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Research Article

TO STUDY THE USE OF NEUTROPHIL-TO-LYMPHOCYTE RATIO AS AN EARLY MARKER OF SEPSIS IN NEONATES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Objectives: To assess the applicability of the neutrophil-to-lymphocyte ratio (NLR) as a diagnostic marker in neonatal sepsis.

Methods: Prospective study done for duration of 2 years, that is, from Jan 2023 to Jan 2025, in 100 neonates admitted with sepsis in the neonatal intensive care unit.

Results: Term (e37 weeks) 50%, Very pre-term (e28–32 weeks) 30%, and extremely pre-term (<28 weeks) 20%. Mean C-reactive protein (CRP) was 14.0 mg/L in neonates, strongly indicated infection and systemic inflammation. Mean NLR (3.1).

Conclusion: NLR is a simple, cost-effective, and accessible inflammatory marker that may support the early diagnosis of neonatal sepsis. While its standalone predictive power is moderate, combining NLR with CRP, and clinical assessment improves diagnostic confidence. Larger studies are recommended to validate optimal cutoff values and explore its prognostic utility.

Keywords: Neonatal sepsis, Neutrophil-to-lymphocyte ratio, Neutrophil-to-lymphocyte ratio, C-reactive protein, Early diagnosis, Biomarkers.

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INTRODUCTION

Neonatal sepsis is a contributing factor for morbidity and mortality of newborn infants, especially in developing countries. Neonatal sepsis can be categorized into early-onset sepsis (EOS), which happens before 72 h of life, and late-onset sepsis (LOS), which happens after 72 h of life [1,2].

The clinical findings of NS range from subclinical infection to severe manifestations of focal or systemic disease and there is often little difference between sepsis that is caused by infectious pathogens or by other non-infectious pathogens, thus making NS difficult to diagnose [3-6].

Diagnosis of NS needs to be done as early as possible to start the appropriate treatment to prevent further morbidity and mortality among infants [7].

The best methods involve using a combination of maternal risk factors, clinical signs and symptoms, and various laboratory markers that are available. Blood culture, which is the gold standard for neonatal sepsis diagnosis, cannot be obtained for all patients, and the results only come out after 24–48 h. Moreover, a negative result does not exclude sepsis as the diagnosis.

Neonatal sepsis could also be diagnosed by isolating the causative agent from blood or any other sterile body fluid, such as cerebrospinal fluid (CSF), urine, as well as pleural, joint, and peritoneal fluids, but culture has a low sensitivity and influenced by many factors.

The most recent diagnostic criteria for neonatal sepsis were published by the *Pediatric Committee* (PDCO) *of the European Medicines Agency* (EMA) in the "*Expert Meeting on Neonatal and Pediatric Sepsis Consensus 2010 Criteria*" [8].

The diagnosis consists of at least 2 clinical features and 2 laboratory findings. The diagnosis is then confirmed by isolating the causative agent from blood or any other sterile body fluid, such as CSF, urine, or pleural, joint, and peritoneal fluids, or by microscopy or polymerase chain reaction [9,10].

Aim of the study

To study the use of Neutrophil-to-lymphocyte ratio (NLR) as an early marker of sepsis in neonates in a tertiary care hospital.

METHODS

Study design

Prospective study.

Place of study

Tagore medical college and hospital.

Duration of study

January 2023 to January 2025.

Sample size

100.

Inclusion criteria

- Neonates aged between 7 and 28 days, diagnosed according to the diagnostic criteria for neonatal sepsis.

Exclusion criteria

- Gestational age <37 weeks, age <7 days, or >28 days
- Newborns with genetic metabolic disorders, chromosomal diseases, or congenital developmental abnormalities
- Newborns with concomitant immune system disorders, hematologic disorders, or impaired liver or kidney function
- Newborns who received antimicrobial or antiplatelet drug therapy before blood sampling
- Positive blood culture without clinical evidence of sepsis, considered as specimen contamination in newborns.

Methodology
A total of 100 newborns were included by a consecutive sampling method. We conducted a 2-year duration, that is, 2023 to 2025, using a cross-sectional design with consecutive sampling in all inborn neonates treated in the neonatal intensive care unit with clinically diagnosed neonatal sepsis. Complete blood count, C-reactive protein (CRP), and blood culture were carried out before giving antibiotics according to the local Clinical Practice Guidelines.

