

Diagnostic Value of Immature to Total Neutrophil Ratio, C-reactive Protein and Clinical Parameters in Early Detection of Neonatal Sepsis: A Cross-sectional Study

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ABSTRACT

Introduction: Neonatal sepsis remains a major cause of neonatal morbidity and mortality worldwide, particularly in resource-limited settings where advanced diagnostic facilities are not readily available. The condition often presents with subtle and non specific clinical features, making early recognition and timely initiation of treatment challenging. Although blood culture is considered the gold standard for diagnosis, its delayed reporting time and limited sensitivity may hinder prompt clinical decision-making. Therefore, readily available clinical and haematological markers are essential for early identification and risk stratification of affected neonates.

Aim: To explore the relationship between Immature to Total neutrophil ratio (IT ratio), C-reactive Protein (CRP), clinical features, laboratory findings and blood culture positivity for early diagnosis of neonatal sepsis.

Materials and Methods: A cross-sectional study was conducted in the Neonatal Intensive Care Unit (NICU) of the Department of Paediatrics at SMBT Institute of Medical Sciences and Research Centre (IMS & RC), located in Dhamangaon, Igatpuri, Nashik, Maharashtra, India from March 2025 to August 2025, including 92 neonates with suspected sepsis. Complete blood count (CBC), I/T ratio, CRP, and blood culture were evaluated. Chi-

square test, Fisher's-exact test, and logistic regression analysis were performed. A p-value <0.05 was considered statistically significant.

Results: The study included 92 neonates, of whom 25 (27.17%) were female and 67 (72.83%) were male. Blood culture was positive in 36 neonates (39.13%). Significant associations with culture positivity were observed for I/T ratio >0.2 (p-value<0.001), CRP, abnormal Total Leucocyte Count (TLC), low Absolute Neutrophil Count (ANC), and thrombocytopenia. Among clinical parameters, abdominal distension ($\chi^2=24.85$; p-value<0.001), low birth weight ($\chi^2=19.63$; p-value=0.0002), prolonged Capillary Refill Time (CRT) ($\chi^2=9.05$; p-value=0.02), direct hyperbilirubinemia ($\chi^2=16.02$; p-value<0.001), and lower gestational age ($\chi^2=22.2$; p-value<0.001) were significantly associated with culture positivity. Hyperglycaemia, hypoglycaemia, increased gastric residue, need for inotropic support, metabolic acidosis, mottling, and sclerema also showed significant associations. On multivariate logistic regression, I/T ratio, CRP, Inotropic support, Sclerema and TLC remained independent predictors of blood culture positivity.

Conclusion: For early detection of sepsis IT ratio is useful parameter along with TLC, inotropic support, sclerema and CRP and has significant association with culture positivity.

Keywords: Blood culture positivity, Haematological parameters, Predictive markers, Risk stratification, Sepsis screening

INTRODUCTION

Neonatal sepsis remains a major contributor to neonatal mortality and morbidity worldwide and often presents with subtle and non specific clinical signs, making early diagnosis challenging [1-4]. Blood culture is considered the gold standard for the diagnosis of neonatal sepsis; however, it is time-consuming, requires a significant volume of blood, has limited sensitivity, and is not readily available in many resource-limited settings [1,2].

Early initiation of antibiotics is crucial to prevent serious complications and reduce mortality among neonates. However, unnecessary or prolonged antibiotic exposure increases the risk of adverse drug effects and contributes to the development of antimicrobial resistance [2]. Consequently, clinicians frequently rely on a combination of maternal history, clinical examination, and biochemical markers to guide early diagnosis and management.

Several perinatal and maternal risk factors have been associated with neonatal sepsis, including prolonged or premature rupture of

membranes, maternal fever, unsterile per vaginal examinations, foul-smelling amniotic fluid, instrumental delivery, and significant interventions at birth. Common clinical features associated with sepsis include lethargy, delayed CRT, mottling, hypothermia or hyperthermia, increased gastric residue, hypo- or hyperglycaemia, and seizures [5].

Multiple biochemical markers have been studied for their diagnostic utility, including CBC, CRP, immature-to-total neutrophil ratio (I/T ratio), platelet count, and ANC, while some newer markers remain largely experimental [5,6]. The accuracy of these investigations may be influenced by factors such as the method of sample collection and maternal conditions [2].

The CRP is one of the most widely studied markers; however, its delayed rise and relatively low sensitivity limit its utility in the early phase of sepsis [2]. Procalcitonin demonstrates an earlier rise during infection, but its high cost and limited availability restrict its routine use in many settings [2]. The I/T ratio is also commonly employed,

although its interpretation may be affected by factors such as maternal hypertension, labor, and oxytocin use [3,7]. While manual estimation of the I/T ratio is labour-intensive and underutilised, it has demonstrated good sensitivity and a high negative predictive value [8]. Low ANC and thrombocytopenia have also been explored as supportive indicators of neonatal sepsis.

