



# Utility of Routine Laboratory Tests for Predicting Gram-Negative Neonatal Sepsis in Low-Resource Settings

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## Abstract

**Background/Aim:** Gram-negative bacteria are increasingly recognised as major pathogens in early-onset neonatal sepsis (EONS). Accurate early prediction of causative agents in EONS remains challenging, particularly in low resources settings where specific diagnostic markers are lacking. This study aimed to evaluate the diagnostic utility of routinely performed laboratory tests in predicting Gram-negative bacterial aetiology in EONS.

**Methods:** Retrospective study included neonates with culture proven EONS who admitted to tertiary hospital in Iraq over 12 months period. Based on result of blood culture, participants were divided into 2 groups: (Group 1): neonates diagnosed with Gram-negative bacterial sepsis, while (Group 2) involved neonates having positive bacterial growth on blood culture. Clinical characteristics and the results of routine septic screen employed included C-reactive protein (CRP) and haematologic parameters obtained from complete blood counts compared between both groups. A receiver operating characteristic curve (ROC) was plotted to evaluate the predictive value of routine laboratory tests for Gram-negative sepsis.

**Results:** A total of eighty neonates included in the study, Gram-negative bacteria accounted for most bacterial isolates 64 (80.0 %), from which *Acinetobacter baumannii* was the most frequent isolates 31 (38.8 %). CRP titre was significantly higher in neonate with Gram-negative bacteria ( $p = 0.003$ ), while each of absolute neutrophile count and N:L ratio was higher in Gram-positive bacteria ( $p = 0.03$ ,  $p = 0.04$ , respectively). On multivariate analysis, CRP was significantly associated with Gram-negative sepsis and on ROC curve CRP titre at cutoff 19.53 demonstrated excellent prediction of Gram-negative EONS with an area under the curve of 0.933 (95% CI: 0.807–1.000;  $p = 0.009$ ).

**Conclusion:** Most EONS cases were due to Gram-negative bacteria, especially *Acinetobacter baumannii*. High CRP with low absolute neutrophil count (ANC) and N:L ratio pointed to Gram-negative sepsis. CRP  $> 19.5$  mg/dL was a strong early predictor for Gram-negative bacterial growth in blood cultures.

**Key words:** *Acinetobacter baumannii*; C- reactive protein; Neonatal sepsis.

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## Introduction

Early onset neonatal sepsis (EONS) represents a common neonatal morbidity and a life-threatening condition with substantial mortality. Though

definition varies in literature, it is mostly defined as bacteraemia that is diagnosed in term neonates during first 72 h of life, or within 7 days

in neonates admitted to intensive care units.<sup>1-3</sup> Gram-negative bacilli including *E. coli*, *Klebsiella*, *Pseudomonas* and *Enterobacter* species account for a substantial and clinically important proportion of culture-proven EONS, particularly in low resource settings.<sup>4</sup>

Diagnosis of Gram-negative bacteria is of critical importance stemming from their tendency to rapidly progress to septic shock and organ dysfunction.<sup>5</sup> Conventional blood culture, the current gold standard, is limited by delayed results and low sensitivity, especially in neonates with prior maternal antibiotic exposure. Clinical signs of Gram-negative bacterial sepsis are nonspecific and frequently overlap with other neonatal conditions, delaying targeted therapy.<sup>6</sup> Consequently, there is a critical need for reliable biomarkers that can accurately and rapidly identify Gram-negative infections in the early stages.<sup>7</sup> Such markers would not only enable timely initiation of appropriate antimicrobial therapy but also help distinguish Gram-negative from Gram-positive aetiologies, improving antibiotic stewardship and reducing the risk of resistance.<sup>8</sup>

A low resource setting is one in which resources required to properly manage critically ill patients are inadequate.<sup>9</sup> As a result, diagnosing EONS is generally restricted to routine laboratory tests, such as blood culture and complete blood count.<sup>10</sup> Among inflammatory biomarkers, C-reactive protein (CRP) is often the only routinely available test to support diagnosis in these settings.<sup>11, 12</sup> Moreover, emerging evidence indicates that the bacterial spectrum associated with EONS in LMICs differs substantially from that described in World Health Organisation (WHO) reports.<sup>13</sup> These disparities highlight the urgent need for context-specific diagnostic criteria that rely on simple and readily accessible laboratory markers to guide clinical decision-making in resource-constrained environments. This study aimed at uncovering the bacterial profile underlying the EONS and identifying possible haematological or laboratory predictors of Gram-negative bacteria in EONS in low resource settings.

