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Neutrophil-to-Lymphocyte Ratio in Neonates: A Predictor of Early-Onset Neonatal Sepsis

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ABSTRACT

Objective: Early-onset neonatal sepsis (EOS) is a common cause of mortality in the neonatal period. The purpose of the study was to determine the value of the neutrophil-to-lymphocyte ratio (NLR) in the early diagnosis of EOS.

Methods: This was a prospective cross-sectional study. A total of 102 newborns with early-onset infection (EOI) were enrolled within the first 72 hours of life in the neonatal intensive care unit of a tertiary referral hospital, the largest pediatric center in central Vietnam.

Results: Among 102 newborns, 32 were identified as having EOS, while the remaining 70 were classified as EOI. The median NLR value in the EOS group (2.7 [2.0–5.6]) was statistically significantly higher than that in the EOI group (1.7 [1.0–2.4], $p < 0.05$). The NLR demonstrated a moderate ability to discriminate between EOS and EOI with the area under the curve (AUC) of 73.6% ($p < 0.05$). An NLR cut-off of 1.87 was found to be optimal, with a sensitivity of 84.4%, a specificity of 60.0%, a positive predictive value of 49.1%, and a negative predictive value of 89.4%. The combination of NLR and C-reactive protein demonstrated the best performance in predicting EOS, with the AUC of 85.3% (95% confidence interval: 76.9–91.5).

Conclusions: This study suggests that NLR can be used as an additional diagnostic marker, alongside C-reactive protein, for the early diagnosis of EOS.

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Introduction

Early-onset neonatal sepsis (EOS) is an infectious disease caused by vertical transmission of pathogens from mother to newborn, with symptoms appearing from birth up to 72 hours after birth.[1] EOS, a severe early-onset neonatal infection with rapid progression and high mortality, requires early diagnosis to improve outcomes.[2] Furthermore, strict adherence to an appropriate antibiotic course for EOS is essential to avoid incomplete treatment and mitigate the risk of severe complications such as delayed diagnosis of bacterial meningitis. Distinguishing EOS from other early-onset infections (EOI) can be challenging in the immediate postnatal period.[1] Blood culture, the gold standard for the diagnosis of EOS, has limitations. It takes at least 48 hours to produce results and its positivity rate is low. Although inflammatory reaction markers such as procalcitonin, Interleukin (IL-1β, IL-4, IL-5, IL-6, IL-8) and tumor necrosis factor are also used, their high cost, limited availability in many facilities, and difficulty in interpreting results due to rapid physiological changes after birth hinder their widespread use.[2] Complete peripheral blood count testing offers several advantages: it is quick, inexpensive, and readily available in most medical facilities. Therefore, identifying an additional index along with white blood cells (WBC) and platelets (PLT) that is easily accessible becomes essential for the early diagnosis of neonatal sepsis, particularly in low settings, such as low-middle-income countries, such as Vietnam. In this context, the neutrophil-to-lymphocyte ratio (NLR) is gaining increasing attention in the diagnosis of EOS. Neutrophils, crucial components of the specific immune response, release inflammatory cytokines and activate T cells.[3,4] During infection, the number of neutrophils increases, while lymphocytes decrease in peripheral blood, leading to a shift in the neutrophil-to-lymphocyte ratio.[2,5,6]

The study aimed to determine the value of the neutrophil to lymphocyte ratio in the early diagnosis of EOS.[1]

Materials and Methods

This was a prospective cross-sectional study that was carried out in the neonatal intensive care unit of a tertiary referral hospital, the largest pediatric center in central Vietnam, between June 2022 and June 2023. A total of 102 neonates were enrolled in the study. All participants met the defined inclusion criteria. Clinical and laboratory data were collected from each neonate.

Neonates had suspected infection of clinical presentation in their first 72 hours after birth and were required to receive a minimum 7-day course of antibiotic treatment.

The neonates exhibited signs suggestive of infection, however, after 48 hours of observation, EOI was ruled out and discontinued antibiotics.[2] Neonates with hematological diseases.

