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Original Research Article

Nucleated Red Blood Cells as a Diagnostic and Prognostic Marker in Term Neonates with Sepsis: A Prospective Observational Study

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Abstract

Background: Neonatal sepsis is a leading cause of neonatal death worldwide, and blood culture, its reference standard, takes 48–72 hours to yield a result. Nucleated red blood cells (NRBCs), released from the marrow in response to hypoxic or inflammatory stress, have been proposed as a rapid, inexpensive adjunct for identifying sepsis and anticipating its course.

Objective: To measure NRBC counts in term neonates with suspected sepsis and to assess their diagnostic accuracy for sepsis and their association with neonatal outcome.

Methods: In this prospective observational study, 73 term neonates admitted to the neonatal intensive care unit (NICU) of Cheluvamba Hospital, Mysuru, with risk factors or clinical features of sepsis were enrolled over 12 months (September 2021–September 2022) by purposive sampling. A sepsis screen, blood culture, and peripheral-smear NRBC count (per 100 white blood cells) were obtained on day 1 of admission, with a repeat NRBC count on day 3. Neonates were classified as proven sepsis (positive blood culture), probable/clinical sepsis (positive screen, negative culture), or no sepsis. An NRBC count greater than 10/100 WBC was considered elevated.

Results: Sepsis was diagnosed in 52 of 73 neonates (71.2%). Mean NRBC counts were significantly higher in the sepsis group than the no-sepsis group on day 1 (10.96±5.56 vs 3.95±2.13; $p<0.001$) and day 3 (12.58±8.02 vs 1.05±2.18; $p<0.001$). At the >10/100 WBC cut-off, NRBC had a sensitivity of 51.9%, specificity of 94.6%, positive predictive value of 87.8%, and negative predictive value of 45.6% for sepsis. Twenty-two neonates (30.1% overall; 42.3% of the sepsis group) died, all of whom belonged to the sepsis group. Mean NRBC counts were significantly higher in neonates who died than in those discharged, on both day 1 (12.68±5.99 vs 7.33±4.92; $p<0.001$) and day 3 (16.77±7.31 vs 6.02±7.02; $p<0.001$).

Conclusion: NRBC count is significantly and consistently elevated in term neonates with sepsis and rises further in those who die. Although its sensitivity for diagnosing sepsis is modest, its high specificity and its association with mortality support its use as a low-cost, rapidly available prognostic adjunct in resource-limited NICUs, best interpreted alongside conventional sepsis-screen parameters rather than as a stand-alone diagnostic test.

Keywords: Neonatal Sepsis; Nucleated Red Blood Cells; Peripheral Smear; Prognosis; Neonatal Mortality.

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Introduction

Neonatal sepsis remains one of the principal causes of neonatal morbidity and mortality, particularly in low- and middle-income countries, accounting for a large share of the world's roughly 2.3 million annual neonatal deaths.[1,2] In India, sepsis-related mortality remains substantial despite improvements in perinatal and neonatal care infrastructure, and delayed recognition continues to be a major contributor to preventable deaths.[3] Clinical signs of sepsis in the newborn are notoriously non-specific, so laboratory support for the diagnosis is essential. Blood culture remains the reference standard, but its turnaround time of 48–72 hours means that antibiotic therapy is almost always

started empirically, before culture confirmation, and often continued unnecessarily in culture-negative infants because clinicians are reluctant to stop it without a reliable early marker to guide de-escalation.[4] Nucleated red blood cells (NRBCs) are erythrocyte precursors that circulate physiologically in the fetus but normally disappear from peripheral blood within the first days after birth in healthy term infants. Their reappearance or persistence at higher-than-expected numbers has been linked to intrauterine hypoxia, chorioamnionitis, and other perinatal stressors, and more recent work suggests that inflammatory cytokines, particularly interleukin-6, can independently drive NRBC release from the marrow even in the absence of hypoxia.[5,6] Because NRBCs can be counted on a routine peripheral smear within an hour, at negligible cost and without additional venipuncture, they have attracted interest as a bedside-available marker that might flag sepsis, or at least flag risk, well before culture results are available.[7,8]

Existing reports on the diagnostic and prognostic value of NRBCs in neonatal sepsis are heterogeneous, with sensitivity estimates ranging from roughly 35% to over 80% across studies, reflecting differences in population, cut-off values, and counting method.[9–12] Comparatively few studies have specifically examined term neonates or tracked NRBC counts serially in relation to survival. We undertook this study to characterise NRBC counts in a cohort of term neonates with suspected sepsis, to determine their accuracy for diagnosing sepsis against the composite reference of blood culture and clinical criteria, and to examine whether the counts on admission and on day 3 were associated with survival to discharge.

