ISSN: 2581 – 3633

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at: https://www.ijmacr.com

Volume – 9, Issue – 4, August – 2026, Page No.: 234 – 248

Correlation of Nucleated Red Blood Cell Count with the Severity and Outcome of Neonatal Sepsis: A Prospective Observational Study

[1]Dr. Pavithra Shree B E, Senior Resident, Department of Paediatrics, Adichunchanagiri Institute of Medical Sciences, B. G. Nagara, Mandya District, Karnataka, India.

[2]Dr. Rajendra Kumar, Professor, Mysore Medical College and Research Institute, Mysuru, Karnataka, India.

Corresponding Author: Dr. Pavithra Shree B E, Senior Resident, Department of Paediatrics, Adichunchanagiri Institute of Medical Sciences, B. G. Nagara, Mandya District, Karnataka, India.

How to citation this article: Dr. Pavithra Shree B E, Dr. Rajendra Kumar, "Correlation of Nucleated Red Blood Cell Count with the Severity and Outcome of Neonatal Sepsis: A Prospective Observational Study", IJMACR– August – 2026, Volume – 9, Issue – 4, P. No. 234 – 248.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: Neonatal sepsis remains a leading cause of neonatal mortality in India, and blood culture, the reference standard, is slow and insensitive. Nucleated red blood cells are released into the circulation in response to erythropoietin and to inflammatory cytokines and are measurable on a routine complete blood count at no additional cost. This study evaluated the correlation of the nucleated red blood cell count with the severity and outcome of neonatal sepsis.

Methods: One hundred and twenty neonates with clinically suspected sepsis and 60 gestation-matched and weight-matched controls were studied prospectively. Nucleated red blood cells were counted manually on a Giemsa-stained peripheral smear and expressed per 100 white cells and as an absolute count, at presentation and at 72 hours. Sepsis was categorised as probable or culture-positive, and severity as sepsis, severe sepsis or septic shock. Neonates with perinatal asphyxia, haemolytic disease and other recognised causes of nucleated red cell elevation were excluded.

Results: The median nucleated red blood cell count was 14 per 100 white cells in septic neonates and 4 in controls ($p < 0.001$), and rose across severity strata from 9 in sepsis to 18 in severe sepsis and 34 in septic shock ($p < 0.001$). The count discriminated mortality with an area under the curve of 0.812 at a cut-off of 22 per 100 white cells, and a count above this threshold independently predicted death (adjusted odds ratio 4.86, $p = 0.003$). A count that failed to fall by 72 hours carried a mortality of 21.4 percent against 6.1 percent where it fell.

Conclusion: The nucleated red blood cell count correlated with the severity and outcome of neonatal sepsis and offers a rapid, inexpensive prognostic adjunct available from a routine smear. It lacks the specificity to serve as a diagnostic test and is interpretable only after exclusion of asphyxia and other causes of erythroblastos is.

Keywords: Neonatal sepsis; Erythroblasts; Biomarkers; Infant, newborn; Intensive care units, neonatal; Prognosis

Introduction

Neonatal sepsis remains among the leading causes of neonatal death in India and in low-income and middle-income countries generally. Its clinical presentation in the newborn is notoriously non-specific, comprising lethargy, poor feeding, temperature instability, respiratory distress or abdominal signs, any of which may equally reflect a non-infectious condition. Because deterioration can be rapid and because the consequences of delayed treatment are severe, empirical antibiotics are commenced in far more neonates than ultimately prove to be infected. The resulting exposure carries its own costs, including the selection of resistant organisms, the disruption of the developing intestinal micro biome with its associated risk of necrotising enterocolitis, prolonged hospitalisation and separation of mother and infant.

Blood culture remains the reference standard for diagnosis but is inadequate as a decision-support tool at the moment when decisions must be made. It requires 48 to 72 hours to yield a result, and its sensitivity in neonatal practice is limited by the small volume of blood obtainable, by the low colony counts characteristic of neonatal bacteraemia and by frequent prior maternal antibiotic exposure. Consequently, a substantial proportion of neonates treated as septic never yield a positive culture, and the category of culture-negative or probable sepsis dominates clinical practice. This gap has driven a prolonged search for haematological and biochemical markers capable of supporting the diagnosis and, equally importantly, of stratifying severity at presentation.

The haematological indices in routine use derive substantially from the classic reference ranges established by Manroe and colleagues, whose analysis of neutrophil dynamics in the newborn established the total neutrophil count, the absolute immature neutrophil count and the immature to total neutrophil ratio as the parameters of interest, while explicitly noting that their predictive value varies with the parameter chosen and with the clinical setting.[1] Subsequent work by Schmutz and colleagues revised these ranges against a larger contemporary dataset.[2]

C-reactive protein and procalcitonin have been extensively studied and are widely used, principally to support the decision to discontinue antibiotics rather than to initiate them, since both rise with a delay after the onset of infection. A range of newer markers including interleukin - 6, CD64 expression and presepsin have shown promise but require assays that are unavailable in most Indian district and medical college units.[3]

Nucleated red blood cells are immature erythroid precursors that are normally confined to the bone marrow after the first days of life. Their appearance in the peripheral circulation reflects accelerated erythropoiesis, driven either by erythropoietin released in response to reduced arterial oxygen tension or by inflammatory cytokines including interleukin-6 and tumour necrosis factor alpha, which promote premature release of precursors from the marrow. Reference ranges

for the neonatal period were established by Christensen and colleagues from a large multihospital dataset, which additionally demonstrated an association between an elevated count on the day of birth and the subsequent development of severe intra ventricular haemorrhage and retinopathy of prematurity.[4] The parameter has the practical advantage of being obtainable from a Giemsa-stained peripheral smear prepared as part of the routine complete blood count, at no additional cost and within minutes, and of being reported automatically by many contemporary haematology analysers.

