IJN
Iranian Journal of Neonatology
http://ijn.mums.ac.ir

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Original Article

Hematological Parameters after One Week of Life among Premature Neonates

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ABSTRACT

Background: Hematological parameters can reflect potential morbidity in premature neonates. Complete blood count (CBC) is a common laboratory examination in neonatal wards, including hematological parameters. Changes may occur during the neonatal period due to gestational age (GA)-related physiological mechanisms. The purpose of this study was to examine CBC in premature neonates at birth and in the first week of life.

Methods: This prospective study was performed in the neonatal ward of a general hospital in Bandung, Indonesia. A total of 53 premature neonates, including 31 males and 22 females, classified as G1 (with 28-31 weeks of gestation) and G2 (with 32-34 weeks of gestation), were examined for CBC. The sequential blood samples of both cord blood at birth and peripheral venous blood in the first week of life (i.e., days 2-7) were drawn. The obtained data were analyzed based on the GA at birth and in the first week of life. The CBC at birth and in the first week of life were also compared.

Results: At birth, younger premature neonates (i.e., G1 group) showed lower platelet counts, compared to the older ones (i.e., G2 group). In the first week of life, the G1 group showed significantly lower platelet counts and eosinophil counts, compared to the G2 group. Both groups demonstrated a significant decrease in hemoglobin, leukocytes, basophils, and neutrophils, but increased platelet counts in the first week of life.

Conclusion: Younger premature neonates indicated lower hematological parameters at birth and in the first week of life. All the premature neonates showed a significant reduction in most hematological parameters in the first week of life.

Keywords: Complete blood count, Gestational age, Premature neonates

Introduction

Preterm birth is an important cause of mortality and a serious cause of childhood-developed problems among survivors around the world. Infection and respiratory disorder due to organ immaturity play an important role in the morbidity and mortality of preterm neonates (1). Some survivors of the neonatal respiratory disorder may develop bronchopulmonary dysplasia and suffer from chronic pulmonary diseases, and some of them may experience neonatal sepsis that may develop into neurodevelopmental disorders.

The global rate of preterm births is about 11% of live births, and preterm birth is the cause of 35% of all neonatal mortalities (1, 2). Preterm birth is defined as all births before 37 completed weeks of gestation or before 259 days from the first day of the last menstrual period. Preterm birth can be classified based on gestational age (GA) into extremely preterm (<28 weeks), very preterm (28-32 weeks), and moderate preterm (32-37 weeks) (1).

Complete blood count (CBC) count is a laboratory test performed on every neonate in the neonatal ward. It provides an overview of each blood cell lineage that indicating whether a

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Please cite this paper as:
Ghrahani R, Ghozali M, Kristianto Sugianli A, Yuniati T, Tina Dewi Judistian R, Setiabudiawan B. Hematological Parameters after One Week of Life among Premature Neonates. Iranian Journal of Neonatology. 2020 Dec: 11(4). DOI: 10.22038/ijn.2020.49341.1860

disease is currently progressing. The CBC is a simple tool for demonstrating hematological parameters that determine the innate immune response during the neonatal period. The innate immune system is predominant in premature neonates because the adaptive immune response has not been completely developed. However, premature neonates have also a deficiency in innate and adaptive immune responses as well as in the synergy between the two systems (2).

The degree of prematurity is a circumstance that significantly alters the hematological parameters at birth. Premature neonates are born with an immature hematological system, and the process has been initiated during the fetal period (3). On the other hand, certain clinical factors, namely infection and respiratory distress syndrome (RDS), may affect the profile of hematological parameters during the neonatal period, particularly among premature neonates. With this background in mind, the present study aimed to evaluate CBC in premature neonates at birth and in the first week of life.