In resource-limited settings, these clinical and biochemical parameters play a vital role in facilitating early diagnosis and timely initiation of treatment. Although various national and international organisations have proposed guidelines for the diagnosis of neonatal sepsis, these recommendations require local adaptation and validation [9,10]. Therefore, the present study was undertaken to evaluate the usefulness of the above-mentioned clinical and laboratory parameters for the early diagnosis of neonatal sepsis.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Neonatal Intensive Care Unit (NICU) of the Department of Paediatrics at SMBT IMS and RC, located in Dhamangaon, Igatpuri, Nashik, Maharashtra, India from March 2025 to August 2025. Institutional Ethics Committee approval was obtained prior to data collection (IEC letter: 1401/SMBT/IMSRC/10/IEC/24/250).

Inclusion criteria: Neonates admitted with suspected sepsis based on clinical and laboratory parameters were included. Blood culture positivity was considered the reference standard outcome. Also, neonates admitted to the NICU during the study period with antenatal risk factors, prematurity, requirement of resuscitation at birth, administration of intravenous fluids, or postnatal risk factors for sepsis was included in the study.

Exclusion criteria: Neonates without risk factors for sepsis and those admitted only for observation or phototherapy were excluded.

Sample size: The sample size was calculated based on the reported proportion of neonates with elevated I/T neutrophil ratio (57.5%) in a previous study by Jethani S et al., [4]. Considering a finite population of 120 neonatal sepsis admissions over three months, with 95% confidence level and 5% margin of error, the required sample size was calculated as 92.

STUDY PROCEDURE

Neonates with antenatal or postnatal risk factors of sepsis admitted to the NICU during the study period were enrolled after obtaining informed parental consent. The I/T ratio, CRP, CBC, and blood culture were performed in neonates with suspected sepsis based on risk factors and clinical features. Data were extracted from NICU case sheets and medical records. Records were reviewed daily, and uniform data collection was ensured.

Parameters studied: Antenatal risk factors evaluated included foul-smelling liquor, prolonged rupture of membranes >24 hours (PROM; leaking per vaginam LPV), and maternal fever >100.4°F [11]. Postnatal risk factors included prematurity and low birth weight requiring NICU admission [11].

Clinical features considered indicative of sepsis included prolonged CRT, hypoglycaemia or hyperglycaemia, and metabolic acidosis [11]. System-specific features such as increased gastric residuals, increased Ryle's tube aspirates (RTA), abdominal distension, direct hyperbilirubinemia, sclerema, and mottling were also assessed [11].

Laboratory criteria evaluated were Total Leukocyte Count (TLC) <5000/mm³, platelet count <1.5 lac/mm³ or >20% fall from baseline, ANC<500/mm³, I/T ratio >0.2, CRP ≥5 mg/L, and blood culture positivity [11].

Demographic details, antenatal history, birth details, clinical features, laboratory findings, and blood culture reports were recorded. Clinical assessments were based on documentation by faculty members. Persistence of clinical signs along with objective measurements such as blood glucose levels, abdominal girth, and gastric aspirates were considered significant indicators of sepsis and extracted from both clinical and nursing records.

Blood samples for I/T ratio were collected on peripheral smears, while samples for CBC and CRP were collected in appropriate vacutainers. Blood culture samples were obtained prior to initiation of antibiotics, either during routine septic screening at 24 hours of life in high-risk neonates or at the onset of clinical signs of sepsis. One milliliter of blood was collected using standard aseptic precautions (spirit-betadine-spirit technique). Blood cultures were processed using the BACTEC system. Positive cultures, without contamination, were sub-cultured on agar plates, followed by Gram staining and antibiotic sensitivity testing using the disc diffusion method. The reporting time for blood culture was up to five days. CBCs were analysed using an automated Coulter method in the central laboratory.

STATISTICAL ANALYSIS

Data were entered into Google Sheets and exported to Microsoft Excel for analysis. Statistical analysis was performed using Epi Info version 7 (Centers for Disease Control and Prevention, USA). Chi-square test and Fisher's-exact test were used to assess the association between clinical/laboratory parameters and blood culture positivity. A p-value <0.05 was considered statistically significant. Multivariate logistic regression analysis was performed with blood culture positivity as the dependent variable and relevant clinical and laboratory parameters as covariates to identify independent predictors.

