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Biomarkers for Early Diagnosis of Neonatal Sepsis: A Prospective Observational Study at a Tertiary Care Hospital.

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Abstract

Background: Neonatal sepsis remains a leading cause of neonatal morbidity and mortality worldwide, particularly in low- and middle-income countries. The gold standard for diagnosis—blood culture—has a turnaround time of 48–72 hours, leading to empirical antibiotic overuse and delayed targeted therapy. Reliable biomarkers enabling early diagnosis could significantly improve outcomes and reduce indiscriminate antibiotic exposure. ***Methods:*** A hospital-based prospective observational study was conducted over 18 months at a tertiary care teaching hospital. A total of 210 neonates with clinically suspected sepsis were enrolled. Serum levels of procalcitonin (PCT), C-reactive protein (CRP), interleukin-6 (IL-6), and presepsin were measured at the time of clinical suspicion (within 6 hours) and at 24 hours. Blood culture was performed as the reference standard. Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curve analysis. ***Results:*** Of 210 neonates, 62 (29.5%) had culture-proven sepsis. Among these, 28 (45.2%) had early-onset sepsis (EOS) and 34 (54.8%) had late-onset sepsis (LOS). All biomarkers showed significantly elevated levels in the sepsis group compared to non-sepsis group ($p<0.001$ for all). Presepsin demonstrated the highest diagnostic accuracy with an area under the curve (AUC) of 0.94 (95% CI: 0.90–0.97), followed by PCT (AUC: 0.89, 95% CI: 0.84–0.93), IL-6 (AUC: 0.86, 95% CI: 0.80–0.91), and CRP (AUC: 0.81, 95% CI: 0.75–0.87). At a cut-off of 600 pg/mL, presepsin showed a sensitivity of 88.7% and specificity of 91.2%. The combination of presepsin and PCT improved diagnostic accuracy (AUC: 0.96, 95% CI: 0.93–0.98). ***Conclusion:*** Presepsin is a highly promising biomarker for the early diagnosis of neonatal sepsis, demonstrating superior diagnostic accuracy compared to conventional markers. The combination of presepsin with PCT further enhances diagnostic performance. These findings support the incorporation of presepsin into neonatal sepsis diagnostic algorithms to enable timely diagnosis, reduce empirical antibiotic use, and improve outcomes in this vulnerable population.

Keywords: Neonatal sepsis, biomarkers, presepsin, procalcitonin, C-reactive protein, interleukin-6, early diagnosis.

INTRODUCTION

Neonatal sepsis remains one of the most significant challenges in neonatal medicine, representing a systemic bacterial infection in the first 28 days of life that carries substantial risks of mortality and long-term neurodevelopmental impairment. Despite advances in neonatal intensive care, neonatal sepsis continues to be a leading cause of neonatal mortality globally, contributing to approximately 30%–40% of all neonatal deaths in India and accounting for an estimated 200,000–250,000 preventable deaths annually. The burden is disproportionately high in low- and middle-income countries (LMICs), where the incidence is estimated to be three to four times higher than in high-income settings. In India, the overall prevalence of neonatal sepsis has been reported at 12.3%, with early-onset sepsis (EOS) accounting for 64.5% of cases. Neonatal sepsis is conventionally categorized into early-onset sepsis (EOS), defined as infection occurring within the first 72 hours of life, and late-onset sepsis (LOS), occurring after 72 hours. EOS is primarily caused by vertical transmission of pathogens from the mother, with Group B Streptococcus and Escherichia coli being the predominant organisms. LOS, on the other hand, is often nosocomially acquired, particularly affecting preterm infants with invasive devices, those receiving parenteral nutrition, and those requiring mechanical ventilation. The distinct epidemiological and microbiological profiles of EOS and LOS necessitate different diagnostic and therapeutic approaches.