Methods

This prospective study was conducted at the Neonatology Division, Department of Child Health, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin Central General Hospital in Bandung, Indonesia, within April-October 2018. The CBC examination of premature neonates (with 28-34 weeks of gestation) was carried out. During the study period, 563 premature neonates (with 28-34 weeks of gestation) were born; however, CBC data were only available for 53 premature neonates due to limited laboratory data and consideration of the maximum volume of blood sampling of premature neonates in the neonatal ward. This led to the inclusion of only newborns with clinical needs for this examination.

Sampling was performed using the convenience sampling method with 28-34 weeks of gestation and availability of CBC data as the inclusion criteria. The exclusion criteria were twins, congenital anomalies, blood transfusion history, and known maternal diseases (e.g., maternal severe infection, hypertension, or diabetes). The subjects were divided into two groups based on the GA at birth, including the G1 group (very preterm; 28-31 weeks) and G2 group (moderate-to-late preterm; 32-34 weeks). Written informed consent was obtained from the parents or caregivers before the neonates were enrolled in the study.

The data were collected by detailed history taking and complete clinical examination, consisting of the data on GA based on the last menstrual period, mode of delivery, birth weight, and signs of respiratory distress. The RDS was verified with more than of the four criteria, including grunting, cyanosis in room air, retraction of the chest wall, respiratory rate more than 60 times per minute, positive radiological findings for RDS, PaO_2 level of 50 mmHg in blood gas analysis, and no improvement in clinical conditions within 24 h (4, 5). Early-onset sepsis (EOS) was defined using the Tollner's scoring system (6).

The cord blood samples at birth (immediately after birth) and peripheral (venous) blood sample in the first week of life (i.e., days 2-7) were collected using standard procedures and analyzed for CBC. The RDS in neonates were identified through posteroanterior chest X-ray, which was then evaluated by a radiologist and confirmed based on the clinical and radiological findings by a pediatrician.

The differences in clinical characteristics and laboratory parameters between the two groups were analyzed by the Student's t-test and Mann-Whitney U test. A comparison of hematological parameters at birth and in the first week of life was carried out using the paired t-test and Wilcoxon signed-rank test. A p-value of less than 0.05 was considered statistically significant for the two-sided tests. The statistical analysis was performed using SPSS software (version 23; SPSS Inc., Chicago). The analyzed data are presented in the tables of distribution frequency. In addition, the ethical clearance was issued by the Health Research Ethics Committee of Faculty of Medicine, Universitas Padjadjaran, Bandung, with an approval number of 303/UN6.KEP/EC/2018.

Results

The clinical characteristics of each group and their exposure over the perinatal period are shown in Table 1. The birth weight of the G1 group was significantly lower than that reported for the G2 group (1359.4±162.5 and 1789.7±280.1 g). No significant difference was observed between the two groups in terms of male/female ratio, APGAR score, delivery method, maternal antibiotics, and steroid exposure. The incidence of EOS and RDS was higher in the G1 group, compared to that of the G2 group, but not significantly.

Figure 1 tabulates the comparison of

Table 1. Clinical characteristics of premature neonates

Variable	G1 n=17	G2 n=36	P-value
Birth weight (mean±SD; g)	1359.4±162.5	1789.7±280.1	0.000†
Gender			
Male	11 (64.70%)	20 (55.56%)	0.740‡
Female	6 (35.30%)	16 (44.44%)	
Delivery method			
CS	8 (47.05%)	26 (72.22%)	0.140‡
Vaginal delivery	9 (52.94%)	10 (27.78%)	
Maternal antibiotics			
Yes	7 (41.2%)	16 (44.4%)	1.00‡
No	10 (58.8%)	20 (55.6%)	
Antenatal steroids			
Yes	6 (35.3%)	10 (27.8%)	0.814‡
No	11 (64.7%)	26 (72.2%)	
EOS			
Yes	10 (58.8%)	12 (33.3 %)	0.079‡
No	7 (41.2%)	24 (66.7%)	
RDS			
Yes	14 (82.4%)	19 (52.8%)	0.077‡
No	3 (17.6%)	17 (47.2%)	

