Validation of C-Reactive Protein in early vs late onset neonatal sepsis and its correlation with blood culture in the diagnosis of neonatal sepsis

✉Paudel L[1], Panthi K[1], Marahatta S[1]
1. Department of Pediatrics, Devdaha Medical College & Research Institute, Rupandehi, Nepal

ABSTRACT

Introduction: Neonatal sepsis is a major cause of morbidity and NICU admission, and early diagnosis is challenging due to nonspecific clinical features. Although blood culture is the gold standard, it has limitations such as low sensitivity, delayed results, and reduced yield after antibiotic exposure. This diagnostic gap highlights the role of adjunct biomarkers like C-Reactive Protein (CRP), which is rapid and requires smaller blood volume. This study aimed to validate CRP in early-onset (EONS) and late-onset neonatal sepsis (LONS) and assess its correlation with blood culture.

Methods: This hospital-based prospective observational study was conducted at Devdaha Medical College from October 15, 2023, to October 14, 2025. Neonates with clinical suspicion of sepsis were included, while those with prior antibiotic exposure, congenital anomalies, or birth weight <1500 g were excluded. Blood culture (2 mL sample) was performed under aseptic conditions prior to antibiotics. CRP was measured at initial evaluation using a semi-quantitative latex agglutination method, with >10 mg/L considered positive. Statistical analysis was done using SPSS; chi-square test and diagnostic validity parameters were calculated. A p-value <0.05 was considered significant.

Results: Among 253 neonates, 188 (74.3%) had EONS and 65 (25.7%) had LONS. CRP positivity was higher in LONS than EONS (49.2% vs 41.5%), but not statistically significant (p=0.347). Blood culture positivity was significantly higher in LONS (18.5% vs 5.9%, p=0.002). CRP showed sensitivity 69.8%, specificity 59.1%, PPV 14.5%, and NPV 95.1%. Sensitivity was higher in LONS (83.3%) than EONS (54.5%), while NPV remained high in both groups. ROC analysis showed AUC of 0.723 (p<0.001).

Conclusion: CRP is a useful adjunct marker with high negative predictive value, making it valuable for ruling out neonatal sepsis. However, due to low specificity and PPV, it should not be used alone and must be interpreted with clinical findings and blood culture, particularly in late-onset sepsis.

Keywords: Blood culture, c-reactive protein, early-onset sepsis, late-onset sepsis, neonatal sepsis.

INTRODUCTION

Neonatal sepsis is a clinical condition with rapid progression of systemic signs and symptoms leading to high morbidity and mortality. It is the 2nd most common cause of neonatal deaths after prematurity in developing countries.[1] In Nepal, it is the 3rd most common cause of death after prematurity and intrapartum complications.[2] One study done in the western part of Nepal has reported the prevalence of neonatal sepsis to be around 20.3%[3]

Neonatal sepsis contributes to high morbidity and mortality rate so prompt diagnosis of neonatal sepsis is of paramount importance. But its diagnosis is difficult to make only on historical or clinical background. Laboratory evaluation is essential in its diagnosis and confirmation. Despite complete blood count, peripheral blood smear, C- Reactive protein, immature neutrophil to leucocyte ratio, Blood culture is the only confirmatory test for the diagnosis of Neonatal Sepsis[4].

Several studies have evaluated C-reactive protein as a screening test for neonatal sepsis and reported variable sensitivity and specificity. Traditionally,

Corresponding author
Dr. Laxman Paudel
Department of Pediatrics
Devdaha Medical College & Research Institute
Email: laxmanpaudel8@gmail.com

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a CRP value of 5 mg/L has been considered a significant predictor of neonatal sepsis.[5] However, higher cut-off value (>10mg/L) are often used in clinical practice to improve specificity and reduce false positive results; we also adopted the latter threshold for determining CRP positivity.

Neonatal sepsis is broadly classified into early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS). EONS typically results from vertical transmission of pathogens from the mother and may be influenced by maternal antibiotic exposure, often leading to lower blood culture positivity. In contrast, LONS is usually acquired from community or hospital environments and is associated with higher rates of both CRP and blood culture positivity. Understanding these differences is important for interpreting diagnostic markers and correlating laboratory findings with clinical patterns.