## Methods

A retrospective study was conducted in the neonatal Intensive Care Unit (NICU) of Central Child Teaching Hospital, Baghdad, Iraq. The research

included neonates with culture-positive early-onset neonatal sepsis, diagnosed between 1 January 2024 and 31 December 2024, who were admitted to the NICU within 72 hours of birth, regardless of clinical presentation. Inclusion criteria comprised (1) neonates admitted within 72 hours of life and (2) laboratory confirmation of early-onset neonatal sepsis via positive blood culture. Exclusion criteria were neonates with negative blood cultures, those presenting with critical congenital malformations or birth defects and cases with incomplete data.

### Patient's recruitment

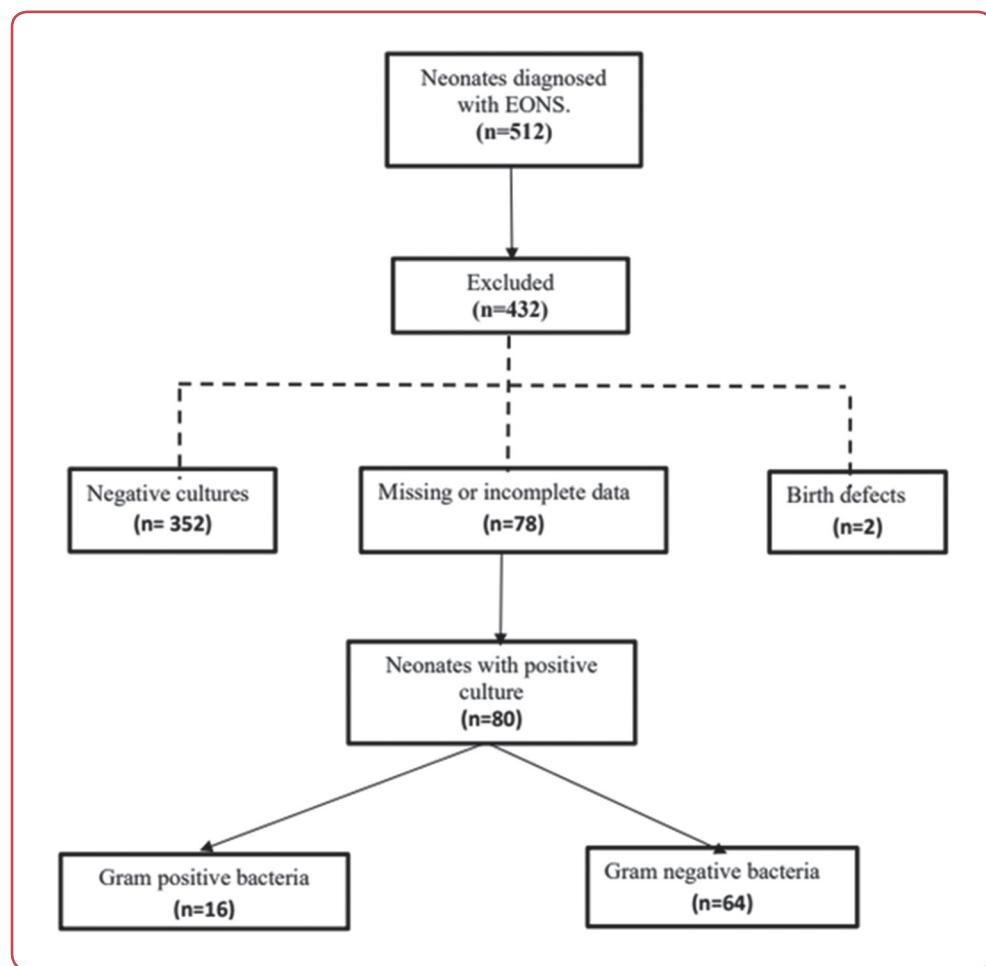
A total of 512 neonates treated with EONS during the study period were initially screened for eligibility. Of these, 432 were excluded due to negative culture results ( $n = 352$ ), incomplete data ( $n = 78$ ), or congenital anomalies ( $n = 2$ ), leaving a final sample of 80 neonates for analysis. The included neonates were then categorised based on blood culture results into Gram-positive ( $n = 16$ ) and Gram-negative ( $n = 64$ ) groups for subsequent analysis (Figure 1). The diagnosis of EONS was made according to the American academy of paediatrics guidelines, in which at least two signs and symptom and two laboratory tests indicative of sepsis were present in addition to positive blood culture.<sup>14, 15</sup>