G1: Gestational age of 28-31 weeks; G2: Gestational age of 32-34 weeks
†Student's t-test; ‡Chi-Square test; SD: Standard deviation; CS: Cesarean section;
EOS: Early-onset sepsis; RDS: Respiratory distress syndrome

hematological parameters at birth in the neonates between the G1 and G2 groups. The platelet count at birth in the G1 group was significantly lower than that reported for the G2 group. No difference in red blood cell (RBC) parameters (i.e., hemoglobin levels, RBC count, red cell distribution width, and hematocrit) was observed between the G1 and G2 groups at birth. However, the white blood cell (WBC) line counts (i.e., neutrophil, eosinophil, monocyte, lymphocyte, and basophil counts) appeared to be lower in the G1 group.

The result of hematological parameter examinations of the two G1 and G2 groups during the first week of life is presented in Figure 2. The eosinophil and platelet counts in the G1 group were significantly lower than those reported for the G2 group at birth. The G1 group showed lower RBC parameters (i.e., hemoglobin levels, RBC count, and hematocrit value) and WBC lines in the first week of life, compared to the G2 group.

At birth and in the first week of life, the G1 group showed lower RBC parameters, WBC lines, and platelet counts than the G2 group (Figure 1 and 2). Figure 3 shows a significant decrease in hemoglobin, hematocrit, total leukocyte count, basophil, and neutrophil in the first week of life (i.e., days 2-7), compared to that reported at birth. However, the platelet count increased in the first week of life.

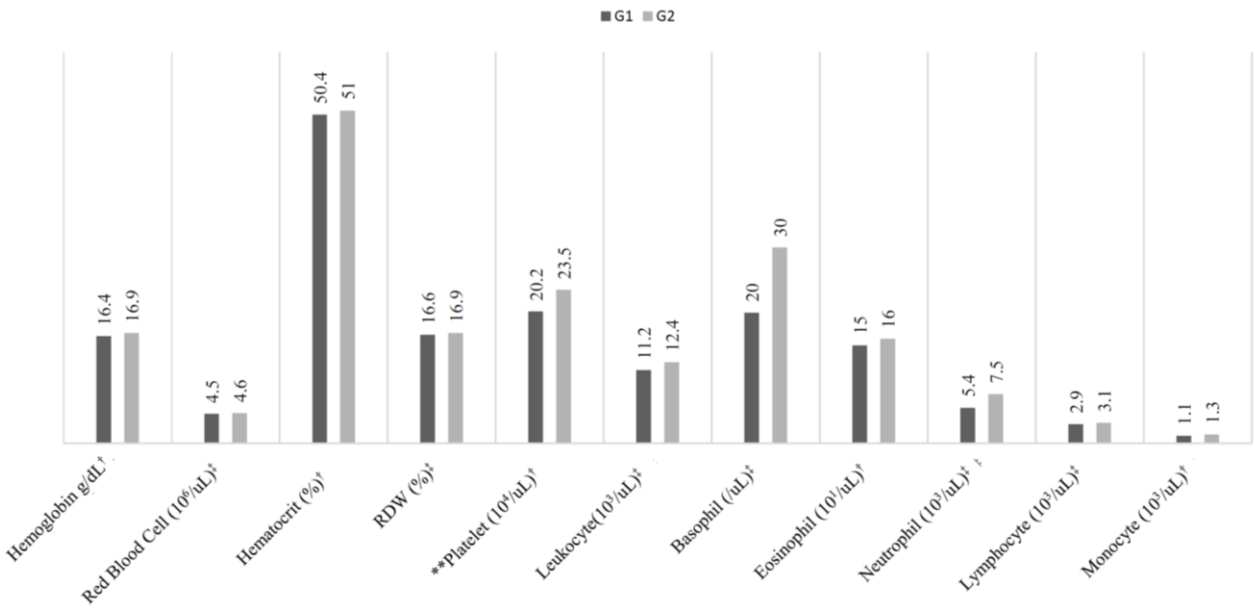

Figure 1. Comparison of hematological parameters at birth between G1 and G2* groups
*Measured from cord blood at birth (immediately after birth)
**P<0.05
G1: Gestational age of 28-31 weeks (very preterm); G2: Gestational age of 32-34 weeks (moderate-to-late preterm)
†Student's t-test
‡Mann-Whitney U test
RDW: Red cell distribution width

