

A clinical study on the diagnostic significance of sepsis markers in neonatal sepsis in a tertiary care center



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ABSTRACT

Background: Sepsis is a major cause of mortality in neonates after prematurity. Timely diagnosis using sepsis markers is crucial for management, especially where blood culture confirmation may be delayed or unavailable. **Aims and Objectives:** The aims and objectives of the study are to identify the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of sepsis markers in a neonate with sepsis. **Materials and Methods:** A hospital-based observational study was conducted from May 2023 to November 2024. 100 neonates (< 28 days old) with clinical features or risk factors suggestive of sepsis presenting to the Neonatal Intensive Care Unit were included. Exclusion criteria were birth asphyxia, birth weight < 1,500 g, gestational age < 32 weeks, or prior antibiotic use. Data collection included clinical assessments and laboratory measurements of total leukocyte count (TLC), platelet count (PLT), C-reactive protein (CRP), and blood cultures. Statistical analysis was performed using the Statistical Package for the Social Sciences version 25. **Results:** One hundred neonates (63 males and 37 females) were enrolled. TLC was abnormal in 32% of cases, thrombocytopenia in 44%, and raised CRP in 89%. Blood culture-confirmed sepsis was found in 18%, probable sepsis in 29%, and suspected sepsis in 53%. TLC sensitivity was 33.3% and specificity was 24.4%, PPV was 8.8% and NPV was 62.5%. PLT sensitivity was 72.2% and specificity was 62.2%. Males and low birth weight/preterm neonates were most affected. **Conclusion:** Combined use of TLC, PLT, and CRP is necessary for screening neonatal sepsis, enabling early intervention and better outcomes.

Key words: Infant; Neonatal screening; Neonatal sepsis; Systematic Inflammatory Response syndrome; Biomarkers; Inflammation mediators; Sensitivity and specificity; Predictive value test; Clinical study; C-reactive protein; Total leukocyte count; Platelet count

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INTRODUCTION

Sepsis remains a significant cause of neonatal mortality and morbidity, especially in low- and middle-income countries.¹ National neonatal perinatal database (NNPD) 2002–2003 report showed the incidence of neonatal sepsis as 30/1,000 live births. After prematurity, sepsis is considered the second leading cause of mortality. The incidence of clinical sepsis is very high in India.²

The presence of signs and symptoms of infection within 1 month of birth is considered neonatal sepsis. Neonatal sepsis can be divided into early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS). EONS occurs within the first 72 h of life. LONS generally occurs after 3 days of birth. Mortality is higher with EONS than LONS.³

On-time diagnosis and management of neonatal sepsis are very essential to prevent mortality. Blood culture is the

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confirmatory method of neonatal sepsis. But among all the neonatal sepsis, only 25–40% have culture positivity. In developing countries like India, with the resource-limited setting and the delay in obtaining culture positivity report (up to 48 h), confirmation of neonatal sepsis can be done based on the clinical presentation and use of sepsis markers.⁴ Hence, this study was taken up to establish the diagnostic significance of sepsis markers in neonatal sepsis.

Aims and objectives

To identify the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of sepsis markers in a neonate with sepsis.

MATERIALS AND METHODS

This study is an observational study done from May 2020 to November 2021. One hundred neonates from the Great Eastern Medical School and Hospital (GEMS) Neonatal Intensive Care Unit, Ragolu, Srikakulam, were enrolled.

Inclusion criteria

Neonates <28 days old with clinical signs or risk factors for sepsis.

Exclusion criteria

Birth asphyxia, birth weight <1,500 g, gestational age <32 weeks, prior antibiotics.

In this observational study, 100 neonates clinically suspected to have sepsis were enrolled. On meeting the inclusion criteria, prior informed, written parental consent was obtained before enrollment in the study. The study has been cleared by the Hospital Ethics Committee and Hospital Research Committee vide reference number M197602128. During the study, a pre-designed and pre-tested pro forma was implemented.

