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Investigation of Changes in Nucleated Red Blood Cells in Neonatal Infection

Hassan Boskabadi[1], Mohammad Hadi Sadeghian[2], Javad Sadeghinasab[1*]

1. Department of Pediatrics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Hematopathology, Ghaem Hospital, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

***Background*:** Early diagnosis is paramount to the treatment of neonatal infections. Nucleated red blood cells (NRBCs) are immature erythrocytes that increase in number due to stress and hypoxia. This study was conducted to determine NRBC count in the peripheral blood and examine its significance as a marker of neonatal infection.

***Methods*:** This case-control study was conducted on 154 infants (78 infants with infection as the case group and 76 infants without infection as the control group) admitted to Ghaem and Emam Reza hospitals, Mashhad, Iran. After a complete physical examination, a series of tests, including blood culture, complete blood count, C-reactive protein test, and peripheral blood smear, were performed on the infants; their NRBCs per 100 white blood cells (WBCs) were also counted.

***Results*:** The mean NRBC counts in the infants with and without infection were 30 and 3 per 100 WBCs, respectively ($P<0.001$). In cases with an NRBC count of more than 10, sensitivity and specificity were reported as 45% and 83%, respectively, and the positive and negative predictive values were 29% and 91%, respectively. The infants' NRBC count was directly correlated with their mortality.

Conclusion: NRBC count in the peripheral blood smear helps with the diagnosis of neonatal infection and can be used in conjunction with other laboratory tests as a simple and convenient method.

Keywords: Diagnosis, Infant, Nucleated red blood cells, Sepsis

Introduction

Neonatal sepsis is a major cause of neonatal hospitalization and mortality; timely diagnosis of neonatal infections can significantly contribute to their treatment. Neonatal sepsis is a serious condition associated with a high mortality rate, and it is considered a major challenge for pediatricians due to its non-specific symptoms and the absence of a definitive diagnostic test (1). The overall incidence rate of neonatal sepsis has been reported as 1-5 cases per 1000 live births. The risk of infection increases with reduced gestational age (2-5). In a study conducted at Ghaem Hospital of Mashhad, Iran, infection (25.3%) was identified as the fourth leading cause of death in infants with the following pattern: sepsis (80%), meningitis (12.2%), and pneumonia (4.8%) (6). The overall mortality rates of sepsis and meningitis were reported as 5-10% and 20-25%, respectively.

According to a report by the World Health Organization (WHO), annually about 2.7 million neonatal deaths occur on a global scale (7), in more than 35% of which infectious agents are implicated. Premature and low-birth-weight (less than 1000 g) neonates are at high risk for infection, experience prolonged hospitalization, and might need catheters or other invasive procedures during their hospitalization (8, 9). Most cases of sepsis occur due to the transmission of pathogens during pregnancy through the maternal genital tract to the amniotic fluid or from the mother to the baby during delivery (10). Maternal chorioamnionitis is also a known factor causing early neonatal sepsis (10).

The symptoms of sepsis include fever, poor feeding, vomiting, dyspnea, and restlessness,

* *Corresponding author*: Javad Sadeghinasab, Department of Pediatrics, Faculty of Medicine, Mashhad University o Medical Sciences, Mashhad, Iran. Tel: 09155337031; Email: sadeghinj921@mums.ac.ir

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and its signs are tachycardia, thermal instability, tachypnea, lethargy, seizure, and convulsion (11). Timely diagnosis of sepsis is important because it can cause irreversible complications among newborns. Treatment should therefore begin with the suspicion of sepsis and the treatment team should not wait for the laboratory results to come back to proceed with the treatment. However, it is worth mentioning that the irrational and indiscriminate administration of antibiotics can cause antibiotic resistance and medication side effects.

Laboratory diagnostic tests for neonatal sepsis include complete blood count with differential (CBC diff), blood culture, cerebrospinal fluid (CSF) culture, and C-reactive protein (CRP) (12). Blood culture is the gold standard for the diagnosis of neonatal sepsis (12-15). Biochemical markers, such as inflammatory cascades (i.e., IL-6 IL-8, acute-phase proteins, and procalcitonin), can help confirm the diagnosis of this condition (11, 16). One of the markers assessed in different studies on infants is nucleated red blood cell (NRBC) count. NRBCs are immature erythrocytes released from the bone marrow as a result of stress. Various studies have found that NRBC count increases in several diseases, such as asphyxia (1).