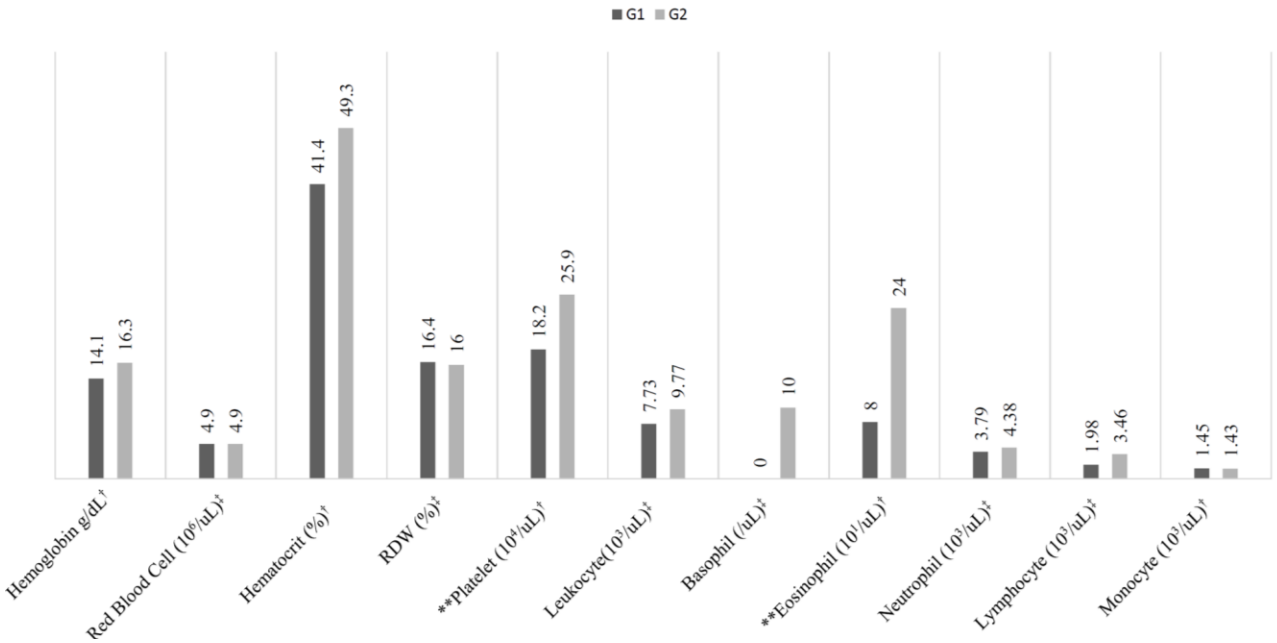

Figure 2. Comparison of hematological parameters in the first week of life between G1 and G2* groups
*Measured from peripheral blood at the first week of life (i.e., days 2-7)
**P<0.05
G1: Gestational age of 28-31 weeks (very preterm); G2: Gestational age of 32-34 weeks (moderate-to-late preterm)
†Student's t-test
‡Mann-Whitney U test
RDW: Red cell distribution width