Given the diagnostic challenges and limitations of blood culture, along with the potential advantages of CRP, this study was conducted to evaluate the diagnostic validity of CRP by determining its sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), and to assess its correlation with blood culture in both early- and late-onset neonatal sepsis.

METHODS

A hospital based prospective observational study was conducted in the NICU of Devdaha Medical College from October 2023 to October 2025 after obtaining ethical approval from the Institutional Review Committee of our institute with IRC no 024/2020. Written informed consent was obtained from parents.

Neonates with clinical suspicion of sepsis (e.g., respiratory distress, poor feeding, lethargy, temperature instability) and birth weight ≥1500 grams were included. Neonates with severe birth asphyxia, congenital heart disease, major congenital anomalies, and birth weight <1500 gm were excluded. Neonates <1500 gm were excluded to reduce confounding factors and homogeneity of the study population. Similarly very low birth weight neonates receive early empirical antibiotics and intensive interventions , which can reduce blood culture yield and introduce bias in evaluating CRP against culture.

Sample size was calculated using the standard formula $n=z^2xp(1-p)/d^2$ where z= 1.96 for a 95% confidence interval, p=prevalence of neonatal sepsis and d= acceptable margin of error (5%). Taking the prevalence of 20.3%[3], the minimum required sample size was 249.

Operational Definitions

Neonates were categorized based on the time of presentation into early-onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS). Early onset neonatal sepsis was defined as sepsis occurring within 72 hours of life, whereas late-onset neonatal sepsis was defined as sepsis occuring after 72 hours of life.

Procedure

Blood samples were collected under aseptic precautions. Approximately 2 mL of blood was obtained for culture prior to antibiotic administration and processed using standard microbiological techniques. Blood culture was performed using an automated BACT/ALERT system. CRP was measured at the time of initial evaluation using a semi-quantitative latex agglutination method, and a value of >10 mg/L was considered positive.

A detailed history and thorough clinical examinations were performed for all enrolled neonates. Relevant investigation reports like complete blood count (CBC), C-reactive protein (CRP), peripheral blood smear, chest X-ray (where indicated) and blood culture were recorded. Sepsis was diagnosed based on clinical suspicion supported by laboratory parameters such as leukocytosis (>11,000 cells/mm^3), leukopenia (<5000 cells/mm^3), presence of band cells and toxic granules in peripheral smear, elevated CRP (>10 mg/L) and/or positive blood culture.[6]

Statistical analysis

Statistical analysis was performed using SPSS software. Chi-square test was applied to assess associations, and diagnostic validity parameters (sensitivity, specificity, Positive Predictive Value-PPV, Negative Predictive Value-NPV) were calculated. Receiver Operating Characteristic (ROC) curve analysis was used to determine diagnostic accuracy. A p-value <0.05 was

Table 1: Demographic Profile of neonates n=253

Variable	Category	Frequency n (%)
Sex	Male	143 (56.5)
	Female	110 (43.5)
Place of Delivery	Hospital	228 (90.1)
	Home	25 (9.9)
Onset of Sepsis	Early onset sepsis	188 (74.3)
	Late onset sepsis	65 (25.7)
Mode of Delivery	Normal Vaginal delivery	126 (49.8)
	LSCS	118 (46.6)
	Instrumental Delivery	9(3.6)
Gestational Age	Term	208 (82.2)
	Preterm	43 (17.0)
	Postterm	2 (0.8)

Table 2: Comparison of CRP Positivity vs Onset of Sepsis

Onset of Sepsis	CRP Positive n (%)	CRP Negative n (%)	Total	p-Value
Early onset sepsis	78 (41.5)	110 (58.5)	188	0.347
Late onset sepsis	32 (49.2)	33 (50.8)	65	
Total	110	143	253	

Table 3: Comparison of Blood Culture Positivity between Early and Late onset sepsis

Type of Sepsis	Blood Culture positive n(%)	Blood Culture Negative n (%)	Total	p-Value
Early onset sepsis	11(5.9)	177 (94.1)	188	0.002*
Late onset sepsis	12 (18.5)	53 (81.5)	65	
Total	23 (9.1)	230 (90.9)	253	

*significant

Table 4: Comparison of CRP with Blood Culture

CRP	Blood Culture		Total
	Culture Negative	Culture Positive	
Negative	136	7	143
Positive	94	16	110
Total	230	23	253

Table 5: Diagnostic Performance of CRP compared with Blood Culture

Diagnostic Parameter	Overall (%)	EONS (%)	LONS (%)
Sensitivity	69.8	54.5	83.3
Specificity	59.1	59.3	58.5
PPV	14.5	7.7	31.3
NPV	95.1	95.5	93.9

considered statistically significant.