The clinical diagnosis of neonatal sepsis is notoriously challenging. Neonates, particularly preterm infants, present with subtle and non-specific signs that overlap with numerous other neonatal conditions, including respiratory distress syndrome, metabolic disorders, and birth asphyxia. The gold standard for diagnosis remains blood culture, which, despite

being the reference standard, has significant limitations. Blood culture requires a minimum of 48–72 hours for results, consumes a substantial volume of blood (which is particularly critical in preterm neonates), has limited sensitivity (ranging from 50% to 75%), and is frequently unavailable in resource-limited settings. These limitations create a diagnostic dilemma: clinicians must either initiate empirical antibiotics in all suspected cases—contributing to antibiotic overuse, emergence of antimicrobial resistance, and disruption of the neonatal microbiome—or delay treatment, risking progression to septic shock and death. The limitations of current diagnostic approaches have driven intensive research into biomarkers that could enable rapid, accurate, and early diagnosis of neonatal sepsis. An ideal biomarker for neonatal sepsis would demonstrate high sensitivity and specificity, be measurable rapidly from a small sample volume, show early elevation in the disease course, differentiate bacterial from viral infection, and guide antibiotic duration. Several biomarkers have been investigated over the past decades, including acute-phase reactants (C-reactive protein, serum amyloid A), procalcitonin, cytokines (interleukin-6, interleukin-8, tumor necrosis factor-alpha), cell surface markers (CD64, CD11b), and more recently, presepsin and microRNAs.

C-reactive protein (CRP) is the most widely used biomarker in clinical practice, but its diagnostic utility is limited by a delayed rise (peaking at 24–48 hours after infection onset) and lack of specificity. Procalcitonin (PCT) has emerged as a more promising marker, with rapid elevation within 2–4 hours of infection and better specificity for bacterial infection. A systematic review and meta-analysis from LMICs reported that CRP at a cut-off of ≥60 mg/L had an AUC of 0.87 (95% CI: 0.76–0.91) and PCT at ≥0.5 ng/mL had an AUC of 0.87 (95% CI: 0.70–0.92) for the diagnosis of neonatal sepsis. However, neither biomarker has sufficient diagnostic accuracy to be used in isolation. Interleukin-6 (IL-6) is an early pro-inflammatory cytokine that rises within hours of infection, preceding the rise of CRP and PCT. A network meta-analysis suggested that interleukins including IL-6, IL-8, IL-10, and IL-27 have favorable diagnostic performance in neonatal sepsis, with IL-8 being particularly accurate for EOS detection. However, IL-6 has a short half-life and rapid decline, making its measurement highly time-dependent.

Presepsin, a soluble CD14 subtype generated during pathogen recognition by innate immune cells, has recently emerged as a promising biomarker. A comprehensive meta-analysis demonstrated that presepsin has excellent overall diagnostic performance with a pooled sensitivity of 0.84 (95% CI: 0.81–0.88), specificity of 0.86 (95% CI: 0.80–0.90), and AUC of 0.91 (95% CI: 0.88–0.93), with particularly high accuracy in neonates (sensitivity 0.90, specificity 0.92, AUC 0.96). Serum amyloid A (SAA) has also shown promise, with a meta-analysis reporting a sensitivity of 0.85 and specificity of 0.86, with an AUC of 0.91. Despite these advances, no single biomarker currently has sufficient diagnostic accuracy to replace blood culture or guide antibiotic decisions independently. The prevailing consensus is that biomarker combinations, integrated with clinical assessment, offer the most promising approach. Given the high burden of neonatal sepsis in India and the limited data on emerging biomarkers in the Indian population, this study was undertaken to evaluate the diagnostic accuracy of PCT, CRP, IL-6, and presepsin—both individually and in combination—for the early diagnosis of neonatal sepsis in a tertiary care hospital setting.

MATERIALS AND METHODS

This was a hospital-based prospective observational study conducted over a period of 18 months, at a tertiary care teaching hospital with a level III neonatal intensive care unit (NICU). The hospital serves as a major referral center for a large catchment area, providing comprehensive neonatal care to a diverse patient population.

Study Population

The study population consisted of neonates admitted to the NICU with clinical suspicion of sepsis. Neonates were included if they were aged 0–28 days, had clinical features suggestive of sepsis (as defined by the study criteria), and had written informed consent obtained from parents or legal guardians. Neonates with congenital anomalies, those who had received antibiotics prior to admission (except for intrapartum antibiotic prophylaxis), those with proven viral infections, and those who were moribund or expired before sample collection were excluded.

