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Nucleated red blood cells (nRBC) as an auxiliary marker of intrauterine infection

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Summary

Background: Nucleated red blood cells (nRBC), or erythroblasts in peripheral blood is associated with the hypoxemic nature of fetal growth. The aim of the present study was to assess the usefulness of determining nRBC in fetal blood within the first 12 hours of life as an auxiliary marker of intrauterine infection.

Material/Methods: The study group (I) comprised 31 newborns with intrauterine infection diagnosed based on anamnesis, physical examination and laboratory diagnostics. The control group (II) comprised 115 healthy term newborns. Venous blood samples were collected and erythrocyte count, as the percentage of white blood cells (WBC) in venous blood smear, was determined at the hospital laboratory. The number of erythroblasts higher than 10 was regarded as elevated.

Results: The mean gestational age at delivery in group I was 36.1 weeks as compared to 37.8 weeks in control group. In group I the mean Apgar score was 7 points and only 56% of newborns with diagnosed congenital infection presented good health condition at birth. In the control group, the mean erythroblast count was very low =4.01 as compared to 16,9 among newborns with congenital infection. In the group of newborns with the signs of intrauterine infection among 15 generalised infection were diagnosed and congenital pneumonia in 20 of newborns. Those with generalised infection presented nRBC level higher than normal (mean 15 blood cells per 100 WBC).

Conclusions: The determination of nRBC count can be used as an auxiliary marker in the diagnostics of congenital infection in preterm and term neonates.

Key words: **intrauterine infection • nucleated red blood cells**

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Background

Nucleated red blood cells (nRBC), or erythroblasts, are the premature forms of erythrocytes that are commonly found in the newborn's blood. Their presence in peripheral blood is associated with the hypoxemic nature of fetal growth [1]. Erythroblasts are generated in fetal bone marrow in response to erythropoetin activity and are deposited as precursor reticulocytes and mature erythrocytes [2,3]. In healthy term neonates, the number of erythroblasts approximates 500 nRBC/mm^3 making up 0.1% neonatal RBC. In the clinical and laboratory practice, nRBC level is expressed as the proportion of WBC in peripheral blood smear [2,3].

The production rate of RBC is dependent on eythropoietin stimulation of stem cells in bone marrow [4,5]. Since erythropoietin does not enter the placenta, its concentration in fetal blood depends on the volume produced by the fetus as well as the factors conducive to fetal hypoxia. Persisting elevated level of erythropoietin (due to prolonged hypoxia) that induces erythropoesis leads to an increased number of circulating erythrocytes, while acute stress triggers release of erythroblasts from bone marrow [5,6].

The increased concentration of erythroblasts in newborn's blood is associated with intrauterine growth restriction (IUGR) [7], maternal cigarette smoking [8], arterial hypertension [3], maternal diabetes [9], severe serologic incompatibility [10] and amnionitis [11].

Aim

The aim of the present study was to assess the usefulness of determining nRBC in fetal blood within the first 12 hours of life as an auxiliary marker of intrauterine infection.

Material and Methods

The project was a prospective study covering 146 newborns delivered at the Clinical Department of Perinatology, Institute of Gynecology and Obstetrics, Medical University of Lodz, between 1 January and 31 December 2002. The study population was divided into two groups. The study group (I) comprised 31 newborns admitted to Intensive Care and Neonatal Pathology Unit with intrauterine infection diagnosed based on anamnesis, physical examination and laboratory diagnostics. The control group (II) comprised 115 healthy term newborns, in good health condition at birth, who developed symptoms requiring blood test within the first 12 hours of life. Venous blood samples were collected and erythrocyte count, as the percentage of white blood cells (WBC) in venous blood smear, was determined at the hospital laboratory. WBC count was corrected by nRBC count. The number of erythroblasts higher than 10 was regarded as elevated. The diagnostic criteria of intrauterine infection included manifestation of at least three of the following symptoms: tachypnoea, cyanosis, apnoea, tachy/bradycardia, hypotension, apathy, restlessness, lack of appetite, food intolerance, fever/hypothermia, intense hepatitis and hepatosplenomegaly. The clinical symptoms were verified with tests for CRP, WBC count, PLT count and I: T ratio. CRP values > 5mg/l, leukocytosis >20 000 or <5000 WBC/ml, PLT count <100 000/ml and I: T ratio were considered indicative of congenital infection.

