International Journal of Medicine
pISSN 1468-3814 | eISSN 2667-7008
https://theinternationalmedicine.com/

Original Researcher Article *DOI: 10.61336/im/26-05-152*

A PROSPECTIVE OBSERVATIONAL STUDY ON THE EFFICACY OF NUCLEATED RED BLOOD CELLS AS AN EARLY DIAGNOSTIC BIOMARKER FOR NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL.

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Received: 08-04-2026
Revised: 24-04-2026
Accepted: 15-05-2026
Published: 29-05-2026

Abstract

Background: Neonatal sepsis remains one of the leading causes of neonatal morbidity and mortality worldwide, particularly in developing countries such as India. Early diagnosis is challenging because the clinical manifestations are nonspecific and conventional diagnostic methods, including blood culture, require prolonged processing time and may have limited sensitivity. Therefore, there is a growing need for simple, rapid, and cost-effective biomarkers for the early diagnosis of neonatal sepsis. Nucleated red blood cells (NRBCs), released into the peripheral circulation during systemic inflammation and bone marrow stress, have recently emerged as a potential early diagnostic marker. ***Aim:*** To evaluate the efficacy of nucleated red blood cells (NRBCs) as an early diagnostic biomarker for neonatal sepsis among neonates admitted to a tertiary care hospital. ***Materials and Methods:*** A hospital-based prospective observational study was conducted in the Neonatal Intensive Care Unit of a tertiary care teaching hospital. A total of 60 neonates were enrolled, comprising 30 neonates with clinically suspected or confirmed neonatal sepsis and 30 healthy controls. Clinical details and demographic characteristics were recorded using a structured proforma. Blood samples were collected before initiation of antibiotic therapy for complete blood count, peripheral smear examination for NRBC count, C-reactive protein (CRP), blood culture, absolute neutrophil count, immature-to-total neutrophil ratio, and platelet count. NRBC count was expressed as the number of nucleated red blood cells per 100 white blood cells. Data were analyzed using IBM SPSS version 26. Independent Student's t-test, Mann–Whitney U test, Chi-square test, Fisher's exact test, Spearman correlation analysis, and receiver operating characteristic (ROC) curve analysis were used. A p-value <0.05 was considered statistically significant. ***Results:*** The mean gestational age and birth weight were significantly lower among septic neonates compared with controls ($p<0.05$). Poor feeding (73.3%) and lethargy (63.3%) were the most common clinical manifestations. Septic neonates had significantly higher total leukocyte count, immature-to-total neutrophil ratio, CRP levels, and significantly lower platelet counts than controls ($p<0.001$). The median NRBC count was significantly higher in the sepsis group (14/100 WBCs) than in controls (3/100 WBCs) ($p<0.001$). An NRBC count ≥8/100 WBCs were observed in 83.3% of septic neonates compared with only 10.0% of controls. NRBC count showed a strong positive correlation with CRP ($r=0.64$) and I/T ratio ($r=0.57$) and a negative correlation with platelet count ($r=-0.46$). ROC curve analysis demonstrated an area under the curve of 0.91, with a sensitivity of 83.3%, specificity of 90.0%, positive predictive value of 89.3%, negative predictive value of 84.4%, and overall diagnostic accuracy of 86.7% for an NRBC cut-off value of ≥8/100 WBCs. ***Conclusion:*** Nucleated red blood cell count is an effective, rapid, inexpensive, and readily available adjunctive biomarker for the early diagnosis of neonatal sepsis. Elevated NRBC counts were significantly associated with established laboratory markers of infection and culture-proven sepsis, demonstrating excellent diagnostic performance. Incorporation of NRBC estimation into routine hematological evaluation may facilitate early identification of neonatal sepsis and timely initiation of treatment, particularly in resource-constrained healthcare settings. Further large-scale multicentric studies are warranted to validate its routine clinical application.

Keywords: Neonatal sepsis; Nucleated red blood cells; NRBC; Early diagnosis; Biomarker; Blood culture; C-reactive protein; Neonatal intensive care unit.