Basic characteristics, such as sex, Gestational age, Birth weight, Lubchenco score, mode of delivery, history of pre-mature rupture of membranes, history of mother with pre-eclampsia/eclampsia, history of pre-natal steroid use, and neonatal sepsis onset.

Complete blood count performed by an automated hematology analyzer and includes white blood count (WBC) differential to evaluate the WBC based on light scattering characteristics. The ANC and absolute lymphocyte count were identified and counted in the WBC differential. The NLR is obtained by dividing the ANC by the absolute lymphocytes count recorded in the medical record manually.

Statistical analysis
Data were processed with the IBM Statistical Package for the Social Sciences Statistics version 25.0 software. Descriptive analysis was used to illustrate the characteristics of the data. A receiver operating characteristic curve analysis was done to assess the NLR cut-off points. Chi-square test was used to analyze the diagnostic value of NLR by calculating the sensitivity, specificity, positive predictive value, and negative predictive value.

RESULTS AND OBSERVATION

In the present study, Term (e37 weeks) 50%, very pre-term (e28–32 weeks) 30%, and extremely pre-term (<28 weeks).

Sex distribution
Males 60%, Females 40%. M: F ratio 2;1 (Table 1).

In the present study, low birth weight (LBW) (<2,500 g) 60% (60/100), very LBW (VLBW) (<1,500 g) 10% (10/100), extremely LBW (ELBW) (<1,000 g) 5% (5/100), and normal 25% (25/100).

Distribution of onset neonatal sepsis
Early-onset neonatal sepsis (EONS) 40% (40/100) and Late-onset neonatal sepsis 60% (60/100).

Distribution of pre-mature rupture of membrane
Pre-mature rupture of membranes noted in 30% cases. (30/100) and absent in 70% (70/100).

Distribution of pre-eclampsia/eclampsia
Pre-mature rupture of membranes noted in 40% cases (40/100) and absent in 60% (60/100).

Distribution of mode of delivery
LSCS in 40% cases (60/100) and Normal delivery in 60%.

Distribution of blood cultures
Resulted in positive growth consisting of *Klebsiella pneumoniae* (35), *Candida albicans* (20), *Serratia marcescens* (5), *Staphylococcus aureus* (10), *Acinetobacter baumannii* (2), *Staphylococcus epidermidis* (10), *Staphylococcus haemolyticus* (8), and *Escherichia coli* (10),

Table 1: Distribution of gestational age

Distribution of gestational age	No. of cases	Percentage
Extremely pre-term (<28 weeks)	20	20
Very pre-term (e28–32 weeks)	30	30
Term (e37 weeks)	50	50
Total	100	100

Logistic regression analysis revealed a significant correlation between high NLR and NS (p<0.0001) (Table 2).

In neonatal sepsis, the NLR can be a useful marker, with higher NLR values generally associated with sepsis. Studies suggest that an NLR above a certain threshold, such as 2.75 or 1.62, may indicate a higher risk of sepsis. However, the sensitivity and specificity of NLR in diagnosing neonatal sepsis vary, and it's important to consider it alongside other clinical and laboratory findings.

In our study, Mean CRP was (14.0 mg/L) in neonates strongly indicated infection and systemic inflammation.

In our study mean NLR (3.1)
The Neutrophil-to-Lymphocyte Ratio is emerging as a simple yet effective marker for early sepsis diagnosis. A mean NLR of 3.1 supports the presence of an inflammatory state and may be useful when combined with CRP and clinical signs. Previous studies suggest that an NLR >2.5 is indicative of sepsis in neonates (Table 3).

Area under the ROC curve	
Area	0.5940
Standard error	0.04131
95% confidence interval	0.5130–0.6750
p-value	0.0216*

ROC: Receiver operating characteristic

The red line represents the line of best fit (linear regression), indicating a positive linear relationship between the two variables. The strong alignment of data points along the line suggests a high degree of correlation (Fig. 1).

In our study, the correlation of NLR and CRP does not show a significant correlation (Table 4).