Evidence linking nucleated red blood cells to neonatal sepsis specifically is accumulating but remains limited. Christou and colleagues, in an observational cohort of 467 critically ill neonates, reported that the count showed significant prognostic power for mortality in septic neonates, with an area under the curve of 0.760 overall and 0.816 among preterm neonates with sepsis, while finding no significant difference in the count between survivors and non-survivors across the cohort as a whole.[5]

That combination of findings is instructive, indicating that the parameter carries prognostic information within a defined diagnostic category while lacking discriminatory power when applied indiscriminately. In critically ill adults, the presence of nucleated red cells in the circulation has been shown to be strongly prognostic of mortality, and the underlying biology is presumed to be shared.[6]

The principal obstacle to clinical application is specificity. Perinatal asphyxia is by far the commonest cause of neonatal erythroblastosis, and counts in asphyxiated neonates exceed those seen in sepsis by a wide margin, with reported diagnostic cut-offs for hypoxia of the order of eleven percent.[5,7] Haemolytic disease of the newborn, maternal diabetes, intrauterine growth restriction with placental insufficiency and maternal smoking all elevate the count independently of infection. Any study seeking to relate the parameter to sepsis must therefore exclude these conditions rigorously, and much of the published literature has not done so consistently, which accounts for a proportion of the heterogeneity in reported results. The present study was designed with precisely this exclusion strategy in mind, and was undertaken to determine whether the nucleated red blood cell count correlates with the severity and the outcome of neonatal sepsis in a population from which the recognised alternative causes of erythroblastosis have been systematically removed.

Aims and Objectives

The study aimed to establish whether a parameter obtainable from a routine peripheral smear carried useful information about the severity and prognosis of neonatal sepsis. The primary objective was to determine the correlation between the nucleated red blood cell count at presentation and the clinical severity of neonatal sepsis, categorised as sepsis, severe sepsis or septic shock. The secondary objectives were to compare the nucleated red blood cell count between neonates with sepsis and gestation-matched and weight-matched control neonates without sepsis, to compare the count between neonates with culture-positive and culture-negative probable sepsis, to determine the diagnostic and prognostic performance of the count for in-hospital mortality by receiver operating characteristic analysis and to identify the optimal cut-off value, and to compare the performance of the count against the C-reactive protein concentration and the immature to total neutrophil ratio for the same outcome. Further objectives were to examine the correlation of the count with the duration of

hospital stay, the requirement for inotropic support and the requirement for mechanical ventilation, to determine whether the count retained an independent association with mortality after adjustment for gestational age, birth weight and the presence of shock, and to examine whether the trend in the count between presentation and 72 hours carried prognostic information additional to that of the presenting value.

Materials and Methods

Study design and setting

This prospective observational study was conducted in the neonatal intensive care unit of the Department of Paediatrics, Adichunchanagiri Institute of Medical Sciences, B. G. Nagara, Mandya District, Karnataka, over an eighteen-month period from January 2024 to June 2025.

Approval of the Institutional Ethics Committee was obtained before the commencement of enrolment, and written informed consent was obtained from a parent or legal guardian of every neonate. The study was conducted in accordance with the principles of the Declaration of Helsinki and with the National Ethical Guidelines for Biomedical and Health Research Involving Children issued by the Indian Council of Medical Research. No blood sample was drawn solely for the purposes of the study; the peripheral smear examined was prepared from the sample obtained for the clinically indicated sepsis screen.

Participants

Cases were neonates of any gestational age admitted to the neonatal intensive care unit with clinically suspected sepsis, defined by the presence of two or more clinical features suggestive of sepsis together with two or more abnormal parameters on the sepsis screen, in whom antibiotics were commenced by the treating team. Controls were neonates admitted for non-infective reasons such as physiological jaundice requiring phototherapy or observation for maternal risk factors without clinical or laboratory evidence of sepsis, matched to cases in a 1:2 ratio by gestational age within two weeks and by birth weight within 250 g.

Because the specificity of the nucleated red blood cell count is the principal threat to the validity of any study of this kind, exclusions were applied rigorously. Neonates were excluded if there was perinatal asphyxia, defined by an Apgar score below 6 at five minutes, the need for prolonged resuscitation, or any stage of hypoxic ischaemic encephalopathy; haemolytic disease of the newborn including Rh or ABO isoimmunisation or a positive direct anti globulin test; maternal diabetes mellitus, whether pre-gestational or gestational; intrauterine growth restriction below the third centile or documented placental insufficiency with abnormal umbilical artery Doppler; maternal smoking or tobacco use; maternal pre-eclampsia with abnormal Doppler studies; any major congenital malformation or chromosomal anomaly; congenital cyanotic heart disease; polycythaemia with a venous haematocrit above 65 percent; any congenital haemolytic anaemia; prior blood transfusion; twin-to-twin transfusion syndrome; and antenatal exposure to erythropoietin. Neonates whose parent or guardian declined consent were also excluded.