### Data collection

For all patients included in the study, three sets of data were collected. First, clinical characteristics were taken from hospital data including sex, gestational age and body weight at birth, mode of delivery, prenatal steroid and outcome. Second, the results of laboratory tests include white blood cell counts (WBC), absolute neutrophil count (ANC) and absolute lymphocyte count (ALC), neutrophil to lymphocyte ratio, platelet count (PLT), platelet distribution width (PDW), mean platelet volume (MPV). In addition, the titre of CRP and results of bacterial growth on blood culture.

### Statistics

Data was analysed by SPSS-26. For continuous variables, normal distribution was assured by the Shapiro-Wilk test and presented as mean  $\pm$  standard deviation, while categorical data presented as frequency and percentage. Binary logistic regression analysis of laboratory parameters was done to elucidate any association with Gram-negative bacterial sepsis cases; an odds ratio with 95 % confidence interval (CI) was cal-



*Figure 1: Flowchart of patients' recruitment in the study*

culated. Receiver operating characteristic (ROC) curve analysis was performed to assess diagnostic performance and the area under the curve (AUROC) was used for comparison. A p-value of  $\leq 0.05$  was regarded as the threshold for statistical significance in all analyses.

## Results

A total of eighty neonates with culture proven neonatal sepsis were included in this study. Gram-negative bacteria accounted for most bacterial isolates 64 (80 %), from which *Acinetobacter baumannii* was the most frequent isolates 31 (38.8 %) followed by *Klebsiella pneumoniae* 20 (25 %). Gram-positive bacteria represent 16 (20 %) of isolates, consisting equally of *Staphylococcus haemolyticus* 8 (10 %) and *Staphylococcus epidermidis* 8 (10 %) (Figure 2).

Most of the neonates were preterm 48 (60 %)

and there was a male predominance 56 (70 %). The average birth weight of the studied cohort was  $2440 \pm 0.80$  g, 56 (70 %) patients were delivered by caesarean section. Regarding clinical outcome, sixty-eight neonates (85 %) were discharged alive, while 12 (15 %) died, yielding a case fatality rate of 15 % in this cohort, (Table 1).

Regarding clinical characteristics, none of the clinical characteristics studied showed statistically significant association with the type of the bacteria in neonates with EONS (Table 2).

CRP titre was significantly higher in neonate with Gram-negative bacteria, while each of absolute neutrophile count and N:L ratio was higher in Gram-positive bacteria. All other haematological parameters studied including ALC, WBC, PLT, PDW and MPV did not demonstrate statistically significant differences based on type of bacteria grown in blood culture, Table 3.

ROC assessment was performed to evaluate the diagnostic performance of CRP titre, ANC and

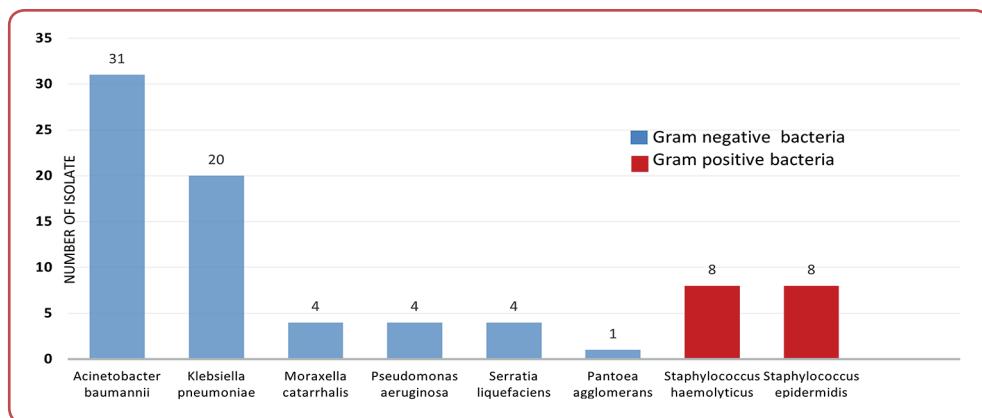


Figure 2: Microbiological profile in neonates with culture-proven sepsis

Table 1: Baseline characteristics of neonates with culture-proven sepsis (n = 80)

