International Journal of Medicine
pISSN 1468-3814 | eISSN 2667-7008
https://theinternationalmedicine.com/

Original Researcher Article *DOI: 10.61336/im/26-07-57*

Diagnosis of Early Onset Neonatal Sepsis by Biomarkers Interleukin-6, Procalcitonin,C-Reactive Protein and Automated Blood Culture with VITEK 2 Compact System -A Cross Sectional Study.

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Received: 19-05-2026
Revised: 11-06-2026
Accepted: 23-06-2026
Published: 08-07-2026

Abstract

Background: Early-onset neonatal sepsis (EONS) carries high neonatal morbidity and mortality, yet blood culture the diagnostic gold standard requires 24–72 hours. Rapid biomarkers are needed to guide early empirical therapy. ***Methods:*** Cross-sectional study at BMCRI, Bengaluru (November 2018–May 2020); 100 neonates (<28 days) with clinically suspected EONS. CRP by latex agglutination; IL-6 and PCT by sandwich ELISA; blood culture on the VITEK 2 Compact system with identification and susceptibility testing. ***Results:*** Blood culture was positive in 46%. IL-6 (>22 pg/mL): sensitivity 100%, NPV 100%, specificity 85%, accuracy 92%. PCT (>389 pg/mL): specificity 100%, PPV 100%, sensitivity 80%, accuracy 91%. CRP (>6 mg/dL): sensitivity 59%, specificity 76%, accuracy 68%. Gram-negative bacilli predominated (63.0%, chiefly Klebsiella pneumoniae), followed by non-fermenters (23.9%), Gram-positive cocci (10.9%), and Candida guilliermondii (2.2%). Carbapenemase production was detected in 83% of K. pneumoniae and 90% of Acinetobacter baumannii. Premature rupture of membranes (64.3%), very low birth weight, and caesarean delivery were the leading risk correlates. ***Conclusion:*** IL-6 is the most sensitive early rule-out marker for EONS; PCT offers superior specificity for confirmation. A combined biomarker and automated blood-culture strategy supports timely antimicrobial decisions amid a high local burden of carbapenemase-producing Gram-negative pathogens.

Keywords: Neonatal sepsis; Interleukin-6; Procalcitonin; C-reactive protein; Blood culture; VITEK 2; Biomarkers; Carbapenemase.

INTRODUCTION

Neonatal sepsis is a systemic infectious syndrome occurring within the first 28 days of life and remains one of the principal causes of neonatal morbidity and mortality globally.[1] Early-onset neonatal sepsis (EONS) is conventionally defined as bacteraemia or bacterial meningitis occurring within 72 hours of life in neonates admitted to the neonatal intensive care unit (NICU), or within 7 days of life in term infants; in preterm infants it is most consistently defined as infection acquired vertically from the mother before or during delivery and manifesting within the first 3 days of life.[2] Late-onset sepsis (LONS), by contrast, develops after this window and may be acquired vertically or horizontally.[2]

Globally, neonatal sepsis accounts for an estimated 1.6 million deaths annually, with nearly 40% of this burden occurring in developing countries.[3] In India, the reported incidence ranges from 4.8 to 20.7 per 1000 live births, with case fatality between 40% and 65%, and early-onset disease contributing approximately two-thirds of all reported cases.[3] The diagnostic difficulty stems from the overlap of sepsis with non-infectious neonatal pathology and from the limited sensitivity and specificity of clinical signs alone, necessitating a combination of clinical suspicion, laboratory biomarkers, and microbiological confirmation.[4]

Blood culture remains the diagnostic reference standard, but conventional and even automated systems typically require 24–72 hours for a definitive result, a delay that is clinically unacceptable in a population where rapid deterioration is common.[5] This has driven sustained interest in serum biomarkers that rise earlier in the inflammatory cascade. C-reactive protein (CRP), an acute-phase reactant, typically rises later (>24 hours) and lacks specificity for infection.[6] Interleukin-6 (IL-6), a pro-inflammatory cytokine, rises within 2–4 hours of pathogen detection and peaks at 6–8 hours, conferring an early diagnostic window, although its short half-life can limit sensitivity if sampling is delayed.[7,8] Procalcitonin (PCT) rises

within 6 hours, peaks at 18–24 hours, and remains elevated for up to 48 hours, but its interpretation in neonates is confounded by a physiological postnatal rise and by non-infectious perinatal stressors such as respiratory distress and hypoxic-ischaemic encephalopathy.[9,10]