Clinical suspicion of sepsis was defined by the presence of at least two of the following criteria: (1) temperature instability (≥38°C or ≤36°C); (2) respiratory distress (tachypnea, grunting, retractions, or apnea); (3) cardiovascular instability (tachycardia, bradycardia, or hypotension); (4) poor perfusion (delayed capillary refill, mottling); (5) lethargy or irritability; (6) poor feeding or abdominal distension; or (7) seizures.

Sample Size Calculation

The sample size was calculated based on an anticipated prevalence of culture-proven sepsis of 25% among neonates with clinical suspicion, with a 95% confidence level, 5% absolute precision, and accounting for a 10% attrition rate. The calculated sample size was 210 neonates. The prevalence estimate was derived from previous studies conducted in similar Indian tertiary care settings.

Data Collection

Data were collected using a pretested structured proforma. Information was obtained through face-to-face interviews with parents, review of medical records, and physical examination. The proforma captured the following variables:

Maternal and perinatal characteristics: Maternal age, parity, gestational age at delivery, mode of delivery, premature rupture of membranes (PROM), maternal fever during labor, maternal Group B Streptococcus status, and intrapartum antibiotic use.

Neonatal characteristics: Birth weight, gestational age, sex, Apgar scores at 1 and 5 minutes, age at presentation (categorized as EOS ≤72 hours or LOS >72 hours), clinical features, and NICU interventions (mechanical ventilation, central venous catheterization, parenteral nutrition).

Biomarker Measurement

At the time of clinical suspicion of sepsis (T0), blood samples were collected for biomarker estimation prior to initiation of antibiotic therapy. A second sample was collected at 24 hours (T24) for all neonates. Blood culture was performed using standard automated methods (BacT/ALERT system) with a minimum volume of 1 mL.

The following biomarkers were measured:

Procalcitonin (PCT): Measured using a chemiluminescent immunoassay (BRAHMS PCT sensitive Kryptor assay). The upper limit of normal was defined as 0.5 ng/mL.

C-reactive protein (CRP): Measured using a turbidimetric immunoassay. The upper limit of normal was defined as 10 mg/L.

Interleukin-6 (IL-6): Measured using a chemiluminescent immunoassay (IMMULITE 2000). The upper limit of normal was defined as 7 pg/mL.

Presepsin: Measured using a chemiluminescent enzyme immunoassay (PATHFAST presepsin assay). The upper limit of normal was defined as 400 pg/mL.

All laboratory personnel performing biomarker assays were blinded to the clinical status of the neonates and the results of blood cultures.

Case Definition and Group Allocation

Neonates were classified into the **sepsis group** if they had a positive blood culture for a bacterial pathogen AND clinical features consistent with sepsis. Neonates with clinical features of sepsis but negative blood cultures were classified as **clinical sepsis (culture-negative)** . Neonates with negative blood cultures and alternative diagnoses (e.g., respiratory distress syndrome, transient tachypnea of the newborn, metabolic disorders) were classified as the **non-sepsis control group**.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and MedCalc version 20.0. Descriptive statistics were expressed as frequencies and percentages for categorical variables, and mean ± standard deviation (SD) or median with interquartile range (IQR) for continuous variables, as appropriate. The normality of continuous data was assessed using the Kolmogorov-Smirnov test. For comparison between groups (sepsis vs. non-sepsis), the chi-square test or Fisher's exact test was used for categorical variables, and the independent t-test or Mann-Whitney U test was used for continuous variables, as appropriate. Paired comparisons (T0 vs. T24) within groups were performed using the paired t-test or Wilcoxon signed-rank test. Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) with 95% confidence intervals (CI) was calculated for each biomarker. Optimal cut-off values were determined using the Youden index (maximum sensitivity + specificity – 1). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR) were calculated for each biomarker at the optimal cut-off.

For combination analysis, logistic regression models were constructed to evaluate the diagnostic accuracy of biomarker combinations. The predicted probability from the logistic regression model was used to generate ROC curves for combination biomarkers. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

The study was approved by the Institutional Ethics Committee of the hospital (IEC No.: 2024/NEO/089). Written informed consent was obtained from parents or legal guardians after explaining the purpose, procedures, risks, and benefits of the study in a language they understood. Confidentiality of the data was maintained throughout the study, and participants were

assured of their right to withdraw at any time without affecting their medical care. All neonates with confirmed or suspected sepsis received appropriate management as per the standard hospital protocol.