Sample size calculation

The sample size was calculated for the primary objective, the difference in the nucleated red blood cell count between severity strata, using the comparison of two independent means as an approximation. On the basis of previously published data the standard deviation of the count was taken as 16 per 100 white cells and the

minimum difference of interest between adjacent severity strata as 9 per 100 white cells:

- n per group = $2 \times (Z_{1-\alpha/2} + Z_{1-\beta})^2 \times \sigma^2 / d^2$
- Substituting $Z_{1-\alpha/2} = 1.96$, $Z_{1-\beta} = 0.84$ for 80 percent power, $\sigma = 16$ and $d = 9$:
- $n = 2 \times (2.80)^2 \times (16)^2 / (9)^2$
- $n = 2 \times 7.84 \times 256 / 81$
- $n = 4014.1 / 81 = 49.6$

Approximately 50 neonates would therefore be required in each of the two smallest severity strata to be compared. Because septic shock was anticipated to account for a minority of admissions and because three severity strata were to be compared, the total case sample was set at 120 neonates with sepsis, with 60 controls recruited in a 1:2 ratios, giving 180 participants in all.

Definitions

Early-onset sepsis was defined as sepsis presenting within 72 hours of birth and late-onset sepsis as that presenting thereafter. Culture-positive sepsis was defined as the growth of a recognised pathogen from a blood culture, and probable sepsis as clinical features with a positive sepsis screen but a sterile blood culture. Severity was categorised in accordance with accepted paediatric criteria as sepsis, denoting the systemic inflammatory response to suspected or proven infection; severe sepsis, denoting sepsis with cardiovascular dysfunction, acute respiratory distress syndrome or dysfunction of two or more other organ systems; and septic shock, denoting sepsis with cardiovascular dysfunction persisting after adequate fluid resuscitation and requiring inotropic support.

The sepsis screen comprised the total leucocyte count, the absolute neutrophil count interpreted against gestation-specific and age-specific reference ranges, the immature to total neutrophil ratio with a value of 0.2 or above taken as abnormal, the micro-erythrocyte sedimentation rate, and C-reactive protein with a value above 10 mg/L taken as abnormal. Two or more abnormal parameters constituted a positive screen.

Nucleated red blood cell estimation

A peripheral blood smear was prepared from the ethylene diaminetetraacetic acid sample obtained for the sepsis screen at the time of presentation, before the administration of the first dose of antibiotic, and again at 72 hours. Smears were stained with Giemsa stain and examined by a single trained observer, unaware of the clinical severity category and of the outcome, using oil immersion microscopy.

Nucleated red blood cells were counted per 100 consecutive white blood cells, and where the count exceeded 10 per 100 white cells at least 200 white cells were counted to improve precision. The corrected total leucocyte count was calculated and the absolute nucleated red blood cell count derived as the count per 100 white cells multiplied by the total leucocyte count and divided by 100, expressed per cubic millimetre. A count above 10 per 100 white cells beyond the first 24 hours of life was regarded as elevated in accordance with published reference ranges. Ten percent of smears were re-examined by a second observer, and the inter-observer agreement was assessed by the intra class correlation coefficient.

Data collection and outcomes

A structured proforma recorded maternal age, parity, antenatal history, mode of delivery, gestational age assessed by the new Ballard score, birth weight, sex, Apgar scores, presenting clinical features, examination findings and the results of all investigations. Blood culture was obtained before the first dose of antibiotic in

every case. Cerebrospinal fluid examination was performed where meningitis was suspected. Outcomes recorded comprised in-hospital mortality, the requirement for inotropic support, the requirement for mechanical ventilation, the duration of hospital stay and the occurrence of complications including disseminated intravascular coagulation, necrotising enterocolitis and meningitis.

Statistical analysis

Data were entered in Microsoft Excel and analysed using IBM SPSS Statistics version 26.0. The nucleated red blood cell count was markedly right-skewed on Shapiro-Wilk testing and was therefore expressed as median with interquartile range and analysed by non-parametric methods throughout. Comparisons between two groups employed the Mann-Whitney U test and comparisons across the three severity strata the Kruskal-Wallis test, with the Dunn test and Bonferroni correction for pairwise comparison where the overall test was significant. Normally distributed continuous variables were expressed as mean with standard deviation and compared by the Student t test.

Categorical variables were compared by the chi-square test or the Fisher exact test. Correlation was assessed by the Spearman rank correlation coefficient. Receiver operating characteristic curves were constructed for the nucleated red blood cell count, the C-reactive protein and the immature to total neutrophil ratio against in-hospital mortality, and areas under the curve were compared by the DeLong method. Variables associated with mortality at p below 0.10 on univariate analysis were entered into a multivariable logistic regression model. A two-tailed p value below 0.05 was considered statistically significant.

Results

Participant characteristics

One hundred and eighty-six neonates with suspected sepsis were screened. Sixty-six were excluded, of whom 28 had perinatal asphyxia or hypoxic ischaemic encephalopathy, 12 had haemolytic disease or a positive direct antiglobulin test, 9 were born to mothers with diabetes, 7 had intrauterine growth restriction below the third centile, 5 had major congenital malformations, and 5 were excluded for other listed reasons. One hundred and twenty neonates with sepsis were enrolled together with 60 matched controls.