All neonates received physical examination and laboratory examination for sepsis screening (blood culture, complete blood count (White blood cell (WBC), Platelet (PLT)), C-reactive protein (CRP), blood glucose, lactate, and blood gas analysis). All neonates in the study were admitted to the hospital on the first day after birth, and blood samples for complete blood count and blood culture were collected immediately prior to antibiotic administration. Based on clinical and laboratory signs, we divided into 2 groups: [1] EOS group and [2] EOI group. EOS was defined according to the criteria proposed by the European Medicines Agency (EMA) (2010) for the diagnosis of sepsis in neonates and children.[7] Sepsis was defined if neonates had the presence of at least two clinical symptoms and at least two laboratory signs in the presence or due to suspected or proven infection (with or without positive blood cultures). The presence of 2 clinical symptoms in neonates suggesting sepsis (including altered body temperature, bradycardia, tachycardia, hypotension, prolonged peripheral perfusion, respiratory instability (apnea, tachypnea), lethargy, irritability, hypotonia, seizures, vomiting, poor sucking, abdominal distension) and ≥ 2 laboratory signs (including thrombocytopenia, leukopenia, leukocytosis, CRP>15mg/l, hypoglycemia, hyperglycemia, metabolic acidosis). The EOI case was defined if the neonates had clinical characteristics suggestive of infection with/without altered leukocyte parameter, CRP or other relevant biochemical parameters for the detection of infection but did not meet the sepsis criteria of sepsis as above.

The following neonates were not considered EOI cases and were excluded from the study group: Neonates with suspected infections at admission showed clinical improvement without antibiotic therapy or those who received initial antibiotic treatment were followed for at least 48 hours after the first dose. If the initial clinical suspicion of infection resolved, and both the blood culture and

CRP level were negative after 48 hours, antibiotic therapy was discontinued.

Categorical variables were presented by frequency, and proportion and analyzed using a Chi-Square, or Fisher's exact test. The Shapiro Wilk test was carried out to check normal distribution of continuous data. We used to mean, and standard deviations (SD) and analyzed using an independent sample t-test (if normally distributed, *p* value of Shapiro Wilk test greater than 0.05). Nonnormally distributed (a *p* value of Shapiro Wilk test less than 0.05) variables were presented as medians (interquartile range) and analyzed using the Mann-Whitney U test. Area Under the Curve (AUC), sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) values were utilized to evaluate the marker of sepsis. The optimal cut-off point was identified using the Youden index (sensitivity + specificity – 1). A combination of biomarkers was also performed to determine if it can improve diagnostic efficacy. With a current sample of 102 neonates, power analyses for the receiver operation characteristic curve (ROC) of NLR were performed to ensure adequate statistical power (98%). A *p* values less than 0.05 were considered statistically significant. IBM SPSS Statistics version 20.0 was used to perform the analysis.

This study was approved by the Institutional Biomedical Ethics Committee of the University of Medicine and Pharmacy, Hue University (Approval No. H2022/088). Written informed consent was obtained from parents regarding the study and the use of their newborns' medical record data before enrollment.

Table 1. Characteristics of study population

Variable		EOS group (n = 32)	EOI group (n=70)	*p* value
		n (%)	n (%)	
Gender	Male	27 (84.4)	48 (68.6)	0.093*
	Female	5 (15.6)	22 (31.4)	
Type of delivery	Vaginal	9 (28.1)	13 (18.6)	0.276*
	Cesarean section	23 (71.9)	57 (81.4)	
Gestational age (weeks)	< 28	1 (3.1)	0 (0.0)	0.023**
	28 - < 32	2 (6.3)	14 (20.0)	
	32 - < 34	3 (9.4)	8 (11.4)	
	34 - < 37	5 (15.6)	22 (31.4)	
	37 - < 42	21 (65.6)	26 (37.2)	
	Median (25^{th} - 75^{th})	38.00 (35.00 – 39.00)	35.00 (32.00 – 38.00)	0.013++
Body weight (grams)	< 1500	3 (9.4)	11 (15.7)	0.349*
	1500 - < 2500	9 (28.1)	28 (40.0)	
	2500 - < 4000	17 (53.1)	28 (40.0)	
	≥ 4000	3 (9.4)	3 (4.3)	
	Means ±SD	2818.12 ± 925.93	2308.57 ± 801.94	0.010+
Laboratory characteristics	WBC ($/mm^3$) Median (25^{th} - 75^{th})	20.30 (12.58–24.12)	12.77 (10.20–16.70)	<0.001++
	NLR Median (25^{th} - 75^{th})	2.72 (2.05 – 5.52)	1.70 (1.00– 2.37)	<0.001++
	PLT ($/mm^3$) Means ±SD	213.72 ± 79.21	244.73 ± 59.47	0.054+
	CRP Median (25^{th} - 75^{th}) (mg/l)	23.75 (14.75– 41.55)	2.85 (0.83 – 10.50)	0.001++

* *Chi Square test;* ** *Fisher exact test;* + *Independent-Samples T Test;* ++ *Mann-Whitney U Test.*

WBC, White blood cell; PLT, Platelet; CRP, C-reactive protein; NLR, Neutrophil to lymphocyte ratio. SD, Standard Deviation.

