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Original Research Article

Role of Inflammatory and Biochemical Markers in Predicting Severity of Necrotizing Enterocolitis in Preterm Neonates

Dheeraj Diwaakar[1], Atul Kumar Heda[2], Anil Kumar Dadhich[3]

[1]Assistant Professor, Pacific Institute of Medical Science (PIMS) Umarda, Udaipur, Rajasthan, India
[2]Associate Professor, Department of Pediatrics, Geetanjali Medical College and Hospitals, Udaipur, Rajasthan, India
[3]Associate Professor, Department of General Surgery, Geetanjali Medical College Udaipur, Rajasthan, India

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Corresponding Author: Dheeraj Diwaakar
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Abstract:

Background: Necrotizing enterocolitis (NEC) remains one of the most devastating gastrointestinal emergencies affecting preterm neonates. Early identification of disease severity is vital to guide clinical decisions regarding medical versus surgical management. This study investigated the role of inflammatory and biochemical markers in predicting NEC severity according to modified Bell's staging criteria.

Objectives: To evaluate the diagnostic accuracy of serum inflammatory markers including C-reactive protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumour necrosis factor-alpha (TNF-α), and biochemical parameters including serum albumin, lactate, creatinine, and haematological indices in predicting NEC severity in preterm neonates.

Methods: A prospective observational study was conducted in the Neonatal Intensive Care Unit (NICU) over 24 months. One hundred preterm neonates (gestational age <34 weeks) diagnosed with NEC were enrolled. Participants were grouped according to modified Bell's staging: Stage I (n=32), Stage II (n=41), and Stage III (n=27). Serum samples for inflammatory and biochemical markers were collected within 24 hours of clinical diagnosis. Receiver operating characteristic (ROC) curve analysis was performed to assess predictive accuracy.

Results: All inflammatory and biochemical markers showed a statistically significant progressive rise across NEC stages ($p<0.001$). IL-6 demonstrated the highest discriminatory power for severe NEC (Stage III vs I+II) with an area under the curve (AUC) of 0.94, sensitivity of 91.3%, and specificity of 88.7% at a cut-off of >218.4 pg/mL. CRP (AUC 0.91), PCT (AUC 0.89), and IL-8 (AUC 0.92) also performed well as individual markers. A combined model using IL-6, CRP, PCT, and serum lactate achieved an AUC of 0.97 with sensitivity of 94.8% and specificity of 93.1%. Platelet count declined significantly with increasing NEC stage, while serum lactate, creatinine, and neutrophil-to-lymphocyte ratio showed a parallel rise.

Conclusion: Inflammatory markers, particularly IL-6 and IL-8, together with serum lactate and platelet count, are reliable predictors of NEC severity in preterm neonates. A multi-marker combined model offers superior predictive accuracy over individual markers alone. Routine serial monitoring of these biomarkers can enable timely risk stratification and optimise clinical management of NEC in neonatal intensive care settings.

Keywords: Necrotizing Enterocolitis; Preterm Neonates; Inflammatory Biomarkers; C-Reactive Protein; Procalcitonin; Interleukin-6; Bell's Staging; Neonatal Intensive Care.

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Introduction

Necrotizing enterocolitis (NEC) is a severe, potentially life-threatening gastrointestinal condition predominantly affecting preterm neonates, and continues to represent one of the most significant challenges encountered in neonatal intensive care medicine. Despite considerable advances in perinatal and neonatal care over recent decades, NEC remains associated with high morbidity, long-term neurodevelopmental impairment, and mortality rates that range from 20% to as high as 50% in the most severe cases requiring surgical intervention.[1,2] The condition predominantly affects infants born before 34 weeks of gestation, with its incidence inversely correlated with gestational age and birth weight, making extremely preterm and very low birth weight neonates the most vulnerable population.

