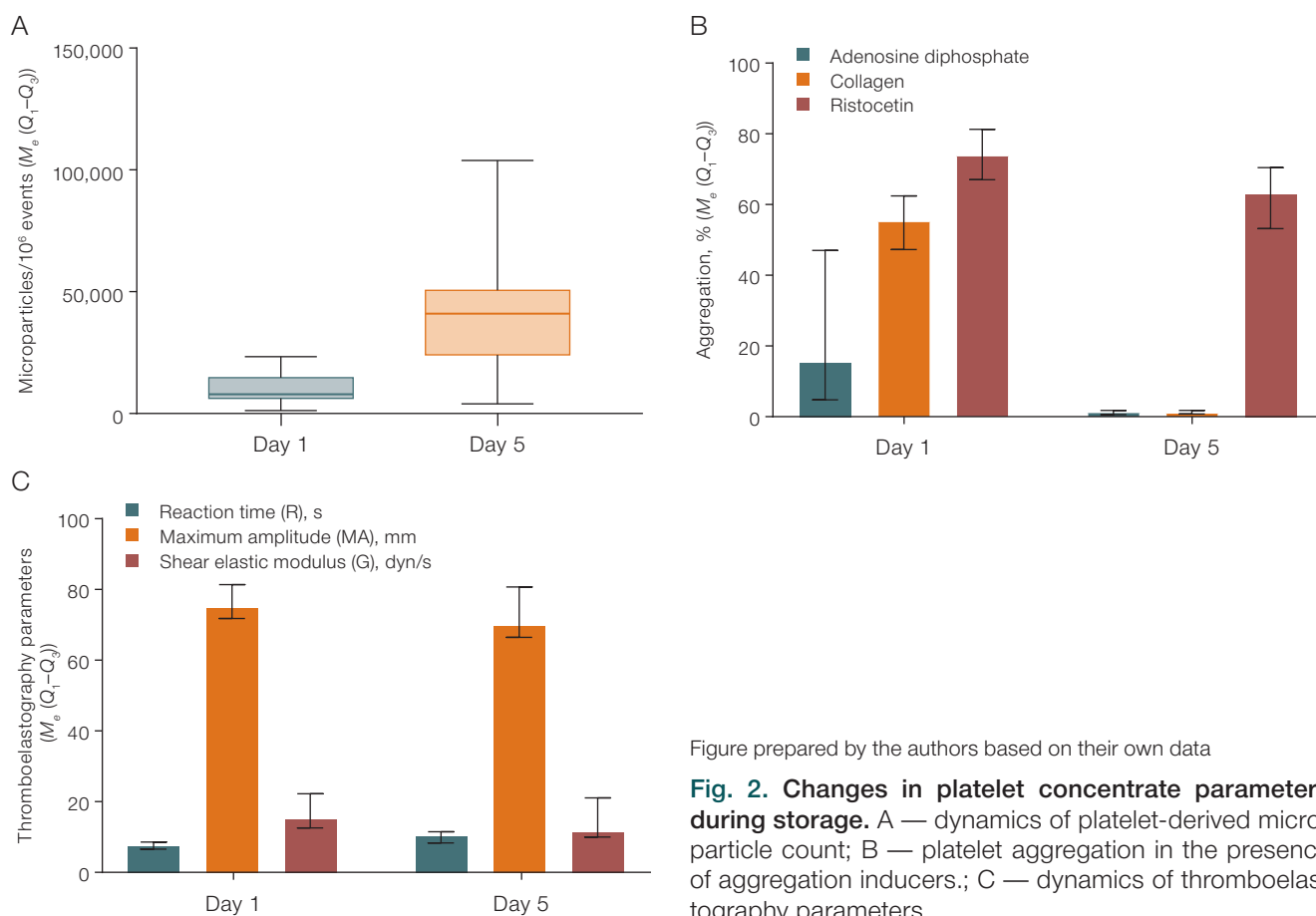


Figure prepared by the authors based on their own data

Fig. 2. Changes in platelet concentrate parameters during storage. A — dynamics of platelet-derived microparticle count; B — platelet aggregation in the presence of aggregation inducers; C — dynamics of thromboelastography parameters

individual characteristics such as age or donor tenure ($p > 0.05$).

During storage of the samples for 5 days, the PMP level in the PC increased 3.5-fold: from 8151 [6404; 14,419] to 44,163 [25,605; 59,976] per 10^6 events ($p < 0.0001$), with an average increase of 33,863 per 10^6 events (Fig. 2A). Such a substantial increase may indicate intense activation and degradation of platelets under storage conditions, which must be taken into account when assessing the functional state of blood component cells. It should be noted that no correlation was found between the PMP content in venous blood samples and either the number of microparticles in the PCs throughout the storage period or their increase by the end of the shelf life ($p > 0.05$).

