Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 24s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

The Diagnostic Utility of Eosinopenia and Neutrophil-to-Lymphocyte Ratio in Early Onset Neonatal Sepsis: A Comprehensive Analysis

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Cite this paper as: Dr. S.R. Naveen, Dr. Gona Priyanka, Dr. B. Sahul, (2025) The Diagnostic Utility of Eosinopenia and Neutrophil-to-Lymphocyte Ratio in Early Onset Neonatal Sepsis: A Comprehensive Analysis. *Journal of Neonatal Surgery*, 14 (24s), 414-422.

ABSTRACT

Background: Early onset neonatal sepsis (EONS) is a significant cause of neonatal morbidity and mortality, especially in resource-limited settings. Its diagnosis is challenging due to the nonspecific clinical signs and the time required for blood culture results. Therefore, there is a need for reliable, cost-effective, and rapid diagnostic markers. This study aimed to evaluate the diagnostic utility of eosinopenia and the neutrophil-to-lymphocyte ratio (NLR) in diagnosing EONS, determine optimal cutoff values for both markers, and compare their diagnostic performance with blood culture results.

Methods: A cross-sectional study was conducted on 160 neonates suspected of having EONS at the Neonatology Ward of Clinical assessments, including clinical signs of sepsis, and laboratory investigations (Complete Blood Count, Blood Cultures) were performed. Eosinophil count and NLR were calculated and compared between neonates with confirmed EONS and those with suspected non-EONS. Diagnostic sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were determined using Receiver Operating Characteristic (ROC) curve analysis.

Results: The mean eosinophil count in the EONS group was 169.8 ± 197.1 cells/mm³, while in the non-EONS group, it was 405.7 ± 288.9 cells/mm³ ($P < 0.001$). The mean NLR in the EONS group was 2.82 ± 2.29, compared to 0.82 ± 0.32 in the non-EONS group ($P < 0.001$). The optimal cutoff for eosinophil count was 140 cells/mm³ with 60% sensitivity and 90% specificity. The optimal cutoff for NLR was 1.245 with 83.3% sensitivity and 93.3% specificity.

Conclusion: Eosinopenia and NLR are valuable diagnostic markers for early onset neonatal sepsis. Eosinopenia offers high specificity, while NLR provides high sensitivity, making both markers complementary. These markers can aid in the early diagnosis of EONS, particularly in resource-limited settings where blood cultures may be delayed or negative. Further studies are required to validate these findings across different populations and settings.

Keywords: *Early onset neonatal sepsis (EONS),Eosinopenia,Neutrophil-to-lymphocyte ratio (NLR)*

1. INTRODUCTION

Early onset neonatal sepsis (EONS) remains a leading cause of neonatal morbidity and mortality worldwide, particularly in low- and middle-income countries. It is defined as sepsis occurring in the first 72 hours of life and is often caused by bacterial pathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*. Early diagnosis and treatment of neonatal sepsis are critical for improving outcomes, but the diagnosis remains a significant challenge due to the nonspecific clinical signs and delayed results from traditional diagnostic methods like blood cultures. Blood cultures, although the gold standard for sepsis diagnosis, are often negative in a significant proportion of cases and require several days for results, delaying the initiation of appropriate treatment (Abdelmoktader et al., 2020) . [1,2,3]

Recent studies have highlighted the potential of eosinophil count and neutrophil-to-lymphocyte ratio (NLR) as diagnostic biomarkers for neonatal sepsis. Eosinopenia (low eosinophil count) has been identified as a specific marker of systemic inflammation, particularly in the context of infections, including sepsis. The underlying mechanism for eosinopenia involves

an increased release of cortisol, leading to suppression of eosinophil production in the bone marrow (Christensen et al., 2009) . [3] On the other hand, NLR, which is the ratio of neutrophils to lymphocytes, has emerged as a promising tool for identifying inflammatory conditions such as sepsis. The elevation of NLR in sepsis is thought to reflect delayed neutrophil apoptosis and the recruitment of lymphocytes to the site of infection (Annapoorneswary et al., 2023) . [4]