Methods

Study design and setting: This was a hospital-based, prospective observational study conducted in the NICU (inborn and outborn) of Cheluvamba Hospital, attached to Mysuru Medical College and Research Institute, Mysuru, Karnataka, over 12 months (September 2021 to September 2022), after institutional ethics committee approval. Written informed consent was obtained from a parent or legal caretaker after the study aims, procedures, and the fact that treatment would not be withheld regardless of consent were explained in the family's own language.

Participants: All term (37–42 weeks' gestation), inborn or outborn neonates admitted with risk factors for sepsis (maternal febrile illness within two weeks of delivery, foul-smelling or meconium-stained liquor, rupture of membranes >24 hours, unclean or repeated per-vaginal examinations, or prolonged labour) or with clinical features suggestive of sepsis (temperature instability, lethargy, poor feeding, poor perfusion, respiratory distress, apnoea, seizures, feed intolerance, or bleeding tendency, among others) were eligible. Neonates with maternal pre-eclampsia/eclampsia, gestational diabetes, intrauterine growth restriction, perinatal asphyxia, preterm or post-term birth, haemolytic anaemia (ABO/Rh incompatibility), maternal smoking, suspected inborn errors of metabolism, or congenital anomalies were excluded, since these conditions independently elevate NRBC counts and could confound the association with sepsis. The sample size of 73 was calculated from an assumed 5% prevalence of sepsis-related neonatal mortality at the study centre, at 95% confidence and 5% precision, using the standard proportion formula; neonates were recruited by purposive sampling.

Procedures: A detailed antenatal, natal, and postnatal history was recorded, together with a systemic examination. Approximately 3 mL of venous blood was drawn at the time of an already-planned venepuncture (for cannulation or another indicated test) for a sepsis screen (total leucocyte count, absolute neutrophil count, and C-reactive protein [CRP]; micro-ESR and the immature-to-total neutrophil ratio could not be performed because the tests were unavailable at the centre), blood culture, and a peripheral smear for NRBC counting.

A positive screen (≥2 abnormal parameters) prompted empirical antibiotics; antibiotics were also continued pending culture in neonates with strong clinical suspicion despite a negative screen. Proven sepsis (positive blood culture) was treated for 14 days, extended to 21 days if meningitis was confirmed on cerebrospinal fluid analysis. Neonates were classified into three groups: proven sepsis (positive blood culture), probable/clinical sepsis (positive screen or strong clinical features with negative culture), and no sepsis (negative culture and negative screen, with an alternative diagnosis established on further evaluation). For this analysis, proven and probable/clinical sepsis were combined into a single sepsis group and compared with the no-sepsis group.

For NRBC counting, a thin peripheral blood film was prepared from EDTA-anticoagulated blood within two hours of collection, air-dried, stained with Leishman stain for 10 minutes, rinsed, and examined under oil immersion. NRBCs were counted per 100 white blood cells; a count greater than 10/100 WBC was taken as abnormal, consistent with cut-offs used in comparable studies.[9,10] The count was repeated on day 3 of admission in the same manner. Blood culture samples were collected under strict aseptic precautions before starting antibiotics.

Statistical Analysis: Data were analysed using SPSS version 22.0 and R version 3.2.2. Continuous variables are presented as mean ± standard deviation and compared using the independent-samples t-test (with Levene's test for homogeneity of variance); categorical variables are presented as number (%) and compared using the chi-square or Fisher's exact test, as appropriate. Sensitivity, specificity, positive predictive value, and negative predictive value of NRBC and CRP for sepsis were calculated against the composite clinical/microbiological reference standard described above. A two-tailed p value <0.05 was considered statistically significant.

Results

Of 73 term neonates enrolled, 44 (60.3%) were male and 29 (39.7%) female (male: female 1.6:1); 23 (31.5%) were inborn and 50 (68.5%) outborn. Fifty-eight neonates (79.5%) were delivered vaginally and 15 (20.5%) by caesarean section. Mean birth weight was 2.62±0.46 kg and mean age at admission 2.95±2.13 days; none of these baseline characteristics differed significantly between the sepsis and no-sepsis groups (all p>0.05). Sepsis (proven or probable/clinical) was diagnosed in 52 neonates (71.2%); the remaining 21 (28.8%) had no sepsis. Among the 33 neonates with positive blood culture, the commonest isolate was methicillin-resistant Staphylococcus aureus (10%), followed by Klebsiella species (8%).

Table 1: NRBC counts on day 1 and day 3 by final diagnosis

Variable	Sepsis (n=52)	No sepsis (n=21)	p value
NRBC/100 WBC, day 1	10.96 ± 5.56	3.95 ± 2.13	<0.001
NRBC/100 WBC, day 3	12.58 ± 8.02	1.05 ± 2.18	<0.001

Using an NRBC cut-off of >10/100 WBC, the test had a sensitivity of 51.9%, specificity of 94.6%, positive predictive value of 87.8%, and negative predictive value of 45.6% for diagnosing sepsis. For comparison, CRP positivity had a sensitivity of 92.3%, specificity of 76.2%, positive predictive value of 90.6%, and negative predictive value of 80.0% in the same cohort. NRBC was therefore markedly more specific but considerably less sensitive than CRP as a stand-alone diagnostic test (a comparison examined in detail in a companion analysis).

