Original Article

A Comparative Study on Diagnostic Efficacy of WBC Parameters, Platelet Count, C-Reactive Protein, and Serum Ferritin in Early Onset and Late Onset Neonatal Sepsis

Naqsh Thakur[1], Prakash M Patil[1]*, Siddu Charki[2], Surekha B Hippargi[1]

[1]Department of Pathology, Shri B.M Patil Medical College Hospital and Research Centre, BLDE Deemed to be University, Ashram Road, Vijayapura, Karnataka, India
[2]Department of Pediatrics, Shri B.M Patil Medical College Hospital and Research Centre, BLDE Deemed to be University, Ashram Road, Vijayapura, Karnataka, India

DOI: 10.21276/APALM.3363

***Corresponding Author**:
Dr. Prakash M Patil
naqshthakur10@gmail.com

Submitted: 28-Apr-2024
Final Revision: 14-May-2024
Acceptance: 20-May-2024
Publication: 01-Jun-2024

Abstract

Background
Early detection of neonatal sepsis is complicated due to vague symptoms and variations based on gestational age. Rapid identification is vital, and low-cost diagnostic tools like WBC parameters, platelet count, serum ferritin, and C-reactive protein (CRP) can speed up time-sensitive diagnosis. This can mitigate antibiotic resistance and overuse, cutting expenses in underdeveloped regions of India.
Material and Methods
Ninety cases of neonates <90 days [clinically suspicious admitted cases of early onset (EOS) and late onset neonatal sepsis (LOS): 30 cases each and 30 controls] were selected post-September 01, 2022, for the cross-sectional comparative study. Blood samples were obtained upon clinical suspicion of sepsis, prior to antibiotic treatment, and the above-mentioned parameters were analyzed.
Results
Preterm births constituted 20% (EOS), 36% (LOS), and 26% (control). Low birth weight occurred in 36% (EOS), 33% (LOS), and 23% (control); females represented 46% (EOS), 53% (LOS), and 53% (control). Culture-positive cases were 14 (EOS) and 19 (LOS). Notable variations emerged in CRP, I/T ratio, and toxic granules. The combined analysis of EOS and LOS indicated CRP's sensitivity at 78%, specificity at 90%, PPV at 94%, and NPV at 68%. I/T ratio exhibited 72% sensitivity, 97% specificity, 98% PPV, and 64% NPV. Toxic granules demonstrated 50% sensitivity, 97% specificity, 97% PPV, and 50% NPV, showcasing their diagnostic potential across various onset patterns.
Conclusion
For early-onset sepsis, the I/T ratio and toxic granules displayed significant utility, while CRP emerged as a standout performer for late-onset cases. On the whole, CRP emerged as the premier standalone marker, adaptable for pairing in early onset and singly for late onset, with commendable efficacy. Remarkable disparities in ferritin values between septic and non-septic groups call for further exploration of ferritin's diagnostic applicability.

Keywords:

early onset sepsis, late onset sepsis, neonatal sepsis, C-reactive protein, haematological parameters, toxic granulations

Introduction

The World Health Organization (WHO) reports that perinatal deaths are the leading cause of childhood mortality in children under 5 years old, particularly in developing nations such as India. Neonatal infections stand out as the primary contributor to perinatal

mortality. In India, as per the National Neonatal Perinatal Database (NNPD, 2020), the prevalence of neonatal sepsis is 18 per 1,000 live births. Neonatal sepsis manifests as a clinical syndrome marked by typical signs and symptoms linked with the presence and multiplication of bacteria in the blood [1].

In essence, early-onset neonatal sepsis refers to infections occurring within 72 hours of birth, while late-onset neonatal sepsis occurs between 72 hours after birth and up to three months of age [2]. Detecting neonatal sepsis early poses challenges due to nonspecific symptoms, which vary based on gestational age, necessitating prompt diagnosis. To achieve this, a combination of white blood cell parameters [including total leucocyte count (T.L.C.), immature to total neutrophil ratio (I/T ratio), absolute neutrophil count (A.N.C.)], platelet count, and C-reactive protein (C.R.P.) estimation can aid in early diagnosis before initiating antibiotic therapy. This approach helps prevent antibiotic resistance and overtreatment, alleviating financial burdens in underdeveloped regions of India [3, 4]. Timely identification and treatment of neonatal sepsis can significantly improve outcomes while minimizing unnecessary antibiotic use through the use of acute-phase reactants and the negative predictive accuracy of various hematological indicators [5]. Therefore, our study aimed to evaluate the diagnostic efficacy of these parameters in both early-onset and late-onset neonatal sepsis.