Nonetheless, few studies have examined the relationship between NRBC count and infection. In a study by Abhishek, NRBC count was significantly higher in infants with early neonatal sepsis than in control group (17). The present study was carried out to examine the relationship between NRBC count and neonatal infection.

Methods

This case-control study was conducted on 154 infants admitted to Ghaem and Imam Reza Hospital, Mashhad, Iran, during 2014-2016. The research project was approved by the Ethics Committee of the university and written informed consents were obtained from the infants' parents. Infants with the clinical signs of infection (i.e., lethargy, apnea, respiratory distress, restlessness, seizure, need for mechanical ventilation, abdominal distention, hypotension, and food intolerance) were assigned to the case group (11).

For the neonates with suspected infection, blood, urine, and CSF cultures were first performed followed by the CBC and CRP tests. Blood glucose, calcium, sodium, and potassium levels were assessed, and blood gas analysis was performed if ordered by the treating physician. After the full examination of the infants by the pediatrician's assistant and requesting immediate treatment and diagnostic measures, the full details of the infants (i.e., birth weight, current weight, age, gender, gestational age, Apgar score, and clinical symptoms), maternal medical history (i.e., age, pregnancy and delivery problems, mode of delivery, and parity), risk factors for infection, and laboratory results were collected and recorded by a pediatric resident.

The routine tests performed included the assessment of infection, jaundice, and CBC, as well as peripheral blood smear. To perform CBC, 1 cc of blood was collected from the subjects and poured into tubes containing EDTA anticoagulant, and then analyzed with Sysmex automated cell counter. In the next step, peripheral blood smears were prepared to control the device results and the cells were morphologically evaluated and separated. It should be mentioned that NRBC count was reported per 100 WBCs observed. After obtaining parental consent, the controls were selected from normal neonates admitted only for unexplained jaundice and with a need for CBC.

The laboratory signs confirming the presence of infection include leukocytosis (WBC > 20000 or leukopenia ≤ 5000), thrombocytopenia (Plt ≤ 150000), and positive CRP (≥ 6 mg/dl). After receiving the test results, the case group was divided into three subgroups, namely definitive infection group (positive blood or CSF cultures), clinical infection group, and possible infection group. The infants with positive blood or CSF culture with at least two clinical symptoms were considered as cases of definitive infection. Infants with at least three clinical symptoms or one clinical symptom plus laboratory signs were considered as cases of clinical infection. Infants with less than three symptoms and no positive laboratory symptoms were assigned to the possible infection group. The exclusion criteria comprised of 5-minute Apgar score of less than 7, congenital malformations, TORCH infections, underlying diseases (e.g., cardiovascular, renal, and gastrointestinal), diabetic mothers or mothers with pre-eclampsia, evidence of hemolytic anemia, and erythroblastosis fetalis.

To analyze the data, Mann-Whitney U test, Student t-test, Chi-square test, and logistic regression were run in SPSS software. The diagnostic value, sensitivity, and specificity of

NRBC count and the ratio of immature neutrophils to total neutrophils (I/T ratio) were evaluated for the differentiation of sick and healthy neonates, definitive diagnosis of infection, and assessment of the relationship of NRBC count with prognosis and mortality. P-values less than 0.05 were considered statistically significant.

Results

The control group consisted of 43 (56.6%) boys and 33 (43.4 %) girls, and the case group consisted of 32 (41%) girls and 46 (59%) boys. The case and control groups were not significantly different in terms of maternal age, gender, and mode of delivery (P>0.05). The only cause of hospitalization in the control group was neonatal jaundice. In the case group, the most common cause of hospital admission was grunting. Table 1 presents the causes of neonatal hospitalization in the case group.

