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The assessment of phagocytic and bactericidal activity of platelets and plasma bactericidal activity in late preterm newborns*

Ocena aktywności fagocytarnej i bakteriobójczej płytek krwi oraz aktywności bakteriobójczej osocza u późnych wcześniaków

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Background:

The aim of the study was to compare the phagocytic and bactericidal properties of blood platelets and the plasma bactericidal activity in 66 late preterm (LPN) and 74 full-term newborns (FTN).

Materials/Methods:

Blood samples were collected from the umbilical artery. Bacteria of the *Staphylococcus aureus* ATCC 6538P were used for the tests.

Results:

Platelet counts in LPN vs FTN were the following: 225 vs 258.5 ($\times 10^3/\mu\text{L}$), $p = 0.003$. The percentage of phagocytic platelets was the following: $\text{Me} = 1.1$ in LPN vs $\text{Me} = 1.1$ in FTN. The phagocytic index was the following: $\text{Me} = 1$ for both LPN and FTN. The phagocytic properties of platelets increased as the birth weight increased. The bactericidal activity of platelets was the following: $\text{Me} = 0$; (average = 0.7) in LPN vs $\text{Me} = 0$; (average = 0.8) in FTN. The median plasma bactericidal activity in LPN was 41.6 vs 43.8, in FTN, $p = 0.027$. The bactericidal capacity of plasma increased with increasing fetal age and birth weight of newborns. sP-selectin was: 63.9 ng/ml in LPN vs 71 ng/ml in FTN, $p = 0.026$. IL-6 in LPN was 3.6 vs 3.9 (pg/ml) in FTN, $p = 0.02$.

Conclusion:

Late preterm newborns have lower defensive capacity against infection than full-term newborns, due to lower platelets count, lower plasma bactericidal activity and lower sP-selectin concentration, which cooperates with neutrophils, monocytes in fighting against infection. All newborns had similar phagocytic and bactericidal properties of platelets.

Keywords:

platelets, phagocytosis, late preterm newborns, infection

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INTRODUCTION

The normal platelet count in healthy newborns is $150\text{--}450 \times 10^3/\mu\text{L}$, similar to adults; however, these platelets differ in terms of morphology and function [19, 26]. They have a less visible tubular structure, a smaller number of granules and a smaller average density of receptors on their surface. During activation, they form a lower quantity of pseudopods, have less aggregation ability, and show increased adhesion capacity [11, 20]. Neonatal blood platelets, especially of those born prematurely, are hyporeactive [11]. In addition to significant functions in hemostasis, thrombocytes act as immune cells, initiate, and modulate inflammatory and immunological reactions and perform phagocytic and bactericidal functions. There are numerous compounds involved in immune processes in platelet granules. Platelet glycoproteins, such as P-selectin, interact with neutrophils, monocytes, lymphocytes, and vascular endothelial cells [17, 21, 22]. The role of platelets in non-specific immunity in infants is not fully understood, especially the ability of platelets to absorb and kill bacteria [1].

Neonates born preterm do not have fully mature immune mechanisms, cellular and humoral immunity, and these problems are exacerbated by the a shortened gestation period. They are more susceptible to infection and less able to fight off infections [2, 4, 14]. Late preterm neonates (LPN) born near term, between 34 (0/7) and 36 (6/7) weeks of pregnancy, constitute the most numerous and a constantly growing category of all premature babies [10, 15]. As they have similar body dimensions to full-term newborns (FTN), they are often treated as fully mature. However, they are a completely different group, characterized by biological immaturity associated with numerous clinical consequences and increased perinatal morbidity and mortality compared to FTN [8]. The consequences of immaturity are disorders of haemostasis, causing intracranial bleeding and increased susceptibility to infection and a tendency to generalize the infection. Thrombocytopenia is more common in premature newborns than in full-term newborns [23]. The aim of the study was to investigate the phagocytic and bactericidal properties of blood platelets in late preterms and healthy newborns with normal body weight delivered at term, without the features of infection. The plasma bactericidal activity, sP-selectin, as the measure of activation of platelets, and IL-6 were determined. The dependence of the examined parameters on the gestational age and birth weight was assessed.