Materials and Methods

Study Design: A cross-sectional comparative study.

Sample Size: A total of 90 cases were included in our study after obtaining ethical clearance from the Chairperson of the Institutional Review Board (Letter No. BLDE(DU)/IEC/680/2022-23, dated 30-08-2022). Sixty cases of suspected neonatal sepsis (30 each for early onset and late onset sepsis) admitted to the NICU, Department of Paediatrics, Shri B.M. Patil Medical College, Hospital and Research Centre, BLDE Deemed to be University, Vijayapura, during the period from September 01, 2022, to December 31, 2023, were selected based on clinical features and/or risk factors. Additionally, 30 controls admitted for other causes (non-infectious diseases or observation) and without clinical suspicion of sepsis in the same department during the same period were included. Written informed consent was obtained from the guardian of every patient participating in the study.

To achieve a power of 85% for detecting a difference in proportions between two groups at a two-sided p-value of 0.05 [5], a sample size of 30 per group was determined using Statulator software and the formula:

$$n = \left(\frac{z_\alpha + z_\beta}{MD}\right)^2 2pq$$

Z = Z statistic at a level of significance

M.D. = Anticipated difference between two proportions

P = Common Proportion

q = 100-p

Inclusion Criteria: Neonates having two or more of the following features/factors were presumed clinically to be suspicious of sepsis: Alteration in behavior and established feeding pattern (refusal to suck/lethargic). Hypo or hyperthermia, convulsions. Diarrhea/vomiting/abdominal distension. Apnea/tachypnea. Any superficial infection (e.g., umbilical sepsis, pyoderma, etc.). Presence of maternal risk factors (e.g., PROM >12 hours duration, foul-smelling liquor, febrile illness during or within two weeks of delivery, etc.).

Exclusion Criteria: Neonates and infants less than 90 days with the following conditions were excluded from the study:

Respiratory distress due to hyaline membrane disease. Hemolytic disease of the newborn.

Study Tools and Methods: Blood samples were collected at the time of clinical diagnosis of suspected sepsis cases before starting antibiotics. EDTA-mixed venous blood was used to estimate total leukocyte count (TLC), platelet count, and neutrophil indices (absolute neutrophil count [ANC], ratio of immature to total neutrophil count [I/T], neutrophils with toxic granulations). Plain vial blood (clotted blood) was used for the estimation of serum CRP and ferritin levels. Hematological parameters, CRP, and ferritin from the controls were also studied. Blood culture was sent for all suspected cases.

TLC, platelet count, and ANC were determined using an automated cell counter (Sysmex XT 2000i) and corroborated with peripheral blood smear (PBS) examination. Other neutrophil indices were determined using PBS examination. In PBS, 100 leukocytes were classified on a wedge-shaped blood film. The percentage of bands and other immature forms of neutrophils were counted, and the ratio of immature neutrophils to total neutrophils was calculated for the I/T ratio. The absolute neutrophil count (ANC) was obtained using the formula:

$$ANC = \frac{TLC\ x\ (\%\ of\ segmented\ neutrophils + \%\ of\ band\ or\ immature\ forms)}{mm^3}$$

Serum CRP was measured using the Vitros 5,1 FS. Serum ferritin was estimated using the Beckman Coulter Access 2 Chemistry Analyzer. The cutoff values of the tests were taken from the literature, standard textbooks, and diagnostic kits.

Statistical Analysis: Statistical analysis was conducted using SPSS® software and JMP software. A Microsoft Excel spreadsheet was used to enter the data. The results were analyzed using appropriate statistical tests (Z-test for two means/Z-test for two proportions) to calculate the p-value and test statistics (t-statistics). The difference between the means was considered statistically significant if the p-value < 0.05 or t-statistic > 1.96.