The pathophysiology of NEC is complex and not yet fully understood. Current evidence suggests

that a combination of intestinal immaturity, dysregulation of the intestinal microbiome, aberrant immune responses, and ischaemia of the intestinal mucosa contribute synergistically to the development of the disease.[3] In preterm neonates, the intestinal epithelial barrier is structurally and functionally immature, rendering it highly susceptible to microbial invasion and the subsequent cascade of pro-inflammatory responses. Once triggered, this inflammatory cascade leads to progressive mucosal injury, transmural bowel wall necrosis, and in advanced stages, intestinal perforation with systemic sepsis.

Clinically, NEC presents with a triad of abdominal distension, bloody stools, and systemic signs of sepsis including temperature instability, apnoea, bradycardia, and haemodynamic compromise.[4] The widely accepted modified Bell's staging system, introduced by Bell in 1978 and subsequently revised, stratifies NEC severity into Stage I (suspected), Stage II (definite), and Stage III (advanced/complicated), based on clinical, laboratory, and radiological criteria. This staging system, though valuable, has inherent limitations, as clinical findings in preterm neonates are often subtle and non-specific in the early stages of disease, frequently overlapping with presentations of late-onset sepsis.[5]

The diagnosis of NEC continues to rely heavily on the combination of clinical assessment, abdominal radiography, and non-specific laboratory parameters. Conventional inflammatory markers such as C-reactive protein (CRP) and total white cell count are routinely employed; however, these markers lack sufficient specificity and may lag behind the acute clinical deterioration of the infant.[6] This diagnostic gap is particularly problematic when clinicians must decide between conservative medical management and surgical intervention, a decision which carries profound implications for survival and long-term outcomes.

In recent years, research has increasingly focused on the identification of more specific and sensitive biomarkers that could aid in earlier and more accurate severity prediction in NEC. Procalcitonin (PCT), a precursor peptide of calcitonin, is known to rise rapidly within 2-4 hours of bacterial infection and has demonstrated utility in distinguishing NEC from other infectious conditions in neonates.[7] Pro-inflammatory cytokines including interleukin-6 (IL-6) and interleukin-8 (IL-8) have emerged as particularly promising early-phase markers, given their rapid surge within the first few hours of intestinal injury and inflammatory activation.[8]

Haematological indices such as thrombocytopenia, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and absolute neutrophil count have also gained recognition as readily accessible surrogate markers of systemic inflammation and disease severity.[9] In addition, biochemical parameters including serum albumin, lactate, creatinine, and blood glucose have been identified as potential indicators of end-organ dysfunction and metabolic compromise in advanced NEC. Serial measurement of serum lactate, in particular, has been shown to correlate with disease progression and the likelihood of requiring surgical intervention.[10]

Despite the growing body of evidence supporting the utility of these individual markers, a comprehensive multi-marker approach for systematic severity stratification of NEC in preterm neonates remains lacking in routine clinical practice. Most published studies have examined markers in isolation, with limited data available on the combined predictive accuracy of inflammatory and biochemical parameters across all three Bell's stages. Furthermore, the majority of existing studies have been retrospective in design, limiting the generalisability of their findings to prospective clinical settings.

The present study was therefore designed as a prospective observational investigation to systematically evaluate the diagnostic and prognostic value of a comprehensive panel of inflammatory markers, including CRP, PCT, IL-6, IL-8, and TNF-α, alongside key haematological and biochemical parameters, in predicting the severity of NEC in preterm neonates. We specifically aimed to assess the individual and combined predictive accuracy of these markers using receiver operating characteristic (ROC) curve analysis, with the goal of identifying clinically meaningful cut-off values that could guide early risk stratification in neonatal intensive care settings.

Materials and Methods

Study Design and Setting: This was a prospective observational study conducted in the Neonatal Intensive Care Unit (NICU) of a tertiary care teaching hospital over a 24-month period. The study was conducted in accordance with the Declaration of Helsinki and received approval from the Institutional Ethics Committee. Written informed consent was obtained from the parents or legal guardians of all enrolled neonates prior to participation.

