To Evaluate Diagnostic Efficacy of Nucleated Red Blood Cells (NRBCs) in Neonatal Sepsis

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ABSTRACT

BACKGROUND
Early diagnosis of sepsis in neonates is cardinal. The isolation of the organism from the body fluids is time-consuming and does not reliably exclude the infection even if it is negative. Nucleated red blood cells (NRBCs) are immature erythrocytes that are elevated in association with foetal distress, hypoxia, and inflammation. The purpose of the study was to evaluate the diagnostic efficacy of nucleated red blood cells (NRBCs) in the detection of neonatal sepsis.

METHODS
This was a prospective study which was conducted in the section of haematology, Department of Pathology of Shri Guru Ram Rai Institute of Health and Medical Sciences, Dehradun, Uttarakhand. A total of 92 term neonates admitted to NICU with clinical features and risk factors of sepsis from October 2020 to March 2021 were included in the study. All study neonates were classified into proven sepsis, probable clinical sepsis and no sepsis group. The NRBC count and haematological parameters were obtained by a fully automated analyzer Sysmex XN1000 and corroborated with a peripheral blood smear. The data obtained were analyzed using one-way ANOVA with Fisher’s LSD multiple comparison tests or t-test using GraphPad Prism 7 software. A P-value of 0.001 was taken to be statistically significant.

RESULTS
Among the 92 cases studied, 23 were culture-positive sepsis, 31 were of probable clinical sepsis or were culture-negative and the rest 38 cases had no sepsis. In the present study, the difference in NRBC count between the sepsis and no sepsis group was statistically significant (P-value < 0.001). The sensitivity, specificity, positive predictive value and negative predictive value of NRBC count for sepsis were found to be 77.78 %, 60.52 %, 73.68 % and 65.71 % respectively. The average NRBC count in the mortality group was higher than in live neonates.

CONCLUSIONS
The NRBC count showed comparable or higher sensitivity than haematological parameters. Higher NRBC counts in the mortality group correlated with adverse neonatal outcomes, hence carrying a prognostic value.

KEY WORDS
Neonatal Sepsis, Nucleated Red Blood Cells, Haematological Parameters
Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremia in the first month of life. World Health Organization estimates that of the four million neonatal deaths all over the world every year, over 35% are due to infection in the neonatal period. The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-03, India) is 30 per 1000 live births. The gold standard for the diagnosis of neonatal sepsis is the growth of pathogenic microorganisms in body fluids. It may be time-consuming and failure to produce an organism does not exclude the diagnosis. This may be due to antenatal antibiotic use and inadequate sampling.

Thus, for the early diagnosis of neonatal sepsis, a diagnostic test is required which is accessible, accurate, cost-effective and minimally invasive to neonates. Systemic inflammatory response syndrome (SIRS) has a central role in the pathogenesis of sepsis. Recently the role of molecular markers like cytokines (interleukin 6, interleukin 8, TNF alpha), cell surface antigens (CD11b, CD64, sCD163) etc are being investigated.

Sepsis is associated with an inappropriate immune response and on account of increased cytokine release, is accompanied by an increased nucleated red blood cell (NRBC) production. Specifically, interleukin-6 (IL-6) is involved. This implies that the foetal inflammatory response and foetal distress have distinct roles in NRBC production and/or release in the peripheral circulation.

Nucleated RBCs are in the peripheral blood of normal infants up to the fifth day of life. At birth, 3-10 NRBCs per 100 WBC are present. Mean value of NRBCs in the first few hours of life in healthy term newborns is about 500 NRBCs/mm3, and a value above 1000 NRBCs/mm3 can be considered elevated. Expressed differently, 0-10 NRBCs/100 WBCs are typical, and values above 10 NRBCs/100 WBC are considered elevated. The present study intended to evaluate the diagnostic efficacy of nucleated red blood cells in neonatal sepsis at a tertiary care teaching hospital.

This was a prospective study which was conducted in the section of haematology, Department of Pathology of Shri Guru Ram Rai Institute of Health and Medical Sciences for a period of 6 months from October 2020 to March 2021.