The infants born to mothers suffering from diabetes, cardiovascular diseases, arterial hypertension, kidney dysfunction and ABO serological conflict were excluded from the study as well as newborns from multiple pregnancies and those with congenital defects.

Statistical analysis was conducted using Student t-test. The differences was regarded as significant when p value was below 0.05.

Results

In group I, the mean gestational age at delivery was 36.1 weeks as compared to 37.8 weeks in control group. The mean newborns' birtweight from I group was 2460g and was lower than in the control group (2970 g). The mean Apgar score was 7 points and only 56% of newborns with diagnosed congenital infection presented good health condition at birth (Apgar 8–10). Among the infants with congenital infections (group I), the signs of IUGR were more prevalent. IUGR newborns made up 14% of this population while in the control group (II) as compared to 5% in control group. In group II all the newborns were born in good health condition and the mean Apgar score was 8.7. In this group, the mean erythroblast count was very low, 4.01 per 100 WBC, which was well below the value consider as normal. Only in two infants, delivered by cesarean section due to fetal distress the number of erythroblasts was found to amount to 20. The clinical parameters for all the 146 newborns examined are displayed in Table 1.

In the first blood test, the mean WBC count in the control group was significantly higher than in the study group (19.4±7.4 vs. 14±7.0) All the other morphological parameters, including RBC, Ht, Hb, and PLT were also had significantly higher except Hb. In group I, the mean PLT count was 150 000±50 000. In group II, normal CRP levels, mean 6.5 mg/l, were observed within the first 24 hours of life, while in group I, the respective levels were almost twice as high, 11 mg/l. Among the infants staying at the Intensive Therapy and Neonatal Pathology Unit, the mean nRBC count was 16.9 per 100 WBC (0–65). Table 2 shows haematological parameters of the study population.

On the first blood testing, 48% of the study group were found to have less than five nRBCs while in the controls this finding referred to as much as 69.6% of infants. Among the newborns with congenital infection 30.4% had more than 6 erythroblasts, up to 65erythroblasts. Detailed data are shown in Table 3.

In the group of newborns with the signs of intrauterine infection among 15 generalised infection were diagnosed and congenital pneumonia in 20 of newborns. Those with generalised infection presented nRBC level higher than normal (mean 15 blood cells per 100 WBC). There were also single cases of enteritis and cerebrospinal meningitis, with nRBC count of 26 and 65, respectively. No fatalities were recorded. The data on the study group are summarised in Table 4.

Discussion

Our data shows that out of 146 infants born at the Department of Perinatology, Medical University of Łódź,

Table 1. Demographic characteristics of the study population.

	Newborns with congenital infection: study group	Newborns with no infection signs: controls	P value
Number of infants n	31	115	
Mean gestational age	36.1	37.8	
Intrauterine growth restriction <percentile	14%	5%	0.220
natural birth/cesarean section	20/11	60/55	0.309
Mean newbrons birthweight	2460 g	2970 g	
Apgar score 8–10	56%	89%	<0.001
N. of newborns with hypoxia = umbilical artery pH ≤7.25	10	17	0.037
PROM >24 hours	9	0	<0.001

Table 2. Haematological parameters for the study population.

	Newborns with congenital infection: study group	Newborns with no infection signs: controls	P value
Number of infants n	31	115	
RBC	4.9±0.7	5.4±0.7	<0.001
WBC	14.2±9.1	19.4±7.4	0.006
Ht	47.5±0.7	50.3± 0.7	<0.001
mean Hb	16.9	17.6	NS
PLT	150 000±50 000	244 000±70 000	<0.001
nRBC	16.9 (0–65)	4.01 (1–20)	<0.001

Table 3. Proportion of infants with elevated nRBC count in each group examined.

	n RBC 0–5	nRBC >6
Study group	48.0% (n=15)	52.0% (n=16)
Control group	69.6% (n=80)	30.4% (n=35)

p=0.028.