RESULTS

The study included 92 neonates, of whom 25 (27.17%) were female and 67 (72.83%) were male. Blood culture positivity was observed in 36 (39.13%) cases. Overall mortality was 24 (26.09%).

A significant relationship was observed between low gestational age and blood culture positivity. Neonates <28 weeks and 29-33 weeks demonstrated significantly higher culture positivity (p-value<0.001). Similarly, low birth weight was significantly associated with blood culture positivity (p-value=0.002) [Table/Fig-1].

Leaking per vaginam >24 hours and maternal fever showed higher proportions of culture positivity but did not reach statistical significance. Foul-smelling liquor was not significantly associated with culture positivity. Among clinical parameters, prolonged CRT, direct hyperbilirubinemia, hyperglycaemia, hypoglycaemia, increased gastric residuals (RTA), ionotropic support, metabolic acidosis, mottling, sclerema, and abdominal distension showed significant associations (p-value<0.05) [Table/Fig-1].

CRP demonstrated a strong graded association with blood culture positivity. Diagnostic accuracy analysis showed that elevated CRP had high specificity and positive predictive value, indicating that markedly raised CRP levels strongly support culture positivity.

Parameters	Category	Culture positive n (%)	Culture negative n (%)	p-value
Birth weight	<1 kg (n=15)	11 (73.3)	4 (26.7)	0.0002
	1-1.5 kg (n=26)	15 (57.7)	11 (42.3)	
	1.5-2.5 kg (n=40)	7 (17.5)	33 (82.5)	
	>2.5 kg (n=11)	3 (27.3)	8 (72.7)	
Gestational age	<28 wk (n=13)	9 (69.2)	4 (30.8)	<0.001
	29-33 wk (n=28)	18 (64.3)	10 (35.7)	
	≥34 wk (n=51)	9 (17.6)	42 (82.4)	
PV leaking	>24h (n=25)	14 (56.0)	11 (44.0)	0.125
	<24h (n=7)	2 (28.6)	5 (71.4)	
	No (n=60)	20 (33.3)	40 (66.7)	
Maternal fever	Yes (n=9)	6 (66.7)	3 (33.3)	0.075
	No (n=83)	30 (36.1)	53 (63.9)	
Foul smelling liquor	Yes (n=11)	4 (36.4)	7 (63.6)	0.84
	No (n=81)	32 (39.5)	49 (60.5)	
Abdominal distension	Yes (n=33)	24 (72.7)	9 (27.3)	<0.001
	No (n=59)	12 (20.3)	47 (79.7)	
Capillary Refill Time (CRT)	>3 sec (n=26)	15 (57.7)	11 (42.3)	0.028
	≤3 sec (n=66)	21 (31.8)	45 (68.2)	
Increased RTA	>50% (n=40)	23 (57.5)	17 (42.5)	0.002
	≤50% (n=52)	13 (25.0)	39 (75.0)	
Iototropic support	Yes (n=37)	30 (81.1)	7 (18.9)	<0.001
	No (n=53)	6 (11.3)	47 (88.7)	
Metabolic acidosis	Yes (n=20)	13 (65.0)	7 (35.0)	0.007
	No (n=72)	23 (31.9)	49 (68.1)	
Hypoglycaemia	Yes (n=43)	29 (67.4)	14 (32.6)	<0.001
	No (n=49)	7 (14.3)	42 (85.7)	
Hyperglycaemia	Yes (n=6)	6 (100)	0	0.006
	No (n=86)	30 (34.9)	56 (65.1)	
Direct hyperbilirubinemia	Yes (n=53)	30 (56.6)	23 (43.4)	<0.001
	No (n=39)	6 (15.4)	33 (84.6)	
Mottling	Generalised (n=53)	33 (62.3)	20 (37.7)	<0.001
	Localised (n=10)	0	10 (100)	
	Not seen (n=29)	3 (10.3)	26 (89.7)	
Sclerema	Yes (n=31)	26 (83.9)	5 (16.1)	<0.001
	No (n=61)	10 (16.4)	51 (83.6)	
Platelet	<1.5×10 ⁵ /mm ³ (n=47)	28 (59.6)	19 (40.4)	<0.001
	≥1.5×10 ⁵ /mm ³ (n=45)	8 (17.8)	37 (82.2)	
TLC	<5000 (n=10)	8 (80.0)	2 (20.0)	0.001
	≥5000 (n=82)	28 (34.1)	54 (65.9)	
CRP	≥5 mg/L (n=31)	26 (83.9)	5 (16.1)	<0.001
	<5 mg/L (n=61)	10 (16.4)	51 (83.6)	
I/T Ratio	>0.2 (n=34)	28 (82.4)	6 (17.6)	<0.001
	≤0.2 (n=58)	8 (13.8)	50 (86.2)	

[Table/Fig-1]: Association of clinical and laboratory parameters with blood culture positivity (N=92). Values expressed as number (percentage); p-values calculated using Chi-square or Fisher's-Exact test as appropriate

However, sensitivity was comparatively lower, suggesting limited reliability in excluding early sepsis when CRP values are normal [Table/Fig-1,2].

Parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
Birth weight <1 kg	30.6	92.9	73.3	66.7	0.67
Gestational age <28 wk	25.0	92.9	69.2	68.3	0.68
PV leaking >24 h	38.9	80.4	56.0	66.7	0.59
Maternal fever	16.7	94.6	66.7	63.9	0.56
Foul liquor	11.1	87.5	36.4	60.5	0.49
Abdominal distension	66.7	83.9	72.7	79.7	0.75
Capillary refill >3 sec	41.7	80.4	57.7	66.7	0.61
Increased RTA	63.9	69.6	57.5	75	0.66
Iototropic support	83.3	87.5	81.1	88.7	0.85
Metabolic acidosis	36.1	87.5	65.0	68.1	0.62
Hypoglycaemia	80.6	75.0	67.4	85.7	0.78
Hyperglycaemia	16.7	100	100	64.4	0.58
Direct hyperbilirubinemia	83.3	58.9	56.6	84.6	0.71
Mottling	91.7	46.4	62.3	85.7	0.69
Sclerema	72.2	91.1	83.9	83.6	0.82
ANC <500	41.7	89.3	71.4	70.4	0.65
Platelet <1.50 lac/mm ³	77.8	66.1	59.6	82.2	0.72
TLC <5000	22.2	96.4	80.0	65.9	0.59
CRP ≥5 mg/L	72.2	91.1	83.9	83.6	0.82
I/T Ratio >0.2	77.8	89.3	82.4	86.2	0.84

[Table/Fig-2]: Diagnostic performance.

PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under ROC curve

An I/T ratio >0.2 was significantly associated with culture positivity (OR 29.17). The I/T ratio demonstrated high sensitivity and high negative predictive value, indicating that a normal I/T ratio effectively reduces the probability of culture-proven sepsis. Although the confidence interval was wide, the strong sensitivity supports its utility as an early screening marker [Table/Fig-1,2].

Severe thrombocytopenia showed high specificity and positive predictive value, suggesting that markedly low platelet counts strongly correlate with culture positivity [Table/Fig-1,2].

The TLC abnormalities demonstrated moderate sensitivity and specificity, supporting their adjunctive role. ANC showed moderate predictive ability and was less reliable as a standalone marker [Table/Fig-1,2].

Prediction of Blood Culture Positivity (Logistic Regression Analysis): Multivariate logistic regression analysis demonstrated that iototropic support and sclerema were independent clinical predictors [Table/Fig-3]. The model showed good discrimination and overall statistical significance.

Hyperglycaemia demonstrated complete separation, with 100% of affected neonates showing blood culture positivity. Due to absence of culture-negative cases in this category, logistic regression produced unstable estimates, and the adjusted odds ratio could not be reliably interpreted.

In the biochemical model, iototropic support, sclerema, I/T ratio, CRP, and TLC remained independent predictors. Receiver Operating Characteristic (ROC) analysis demonstrated good discriminative ability of I/T ratio and CRP, with acceptable Area Under the Curve (AUC) values. Inclusion of I/T ratio significantly improved model performance. The combined model showed improved overall diagnostic accuracy compared to individual parameters alone.

Variables	Adjusted OR	95% CI	p-value
Birth weight	0.5384	0.05-5.88	0.6118
Gestational age	1.1452	0.20-6.36	0.8769
Abdominal distension	4.6160	0.72-29.23	0.1044
Capillary Refill Time (CRT)	0.8973	0.28-2.83	0.8534
Increased RTA	1.6958	0.20-14.26	0.6270
Iontropic support	6.8800	1.04-45.47	0.0453
Metabolic acidosis	0.1789	0.02-1.50	0.1132
Hypoglycaemia	1.6190	0.35-7.32	0.5317
Hyperglycaemia	Unstable	-	0.9698
Direct hyperbilirubinemia	2.1312	0.29-15.58	0.4559
Mottling	0.4841	0.06-3.88	0.4947
Sclerema	13.0510	1.82-93.19	0.0104
ANC	2.10	0.97-4.55	0.060
Platelet	1.09	0.66-1.83	0.722
TLC	0.56	0.33-0.97	0.037
CRP	1.58	1.08-2.31	0.018
I/T Ratio	47.48	4.47-504.64	0.001

[Table/Fig-3]: Combined multivariable logistic regression.