MATERIAL AND METHODS

The study group included 66 neonates born late preterm between 34 and 36 weeks of pregnancy with weight ranging from 1.650 g to 3.150 g (Me: 2450, Q1 = 2000; Q = 2600). Seventy-four healthy, full-term newborns, born between 38 and 40 weeks of pregnancy, with body weight ranging from 2.650 g to 4.250 g (Me: 3415, Q1 = 3000; Q3 = 3700) were included in the control group. Newborns with clinically and laboratory confirmed congenital infection and infants of mothers who received antiplatelet medication or blood products during the last 10 days of gestation were excluded from the study. Sensitive markers of inflammation, IL-6 and sP-selectin [Human P-selectin/CD62P R & D Systems] were determined in every newborn.

Blood for laboratory tests was taken from the umbilical artery. In order to avoid platelet activation, the first 0.5 ml of blood was rejected. The next portion of blood was collected directly into haematological tubes with EDTA and in tubes with heparine.

Parameters such as platelet count (PLT), percentage of phagocytic platelets, phagocytic index of platelets, bactericidal activity of platelets, plasma bactericidal activity, were examined. *Staphylococcus aureus* ATCC 6538P bacteria were used for the research [9, 12, 13].

The normality of the distribution was verified by Kolmogorov-Smirnov tests with the Lilliefors correction and the Shapiro-Wilk test. There was no normality of the distribution of the quantitative variables found. Therefore, to compare quantitative variables between two groups, a nonparametric Mann-Whitney U test was used. In order to analyze the correlation between quantitative variables, a Spearman correlation coefficient was calculated. Statistically significant results were found at $p < 0.05$. The analysis was performed using STATISTICA 13 software.

RESULTS

The average birth weight in the study group was 2450g (min: 1,650g, max: 3.150g) and 3415 g (min: 2.650 g, max: 4.250 g) in the control group. Our study showed a statistically significant lower PLT count in a group of late preterm newborns: median Me = $225 \times 10^3/\mu\text{L}$ (Q1 = $188 \times 10^3/\mu\text{L}$; Q3 = $267 \times 10^3/\mu\text{L}$) in comparison with full-term newborns: Me = $258.5 \times 10^3/\mu\text{L}$ (Q1 = $222 \times 10^3/\mu\text{L}$; Q3 = $300 \times 10^3/\mu\text{L}$),

Table 1. Comparison of birth weight, PLT and parameters evaluating phagocytic and bactericidal activity of platelets between preterm newborns born near term, between 34 and 36 weeks of pregnancy (study group) and full-term newborns (control group)

	Study group LPN N = 66	Control group FTN N = 74	p
Birth weight (g)	Me = 2450 (Q1 = 2000; Q3 = 2600)	Me = 3415 (Q1 = 3000; Q3 = 3700)	<0.001***
PLT (x10 ³ /μL)	Me = 225 (Q1 = 188; Q3 = 267)	Me = 258.5 (Q1 = 222; Q3 = 300)	0.003**
Percentage of phagocytic platelets	Me = 1.1 (Q1 = 1; Q3 = 1.2)	Me = 1.1 (Q1 = 1; Q3 = 1.2)	NS
Phagocytic index	Me = 1 (Q1 = 1; Q3 = 1.1)	Me = 1 (Q1 = 1; Q3 = 1.1)	NS
Bactericidal activity of platelets	Me = 0, M = 0.7 (Q1 = 0; Q3 = 1.2)	Me = 0, M = 0.8 (Q1 = 0; Q3 = 1.6)	NS
Plasma bactericidal activity	Me = 41.6 (Q1 = 35.4; Q3 = 45.9)	Me = 43.8 (Q1 = 38.3; Q3 = 48.4)	0.027*
sP-selectin concentration (ng/ml)	Me = 63.9 (Q1 = 50.8; Q3 = 78)	Me = 71 (Q1 = 58.4; Q3 = 83.5)	0.026*
Interleukin 6 concentration (pg/ml)	Me = 3.6 (Q1 = 2.9; Q3 = 4.6)	Me = 3.9 (Q1 = 3.4; Q3 = 4.9)	0.02*

p=0.003 (Table 1). Moreover, the PLT count increased with the number of completed weeks of pregnancy and birth weight. The research revealed an average positive correlation between fetal age and PLT ($R = 0.33$, $p < 0.001$) and between birth weight and PLT ($R = 0.29$, $p < 0.001$), R (Spearman's rank correlation coefficient).

The platelet phagocytic capacity determined by the percentage of phagocytic platelets and the phagocytic index were similar in both groups (Table 1).