On admission, as per the hospital protocol, detailed information was noted in the medical case sheet. When sepsis was suspected, clinical features were noted. Investigations such as complete blood picture, C-reactive protein (CRP), and blood culture were done. Various biomarkers are used as diagnostic tools for neonatal sepsis. However, the gold standard for the detection of sepsis is blood culture, but blood culture takes 2–3 days for a diagnosis. This study evaluates the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the sepsis markers, i.e., total leukocyte count, CRP, and platelet count (PLT). Data were analyzed with Microsoft Excel and the Statistical Package for the Social Sciences version 25. Statistical analysis was done using the Chi-square test and analysis of variance. Results were expressed in terms of mean, percentages, and depicted

as tables, graphs. $P < 0.05$ was considered statistically significant.

RESULTS

All 100 neonates enrolled survived without any mortality.

Demographics

Regarding gender among 100 neonates, 63% were male and 37% were female. Birth weight ≤ 2.5 kg was seen in 71% and > 2.5 kg in 29%. The average birth weight of preterm neonates was 2336.76 ± 148.23 g, and term neonates were 2661.03 ± 129.16 g. The average birth weight of all (100) neonates was 2430.80 ± 205.238 g. Preterm babies ≤ 37 weeks were seen in 62% and > 37 weeks were seen in 38%. The average gestational age of the preterm neonates was 34.29 ± 0.67 weeks, term neonates were 37.88 ± 0.73 weeks, and the average gestational age of total neonates was 35.65 ± 1.88 weeks. Cesarean delivery was done in 57%; indications for cesarean were fetal distress (16%), meconium-stained liquor (12%), Cephalo pelvic disproportion (CPD) (10%), non-reactive non-stress test (NST) (9%), non-progression of labor (8%), and compound presentation (2%) as shown in Figure 1.

Risk factors

Premature rupture of membranes (> 18 h) was seen in 34%, multiple vaginal examinations (> 3): In 20%, meconium-stained liquor in 21%, febrile illness in the mother in 17%, and foul-smelling liquor was seen in 8% neonatal mothers.

In this study [Figure 2], among the total neonates, regarding the manifestation of sepsis, 82% of the cases had shock, 9% of cases had congenital pneumonia, 4% of cases had meningitis, and 5% of the cases had necrotizing enterocolitis (NEC).

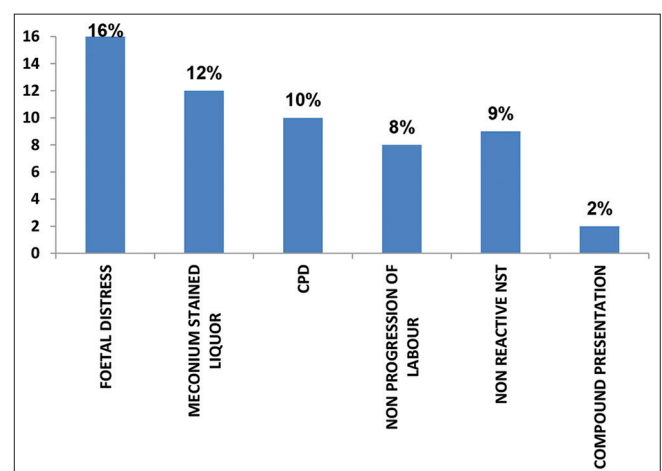


Figure 1: Distribution of study population based on indication for cesarean

In this study, EONS was observed among 56% of the neonates, and LONS was observed among 44% of neonates.

The average duration of EONS was 18 ± 9.12 h, and LONS was 168.1 ± 74.35 h.

In this study, blood culture was positive in only 18% of the cases and was negative in 82% of cases.

Laboratory findings

Total leukocyte count (TLC) was abnormal in 32%, PLT ≤ 1.5 L in 44%, and CRP was positive in 89%. Blood culture was positive in 18% cases (Staph 7%, *Escherichia coli* 4%, *Klebsiella* 3%, others <2%).

Of the total cases, only 18% of the cases had culture-proven sepsis, 29% of cases had probable sepsis, and 53% of the cases had suspected sepsis.