Results

Out of a total of 30 neonates in each group, preterm (less than 37 completed weeks of gestational age) neonates were 6 (20%), 11 (36%), and 8 (26%) in the EOS, LOS, and Control groups, respectively. Low birth weight (less than 2.5 kg) neonates were 11 (36%), 10 (33%), and 7 (23%) in the EOS, LOS, and Control groups, respectively. Female neonates were 14 (46%), 16 (53%), and 16 (53%) in the EOS, LOS, and Control groups, respectively.

Of all selected cases, 33 were found to be culture positive, with 14 in the early onset sepsis group and 19 in the late onset sepsis group. E. coli was the predominant bacteria grown (n=8) in the early onset group, whereas coagulase-negative staphylococcus was the predominant bacteria in the late onset group (n=9).

Table 1: Comparison between mean values and proportions of all the tests performed in all the three groups.

Tests	EOS (n=30)	LOS (n=30)	Control (n=30)
TLC (/mm^3)	13876.67	11553.33	12250.0
ANC (/mm^3)	10487.37	7918.36	8841.9
I/T ratio	0.225	0.21	0.097
Platelets (/mm^3)	2.21	1.86	2.42
CRP (mg/dl)	2.03	2.74	0.50
Ferritin (ng/ml)	73.39	88.44	51.79
Proportion of neonates with Toxic granulations. (TG)	16/30 (0.53)	14/30 (0.47)	01/30 (0.033)

The most significant difference in the means and proportion between EOS and Controls was found in I/T ratio (p <0.0001, t-stats:9.1) followed by CRP (p<0.0001, t-stats:5.3) and TG (p<0.0001, t-stats=4.27).

Table 2: Comparison between mean values of the tests in EOS & Control groups.

Tests	EOS (n=30)		Control (n=30)		P value
	Mean Value	S.D.	Mean Value	S.D.	
TLC (/mm^3)	13876.67	7510.62	12250.0	4561.14	0.31
ANC (/mm^3)	10487.37	6277.55	8841.9	3859.12	0.22
I/T	0.225	0.059	0.097	0.054	<0.0001
Platelets (/mm^3)	2.21	1.31	2.42	0.91	0.47
CRP (mg/dl)	2.03	1.55	0.50	0.31	<0.0001
Ferritin (ng/ml)	73.39	35.01	51.79	18.38	0.004

Here the most significant difference was found in I/T ratio ($p<0.00001$, t-stats: 7.32) followed by CRP (<0.0001, t-stats: 5.8) and ferritin ($p<0.0001$, t-stats: 4.2).

Table 3: Comparison between mean values of the tests in LOS & Control groups.

Tests	LOS (n=30)		Control (n=30)		P value
	Mean Value	S.D.	Mean Value	S.D.	
TLC (/mm^3)	11553.33	8085.52	12250.0	4561.14	0.68
ANC (/mm^3)	7918.37	6397.15	8841.9	3859.12	0.501
I/T	0.210	0.065	0.097	0.054	<0.0001
Platelets (/mm^3)	1.86	1.0	2.42	0.91	0.027
CRP (mg/dl)	2.74	2.09	0.50	0.31	<0.0001
Ferritin (ng/ml)	88.44	43.07	51.79	18.38	<0.0001

The difference in the mean values and proportions of the tests done between EOS and LOS groups are insignificant as p values are >0.05 for each mean or proportion difference between the two groups of neonatal sepsis.

Table 4: Comparison between mean values of the tests in EOS & LOS groups.

Tests	EOS (n=30)		LOS (n=30)		P value
	Mean Value	S.D.	Mean Value	S.D.	
TLC (/mm^3)	13876.67	7510.62	11553.33	8085.52	0.25
ANC (/mm^3)	10487.37	6277.55	7918.37	6397.15	0.12
I/T	0.225	0.059	0.210	0.065	0.35
Platelets (/mm^3)	2.21	1.31	1.86	1.0	0.25
CRP (mg/dl)	2.03	1.55	2.74	2.09	0.14
Ferritin (ng/ml)	73.39	35.01	88.44	43.07	0.14

Using standard cut-off values, four tests proved to be statistically significant in differentiating between septic and non-septic babies: CRP, I/T ratio, toxic granules in the neutrophils, and TLC in decreasing order (Table 5). Platelets and ANC were statistically insignificant in differentiating between the groups.

