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Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio as Biomarkers in Early-Onset Neonatal Sepsis in Full-Term Neonates: A Prospective Observational Study

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ABSTRACT

Background: Early-onset neonatal sepsis (EOS) is a leading cause of neonatal morbidity and mortality in developing countries. Blood culture, the gold standard, is slow and often low-yield in resource-limited settings. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), derived from a routine complete blood count, may serve as rapid, inexpensive adjuncts. We assessed their association with perinatal risk factors and their diagnostic and prognostic performance in full-term neonates with EOS.
Methods: In this one-year prospective observational study at the neonatal intensive care units of two tertiary hospitals in Kota, India, 150 term neonates (≥37 weeks) with suspected or confirmed EOS were enrolled. NLR, PLR, C-reactive protein (CRP) and immature-to-total neutrophil (I/T) ratio were measured at admission. Diagnostic performance was assessed by receiver operating characteristic (ROC) analysis and predictors of culture positivity and mortality by logistic regression.
Results: Forty neonates (26.7%) were culture-positive. NLR (4.1 vs 1.9) and PLR (122 vs 64) were higher with culture positivity (both $p<0.001$). Optimal cut-offs were NLR ≥1.8 (AUC 0.82) and PLR ≥75 (AUC 0.76); CRP had the highest AUC (0.85). Chorioamnionitis (OR 6.25) and prolonged rupture of membranes (OR 2.18) were the strongest risk factors, and Klebsiella spp. the commonest isolate (37.5%). Fifteen neonates (10.0%) died. NLR ≥1.8 independently predicted culture-positive sepsis (adjusted OR 4.1) and mortality (adjusted OR 3.6), as did thrombocytopenia (adjusted OR 2.8).
Conclusion: NLR and PLR are significantly elevated in EOS and add diagnostic and prognostic value alongside CRP and clinical assessment. These accessible, low-cost markers suit early sepsis identification and risk stratification in resource-limited neonatal care.

Keywords: *Neonatal sepsis; early-onset sepsis; neutrophil-to-lymphocyte ratio; platelet-to-lymphocyte ratio; haematological biomarkers; neonatal intensive care; India.*

INTRODUCTION

Neonatal sepsis is a systemic inflammatory response to invasive microbial infection in the neonatal period, characterised by bacteraemia with multisystem involvement, and remains a major contributor to neonatal morbidity and mortality worldwide.[1-3] It is classified into early-onset sepsis (EOS), occurring within the first 72 hours through vertical transmission, and late-onset sepsis, developing after 72 hours. This temporal distinction is clinically important because pathogen profiles, pathogenesis, and preventive strategies differ between the two forms.[4]

Global estimates indicate approximately 2824 cases of neonatal sepsis per 100 000 live births, with case fatality rates of 11%–19%.[5] The burden is disproportionately higher in south Asia and sub-Saharan Africa, where perinatal healthcare and infection-control infrastructure remain limited. In India, neonatal sepsis accounts for approximately 25%–30% of neonatal

deaths, with a hospital-based NICU incidence of 30–50 per 1000 live births.[6] Gram-negative organisms predominate in Indian EOS and are associated with high mortality and antimicrobial resistance.

EOS presents with non-specific manifestations—lethargy, poor feeding, respiratory distress and temperature instability—which overlap with normal neonatal transitional physiology, complicating early recognition.[7] Blood culture remains the gold standard but requires prolonged incubation and may be affected by prior antibiotics or a low bacterial load. Procalcitonin shows physiological elevation in early neonatal life, reducing its specificity, while C-reactive protein (CRP) rises later in the inflammatory course, limiting its early diagnostic value.[8]

Neonatal sepsis produces characteristic haematological alterations: infection induces neutrophilia with increased immature forms, relative lymphopenia from redistribution and apoptosis, and thrombocytopenia from platelet activation and consumption.[9] Because sepsis simultaneously alters neutrophil, lymphocyte and platelet counts, derived ratios may reflect the overall inflammatory state better than isolated cell counts. The neutrophil-to-lymphocyte ratio (NLR) represents the balance between innate inflammatory activation and adaptive immune regulation, whereas the platelet-to-lymphocyte ratio (PLR) reflects platelet-mediated inflammatory activation combined with lymphocyte suppression. Both are derived from a routine complete blood count (CBC), making them inexpensive and rapidly available.[10]