Table 1. Etiology of neonatal hospitalization in the case group

Cause of hospitalization	Number	Percentage
Grunting	42	55
Apnea	19	25.6
Lethargy	3	3.84
Very low birth weight	3	3.84
Restlessness	2	2.56
Poor feeding	2	2.56
Abdominal distention	2	2.56
Vomiting	1	1.28
Total	78	100

The case and control groups were compared in terms of NRBC count. The NRBC counts were reported as 3 and 30 per 100 WBCs in infants without and with infection, respectively. The minimum and maximum NRBC counts were 0 and 35 in the control group and 0 and 770 in the case group, respectively. Mann-Whitney U test was run to compare the means between the case and control groups; according to the test results, the difference between the two groups was statistically significant (P<0.0001). A comparison was made between the case and control groups in terms of the dispersion parameters of NRBCs, which is summarized in Table 2.

The total number of positive blood cultures in the case group, which indicates a definite infection, was 20 (26%). The rest of the subjects had clinical sepsis (n=24) or possible sepsis (n=34). To assess the relationship between NRBC count and prognosis, the infants were divided into three groups as follows: Group 1: infants discharged with complete recovery and with no side-effects; Group 2: neonates discharged with long-term hospital complications including retinopathy of prematurity (ROP) or broncho-pulmonary dysplasia (BPD); and Group 3: deceased infants.

Table 2. Dispersion parameters of nucleated red blood cells in the case and control groups

Group	Control	Case
Variance	26.4	8562.4
Standard deviation	5.13	92.5

The regression curve for ANOVA was used to investigate the relationship between NRBC count and prognosis (Figure 1). According to the results, there was a significant relationship between prognosis and NRBC count (P=0.002), and prognosis worsened as NRBC count increased.

Of all the infants in the case group, 24 (30.7%) died. The regression curve was used to investigate the association between mortality and NRBC count. According to this test, there is a strong relationship between mortality and NRBC count (P<0.0001).

To assess the sensitivity, specificity and positive predictive value of NRBC in the prediction of neonatal infection, statistical tests was performed and compared with the gold standard test.

The data analysis showed that if the cut-off point for the NRBC count is taken to be 10, its sensitivity, specificity, and positive and negative predictive values for the diagnosis of neonatal sepsis will be 45%, 83%, 29%, and 91%, respectively.

In the present study, nine infants were born to mothers with premature rupture of membranes (PROM). Mann-Whitney U test reflected a significant relationship between PROM and NRBC count (P=0.048); however, the association was not very strong.

Examining the CRP rates in the case group and their comparison with the gold standard showed that, considering the diagnostic cut-off point of CRP as 15 mg/dL, the sensitivity and specificity of the test for the diagnosis of sepsis would be 77.8% and 81.2%, respectively. The receiver operating characteristic (ROC) curve was plotted for each test so as to measure the CRP values, I/T ratio, and NRBC count for the diagnosis of sepsis (Figure 2).

The mean platelet counts in the case and control groups were 211,000 and 301,000 per mm^3, and the difference between them was statistically significant (P<0.001). There was also an inverse and significant relationship between platelet count and neonatal mortality rate (P<0.004).