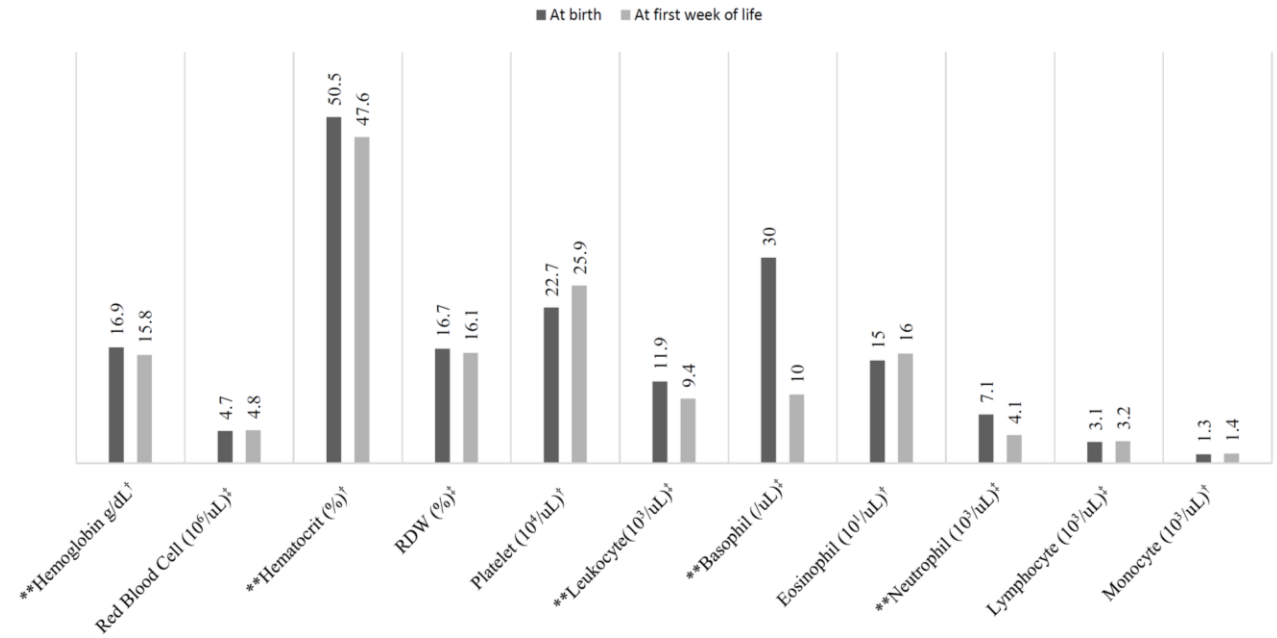

Figure 3. Comparison of hematological parameters at birth and in the first week of life in premature neonates*
*At birth: measured from cord blood (immediately after birth); at the first week of life: measured from peripheral blood at the first week of life (i.e., days 2-7)
**P<0.05
RDW: Red cell distribution width
†Paired t-test
‡Wilcoxon signed-rank test (based on positive ranks)

Discussion

Prematurity is the leading cause of mortality during the neonatal period. Organ immaturity accompanied by infections and respiratory disorders play an important role in morbidities and mortalities during the neonatal period (1). The CBC count is proposed as a simple tool for the evaluation of the innate immune response during the neonatal period. Immune cells, especially WBCs, are important parts of the innate immune system. Leukocytes providing innate immunity are derived from the myeloid lineage. These cells include highly phagocytic motile neutrophils, monocytes and tissue macrophages, eosinophils, and natural killer cells providing the first line of defense against most pathogens (2).

The innate immune system is dominant in premature neonates because the adaptive immune response has not been completely developed. However, premature neonates may have deficiencies in the innate and adaptive immune responses as well as in the synergy between these two systems (2). The degree of prematurity is of specific importance to the hematological parameters at birth. Premature neonates are born with an underdeveloped and immature hematological system since its development was interrupted due to the early birth (3). On the other hand, some clinical factors, including infection and RDS, may influence the hematological parameters during the neonatal period, especially among premature neonates.