RESULTS

Baseline Characteristics of the Study Population

A total of 210 neonates with clinical suspicion of sepsis were enrolled in the study. The mean gestational age was 34.2 ± 4.1 weeks (range: 26–41 weeks), and the mean birth weight was 2,184 ± 742 grams (range: 680–3,800 grams). Preterm neonates (<37 weeks) constituted 58.6% (123/210) of the study population, and 45.7% (96/210) had low birth weight (<2,500 grams). The male-to-female ratio was 1.2:1.

Blood culture was positive in 62 neonates (29.5%), of whom 28 (45.2%) had EOS (≤72 hours) and 34 (54.8%) had LOS (>72 hours). The most common organisms isolated were Klebsiella pneumoniae (22.6%), Staphylococcus aureus (19.4%), Escherichia coli (17.7%), Enterobacter species (11.3%), and Coagulase-negative staphylococci (9.7%). A total of 148 neonates (70.5%) had negative blood cultures, of whom 92 (62.2%) had clinical sepsis with alternative evidence of infection (elevated inflammatory markers, clinical response to antibiotics) and 56 (37.8%) had alternative non-infectious diagnoses.

Table 1 compares the baseline characteristics between the sepsis group and the non-sepsis group.

Table 1: Baseline characteristics of the study population

Characteristic	Sepsis Group (n=62)	Non-Sepsis Group (n=148)	p-value
Gestational age (weeks), mean ± SD	32.8 ± 4.5	34.8 ± 3.8	0.002*
Birth weight (grams), mean ± SD	1,945 ± 698	2,284 ± 742	0.003*
Preterm (<37 weeks), n (%)	45 (72.6%)	78 (52.7%)	0.008†
Low birth weight (<2,500 g), n (%)	38 (61.3%)	58 (39.2%)	0.004†
Male sex, n (%)	34 (54.8%)	78 (52.7%)	0.784†
EOS (≤72 hours), n (%)	28 (45.2%)	-	-
LOS (>72 hours), n (%)	34 (54.8%)	-	-
Maternal age (years), mean ± SD	26.4 ± 5.2	27.1 ± 4.8	0.352*
PROM (>18 hours), n (%)	22 (35.5%)	28 (18.9%)	0.010†
Maternal fever, n (%)	15 (24.2%)	18 (12.2%)	0.030†
Mechanical ventilation, n (%)	28 (45.2%)	31 (20.9%)	<0.001†
Central venous catheter, n (%)	18 (29.0%)	22 (14.9%)	0.017†
Parenteral nutrition, n (%)	24 (38.7%)	26 (17.6%)	0.001†

*Independent t-test; †Chi-square test; SD: standard deviation; EOS: early-onset sepsis; LOS: late-onset sepsis; PROM: premature rupture of membranes

Neonates in the sepsis group had significantly lower gestational ages (p=0.002), lower birth weights (p=0.003), higher rates of prematurity (p=0.008), and higher rates of low birth weight (p=0.004) compared to the non-sepsis group. Sepsis was also associated with higher rates of prolonged rupture of membranes (p=0.010), maternal fever (p=0.030), mechanical ventilation (p<0.001), central venous catheterization (p=0.017), and parenteral nutrition (p=0.001).

Biomarker Levels at Presentation and 24 Hours

Table 2 presents the biomarker levels at presentation (T0) and at 24 hours (T24) in the sepsis and non-sepsis groups.

Table 2: Biomarker levels at presentation and 24 hours

Biomarker	Time Point	Sepsis Group (n=62) Median (IQR)	Non-Sepsis Group (n=148) Median (IQR)	p-value†
PCT (ng/mL)	T0	4.82 (1.24–12.65)	0.28 (0.12–0.62)	<0.001
	T24	3.96 (0.98–10.42)	0.32 (0.14–0.58)	<0.001
CRP (mg/L)	T0	28.4 (12.6–56.8)	6.2 (2.8–14.5)	<0.001
	T24	42.6 (18.4–78.2)	8.4 (3.2–16.8)	<0.001
IL-6 (pg/mL)	T0	68.5 (24.2–156.4)	6.8 (2.4–14.2)	<0.001
	T24	24.6 (8.4–68.2)	5.2 (2.0–12.6)	<0.001
Presepsin (pg/mL)	T0	1,245 (684–2,846)	264 (148–412)	<0.001
	T24	986 (456–2,124)	286 (156–428)	<0.001

†Mann-Whitney U test; IQR: interquartile range; PCT: procalcitonin; CRP: C-reactive protein; IL-6: interleukin-6; T0: presentation; T24: 24 hours

All biomarkers were significantly elevated in the sepsis group compared to the non-sepsis group at both time points (p<0.001 for all). Among the biomarkers, presepsin showed the most marked elevation, with a median level of 1,245 pg/mL in the sepsis group compared to 264 pg/mL in the non-sepsis group at presentation.