Despite growing evidence, the combined evaluation of NLR and PLR in full-term neonates with EOS is limited, particularly in resource-constrained Indian settings. Most studies include heterogeneous preterm and term populations, focus on late-onset or mixed sepsis, or emphasise diagnostic performance without examining outcomes and perinatal risk factors. The present study addresses these gaps by evaluating NLR and PLR in EOS among full-term neonates and by examining their relationship with clinical characteristics, microbial profile and neonatal outcomes.

The primary objective was to estimate NLR and PLR in EOS among full-term neonates at a tertiary-care hospital and to assess their diagnostic performance against blood-culture results. The secondary objectives were to study the perinatal risk factors associated with EOS, to identify the causative organisms and their antibiotic-sensitivity patterns, and to evaluate clinical outcomes—including the prognostic value of NLR, PLR and thrombocytopenia in predicting mortality.

METHODS

Study design and setting: A prospective observational study was conducted over one year after obtaining Institutional Ethics Committee approval. It was performed in the neonatal intensive care units (NICUs) of the Department of Paediatrics at JK Lon Mother and Child Hospital and New Medical College and Hospital, Government Medical College, Kota, Rajasthan, India.

Selection of participants: Term neonates (gestational age ≥37 weeks) with suspected or confirmed EOS were enrolled. Suspected EOS was defined as a positive sepsis screen (two or more of: total leucocyte count <5000/mm³; absolute neutrophil count below the Manroe chart cut-off for age; quantitative CRP >1 mg/dl; and immature-to-total neutrophil [I/T] ratio >0.2). Confirmed EOS was defined as a positive blood culture. Neonates delivered vaginally or by caesarean section were included. Exclusion criteria were gestational age <37 weeks, late-onset sepsis and refusal of parental consent.

Sample size: The sample size was calculated using the formula [$n = z^2 \times p \times (1-p)/e^2$], where z=1.96, p=0.17 (based on the reported prevalence of EOS among NICU admissions at referral centres in India)[6] and e=0.06, yielding n=150.

Data collection and laboratory methods: A detailed history was obtained to identify maternal and neonatal risk factors as per National Neonatology Forum guidelines. After informed consent, simultaneous blood samples were drawn for haematological studies, quantitative CRP and blood culture, followed by initiation of empirical antibiotics. Complete blood counts were performed on an automated haematology analyser. NLR and PLR were calculated as the ratio of neutrophil count to lymphocyte count and of platelet count to lymphocyte count, respectively. CRP was measured by immunoturbidimetric assay. Blood cultures were processed using a semi-automated culture system (BACTEC 9050, Becton Dickinson) and subsequently plated on solid media. Antimicrobial-sensitivity testing was performed by the disc-diffusion method for culture-positive cases.

Outcome measurement and follow-up: ROC curve analysis was performed to determine optimal cut-off values for NLR, PLR, CRP and I/T ratio using the Youden index. In addition, the lower protocol-defined thresholds (NLR ≥0.1, PLR ≥7), used in some institutional screening protocols to maximise sensitivity, were evaluated for specificity. Sensitivity, specificity, and positive and negative predictive values were calculated. Neonates were followed until discharge, death or discharge against medical advice; no enrolled neonate was lost to follow-up during the hospital stay.

Statistical analysis: All analyses were performed using SPSS version 24.0. The Student t-test, chi-square test with Yates continuity correction, Fisher exact test and Fisher–Freeman–Halton test were used for comparisons. Continuous data are presented as mean ± SD and categorical data as number and percentage. Univariate logistic regression identified candidate

predictors of culture positivity and mortality; significant variables were entered into multivariate logistic regression. A p value <0.05 was considered statistically significant.