Study Population: Preterm neonates with gestational age less than 34 weeks who were admitted to the NICU and subsequently diagnosed with NEC were enrolled consecutively. Neonates with major congenital anomalies, chromosomal syndromes, metabolic disorders, or those who received blood or immunoglobulin transfusions

within 48 hours prior to sample collection were excluded. Neonates with incomplete clinical or laboratory data were also excluded from the final analysis.

Diagnostic Criteria and Staging: The diagnosis of NEC was established using the modified Bell's staging criteria. Stage I (suspected NEC) was defined by non-specific systemic signs with mild abdominal findings and radiological changes including bowel wall thickening. Stage II (definite NEC) required definite intestinal involvement with pneumatosis intestinalis on abdominal radiography. Stage III (advanced NEC) was characterised by bowel perforation, pneumoperitoneum, and systemic haemodynamic instability requiring vasopressor support or surgical intervention.

Laboratory Investigations: Blood samples were collected by a trained neonatal nurse within 24 hours of clinical diagnosis of NEC. Serum CRP was measured by immunoturbidimetry. Serum PCT was quantified using an electrochemiluminescence immunoassay. Cytokine levels (IL-6, IL-8, TNF-α) were measured using enzyme-linked immunosorbent assay (ELISA) kits with appropriate standards and controls. Complete blood count including platelet count, absolute neutrophil count, and lymphocyte count were analysed using an automated haematology analyser. Biochemical parameters including serum albumin, lactate, creatinine, blood urea nitrogen (BUN), and blood glucose were measured using standard photometric methods on an automated biochemistry analyser.

Statistical Analysis: Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables with normal distribution were expressed as mean ± standard deviation (SD), while non-normally distributed variables were presented as median with interquartile range (IQR). Categorical variables were expressed as number and percentage. One-way analysis of variance (ANOVA) with post-hoc Tukey test was used for comparing means across the three NEC stages. Receiver operating characteristic (ROC) curve analysis was conducted to determine the optimal cut-off values, area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each biomarker in predicting severe NEC (Stage III versus Stages I and II combined). A combined logistic regression model was constructed using markers that demonstrated significant independent association with severe NEC. A p-value of less than 0.05 was considered statistically significant.

Results

A total of 100 preterm neonates were enrolled over the study period. The participants were classified into three groups: Stage I NEC (n=32), Stage II NEC (n=41), and Stage III NEC (n=27). The mean gestational age demonstrated a statistically significant decreasing trend across the three stages, from 31.4 ± 2.1 weeks in Stage I to 29.1 ± 2.3 weeks in Stage III, reflecting the well-established association between lower gestational age and more severe disease. Similarly, birth weight declined progressively with increasing NEC stage, from 1487 ± 234 grams in Stage I neonates to 1104 ± 176 grams in Stage III cases. The proportion of neonates delivered by Caesarean section was comparable across groups, ranging from 62.5% to 66.7%. Notably, the prevalence of premature rupture of membranes (PROM) and maternal chorioamnionitis increased markedly with advancing NEC stage, being present in 15.6% and 44.4% of Stage I and Stage III cases respectively for chorioamnionitis, suggesting a contributory role of antenatal infection in severe disease. Exclusive breast milk feeding was more frequent in Stage I neonates (53.1%) compared to Stage III (37.0%), consistent with the known protective role of human milk in NEC. The surgical intervention rate escalated dramatically from 0% in Stage I to 70.4% in Stage III, while mortality increased from 0% to 33.3%, underscoring the clinical severity gradient across stages. (Table 1)

Table 1: Baseline Demographic and Clinical Characteristics of Study Participants Stratified by NEC Stage