**Inclusion Criteria**

All term neonates admitted to NICU with clinical features or risk factors of sepsis within the first 28 days of life were included in the study. The onset of symptoms within the first 72 hours of life was labelled as early-onset sepsis (EOS) and after 72 hours as late-onset sepsis (LOS).

**Exclusion Criteria**

- Neonates with inborn errors of metabolism.
- Neonates with congenital anomalies.
- Birth asphyxia
- Maternal preeclampsia and edema
- Gestational diabetes mellitus
- Placenta previa, abortion or infarct.
- Pre-term / Post-term babies.
- Haemolytic disease of the newborn (ABO/RH incompatibility).
- Intrauterine growth retardation

All the neonates selected for the study were evaluated in particular, comprising detailed history including maternal details and risk factors for sepsis, clinical examination, and relevant investigations.

**Clinical Criteria for Neonatal Sepsis**

Neonates present with feeding problems, respiratory distress, lethargy, irritability, convulsions, abdominal distension, recurrent attacks of apnoea or cyanotic spells, vomiting, poor cry and tachypnoea. Neonates with maternal history of fever within 2 weeks before delivery, foul-smelling or meconium-stained liquor, rupture of membranes >24 hours, single unclean or more than 3 sterile vaginal examination during labour and prolonged labour.

Venous, arterial or umbilical cord blood samples were collected in EDTA anticoagulated vacutainers using all aseptic precautions. Following investigations were done in all the cases.

Complete blood count and NRBC count were performed by automated quantitative haematology analyzer SYSMEX XN1000 in all the cases. Peripheral blood smears were prepared and stained by using Leishman stain. The test values obtained by fully automated quantitative haematology analyzer SYSMEX XN1000 were corroborated with a peripheral blood examination. Degenerative changes in neutrophils like toxic granules and cytoplasmic vaculations were looked for in all the cases.

**Sepsis Screen**

The components of the sepsis screen included total leukocyte count (TLC), absolute neutrophil count (ANC), immature / total neutrophil ratio (I/T), micro ESR and C-reactive protein (CRP). Due to the non-availability of the micro-ESR test, it was not done in any of the patients. Following cut off values of NRBC and other haematological parameters were taken into consideration (Table 1).

<table>
<thead>
<tr>
<th>Components</th>
<th>Cut off value</th>
</tr>
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<tbody>
<tr>
<td>Nuclated RBC</td>
<td>&gt;10/100 WBC</td>
</tr>
<tr>
<td>Total Leukocyte Count</td>
<td>≤ 5000/mm or ≥ 25000, 30000 and 21000/mm at birth, 12-24 hr and 2 days onwards, respectively</td>
</tr>
<tr>
<td>Immature to Total neutrophil ratio (I/T)</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Absolute Neutrophil count (ANC)</td>
<td>Low counts as per Marnie’s Charts(14) for term and Mouzin’s charts(15) for very low birth weight infants</td>
</tr>
<tr>
<td>C-reactive protein (CRP)</td>
<td>&gt;6mg/dl(16)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>≤ 150,000/mm(17)</td>
</tr>
</tbody>
</table>

**Table 1. Cut Off Values for NRBC and Haematological Parameters**

A positive screen was taken if two or more parameters were abnormal. If negative with high clinical suspicion, sepsis screen tests were repeated within 12 hours. If the screen was still negative, the diagnosis of clinical sepsis was excluded.
Statistical Analysis

Mean and Standard Deviations were calculated from the data obtained. The data were analyzed using one-way ANOVA with Fisher’s LSD multiple comparison tests or t-test using GraphPad Prism 7 software for sensitivity, specificity and positive predictive value (PPV) and negative predictive values (NPV) of haematological parameters and NRBCs. A P-value of 0.001 was taken to be statistically significant.

RESULTS

A total of 92 consecutive neonates were enrolled in the study. Out of which, 72 neonates (78.26 %) were < 72 hours old and were suspected to have early onset sepsis (EOS). The remaining 20 (21.73 %) were > 72 hours old presenting with clinical features of sepsis and were suspected to have late-onset sepsis (LOS). Of 92 neonates, 55 (59.8 %) were males and 37 (40.2 %) were females. (Table 2). 52 neonates were born by normal vaginal delivery (NVD) and 40 by lower segment caesarean section (LSCS).