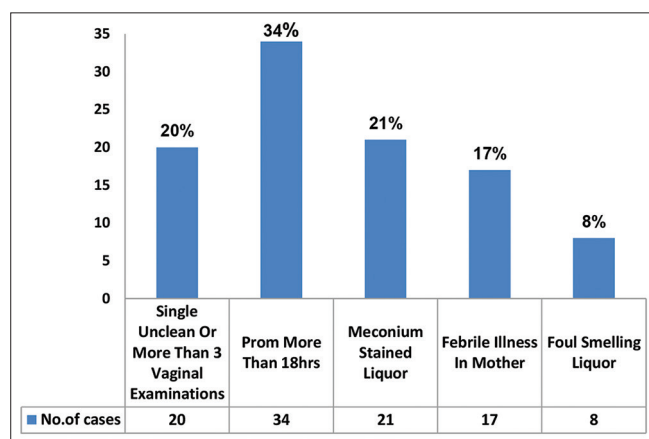


Figure 2: Distribution of risk factors for sepsis

The association between gender and sepsis was statistically not significant ($P=0.294$).

In this study, 16 (25.8%) neonates of ≤ 37 weeks and 2 (5.3%) neonates of >37 weeks had culture proven sepsis, 20 (32.3%) neonates of ≤ 37 weeks, and 9 (23.7%) neonates of >37 weeks had probable sepsis, and 26 (41.9%) neonates of ≤ 37 weeks and 27 (71.1%) neonates of >37 weeks had suspected sepsis. The association between gestational age and sepsis was statistically significant ($P=0.007$).

In this study, 16 (22.5%) neonates of ≤ 2.5 kg birth weight and 2 (6.9%) neonates of >2.5 kg birth weight had culture proven sepsis, 23 (32.4%) neonates of ≤ 2.5 kg birth weight and 6 (20.7%) neonates of >2.5 kg birth weight had probable sepsis, and 32 (45.1%) neonates of ≤ 2.5 kg birth weight and 21 (72.4%) neonates of >2.5 kg birth weight had suspected sepsis. The association of birth weight with sepsis was statistically significant ($P=0.036$).

In Table 1, lower bound values were lowest in the culture-proven sepsis and higher bound values were observed in suspected sepsis. Thus, leukopenia, an adverse marker of sepsis, was observed in the study that differentiated the culture-proven sepsis and suspected sepsis. This difference reached levels of the highest statistical significance ($P=0.0001$).

In Table 2, lower bound values were lowest in the culture-proven sepsis and higher bound values were observed in suspected sepsis. Thus, thrombocytopenia was a significant marker in diagnosing proven and probable sepsis. This trend reached levels of the highest statistical significance ($P<0.0001$).

Table 1: Mean total leukocyte count (TLC) versus neonatal sepsis

Mean TLC	n	Mean $\pm$ SD	95% confidence interval for mean		P-value
			Lower bound	Upper bound	
Suspected sepsis	53	12515.09 $\pm$ 2554.73	11810.92	13219.27	0.0001 (highly sig.)
Probable sepsis	29	7855.17 $\pm$ 4868.6	6003.26	9707.09	
Culture-proven sepsis	18	7805.56 $\pm$ 5534.11	4953.50	10557.6	
Total	100	10316.00 $\pm$ 4571.64	9408.89	11223.1	

SD: Standard deviation

Table 2: Mean platelet count (PLT) versus neonatal sepsis

Mean PLT	n	Mean $\pm$ SD	95% confidence interval for mean		P-value
			Lower	Upper	
Suspected sepsis	53	202113.21 $\pm$ 39406.003	191251.57	140359.60	0.0001 (highly significant)
Probable sepsis	29	99172.41 $\pm$ 31399.578	87228.65	111116.17	
Culture-proven sepsis	18	95833.33 $\pm$ 42726.559	74585.92	117080.75	
Total	100	153130.00 $\pm$ 64359.887	140359.60	165900.40	

SD: Standard deviation

Table 3: Mean CRP levels versus neonatal sepsis

Mean CRP	n	Mean±SD	95% confidence interval for mean		P-value
			Lower bound	Upper bound	
Suspected sepsis	53	14.47±6.863	12.58	16.36	0.0001 (highly significant)
Probable Sepsis	29	48.00±28.864	37.02	58.98	
Culture-proven sepsis	18	78.17±35.499	60.51	95.82	
Total	100	35.66±33.019	29.11	42.21	