Within the sepsis group, PCT showed a decreasing trend from T0 to T24 (median: 4.82 to 3.96 ng/mL, p=0.042), while CRP showed an increasing trend (28.4 to 42.6 mg/L, p<0.001). IL-6 showed a significant decline (68.5 to 24.6 pg/mL, p<0.001), consistent with its short half-life. Presepsin also showed a decline (1,245 to 986 pg/mL, p=0.038).

Diagnostic Accuracy of Individual Biomarkers

Table 3 presents the diagnostic accuracy of individual biomarkers at presentation (T0) for the diagnosis of neonatal sepsis.

Table 3: Diagnostic accuracy of individual biomarkers at presentation

Biomarker	AUC (95% CI)	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR
Presepsin	0.94 (0.90–0.97)	600 pg/mL	88.7	91.2	80.9	94.9	10.08	0.12
PCT	0.89 (0.84–0.93)	1.2 ng/mL	83.9	87.8	74.3	92.3	6.89	0.18
IL-6	0.86 (0.80–0.91)	32 pg/mL	80.6	84.5	68.5	90.2	5.20	0.23
CRP	0.81 (0.75–0.87)	18 mg/L	75.8	78.4	62.7	87.4	3.51	0.31

AUC: area under the curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; PLR: positive likelihood ratio; NLR: negative likelihood ratio; PCT: procalcitonin; CRP: C-reactive protein; IL-6: interleukin-6

Presepsin demonstrated the highest diagnostic accuracy with an AUC of 0.94 (95% CI: 0.90–0.97), significantly higher than PCT (AUC: 0.89, 95% CI: 0.84–0.93; p=0.041), IL-6 (AUC: 0.86, 95% CI: 0.80–0.91; p=0.008), and CRP (AUC: 0.81, 95% CI: 0.75–0.87; p<0.001). At an optimal cut-off of 600 pg/mL, presepsin had a sensitivity of 88.7% and specificity of 91.2%, with a positive likelihood ratio of 10.08 and negative likelihood ratio of 0.12.

PCT at a cut-off of 1.2 ng/mL showed a sensitivity of 83.9% and specificity of 87.8%. IL-6 at 32 pg/mL had a sensitivity of 80.6% and specificity of 84.5%. CRP at 18 mg/L had the lowest diagnostic accuracy with a sensitivity of 75.8% and specificity of 78.4%.

Diagnostic Accuracy of Biomarker Combinations

Table 4 presents the diagnostic accuracy of biomarker combinations at presentation.

Table 4: Diagnostic accuracy of biomarker combinations

Biomarker Combination	AUC (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Presepsin + PCT	0.96 (0.93–0.98)	91.9	93.2	85.1	96.4
Presepsin + IL-6	0.95 (0.91–0.97)	90.3	92.6	83.6	95.6
Presepsin + CRP	0.94 (0.91–0.97)	88.7	91.9	82.1	94.9
PCT + IL-6	0.91 (0.86–0.95)	85.5	88.5	75.7	93.2
PCT + CRP	0.90 (0.85–0.94)	83.9	88.1	74.3	92.6
All four biomarkers	0.96 (0.93–0.98)	93.5	94.6	87.9	97.1

AUC: area under the curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; PCT: procalcitonin; CRP: C-reactive protein; IL-6: interleukin-6

The combination of presepsin and PCT showed excellent diagnostic accuracy with an AUC of 0.96 (95% CI: 0.93–0.98), sensitivity of 91.9%, and specificity of 93.2%. The combination of all four biomarkers yielded the highest sensitivity (93.5%) and specificity (94.6%) with an AUC of 0.96 (95% CI: 0.93–0.98). However, the difference between the presepsin+PCT combination and the four-biomarker combination was not statistically significant (p=0.342).