RESULTS

Total of 253 neonates were enrolled in the study. The demographic and baseline characteristics were summarized in the table 1 below.

Based on the timing of onset, 188 (74.3%) were classified as early onset sepsis and 65 (25.7%) as late onset sepsis.

Blood culture was taken as the gold standard for

diagnosis of neonatal sepsis. CRP was considered positive at a value of >10 mg/L.

There was no statistical significance between CRP positivity and type of sepsis (EONS vs LONS). But CRP positivity was higher in late-onset sepsis than early-onset neonatal sepsis.

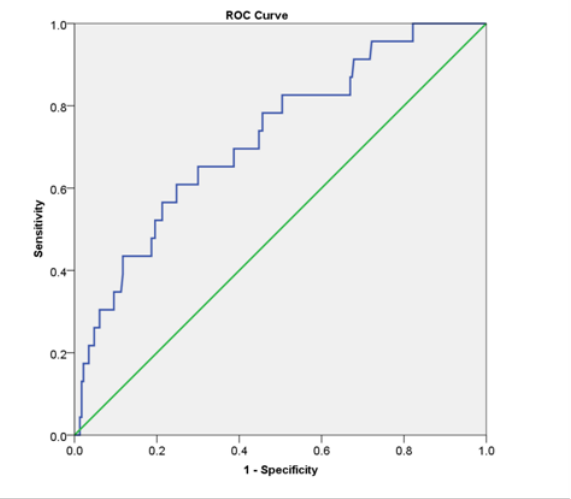
There was statistically significant differences between blood culture positivity and type of sepsis (early vs late onset sepsis). Culture positivity was significantly higher in late than early onset sepsis (18.8% vs 5.9%).

When compared with blood culture as gold standard, CRP demonstrated a sensitivity of 69.8%, specificity of 59.1%, PPV 14.5% and NPV 95.1%

In early onset sepsis, CRP demonstrated moderate sensitivity and specificity (54.5% vs 59.3%). PPV was low 7.7% with high NPV 95.5% indicating good ability to exclude culture proven sepsis.

In LONS, CRP demonstrated higher sensitivity (83.3%) with moderate specificity (58.5%). CRP demonstrated better sensitivity in late onset sepsis but high NPV in both groups supporting its role as a rule out marker rather than a confirmatory test.

ROC Curve



Interpretation: The ROC curve showed that CRP is the good predictor of neonatal sepsis with AUC (0.723) and found to be statistically significant (p<0.001).

DISCUSSION

Neonatal sepsis remains a major cause of morbidity and mortality in Nepal, and its early diagnosis continues to be a challenge due to nonspecific clinical presentation and limited availability of rapid and reliable confirmatory tests. The present study evaluated the diagnostic utility of C-reactive protein (CRP) and its correlation with blood culture in early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS).

A male preponderance was observed in this study with a male-to-female ratio of 1.3:1, which is consistent with findings from studies by Khanal et al.[3] and Ansari et al.[7] The predominance of male neonates may be attributed to sex-related immunological differences, making male infants more susceptible to infections.

In this study, preterm neonates constituted 17% of the study population. Since this does not represent a high proportion, no over interpretation is made regarding prematurity. Additionally, only neonates with birth weight ≥1500 grams were included as per study criteria; therefore, comparisons related to low birth weight neonates were not considered to avoid inconsistency.

Similarly, early-onset sepsis constituted the majority of cases (74.3%), while late-onset sepsis accounted for 25.7%. Blood culture positivity was significantly higher in late-onset sepsis compared to early-onset sepsis (18.5% vs 5.9%, p = 0.002). This finding is consistent with studies by Lamichhane et al.[8] and Bhatia et al.[9] The higher culture positivity in LONS may be due to increased exposure to nosocomial or community-acquired pathogens and higher bacterial load, whereas EONS may be influenced by maternal antibiotic exposure and lower bacteremia levels, leading to reduced culture yield.