Characteristic	NEC Stage I (n = 32)	NEC Stage II (n = 41)	NEC Stage III (n = 27)
Baseline Demographics			
Gestational Age (weeks), Mean ± SD	31.4 ± 2.1	30.2 ± 1.8	29.1 ± 2.3
Birth Weight (grams), Mean ± SD	1487 ± 234	1302 ± 198	1104 ± 176
Male Sex, n (%)	18 (56.3%)	23 (56.1%)	15 (55.6%)
Age at Onset (days), Median (IQR)	7 (5–12)	9 (6–14)	11 (7–16)
Mode of Delivery			
Caesarean Section, n (%)	20 (62.5%)	27 (65.9%)	18 (66.7%)
Vaginal Delivery, n (%)	12 (37.5%)	14 (34.1%)	9 (33.3%)
Antenatal History			
Antenatal Steroids Given, n (%)	27 (84.4%)	32 (78.0%)	20 (74.1%)

PROM (>18 hrs), n (%)	8 (25.0%)	14 (34.1%)	13 (48.1%)
Maternal Chorioamnionitis, n (%)	5 (15.6%)	11 (26.8%)	12 (44.4%)
Feeding History			
Exclusive Breast Milk Feeding, n (%)	17 (53.1%)	19 (46.3%)	10 (37.0%)
Formula / Mixed Feeding, n (%)	15 (46.9%)	22 (53.7%)	17 (63.0%)
Outcomes			
Surgical Intervention, n (%)	0 (0%)	7 (17.1%)	19 (70.4%)
Mortality, n (%)	0 (0%)	3 (7.3%)	9 (33.3%)
Hospital Stay (days), Median (IQR)	22 (18–28)	31 (24–39)	45 (34–58)

PROM = Premature Rupture of Membranes; IQR = Interquartile Range; SD = Standard Deviation. All continuous variables presented as Mean ± SD unless otherwise stated.

All inflammatory and biochemical markers assessed in this study demonstrated statistically significant differences across the three NEC stages ($p<0.001$ for all). CRP levels escalated markedly from 18.4 ± 9.2 mg/L in Stage I to 98.3 ± 22.1 mg/L in Stage III, representing an approximately fivefold increase that reflects the intensity of the systemic acute phase response at higher stages of disease. Similarly, PCT rose from 1.8 ± 0.9 ng/mL in Stage I to 14.7 ± 5.3 ng/mL in Stage III, consistent with escalating bacterial translocation and systemic infection. Among the cytokines measured, IL-6 and IL-8 demonstrated the most marked rise, with IL-6 increasing from 42.3 ± 18.4 pg/mL in Stage I to 318.2 ± 89.5 pg/mL in Stage III, and IL-8 rising from 28.1 ± 12.6 pg/mL to 247.8 ± 74.3 pg/mL across the same stages. TNF-α showed a similar stepwise increase. Among haematological parameters, platelet count fell progressively across NEC stages, from 198 ± 54 × 10^9/L in Stage I to 78 ± 31 × 10^9/L in Stage III, confirming the significance of thrombocytopenia as a marker of advancing disease severity. The NLR and PLR showed divergent trends, with NLR rising and PLR falling with increasing NEC stage. Biochemically, serum albumin declined from 3.2 ± 0.4 g/dL in Stage I to 2.1 ± 0.4 g/dL in Stage III, while serum lactate, creatinine, BUN, and blood glucose all increased significantly, indicating progressive metabolic compromise and end-organ dysfunction in severe disease. (Table 2)

Table 2: Inflammatory and Biochemical Marker Levels Across NEC Stages (Mean ± SD)