Diagnostic Accuracy by Sepsis Type (EOS vs. LOS)

Table 5 presents the diagnostic accuracy of biomarkers for EOS and LOS separately.

Table 5: Diagnostic accuracy of biomarkers for EOS and LOS

Biomarker	EOS (n=28)	LOS (n=34)
	AUC (95% CI)	AUC (95% CI)
Presepsin	0.95 (0.90–0.98)	0.93 (0.88–0.97)
PCT	0.91 (0.86–0.95)	0.87 (0.82–0.92)
IL-6	0.88 (0.82–0.93)	0.84 (0.78–0.89)
CRP	0.83 (0.76–0.88)	0.79 (0.72–0.85)

EOS: early-onset sepsis; LOS: late-onset sepsis; AUC: area under the curve; CI: confidence interval; PCT: procalcitonin; CRP: C-reactive protein; IL-6: interleukin-6

Presepsin demonstrated excellent diagnostic accuracy for both EOS (AUC: 0.95) and LOS (AUC: 0.93), with no significant difference between the two groups (p=0.382). PCT also showed good accuracy for both EOS (AUC: 0.91) and LOS (AUC: 0.87). IL-6 showed higher accuracy for EOS (AUC: 0.88) compared to LOS (AUC: 0.84), consistent with its role as an early inflammatory mediator.

DISCUSSION

This prospective observational study evaluated the diagnostic accuracy of four biomarkers—presepsin, PCT, CRP, and IL-6—for the early diagnosis of neonatal sepsis in a tertiary care hospital setting. Our findings demonstrate that presepsin is a highly promising biomarker with superior diagnostic accuracy compared to conventional markers, and that the combination of presepsin with PCT further enhances diagnostic performance.

The prevalence of culture-proven sepsis in our study population was 29.5%, which is consistent with previous reports from Indian tertiary care settings. A study from southern Odisha reported a prevalence of 12.3%, while the prevalence varies considerably based on the population studied and the diagnostic criteria employed. The predominance of Gram-negative organisms (Klebsiella pneumoniae, Escherichia coli, Enterobacter species) in our study is consistent with the evolving microbiological landscape in Indian NICUs, where Gram-negative pathogens have become increasingly prevalent.

Our finding that presepsin has the highest diagnostic accuracy (AUC: 0.94) among the evaluated biomarkers is consistent with recent meta-analyses. A comprehensive meta-analysis by Peng et al. reported that presepsin has excellent overall diagnostic performance with an AUC of 0.91 across all populations, with particularly high accuracy in neonates (sensitivity 0.90, specificity 0.92, AUC 0.96). The slightly lower AUC in our study (0.94) may reflect the specific characteristics of our study population or the use of different assay platforms. Nevertheless, the diagnostic accuracy of presepsin in our study was superior to that of PCT (AUC: 0.89), IL-6 (AUC: 0.86), and CRP (AUC: 0.81).

The mechanistic basis for presepsin's superior performance lies in its biology. Presepsin is a soluble fragment of CD14, a receptor expressed on the surface of monocytes and macrophages that plays a critical role in the recognition of bacterial lipopolysaccharide. During bacterial infection, the binding of pathogens to CD14 triggers the release of presepsin into the circulation, with levels rising rapidly and proportionally to the severity of infection. This early and specific response to bacterial pathogens explains why presepsin outperforms downstream markers like CRP, which rises later, and IL-6, which has a short half-life and rapid decline.

Our finding that PCT has good diagnostic accuracy (AUC: 0.89) is consistent with the extensive literature on PCT in neonatal sepsis. A systematic review and meta-analysis from LMICs reported that PCT at a cut-off of ≥0.5 ng/mL had an AUC of 0.87 (95% CI: 0.70–0.92). The slightly higher AUC in our study may reflect the use of a higher cut-off (1.2 ng/mL) optimized for our population. PCT has several advantages over CRP: it rises earlier (within 2–4 hours of infection), shows a more rapid decline with clinical improvement, and has better specificity for bacterial infection. However, PCT has limitations in neonates, as it shows physiological elevation in the first 48–72 hours of life, complicating interpretation in EOS.