CRP positivity was observed in 43.5% of neonates overall and was slightly higher in LONS (49.2%) compared to EONS (41.5%); however, this difference was not statistically significant (p = 0.347). This suggests that while CRP may be elevated in established infection, it does not reliably differentiate between early- and late-onset sepsis. This may be explained by delayed hepatic synthesis of CRP in the early phase of infection and the influence of non-infectious inflammatory conditions commonly seen in neonates.

In the present study, CRP was evaluated

against blood culture as the gold standard and demonstrated moderate sensitivity (69.8%) and specificity (59.1%), with a low positive predictive value (14.5%) but a high negative predictive value (95.1%). These findings indicate that CRP has limited utility as a confirmatory diagnostic test due to its low PPV, but it is valuable as a screening tool owing to its high NPV. Similar observations have been reported by Lamichhane et al.[8], Bhatia et al.[9], El-Sonbaty et al.[10], and Hissamudin et al.[11], emphasizing that CRP should be used as an adjunct rather than a standalone diagnostic marker.

Subgroup analysis revealed that CRP had lower sensitivity (54.5%) and very low positive predictive value (7.7%) in early-onset sepsis, indicating poor performance in detecting culture-proven infection in the early phase. This may be due to the time-dependent nature of CRP synthesis, which typically rises 6–12 hours after the onset of infection, leading to false-negative results when measured early. In contrast, CRP demonstrated higher sensitivity (83.3%) and improved positive predictive value (31.3%) in late-onset sepsis, reflecting a more established inflammatory response. These findings are consistent with studies by Shrestha et al.[12], Meisner et al.[13], and Hofer et al.[14], which highlight better diagnostic performance of CRP in later stages of infection.

The high negative predictive value of CRP observed in both EONS and LONS (95.5% vs 93.9%) suggests that CRP is particularly useful in ruling out sepsis. This is especially relevant in resource-limited settings where rapid and reliable culture facilities may not be readily available. A negative CRP result can aid clinicians in safely withholding or discontinuing unnecessary antibiotic therapy, thereby reducing hospital stay, healthcare costs, and the risk of antimicrobial resistance.

Receiver Operating Characteristic (ROC) curve analysis in this study demonstrated an Area Under the Curve (AUC) of 0.723, indicating moderate diagnostic accuracy of CRP in distinguishing septic from non-septic neonates. Comparable AUC values (~0.70) have been reported by Hofer et al.[14], while Benitz et al.[15] demonstrated improved diagnostic accuracy with serial CRP measurements, suggesting that repeated testing may enhance clinical utility, so it can be used as an adjunct in the diagnosis of sepsis.

Overall, the findings of this study indicate that CRP has moderate sensitivity and limited specificity, with poor ability to confirm neonatal sepsis when used alone. However, its high negative predictive value and better performance in late-onset sepsis support its role as a useful screening and adjunctive tool in the diagnostic workup of neonatal sepsis.

This study has several limitations. Blood culture, though used as the gold standard, has low sensitivity and may yield false-negative results due to prior antibiotic exposure or inadequate sample volume, affecting diagnostic accuracy estimates. CRP was measured once using a semi-quantitative method; lack of serial measurements may have reduced sensitivity, especially in early-onset sepsis. Exclusion of neonates with birth weight <1500 gm limits generalizability to high-risk populations. The single-center design reduces external validity. Additionally, formal power analysis and detailed evaluation of confounding factors such as maternal antibiotic use and perinatal risk factors were not performed, which may influence the findings.

CONCLUSIONS

CRP has moderate diagnostic accuracy with high negative predictive value, making it a useful screening and rule-out marker in neonatal sepsis, particularly in late-onset cases. However, because of its moderate specificity, positive predictive value, and high negative predictive value, it should not be relied upon as a standalone diagnostic test. Instead, it should be used as part of antibiotic stewardship and interpreted in conjunction with clinical findings and blood culture results.

Conflict of Interest: None.

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Data availability statement: The data are

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