The obtained results of the present study showed that the birth weight of younger premature neonates was lower than that of the older subjects. This is appropriate to the neonatal physiology that the gestational weight gain of premature neonates is in line with the GA (7). Because innate and adaptive immune responses are not fully developed in premature neonates, they were susceptible to EOS (8). These neonates are prone to infection due to defective neutrophil actions (e.g., phagocytosis, oxygen radical formation, and intracellular pathogen destruction), which becomes a risk factor for the progress of sepsis (2). This fact was evident in the current study indicating that the occurrence of EOS was higher in younger premature neonates.

The RDS morbidities appeared to be higher in younger premature neonates in the present study. The possible explanation is that premature neonates must acclimatize to the extra-uterine environment in order to survive and face potential complications caused by relatively immature lungs, especially the surfactant deficiency. The frequency of RDS increased with reduced GA due to a lower level of surfactant secretion (9). The inflammatory process due to immaturity, potential for infection, and RDS development will affect the hematological parameters in the neonatal period.

Hematological Parameters at Birth Based on Gestational Age

The results of the present study showed that the platelet count in the G1 group was lower than that reported for the G2 group at birth (Figure 1), which is consistent with the findings of studies conducted by Roudil et al. and Christensen et al. (3, 10). Platelet physiology during the neonatal period includes an inverse correlation between the number of megakaryocytes and GA. Platelet count changes along the gestational age and it increases with gestational age (11). Although our study only included subjects in 28-34 weeks of gestation, this increasing trend in platelet count was observed, with G2 platelet count was higher than the G1.

The present study also showed that the hematological parameters between the two groups were different in several aspects at the time of birth. For instance, the hemoglobin level, RBC, hematocrit count, and red cell distribution width at birth are lower in premature neonates with younger GA. According to the literature, it was shown that RBC parameters increase simultaneously with GA (3, 12).

A similar pattern is demonstrated by the WBC lines (i.e., basophil, eosinophil, neutrophil, lymphocyte, and monocyte counts) at birth, which appears to decrease in younger premature neonates. A previous study supports a similar condition indicating that the total leukocyte count and WBC lines at birth are lower in younger premature neonates. This condition may play an important role in infections during the neonatal period, which inversely increases with GA (13). The reason is that the low levels of granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor lead to reduced neutrophils, monocytes, and their precursors in premature neonates (2, 14, 15).

Hematological Parameters in the First Week of Life According to Gestational Age

In the present study, a lower trend of eosinophil and platelet counts was observed in

the younger premature neonates, as shown in Figure 2. The higher trend of platelet is similar to previous studies reporting that platelet count increases with an increase in GA (3, 12). The results of the present study also demonstrated that younger premature neonates had lower RBC parameters than WBC lines in the first week of life. The degree of prematurity is a circumstance that markedly affects the CBC at birth. A previous study showed that all bloodlines increase with GA (3). There was no difference in basophil count between the two groups. Lymphocyte count was lower in G1; however, the monocyte count was slightly higher in this group. Roudil et al. also reported visible monocytosis in the first three days of life in premature neonates (3).

Comparison of Hematological Parameters of Premature Neonates at Birth and in the First Week of Life

The results of the present study showed a decreasing trend of RBC parameters and WBC lines in the first week of life; however, an increasing trend was observed in the platelet count. The RBC parameters in previous studies have been demonstrated to decline after birth in all premature neonates (16-18). Physiologically, the hemoglobin level increases at birth but continues to decline with increasing postnatal age. The most logical description is that early net fluid moves with the extravascular fluid motion into the vascular space, resulting in decreasing levels of hemoglobin or hematocrit (16). Another suggested reason is that the physiological factors are the main pathogenesis of anemia in premature neonates.

Premature neonates are born when iron transferred from the mother gets depleted and ends the process of fetal erythropoiesis. The lower hemoglobin in premature neonates, compared to that of mature ones, reduces plasma erythropoietin (EPO) response to anemia. On the other hand, the liver is the main site of EPO development in premature neonates who are less prone to tissue hypoxia, anemia, and decreasing trend of hematocrit level. (17, 18). Christensen et al. (2009) demonstrated the partial interruption of fetal hematopoiesis due to preterm birth. Hepatic hematopoiesis abruptly decreased at birth and is replaced by immature bone marrow hematopoiesis.