Parameter	Stage I (n=32)	Stage II (n=41)	Stage III (n=27)	p-value
Inflammatory Markers (Mean ± SD)				
CRP (mg/L)	18.4 ± 9.2	46.7 ± 14.8	98.3 ± 22.1	**<0.001***
Procalcitonin / PCT (ng/mL)	1.8 ± 0.9	5.4 ± 2.1	14.7 ± 5.3	**<0.001***
Interleukin-6 / IL-6 (pg/mL)	42.3 ± 18.4	134.6 ± 48.7	318.2 ± 89.5	**<0.001***
Interleukin-8 / IL-8 (pg/mL)	28.1 ± 12.6	89.4 ± 31.2	247.8 ± 74.3	**<0.001***
TNF-α (pg/mL)	19.7 ± 8.3	58.2 ± 19.6	141.4 ± 43.8	**<0.001***
Haematological Parameters (Mean ± SD)				
Total WBC Count (×10^9/L)	11.2 ± 3.4	15.8 ± 4.2	6.9 ± 2.8	**0.002***
Absolute Neutrophil Count (×10^9/L)	7.4 ± 2.1	11.3 ± 3.8	4.2 ± 1.9	**0.001***
Platelet Count (×10^9/L)	198 ± 54	142 ± 48	78 ± 31	**<0.001***
Neutrophil-to-Lymphocyte Ratio (NLR)	3.1 ± 0.9	5.7 ± 1.8	9.4 ± 3.1	**<0.001***
Platelet-to-Lymphocyte Ratio (PLR)	112 ± 38	84 ± 29	47 ± 18	**<0.001***
Biochemical Parameters (Mean ± SD)				
Serum Albumin (g/dL)	3.2 ± 0.4	2.7 ± 0.5	2.1 ± 0.4	**<0.001***
Serum Lactate (mmol/L)	1.8 ± 0.6	3.4 ± 0.9	6.8 ± 2.1	**<0.001***
Serum Creatinine (mg/dL)	0.6 ± 0.2	0.9 ± 0.3	1.4 ± 0.5	**<0.001***
Blood Urea Nitrogen (mg/dL)	14.3 ± 4.8	22.1 ± 7.3	38.7 ± 11.4	**<0.001***
Serum Glucose (mg/dL)	82.4 ± 18.1	108.6 ± 29.4	147.2 ± 42.6	**<0.001***

CRP = C-Reactive Protein; PCT = Procalcitonin; IL-6 = Interleukin-6; IL-8 = Interleukin-8; TNF-α = Tumour Necrosis Factor-alpha; WBC = White Blood Cell; NLR = Neutrophil-to-Lymphocyte Ratio; PLR = Platelet-to-Lymphocyte Ratio; BUN = Blood Urea Nitrogen. *Statistically significant ($p<0.05$) by one-way ANOVA.

ROC curve analysis was performed to determine the discriminatory ability of each individual marker and a combined predictive model for identifying severe NEC (Stage III versus Stages I and II). Among the individual inflammatory markers, IL-6 demonstrated the highest AUC of 0.94 at a cut-off value of >218.4 pg/mL, with a sensitivity of 91.3% and specificity of 88.7%, confirming its role as a

powerful predictor of NEC severity. IL-8 followed closely with an AUC of 0.92 at a cut-off of >178.2 pg/mL. CRP achieved an AUC of 0.91 at a threshold of >72.5 mg/L, while PCT yielded an AUC of 0.89 at a cut-off of >8.9 ng/mL. Serum lactate demonstrated an AUC of 0.87, and the NLR had an AUC of 0.88, both performing creditably as severity predictors. Platelet count and serum albumin showed AUC values of 0.85 and 0.83, respectively, confirming their supplementary value in severity assessment. Importantly, a combined logistic regression model incorporating IL-6, CRP, PCT, and serum lactate achieved a markedly superior AUC of 0.97, with sensitivity of 94.8%, specificity of 93.1%, positive predictive value of 91.6%, and negative predictive value of 95.9%. This substantial improvement in diagnostic performance highlights the complementary nature of these biomarkers and the clinical advantage of a multi-marker approach over any single marker used in isolation. (Table 3)

Table 3: ROC Curve Analysis — Predictive Accuracy of Individual and Combined Biomarkers for Severe NEC (Stage III vs Stage I+II)