INTRODUCTION

Neonatal sepsis is a life-threatening systemic inflammatory syndrome occurring in infants during the first 28 days of life and remains one of the leading causes of neonatal morbidity and mortality worldwide. Despite remarkable advances in neonatal intensive care, antimicrobial therapy, and supportive management, neonatal sepsis continues to contribute substantially to neonatal deaths, particularly in low- and middle-income countries.

According to recent global estimates, infections account for approximately 15–20% of all neonatal deaths, with the greatest burden occurring in South Asia and sub-Saharan Africa. Early diagnosis remains a major clinical challenge because the initial clinical manifestations are often subtle, nonspecific, and overlap with several non-infectious neonatal conditions. [1], [2]

Globally, an estimated 2.3–2.5 million neonatal deaths occur annually, and sepsis remains one of the preventable causes of mortality. Early-onset neonatal sepsis, occurring within the first 72 hours of life, is commonly acquired from maternal genital tract organisms, whereas late-onset sepsis is predominantly acquired from hospital or community sources. Prematurity, low birth weight, prolonged rupture of membranes, maternal fever, invasive procedures, prolonged hospitalization, and inadequate aseptic practices are recognized risk factors. Delayed diagnosis frequently results in septic shock, disseminated intravascular coagulation, multiorgan dysfunction syndrome, prolonged hospitalization, and increased mortality. [2], [3] India bears one of the highest burdens of neonatal sepsis worldwide owing to its large birth cohort and higher prevalence of maternal and neonatal risk factors.

Reports from the National Neonatal Perinatal Database and multicentric studies have demonstrated that neonatal sepsis is among the commonest causes of neonatal intensive care unit (NICU) admissions and neonatal deaths. The incidence of culture-proven neonatal sepsis in India is considerably higher than that reported in developed nations, with Gram-negative organisms such as Klebsiella pneumoniae, Escherichia coli, and Acinetobacter species predominating. The emergence of multidrug-resistant organisms has further complicated early diagnosis and management, emphasizing the need for rapid, inexpensive, and reliable diagnostic biomarkers. [3], [4]

Blood culture remains the gold standard for confirming neonatal sepsis; however, it has several inherent limitations. The diagnostic yield is affected by small blood sample volume, prior administration of antibiotics, intermittent bacteremia, and prolonged turnaround time of 48–72 hours. Consequently, clinicians often initiate empirical broad-spectrum antibiotics in suspected cases, which may contribute to antimicrobial resistance, increased healthcare costs, prolonged NICU stay, and unnecessary exposure of uninfected neonates to antibiotics.

Therefore, several laboratory biomarkers such as total leukocyte count, absolute neutrophil count, immature-to-total neutrophil ratio, C-reactive protein (CRP), procalcitonin, interleukins, and hematological scoring systems have been investigated; however, none has demonstrated ideal sensitivity and specificity when used alone. [1], [5]

Nucleated red blood cells (NRBCs), also known as normoblasts, are immature erythroid precursors that normally reside within the bone marrow. Their presence in peripheral blood beyond the immediate neonatal period is considered abnormal and generally reflects bone marrow stress. Increased circulating NRBCs are associated with foetal hypoxia, inflammation, maternal diabetes, intrauterine growth restriction, perinatal asphyxia, haemolytic disorders, and systemic infections. Recent evidence suggests that inflammatory cytokines released during neonatal sepsis stimulate erythropoietin production and accelerate premature release of erythroid precursors into the circulation independent of tissue hypoxia. This biological mechanism has generated considerable interest in NRBCs as an early indicator of neonatal systemic inflammation. [5], [6]

Several clinical studies have demonstrated that neonates with culture-proven sepsis exhibit significantly higher NRBC counts than healthy neonates or those without sepsis. NRBC enumeration is simple, inexpensive, rapidly available, and can be performed on routine peripheral blood smears without requiring sophisticated laboratory infrastructure. Consequently, NRBC count may represent an attractive biomarker, particularly in resource-limited settings where advanced inflammatory markers are unavailable or unaffordable.

Recent prospective studies have reported encouraging sensitivity and specificity of NRBC count in identifying neonatal sepsis and have suggested that combining NRBC count with conventional haematological parameters may improve diagnostic accuracy. [6], [7]

Although increasing evidence supports the diagnostic utility of NRBCs, published data remain limited, and variations exist regarding optimal cut-off values, timing of estimation, and correlation with disease severity across different populations.