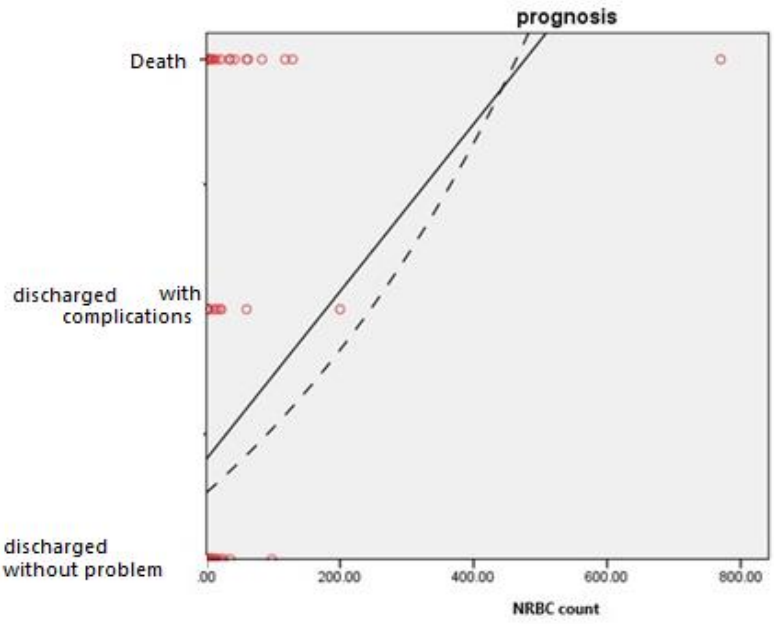

Figure 1. Relationship between the nucleated red blood cells count and prognosis

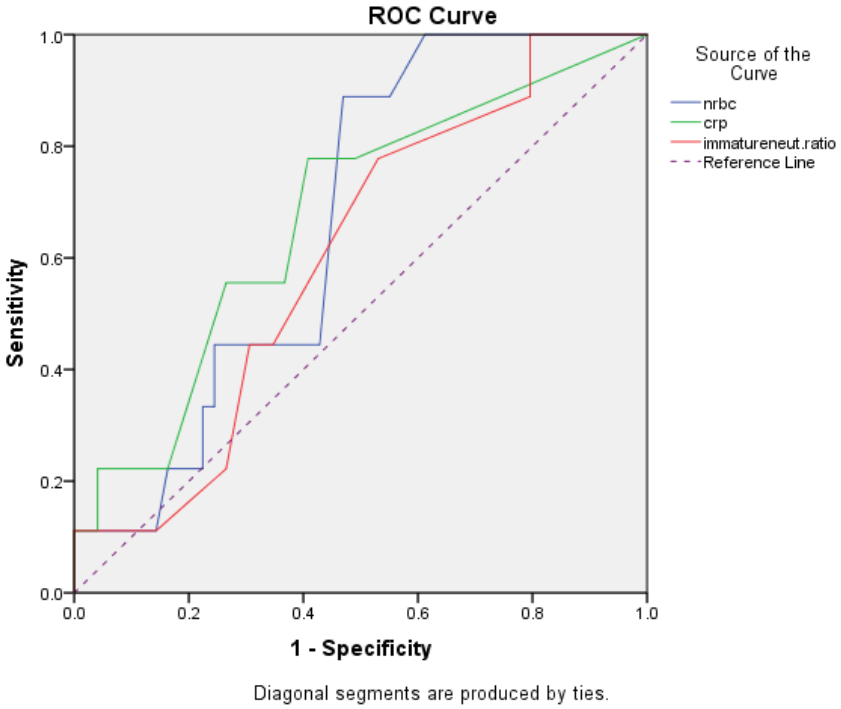

Figure 2. Receiver operating characteristic curve for the immature neutrophils to total neutrophils ratio, C-reactive protein, and nucleated red blood cell count/100 white blood cells

Discussion

According to the results of this study, NRBC count is a simple and valuable marker for the diagnosis of neonatal infection. Neonatal infection is a prevalent problem throughout the world. According to a WHO report, annually about 2.7 million neonatal deaths occur worldwide, with more than 35% of them being caused by infectious agents (7). Premature and

low-birth-weight (less than 1000 grams) neonates are at higher risk for infection(1, 10). Different diagnostic tests have been developed for neonatal infection. Some of these tests are readily available and some are expensive and not available in all healthcare centers. The most valuable tests are those that are easily accessible, accurate, and cost-effective and cause minimum harm to patients. The laboratory methods used for the diagnosis of sepsis include direct (e.g., blood, urine, and CSF cultures) and indirect (e.g., leukocyte count, I/T ratio, ESR, and CRP) methods (11).

NRBCs, which are the precursors of erythrocytes, are released from the bone marrow in response to stress. Studies have so far examined the role of NRBCs in the diagnosis of neonatal diseases. An increase in the NRBC count has been associated with conditions such as asphyxia, gestational diabetes, and adult sepsis (18). Former studies have also investigated the role of NRBCs in the prognosis of sepsis. In the present study, the mean NRBC count was about ten times higher in the infection group than in the control group.