Ethical considerations: The study was approved by the Institutional Ethics Committee, Government Medical College, Kota (Ethical Clearance Certificate/Office of Principal GMC & Controller AG Hosp, Kota Letter no. F.3( )Acad/Ethical Clearance/Batch 2023/2025/05; Dated: 24.02.2025; S.No.77) , and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parents or guardians of all enrolled neonates.

RESULTS

Socio-demographic and baseline characteristics: Of 150 enrolled neonates, 84 (56.0%) were male and 66 (44.0%) female. Maternal age clustered predominantly in the 25–30 years range (50.0%), followed by 18–24 years (30.0%) and >30 years (20.0%). The mean birth weight was 2500 ± 400 g and the mean admission weight 2450 ± 420 g. The mean gestational age was 38.6 ± 1.1 weeks by last menstrual period and 38.4 ± 1.0 weeks by Ballard scoring. The mean age at presentation was 18 ± 10 hours, and 60.0% presented within the first 24 hours. The majority were inborn (73.3%) and delivered vaginally (60.0%). Baseline characteristics are summarised in Table 1.

Perinatal risk factors and clinical profile: Premature rupture of membranes (PROM) >24 hours was the most frequent perinatal risk factor, present in 51 (34.0%) cases, followed by prolonged labour in 36 (24.0%), foul-smelling liquor in 27 (18.0%), severe perinatal asphyxia (Apgar <4 at 1 minute) in 24 (16.0%) and chorioamnionitis in 12 (8.0%). On admission, 50.0% of neonates were lethargic and 10.0% comatose; poor cry or suck was observed in 53.3%. Neonatal reflexes were weak or absent in 56.7%, central or peripheral cyanosis was present in 26.7% and delayed capillary refill time (CRT) in 36.7%.

Haematological and inflammatory parameters: Overall mean values in the cohort were: TLC 9100/mm³, ANC 4980/mm³, ALC 2240/mm³, I/T ratio 0.22, platelets 188 400/mm³, CRP 2.22 mg/dl, NLR 2.49 and PLR 83. Culture-positive neonates (n=40) had significantly higher ANC (6200 vs 4300/mm³; p<0.001), NLR (4.1 vs 1.9; p<0.001), PLR (122 vs 64; p<0.001), CRP (4.2 vs 1.1 mg/dl; p<0.001) and I/T ratio (0.30 vs 0.17; p<0.001), with lower ALC (1600 vs 2600/mm³; p<0.001) and platelet counts (150 000 vs 210 000/mm³; p<0.001) than culture-negative neonates (n=110). TLC also differed significantly (8200 vs 9600/mm³; p=0.02). Details are given in Table 2.

Microbiological profile: Among the 40 culture-positive neonates, *Klebsiella* spp. was the commonest isolate (15; 37.5%), followed by *Escherichia coli* (9; 22.5%), *Acinetobacter* spp. (6; 15.0%), coagulase-negative staphylococci (CoNS; 5; 12.5%), *Staphylococcus aureus* (3; 7.5%) and *Enterococcus* spp. (2; 5.0%). Gram-negative organisms accounted for 75% of all isolates. *Klebsiella* was sensitive to amikacin and cefoperazone–sulbactam; *E. coli* to meropenem and amikacin; *Acinetobacter* to colistin; CoNS and *Enterococcus* to vancomycin; and *S. aureus* to linezolid. The case fatality rate among culture-positive neonates was 12.5% (5 deaths).

Table 1: Baseline characteristics of study neonates (n=150)

Variable	Category	n (%)
Sex	Male	84 (56.0)
	Female	66 (44.0)
Place of delivery	Inborn	110 (73.3)
	Outborn	40 (26.7)
Mode of delivery	Vaginal	90 (60.0)
	LSCS	60 (40.0)
Age at presentation	0–24 h	90 (60.0)
	25–48 h	40 (26.7)
	49–72 h	20 (13.3)
Mean birth weight (g)	2500 ± 400	—
Mean admission weight (g)	2450 ± 420	—
GA by LMP (weeks)	38.6 ± 1.1	—
GA by Ballard score (weeks)	38.4 ± 1.0	—

GA=gestational age; LMP=last menstrual period; LSCS=lower-segment caesarean section. Continuous variables are expressed as mean ± SD.