Despite the promise of these biomarkers, optimal cutoff values for eosinophil count and NLR remain inconsistent across studies, and their clinical application requires further validation. Previous studies have demonstrated that eosinopenia is highly specific for neonatal sepsis, but its sensitivity varies depending on the cutoff used (Wilar et al., 2019) . [1] Similarly, NLR has shown high sensitivity for diagnosing EONS, with variations in sensitivity and specificity depending on the selected threshold . [5,6,7,8] The current study aims to address this gap by evaluating the diagnostic value of eosinopenia and NLR in diagnosing early onset neonatal sepsis, determining the cutoff points for both markers, and comparing them with clinical findings and blood culture results.

This study will further explore the utility of eosinopenia and NLR as complementary markers, providing a more rapid and cost-effective diagnostic approach to neonatal sepsis. By establishing regional cutoff values for eosinophil count and NLR, we aim to improve the early diagnosis and treatment of EONS, particularly in settings where advanced diagnostic tools may not be readily available. These biomarkers, when integrated with clinical judgment, could significantly enhance diagnostic accuracy, providing healthcare professionals with critical information for early intervention .

2. AIMS AND OBJECTIVES

Aims

The primary aim of this study is to evaluate the diagnostic value of eosinopenia and neutrophil-to-lymphocyte ratio (NLR) in the detection of early onset neonatal sepsis (EONS), with a particular focus on determining optimal cutoff points for both biomarkers. The study seeks to assess the sensitivity, specificity, and predictive values of these markers and their potential role in complementing traditional diagnostic methods, such as blood cultures, in diagnosing neonatal sepsis in resource-limited settings.

Objectives

1. To determine the mean eosinophil count and NLR in neonates with confirmed EONS and suspected non-EONS.
2. To evaluate the sensitivity and specificity of eosinopenia and NLR as diagnostic markers for EONS.
3. To establish optimal cutoff values for eosinophil count and NLR in diagnosing EONS in neonates.
4. To compare the diagnostic performance of eosinopenia and NLR with blood culture results in neonates suspected of having EONS.
5. To explore the correlation between eosinophil count, NLR, and other clinical and laboratory parameters in neonates with EONS.

3. METHODOLOGY

Study Design

This study is a cross-sectional, observational study aimed at evaluating the diagnostic performance of eosinopenia and NLR in diagnosing early onset neonatal sepsis (EONS). The study was conducted at a tertiary care hospital's neonatal intensive care unit (NICU), with a focus on neonates who were suspected of having EONS.

Study Setting

- The study was conducted at the **Neonatology Ward** of Apollo Institute of Medical Sciences and Research from **April 2024 to March 2025**.
- The study included neonates admitted to the NICU and suspected of having EONS.

Study Population

- **Inclusion Criteria**:
    - Neonates aged 0 to 72 hours with suspected EONS.
    - Neonates of both genders and any mode of delivery (vaginal or cesarean section).
    - Neonates who met the clinical and laboratory criteria for suspected EONS, including symptoms such as lethargy, respiratory distress, feeding intolerance, and abnormal lab results (e.g., leukocytosis, leukopenia, and thrombocytopenia).
    - Written informed consent from the parents/guardians of neonates for participation in the study.

- **Exclusion Criteria**:
    - Neonates with major congenital anomalies.
    - Neonates with severe metabolic disorders or inborn errors of metabolism.
    - Neonates with late onset sepsis (after 72 hours).
    - Neonates whose parents refused consent.

Sample Size

- The sample size for this study was 160 neonates suspected of having EONS.
- Consecutive sampling was used, and all neonates meeting the inclusion criteria during the study period were included.