Table 2: Outcome and its association with NRBC count

Variable	Discharged (n=51)	Death (n=22)	p value
NRBC/100 WBC, day 1	7.33 ± 4.92	12.68 ± 5.99	<0.001
NRBC/100 WBC, day 3	6.02 ± 7.02	16.77 ± 7.31	<0.001

Twenty-two of 73 neonates (30.1%) died; all 22 belonged to the sepsis group, giving a case-fatality rate of 42.3% among septic neonates and an overall mortality directly attributable to sepsis in this cohort (p<0.001 for outcome by diagnosis). As shown in Table 2, mean NRBC counts on both day 1 and day 3 were significantly higher among neonates who died than among those discharged, and — unlike the discharged group, in which the day-3 mean was slightly lower than day 1 — the mean count continued to rise between day 1 and day 3 in the neonates who ultimately died, a trend explored further in a dedicated analysis of serial NRBC kinetics.

Discussion

In this cohort of 73 term neonates, NRBC counts were consistently and significantly higher in neonates with sepsis than in those without, on both day 1 and day 3 of admission, and rose further still in neonates who subsequently died. These findings are broadly consistent with the wider literature, though the reported diagnostic accuracy of NRBC varies considerably between studies. Rathi et al. reported a sensitivity of 86.2% and specificity of 51.1% for NRBC in neonatal sepsis, essentially the mirror image of the sensitivity–specificity trade-off seen in our cohort (51.9% and 94.6%, respectively).[9] Abhishek and Sanjay reported a sensitivity of 35% and specificity of 53.4%,[10] while Boskabadi et al. reported 45% and 83%.[11] This spread most likely reflects differences in counting method (manual smear versus automated analyser), the NRBC cut-off applied, and the case-mix of early- versus late-onset sepsis across studies, and underscores that NRBC accuracy is not a fixed test property but depends heavily on local methodology. The prognostic signal in our data was more consistent across studies than the diagnostic one. Kulandaivel et al., in a similar Indian cohort, found markedly higher day-3 NRBC counts in neonates who died (mean 22.4) than in survivors (mean 3.47), a trajectory that parallels the rising counts we observed in non-survivors.[12] Outside the neonatal period, elevated NRBCs have repeatedly predicted mortality in adult critical illness, including surgical sepsis, ARDS, and COVID-19,[13-15] and a recent narrative review concluded that NRBCs are one of the more consistently reproducible mortality markers across diverse critically ill populations, even though their diagnostic performance for the underlying

condition is more variable.[16] Mechanistically, this is plausible: NRBC release reflects cumulative marrow stress from hypoxia and cytokine exposure, which should track illness severity and physiological reserve more closely than it tracks the binary presence or absence of infection.[5,6] Our data also illustrate why NRBC is better positioned as a complement to, rather than a replacement for, existing sepsis-screen parameters. CRP, an acute-phase reactant synthesised by the liver in response to interleukin-6 and other cytokines, was considerably more sensitive in our cohort, consistent with most published comparisons.[17] A test combining a highly sensitive marker such as CRP with a highly specific one such as NRBC could in principle reduce both missed diagnoses and unnecessary antibiotic courses, though this would need to be tested prospectively rather than assumed from cross-sectional accuracy figures alone.

Strengths of this study include its prospective design, uniform application of inclusion and exclusion criteria to minimise confounding by conditions that independently raise NRBC counts, and serial (day 1 and day 3) rather than single-time-point sampling. Limitations include the modest sample size, use of manual smear counting rather than an automated haematology analyser (which is more reproducible but not universally available in resource-limited settings), the unavailability of micro-ESR and immature-to-total neutrophil ratio for the sepsis screen, and the restriction to term neonates, which limits generalisability to preterm infants, in whom baseline NRBC counts are physiologically higher.[18] As an observational, single-centre study, it cannot establish that NRBC elevation is causally related to mortality rather than simply a marker of overall illness severity.

Conclusion

NRBC count is significantly elevated in term neonates with sepsis and rises further in those who do not survive, supporting its role as an inexpensive, rapidly available prognostic adjunct — most useful for risk-stratifying neonates already suspected of sepsis rather than as a stand-alone screening test, given its modest sensitivity.

Larger, multicentric studies using automated NRBC quantification and pre-specified cut-offs derived from receiver operating characteristic analysis are needed before NRBC can be recommended for routine clinical decision-making.

Declarations

Ethics approval: Institutional Ethics Committee, Mysuru Medical College and Research Institute. Informed consent was obtained from parents/legal guardians of all participants. Conflicts of interest: none declared. Funding: none. Data availability: the dataset analysed is available from the corresponding author on reasonable request.

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