The percentage of phagocytic platelets did not differ significantly between both groups of newborns: Me = 1.1 (Q1 = 1; Q3 = 1.2) vs Me = 1.1 (Q1 = 1; Q3 = 1.2) in the study and the control group, respectively. A similar relationship was found in the phagocytic index in the study group: Me = 1 (Q1 = 1; Q3 = 1.1) vs the control group: Me = 1 (Q1 = 1; Q3 = 1.1). Phagocytic index = number of phagocytized bacteria/number of phagocytizing platelets. Both of these parameters did not depend on gestational age. Phagocytic properties of platelets were increased with the increase in birth weight. A correlation between the phagocytic index and birth weight (g) ($R = 0.25$, $p = 0.003$) was found (Fig. 1). A positive correlation between the percentage of phagocytic platelets and the phagocytic index was obtained in all newborns ($R = 0.63$, $p < 0.001$). The bactericidal activity of platelets (the number of bacteria killed by platelets) was similar in both study groups of newborns: Me = 0 (Q1 = 0; Q3 = 1.2) (average = 0.7) vs Me = 0 (Q1 = 0; Q3 = 1.6) (average = 0.8) in the study and control group, respectively (Table 1). The gestational age of the newborn did not affect the ability of the platelets to kill bacteria.

The plasma bactericidal activity (the number of bacteria killed by plasma) against *Staphylococcus aureus* was significantly different in both groups of newborns: Me = 41.6 (Q1 = 35.4; Q3 = 45.9) vs Me = 43.8 (Q1 = 38.3; Q3 = 48.4), $p = 0.027$ (Table 1) and increased with fetal age and birth weight. We found a positive, weak correlation between the plasma bactericidal activity and birth weight ($R = 0.24$, $p = 0.005$) and the plasma bactericidal activity and gestational age ($R = 0.26$, $p = 0.002$) (Fig. 2).

The concentration of sP-selectin was significantly lower ($p = 0.026$) in the group of late preterm newborns Me = 63.9 ng/ml (Q1 = 50.8 ng/ml; Q3 = 78 ng/ml) compared to full-term newborns Me = 71.1 ng/ml (Q1 = 58.4 ng/ml; Q3 = 83.5 ng/ml) (Table 1). sP-selectin concentration increased with each completed week of pregnancy: a positive, weak correlation between concentration of sP-selectin and fetal age was found ($R = 0.19$, $p = 0.03$).

Our study showed that the concentration of Interleukin-6 was statistically lower ($p = 0.02$) in the study group of late preterms: Me = 3.6 (Q1 = 2.9; Q3 = 4.6) in comparison with healthy neonates born at term: Me = 3.9 (Q1 = 3.4; Q3 = 4.9) (Table 1). A positive, weak correlation between the concentration of Interleukin-6 and fetal age was found ($R = 0.18$, $p = 0.04$).

DISCUSSION

Our study showed that late preterm newborns had a lower number of platelets, lower plasma bactericidal activity and lower concentration of sP-selectin and Interleukin-6 in

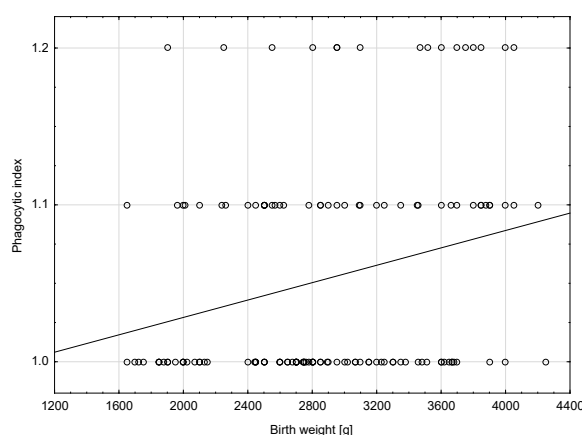


Fig. 1. Correlation between the phagocytic index and birth weight (g) ($R = 0.25$, $p = 0.003$)

comparison with neonates born at term. Similar results of the study were found by many other investigators [18, 24, 26]. Decreased PLT counts may indicate impaired thrombopoiesis in LPN, which causes thrombocytopenia [25]. The main role in the regulation of these mechanisms is played by thrombopoietin hormone (TPO), whose concentration, due to an immature liver, may be insufficient for proper platelet production during this period of life. Thrombocytopenia is closely related to the increased risk of intraventricular bleeding in LPN, which is associated with hemostatic disorders, in which platelets play an important role. In addition, low blood platelet counts can lead to the weakening of defensive capabilities against pathogens in the newborn's organism, and thrombocytes are involved in the development of non-specific immunity. The most serious consequence of this may be the development of sepsis [5, 28].