Results

During the study period, 102 neonates met the selection criteria. Among these, 32 neonates (accounting for 31.4%) were in the EOS group (including 3 cases of proven sepsis and 29 cases of clinical sepsis). The remaining 70 neonates (accounting for 68.6%) belonged to the EOI group. In the EOS group, only 3 blood cultures were positive, identifying 3 types of bacteria. *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*.

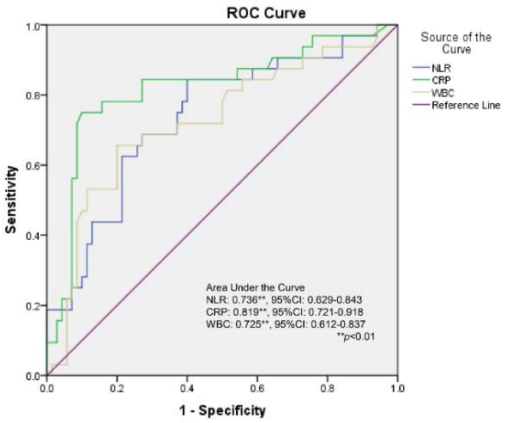

Figure 1. ROC curves for correlation of WBC, CRP, NLR for predicting early -onset neonatal sepsis in total population. WBC, White blood cell; CRP, C-reactive protein; NLR, Neutrophil to lymphocyte ratio.

The proportion of male babies was higher than that of female babies. The rates of cesarean section were higher than the rates of vaginal delivery, with rates of 71.9% and 81.4% in the EOS and EOI groups, respectively. Full-term newborns also accounted for the main proportion in both the EOS and EOI groups (65.6% and 37.2%, respectively). The median gestational age of the study group was 36.0 weeks (32.8 – 39.0 weeks). The mean birth weight of the study group was 2468.0 grams (± 871.2 g). The basic characteristics of the study population are presented in **Table 1.**

In the EOS group, both the median white blood cell count (WBC) (20.300 /mm^3 vs 12.800/mm^3) and the CRP level (23.4 mg/L vs. 2.9 mg/L) were significantly higher compared to the EOI group ($p < 0.05$ for both). Similarly, the NLR ratio in the EOS group (2.7) was significantly higher than in the EOI group (1.7) ($p < 0.05$). Details are shown in **Table 1**.

ROC curve analysis was performed to determine the ability to identify neonatal sepsis for WBC, CRP and NLR values. Peripheral blood NLR demonstrated good performance in discriminating between neonates with EOS and EOI with an area of the ROC curve (AUC) of 0.736 ($p<0.05$). The 95% confidence interval for the AUC was 0.629 to 0.842 ($p < 0.05$) (**Table 2** and **Figure 1).**

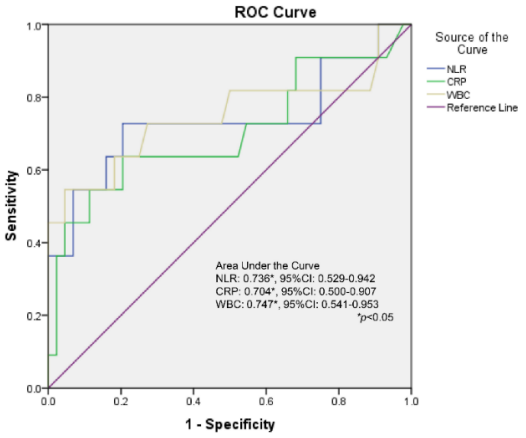

Figure 2. ROC curves for correlation of WBC, CRP, NLR for predicting early -onset neonatal sepsis in preterm group. WBC, White blood cell; CRP, C-reactive protein; NLR, Neutrophil to lymphocyte ratio

The AUC of NLR was lower than CRP (0.820) and higher than WBC (0.725). The optimal cut-off point for NLR was 1.87, with Se (84.4%) and Sp (60.0%); PPV of 49.1%; and NPV of 89.4% (**Table 2**). In the preterm group, ROC analysis showed that NLR and WBC presented good performance in predicting EOS (NLR: AUC [95%CI=0.736 (0.529-0.942)], p=0.016; WBC: AUC=0.747 [95%CI=0.541-0.953], p=0.012), which was higher than the performance of CRP (AUC [95%CI] =0.704 [0.500-0.907], p=0.038) **(Figure 2).**