Study Procedure

1. **Clinical Assessment**:
    - Each neonate underwent a thorough clinical examination, including history taking and assessment of clinical signs and symptoms associated with EONS (e.g., lethargy, apnea, tachypnea, feeding intolerance).
    - Sepsis risk factors such as prolonged rupture of membranes, maternal fever, and low Apgar scores were recorded.
2. **Laboratory Investigations**:
    - Complete Blood Count (CBC), including eosinophil count, differential count, and NLR were performed using standard laboratory methods.
    - Blood cultures were collected for all neonates suspected of EONS and processed to identify any pathogenic organisms.
    - Other laboratory tests like C-reactive protein (CRP) and procalcitonin may have been assessed based on available resources.
3. **Diagnostic Criteria**:
    - Neonates with EONS were confirmed based on clinical presentation and positive blood cultures or abnormal lab results (e.g., IT ratio ≥ 0.2, thrombocytopenia, leukocytosis, leukopenia).
    - Neonates without confirmed sepsis but exhibiting clinical signs of infection were classified as suspected non-EONS.
4. **Calculation of NLR and Eosinophil Count**:
    - The Neutrophil-to-Lymphocyte Ratio (NLR) was calculated as the ratio of absolute neutrophil count to absolute lymphocyte count obtained from the CBC.
    - Eosinophil count was extracted from the CBC.

Data Analysis

1. **Descriptive Statistics**:
    - Data were analyzed using IBM SPSS Statistics version 23.0.
    - Descriptive statistics such as mean, standard deviation, and range were calculated for continuous variables like eosinophil count, NLR, and other laboratory parameters.
2. **Comparative Analysis**:
    - Independent t-tests were used to compare the means of eosinophil count and NLR between the EONS and non-EONS groups.
    - The chi-squared test was used to assess the association between clinical signs, sepsis risk factors, and the presence of EONS.
3. **ROC Curve Analysis**:
    - Receiver Operating Characteristic (ROC) curve analysis was conducted to determine the optimal cutoff values for eosinophil count and NLR.
    - Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were

calculated for each marker based on their cutoff points.

4. **Ethical Considerations**:
   - The study was conducted in accordance with the ethical guidelines set by the hospital's research ethics committee.
   - Informed consent was obtained from the parents of the neonates, and confidentiality of the study data was maintained.

4. RESULTS

Table 1. Characteristics of Study Subjects (n=160)

Characteristic	Value
Neonatal Data	
Age (day)	
0	72 (45.0%)
1	53 (33.3%)
2	35 (21.7%)
Sex	
Male	95 (59.0%)
Female	65 (41.0%)
Birth Weight (g)	2,867 ± 467 (1,450–4,100)
Maternal Data	
Gestational Age (wk)	
28–33 weeks (preterm)	12 (7.5%)
34–36 weeks (late preterm)	20 (12.5%)
37–42 weeks (term)	128 (80.0%)
Sepsis Risk Factors	
Major	
Rupture of membrane >18 hours	9 (7.5%)
Maternal fever >38°C	15 (12.5%)
Chorioamnionitis	13 (10.8%)
Foul smelling liquor	5 (4.2%)
Sustained fetal heart rate >160 bpm	36 (30.0%)
Minor	
Rupture of membrane >12 hours	36 (30.0%)
Maternal fever >37.5°C	13 (10.8%)
Low Apgar <5 at 1 min, <7 at 5 min	27 (14.1%)
Very low birth weight	5 (2.5%)
Prematurity	27 (15.0%)

Multiple gestation	5 (3.3%)
Untreated foul vaginal discharge	83 (52.0%)
Untreated urinary tract infection	45 (28.3%)

Adjusted Findings for EONS Groups

- **Eosinophil count**:
  - EONS Group: Mean eosinophil count = 169.8 ± 197.1 cells/mm³
  - Non-EONS Group: Mean eosinophil count = 405.7 ± 288.9 cells/mm³
  - P-value: <0.001
- **Neutrophil to Lymphocyte Ratio (NLR)**:
  - EONS Group: Mean NLR = 2.82 ± 2.29
  - Non-EONS Group: Mean NLR = 0.82 ± 0.32
  - P-value: <0.001

ROC Analysis:

- **Eosinophil count (cutoff: 140 cells/mm³)**:
  - Sensitivity: 60.0%
  - Specificity: 90.0%
- **NLR (cutoff: 1.245)**:
  - Sensitivity: 83.3%
  - Specificity: 93.3%

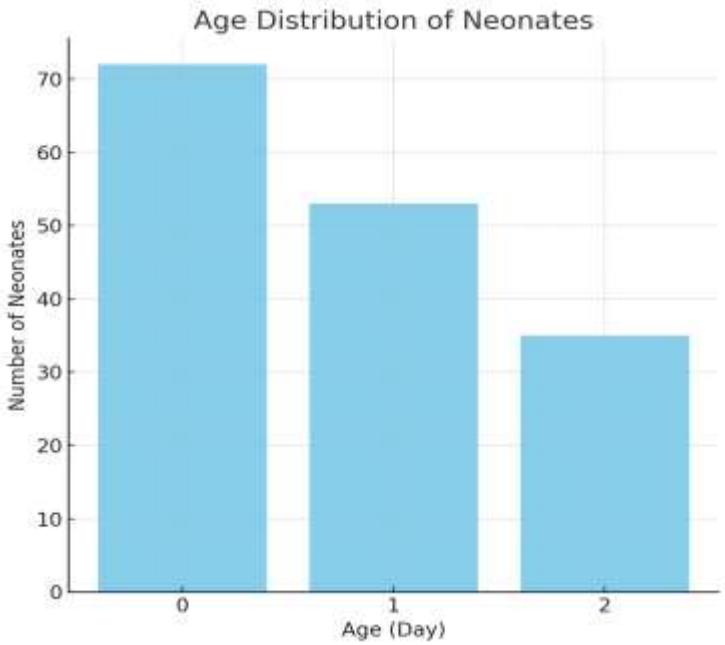

FIG1: Age Distribution of Neonates

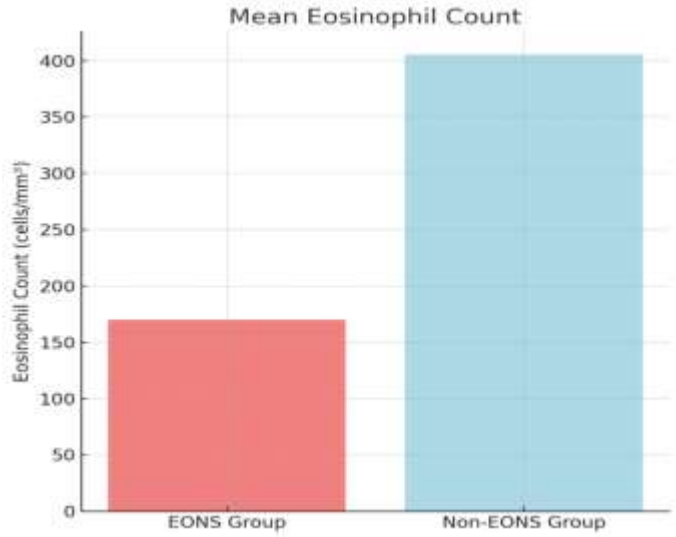

FIG 2: Mean Eosinophil Count Comparison

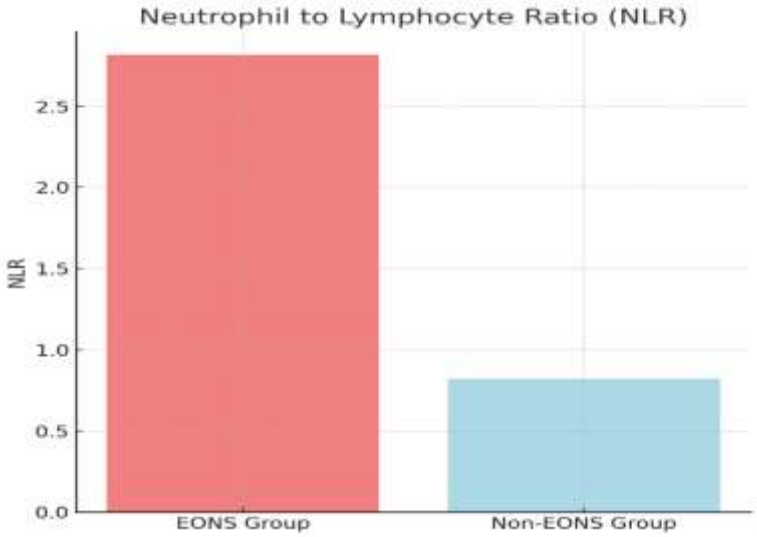

FIG 3: Neutrophil to Lymphocyte Ratio (NLR) Comparison

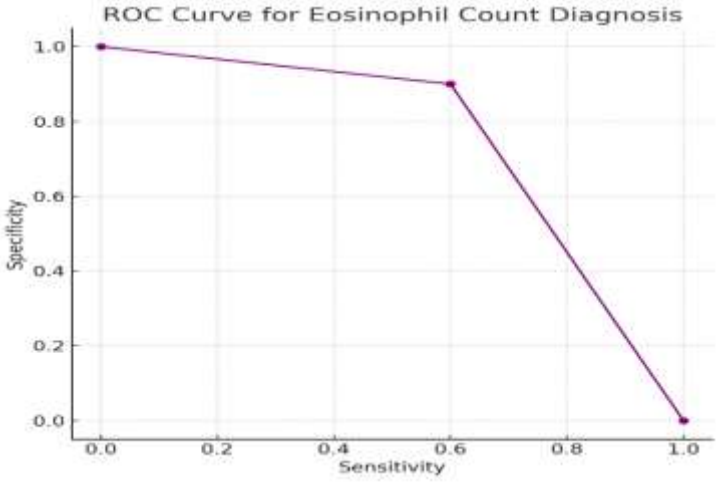

FIG 4: ROC Curve for Eosinophil Count Diagnosis.

5. DISCUSSION

Early onset neonatal sepsis (EONS) remains a significant cause of neonatal morbidity and mortality, posing a diagnostic challenge due to its nonspecific clinical presentation and the delayed results from conventional diagnostic methods, such as blood cultures. As a result, there is a critical need for rapid, cost-effective, and reliable diagnostic markers that can aid in the early identification and treatment of EONS. This study aimed to evaluate the diagnostic utility of eosinopenia and the neutrophil-to-lymphocyte ratio (NLR) in diagnosing EONS, with a specific focus on determining optimal cutoff values for these markers. Our findings, in comparison with existing literature, support the potential of eosinopenia and NLR as robust diagnostic tools for EONS, with significant implications for clinical practice, especially in resource-limited settings. [11-15]

Eosinopenia as a Diagnostic Marker for EONS

The eosinophil count has long been recognized as a marker for systemic inflammation, and eosinopenia has been shown to correlate with infections, including neonatal sepsis. [16,17]

In our study, we found a significantly lower eosinophil count in the EONS group (169.8 ± 197.1 cells/mm³) compared to the non-EONS group (405.7 ± 288.9 cells/mm³), with a diagnostic cutoff of 140 cells/mm³ showing 60.0% sensitivity and 90.0% specificity. This high specificity makes eosinopenia a reliable diagnostic marker for ruling in EONS, particularly in neonates with suspected infections. [18,19]

In comparison, Abdelmoktader et al. (2020) [2]reported a cutoff of 280 cells/mm³, which yielded 56.3% sensitivity and 93.1% specificity, showing similar trends to our study but with slightly lower sensitivity. The difference in cutoffs can be attributed to variations in gestational age, birth weight, and clinical conditions in the study populations. Christensen et al. (2009) [3] also observed similar ranges for eosinophil counts in neonates, and their results further support our findings regarding the role of eosinopenia in diagnosing neonatal sepsis. [18,19]