| Variables        | N (%)        |
|------------------|--------------|
| Gestational age  |              |
| Preterm          | 48 (60.0)    |
| Term             | 24 (40.0)    |
| Sex              |              |
| Male             | 56 (70.0)    |
| Female           | 24 (30.0)    |
| Birth weight (g) |              |
| Mean ± SD        | 2440 ± 0.80* |
| Range            | 1200-4500    |
| Mode of delivery |              |
| Caesarean        | 56 (70.0)    |
| Vaginal          | 24 (30.0)    |
| Outcome          |              |
| Discharge        | 68 (85.0)    |
| Death            | 12 (15.0)    |

N:L ratio. CRP titre at cutoff 19.53 demonstrated excellent prediction of Gram-negative bacterial sepsis with an area under the curve (AUC) of 0.933 (95 % CI: 0.807-1.000; p = 0.009). In contrast, ANC (AUC = 0.250; 95 % CI: 0.004-0.496; p = 0.134) and N:L ratio (AUC = 0.275; 95 % CI: 0.000-0.563; p = 0.177) showed poor diagnostic ability, performing worse than chance (Figure 3).

In the multiple logistic regression model, using an enter method revealed that CRP (OR = 0.896, 95 % CI: 0.761-1.054, p = 0.001) remained significant predictors of Gram-negative bacteraemia after adjusting for the other confounding variables, which were either found to be statistically significant in the univariate analysis (Table 4)

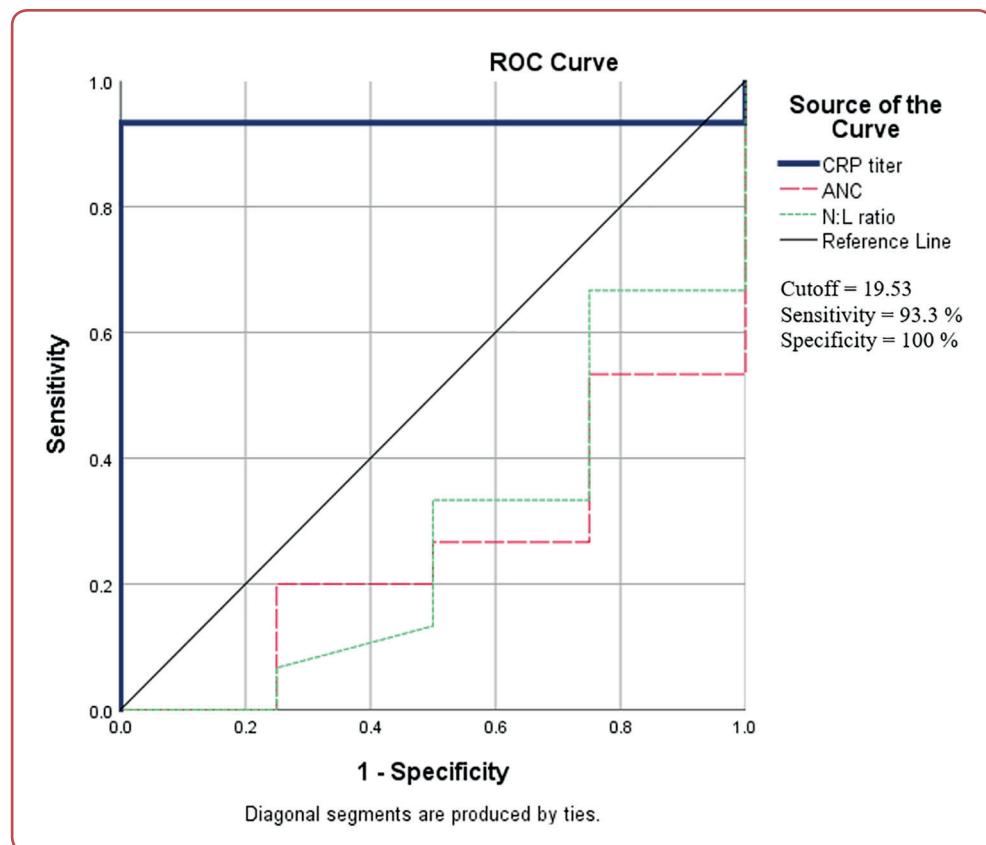
Table 2: Association of the clinical characteristics with type of bacteria