CRP: C-reactive protein, SD: Standard deviation

Table 4: Platelet count (PLT) versus blood culture positivity

PLT count	Blood culture				Total	
	Positive		Negative		No.	Percentage
	No.	Percentage	No.	Percentage		
≤LTce	13	72.2	31	37.8	44	44
<1.5L	5	27.8	51	62.2	56	56
Total	18	18	82	82	100	100

Pearson Chi-square 7.096; $P=0.008$ (significant)**Table 5: CRP levels versus blood culture positivity**

CRP	Blood culture				Total	
	Positive		Negative			
	No.	Percentage	No.	Percentage	No.	Percentage
Positive	15	83.3	74	90.2	89	89
Negative	3	16.7	8	9.8	11	11
Total	18	18	82	82	100	100

Pearson Chi-square 0.72; $P=0.396$ (no significance). CRP: C-reactive protein

In Table 3, lower bound values were lowest in the suspected sepsis and higher bound values were found in culture-proven sepsis. Thus, raised CRP levels were a significant marker in diagnosing proven and probable sepsis. This trend reached levels of the highest statistical significance ($P<0.0001$).

In this study, abnormal TLC was observed in 66.7% of the culture-positive sepsis and 24.4% of culture-negative cases. This abnormal trend of TLC in proven sepsis showed highly statistical significance ($P=0.0001$).

The TLC of this study had a moderate NPV (62.5%) with low sensitivity (33.3%), low specificity (24.4%), and low PPV (8.8%).

In this study, thrombocytopenia occurred in 72.2% of the culture-positive sepsis, and 37.8% of culture-negative cases. This increased trend of thrombocytopenia in proven sepsis showed statistical significance ($P=0.008$) as shown in Table 4.

In this study, platelets had a high NPV (91.1%), moderate sensitivity (72.2%), moderate specificity (62.2%), and a low PPV (29.5%) in the prediction of sepsis.

In Table 5, increased CRP levels were observed in 83.3% of the culture-positive sepsis and 90.2% of culture-negative cases. This association between CRP levels and culture did not show statistical significance ($P=0.396$).

In this study, CRP levels had high sensitivity (83.3%), moderate NPV (72.7%), low specificity (9.8%), and low PPV (16.9%) in the prediction of sepsis.

DISCUSSION

Sex

In this study, males were more (63%) compared to females (37%) which showed a male preponderance with a male-to-female ratio of 1.7:1. Similar male preponderance was reported by studies done by Bhalodia et al.,⁵ (66.7%), Vinay et al.,⁶ (66.6%), Morad et al.,⁷ (66%), Sorsa⁸ (65.3%), Mittal et al.,⁹ (58.8%), Sengupta et al.,¹⁰ (58.2%), and Chacha et al.,¹¹ (51.2%).

Birth weight

In the present study, low birth weight was reported in the majority (71%) of the study population. Similar reports were obtained in the study by Vinay et al.,⁶ (70%), Mittal et al.,⁹ (56.1%), Sengupta et al.,¹⁰ (56.6%), and Morad et al.,⁷ (54%). Contrast findings were seen in Chacha et al.,¹¹ (29.8%) and Sorsa study⁸ (24.1%).

Gestational age

In this study, more (62%) preterm babies were seen than term babies (38%). Similar to this finding, preterm babies were more in Vinay et al.,⁶ (68.4%), Choudhary et al.,¹² (76%) studies, whereas preterm babies were less in the study by Chacha et al.,¹¹ (22.6%), Sorsa study⁸ (22.9%), Bhalodia et al.,⁵ (26.7%), Morad et al.,⁷ (38%).

Mode of delivery

Of the total babies, 57% of the neonates were delivered by cesarean section (57%) and 43% were delivered by normal vaginal delivery. Whereas studies by Chacha et al.¹¹ (22%), Sorsa study⁸ (24.1%), and Morad et al.⁷ (46%), cesarean sections were less compared to normal deliveries.