Discussion

Neonatal sepsis remains a grave concern, particularly in developing nations like India, where maternal risk factors such as maternal infections and premature rupture of membranes, along with neonatal risk factors like poor cord care and low birth weight, contribute to its severity. Neonates may present various symptoms, including respiratory distress, hypothermia, irritability, and seizures, posing challenges for early diagnosis [6, 7].

Given the high mortality and morbidity rates among neonates, there's a critical need for diagnostic markers with high sensitivity and specificity. While studies worldwide have examined CRP and other hematological parameters in neonatal sepsis, no single marker possesses all the ideal characteristics for diagnosis. While some studies, like that by Saboohi et al. [1], emphasize the utility of the immature to total neutrophil ratio (I/T ratio) for early-onset sepsis, others, such as Rohil et al. [3], highlight the superior accuracy of C-Reactive Protein in identifying late-onset neonatal sepsis, particularly in preterm newborns. Jethani et al. [5], in their analysis of 80 cases, found elevated levels of I/T ratio, absolute neutrophil count, and C-Reactive Protein in neonatal sepsis cases compared to controls.

Table 5: Tests with their results (using standard cut offs values) in the three groups

Tests	EOS (n=30)	LOS (n=30)	EOS+LOS (n=60)	Control (n=30)	P value &(t-stats*)
TLC					
Positive test (<5000/mm^3)	05 (16.6%)	08 (26.6%)	13 (21.6%)	01 (3.3%)	0.023
Negative test (>/= 5000/mm^3)	25	22	47	29	(t-stats=2.26)
ANC					
Positive test (<1750/mm^3)	02 (6.7%)	04 (13.3%)	06 (10%)	00 (0.0%)	0.073
Negative test (>1750/mm^3)	28	26	54	30	(t-stats=1.79)
I/T Ratio					
Positive test (>0.2)	23 (76.6%)	20 (66.7%)	43 (71.6%)	01 (3.3%)	<0.00001
Negative test (<0.2)	07	10	17	29	(t-stats=6.08)
Toxic Granules					
Positive test (Present)	16 (53.3%)	14 (46.6%)	30 (50%)	01 (3.3%)	0.0001
Negative test (Absent)	14	16	30	29	(t-stats=4.42)
Platelet Count					
Positive test (<100000/mm^3) Negative test (>100000/mm^3)	07 (23.3%) 23	05 (16.6%) 25	12 (20%) 48	02 (6.7%) 28	0.082 (t-stats=1.73)
CRP					
Positive test (>1.0mg/dl)	21 (70%)	26 (86.6%)	47 (78.3%)	03 (10%)	<0.00001
Negative test(<1.0mg/dl)	09	04	13	27	(t-stats=6.11)

Table 6: Sensitivity, Specificity, Positive & Negative Predictive values of the tests for sepsis (both EOS + LOS)

Tests	Sensitivity	Specificity	PPV	NPV
TLC	22%	97%	93%	38%
ANC	10%	100%	100%	36%
I/T	72%	97%	98%	64%
TG	50%	97%	97%	50%
Platelets	20%	93%	86%	37%
CRP	78%	90%	94%	68%
CRP + I/T	63%	100%	100%	58%

Table 7: Sensitivity, Specificity, Positive & Negative Predictive values of the tests for EOS & LOS

Tests	Sensitivity		Specificity		PPV		NPV	
	EOS	**LOS**	**EOS**	**LOS**	**EOS**	**LOS**	**EOS**	**LOS**
TLC	17%	27%	97%	97%	83%	89%	54%	57%
ANC	07%	13%	100%	100%	100%	100%	52%	54%
I/T	77%	67%	97%	97%	96%	95%	81%	74%
TG	53%	47%	97%	97%	94%	93%	67%	64%
Platelets	23%	17%	93%	93%	78%	71%	55%	53%
CRP	70%	87%	90%	90%	88%	90%	75%	87%