| Variables           | Gram-positive<br>n = 16 (%) | Gram-negative<br>n = 64 (%) | p-value |
|---------------------|-----------------------------|-----------------------------|---------|
| Gestational age     |                             |                             |         |
| Preterm             | 16 (100.0)                  | 40 (62.5)                   | 0.245   |
| Term                | 0 (0.0)                     | 24 (37.5)                   |         |
| Sex                 |                             |                             |         |
| Male                | 12 (75.0)                   | 44 (68.8)                   | 0.807   |
| Female              | 4 (25.0)                    | 20 (31.2)                   |         |
| Birth weight        |                             |                             |         |
| Low birth weight    | 8 (50.0)                    | 30 (46.9)                   | 0.893   |
| Normal birth weight | 8 (50.0)                    | 34 (53.1)                   |         |
| Mode of delivery    |                             |                             |         |
| Caesarean           | 12 (75.0)                   | 48 (75.0)                   | 0.880   |
| Vaginal             | 4 (25.0)                    | 16 (25.0)                   |         |
| Prenatal steroid    |                             |                             |         |
| Present             | 12 (75.0)                   | 32 (50.0)                   | 0.375   |
| Absent              | 4 (25.0)                    | 32 (50.0)                   |         |
| Outcome             |                             |                             |         |
| Discharge           | 12 (75.0)                   | 56 (87.5)                   | 0.531   |
| Death               | 4 (25.0)                    | 8 (12.5)                    |         |

Table 3: Comparison of haematological indices with type of bacteria in neonates with culture proven sepsis

| Variables | Gram-positive<br>n = 16 (%) | Gram-negative<br>n = 64 (%) | p-value |
|-----------|-----------------------------|-----------------------------|---------|
| CRP       | 16.96 ± 2.59                | 16.96 ± 2.59                | 0.003   |
| WBC       | 10.73 ± 2.34                | 10.73 ± 2.34                | 0.947   |
| ANC       | 23.73 ± 13.62               | 23.73 ± 13.62               | 0.030   |
| ALC       | 2.76 ± 0.83                 | 2.76 ± 0.83                 | 0.603   |
| N:L ratio | 9.80 : 14.10                | 9.80 : 14.10                | 0.040   |
| PLT       | 233.25 ± 76.45              | 233.25 ± 76.45              | 0.731   |
| PDW       | 13.70 ± 2.39                | 13.70 ± 2.39                | 0.103   |
| MPV       | 10.43 ± 1.16                | 10.43 ± 1.16                | 0.805   |

CRP: C-reactive protein; WBC: white blood cells count; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; PLT: thrombocyte count; N:L ratio: neutrophil lymphocyte ratio; PLT: thrombocyte count; PDW: platelet distribution width; MPV: mean platelet volume;



**Figure 3:** Receiver operating characteristic (ROC) curve analysis of CRP, N:L ratio and ANC for predicting Gram-negative bacterial growth in EONS

CRP: C-reactive protein; ANC: absolute neutrophil count; N:L ratio: neutrophil-lymphocyte ratio; EONS: early-onset neonatal sepsis;

**Table 4:** Univariate and multivariate analysis for predicting Gram-negative bacteria in early-onset neonatal sepsis (EONS)

| Variables | Univariate analyses |            |             | Multivariate analyses |            |             |
|-----------|---------------------|------------|-------------|-----------------------|------------|-------------|
|           | p-value             | Odds ratio | 95 % CI     | p-value               | Odds ratio | 95 % CI     |
| CRP       | < 0.001             | 0.905      | 0.784-1.045 | 0.001                 | 0.896      | 0.761-1.054 |
| ANC       | 0.046               | 1.066      | 0.962-1.181 | 0.060                 | 1.710      | 1.120-1.998 |
| N:L ratio | 0.035               | 1.209      | 0.839-1.743 | 0.460                 | 1.016      | 0.935-1.234 |

CRP: C-reactive protein; ANC: absolute neutrophil count; N:L ratio: neutrophil-lymphocyte ratio;

## Discussion

The current data indicates a predominance of Gram-negative bacteria in EONS, as it accounted for 64 (80 %) of bacterial isolates with *Acinetobacter baumannii* as the most frequent 31 (38.8 %). Higher titres of CRP and lower ANC and N:L ratio were significantly associated with Gram-negative bacterial growth in blood cultures of neonates with EONS. Further analyses by ROC curve indicate that a CRP titre at 19.53 mg/dL was associated with 93.3 % sensitivity and

100 % specificity in predicting Gram-negative bacteria in EONS.