The mean gestational age was 34.2 ± 3.4 weeks among cases and 34.6 ± 3.2 weeks among controls (t = 0.75, p = 0.454), and the mean birth weight was 2148 ± 612 g and 2214 ± 588 g respectively (t = 0.69, p = 0.492), matching having been performed on both. Seventy cases (58.3 percent) and 34 controls (56.7 percent) were male (χ^2 = 0.04, p = 0.833). Early-onset sepsis accounted for 68 cases (56.7 percent) and late-onset sepsis for 52 cases (43.3 percent). Blood culture was positive in 42 cases (35.0 percent), the commonest isolates being Klebsiella pneumoniae in 14, coagulase-negative staphylococci in 9, Escherichia coli in 7, Staphylococcus aureus in 6, Acinetobacter species in 4 and Candida species in 2. Baseline characteristics are presented in Table 1.

Nucleated red blood cell counts in cases and controls

The median nucleated red blood cell count at presentation was 14 per 100 white cells with an interquartile range of 7 to 26 among septic neonates and 4 per 100 white cells with an interquartile range of 2 to 7 among controls, a highly significant difference (U = 741.5, p < 0.001). The median absolute count was 1,624 per cubic millimetre with an interquartile range of 812 to 3,146 among cases and 428 per cubic millimetre with an

interquartile range of 214 to 764 among controls (U = 782.0, p < 0.001). A count above 10 per 100 white cells was present in 74 cases (61.7 percent) and 6 controls (10.0 percent) ($\chi^2 = 44.62$, p < 0.001).

The count did not differ significantly between culture-positive and culture-negative probable sepsis, with medians of 16 and 13 per 100 white cells respectively (U = 1508.5, p = 0.284), nor between early-onset and late-onset sepsis, with medians of 15 and 13 (U = 1642.0, p = 0.471). The inter-observer intraclass correlation coefficient for the manual count was 0.94 with a 95 percent confidence interval of 0.86 to 0.98. These data are set out in Table 2.

Correlation with severity

Among the 120 septic neonates, 58 (48.3 percent) were categorised as sepsis, 41 (34.2 percent) as severe sepsis and 21 (17.5 percent) as septic shock. The median nucleated red blood cell count rose progressively across these strata, being 9 per 100 white cells with an interquartile range of 5 to 14 in sepsis, 18 with an interquartile range of 11 to 28 in severe sepsis, and 34 with an interquartile range of 22 to 52 in septic shock. The Kruskal-Wallis test confirmed a highly significant difference across strata (H = 46.28, degrees of freedom 2, p < 0.001), and all three pairwise comparisons remained significant after Bonferroni correction (sepsis versus severe sepsis p < 0.001, severe sepsis versus septic shock p = 0.002, sepsis versus septic shock p < 0.001).

Spearman rank correlation confirmed a positive association between the count and severity category (rho = 0.612, p < 0.001). The count also correlated positively with C-reactive protein (rho = 0.428, p < 0.001), with the immature to total neutrophil ratio (rho = 0.386, p < 0.001) and with the duration of hospital stay (rho = 0.324, p < 0.001), and negatively with gestational age (rho = −0.286, p = 0.002) and with birth weight (rho = −0.264, p = 0.004). Severity data appear in Table 3.

Outcome and discriminatory performance

Twenty-two of the 120 septic neonates died, an in-hospital mortality of 18.3 percent. The median nucleated red blood cell count was 32 per 100 white cells with an interquartile range of 22 to 54 among non-survivors and 11 per 100 white cells with an interquartile range of 6 to 19 among survivors (U = 412.5, p < 0.001).

On receiver operating characteristic analysis the nucleated red blood cell count discriminated in-hospital mortality with an area under the curve of 0.812 with a 95 percent confidence interval of 0.716 to 0.908 (p < 0.001). The Youden index identified an optimal cut-off of 22 per 100 white cells, at which the sensitivity was 77.3 percent, the specificity 76.5 percent, the positive predictive value 42.5 percent and the negative predictive value 93.8 percent. C-reactive protein achieved an area under the curve of 0.748 with a 95 percent confidence interval of 0.634 to 0.862, and the immature to total neutrophil ratio 0.706 with a 95 percent confidence interval of 0.586 to 0.826. Neither differed significantly from the nucleated red blood cell count on DeLong comparison (p = 0.362 and p = 0.184 respectively). The combination of the nucleated red blood cell count with C-reactive protein achieved an area under the curve of 0.856 with a 95 percent confidence interval of 0.772 to 0.940, which was significantly superior to C-reactive protein alone (p = 0.024).

On multivariable logistic regression, a nucleated red blood cell count above 22 per 100 white cells remained independently associated with in-hospital mortality after adjustment (adjusted odds ratio 4.86, 95 percent confidence interval 1.72 to 13.73, p = 0.003), as did the

presence of septic shock (adjusted odds ratio 5.42, 95 percent confidence interval 1.86 to 15.79, p = 0.002). Gestational age below 32 weeks and birth weight below 1500 g did not retain independent significance. Outcome and discrimination data are presented in Tables 4 and 5.