OR: Odds ratio; CI: Confidence interval; Overall model likelihood ratio $\chi^2=65.25$; $p<0.001$

DISCUSSION

In addition to culture positivity rates, individual maternal risk factors were evaluated. The blood culture positivity rate in the present study was 39.13%, though lower than Kayange N et al., (47-51%), comparable to reports by Kartik K et al., (28%) and higher than that reported by Jatsho J et al., (14%) [12-14].

Maternal fever was not significantly associated with blood culture positivity, possibly reflecting the relatively small number of cases with documented maternal fever in the study population. The lack of association with foul smelling liquor may partly reflect the limited number of exposed cases, thereby reducing statistical power ($n=11$) and the influence of other dominant risk factors such as prematurity and low birth weight.

Low birth weight and lower gestational age were associated with culture positivity in univariate analysis. However, these variables did not remain independent predictors in multivariate analysis. Similar associations have been reported by Kayange N et al., and Jatsho J et al., [12,14], whereas Gandra S et al., did not find significant associations with perinatal risk factors [5].

Several clinical features demonstrated significant associations with culture positivity. Among these, ionotropic support and sclerema emerged as independent predictors, indicating that haemodynamic instability and severe systemic involvement are strong markers of culture-proven sepsis. Among biochemical parameters, I/T ratio demonstrated high sensitivity and negative predictive value, supporting its role as an early screening tool. CRP showed higher specificity and positive predictive value, indicating greater confirmatory utility when elevated. These findings are consistent with Gandra S et al., and partially aligned with Zhou B et al., who reported strong association with CRP but limited association with TLC [5,15]. Amber S et al., did not observe significant association of I/T ratio and CRP [16].

Beyond clinical predictors, biochemical markers also demonstrated significant diagnostic performance. Thrombocytopenia showed strong specificity, consistent with reports by Das M et al., and Mevundi GN et al., [17,18]. Low TLC and low ANC were also

associated with culture positivity, similar to findings by Gandra S et al., [5]. However, ANC did not retain independent significance in multivariate analysis, suggesting limited standalone diagnostic utility. In contrast, Jethani S et al., observed elevated ANC in septic neonates, although I/T ratio and CRP were also raised [4]. Newman TB et al., likewise reported an association between I/T ratio, ANC, and culture positivity [19]. The differing findings may reflect variation in disease severity and study populations.

The combined logistic regression model demonstrated good overall predictive capability (p -value <0.001). The strongest independent biochemical predictors were CRP and I/T ratio, followed by TLC, ionotropic support, Sclerema. The integration of clinical predictors (ionotropic support, sclerema) further improved risk stratification. Shrayya S et al., identified CRP, gestational age, and birth weight as predictors [20]. In contrast, gestational age and birth weight did not remain independent predictors after adjustment in this analysis.

Overall, these findings suggest that I/T ratio serves as a sensitive early screening parameter, while CRP and severe thrombocytopenia provide greater confirmatory value. A combined-marker approach enhances diagnostic accuracy compared to reliance on a single parameter.

Limitation(s)

The present study was conducted at a single center which may limit generalisability. The cross-sectional design precludes causal inference. Blood cultures were obtained only from high-risk neonates or those with clinical suspicion of sepsis, in accordance with the study objective, and not from well neonates. The predictive model requires external validation before broader application. Long-term neonatal outcomes were not assessed, and the duration of the study was limited. Laboratory personnel were not blinded to clinical data, and interobserver variability in I/T ratio estimation was not evaluated. These factors may have influenced the observed associations. Future studies with larger, multicentric cohorts and longer follow-up are warranted to validate and refine the predictive model.

CONCLUSION(S)

The CRP, I/T ratio, Ionotropic support, Sclerema, and TLC demonstrated strong associations with blood culture positivity and should be considered key components of the initial diagnostic workup and risk stratification in neonates with suspected sepsis. Among clinical variables, sclerema and the need for ionotropic support emerged as significant independent predictors, reflecting greater disease severity. The combined clinical and hematological model facilitates stratification of neonates into higher- and lower-risk categories and may serve as a practical bedside decision-support tool, particularly in resource-limited settings. The I/T ratio showed high sensitivity and negative predictive value, supporting its role as an early screening marker, while CRP demonstrated higher specificity, reinforcing its confirmatory value when elevated. Although I/T ratio estimation require manual assessment, it remains cost-effective and feasible in most settings. These parameters can assist in early escalation of treatment while awaiting culture reports; however, they cannot replace blood culture as the definitive diagnostic method.

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