

Comparison of absolute neutrophil count between premature and term infants

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ABSTRACT

Background Neutrophils are very important in the body defense against bacterial infection. Absolute neutrophil count (ANC) could be used for the recognition of early-onset bacterial sepsis in neonates.

Objective The aim of this study was to compare the value of ANC between premature and term infants, to assess the prevalence of early-onset neutropenia in premature infants and its relationship with prematurity, and to find out the correlation between gestational age and ANC.

Methods A cross-sectional study was conducted during February to May 2003. Subjects were newborn infants with gestational age of less than 37 weeks who were born in Adam Malik and Pirngadi Hospitals, Medan. Newborn infants with severe asphyxia (5-minute Apgar score of less than 4), fever, seizure, and maternal hypertension were excluded. Complete blood count was done by means of automatic cell counter (Micros[®], Germany). Term healthy infants were used as control subjects.

Results ANC differed significantly between both groups ($p=0.0001$). The prevalence of early-onset neutropenia in premature infants was 9% (95%CI 0.065;0.21). Prematurity was related with the incidence of neutropenia with a prevalence ratio of 1.1. There was a weak positive correlation between gestational age and ANC with an r -value of 0.49 ($p=0.0001$).

Conclusions ANC in premature infants differs from that in term infants. The prevalence of early-onset neutropenia in premature infants was 9% (95%CI 0.065;0.21). Prematurity is related with the incidence of early-onset neutropenia in newborn infants. There is a correlation between gestational age and ANC [**Paediatr Indones 2004;44:197-200**].

Keywords: absolute neutrophil count, premature infant, term infant, neutropenia.

Complete blood count examination and its differentiation are often used for diagnostic evaluation in premature infants. Absolute neutrophil count (ANC) could be used to recognize early-onset bacterial sepsis in neonates.

Neutrophils are very important in the body defense against bacterial infection. Early-onset neutropenia affects 6 to 58% of premature infants within the first week of life.¹ There are some factors related to early-onset neutropenia in premature infants, such as sepsis, severe asphyxia, periventricular hemorrhage, and maternal hypertension.¹⁻⁴

Premature or low birth weight infants usually experience immunodeficiency, so that neutropenia represents a high risk of infection in the perinatal period.⁴ Some previous studies demonstrated the relationship between neutropenia and sepsis in the perinatal period and between total neutrophil count and gestational age.^{1,2,4} ANC has a good sensitivity in diagnosing neonatal sepsis, so it can be used as a screening test to improve the recognition of this disease.¹ The aims of this study were to compare the value of ANC between premature and term infants, to assess the prevalence of early-onset neutropenia in premature infants and its relations with prematurity, and to find out the correlation between gestational age and ANC.

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Methods

A cross-sectional study was conducted at the Division of Neonatology, Adam Malik and Pirngadi Hospitals, Medan, from February until May 2003. Subjects were recruited consecutively from all premature infants (gestational age of <37 weeks) and term infants (gestational age of 37-42 weeks), as control subjects, born in the hospital during the study period. The gestational age was estimated from the first day of the last menstruation. Infants with severe asphyxia (5-minute Apgar score of <4), fever (temperature of >37.5°C), seizure, or maternal hypertension were excluded. Using the formula for the estimation of difference between two proportions,⁵ the sample size was 43 subjects for each group.

Maternal data (age, parity, first day of the last menstrual period, mode of delivery, body weight, and blood pressure) were retrieved from medical records. Infants' data recorded were sex, body weight measured by Tanita^(R) baby scale, body length measured by stadiometer, rectal temperature by mercury thermometer, and 1-minute and 5-minute Apgar scores. Blood samples for complete blood and differential counts were taken on the second day of life by femoral vein puncture. Complete blood count was done by automatic cell counter of ABX Micros^(R) (Germany). Differential count was assessed by a clinical pathologist using Giemsa-stained blood smear. Absolute neutrophil count (ANC) was calculated by multiplying the percentage of band and segmented neutrophils with total leukocytes.