The diagnostic accuracy of IL-6 in our study (AUC: 0.86) is consistent with previous reports. A meta-analysis reported that IL-6 had a pooled sensitivity of 83% and specificity of 87% in umbilical cord blood for EOS diagnosis. The higher accuracy of IL-6 for EOS (AUC: 0.88) compared to LOS (AUC: 0.84) in our study reflects its role as an early inflammatory mediator that rises rapidly in response to infection but declines quickly due to its short half-life. The time-dependent nature of IL-6 measurement is a practical limitation, as levels may have already declined by the time clinical suspicion arises.

CRP showed the lowest diagnostic accuracy among the evaluated biomarkers (AUC: 0.81), consistent with its limitations in neonatal sepsis. CRP rises later (peaking at 24–48 hours) and is less specific for bacterial infection. In our study, CRP levels were significantly higher at 24 hours than at presentation in the sepsis group, reflecting the delayed rise of this acute-

phase reactant. While CRP remains a useful marker for monitoring response to therapy, its utility for early diagnosis is limited.

The finding that biomarker combinations improve diagnostic accuracy is consistent with the emerging consensus in the field. The combination of presepsin and PCT achieved an AUC of 0.96, with a sensitivity of 91.9% and specificity of 93.2%, significantly higher than any individual biomarker. This is not surprising given that presepsin and PCT reflect different aspects of the host response to infection—presepsin is an early marker of monocyte activation, while PCT reflects the systemic inflammatory response. The combination of all four biomarkers yielded the highest sensitivity (93.5%) and specificity (94.6%), though this did not represent a statistically significant improvement over the presepsin+PCT combination.

The implications of these findings for clinical practice are substantial. The high negative predictive value of presepsin (94.9% at 600 pg/mL) means that a negative presepsin result can reliably rule out sepsis, potentially enabling clinicians to avoid unnecessary antibiotic exposure in low-risk neonates. The positive likelihood ratio of 10.08 means that a positive presepsin result substantially increases the probability of sepsis, supporting the initiation of appropriate antimicrobial therapy. The combination of presepsin and PCT, with its excellent diagnostic accuracy (sensitivity 91.9%, specificity 93.2%), could serve as a powerful rule-in/rule-out tool in clinical algorithms.

The WHO has called for rapid, low-volume, point-of-care tests that can be used in both hospital and primary care environments for neonatal sepsis diagnosis. The availability of point-of-care testing for biomarkers like CRP, PCT, and IL-6 has been demonstrated to be feasible, with a mean time of 12 ± 3 minutes for POC estimation compared to 366 ± 61 minutes for standard techniques. While point-of-care testing for presepsin is not yet widely available, the development of such assays would represent a significant advance, particularly in resource-limited settings where laboratory infrastructure is limited.

CONCLUSION

This study demonstrates that presepsin is a highly promising biomarker for the early diagnosis of neonatal sepsis, with superior diagnostic accuracy compared to conventional markers including PCT, CRP, and IL-6. At an optimal cut-off of 600 pg/mL, presepsin showed a sensitivity of 88.7% and specificity of 91.2%, with an AUC of 0.94. The combination of presepsin with PCT further improved diagnostic accuracy, achieving an AUC of 0.96 with a sensitivity of 91.9% and specificity of 93.2%.

These findings support the incorporation of presepsin into neonatal sepsis diagnostic algorithms to enable timely diagnosis, reduce empirical antibiotic use, and improve outcomes in this vulnerable population. Given the high burden of neonatal sepsis in India and other LMICs, the development of affordable, point-of-care assays for presepsin should be a priority. Future research should focus on: (1) validating these findings in larger, multi-center studies across diverse populations; (2) evaluating the utility of biomarker combinations for guiding antibiotic duration; (3) assessing the cost-effectiveness of biomarker-based diagnostic algorithms; and (4) exploring the role of emerging biomarkers including serum amyloid A, resistin, and microRNAs in neonatal sepsis diagnosis.

Ultimately, the goal of biomarker research in neonatal sepsis is not to replace clinical judgment but to provide clinicians with rapid, reliable, and actionable information to support decision-making. With continued research and technological advances, biomarker-based diagnostics have the potential to transform the management of neonatal sepsis, reducing the twin burdens of antibiotic overuse and delayed treatment in this most vulnerable population.

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