Trend in count between presentation and 72 hours

Paired counts at presentation and at 72 hours were available for 108 of the 120 septic neonates, the remainder having died or been discharged before the second sample. The count fell by 25 percent or more in 66 neonates (61.1 percent), remained within 25 percent of the presenting value in 27 neonates (25.0 percent) and rose by 25 percent or more in 15 neonates (13.9 percent). Mortality differed markedly across these categories, being 4 of 66 (6.1 percent) where the count fell, 5 of 27 (18.5 percent) where it was static and 4 of 15 (26.7 percent) where it rose. Taking the static and rising categories together, mortality was 9 of 42 (21.4 percent) where the count failed to fall by 72 hours compared with 4 of 66 (6.1 percent) where it fell ($\chi^2 = 5.71$, p = 0.017). Among neonates whose presenting count exceeded 22 per 100 white cells, a subsequent fall was associated with survival in 18 of 21 (85.7 percent) compared with 8 of 17 (47.1 percent) in whom it did not fall ($\chi^2 = 6.49$, p = 0.011). Trend data appear in Table 6.

Table 1: Baseline characteristics of septic neonates and matched controls (N = 180)

Variable	Sepsis (n = 120)	Controls (n = 60)	Test statistic	p value
Gestational age, weeks (mean ± SD)	34.2 ± 3.4	34.6 ± 3.2	t = 0.75	0.454
Gestational age <32 weeks, n (%)	34 (28.3)	15 (25.0)	$\chi^2 = 0.22$	0.638
Birth weight, g (mean ± SD)	2148 ± 612	2214 ± 588	t = 0.69	0.492
Birth weight <1500 g, n (%)	28 (23.3)	12 (20.0)	$\chi^2 = 0.25$	0.615
Male sex, n (%)	70 (58.3)	34 (56.7)	$\chi^2 = 0.04$	0.833
Caesarean delivery, n (%)	62 (51.7)	29 (48.3)	$\chi^2 = 0.17$	0.677
Outborn, n (%)	38 (31.7)	14 (23.3)	$\chi^2 = 1.34$	0.247
Prolonged rupture of membranes >18 h, n (%)	31 (25.8)	6 (10.0)	$\chi^2 = 6.16$	0.013
Foul-smelling liquor, n (%)	18 (15.0)	2 (3.3)	$\chi^2 = 5.44$	0.020
Onset of sepsis, n (%)				
Early onset (<72 h)	68 (56.7)	—	—	—
Late onset (≥72 h)	52 (43.3)	—	—	—
Blood culture positive, n (%)	42 (35.0)	0 (0.0)	—	—
Klebsiella pneumoniae	14	—		
Coagulase-negative staphylococci	9	—		
Escherichia coli	7	—		
Staphylococcus aureus	6	—		
Acinetobacter species	4	—		
Candida species	2	—		

SD, standard deviation. Controls were matched to cases by gestational age within two weeks and by birth weight within 250 g in a 1:2 ratio.

Table 2: Nucleated red blood cell counts in septic neonates and controls (N = 180)

Parameter	Sepsis (n = 120)	Controls (n = 60)	Test statistic	p value
NRBC per 100 WBC, median (IQR)	14 (7–26)	4 (2–7)	U = 741.5	<0.001
Absolute NRBC, /mm³, median (IQR)	1624 (812–3146)	428 (214–764)	U = 782.0	<0.001
NRBC >10 per 100 WBC, n (%)	74 (61.7)	6 (10.0)	$\chi^2 = 44.62$	<0.001
NRBC >22 per 100 WBC, n (%)	40 (33.3)	1 (1.7)	$\chi^2 = 22.85$	<0.001
Within sepsis group				
Culture-positive (n = 42), median (IQR)	16 (8–30)	—	U = 1508.5	0.284
Culture-negative (n = 78), median (IQR)	13 (7–24)	—		
Early onset (n = 68), median (IQR)	15 (8–28)	—	U = 1642.0	0.471
Late onset (n = 52), median (IQR)	13 (7–24)	—		
C-reactive protein, mg/L, median (IQR)	38 (16–72)	3 (2–5)	U = 604.0	<0.001
Immature to total neutrophil ratio, median (IQR)	0.24 (0.16–0.34)	0.08 (0.05–0.12)	U = 812.5	<0.001
Inter-observer ICC for manual NRBC count	0.94 (0.86–0.98)	—	—	—

NRBC, nucleated red blood cells; WBC, white blood cells; IQR, interquartile range; ICC, intra class correlation coefficient.

Table 3: Nucleated red blood cell count by clinical severity of sepsis (n = 120)

Parameter	Sepsis (n = 58)	Severe sepsis (n = 41)	Septic shock (n = 21)	Test statistic	p value
NRBC per 100 WBC, median (IQR)	9 (5–14)	18 (11–28)	34 (22–52)	H = 46.28	<0.001
Absolute NRBC, /mm³, median (IQR)	946 (524–1682)	2148 (1204–3428)	4126 (2384–6218)	H = 42.16	<0.001
NRBC >22 per 100 WBC, n (%)	6 (10.3)	16 (39.0)	18 (85.7)	$\chi^2 = 42.18$	<0.001
C-reactive protein, mg/L, median (IQR)	24 (12–42)	48 (28–82)	86 (52–134)	H = 32.64	<0.001
Inotropic support, n (%)	0 (0.0)	8 (19.5)	21 (100.0)	$\chi^2 = 96.42$	<0.001
Mechanical ventilation, n (%)	4 (6.9)	17 (41.5)	19 (90.5)	$\chi^2 = 55.28$	<0.001
Hospital stay, days, median (IQR)	8 (6–11)	12 (9–16)	9 (5–18)	H = 14.82	0.001
Mortality, n (%)	2 (3.4)	8 (19.5)	12 (57.1)	$\chi^2 = 32.46$	<0.001

NRBC, nucleated red blood cells; WBC, white blood cells; IQR, interquartile range; H, Kruskal-Wallis test statistic. All pair wise comparisons of NRBC count between severity strata remained significant after

Bonferroni correction (sepsis vs severe sepsis $p < 0.001$; severe sepsis vs septic shock $p = 0.002$; sepsis vs septic shock $p < 0.001$). Spearman correlation of NRBC count with severity category rho = 0.612, $p < 0.001$.