<table>
<thead>
<tr>
<th>Age of Neonate</th>
<th>Sepsis (1A+1B)</th>
<th>No Sepsis (2)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;72hrs</td>
<td>44</td>
<td>28</td>
<td>72(78.26%)</td>
</tr>
<tr>
<td>&gt;72hrs</td>
<td>9</td>
<td>11</td>
<td>20(21.73%)</td>
</tr>
<tr>
<td>Gender Distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (M)</td>
<td>33</td>
<td>27</td>
<td>60(65.21%)</td>
</tr>
<tr>
<td>Female (F)</td>
<td>21</td>
<td>11</td>
<td>32(34.79%)</td>
</tr>
</tbody>
</table>

Table 2. Age and Gender Distribution

The most common maternal risk factor for sepsis was foul-smelling liquor and meconium-stained liquor (21 cases each) followed by maternal fever (16 cases), premature rupture of membranes (11 cases) and 5 cases had a history of unclean vaginal examination (PV >3). The most common symptomatology among sepsis group neonates was respiratory distress and refusal to feed.

Of the 92 neonates, 23 cases were culture-positive sepsis, 31 cases were of probable clinical sepsis or culture-negative and the rest 38 cases were negative for sepsis (no sepsis). Out of 54 cases in the sepsis group, 43 neonates were of low birth weight with a mean of 2.41 kg ± 0.41 SD. The mean birth weight of cases without sepsis was 2.75 kg ± 0.44 SD.

Among the culture-positive cases, Klebsiella pneumoniae (21.74 %) was the most common organism isolated followed by Staphylococcus aureus (13.04 %) and Escherichia coli (13.04 %) (Table 3).

The NRBCs were found to be elevated in 57 cases and were not elevated in 35 cases. The NRBCs >10/100 WBC were observed in 20 culture-positive cases, 22 culture-negative cases, and 15 cases without sepsis. Mean values of NRBCs in each group are tabulated in Table 4. The difference in NRBC count among the sepsis group (1A culture-positive and 1B probable sepsis) and no sepsis group (group 2) was statistically significant. (P-value < 0.001)

Nine (9.8 %) neonates expired of which 3 had sepsis screen positive and 6 were culture positive. The average NRBC count in the mortality group was 22.8 ± 12.83, which was higher than the mean observed in the live neonates. NRBC count decreased with the maturity of the neonate.

We compared other haematological parameters between sepsis (blood culture positive and negative blood culture with positive sepsis screen) and no sepsis group (blood culture as well as sepsis screen negative) (Table -5, 7). The results of various index tests obtained in each study group are tabulated in Table 6.
In our study, the incidence of neonatal sepsis was higher in males 61.11 % (33/54) than in female neonates. Other studies by Chandra et al. and Antoniette et al. have reported similar findings.[18,19] This is probably because the factors regulating the synthesis of gamma globulin are situated on the X-chromosome and male has only one X-chromosome.[18]

In the Indian scenario, high male: female birth ratio adds to the high rate of neonatal sepsis in male children.[20]

Out of 54 neonates from the sepsis group, 43 (79.6 %) were low birth weight. The difference between the weights of the sepsis and no sepsis group was statistically significant (P-value < 0.001). The incidence of sepsis was high in the low-birth-weight neonates as evident in other studies.[20,21] According to Barbara Stoll et al. the rate of infection is inversely proportional to the birth weight, and low 1&5 levels due to impaired cellular immunity in the very low birth weight neonates contribute to increased susceptibility to infections in these neonates.[22]

69.56 % (16/23) of neonates presented within 72 hours of birth in culture-positive cases and 56.52 % of them (13/23) presented within 24 hours of birth. Respiratory distress (73.6 %) was the most frequent symptom associated with sepsisemia. Meconium-stained liquor and maternal fever were the most frequent maternal risk factors reported.