Table 2: Comparison of haematological parameters between culture-positive and culture-negative neonates

Parameter	Culture-positive (n=40)	Culture-negative (n=110)	*p* value
TLC (/mm³)	8200	9600	0.02
ANC (/mm³)	6200	4300	<0.001
ALC (/mm³)	1600	2600	<0.001
I/T ratio	0.30	0.17	<0.001
Platelets (/mm³)	150 000	210 000	<0.001
CRP (mg/dl)	4.2	1.1	<0.001
NLR	4.1	1.9	<0.001
PLR	122	64	<0.001

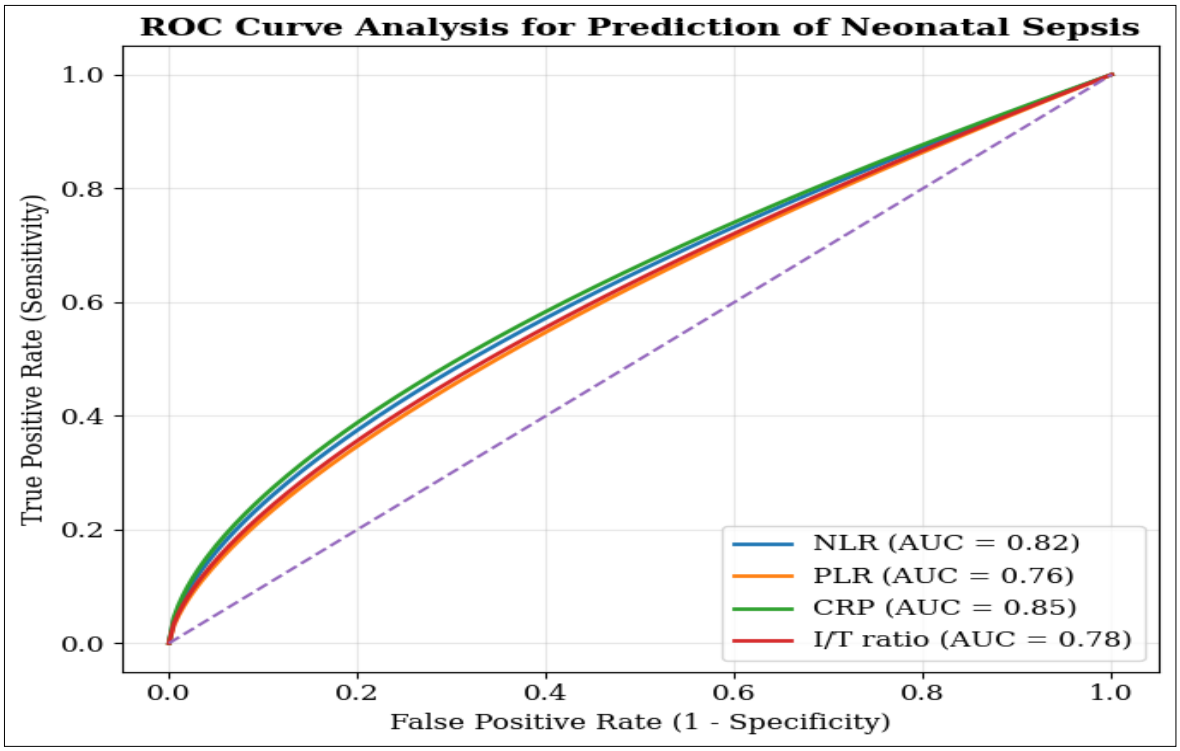
ALC=absolute lymphocyte count; ANC=absolute neutrophil count; CRP=C-reactive protein; I/T=immature-to-total neutrophil ratio; NLR=neutrophil-to-lymphocyte ratio; PLR=platelet-to-lymphocyte ratio; TLC=total leucocyte count.