Table 4. Mean nRBC count within the first 12 hours of life according to final clinical diagnosis.

Clinical diagnosis	Mean nRBC count
Congenital pneumonia n=20	10
Generalised infection n=15	15
Cerebrospinal meningitis n=1	65
NEC n=1	26

31 newborns were treated at the Intensive Care and Neonatal Pathology Unit with diagnosed intrauterine infection. In this group, 52% of newborns had an increased number of erythroblasts in blood test performed within the first 12 hours of life.

The level of nRBC in the newborns depends on their fetal age. In term infants who did not suffer from intrauterine fetal anoxia, the mean number of erythroblasts rarely exceeds 10 per 100 WBC [1,2]. In the reference population of healthy eutrophic newborns, that value was even lower and equaled 4.01/100 WBC.

Asphyxia is thought to be the main factor determining the increase in nRBC count both in fetal and infant blood [8,12–14]. Elevated level of nRBC in fetal blood is connected with increased concentration of erythropoetin, which induces erythropoesis. Tissue hypoxia leads to an increased production rate of erythropoetin in the liver and stimulates early release of RBC from bone marrow to the cardiovascular system [4,5,15]. The increase in the number of nRBC in newborn's blood may also be connected with intrauterine growth restriction [7], severe serological incompatibility [5] as well as maternal cigarette smoking [8] or diabetes [9].

Another factor that may contribute to an increased number of erythroblasts in newborn's blood directly after delivery is

the intrauterine infection [14]. In our project, the increase in nRBC count was higher among the infants with infection signs. The mean value was 16.9 nRBC/100 WBC, i.e. almost four times as high as in the group of healthy newborns. Acute chorioamnionitis results in an increase in erythropoietin concentration as well as in the number of erythroblasts in newborn's blood. Maier et al. reported a significantly higher level of erythropoietin concentration in newborns from pregnancies with the signs of placental inflammation in histopathology [14]. Leikin et al. observed an increase in nRBC count also in the cases where placental infection was indicated only by histological findings but no clinical symptoms were found [11]. Elevated level of nRBC was reported in premature newborns from pregnancies complicated with intrauterine infection without acidosis or hypoxemia [16]. Salafia et al. postulated that such an increase in nRBC count may be due to fetal response to inflammatory environment within the uterus when no concomitant hypoxia is present [17].

This assumption was confirmed by the outcomes of this project. Only 10 newborns (32%) were diagnosed with hypoxia, based on pH analysis of umbilical artery blood. Nicolini et al. as well as Bernstein did not find any differences in pH results for umbilical artery in their populations of newborns [18,19]. Blood analysis from the umbilical artery, especially BE and pH tests, is very helpful in monitoring the labour period. If anoxia develops earlier, these values remain at the normal level during delivery [19–21].

Hypoxia and intrauterine infection may affect the production rate of nRBC in the fetus as well as the Apgar score at birth. According to Hanion-Lundberg and Kirby [5] an inverse proportion can be found between Apgar score and the number of nRBC [6]. Our study supports this observation because in the infants with infection and elevated nRBC count and in healthy infants with nRBC count above the mean value for this group, the mean Apgar score was 7.2 points, and 56% of the infants presented good health condition at birth (Apgar 8–10).

In the blood test performed within the first 12 hours of life, no significant differences in WBC and RBC counts were found between the groups examined. The number of platelets was lower among the infants with infection signs amounting to 150 000±50 000,. In chronic hypoxia, increased erythropoiesis frequently hinders thrombocytopoiesis, which leads to a decreased level of thrombocytes after delivery [20,21]. In the study group of infants with congenital infection, a lower number of platelets and a higher number of nRBC could be found but with no signs of increased erythropoiesis in haematocrit test. Infected newborns presented higher levels of nRBC after delivery than those with no infection signs. In the case of infections turning into pneumonia, the average number of nRBC approximated the upper normal value and in generalised infections it was slightly above the normal level.

CONCLUSIONS

The determination of nRBC count can be used as an auxiliary marker in the diagnostics of congenital infection in preterm and term neonates. Moreover, it is a non-invasive method that helps elicit additional information to identify the high risk population.

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