Culture positivity in the present study was 25 % (23/92). Priyanka et al. reported 27 % positivity.[23] The low yield of culture may be attributed to the administration of antibiotics during the last trimester, before delivery or in intrapartum and at-risk cases.[24] We had 33.69 % (31/92) neonates in the probable sepsis group who had sepsis scores positive but were culture negative.

In our study, gram-negative organisms were the predominant causative organisms comprising 60.86 % (14/23) of culture-positive cases. Similar findings were observed by Patel et al.[25] and Mondal et al.[24]

The sensitivity, specificity, positive predictive value and negative predictive value of NRBC count for sepsis were found to be 77.78 %, 60.52 %, 73.68 % and 65.71 % respectively. These were comparable to various other studies (Table-8)

Dulay et al. demonstrated that cytokines released in sepsis have an important role in stimulating NRBC production independent of hypoxia. In the study, elevated NRBC was demonstrated in E05 along with significantly elevated IL-6. They found no increase in the level of umbilical cortisol or erythropoietin.[27]

Table 8: Comparative performance of NRBCs in various studies

A mortality of 16.67 % was reported in the sepsis group (9/54) in the present study. NNPD (2002-2003) reported neonatal mortality of 18.6 % due to infection.[3] The average NRBC count was on the higher side in the mortality group with a mean ± SD of 22.8 ± 12.83. Higher NRBC count correlated with adverse neonatal outcomes. In the study by Kil, conducted on very low birth weight infants, it was observed that an increase in the NRBC count, had a significant correlation with severe intraventricular haemorrhage, necrotizing enterocolitis, and perinatal death.[30]

The difference in haemoglobin in cases with sepsis and without sepsis was statistically significant (P-value < 0.001). In a study by Maabood et al., haemoglobin level was reported to be lower in the sepsis group compared to controls (10.1 g/dl vs. 13.8 g/dl).[31]

In the present study, the sensitivity of TLC was low (56.60 %) comparable to other studies.[32,33] The specificity of ANC was high (89.47 %) with high PPV (89.75 %) and sensitivity was low (64.81 %). Our results were comparable with Buch et al. and Priyanka T et al.[23] In some studies, neutropenia was more significant.[23]

Increased IT ratio showed high sensitivity (72.2 %), specificity (89.47 %), PPV (79.06 %) and moderately high NPV (69.38 %) which was comparable to other published results. It is the single most helpful test in diagnosing neonatal sepsis, as high NPV rules out sepsis.[23]

In the case of CRP, multiple studies have reported higher sensitivities.[34] We also reported a sensitivity of 77.78 % and specificity of 65.78 % compared with other studies. Himayun concluded that a single CRP value done at the time of admission lacked sensitivity. Sensitivity increased with serial testing, serial reducing values indicate discontinuation of antibiotic therapy.[35]

The thrombocytopenia lacked sensitivity (37.04 %) with high specificity (81.51 %) comparable to other studies.[3] Thrombocytopenia is thought to be due to increased platelet destruction, sequestration secondary to infections, failure in platelet production due to reduced megakaryocytes or damaging effects of endotoxin.[36] Thrombocytopenia was found in 56.5 % of culture-positive cases in our study.

The degenerative changes in neutrophils lacked sensitivity (58.82 %) but had high specificity of 89.47 %. The presence of toxic granules indicates the production of unusual PMNs during infection and stress-induced leucopoiesis as they are never seen in healthy babies. Their presence invariably indicates sepsis, but their count is not always increased.[36]

The variations in the results shown by different studies may be due to the differences in blood sampling time, the severity of infections, the age of neonates and reduced sensitivity of these tests in the first week of life.[23]
CONCLUSIONS

NRBC count showed comparable or higher sensitivity in contrast to other haematological parameters of sepsis screen. Higher NRBC counts in the mortality group correlated with adverse neonatal outcomes, hence carrying a prognostic value. NRBC count can be used as a simple, rapid and cost-effective test in the detection of early neonatal sepsis. A combination of NRBC count with other haematological parameters including components of sepsis screen can improve sensitivity and specificity as no single haematological parameter at present is sufficiently accurate or reliable in identifying an infected infant.

REFERENCES