Data were recorded and analyzed using SPSS for Windows ver. 10.0. Statistical comparison between quantitative variables was done using independent t-test. Hypothesis testing for proportion was performed by chi-square test for independent groups. To assess relative risk, we calculated the prevalence ratio. Correlation between gestational age and ANC was analyzed by Pearson correlation. A p value of <0.05 was considered significant.

Results

Forty-four premature infants (20 males, 23 females) and 43 term infants (30 males, 13 females) were included in this study. The characteristics of infants and mothers are shown in **Table 1**.

The hemoglobin level was not significantly different between both groups ($p=0.193$). The leukocyte count of premature infants was significantly lower than that of term infants ($p=0.0001$, 95%CI 2433;6680). The ANC was significantly lower in premature compared to term infants ($p=0.0001$, 95%CI 2127;5474) (**Table 2**).

We found 4 (9%) premature infants with neutropenia but no term infants had it ($p=0.043$) (data not shown). The prevalence ratio of neutropenia between premature and term infants was 1.1 (95%CI 1.002;1.208). There was a weak correlation between gestational age and ANC with an r-value of 0.49 ($p=0.0001$) as seen in **Figure 1**.

Discussion

The incidence of early-onset neutropenia in premature infants is associated with sepsis, maternal hypertension, severe asphyxia, and periventricular hemorrhage.¹⁻⁴ In this study, we excluded factors

TABLE 1. CHARACTERISTICS OF INFANTS AND MOTHERS.

Characteristics	Term infants Mean (SD)	Prem. infants Mean (SD)
Mothers		
Age(year)	30.2 (5.7)	28.4 (5.1)
Body weight (kg)	63.1 (10.3)	62.2 (5.7)
Parity	2.53 (1.5)	2.23 (1.6)
Systolic blood pressure (mmHg)	122.3 (9.2)	122. (7.3)
Diastolic blood pressure (mmHg)	81.2 (6.9)	77.7 (9.1)
Infants		
Body weight (g)	3215.1 (443.5)	1878.4 (414.8)
Body length (cm)	48 (2.1)	41.9 (3.2)
Rectal temperature (°C)	36.4 (0.3)	36.6 (0.3)
Apgar score at 1 minute (median)	7	6
Apgar score at 5 minutes (median)	8	9

TABLE 2. AVERAGE VALUE OF HEMOGLOBIN, LEUKOCYTE, AND ABSOLUTE NEUTROPHIL COUNT ON THE 2ND DAY OF LIFE ACCORDING TO GESTATIONAL AGE

Value	Premature infants Mean (SD)	Term infants Mean (SD)	p
Hb (g/dl)	13.9 (2.3)	13.3 (1.8)	0.193
Leukocyte (per μ l)	10240 (4953)	14797 (5008)	0.0001
ANC (per μ l)	6517 (3684)	10319 (4156)	0.0001

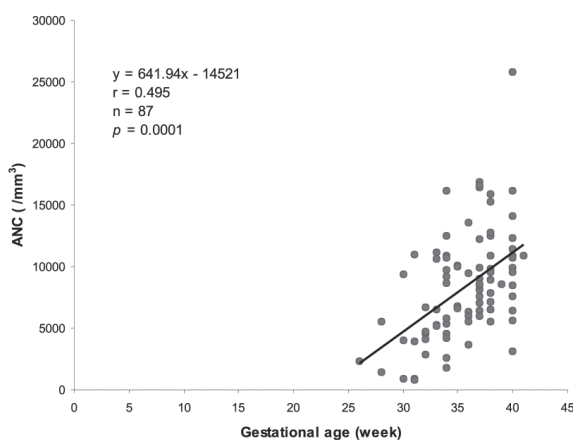


FIGURE 1. CORRELATION BETWEEN GESTATIONAL AGE AND ANC

related to the incidence of neutropenia, such as maternal hypertension, severe asphyxia, seizure (to exclude the possibility of periventricular hemorrhage), and fever, so that the neutropenia found in this study was most probably caused by prematurity only.