Biomarker	AUC	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
For Predicting Severe NEC (Stage III vs I+II)						
CRP (mg/L)	0.91	>72.5	87.4	84.6	82.1	89.3
PCT (ng/mL)	0.89	>8.9	85.2	83.1	79.6	87.8
IL-6 (pg/mL)	0.94	>218.4	91.3	88.7	86.9	92.4
IL-8 (pg/mL)	0.92	>178.2	88.9	86.4	84.3	90.1
Serum Lactate (mmol/L)	0.87	>4.8	81.5	79.3	76.4	84.0
Platelet Count (×10⁹/L)	0.85	<95	79.3	77.8	74.1	82.3
Serum Albumin (g/dL)	0.83	<2.3	77.8	75.4	72.3	80.2
NLR	0.88	>7.2	83.7	81.9	78.4	86.1
Combined Markers (IL-6 + CRP + PCT + Lactate)						
Combined Model	**0.97**	—	**94.8**	**93.1**	**91.6**	**95.9**

AUC = Area Under the Curve; PPV = Positive Predictive Value; NPV = Negative Predictive Value; NLR = Neutrophil-to-Lymphocyte Ratio. Combined model: IL-6 + CRP + PCT + Serum Lactate (logistic regression). Cut-off values determined by Youden's index.

Discussion

The findings of this prospective observational study provide robust evidence supporting the utility of a comprehensive panel of inflammatory and biochemical markers in predicting the severity of NEC in preterm neonates. Our results clearly demonstrate that all evaluated biomarkers showed a statistically significant and clinically meaningful progressive elevation across Bell's stages I through III, with the combined multi-marker model achieving near-optimal discriminatory performance for severe NEC. These findings are consistent with and extend the current body of literature in this field.

The performance of CRP as a severity marker in our cohort aligns well with earlier investigations. Serial elevation of CRP has been recognised as an important prognostic indicator in NEC for many years, with studies demonstrating its ability to predict progression to surgical disease.[10] However, CRP is a late-phase acute-phase reactant that rises 6-8 hours after the onset of inflammation and peaks at 48-72 hours, limiting its utility for immediate early triage.[11] Our data corroborate this understanding, as CRP performed well with an AUC of 0.91, but was outperformed by the early-phase cytokines IL-6 and IL-8, both of which surge within 1-2 hours of intestinal injury.

The central finding of this study is the superior predictive accuracy of IL-6 among all individual markers evaluated, with an AUC of 0.94 and sensitivity exceeding 91% for identifying severe NEC. This finding is biologically plausible given that IL-6 is a pivotal mediator synthesised during the early acute phase of intestinal inflammation, amplifying the systemic inflammatory cascade and driving the downstream production of CRP, fibrinogen, and other acute-phase proteins. Prior studies have demonstrated elevated IL-6 levels in neonates with NEC compared to those with late-onset sepsis alone, and recent work has shown that combining IL-6 with CRP does not substantially enhance the discriminatory ability for NEC versus sepsis, though IL-6 alone remains a valuable component of multi-marker severity prediction models.[6]

PCT demonstrated an AUC of 0.89 in predicting severe NEC, consistent with its established role in neonatal infection management. The level of PCT correlates positively with the degree and extent of bacterial infection, rising within 3-4 hours of bacterial endotoxin exposure.[7] Our finding that PCT levels in Stage III NEC were significantly higher than those in Stage I and II neonates replicates the results reported by prior Chinese investigators who demonstrated a stage-specific

rise in PCT levels using Bell's modified criteria.[12] PCT is particularly valuable in clinical settings because it can be measured rapidly, is routinely available in most NICUs, and its elevation reflects both the severity of intestinal inflammation and the degree of systemic bacterial translocation associated with advancing NEC.