Time of onset of sepsis

Early onset of neonatal sepsis was observed among 56% of the neonates, and late onset of neonatal sepsis was observed among 44% of neonates. Similar to this finding in Sorsa study⁸, early onset of neonatal sepsis was seen in 61.2% of cases, and in 38.8% of cases, late onset of neonatal sepsis was reported, and Vinay et al.,⁶ also reported EONS in 90% of cases.

In contrast to this study finding, in the study by Choudhary et al.,¹² EONS was present in 27% of cases, while late-onset neonatal sepsis was present in 73% of cases.

Blood culture

In this study, blood culture was positive in only 18% of the cases and was negative in 82% of cases. Similar findings were reported by Choudhary et al.,¹² (17%), Swamy et al.,¹³ (20%), Chacha et al.,¹¹ (20.3%), Bhalodia et al.,⁵ (38%), whereas the higher incidence of positive blood culture was reported in the studies by Sriram¹⁴ (50.4%) and Vinay et al.,⁶ (80%).

Staphylococcal species were present in 7% of the cases, followed by *E. coli* (4%), *Klebsiella* (3%), whereas Actinobacteria species, Haemophilus, Methicillin-resistant *Staphylococcal aureus*, and *Pseudomonas aeruginosa* were observed in each 1% of the cases. Staphylococcal species were the predominant organisms. Similar to this study, *S. aureus* was the most common organism in the studies by Procianoy and Silveira¹⁵, Misquith et al.,¹⁶ and Hemalata.¹⁷

CRP was positive in 89% and was negative in 11% of cases. Similar to this finding in Sriram study,¹⁴ CRP was positive in 88.7% cases and negative in 11.3% cases. In contrast to this finding, Gurpreet Singh Chhabra et al.¹⁸ studies had 3% CRP positivity.

Total white blood cells (WBC) count versus blood culture positivity

In this study, abnormal TLC was observed in 66.7% of the culture-positive sepsis and 24.4% of the culture-negative cases. The TLC of this study had reported 33.3% Sensitivity, which was in accordance with studies by Hemalata et al.,¹⁷ (23.63%), Swamy et al.¹³ (20%), and Makkar et al.¹⁹ (43.18%).

In this study, TLC had shown a specificity of 24.4%, which was lower than other studies by Hemalata et al.¹⁷ (71.27%), Bhalodia et al.,⁵ (74.5%), and Majumdar et al.,²⁰ (85%).

This variation might be due to different selection criteria used while selecting the participants and different levels of infections in neonates.

In this study, TLC had a low PPV (8.8%), which was similar to the study by Hiral and Bharti²¹ (15.38%). Contrast results were obtained in the studies by Punyashetty and Patil²² (87.5%), Makkar et al.,¹⁹ (86.36%), and Narasimha and Harendra Kumar²³ (80%).

The TLC of this study had a moderate NPV (62.5%), which was in accordance with Makkar et al.,¹⁹ (56.89%), Majumdar et al.,²⁰ (87%), and Bhalodia et al.,⁵ (87%) studies.

PLT versus blood culture positivity

This study had high sensitivity (72.2%) for platelets which was in accordance with Makkar et al.,¹⁹ (70.45%), Majumdar et al.,²⁰ (70%), Hiral and Bharti²¹ (73.68%), and Mittal et al.,⁹ (83.08%).

For platelets, moderate specificity (62.2%) was observed in this study which was in line with studies by Bhalodia et al.,⁵ (55.9%), Hiral and Bharti²¹ (53.09%), and Narasimha and Harendra Kumar²³ (75%). Low PPV (29.5%) was reported which was in concordance with Khair et al.,²⁴ (31%), Hiral and Bharti²¹ (26.92%), Mittal et al.,⁹ (35.53%), and Swamy et al.,¹³ (21.4%).

In this study, platelets had a high NPV (91.1%), which was similar to studies of Hiral and Bharti²¹ (89.58%), Punyashetty and Patil²² (93.5%), Khair et al.,²⁴ (94%), Majumdar et al.,²⁰ (95%), and Narasimha and Harendra Kumar²³ (85.71%).