The prevalence of neutropenia in premature infants in this study was 9% (95%CI 6.5;21). A previous study reported that the prevalence of neutropenia in premature infants in the first week of life was approximately 6% to 58%. Of early-onset neutropenia occurring in the first week of life, half of it started on the first day of life and approximately two-third lasted for less than 1 week.¹ In this study, blood samples were taken on the second day of life pointing to the peak incidence of neutropenia in premature infants.

According to Manroe, neutrophil count increases and reaches its highest level at 12-14 hours of life and then decreases to the lowest level at 72 hours of life.⁶ According to Mouzinho, the neutrophil count reaches its highest level at 22-24 hours of life, then decreases and stabilizes at 120 hours.⁷

In this study, ANC of premature infants differed significantly from that of full-term infants, which is similar to the study of Xanthou.⁸ Manroe reported that besides bacterial infection, other perinatal factors that can cause alteration of neutrophil count were maternal hypertension, fever during delivery, hemolytic disease, and periventricular hemorrhage.⁶ Mouzinho reported that the neutrophil count of premature neonates with birth weight

of <1500 g differed significantly from that of larger and older neonates.⁷ We found a weak positive correlation between gestational age and ANC.

Neutropenia in neonates is probably caused by decreased supply from the bone marrow, but can also represent a severe infection, such as septicemia.⁹ The neutrophil storage of a newborn infant is 20-30% of that in an adult. Neutrophils are much more likely to be depleted by a neonatal infection, which is the major host factor contributing to poor outcome in bacterial sepsis.¹⁰ Neonates are prone to neutropenia because the bone marrow neutrophil storage is small, neutrophil reserve is released too rapidly during stress, the production is near maximum, and the marrow stem cells are unable to compensate for the increased cellular demand of infection.¹¹

Neutrophils are derived from pluripotent stem cell progenitors in the bone marrow. Upon stimulation by colony stimulating factors, such as stem cell factor, granulocyte macrophage colony stimulating factor (GM-CSF), and granulocyte colony stimulating factor (G-CSF), the proliferation and development into mature segmented neutrophils occur in the bone marrow. The first three stages of neutrophil maturation – myeloblast, promyelocyte, and myelocyte – involve young actively dividing cells. After the myelocyte stage, the cells lose their ability to divide and form metamyelocytes, band cells, and finally segmented polymorphonuclear neutrophils. The release of neutrophils from the bone marrow into the circulation is influenced by many factors, i.e., neutrophil deformability, cell releasing factor, blood stream passing through the bone marrow, and cell location in relation to vein. Chemotactic substances, such as C5a, interleukin-8 (IL-8), monocyte chemotactic factor, leukotrienes, and bacterial peptides induce the migration of circulating neutrophils to sites of infection and inflammation.^{9,12}

Neutrophils function as body defense against bacterial infection.¹ Chemotaxis of neutrophils is limited in neonates and its low aggregation results in delayed response to infection.¹³ Levy *et al* reported that neutrophils of the newborn are selectively deficient in BPI (*bactericidal permeability-increasing protein*), a central effector of antibacterial activity against Gram-negative bacteria. This could contribute to the increased incidence of Gram-negative sepsis among newborns.¹⁴

There is a correlation between the severity of neutropenia and infection.^{11,13,14} Prematurity is the single most significant factor correlated with neonatal sepsis, and the risk of sepsis increases in proportion to the decrease in birth weight.¹⁵ Neutropenia in premature infants correlates significantly with an increased risk of early-onset sepsis in the newborn.⁴

We conclude that ANC in premature infants differs from that in term infants. The prevalence of early-onset neutropenia in premature infant was 9%. Prematurity is related with the incidence of early-onset neutropenia in newborn infants. There is a correlation between gestational age and ANC.

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