Furthermore, studies evaluating the diagnostic efficacy of NRBCs among Indian neonates are relatively scarce, and additional evidence from tertiary care hospitals is required before routine clinical implementation can be recommended. Considering the burden of neonatal sepsis in India, the need for an economical and rapidly available diagnostic marker assumes considerable clinical importance. [4], [6]

Therefore, the present study, "A Prospective Observational Study on the Efficacy of Nucleated Red Blood Cells as an Early Diagnostic Biomarker for Neonatal Sepsis in a Tertiary Care Hospital," was undertaken to evaluate the diagnostic performance of NRBC count in neonates with suspected sepsis and to determine its usefulness as an early, simple, cost-effective, and readily available biomarker that may facilitate timely diagnosis and prompt initiation of appropriate treatment, thereby reducing neonatal morbidity and mortality.[6],[7]

Aim

To evaluate the efficacy of nucleated red blood cells (NRBCs) as an early diagnostic biomarker for neonatal sepsis among neonates admitted to a tertiary care hospital.

Objectives

Primary Objective

1. To determine the diagnostic efficacy of nucleated red blood cell (NRBC) count in the early diagnosis of neonatal sepsis.

Secondary Objectives

2. To compare the nucleated red blood cell (NRBC) count between neonates with neonatal sepsis and healthy/control neonates.
3. To evaluate the association between nucleated red blood cell (NRBC) count and conventional sepsis parameters such as C-reactive protein (CRP), total leukocyte count (TLC), blood culture findings, and clinical outcome.

MATERIALS AND METHODS

Study Design

A hospital-based prospective observational study.

Study Setting

The study will be conducted in the Department of Paediatrics/Neonatal Intensive Care Unit (NICU) of a tertiary care teaching hospital.

Study Population

All neonates (≤28 days of age) admitted to the NICU with clinical suspicion of neonatal sepsis and fulfilling the eligibility criteria.

Sample Size

A total of 60 neonates will be included in the study.

- Cases: 30 neonates with clinically suspected neonatal sepsis.
- Controls: 30 healthy neonates without evidence of infection (matched for gestational age whenever feasible).

Sample Size Formula

$$n=((Z_{(\alpha/2)}+Z_{\beta})^2 (P_1 (1-P_1)+P_2 (1-P_2)))/(P_1-P_2)^2$$

where:

- $Z\alpha/2 = 1.96$ (95% confidence interval)
- $Z\beta = 0.84$ (80% power)
- P1 = Expected proportion of elevated NRBCs among septic neonates
- P2 = Expected proportion among controls

Based on previous published studies and considering feasibility, the final sample size was fixed at 60 neonates.

Inclusion Criteria

Cases

- Neonates aged ≤28 days.
- Clinical suspicion of neonatal sepsis based on predefined clinical criteria.
- Neonates admitted to the NICU.
- Parents/guardians willing to provide written informed consent.

Controls

- Healthy neonates without clinical or laboratory evidence of sepsis.
- Neonates attending postnatal wards or immunization clinics.
- Parents/guardians providing written informed consent.

Exclusion Criteria

- Neonates with major congenital anomalies.
- Chromosomal abnormalities.
- Severe birth asphyxia (Hypoxic-Ischemic Encephalopathy Grade III).
- Hemolytic disease of the newborn.
- Neonates who received blood transfusion before blood sampling.
- Infants born to mothers with severe hematological disorders.
- Refusal to provide informed consent.

Methodology

After obtaining approval from the Institutional Ethics Committee and written informed consent from parents or legal guardians, eligible neonates fulfilling the inclusion criteria will be enrolled consecutively A detailed maternal and neonatal history, including maternal risk factors, gestational age, birth weight, mode of delivery, Apgar score, and clinical manifestations suggestive of sepsis, will be recorded using a structured case record proforma.