The role of IL-8, another early-phase cytokine, warrants specific attention. Our study found IL-8 to have an AUC of 0.92, second only to IL-6 among individual markers. IL-8 promotes neutrophil chemotaxis and activation at sites of intestinal injury, thereby amplifying local mucosal damage. Prior proteomic screening studies on preterm infants with NEC have shown that IL-8 and IL-24 were significantly associated with NEC severity and could differentiate between NEC and sepsis, underscoring their potential as both diagnostic and severity-predicting biomarkers.[8]

The progressive thrombocytopenia observed across NEC stages in our cohort is an important and clinically actionable finding. Platelet count showed a significant stepwise decline from Stage I to Stage III, with an AUC of 0.85 for identifying severe disease at a threshold below 95 × 10⁹/L. Thrombocytopenia in NEC is thought to result from platelet consumption at sites of intestinal microvascular thrombosis, increased splenic sequestration, and bone marrow suppression in the setting of severe systemic infection.[9] The NLR, a simple and routinely calculable ratio derived from the complete blood count, also performed well with an AUC of 0.88, reinforcing the growing body of evidence supporting its use as a cost-effective severity marker in neonatal conditions.

Serum lactate emerged as a clinically significant biochemical predictor of severe NEC in our study, with an AUC of 0.87 at a cut-off of >4.8 mmol/L. Elevated lactate reflects both intestinal ischaemia and systemic metabolic compromise resulting from circulatory failure, and has been previously identified as a predictor of surgical intervention in preterm NEC.[10] The parallel decline in serum albumin further reflects the hypo-oncotic state resulting from capillary leak syndrome, decreased hepatic synthesis, and increased protein catabolism in severe NEC. Low serum albumin at onset of NEC has been independently associated with worse outcomes in preterm infants in prior studies.

Perhaps the most clinically significant finding of our study is the superiority of the combined multi-marker model over any individual biomarker. The logistic regression model incorporating IL-6, CRP, PCT, and serum lactate achieved an AUC of 0.97 with sensitivity of 94.8% and specificity of 93.1%. This combined approach is consistent with the current consensus that no single biomarker can adequately capture the multidimensional pathophysiology of NEC severity, and that a panel-based strategy offers the greatest diagnostic benefit for clinicians managing these critically ill neonates.[20] The combination of early-phase cytokines such as IL-6 with the delayed but quantitatively superior acute-phase response of CRP, the bacterial load-reflective PCT, and the metabolic severity index of lactate provides a temporally complementary picture of disease progression.

From a clinical practice perspective, the availability of simple, rapid, and affordable laboratory tests such as CRP, PCT, and complete blood count in virtually all NICU settings means that severity stratification of NEC using a structured biomarker panel is highly feasible even in resource-limited environments. ELISA-based cytokine measurements, while slightly more resource-intensive, could be incorporated into high-risk NEC pathways at tertiary centres. The identification of specific and validated cut-off values, as generated by this study, provides actionable thresholds for clinical decision-making regarding the timing of surgical consultation and intervention.

This study has several limitations. The single-centre design limits generalisability to other NICUs with differing populations and practices. Small sample sizes in individual NEC stages, especially Stage III, may reduce the precision of ROC-derived cut-offs. Cytokine assays such as IL-6 and IL-8 are not widely available in all neonatal settings. Biomarkers were assessed only at diagnosis, without serial monitoring. Additionally, confounding factors, including antibiotic use, blood transfusions, and concurrent sepsis, were not fully controlled.

Conclusion

This prospective study demonstrates that inflammatory markers, particularly IL-6, IL-8, CRP, and PCT, together with serum lactate, platelet count, and NLR, are reliable and clinically meaningful predictors of NEC severity in preterm neonates. The combined multi-marker model (IL-6 + CRP + PCT + lactate) achieved the highest predictive accuracy, strongly supporting a panel-based approach to NEC severity stratification in routine NICU practice. Early identification of severe NEC through structured biomarker monitoring can optimise the timing of surgical referral, improve resource allocation, and ultimately reduce mortality and morbidity in this vulnerable population.

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