Table 4: Comparison of survivors and non-survivors among septic neonates (n = 120)

Variable	Survivors (n = 98)	Non-survivors (n = 22)	Test statistic	p value
NRBC per 100 WBC, median (IQR)	11 (6–19)	32 (22–54)	U = 412.5	<0.001
Absolute NRBC, /mm³, median (IQR)	1284 (642–2416)	3862 (2214–6484)	U = 438.0	<0.001
NRBC >22 per 100 WBC, n (%)	23 (23.5)	17 (77.3)	$\chi^2 = 23.42$	<0.001
Gestational age, weeks (mean ± SD)	34.6 ± 3.2	32.4 ± 3.6	t = 2.84	0.005
Birth weight, g (mean ± SD)	2216 ± 588	1846 ± 634	t = 2.61	0.010
C-reactive protein, mg/L, median (IQR)	32 (14–62)	78 (44–118)	U = 546.0	<0.001
Immature to total neutrophil ratio, median (IQR)	0.22 (0.14–0.31)	0.34 (0.24–0.46)	U = 618.5	<0.001
Septic shock, n (%)	9 (9.2)	12 (54.5)	$\chi^2 = 25.84$	<0.001
Culture-positive sepsis, n (%)	31 (31.6)	11 (50.0)	$\chi^2 = 2.71$	0.100
Mechanical ventilation, n (%)	22 (22.4)	18 (81.8)	$\chi^2 = 29.16$	<0.001

NRBC, nucleated red blood cells; WBC, white blood cells; IQR, interquartile range; SD, standard deviation.

Table 5: Discriminatory performance for in-hospital mortality and multivariable analysis (n = 120)

Parameter	AUC	95% CI	Cut-off	Sens (%)	Spec (%)	p
NRBC per 100 WBC	0.812	0.716–0.908	>22	77.3	76.5	<0.001
C-reactive protein	0.748	0.634–0.862	>52 mg/L	68.2	73.5	<0.001
Immature to total neutrophil ratio	0.706	0.586–0.826	>0.28	63.6	69.4	0.002
NRBC combined with CRP	0.856	0.772–0.940	—	81.8	79.6	<0.001
Multivariable logistic regression						
NRBC >22 per 100 WBC	—	aOR 4.86 (1.72–13.73)	—	—	—	0.003
Septic shock	—	aOR 5.42 (1.86–15.79)	—	—	—	0.002
Gestational age <32 weeks	—	aOR 1.94 (0.68–5.54)	—	—	—	0.215
Birth weight <1500 g	—	aOR 1.72 (0.59–5.02)	—	—	—	0.322
Culture-positive sepsis	—	aOR 1.48 (0.54–4.06)	—	—	—	0.446

NRBC, nucleated red blood cells; WBC, white blood cells; AUC, area under the curve; CI, confidence interval; Sens, sensitivity; Spec, specificity; CRP, C-reactive protein; aOR, adjusted odds ratio. At the cut-off of 22 per 100 WBC the positive predictive value was 42.5 percent and the negative predictive value 93.8 percent. DeLong comparison against NRBC: CRP p = 0.362; I:T ratio p = 0.184; NRBC with CRP versus CRP

alone p = 0.024. Model: overall χ^2 = 38.6, df 5, p < 0.001; Hosmer - Lemeshow p = 0.584; Nagelkerke R^2 = 0.428.

Table 6: Trend in nucleated red blood cell count between presentation and 72 hours and associated mortality (n = 108)

Trend category	n (%)	Median NRBC at 0 h	Median NRBC at 72 h	Mortality, n (%)
Fell by ≥25%	66 (61.1)	13 (7–22)	5 (3–9)	4 (6.1)
Static (within ±25%)	27 (25.0)	16 (9–28)	15 (8–26)	5 (18.5)
Rose by ≥25%	15 (13.9)	22 (14–38)	38 (24–62)	4 (26.7)
Static or rising combined	42 (38.9)	18 (10–32)	22 (12–42)	9 (21.4)
Comparison: fell versus not fell			χ^2 = 5.71	p = 0.017
Among those with 0 h NRBC >22 (n = 38)				
Subsequent fall	21 (55.3)	—	—	3 (14.3)
No fall	17 (44.7)	—	—	9 (52.9)
Comparison within this subgroup			χ^2 = 6.49	p = 0.011

NRBC, nucleated red blood cells, expressed per 100 white blood cells as median with interquartile range. Paired counts were available for 108 of 120 neonates; 12 died or were discharged before the 72-hour sample. Thirteen of the 22 deaths occurred among neonates with paired counts; the remaining nine died before the 72-hour sample could be obtained and are therefore not represented in this table.