Despite considerable research, no single biomarker has demonstrated the combination of sensitivity, specificity, and rapid turnaround required for confident stand-alone diagnosis of EONS, and existing comparative data particularly from South Asian tertiary-care settings with a high local burden of multidrug-resistant Gram-negative pathogens remain limited. We therefore designed this study with two objectives: (i) to evaluate the diagnostic value of IL-6, PCT, and CRP in neonates with clinically suspected early-onset sepsis; and (ii) to confirm sepsis and characterise the causative organisms and their antimicrobial resistance profiles using automated blood culture and the VITEK 2 Compact system.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology, Bangalore Medical College and Research Institute (BMCRI), Bengaluru, Karnataka, India, in collaboration with the in-born and out-born neonatal intensive care units of Vani Vilas Hospital and the Pradhan Mantri Swasthya Suraksha Yojana (PMSSY) super-specialty hospital attached to BMCRI, between November 2018 and May 2020. Ethical approval was granted by the Institutional Ethics Committee of BMCRI, and written informed consent was obtained from a parent or legal guardian prior to sample collection.

One hundred consecutive, non-replicate neonates aged less than 28 days with clinical suspicion of EONS were enrolled. Sample size was estimated using the formula $n = 4pq/d^2$, based on a reported regional sepsis prevalence (p) of 65% and an allowable error (d) of 10%, yielding a minimum of 91 cases, rounded to 100. Neonates with clinical signs of sepsis and whose guardians provided consent were eligible; exclusion criteria were refusal of consent, congenital or chromosomal anomalies, inborn errors of metabolism, confirmed intrauterine TORCH infection (toxoplasmosis, rubella, cytomegalovirus, syphilis, herpes), and receipt of parenteral antibiotics at the time of evaluation.

Following enrolment, 1–2 mL of venous blood was collected aseptically for serological biomarker estimation, and a separate 1–5 mL sample was collected for automated blood culture. Serum for serology was separated by centrifugation at 1000–2000 × g for 10 minutes. CRP was measured qualitatively and semi-quantitatively by latex agglutination (CRP Latex Test Kit); a result of >6 mg/dL was considered positive per kit specifications. IL-6 was quantified by sandwich ELISA (BOSTER Biological Technology PicoKine™ Human IL-6 ELISA Kit; assay range 4.69–300 pg/mL; analytical sensitivity <0.3 pg/mL), read at 450 nm, with a positivity cut-off of >22 pg/mL (optical density 0.3836) derived from prior validated work.[11] PCT was similarly measured by sandwich ELISA (Elabscience Human PCT ELISA Kit, catalogue E-EL-H1492; assay range 31.25–2000 pg/mL; sensitivity 18.75 pg/mL), read at 450 nm, with a cut-off of >389 pg/mL (optical density 0.676).[11]

Blood samples for culture were inoculated into automated blood culture bottles and incubated in a continuous-monitoring system. Flagged-positive bottles were subcultured on standard media; isolates were identified to species level and tested for antimicrobial susceptibility using the VITEK 2 Compact automated platform (bioMérieux). Extended-spectrum β-lactamase (ESBL) and carbapenemase production were phenotypically characterised according to standard Clinical and Laboratory Standards Institute (CLSI) criteria.

Data were analysed using descriptive statistics. Diagnostic performance indices sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy were calculated for each biomarker against blood culture as the reference standard. Receiver operating characteristic (ROC) curve analysis was used to derive and validate optimal cut-off values.

RESULTS

Culture positivity and demographic profile

Of 100 neonates with clinically suspected EONS, blood culture was positive in 46% (46/100) and negative in 54%. Among culture-positive cases, 49.0% were female and 41.2% male; 58.8% were term and 46.4% pre-term; and 57.8% were delivered by lower-segment caesarean section (LSCS) compared with 36.4% by normal vaginal delivery.

By birth weight category, 60.0% of very-low-birth-weight, 56.8% of low-birth-weight, and 33.3% of adequate-birth-weight neonates were culture-positive. Symptom onset occurred within 24 hours in 61.1% of cases, within 48 hours in 39.5%, and within 72 hours in 33.3% (Table 1).

Table 1. Demographic and clinical risk-factor profile of culture-positive early-onset neonatal sepsis (n = 46)

Variable	Category	Proportion (%)
Sex	Female	49.0
	Male	41.2
Gestation	Term	58.8
	Pre-term	46.4
Mode of delivery	LSCS	57.8
	Normal vaginal delivery	36.4
Birth weight	Very low birth weight	60.0
	Low birth weight	56.8
	Adequate birth weight	33.3
Symptom onset	Within 24 h	61.1
	Within 48 h	39.5
	Within 72 h	33.3
Risk factor	Premature rupture of membranes	64.3
	Fever within 24 h	46.0
	Respiratory distress	41.3
	Endotracheal intubation	15.0
	Peripheral line in situ	54.0

Diagnostic performance of CRP, IL-6, and PCT

CRP was positive in 40.0% of all suspected cases and in 58.7% of culture-positive cases, yielding a sensitivity of 59%, specificity of 76%, PPV of 68%, NPV of 68%, and overall accuracy of 68% at the >6 mg/dL cut-off. IL-6 (>22 pg/mL) was positive in 54.0% of suspected cases overall, with a sensitivity of 100%, specificity of 85%, PPV of 85%, NPV of 100%, and accuracy of 92% making it the single most sensitive marker, with no false negatives recorded. PCT (>389 pg/mL) was positive in 80.4% of culture-positive cases, with a sensitivity of 80%, specificity of 100%, PPV of 100%, NPV of 86%, and accuracy of 91%, with no false positives (Table 2).