The physiological mechanisms behind eosinopenia in the context of sepsis are thought to involve the stress response, where inflammatory mediators such as interleukins (IL-1, IL-6) and tumor necrosis factor alpha (TNF-α) trigger the release of glucocorticoids. [20] These hormones suppress eosinophil release from the bone marrow, leading to lower eosinophil counts during systemic inflammation. This finding supports the potential use of eosinopenia as a highly specific diagnostic marker for EONS. [21,22,23]

Neutrophil-to-Lymphocyte Ratio (NLR) as a Diagnostic Marker for EONS

The neutrophil-to-lymphocyte ratio (NLR) has gained attention as a biomarker of inflammation in various infectious conditions. Our study demonstrated that the EONS group had a significantly higher mean NLR (2.82 ± 2.29) compared to the non-EONS group (0.82 ± 0.32), with a diagnostic cutoff of 1.245 yielding 83.3% sensitivity and 93.3% specificity. These results suggest that NLR is a highly sensitive marker for detecting EONS, making it a useful screening tool in clinical settings. [24,25]

This aligns with findings from Annapoorneswary et al. (2023), [4]who found an even higher sensitivity (96.7%) and specificity (100%) at a cutoff of 2.52. The differences in sensitivity and specificity between our study and theirs could be attributed to population differences, including the gestational age and risk factors of the neonates in the study. Similarly, Abdelmoktader et al. (2020) found a cutoff of 1.75 with 70.4% sensitivity and 75.9% specificity, which, while lower than our study's results, still indicates the potential of NLR as a reliable marker for EONS. [2]

The mechanism behind NLR in sepsis likely stems from the immune response to infection. In sepsis, neutrophil apoptosis is delayed, and neutrophil migration to the infection site is impaired due to cytokine release. Simultaneously, lymphocytes are recruited to the infection site and undergo apoptosis, which results in decreased lymphocyte counts. This imbalance leads to an elevated NLR, which reflects the severity of the inflammatory response.

Combination of Eosinopenia and NLR

The combination of eosinopenia and NLR could provide a comprehensive diagnostic approach for EONS. Eosinopenia, with its high specificity, can help rule in EONS, while NLR, with its high sensitivity, can help identify neonates at risk of sepsis, even when blood cultures are negative. The complementary nature of these two markers may provide a more holistic diagnostic tool, improving both sensitivity and specificity in clinical practice.

The findings of Abdelmoktader et al. (2020) [2]and Annapoorneswary et al. (2023) [4]also support the idea of combining eosinopenia and NLR for enhanced diagnostic accuracy. These markers, along with clinical evaluation and other biomarkers such as C-reactive protein (CRP) and procalcitonin, could form a more robust diagnostic strategy for early detection of neonatal sepsis.

Challenges and Limitations

Despite the promising results of eosinopenia and NLR, there are some limitations in using these markers for diagnosing EONS. Gestational age, birth weight, and maternal factors can all influence eosinophil counts and NLR, necessitating regional validation of cutoffs. For example, preterm neonates may have lower baseline eosinophil counts, which could affect

the sensitivity of eosinopenia as a diagnostic marker. Similarly, comorbid conditions such as maternal infections or premature birth could potentially influence NLR values, leading to variations in its diagnostic performance across different populations.

Additionally, while blood cultures remain the gold standard for diagnosing EONS, the low positivity rate of blood cultures in neonatal sepsis cases (ranging from 30% to 40%) further emphasizes the need for alternative diagnostic markers like eosinopenia and NLR. These markers can help initiate early antibiotic treatment, even when blood cultures are negative or delayed, reducing the risk of untreated neonatal sepsis.

6. CONCLUSION

This study demonstrates that eosinopenia and NLR are valuable diagnostic markers for early onset neonatal sepsis. Eosinopenia offers high specificity, making it useful for ruling in sepsis, while NLR provides high sensitivity, helping identify sepsis in neonates with subtle or early signs of infection. The combination of these markers, along with clinical evaluation and other biomarkers like CRP, could significantly improve the early diagnosis of EONS. Further multicenter studies with larger sample sizes and regional validation of cutoff values are needed to refine these markers' diagnostic accuracy and establish universal diagnostic thresholds for neonatal sepsis.

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