Discussion

This prospective observational study found that the nucleated red blood cell count at presentation was significantly higher in neonates with sepsis than in matched controls, that it rose progressively and significantly across categories of increasing clinical severity, and that it discriminated in-hospital mortality with an area under the curve of 0.812, remaining independently associated with death after adjustment for gestational age, birth weight and the presence of shock. A failure of the count to fall by 72 hours identified a group with a mortality of 42.9 percent.

These findings are broadly concordant with the limited published literature. Christou and colleagues, in a cohort of 467 critically ill neonates, reported that the count showed significant prognostic power for mortality specifically among septic neonates, with an area under the curve of 0.760 overall and 0.816 among preterm septic neonates.[5] The area under the curve of 0.812 observed here lies between those two figures. Of particular interest is the parallel observation in that study that no significant difference in the count was found between survivors and non-survivors across the critically ill cohort as a whole, despite the significant prognostic performance within the septic subgroup. This apparent paradox is explained by the heterogeneity of causes of erythroblastosis in an unselected critically ill population, in which markedly elevated counts arising from perinatal hypoxia obscure the signal attributable to sepsis. The design of the present study, which excluded asphyxia, haemolytic disease, maternal diabetes and growth restriction, was intended precisely to avoid this dilution, and the resulting performance is correspondingly better. The corollary is important and limits the applicability of

these findings: the count is interpretable as a marker of sepsis severity only after these alternative causes have been excluded, which in practice means it cannot be applied at the bedside to an undifferentiated sick neonate without clinical judgement about the perinatal history.

The biological explanation for the association is plausible and is likely to involve two mechanisms operating together. Inflammatory cytokines released in sepsis, particularly interleukin-6 and tumour necrosis factor alpha, promote the premature release of erythroid precursors from the marrow, and their concentrations rise with the severity of the inflammatory response, which would account for the graded relationship with severity observed here. In parallel, the tissue hypoxia accompanying septic shock stimulates erythropoietin release, providing a second and independent stimulus to erythroblastosis, which would explain why the highest counts were observed in the septic shock stratum with a median of 34 per 100 white cells. The observation that the count correlated with C-reactive protein and with the immature to total neutrophil ratio, both markers of inflammatory intensity, supports the first mechanism, while its steep rise in the shock stratum supports the second.

The prognostic value of the trend between presentation and 72 hours is arguably the most clinically useful finding of this study and has received little attention in the published literature. That mortality was 6.1 percent where the count fell and 21.4 percent where it did not is a substantial difference, and it is consistent with the interpretation of the count as an index of the intensity of the ongoing inflammatory and hypoxic stimulus rather than as a static marker of infection. A repeat smear at 72 hours costs nothing beyond the technician time already committed to the routine count and may identify the neonate in whom the current therapeutic approach is failing.

Several important caveats must temper any enthusiasm. First, the parameter is prognostic and not diagnostic. The count did not differ between culture-positive and culture-negative probable sepsis, which is unsurprising given that culture negativity in neonatal practice reflects sampling limitations rather than the absence of infection, but it also means the count cannot be used to determine which neonates are truly infected and therefore cannot guide the decision to start or withhold antibiotics. Second, the positive predictive value at the optimal cut-off was only 42.5 percent, so a high count does not reliably predict death. The negative predictive value of 93.8 percent is the more useful figure, and the parameter is therefore better employed to identify neonates at low risk than to identify those at high risk. Third, the manual count is observer-dependent, and although the inter-observer intraclass correlation coefficient of 0.94 achieved here was excellent, it was obtained with a single trained observer under study conditions, and reproducibility in routine practice with rotating staff may be lower. Automated counts reported by contemporary analysers may not be interchangeable with manual counts, as noted in the reference range work of Christensen and colleagues.[4]

The comparison with established markers is worth stating plainly. The nucleated red blood cell count did not perform significantly better than C-reactive protein or the immature to total neutrophil ratio for the prediction of mortality, and any claim of superiority would be unsupported by these data. Its advantages are practical rather than statistical: it is available from a smear already being prepared, requires no reagent, is reported within minutes, and adds prognostic

information to C-reactive protein when the two are combined, the combination achieving an area under the curve significantly superior to C-reactive protein alone. In a district or medical college unit where interleukin-6, presepsin and CD64 assays are unavailable, that practical profile has real value.[3]

Limitations

This study has several limitations. It was conducted at a single centre with a modest sample size, and the septic shock stratum contained only 21 neonates, so the estimates within that stratum are imprecise. The rigorous exclusion criteria, while necessary to isolate the effect of sepsis, produced a study population that is not representative of the undifferentiated sick neonate to whom a clinician would wish to apply the test, and the performance reported here should be regarded as an upper bound. The manual count, though performed by a blinded and trained observer with excellent reproducibility, remains subject to sampling error, particularly at low counts. Erythropoietin, interleukin-6 and other cytokines were not measured, so the proposed mechanism was not tested directly. Sepsis was defined clinically with laboratory support, and the majority of cases were culture-negative, which is representative of Indian neonatal practice but means that a proportion of participants may not have been infected. Only two time points were sampled, so the full kinetics of the count were not characterised. Follow-up ended at discharge, so no comment can be made on neurodevelopment al outcome, which is relevant given the established association between elevated counts and intraventricular haemorrhage and retinopathy of prematurity. Finally, the study did not assess whether knowledge of the count would alter management or improve outcome, which is the question that ultimately determines clinical utility.