Analysis of inflammatory markers between early and late onset groups, no statistically significant differences were observed in the levels of NLR, Procalcitonin, or CRP. The Mann–Whitney U test results indicate that these markers do not significantly vary with the timing of onset,

Table 2: Distribution of birth weight

Distribution of birth weight	No. of cases	Percentage
Normal weight	25	25
Low birth weight (<2,500 g)	60	60
Very low birth weight (<1,500 g)	10	10
Extremely low birth weight (<1,000 g)	05	05
Total	100	100

Table 3: Distribution of DLC

Distribution of DLC	No. of cases
Total leucocyte count,/mm^3	26200/cumm
Mean neutrophil count,/mm^3	78.5
Mean lymphocyte count	14.8
MEAN CRP	14.0
Mean NLR ratio	3.1

NLR: Neutrophil-to-lymphocyte ratio, CRP: C-reactive protein

Table 4: Spearman correlation for NLR as a marker with CRP

Spearman correlation	NLR	CRP	r-value	p-value
Mean±SD	7.019±4.403	7.260±3.060	0.01365	0.8927 ns

Spearman correlation, *P*<0.05, *: Significant, ns: Non-significant,
NLR: Neutrophil-to-lymphocyte ratio, CRP: C-reactive protein, SD: Standard deviation

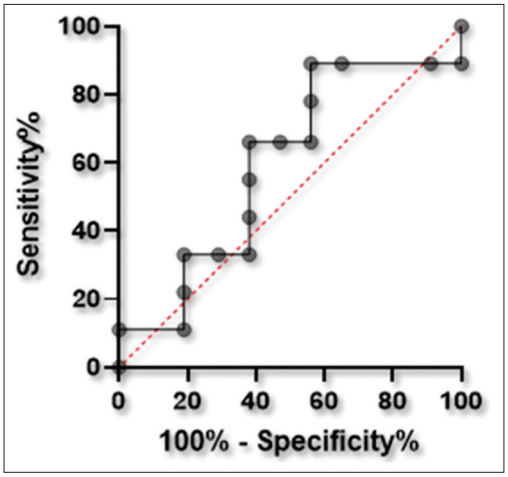

Fig. 1: Receiver operating curve of the neutrophil-to-lymphocyte ratio as a marker with C-reactive protein

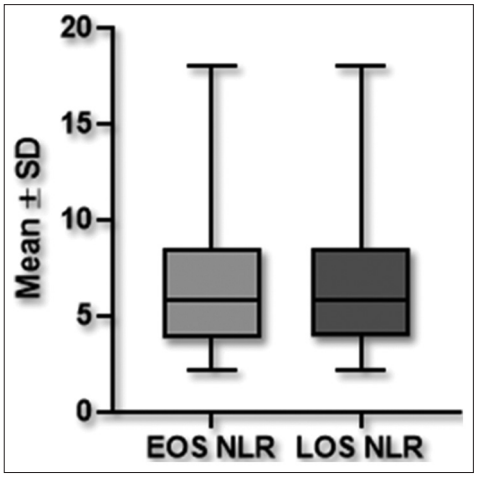

Fig. 2: Box-and-whisker plot between neutrophil-to-lymphocyte ratio in early and late onset sepsis

Table 5: Comparison of NLR, CRP, and PCT among two groups

Indicator	Early onset	Late onset	U	p-value
NLR	6.798±4.340	7.167±4.475	1,125	0.6006ns
Procalcitonin	6.034±6.805	6.830±6.364	1,030	0.2331ns
CRP	7.150±3.118	7.333±3.046	1,157	0.7585ns

Mann–Whitney test, *P*<0.05, *: Significant, ns: Non-significant,
NLR: Neutrophil-to-lymphocyte ratio, CRP: C-reactive protein

suggesting limited utility of these parameters in distinguishing between early, and late onset presentations (Table 5).

The box plot demonstrates no substantial difference in the distribution of the studied parameter between the early and late onset groups. The overlapping interquartile ranges and similar medians support the conclusion that the variable is not significantly associated with the timing of onset (Fig. 2).

The box plot comparison of CRP levels shows no significant difference between early and late onset groups. Both groups display similar median values and comparable variability, supporting the conclusion that CRP is not significantly associated with the timing of onset in the studied population (Fig. 3).