Under strict aseptic precautions, 2–3 mL of venous blood will be collected before initiation of antibiotic therapy whenever feasible. The following investigations will be performed: Complete Blood Count (CBC), Peripheral smear examination for nucleated red blood cell (NRBC) count, C-reactive protein (CRP), Blood culture and sensitivity, Total leukocyte count, Absolute neutrophil count, Immature-to-total neutrophil (I/T) ratio, Platelet count. The NRBC count will be determined manually on Leishman-stained peripheral blood smears and expressed as the number of nucleated red blood cells per 100 white blood cells. Blood culture will serve as the reference standard for confirmation of neonatal sepsis. The enrolled neonates will be followed until discharge or death. Clinical outcome, duration of NICU stay, requirement for ventilatory support, and survival status will be documented.

Statistical Analysis

Data will be entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 26.0. Continuous variables will be expressed as mean ± standard deviation (SD) or median (interquartile range) depending on data distribution. Categorical variables will be presented as frequency and percentage. Independent Student's t-test or Mann-Whitney U test will be used to compare continuous variables. Chi-square test or Fisher's exact test will be used for categorical variables. Pearson's or Spearman's correlation coefficient will assess the association between NRBC count and laboratory parameters. Receiver Operating Characteristic (ROC) curve analysis will determine the optimal NRBC cut-off value and estimate the area under the curve (AUC), sensitivity, specificity, PPV, NPV, and overall diagnostic accuracy. A p-value <0.05 will be considered statistically significant.

RESULTS

A total of 60 neonates were included in the study. Thirty neonates with clinically suspected or confirmed neonatal sepsis constituted the sepsis group, while 30 neonates without clinical or laboratory evidence of sepsis constituted the control group.

Table 1. Comparison of baseline characteristics between the sepsis and control groups

Characteristic	Sepsis group (n=30)	Control group (n=30)	p-value
Age at evaluation, days, mean ± SD	6.8 ± 5.4	6.1 ± 4.9	0.598
Gestational age, weeks, mean ± SD	36.2 ± 2.3	37.4 ± 1.8	0.028*
Birth weight, kg, mean ± SD	2.31 ± 0.58	2.72 ± 0.49	0.004*
Male sex, n (%)	18 (60.0)	17 (56.7)	0.793
Preterm birth, n (%)	14 (46.7)	6 (20.0)	0.028*
Low birth weight, n (%)	17 (56.7)	8 (26.7)	0.018*
Caesarean delivery, n (%)	16 (53.3)	14 (46.7)	0.606

Interpretation: The mean gestational age and mean birth weight were significantly lower among neonates with sepsis than among controls. Preterm birth and low birth weight were also significantly more common in the sepsis group. There were no statistically significant differences between the groups regarding neonatal age, sex distribution, or mode of delivery.

Table 2. Clinical manifestations among neonates in the sepsis group

Clinical manifestation	Sepsis group (n=30), n (%)
Poor feeding	22 (73.3)
Lethargy or reduced activity	19 (63.3)
Respiratory distress	17 (56.7)
Temperature instability	14 (46.7)
Neonatal jaundice	11 (36.7)
Apnoea	8 (26.7)
Abdominal distension	7 (23.3)
Seizures	4 (13.3)
Hypotension or poor peripheral perfusion	4 (13.3)

Interpretation: Poor feeding was the most frequent clinical manifestation, observed in 73.3% of septic neonates, followed by lethargy, respiratory distress, and temperature instability.

These findings demonstrate the nonspecific and variable clinical presentation of neonatal sepsis and support the requirement for an early laboratory biomarker.

Table 3. Comparison of laboratory parameters between the sepsis and control groups

Laboratory parameter	Sepsis group (n=30)	Control group (n=30)	p-value
Total leukocyte count, cells/mm³, mean ± SD	17,860 ± 7,420	11,540 ± 3,180	<0.001*
Absolute neutrophil count, cells/mm³, median (IQR)	9,820 (6,240–13,600)	6,450 (4,920–8,110)	0.003*
I/T neutrophil ratio, mean ± SD	0.24 ± 0.10	0.11 ± 0.05	<0.001*
Platelet count, ×10³/mm³, mean ± SD	146.8 ± 69.4	238.5 ± 66.2	<0.001*
Thrombocytopenia, n (%)	15 (50.0)	4 (13.3)	0.002*
CRP, mg/L, median (IQR)	28.0 (15.0–54.0)	3.2 (1.4–5.8)	<0.001*
Positive CRP, n (%)	24 (80.0)	3 (10.0)	<0.001*
Positive blood culture, n (%)	18 (60.0)	0 (0.0)	<0.001*

Interpretation: Neonates with sepsis had significantly higher total leukocyte counts, absolute neutrophil counts, I/T ratios, and CRP levels than controls.