Conclusion

The nucleated red blood cell count at presentation was significantly higher in neonates with sepsis than in matched controls, rose progressively across categories of increasing severity from a median of 9 per 100 white cells in sepsis to 34 in septic shock, and discriminated in-hospital mortality with an area under the curve of 0.812 at an optimal cut-off of 22 per 100 white cells. A count above this threshold remained independently associated with death after adjustment, and a failure of the count to fall by 72 hours identified a group with a mortality of 21.4 percent against 6.1 percent in those whose count fell.

The nucleated red blood cell count is therefore best regarded as a prognostic adjunct rather than a diagnostic test. It cannot distinguish infected from uninfected neonates, it did not outperform C-reactive protein or the immature to total neutrophil ratio statistically, and its positive predictive value for death is low. What it offers is a high negative predictive value, a graded relationship with severity, and prognostic information from the trend over the first 72 hours, all obtainable from a Giemsa-stained smear at no additional cost and within minutes of the sample reaching the laboratory. These properties suit it to resource-constrained units in which newer inflammatory markers are unavailable. Its interpretation depends absolutely on the prior exclusion of perinatal asphyxia, haemolytic disease, maternal diabetes and growth restriction, without which the count reflects hypoxia rather than infection. Larger multicentre studies incorporating cytokine measurement, automated counting and neurodevelopmental follow-up are required before the parameter can be recommended for routine prognostic use.

References

1. Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. I. Reference values for neutrophilic cells. J Pediatr. 1979; 95(1):89-98.
2. Schmutz N, Henry E, Jopling J, Christensen RD. Expected ranges for blood neutrophil concentrations of neonates: the Manroe and Mouzinho charts revisited. J Perinatol. 2008; 28(4):275-81.
3. Sharma D, Farahbakhsh N, Shastri S, Sharma P. Biomarkers for diagnosis of neonatal sepsis: a literature review. J Matern Fetal Neonatal Med. 2018; 31 (12): 16 46-59.
4. Christensen RD, Henry E, Andres RL, Bennett ST. Reference ranges for blood concentrations of nucleated red blood cells in neonates. Neonatology. 2011; 99(4): 289-94.
5. Christou E, Karaolia D, Tsekoura E, Vraila V, Papadopoulou-Legbelou K, Sarafidis K, et al. Nucleated red blood cells: could they be indicator markers of illness severity for neonatal intensive care unit patients? Children (Basel). 2020;7(11):197.
6. Menk M, Giebelhaeuser L, Vorderwuelbecke G, Gassner M, Graw JA, Weiss B, et al. Nucleated red blood cells as predictors of mortality in patients with acute respiratory distress syndrome (ARDS): an observational study. Ann Intensive Care. 2018;8(1):42.
7. Boskabadi H, Maamouri G, Sadeghian MH, Ghayour-Mobarhan M, Heidarzade M, Shakeri MT, et al. Early diagnosis of perinatal asphyxia by nucleated red blood cell count: a case-control study. Arch Iran Med. 2010; 13(4):275-81.
8. Newman TB, Puopolo KM, Wi S, Draper D, Escobar GJ. Interpreting complete blood counts soon after birth in newborns at risk for sepsis. Pediatrics. 2010; 126(5):903-9.
9. Goldstein B, Giroir B, Randolph A; International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med. 2005; 6(1):2-8.
10. Shane AL, Sanchez PJ, Stoll BJ. Neonatal sepsis. Lancet. 2017;390(10104):1770-80.
11. Hornik CP, Fort P, Clark RH, Watt K, Benjamin DK Jr, Smith PB, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early Hum Dev. 2012;88 Suppl 2:S69-74.
12. Investigators of the Delhi Neonatal Infection Study (DeNIS) Collaboration. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. Lancet Glob Health. 2016;4(10):e752-60.
13. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. J Pediatr. 1991; 119 (3):417-23.
14. Hebbar S, Misha M, Rai L. Significance of maternal and cord blood nucleated red blood cell count in pregnancies complicated by preeclampsia. J Pregnancy. 2014;2014:496416.
15. Perrone S, Vezzosi P, Longini M, Marzocchi B, Paffetti P, Bellieni CV, et al. Nucleated red blood cell count in term and preterm newborns: reference values at birth. Arch Dis Child Fetal Neonatal Ed. 2005; 90(2): F174-5.
16. Dulay AT, Buhimschi IA, Zhao G, Bahtiyar MO, Thung SF, Cackovic M, et al. Nucleated red blood cells are a direct response to mediators of inflammation in

newborns with early-onset neonatal sepsis. Am J Obstet Gynecol. 2008;198(4):426.e1-9.

17. Cremer M, Roll S, Graf C, Weimann A, Buhrer C, Dame C. Nucleated red blood cells as marker for an increased risk of unfavorable outcome and mortality in very low birth weight infants. Early Hum Dev. 2015; 91 (10):559-63.

18. Ghosh B, Mittal S, Kumar S, Dadhwal V. Prediction of perinatal asphyxia with nucleated red blood cells in cord blood of newborns. Int J Gynaecol Obstet. 2003; 81(3):267-71.