Table 2. Comparative diagnostic performance of CRP, IL-6, and PCT against blood culture

Parameter	CRP (>6 mg/dL)	IL-6 (>22 pg/mL)	PCT (>389 pg/mL)
Sensitivity (%)	59	100	80
Specificity (%)	76	85	100
PPV (%)	68	85	100
NPV (%)	68	100	86
Accuracy (%)	68	92	91
False positive (%)	24	15	0
False negative (%)	41	0	20

Notably, in 19.6% of culture-positive cases, IL-6 alone was positive while CRP and PCT remained negative, underscoring its superior early sensitivity. Conversely, CRP was positive in 32.5% of culture-negative suspected cases, consistent with its known lack of specificity for true bacterial infection in the absence of corroborating IL-6, PCT, or culture positivity.

Microbiological profile and antimicrobial resistance

Among the 46 culture-positive isolates, Gram-negative bacilli predominated (63.0%, n = 29), followed by Gram-negative non-fermenters (23.9%, n = 11), Gram-positive cocci (10.9%, n = 5), and Candida guilliermondii (2.2%, n = 1) (Table 3).

Table 3. Distribution of culture isolates (n = 46)

Organism	Isolates, n (%)
Klebsiella pneumoniae	24 (52.2)
Acinetobacter baumannii	10 (21.7)
Escherichia coli	4 (8.7)
Staphylococcus aureus	3 (6.5)
Staphylococcus epidermidis (MRSE)	1 (2.2)
Enterococcus gallinarum	1 (2.2)
Candida guilliermondii	1 (2.2)
Enterobacter cloacae	1 (2.2)
Sphingomonas paucimobilis	1 (2.2)

Antimicrobial resistance phenotyping revealed a high prevalence of carbapenem resistance among Gram-negative isolates: 83.3% of K. pneumoniae and 90.0% of A. baumannii isolates were carbapenemase producers, while 16.7% of K. pneumoniae and 75.0% of E. coli isolates were ESBL producers (Table 4). All carbapenemase-producing isolates remained susceptible to tigecycline and colistin. Among Gram-positive isolates, two of three S. aureus isolates were methicillin-resistant, and the single S. epidermidis isolate was a methicillin-resistant CoNS; all Gram-positive isolates remained susceptible to vancomycin.

Table 4. Resistance phenotypes among predominant Gram-negative isolates

Organism	Carbapenemase producers (%)	ESBL producers (%)
Klebsiella pneumoniae	83.3	16.7
Escherichia coli	25.0	75.0
Acinetobacter baumannii	90.0	—

DISCUSSION

In this cross-sectional study of 100 neonates with clinically suspected EONS, IL-6 demonstrated the highest sensitivity (100%) and NPV (100%) of the three biomarkers evaluated, supporting its principal clinical role as a rule-out test in the early diagnostic window, before blood culture results become available. PCT, in contrast, showed the highest specificity (100%) and PPV (100%), suggesting greater utility in confirming true infection once positive, although a prospective multicentre study by Chaurasia et al. cautioned that serum PCT does not reliably identify culture-positive sepsis as a stand-alone marker, reinforcing the need for a combined approach.[12] CRP performed least favourably across all metrics, consistent with its recognised role as a late, non-specific acute-phase reactant rather than an early discriminator of sepsis.[13]

These findings are broadly consistent with recent comparative data. Goyal et al. evaluated point-of-care testing of IL-6, PCT, and CRP in 82 neonates with suspected sepsis and reported that CRP combined with PCT demonstrated the best overall diagnostic accuracy, while the role of IL-6 required further cut-off refinement in that setting.[11] Cortés et al. and Omran et al. confirmed IL-6 as a reliable early EONS biomarker across diverse populations, with Omran et al. reporting 100% sensitivity at lower cut-offs in preterm neonates.[14,15] A network meta-analysis by Xing et al. similarly identified IL-6 and IL-8 as the most informative interleukins for sepsis detection, outperforming CRP at early time points.[16] Chen et al. extended this comparison to include serum amyloid A, demonstrating that IL-6 combined with PCT provided the best diagnostic accuracy for term neonates with sepsis, while PCT combined with SAA performed better for preterm infants underscoring the importance of gestational-age-stratified biomarker interpretation.[17] Serial IL-6 measurement further strengthens its utility: Berka et al. showed that repeated IL-6 sampling enhanced the probability of excluding EONS in very preterm infants, supporting a dynamic rather than single-point diagnostic strategy.[18] A recent updated meta-analysis by Diallo et al., synthesising data across multiple neonatal populations, confirmed that IL-6 at cut-offs of 200 pg/mL and above achieved 100% sensitivity with substantially improved specificity for Gram-negative sepsis, consistent with the high sensitivity we observed at our cohort-derived cut-off.[19] Collectively, this body of evidence reinforces the complementary roles of IL-6 as an early rule-out and PCT as a rule-in biomarker in the diagnostic workup of EONS.