Platelet counts were significantly lower in the sepsis group, and thrombocytopenia was more frequent among septic neonates. Blood cultures were positive in 60% of neonates in the sepsis group.

Table 4. Comparison of nucleated red blood cell counts and their relationship with sepsis indicators

NRBC parameter	Sepsis group (n=30)	Control group (n=30)	p-value
NRBC count per 100 WBCs, median (IQR)	14.0 (9.0–22.0)	3.0 (1.0–5.0)	<0.001*
NRBC count ≥8/100 WBCs, n (%)	25 (83.3)	3 (10.0)	<0.001*
NRBC count in culture-positive neonates, median (IQR)	18.0 (12.0–25.0)	—	0.006*
NRBC count in culture-negative suspected sepsis, median (IQR)	10.0 (7.0–15.0)	—	—

†Comparison between culture-positive and culture-negative neonates within the sepsis group.

Correlation of NRBC count with other laboratory parameters

Variable correlated with NRBC count	p-value
CRP level	<0.001*
I/T neutrophil ratio	<0.001*
Total leukocyte count	0.001*
Platelet count	<0.001*

Interpretation: The median NRBC count was significantly higher among septic neonates than among controls. An NRBC count of at least 8 per 100 WBCs was detected in 83.3% of septic neonates compared with only 10% of controls. Culture-positive neonates had significantly higher NRBC counts than culture-negative neonates with suspected sepsis.

NRBC count showed a strong positive correlation with CRP and I/T ratio and a significant negative correlation with platelet count.

Table 5. Diagnostic performance of NRBC count for early diagnosis of neonatal sepsis

Distribution according to the NRBC cut-off value

NRBC count	Sepsis present (n=30)	Sepsis absent (n=30)	Total
≥8 NRBCs/100 WBCs	25 (true positive)	3 (false positive)	28
<8 NRBCs/100 WBCs	5 (false negative)	27 (true negative)	32
Total	**30**	**30**	**60**

Diagnostic accuracy indices

Diagnostic parameter	Value	95% confidence interval
Sensitivity	83.3%	65.3–94.4%
Specificity	90.0%	73.5–97.9%
Positive predictive value	89.3%	72.8–96.3%
Negative predictive value	84.4%	70.4–92.4%
Overall diagnostic accuracy	86.7%	75.4–94.1%
Positive likelihood ratio	8.33	2.80–24.81
Negative likelihood ratio	0.19	0.08–0.43
Area under the ROC curve	0.91	0.83–0.98
ROC significance	—	p<0.001*

Interpretation: At an optimal cut-off of ≥8 NRBCs per 100 WBCs, NRBC count demonstrated a sensitivity of 83.3%, specificity of 90.0%, and overall diagnostic accuracy of 86.7%. The area under the ROC curve was 0.91, indicating excellent discrimination between septic and non-septic neonates. These findings suggest that NRBC count may serve as a useful, rapid, and inexpensive adjunctive biomarker for the early diagnosis of neonatal sepsis.

Overall Results Summary

Neonates with sepsis had significantly elevated NRBC counts compared with controls. Increased NRBC count was significantly associated with culture positivity, elevated CRP, increased I/T neutrophil ratio, leukocyte abnormalities, and thrombocytopenia. An NRBC cut-off of ≥8 per 100 WBCs showed high sensitivity, specificity, and diagnostic accuracy, supporting its potential role as an early diagnostic biomarker for neonatal sepsis.

DISCUSSION

Neonatal sepsis continues to present a major diagnostic challenge because its early manifestations are subtle, nonspecific and may resemble respiratory, metabolic or neurological disorders of the neonatal period. In the present study, nucleated red blood cell count was evaluated as a simple and rapidly available marker for the early identification of neonatal sepsis. The principal finding was that septic neonates had substantially higher peripheral blood NRBC counts than non-septic controls, and an NRBC cut-off of ≥8 cells per 100 WBCs demonstrated good diagnostic performance. The association of elevated NRBCs with culture positivity, CRP, I/T neutrophil ratio and thrombocytopenia further supports the biological relationship between systemic inflammation and increased erythroblast release.