Diagnostic performance of NLR and PLR: ROC analysis showed good diagnostic performance for all markers (Table III). CRP had the highest AUC of 0.85 (95% CI 0.79–0.91), followed by NLR at 0.82 (0.75–0.89), I/T ratio at 0.78 (0.71–0.85) and PLR at 0.76 (0.68–0.84). The optimal cut-offs derived by the Youden index were NLR ≥1.8 (sensitivity 81%, specificity 69%), PLR ≥75 (sensitivity 74%, specificity 71%), CRP >2.0 mg/dl (sensitivity 85%, specificity 78%) and I/T ratio >0.20 (sensitivity 70%, specificity 75%). The lower institutional screening thresholds (NLR ≥0.1, PLR ≥7), designed to maximise sensitivity, yielded a sensitivity of 100% for both markers but a specificity of only 2% and 5%, respectively, confirming their inadequacy as standalone diagnostic criteria.

Table 3: Receiver operating characteristic (ROC) analysis for NLR, PLR, CRP and I/T ratio (n=150)

Marker	AUC (95% CI)	*p*	Optimal cut-off	Sensitivity	Specificity
NLR	0.82 (0.75–0.89)	0.001	≥1.8	81%	69%
PLR	0.76 (0.68–0.84)	0.001	≥75	74%	71%
CRP	0.85 (0.79–0.91)	0.001	>2.0 mg/dl	85%	78%
I/T ratio	0.78 (0.71–0.85)	0.001	>0.20	70%	75%

AUC=area under the ROC curve; CI=confidence interval; CRP=C-reactive protein; I/T=immature-to-total neutrophil ratio; NLR=neutrophil-to-lymphocyte ratio; PLR=platelet-to-lymphocyte ratio.



ROC Curve Analysis of NLR, PLR, CRP, and I/T Ratio for Prediction of Neonatal Sepsis

Table 4: Clinical outcomes and prognostic analysis (n=150)

Parameter	Survivors (n=128)	Non-survivors (n=15)	*p* value
Mean NLR	2.4	6.2	0.001
Mean PLR	80	145	0.001
Mean CRP (mg/dl)	1.9	6.1	0.001
Thrombocytopenia — mortality	6.3%	21.1%	0.002

CFR=case fatality rate; CRP=C-reactive protein; LAMA=left against medical advice; NLR=neutrophil-to-lymphocyte ratio; PLR=platelet-to-lymphocyte ratio. Culture-positive CFR 12.5% (5/40); culture-negative CFR 9.1% (10/110). Overall, 128 (85.3%) neonates were discharged, 15 (10.0%) died and 7 (4.7%) left against medical advice.

Risk factors and clinical outcomes: Perinatal risk factors significantly associated with culture-positive sepsis included chorioamnionitis (OR 6.25; 95% CI 1.60–24.4; p=0.008), severe perinatal asphyxia (OR 4.00; 1.08–14.8; p=0.04), foul-smelling liquor (OR 2.50; 1.03–6.06; p=0.04) and PROM >24 hours (OR 2.18; 1.07–4.44; p=0.03). Clinical features associated with culture-positive sepsis included absent neonatal reflexes (OR 4.5), central cyanosis (OR 3.9), lethargy or coma (OR 3.8), delayed CRT (OR 3.5) and poor cry or feeding (OR 3.2) (p<0.001 for all). Multivariate logistic regression identified NLR ≥1.8 (adjusted OR 4.1; 95% CI 1.9–8.8; p<0.001), CRP >1 mg/dl (adjusted OR 5.0; 95% CI 2.3–10.9; p<0.001) and chorioamnionitis (adjusted OR 3.2; 95% CI 1.1–9.0; p=0.03) as independent predictors of culture-positive sepsis.