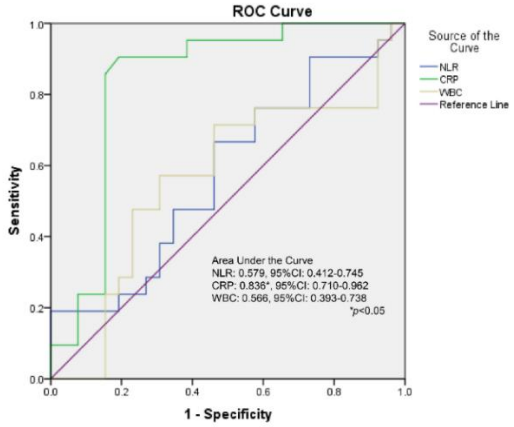

Figure 3. ROC curves for correlation of WBC, CRP, NLR for predicting early -onset neonatal sepsis in term group. WBC, White blood cell; CRP, C-reactive protein; NLR, Neutrophil to lymphocyte ratio

In the term group, CRP exhibited the highest AUC value of 0.836, while NLR and WBC achieved AUC values of 0.579 and 0.566, respectively **(Figure 3)**.

Based on these findings, we further analyzed and compared the performance of NLR with combinations of biomarkers (NLR and CRP; NLR and WBC; and NLR, CRP, and WBC). Our results indicated that the combination of NLR and CRP yielded the best performance in predicting EOS, with an AUC of 0.853 (95% CI: 0.769-0.915) ($p<0.001$), corresponding to a Se of 65.6%, Sp of 94.3%, PPV of 84.0%, and NPV of 85.7%. This combination yielded higher AUC values than individual biomarkers (CRP or NLR) and even outperformed the combination of all three biomarkers (NLR, CRP, and WBC), which had an AUC of 0.833 (95% CI: 0.747-0.899) **(Table 2).**

Table 2. The performance of biomarkers in diagnosing of early -onset neonatal sepsis

Biomarkers *(Cut-off point)*	**AUC (95%CI)**	**p-value**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
NLR (1.87)	0.736 (0.629-0.843)	<0.001	84.4	60.0	49.1	89.4
WBC (17.15)	0.725 (0.612-0.837)	<0.001	65.6	80.0	60.0	83.6
CRP (15.9)	0.819 (0.721-0.918)	<0.001	75.0	90.0	77.4	88.7
NLR and CRP	0.853 (0.769-0.915)	<0.001	65.6	94.3	84.0	85.7
NLR and WBC	0.765 (0.670-0.843)	<0.001	59.4	84.3	63.3	81.9
NLR, CRP, and WBC	0.833 (0.747-0.899)	<0.001	50.0	98.6	94.1	81.2

NLR, Neutrophil-lymphocyte ratio; WBC, White blood cell; CRP, C-reactive protein; AUC, area under the curve. CI, Confidence Interval; PPV, positive predictive value; NPV, negative predictive value.

Discussion

This study supports the inclusion of NLR as a valuable diagnostic marker for early-onset neonatal sepsis (EOS). In neonatal care, diagnostic tests are essential for identifying EOS in both asymptomatic high-risk neonates and symptomatic neonates. While blood cultures are commonly used, this study highlights their limitations, particularly in culture-negative cases with strong clinical signs of infection (only 3 out of 102 cases showed positive results). Early recognition of EOS is crucial due to its rapid progression and high mortality rate. Therefore, clinicians should consider a combination of clinical signs, symptoms, and diagnostic tools, including NLR, to ensure timely and appropriate treatment.

Neutrophils and lymphocytes play an important role in the fight against infections. When infected, neutrophils adhere to the wall of the vessel, resulting in increased neutrophil synthesis in the bone marrow and leading to the release of many immature leukocytes in the blood.[3] The role of increasing the number of WBC in peripheral blood, along with prolonging the life of leukocytes, is to increase the body's immune response by destroying bacteria through phagocytosis, cytokine release, and activation of T-cell metabolism.[4] However, high levels of inflammatory cytokines released due to sepsis can cause tissue damage and impair organ function. This triggers the release of anti-inflammatory cytokines that induce lymphocyte death and a decrease in lymphocyte numbers. Lymphopenia is part of the host's normal immune response to stop or control excessive inflammation, with the goal of preventing progressive tissue damage. Therefore, the body controls immune-mediated tissue damage by inducing lymphocyte apoptosis.[3,4] Migration of lymphocytes to the site of infection may contribute to lymphocytopenia in sepsis, particularly during the first 24 hours. Consequently, an increase in the number of neutrophils and a decrease in the number of lymphocytes leads to an increase in the NLR and can serve as a biomarker to diagnose and evaluate the severity of sepsis, contributing to early and effective treatment. Previous studies have demonstrated that NLR was a marker for the diagnosis and assessment of various other infections, including COVID-19, RSV, and Influenza.[8-11] Our results showed that the median NLR ratio in the EOS group was also higher than in the EOI group ($p<0.05$). Several studies