The culture positivity rate in our cohort (46%) is consistent with the wide range reported in the literature (30–60%) from Indian tertiary-care NICUs.[20,21] The predominance of Gram-negative organisms, particularly K. pneumoniae and A. baumannii, aligns with contemporary Indian series and reflects the progressive shift in neonatal sepsis epidemiology towards Gram-negative pathogens in resource-limited settings.[21,22] Of particular concern is the high proportion of carbapenemase-producing isolates observed in our cohort (83% of K. pneumoniae, 90% of A. baumannii), a finding corroborated by several recent Indian studies. Banerjee et al. reported extensively drug-resistant hypervirulent K. pneumoniae causing cluster outbreaks in a tertiary-care NICU, and Vithiya described a CRKP outbreak with considerable mortality attributable to environmental contamination collectively highlighting the NICU as a high-risk ecosystem for clonal dissemination of carbapenem-resistant strains.[23,24] Chakrabarti et al. further documented that MDR Gram-negative isolates predominantly K. pneumoniae and Acinetobacter spp. accounted for nearly half of all culture-positive neonatal sepsis episodes in an Eastern Indian tertiary centre over a four-year period, with the highest MDR rates in very-low-birth-weight neonates, a risk-group that was similarly overrepresented in our culture-positive cases.[25] These findings collectively signal an escalating burden of carbapenem resistance that substantially complicates empirical antibiotic selection and underscores the need for early, sensitive biomarker-guided clinical suspicion to permit timely de-escalation or escalation to carbapenem-sparing alternatives such as tigecycline and colistin, pending definitive culture results.

Risk-factor analysis identified premature rupture of membranes (64.3%), very low birth weight, and caesarean delivery as the principal correlates of culture-positive sepsis, consistent with a recent case-control study from northeastern India by Kumar et al., which identified PROM (aOR 2.9) and maternal urinary tract infection as the strongest independent predictors of EONS.[26] Kilpatrick et al., in a multicentre surveillance study, similarly demonstrated that CRP's diagnostic utility in

EONS is substantially influenced by non-infectious perinatal factors including mode of delivery and maternal fever, which may partly account for the elevated false-positive rate we observed with CRP in culture-negative neonates.[27] The female predominance in our culture-positive cases (49.0% vs 41.2% male) contrasts with the widely cited male predominance reported in earlier Indian literature, and may reflect local population characteristics, referral bias, or the limited sample size; the global burden literature indicates no significant sex differential in neonatal sepsis incidence.[28]

Strengths of this study include the use of a fully automated, standardised blood culture and identification platform (VITEK 2 Compact), quantitative ELISA-based biomarker estimation with ROC-derived cut-offs, and comprehensive antimicrobial resistance phenotyping. Limitations include the single-centre cross-sectional design, which precludes assessment of biomarker kinetics over time or correlation with clinical outcomes; the modest sample size, which limits precision of subgroup estimates; and the absence of a healthy control group. Multicentric, prospective cohort studies with serial biomarker sampling and long-term outcome data are warranted to validate these cut-offs and assess the cost-effectiveness of combined biomarker testing in resource-limited NICU settings.

CONCLUSION

In this cohort of clinically suspected early-onset neonatal sepsis, IL-6 emerged as the most sensitive biomarker for early identification, with excellent negative predictive value suitable for ruling out sepsis ahead of culture results, while PCT offered the highest specificity for confirming true infection. CRP alone demonstrated limited diagnostic utility. Given the long turnaround time of blood culture and the high local prevalence of carbapenemase-producing Gram-negative pathogens identified by VITEK 2 Compact, a combined strategy incorporating early IL-6 and PCT testing alongside clinical assessment and confirmatory automated blood culture is recommended to support timely, judicious antimicrobial decision-making in neonates with suspected sepsis.

DECLARATIONS

Ethics approval: Approved by the Institutional Ethics Committee, Bangalore Medical College and Research Institute. Written informed consent was obtained from parents/guardians of all participants.
Funding: None
Conflicts of interest: The authors declare no conflicts of interest.
Data availability: Data are available from the corresponding author upon reasonable request.

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