Among the 150 neonates, 128 (85.3%) were discharged after recovery, 15 (10.0%) died and 7 (4.7%) left against medical advice (LAMA). Of the 15 deaths, 5 occurred among culture-positive neonates (CFR 12.5%) and 10 among culture-negative neonates with severe clinical sepsis (CFR 9.1%); Gram-negative organisms accounted for 80% of culture-positive deaths. Non-survivors had significantly higher mean NLR (6.2 vs 2.4), PLR (145 vs 80) and CRP (6.1 vs 1.9 mg/dl) than survivors (p=0.001). Neonates with thrombocytopenia had a mortality of 21.1% versus 6.3% in those with normal platelet counts (p=0.002). On multivariate analysis, NLR ≥1.8 (adjusted OR 3.6; 95% CI 1.4–9.2; p=0.007) and thrombocytopenia (adjusted OR 2.8; 95% CI 1.0–7.9; p=0.04) were independent predictors of mortality, whereas CRP did not retain significance after adjustment. These outcomes are summarised in Table 4.

DISCUSSION

This prospective observational study evaluated NLR and PLR as biomarkers of EOS in 150 full-term neonates at a tertiary-care centre in India. Both ratios were significantly elevated in culture-positive neonates and showed good diagnostic performance, supporting prior evidence while extending the analysis to prognostic significance and perinatal risk-factor associations.

The male predominance (56.0%) and early presentation (60.0% within 24 hours) align with previous literature. Panda et al. and Binny et al. similarly reported male predominance among culture-positive neonates, which they attributed to biological susceptibility related to X-linked immune factors.[11,12] The relatively lower admission weight than birth weight probably reflects early disease impact, and the high proportion of inborn and vaginally delivered neonates reflects institutional delivery patterns with early neonatal surveillance.

PROM >24 hours was the most frequent perinatal risk factor (34.0%), followed by prolonged labour (24.0%) and foul-smelling liquor (18.0%). Chorioamnionitis, though less frequent (8.0%), emerged as the strongest independent risk factor on multivariate analysis (adjusted OR 3.2), consistent with Kumar et al., who identified chorioamnionitis as the dominant risk factor for EOS.[13] Its high odds ratio reflects its role as a direct marker of ascending bacterial infection. The loss of significance of PROM on multivariate analysis suggests that its effect is mediated through downstream variables such as chorioamnionitis and direct inflammatory activation.

The mean NLR in culture-positive neonates was 4.1, comparable to the 3.88 ± 1.78 reported by Panda et al.[11] and consistent with Binny et al., who demonstrated NLR as a strong predictor in culture-positive cases.[12] The elevated PLR of 122 in confirmed sepsis aligns with Arcagok and Karabulut, who first demonstrated PLR as a simple, inexpensive parameter for EOS identification.[14] The meta-analysis by Bai et al. confirmed that both NLR and PLR show good sensitivity and specificity, with NLR marginally more accurate—consistent with our ROC findings.[15]

Diagnostic performance in our cohort was robust. NLR at a cut-off of ≥1.8 yielded an AUC of 0.82 (sensitivity 81%, specificity 69%), while PLR ≥75 yielded an AUC of 0.76 (sensitivity 74%, specificity 71%). These findings agree with the meta-analysis by Chen et al. demonstrating a strong diagnostic association for NLR in neonatal sepsis.[16] The superiority of CRP (AUC 0.85) reinforces its established role, while the combination of NLR, PLR, CRP and I/T ratio provides a more comprehensive diagnostic panel than any single marker. The lower institutional screening thresholds (NLR ≥0.1, PLR ≥7) proved diagnostically inadequate, with near-zero specificity, emphasising that clinically meaningful threshold selection is essential to reduce false positives and unnecessary antibiotic exposure.

The microbiological findings show predominant Gram-negative infection, with *Klebsiella* spp. (37.5%) and *E. coli* (22.5%) accounting for most isolates—consistent with contemporary Indian EOS literature.[17] The sensitivity pattern, with *Klebsiella* susceptible to amikacin and cefoperazone–sulbactam and *E. coli* to meropenem, reflects local resistance patterns that deviate from standard first-line therapy and underscores the importance of local antibiogram-guided empirical strategies. The susceptibility of *Acinetobacter* only to colistin is particularly concerning, reflecting increasing carbapenem resistance in Indian NICUs.