CRP versus blood culture positivity

In this study, in 83.3% of the culture-positive sepsis and 90.2% of culture-negative cases, increased CRP levels were reported. This increased CRP levels in culture-proven sepsis did not show statistical significance ($P=0.396$).

In this study, CRP levels had high sensitivity (83.3%), which was similar to Patel et al.,²⁵ (81.7%), Vinay et al., study⁶ (81.2%), Sharma et al.,²⁶ (80%), and Swamy et al.,¹³ (90%).

CRP levels in this study had low specificity (9.8%), which was less than studies of Swamy et al.¹³ (47.5%). This variation could be because of the different methodologies used to measure CRP and the cut-off used.

CRP levels in this study had low PPV (16.9%), which was similar to studies of Chacha et al.¹¹ (37.5%) and Swamy et al.¹³ (30%).

In this study, CRP levels had a moderate NPV (72.7%), which was similar to studies of Sucilathangam G et al.,²⁷ (78.1), Chacha et al.,¹¹ (84.5%), and Swamy et al.,¹³ (95%). Comparison of sensitivity, specificity, PPV,

Table 6: Comparison of sensitivity, specificity, PPV, and NPV with other studies

Test	Authors	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Total WBC count	Khair et al., ²⁴ (2010)	50	91	43	93
Total WBC count<5,000/>20,000	Narasimha and Harendra Kumar ²³ (2011)	10.5	91.66	80	24.4
	Makkar et al., ¹⁹ (2013)	43.18	86.36	86.36	56.89
	Majumdar et al., ²⁰ (2013)	45	85	40	87
	Bhalodia et al., ⁵ (2017)	66.7	74.5	48	87
	Punyashetty and Patil ²² (2016)	100	90.62	87.5	100
	Hiral and Bharti ²¹ (2019)	10.53	86.42	15.38	80.46
	Swamy et al., ¹³ (2020)	20	90	33.3	81.8
	Hemalata ¹⁷ (2018)	23.63	71.27	35.83	-
	Present study	33.3	24.4	8.8	62.5
Platelets≤1.5L	Narasimha and Harendra Kumar ²³ (2011)	47.36	75	85.71	31
	Khair et al., ²⁴ (2010)	60	82	31	94
	Makkar et al., ¹⁹ (2013)	70.45	93.9	93.9	72.3
	Majumdar et al., ²⁰ (2013)	70	80	40	95
	Bhalodia et al., ⁵ (2017)	56.3	55.9	56	58
	Punyashetty and Patil ²² (2016)	91.3	100	100	93.5
	Mittal et al., ⁹	83.08	20.33	35.53	69.4
	Hiral and Bharti ²¹ (2019)	73.68	53.09	26.92	89.5
	Swamy et al., ¹³ (2020)	60	45	21.4	81.8
	Hemalata ¹⁷ (2018)	34.6	78.7	52.5	63.9
	Present study	72.2	62.2	29.5	91.1
CRP levels (>6 mg/dL)	Patel et al., ²⁵ (2014)	81.7	88	95.7	59.5
	Vinay et al., study ⁶ (2015)	81.2	50	86.6	40
	Chacha et al., ¹¹ (2014)	40.4	82.7	37.5	84.5
	Sharma et al., ²⁶ (2013)	80	93	-	-
	Swamy et al., ¹³	90	47.5	30	95
	Sucilathangam G et al., ²⁷ (2012)	50	69.4	38.8	78.1
	Sriram study ¹⁴	52.0	61.5	91.4	14
	Present study	83.3	9.8	16.9	72.7

PPV: Positive predictive value, NPV: Negative predictive value, WBC: White blood cells, CRP: C-reactive protein

and NPV of TLC, Platelet count and CRP of this study compared to other studies shown in Table 6.

Limitations of the study

1. Small sample size 100 only
2. Single centre study

CONCLUSION

It is proven that no single individual test is better than others in detecting neonatal sepsis. Hence, the conjunction of tests such as total WBC, platelets, and CRP can be utilized for better sepsis screening, timely management and to reduce the duration of hospital stay and to improve appropriate antibiotic utilization.

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