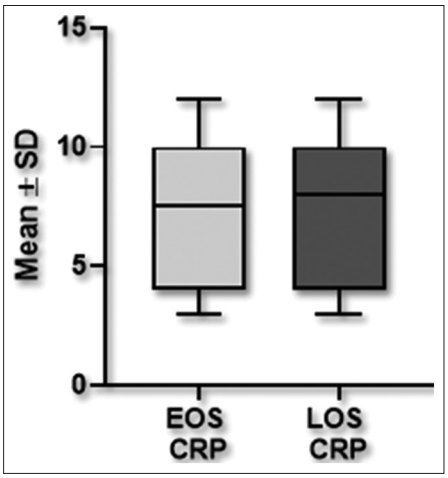

Fig. 3: Box-and-whisker plot between C-reactive protein in early and late onset sepsis

DISCUSSION

Neonatal sepsis continues to be a major contributor to illness and death among newborns, especially in low-resource healthcare environments. Prompt diagnosis is essential to initiate antibiotic therapy without delay and improve clinical outcomes. However, traditional methods, such as blood culture are often slow, potentially hindering timely treatment decisions. Recently, hematological parameters, such as the NLR have gained attention as potential early indicators of sepsis, due to their affordability, availability, and quick reporting time. This study investigates the effectiveness of NLR as a dependable early marker for neonatal sepsis in a tertiary care teaching hospital and examines its association with clinical presentations and microbiological findings.

Comparative studies related to the distribution of gestational age

In the present study, half of the neonates (50%) were term (e37 weeks), while 30% were very pre-term (28–32 weeks), and the remainder were extremely pre-term (<28 weeks). In comparison, the study by Panda *et al.* [10] reported 16 term neonates, seven late preterm, and 18 neonates with gestational age <34 weeks. While both studies included a mix of term and pre-term infants, the present study had a higher proportion of extremely pre-term cases, whereas Panda *et al.* [10] had larger representation of late pre-term and moderately pre-term infants (Table 6).

Comparative studies related to sex distribution

In the present study, males constituted 60% of the neonates and females 40%, resulting in a male-to-female ratio of 2:1. A similar male predominance was observed in the studies by Binny *et al.* [11] (53.5%) and Wilar *et al.*, [12] (58.3%). This consistent trend across studies may be attributed to the increased vulnerability of male neonates to perinatal complications, which has been well-documented in neonatal literature. Biological factors, including differences in lung maturity, immune response, and hormonal influences, may contribute to the higher susceptibility of male infants to sepsis and other neonatal morbidities, thus leading to their overrepresentation in clinical studies.

Comparative studies related to the distribution of birth weight

In the present study, LBW (LBW; <2,500 g) was observed in 60% of neonates, with 10% classified as VLBW (VLBW; <1,500 g), and 5% as ELBW (ELBW; <1,000 g). Only 25% had normal birth weight (g2,500 g). In comparison, the study by Binny *et al.* [11] reported a much lower median birth weight of 906.0 g (±572), reflecting a cohort dominated by ELBW and VLBW infants. In addition, 23.6% of neonates in their study were identified as growth-restricted, highlighting a higher prevalence of fetal growth impairment.

Table 6: Comparative studies related to the distribution of NLR

Study	Key findings	Conclusion
Büyüktiryaki *et al.* (2018) [14]	NLR was significantly higher in neonates with proven sepsis compared to controls.	NLR is a simple, cost-effective inflammatory marker for early diagnosis of neonatal sepsis.
Wang *et al.* (2020) [15]	NLR>2.5 showed good sensitivity and specificity in predicting early-onset sepsis.	NLR can be used as a supportive diagnostic tool, especially in early-onset cases.
Davis *et al.* (2019) [16]	NLR, along with CRP and PCT, improved diagnostic accuracy.	Combined use enhances predictive value and supports clinical decisions.
Patel *et al.* (2021) [17]	NLR showed correlation with the severity of sepsis and the need for NICU support.	Higher NLR was associated with adverse outcomes and greater clinical severity.
Sharma *et al.* (2022) [18]	In a tertiary care study, the mean NLR was significantly elevated in culture-positive neonates.	Suggested inclusion of NLR in routine screening for suspected sepsis.
Present study	A mean NLR of 3.1 supports the presence of an inflammatory state and may be useful when combined with CRP and clinical signs.	Statistically significant association between elevated NLR and neonatal sepsis ($p<0.0001$), supporting its diagnostic value.