Traditionally, group *B streptococcus* was the leading pathogen in EONS, controlled in high-income settings by universal maternal screening and intrapartum antibiotic prophylaxis.<sup>16</sup> However, in other parts of the world and most notably in preterm neonates' other organisms surpass GBS as major cause of neonatal sepsis.<sup>17</sup> Recent studies indicate a noticeable shift in the microbiologic landscape of early-onset neonatal sepsis, with Gram-negative bacteria increasingly rivalling or

surpassing Gram-positive organisms as leading pathogens. A large meta-analysis in Iran found that Gram-negative bacteria accounted for about 53.6 % of neonatal sepsis cases (including early onset), with *Klebsiella pneumoniae* and *Escherichia coli* being the most prevalent.<sup>18</sup>

In this study *Acinetobacter baumannii* was the leading causative organism isolated from neonates with EONS. It is an increasingly recognised cause of EONS, particularly in low resource settings and in neonatal care units that experience environmental contamination or infection-control lapses. This organism is often multidrug-resistant, present early in life (within 48–72 hours) and are associated with high morbidity and mortality and intensive-care needs.<sup>19,20</sup> Preterm neonates are specifically liable for sepsis with *Acinetobacter baumannii* and 60 % of sample studied in this study was premature. In a previous study, pure growth of *Acinetobacter* recovered from blood culture obtain at first day of life in 65 % of neonate with EONS caused by *Acinetobacter baumannii* that show a trend of early acquisition of this pathogen which rise the possibility of vertical transmission from the mother or very early contamination at the delivery room.<sup>21</sup> Recent Iraqi studies demonstrated an increasing prevalence of this pathogen in neonatal sepsis over different Iraqi governorates,<sup>22–24</sup> these findings underscore the need to update current management protocols and to develop unified national guidelines tailored to the prevailing bacterial profile.

Early prediction of Gram-negative bacterial sepsis as the cause of EONS is crucial for selecting appropriate empirical antibiotic coverage and for anticipating disease progression and prognosis. In the present study, three laboratory markers showed a significant association with Gram-negative bacterial growth in blood cultures: elevated CRP levels as a biochemical marker and a reduced ANC and N:L ratio as a haematologic parameter. Previous studies demonstrate that N:L ratio had higher diagnostic accuracy than CRP, but this is not without a drawback, as its cutoff level is different between studies and should be changed with every day of life. On the other hand, although CRP level is affected by postnatal age and mode of delivery, reliable reference cutoff values are well demonstrated by literature.<sup>25,26</sup>

There is no single cutoff value for CRP demonstrated in previous studies for Gram-negative neonatal sepsis, with value  $\geq 10$  mg/L lin-

ked with higher possibility of neonatal sepsis in general.<sup>8</sup> Güneş et al demonstrated a high diagnostic accuracy of CRP in identification of Gram-negative bacteria in neonatal sepsis which further enhanced by taking CRP/albumin ratio.<sup>27</sup> In the current study, CRP had a 93.3 % sensitivity and 100 % specificity for predicting Gram-negative bacteria at a cutoff titre of 19.53. This finding is of paramount importance as rapid and reliable identification of Gram-negative bacteria enables timely initiation of appropriate antibiotics with subsequent improved survival rates.

The results of the present study are constrained by the small sample size and a single -centre setting, that may limit the generalisability of the results. Nevertheless, this study contributes to the growing body of evidence on the bacterial profile of EONS in Iraqi neonatal units and highlights a promising biomarker for the diagnosis of Gram-negative sepsis. Future multicentre studies with large cohort are warranted to validate these findings to facilitate the adoption of standardised criteria for early detection of Gram-negative bacterial sepsis in neonates admitted to intensive care units

## Conclusion

Gram-negative bacteria, predominantly *Acinetobacter baumannii*, accounted for most culture proven EONS cases. Elevated CRP, along with lower ANC and N:L ratio, were significantly associated with Gram-negative culture isolation. A CRP threshold of 19.53 demonstrated excellent predictive accuracy, highlighting its value as an early diagnostic marker for Gram-negative bacteraemia.

## Ethics

Study approval was obtained from the College of Medicine's Ethical Committee, decision No IBR 7, dated 20 September 2025.

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None.

## Conflicts of interest

The authors declare that there is no conflict of interest.

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## Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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