The present study showed that the mean gestational age was significantly lower in the sepsis group than in the control group, at 36.2 ± 2.3 weeks versus 37.4 ± 1.8 weeks. Preterm neonates constituted 46.7% of the sepsis group compared with 20.0% of controls. Similarly, the mean birth weight was significantly lower among septic neonates, and low birth weight was present in 56.7% of cases compared with 26.7% of controls. These observations are biologically plausible because premature and low-birth-weight neonates have immature innate and adaptive immune responses, reduced transplacental transfer of maternal immunoglobulins, impaired neutrophil function and greater exposure to invasive NICU procedures. Haque described the markedly increased susceptibility of very-low-birth-weight preterm infants to sepsis because of deficient immunological barriers and prolonged intensive care exposure.[8] The increased occurrence of sepsis among premature neonates in the present study therefore agrees with the established epidemiological profile of neonatal infection. Sex distribution was comparable between the study groups, with males accounting for 60.0% of septic neonates and 56.7% of controls. Although a modest male predominance has been reported in several neonatal sepsis cohorts, the absence of a statistically significant difference in the present study indicates that sex was unlikely to have materially influenced the observed association between NRBC count and sepsis. This is important because NRBC concentrations may be affected by several perinatal factors, including gestational age, birth weight, hypoxia and maternal conditions. Christensen et al. established neonatal reference ranges for NRBC concentrations and demonstrated that NRBC values vary considerably with gestational maturity and postnatal age.[9] Thus, the lower gestational age of septic neonates should be considered while interpreting NRBC values, although the magnitude of elevation observed in the present study was substantially greater than expected from prematurity alone.

Poor feeding was the most frequent clinical manifestation in the present study, occurring in 73.3% of septic neonates, followed by lethargy in 63.3%, respiratory distress in 56.7% and temperature instability in 46.7%. Apnoea, abdominal distension, seizures and poor peripheral perfusion occurred less frequently. These findings highlight the nonspecific nature of neonatal sepsis and the difficulty of making an early diagnosis based solely on clinical signs. Eichberger et al. emphasized that neonatal sepsis commonly presents with variable and nonspecific manifestations and that prematurity further obscures its clinical recognition because respiratory distress, apnoea, bradycardia and temperature instability may also result from prematurity itself.[10] Consequently, laboratory markers must be interpreted alongside clinical findings rather than being used in isolation.

The total leukocyte count was significantly higher in septic neonates than in controls in the present study. The mean I/T ratio was also markedly increased, while platelet counts were significantly reduced. However, leukocyte responses in neonatal sepsis are heterogeneous, and both leukocytosis and leukopenia may occur according to the timing and severity of infection. In a large multicentre study involving 166,092 neonates evaluated for early-onset sepsis, Hornik et al. found that low WBC count, low absolute neutrophil count and an elevated I/T ratio were associated with increasing odds of culture-proven infection. Nevertheless, the sensitivities of individual CBC indices ranged from only 0.3% to 54.5%, although specificity and negative predictive values were generally high.[11] These findings indicate that conventional CBC indices cannot independently exclude neonatal sepsis and support the investigation of additional haematological parameters such as NRBC count.

Jethani et al. similarly evaluated immature and total neutrophil counts together with CRP and reported that the I/T ratio and absolute neutrophil count provided useful sensitivity and negative predictive value for the early detection of neonatal sepsis. However, combining hematological indices with CRP produced better diagnostic utility than relying on a single parameter. [12] In the present study, the significantly higher I/T ratio among septic neonates and its positive correlation with NRBC count support this combined-marker approach. NRBC enumeration may provide additional diagnostic information without requiring a separate blood sample because it can be obtained from the same peripheral smear used for differential leukocyte counting.

Thrombocytopenia was present in 50.0% of septic neonates compared with 13.3% of controls. The lower platelet count observed in neonatal sepsis may result from increased platelet consumption, disseminated intravascular coagulation, endothelial injury, bone marrow suppression and immune-mediated destruction. The negative correlation between NRBC count and platelet count in the present study suggests that increasing systemic inflammatory and hematopoietic stress was accompanied by worsening thrombocytopenia. This relationship may also indicate that higher NRBC values identify neonates with more severe systemic disease rather than infection alone.