On the day of platelet concentrate collection, sufficient aggregation levels were recorded in the presence of all three inducers (ADP, collagen, ristocetin), indicating preserved platelet function (Fig. 2B). By the fifth day of storage, a significant decrease in aggregation parameters was observed, especially in the presence of the aggregation inducers ($p < 0.001$): aggregation in the ADP presence decreased from 15.4 [4.7; 47.0] to 1.2 [0.6; 1.8]%, and in the presence of collagen — from 55.2 [47.2; 62.5] to 1.1 [0.8; 1.8]%. A smaller decrease in aggregation activity was observed in the presence of

ristocetin (from 73.8 [67.1; 81.3] to 63.2 [53.2; 70.4]%, $p < 0.001$).

On the first day of PC storage, the thromboelastography parameters (R, MA, G) corresponded to levels indicating sufficient hemostatic potential of the component (Fig. 2C). By the fifth day of storage, statistically significant changes in these parameters were recorded. R increased from 7.5 [6.6; 8.6] min to 10.5 [8.4; 15.5] min ($p < 0.001$); MA values decreased from 75.2 [71.8; 81.4] mm to 70.0 [66.6; 80.8] mm ($p = 0.008$), and G decreased from 15.3 [12.6; 22.3] dyn/s to 11.6 [10.0; 21.1] dyn/s ($p = 0.033$). Thus, changes were observed for all parameters; however, MA, which to a greater extent reflects the platelet component of hemostasis, despite a significant decrease, was characterized by a less pronounced dynamic — a reduction of 6.7% over 5 days of storage.

An inverse correlation was found between the baseline PMP level in donor blood and the increase in R ($r = -0.490$, $p = 0.028$), demonstrating an association between higher microparticle level and a less pronounced increase in clot formation time during storage. These data may indicate a link between a higher baseline PMP level and better preservation of the hemostatic potential of the PC. Possible mechanisms may include sufficient resistance of cells to apoptosis.

DISCUSSION

Microparticles are a heterogeneous population of membrane vesicles formed from cells of various origins during their life cycle, activation, and apoptosis [20]. The PMPs proportion in the bloodstream reaches up to 80%; microparticles of other origins (leukocytes, erythrocytes, endothelial cells) constitute a smaller fraction [21]. Such a substantial predominance, combined with high biological activity, may determine the potential contribution of PMPs to the overall procoagulant properties of blood components. The hemostatic potential of PMPs is 50–100 times higher than the procoagulant activity of platelets, and therefore they play an important role in blood coagulation [22]. The present study clearly demonstrates the PMPs accumulation during the storage of blood components, which is consistent with data from Russian publications [23, 24]. A significant increase in PMPs may indicate the preservation of the hemostatic properties of blood components [25, 26].

In the presence of all studied aggregation inducers on the day of component collection, high values of aggregometry parameters were recorded; however, by the 5th day of observation, a significant decrease was noted. The decrease in the degree of aggregation in the ristocetin presence was statistically significant but was less pronounced compared to the effects of ADP and collagen, which is due to the specific mechanism of action of this inducer. Unlike direct aggregation inducers (ADP or collagen), ristocetin acts indirectly by enhancing the binding of von Willebrand factor to GPIIb receptors on platelets. Moreover, the results of the ristocetin test are more resistant to degradation processes, since GPIIb receptors are preserved on platelets for a longer period during sample storage [27]. The obtained data are consistent with the expected dynamics of reduced platelet viability during storage. It is important to note that there are currently no unified reference values for aggregation activity for platelet count, as standard methods are validated for platelet-rich plasma or whole blood. Therefore, the assessment of functional status in the present study was performed in a comparative manner (within-group dynamics) [28].

According to the thromboelastography results during the PC storage, an overall decrease in the hemostatic potential of the blood component was revealed, which manifested as an increase in clot formation time

and a decrease in maximum amplitude and clot elasticity parameters. At the same time, in samples with high PMP level, faster clot formation was observed, indicating the PMPs role in procoagulant activity.

The obtained results extend our previously published data, demonstrating for the first time the absence of a significant association between the baseline PMP level and donor characteristics (sex, age, donor experience) in the studied cohort. In a number of similar studies, despite mentions of a possible influence of donor factors, no reliable evidence of an association between PMPs and the parameters of the examined donors was obtained [29, 30]. Moreover, these studies pay particular attention to other factors that have a greater impact on PMP level: collection methods, component processing methods, and the addition of additive solution [2, 31].

CONCLUSION

PMPs are a marker reflecting the platelets state during the collection and storage stages of PCs. Their level can serve both as an indicator of platelet activation and as a marker of functional activity.

In our study, it was confirmed that a pronounced accumulation of PMPs occurs during the PCs storage, which is consistent with the literature data and can be considered as an additional criterion for assessing the component quality. The recorded results demonstrate that a higher baseline PMP level in the donor's blood is associated with a lesser degradation of the hemostatic potential of the PC during storage, which is reflected in a less pronounced increase in clot formation time. These data suggest a possible prognostic significance of the baseline PMP level in the donor with respect to the resistance of platelets to storage conditions and the preservation of the functional and procoagulant potential of the component. The analysis did not reveal any statistically significant correlations between the individual characteristics of the donors and the PMP level, suggesting that the technological factors of blood component collection and processing have a dominant influence on the microparticle formation process. At the same time, the PMP level in peripheral blood is of significant scientific interest and requires further in-depth study, including in the context of a personalized approach to donor selection and prediction of the quality of the resulting blood components.

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