Although the population and infection site differed, a contemporaneous study of culture-confirmed paediatric urinary tract infections from the same institution also demonstrated predominance of Gram-negative organisms, particularly *E. coli* (66.3%) and *Klebsiella* spp. (19.3%), together with substantial resistance to commonly used cephalosporins and fluoroquinolones.[18] These findings further emphasise the importance of periodic local antimicrobial-surveillance; however, susceptibility patterns from older children with urinary infections should not be extrapolated directly to neonatal bloodstream infections.

The prognostic findings are a clinically important contribution of this study. Non-survivors had substantially higher NLR (6.2), PLR (145) and CRP (6.1 mg/dl). On multivariate analysis, elevated NLR and thrombocytopenia were independent predictors of mortality, whereas CRP did not retain significance after adjustment. These results corroborate Bafna et al., who showed that an elevated admission NLR was associated with increased mortality and that a decreasing NLR during treatment predicted improved prognosis.[19] The thrombocytopenia-associated mortality (21.1% vs 6.3%) reinforces the role of platelet dynamics as a marker of disease severity, reflecting coagulopathic and immune-dysregulatory processes in severe neonatal sepsis. That CRP was the strongest diagnostic marker yet NLR the stronger prognostic marker suggests that these biomarkers capture different phases of the inflammatory response: CRP reflects early acute-phase activation, whereas NLR better captures the sustained immune dysregulation associated with fatal outcomes.

The overall favourable outcome (85.3% recovery), with 10.0% total mortality—12.5% CFR in culture-positive and 9.1% CFR in culture-negative neonates—reflects the impact of timely diagnosis and NICU-based management in a tertiary setting. The presence of LAMA cases (4.7%) represents a healthcare-access and compliance challenge relevant to the Indian context that is often under-reported in biomarker studies.

Limitations

This study has certain limitations. The prospective observational design, although more robust than a cross-sectional approach, does not permit controlled assessment of serial trends in inflammatory markers during illness progression. The population was restricted to term neonates at tertiary NICUs, limiting generalisability to preterm neonates and to primary or secondary care settings. Blood-culture sensitivity is inherently limited by prior antibiotic exposure and low bacterial load, potentially underestimating true culture positivity. Single time-point measurement of haematological parameters may not capture the evolving inflammatory response. Finally, although ROC-derived cut-offs improved specificity over protocol thresholds, external validation in larger, diverse cohorts is required before clinical standardisation.

In summary, NLR and PLR are readily available, low-cost adjuncts that add diagnostic and prognostic value to CRP and clinical assessment in the early recognition and risk stratification of EOS in resource-limited settings.

CONCLUSION

NLR and PLR are significantly elevated in early-onset neonatal sepsis and show meaningful diagnostic and prognostic value when used alongside CRP, I/T ratio and clinical assessment. In this cohort of 150 full-term neonates, ROC-derived cut-offs of NLR ≥1.8 and PLR ≥75 provided balanced sensitivity and specificity for identifying culture-positive EOS, whereas lower institutional thresholds were diagnostically inadequate; CRP showed the highest discriminative ability. Chorioamnionitis and PROM were the dominant perinatal risk factors, and Gram-negative organisms—particularly *Klebsiella* and *E. coli* with evolving resistance—predominated microbiologically. Elevated NLR and thrombocytopenia independently predicted mortality, establishing their prognostic value beyond diagnosis.

These findings support incorporating NLR and PLR into routine NICU sepsis-screening protocols as accessible, cost-effective adjuncts, using optimised cut-offs and combined panel assessment (NLR, PLR, CRP and I/T ratio) rather than any single marker. Serial monitoring of these indices, close observation of neonates with elevated NLR and thrombocytopenia, and periodic review of local microbiological patterns to guide empirical therapy are recommended. Larger multicentric prospective studies are needed to validate these thresholds across diverse neonatal populations.

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