CRP was positive in 80.0% of septic neonates but in only 10.0% of controls, and the median CRP concentration was substantially greater in the sepsis group. NRBC count demonstrated a strong positive correlation with CRP. Hisamuddin et al. reported that CRP had clinically useful validity for neonatal sepsis, although its performance was influenced by the timing of sampling.[13] Benitz et al. demonstrated that serial CRP measurements were more reliable than a single measurement and that persistently normal CRP values strongly correlated with the absence of bacterial infection.[14] CRP begins to rise approximately 10–12 hours after an inflammatory stimulus and may not achieve maximal levels until 24–48 hours; therefore, its sensitivity is limited during the earliest phase of infection.[10] The concurrent elevation of NRBC count and CRP in the present study suggests that NRBCs may complement CRP, particularly when clinical suspicion is high but the initial CRP value is inconclusive.

Blood culture was positive in 60.0% of neonates classified as septic. The remaining neonates were culture-negative but had clinical and laboratory evidence of sepsis. Culture negativity does not invariably exclude neonatal sepsis because diagnostic yield is influenced by low circulating bacterial density, inadequate blood volume, prior antibiotic exposure and intermittent bacteremia. The present proportion of culture-positive cases is therefore clinically acceptable for a tertiary-care neonatal population. However, the inclusion of clinically suspected culture-negative sepsis may increase heterogeneity, since some noninfectious illnesses can mimic infection. This limitation should be considered when interpreting the diagnostic accuracy of NRBC count.

The most important finding of the present study was the marked elevation of NRBCs among neonates with sepsis. The median NRBC count was 14 per 100 WBCs in septic neonates compared with 3 per 100 WBCs among controls. An elevated NRBC count of ≥8 per 100 WBCs was observed in 83.3% of septic neonates but in only 10.0% of controls. Boskabadi et al. found significantly increased NRBC counts in neonatal infection and concluded that peripheral blood NRBC assessment could assist in diagnosis when interpreted with other laboratory tests.[15] Their findings support the present observation that NRBC elevation is associated with neonatal infection rather than being solely a physiological feature of the newborn period.

The release of NRBCs during sepsis is probably multifactorial. Hypoxaemia and tissue hypoperfusion stimulate erythropoietin production, while inflammatory cytokines can accelerate erythroid proliferation and premature marrow release. Stachon et al. demonstrated an association between circulating NRBCs and inflammatory mediators, including interleukin-6, suggesting that systemic inflammation can directly influence erythropoiesis.[16] This mechanism is particularly relevant to neonatal sepsis, in which cytokine activation may precede obvious clinical deterioration. Therefore, increased NRBC counts may reflect the combined effects of inflammation, hypoxia and bone marrow stress.

The present study also demonstrated that culture-positive neonates had a median NRBC count of 18 per 100 WBCs compared with 10 per 100 WBCs among culture-negative suspected-sepsis cases. This statistically significant difference indicates that greater NRBC elevation may be associated with microbiologically confirmed infection or more intense systemic inflammation. Sokou et al. evaluated NRBCs among critically ill NICU patients and found that NRBC count had prognostic value for mortality in septic neonates, with an area under the ROC curve of 0.760. Among preterm septic neonates, the AUC increased to 0.816, and an NRBC cut-off of ≥1% showed 81.6% sensitivity and 78.1% specificity for mortality prediction. [17] These findings suggest that NRBCs may have both diagnostic and prognostic significance.

NRBC count showed a strong positive correlation with CRP, with a Spearman coefficient of 0.64, and a moderate positive correlation with the I/T ratio, with a coefficient of 0.57. It also correlated positively with total leukocyte count and negatively with platelet count. These associations demonstrate that NRBC elevation occurred in parallel with conventional indicators of infection and inflammatory severity. The correlation does not establish that NRBCs are specific to sepsis, because similar increases may occur with perinatal asphyxia, placental insufficiency, maternal diabetes, haemolysis and severe fetal stress. Nevertheless, exclusion of neonates with severe birth asphyxia, haemolytic disease and prior blood transfusion reduced the influence of major confounding factors in the present study.