In a study conducted on preterm infants with early sepsis caused by maternal chorioamnionitis, a significant difference was observed between healthy and infected groups. By excluding the effect of erythropoietin (EPO), cortisol, and acid-base disorders, the researchers finally came to the conclusion that inflammation alone plays an independent role in increasing NRBC count in preterm infants (19). Sepsis is an inflammatory reaction that places the body under stress. In this condition, the inflammation associated with stress causes the production of inflammatory mediators, including interleukins (11). In a number of studies, selective erythroid hyperplasia was observed in the bone marrow by administering interleukin-6 to adult animals after 12 hours (during this period, this event could not have been caused by the production of EPO). Similarly, an increased NRBC count associated with the elevated production of interleukins 3, 6 and 12 has been reported in critically-ill patients (12). There is therefore, a direct and positive correlation between the increased production of interleukin-6 (an inflammatory mediator) and a rise in NRBC count (19).

To eliminate the effect of asphyxia, the neonates with a 5-minute Apgar score of below 7 were excluded from the present study. According to the regression curve and the ANOVA test, the relationship between NRBC count and 5-minute Apgar score was significant. Boskabadi and Hermansen examined changes in NRBC count in neonates with asphyxia in two separate studies and a significant relationship was observed between NRBC count and asphyxia in both studies. Moreover, NRBC count was found to have a positive correlation with the severity of asphyxia, which confirms the present findings (17, 20).

In the present study, a strong correlation was observed between infant mortality and NRBC count. The diagnostic cut-off point for NRBC count to predict infant mortality was higher than 7 per 100 WBCs. This cut-off point yielded a sensitivity of 65% and a specificity of 85%, while its positive and negative predictive values were 44% and 93%, respectively. In the study by Abhishek, NRBC count was also significantly higher in infants with early neonatal sepsis than in controls (17). In the study by Kil conducted on very-low-birth-weight infants, NRBC count was significantly associated with neonatal morbidities, such as intraventricular hemorrhage and necrotizing enterocolitis, and neonatal mortality (21).

Cremer and Baschat studied the relationship between NRBC count and the prognosis of premature infants in two separate studies. They found a direct correlation between high NRBC count and poor prognosis in infants (22, 23). Shah and Desai also found in two separate studies on adults that increased NRBC count is directly correlated with worsened prognosis in adult patients with sepsis (24, 25). Nonetheless, in a study by Wirbelauer, NRBC count was not significantly correlated with the incidence of infections (26).

In the present study, considering the diagnostic cut-off point of NRBC count being 20 per 100 WBCs, the sensitivity and specificity of the test for the diagnosis of sepsis were 33% and 88%, respectively. If the diagnostic cut-off point for the CRP is taken as 15 mg/dL, its sensitivity, specificity, and negative predictive value for sepsis diagnosis will be 77.8%, 81.2%, and 97.8%, respectively. If the diagnostic cut-off point for the I/T ratio is 0.51, its sensitivity, specificity, and negative predictive value will be 83%, 77%, and 98.9%, respectively. In the study by Borna et al., the sensitivity of CRP for the diagnosis of neonatal sepsis was 79% and its specificity was 85%. The positive predictive value of CRP was also reported as 36% (27). In other studies, low sensitivity and high negative

predictive values were reported for CRP, while its cut-off point for the diagnosis of neonatal sepsis was 9.5 mg/dL. As for I/T ratio, some studies reported the sensitivity of 90% and negative predictive value of 98% (27).

In the present study, the diagnostic value of NRBC count was higher than those of I/T ratio and CRP. After plotting the ROC chart, the areas under the NRBC, I/T ratio, and CRP curves were calculated as 0.682, 0.653 and 0.67, respectively. In terms of accuracy, NRBC count was more accurate than CRP test and CRP test was more accurate than I/T ratio.

Hemoglobin levels in the case and control groups were 15.114 and 15.119 g/dL, respectively, in the present study; however, the difference was not significant. In a study by Maabood et al., hemoglobin level was reported to be lower in sepsis group compared to controls (10.1 g/dL vs. 13.8 g/dL) (27, 28).

The limitation of this study lies in the selection of healthy preterm infants. Even after performing ANCOVA and removing the variable of gestational age, NRBC count was still significantly different between the case and control groups.

Conclusion

Based on the results of this study, NRBC count is a simple, available, and fast-responding capability factor that can help with the early diagnosis of neonatal infections. In conjunction with other laboratory tests, this test can contribute to the diagnosis of infections, even before the blood culture results are known.

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