NLR: Neutrophil-to-lymphocyte ratio, CRP: C-reactive protein, NICU: Neonatal intensive care unit

Comparative studies related to the distribution of blood cultures

In the present study, blood cultures showed positive growth in multiple cases, with *K. pneumoniae* being the most common isolate (n=35), followed by *C. albicans* (n=20), *S. aureus* (n=10), *Escherichia coli* (n=10), *S. epidermidis* (n=10), *S. haemolyticus* (n=8), *S. marcescens* (n=5), and *Acinetobacter baumannii* (n=2). In comparison, Sumitro *et al*. [13] reported a predominance of gram-negative organisms (75.0%) in neonatal sepsis, with extended-spectrum β-lactamase-producing *K. pneumoniae* accounting for 61.5% of positive isolates. Similarly, Wilar *et al*. observed positive blood cultures in 84 cases, with major pathogens, including *K. pneumoniae* (n=26), *C. albicans* (n=10), *S. marcescens* (n=9), *S. aureus* (n=9), *Acinetobacter baumannii* (n=6), *S. epidermidis* (n=6), *S. haemolyticus* (n=4), and *E. coli* (n = 4), along with rare isolates, such as *Candida pelliculosa*, *Salmonella spp*., *Enterobacter aerogenes*, *Enterococcus faecium*, *Candida tropicalis*, *Listeria monocytogenes*, *Micrococcus luteus*, and *Pseudomonas aeruginosa*. Panda *et al*. [10] investigated 41 cases of culture-confirmed neonatal sepsis and reported that gram-negative bacteria accounted for the majority (63.41%) of infections. Gram-positive organisms were identified in 29.26% of cases, while fungal pathogens were responsible for 7%. These findings align with broader trends showing gram-negative organisms – especially – *K. pneumonia*e – as leading causative agents in neonatal sepsis. This highlights the critical need for empiric antimicrobial regimens to be guided by local pathogen prevalence and resistance profiles.

Comparative studies related to the distribution of the onset of sepsis

In the present study, EONS was observed in 40% of cases (40 out of 100), while late-onset sepsis accounted for the remaining 60% (60 out of 100). A comparable pattern was observed in the study conducted by Binny *et al*., where 44.2% of neonates (n=895) were diagnosed with EOS, while the remaining 55.8% (n=1129) developed late-onset sepsis. In contrast, findings from Wilar *et al*. indicated a predominance of early-onset cases, with 80.9% (n=68) presenting within the first 72 h of life, and only 19.1% (n=16) classified as late-onset. These disparities in the timing of sepsis onset across studies may stem from variations in neonatal care environments, maternal risk profiles, delivery practices, or infection control measures employed in different institutions.

CONCLUSION

This prospective investigation aimed to explore the role of the NLR in the early identification of neonatal sepsis in a tertiary care setting. The study enrolled 100 neonates, among whom 60% had LBW. Late-onset sepsis (LOS) was more frequent than EOS, accounting for 60% and 40% of cases, respectively. *K. pneumoniae* was identified as the most commonly isolated pathogen in blood culture results.

The average NLR value observed was 3.1, while the mean CRP level was 14.0 mg/L – both elevated in affected neonates, reflecting underlying inflammatory activity. Logistic regression demonstrated a statistically significant relationship between increased NLR and the presence of sepsis ($p<0.0001$), highlighting the diagnostic value of NLR in this context. However, Spearman's correlation analysis between NLR and CRP yielded no significant association ($r=0.01365$, $p=0.8927$), suggesting that these markers may act independently in reflecting the inflammatory process</AQ7>

In summary, NLR appears to be a simple, cost-effective, and supportive inflammatory marker for early diagnosis of neonatal sepsis. While its standalone sensitivity may be moderate, its use alongside CRP, clinical signs, and microbiological findings can enhance early sepsis detection in neonates.

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