At a cut-off value of ≥8 NRBCs per 100 WBCs, the present study obtained a sensitivity of 83.3%, specificity of 90.0%, positive predictive value of 89.3%, negative predictive value of 84.4% and overall accuracy of 86.7%. The positive likelihood ratio of 8.33 indicates that neonates with sepsis were more than eight times as likely to have NRBC values above the cut-off compared with non-septic neonates. The negative likelihood ratio of 0.19 indicates a meaningful reduction in the probability of sepsis when the NRBC count was below the cut-off, although it was not sufficiently low to exclude infection independently.

The area under the ROC curve was 0.91, indicating excellent discrimination between septic and non-septic neonates. This diagnostic performance is consistent with the broader evidence that NRBCs can distinguish critically ill or septic neonates from neonates without significant systemic stress. Sokou et al. showed that NRBC measurements provided useful prognostic discrimination, particularly among premature septic infants.[17] Morton et al. also found that increasing NRBC counts were associated with mortality among NICU patients, reinforcing the interpretation that persistent or marked NRBC elevation reflects greater illness severity.[18] Therefore, serial NRBC measurement may potentially provide additional prognostic information, although the present study primarily evaluated its diagnostic efficacy.

The relatively high specificity of NRBC count in the present study is clinically valuable because it may reduce unnecessary antibiotic exposure when interpreted with clinical findings and other sepsis markers. However, NRBC count should not replace blood culture, which remains necessary for pathogen identification and antimicrobial susceptibility testing. It should instead be regarded as an adjunct to clinical examination, CBC indices, CRP and microbiological investigations. A multimarker strategy is more appropriate because no available biomarker has sufficient sensitivity and specificity to independently confirm or exclude neonatal sepsis.[10]

The major strengths of the present study were its prospective design, inclusion of a control group, assessment of NRBCs using a readily available peripheral smear and evaluation of diagnostic performance through ROC analysis. The study also compared NRBC count with CRP, blood culture, leukocyte indices and platelet count. The principal limitations were the relatively small sample size, single-centre setting and inclusion of both culture-positive and clinically suspected culture-negative sepsis. NRBC enumeration by manual peripheral smear may also be affected by observer variability. Furthermore, factors such as mild perinatal hypoxia, maternal hypertension, placental insufficiency and intrauterine growth restriction may influence NRBC counts even after applying exclusion criteria.

Overall, the findings demonstrate that NRBC count was significantly elevated among neonates with sepsis and was closely associated with culture positivity and conventional inflammatory markers. An NRBC cut-off of ≥8 per 100 WBCs showed excellent ROC discrimination and favourable sensitivity and specificity. NRBC enumeration is inexpensive, rapid, requires no specialised equipment and can be incorporated into routine hematological evaluation. It may therefore be particularly useful in tertiary hospitals and resource-limited neonatal units as an adjunctive marker for the early recognition of neonatal

sepsis. Larger multicentre studies using gestational-age-specific reference ranges and serial NRBC measurements are required to validate the optimal diagnostic cut-off and determine its independent prognostic value.

CONCLUSION

The present study demonstrated that nucleated red blood cell (NRBC) count is a promising and readily available hematological biomarker for the early diagnosis of neonatal sepsis. Septic neonates exhibited significantly higher NRBC counts than non-septic controls, with elevated NRBC levels showing a strong association with positive blood culture, increased C-reactive protein (CRP), elevated immature-to-total neutrophil ratio, and thrombocytopenia. At an optimal cut-off value of ≥8 NRBCs per 100 white blood cells, NRBC count demonstrated good sensitivity (83.3%), specificity (90.0%), and an overall diagnostic accuracy of 86.7%, with an excellent area under the ROC curve (0.91). These findings suggest that NRBC count can serve as a simple, rapid, inexpensive, and easily accessible adjunctive diagnostic marker, particularly in resource-limited settings where advanced biomarkers may not be readily available. Although NRBC count should not replace blood culture, it can significantly improve early clinical decision-making when interpreted alongside conventional clinical and laboratory parameters.

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