Our research showed that LPN and FTN have similar phagocytic activity of platelets. Saving noted in his study that platelets in newborns have fewer pseudopods and microtubules than in adults, which could indirectly affect the immaturity of phagocytosis. He did not observe such a difference between premature babies and full-term newborns [16]. This may also suggest that phagocytic activity is similar in almost full-term neonates and those born at term. The bactericidal abilities of platelets did not differ statistically in both study groups. Białowas showed that eutrophic newborns have even three times lower platelet bactericidal activity than adults [1]. This may be related to impaired degranulation of platelet dense granules (in comparison to adults) [11], in which antibacterial enzymes – thrombocidines, which kill microorganisms – are stored. In addition, Urban and co-authors found that the number of dense granules in thrombocytes increases with age, beginning their observations in fetuses and ending in adults [20].

The reason for the weakened plasma bactericidal activity in the late preterm newborns, showed in the study, may be the impairment of the function of the cells involved in

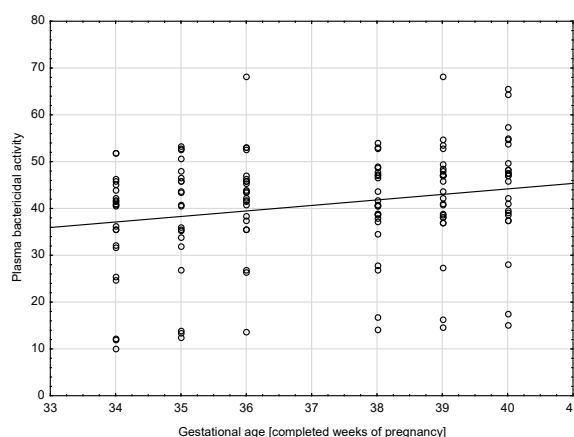


Fig. 2. Correlation between the plasma bactericidal activity and gestational age (completed weeks of pregnancy) ($R = 0.26$, $p = 0.002$)

the immunological processes. Compared to adults, newborns (premature and full-term) have a worse response of lymphocytes and monocytes, belonging to phagocytic cells, to the presence of pathogens and inflammation [4]. This may result in greater susceptibility to infection and mortality of nearly full-term neonates in relation to those born at term [8]. Prematurity is associated with an immature immune system, including delayed recruitment of neutrophils and monocytes into infected tissues and reduced cytotoxicity of NK cells [2]. Glasser and other authors found that late preterm and full-term neonates exhibit lower neutrophil, monocyte and lymphocyte counts [6]. sP-selectin is a marker of platelets activation [17]. Lower concentration of this parameter in LPN may indicate a decrease in platelet activity, which is associated with impairment of their function, including phagocytic or defense against pathogens. It could, to a certain extent, explain the greater susceptibility of this group of newborns to infections. Wasiluk achieved different results in her study; namely, the expression of P-selectin on the surface of platelets was almost twice as high in late preterm infants as in neonates born at term of birth [22]. In our study, the concentration of sP-selectin increased with fetal age. A similar relationship was found by Yang [27]. Interleukin-6 plays an important role in the development of diseases with infectious, inflammatory, and traumatic etiology and may increase dramatically in these clinical conditions.

According to this, IL-6 is considered to be a very sensitive marker of inflammation [7]. The IL-6 concentration measured before and after delivery is a good marker for predicting congenital infection in the newborn. In the study, late premature newborns were characterized by a lower number of IL-6 producing cells in this group of newborns. They can also be less mature and active than full-term neonates. We noted a positive correlation between the concentration of Interleukin-6 and fetal age, unlike other investigators, who found a negative relationship [3]. Our research allowed us to better understand the platelet function of late preterm newborns.

CONCLUSIONS

The conducted research allowed us to draw the following conclusions and practical implications:

- Late preterms have a lower platelet count PLT and lower plasma bactericidal activity, which may indicate weaker non-specific immunity compared to full-term infants.
- Neonates born near term and at term of delivery have similar phagocytic and bactericidal properties of platelets.
- The plasma bactericidal activity increases with fetal age and birth weight of newborns.
- The percentage of phagocytic platelets and the platelet phagocytic index increases with an increase in birth weight of newborns.
- Late preterms had lower plasma levels of sP-selectin and IL-6 than infants born at term.

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The authors have no potential conflicts of interest to declare.