support our observation, [6,12-14] Notably, studies reported varying average NLR values for EOS: Birol Karabulut and Silem Ozdem Alatas (2021) found 3.16 ± 1.72,[12] or Santosh K. Panda et al. (2020) showed 3.88± 1.78,[13] Kurt et al. (2022) reported 1.95 ± 2.12,[14] Rocky Wilar (2019) reported 2.82±2.29,[15] while Tiewei Li et al. (2020) revealed 1.65 (0.85-3.07) in the sepsis group and 1.07 (0.75- 1.84) in infection group.[6] Although many studies suggest that WBC may not be specific for diagnosing EOS, NLR, derived from WBC count, remains a valuable tool in its evaluation. Peripheral blood NLR demonstrated a good ability to discriminate between neonates with sepsis (EOS) and those without (EOI) with an AUC of 73.6% ($p < 0.05$), indicating a fair level of significance. The number of leukocytes can vary depending on gestational age. To minimize this factor, we conducted a separate analysis of NLR in both term and preterm neonates. Our findings indicated that NLR has some value in the early diagnosis of EOS, although its performance is not as strong as CRP in term group. We also observed that NLR and WBC were more effective predictors of EOS in preterm group, while CRP was a better predictor in term group. Previous research has revealed that gestational age is a significant factor influencing serum CRP levels in the early postnatal period. Studies have shown a positive correlation between gestational and CRP, and preterm neonates generally exhibit a lower and shorter CRP response compared to term neonates. Therefore, the use of CRP in diagnosing of EOS in preterm neonates was less reliable than in term neonates.[16,17] To enhance diagnostic accuracy, we compared NLR with combinations of other biomarkers (NLR and CRP; NLR and WBC; and NLR, CRP, and WBC). We found that combining NLR with CRP significantly improved diagnostic efficacy, outperforming both individual biomarkers (CRP or NLR) and even the combination of all three biomarkers (NLR, CRP, and WBC). These biomarkers are widely available and easily obtained in most healthcare settings. Previous studies have shown variations in the NLR cut-off values for diagnosing EOS. In China, Tiewei Li et al. reported a cutoff of 1.62 for the prediction of neonatal sepsis, achieving 51% Se and 75% Sp, the AUC indicated good discriminatory power (AUC = 63%, 95% CI 60%–66%, $P < 0.001$).[6] Similarly, Santosh K. Panda et al. in India found a cut-off of >1.7 for diagnosing EOS, producing a Se of 68.3% and specificity of 46.2%. The AUC for NLR in their study was 62.3%.[10] Rocky Wilar's research showed a cut-off of 1.24 for NLR in the non-EOS group, demonstrating 83.3% Se and 93.3% Sp.[15] Birol Karabulut and Silem Ozdem Alatas reported an NLR AUC of 89.1%. They found a cut-off level of 1.42 with 88% Se, 84% Sp, 84.6% PPV, and 87.5% NPV.[12] The wide ranges of cut-off values, sensitivity, and specificity of NLR reported in studies may be associated with variations in the basic characteristics of the study groups, including gestational age, birth weight, day after birth, and the criteria used to diagnose neonatal sepsis. None of these studies showed a combination of biomarkers in predicting EOS.

Our study has several limitations. First, the diagnosis of EOS was based on clinical characteristics and all case of EOS was not culture-confirmed infections. Second, NLR was only analysed before treament. Serial measurements of NLR and the change in EOS would provide more comprehensive data on their relationship and could help elucidate the dynamic correlation between these factors.

Conclusion

NLR shows promise as a tool for diagnosing EOS, but it should be used in conjunction with clinical evaluation and other laboratory tests, such as CRP, to improve accuracy and timeliness of diagnosis

Declarations

Conflict of interest

We have no conflicts of interest to disclose.

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