The findings of the current study showed a decrease in leukocyte count in the first week of life. On the contrary, a previous study reported that premature neonates with a GA of 28-32 weeks showed an increase in leukocyte count during the first month of life. This increase is not observed in the older group (with more than 32 weeks of gestation) (3).

The eosinophil count appeared to slightly increase in the first week of the subjects' life. Since eosinophils also have immune modulation functions in addition to their role in antigen-antibody complex phagocytosis and parasite destruction, the high eosinophil count in preterm newborns may potentially make them susceptible to eosinophil-mediated tissue injuries. However, the evidence on the role of high eosinophil count during the neonatal period is still insufficient; therefore, the clinical interpretation of this condition remains unclear. Juul et al. (2005) reported that the incidence and degree of eosinophilia relate to immaturity, as well as correlate to several morbidities during the neonatal period (e.g., necrotizing enterocolitis, infection, and packed RBC transfusion effect) (19).

A similar pattern was observed in the monocyte count with a slight increase during the first week of life. Monocytes, which are categorized as leukocytes, play an important role in the innate immune system. Monocytes will differentiate into dendritic cells or become macrophages in the mucosa and produce inflammatory mediators if infection occurs. In premature neonates, monocyte dysfunction may occur leading to impaired innate immune responses although the pathophysiological mechanism is not well known.

Blood neutrophil levels during the first 72 h (including in the umbilical cord) in premature neonates (with 28-36 weeks of gestation) tend to decrease after birth as demonstrated in a previous study (12). In the present study, a similar condition was observed with neutrophil levels that were lower in the first week of life as opposed to the levels at birth. Neutrophil count in CBC must be evaluated in order to predict several clinical conditions. The interpretation of the ratio of immature neutrophils to total neutrophils (I:T ratio) should indicate various clinical conditions (20).

Platelet count increases in the first week of life in premature neonates as shown in Figure 3. This is similar to previous studies indicating the mean postnatal platelet count of premature neonates with 29-34 weeks of gestation to continuously increase in the first 2-3 weeks of life (3, 10). This explains that TPO is a major regulator of megakaryopoiesis. The TPO increased after birth

with the highest increase on the second day and then slowly decreased to the cord blood level at birth by the end of the first month. Several conditions will lead to temporary elevations in platelet counts, such as iron-deficiency anemia and infections, which may be mediated by the increased production of TPO or other thrombopoiesis factors (12). Platelets play an active role in both innate and adaptive immunity and act as the primary mediators.

Limitations

There were several limitations in the present study, including the limited number of enrolled neonates. The second limitation was the limited availability of the complete hematological analyses of premature neonates. The volume of blood samples drawn from the premature neonates was restricted by the neonatologist team to minimize blood loss. Blood tests are only conducted when the clinical signs prescribe performing these tests.

Conclusion

The hematological parameters of premature neonates depend on GA and postnatal age. The younger premature neonates showed lower RBC and WBC line counts at birth and in the first week of life than the older ones. The premature neonates demonstrated a significant reduction in hematological parameters in the first week of life. It is suggested to carry out further studies focusing on hematological parameters while considering the physiological factors and morbidities caused by prematurity.

Acknowledgments

The authors would like to express their gratitude to the Neonatology Division, Department of Child Health and Department of Clinical Pathology, Faculty of Medicine, Universitas Padjadjaran, and Dr. Hasan Sadikin Central General Hospital in Indonesia for their support for data collection.

Conflicts of interest

The authors declare that there is no conflict of interest.

Funding

“Lecturer Competency Grant” under Tetty Yuniati (855/UN6.3.1/PL/2017) and “Academic Leadership Grant” under Budi Setiabudiawan (8551UN6.3. I/PU2017) were obtained from Universitas Padjadjaran 2017.

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