The main aim of our study was to delineate differences in these parameters between neonatal sepsis groups and explore ferritin's potential as a sepsis marker. In our study, C-reactive protein (CRP) exhibited the most significant difference between the means of septic and non-septic neonates, particularly noticeable between the Late-Onset Sepsis group and the non-infected group (Table 3). Similarly, the Immature to Total Neutrophil Ratio (I/T ratio) showed a significant difference between septic and non-septic neonates, especially between the Early-Onset Sepsis (EOS) group and the non-infected group (Table 2). Ferritin levels also displayed a notable distinction between septic and non-septic neonates, particularly pronounced between the LOS group and the non-infected group in our study (Table 3). However, while most tests showed differences in mean values between EOS and LOS groups, these differences did not reach statistical significance (Table 4).

CRP demonstrated the highest sensitivity at 78%, followed by the I/T ratio at 72%, although CRP's specificity was not optimal (Table 6). The Absolute Neutrophil Count (ANC) exhibited the highest specificity at 100% but the lowest sensitivity at 10% among the tests. ANC, I/T ratio, and toxic granulations all displayed high specificity of 100%, 97%, and 97% respectively (Table 7). Toxic granulations in neutrophils showed an average sensitivity of 50% and high specificity of 97%, consistent with findings from previous studies [7]. ANC also had the highest positive predictive accuracy of 100%, followed by I/T ratio and toxic granulation, having 98% and 97% respectively (Table 6). When combining results from two tests, specificity and positive predictive accuracy increased, albeit at the expense of decreased sensitivity. The combination of CRP with the I/T ratio was particularly effective in distinguishing between septic and non-septic neonates, with a sensitivity of 63%, specificity of 100%, and positive predictive accuracy of 100% (Table 6).

In our study, the I/T ratio was the most sensitive test in the EOS group, with a sensitivity of 77%, and CRP was the most sensitive test in the LOS group, with a sensitivity of 87% (Table 7), both with a specificity of over 90%. Combining CRP with the I/T ratio further increased specificity to 100%. Leucopenia was more common in the LOS group than in the EOS group, contrasting with findings from a study conducted by Alexejew B et al. [9]. Additionally, ANC was below the cutoff value in only 10% of septic babies in our study, lower than in most other studies [7, 8, 10]. In the present study, ferritin level was found to be significantly elevated in the septic group of neonates (in both EOS and LOS, more in the latter group) than in the non-septic neonates (control group). Godula-Stuglik U et al. [10] made a similar observation, finding levels of ferritin to be higher in neonates with septicemia and pneumonia than in controls. As there is no cutoff value for ferritin in neonatal sepsis, sensitivity, specificity, and other metrics could not be calculated. However, if we had taken the mean value of ferritin in the control group of the present study (51.8) as the cutoff value, then sensitivity, specificity, positive predictive accuracy, and negative predictive accuracy would be 75%, 57%, 78%, and 53% respectively. Ferritin was raised in 85.1% (n=40) of septic cases in which CRP was also raised (n=47). Further exploration of ferritin's diagnostic utility is warranted to better understand its role in the early detection and management of sepsis in neonates.

A total of seven diagnostic parameters were assessed in our study to determine their changes in neonatal sepsis and their efficacy when used alone and in combination. The I/T ratio, CRP, and toxic granulations were identified as useful due to their acceptable efficacy and cost-effectiveness, particularly in developing countries where neonatal sepsis is a significant cause of neonatal mortality.

Limitations of the study: While the study offers valuable insights into diagnosing neonatal sepsis, it's essential to recognize its limitations. The relatively small sample size of 90 cases could restrict the broad applicability of the findings. Additionally, selecting cases from a single region or hospital may introduce bias, affecting the representativeness of the study population.

Although the study examines various biomarkers, it may not cover the full spectrum of potential diagnostic markers for neonatal sepsis. Exploring additional biomarkers such as procalcitonin or Interleukin-6, or molecular diagnostics, could provide a more comprehensive understanding. Unaccounted confounding factors like maternal health status or comorbidities might impact diagnostic parameters. Lastly, the absence of universally accepted cut-off values for ferritin in neonatal sepsis complicates interpretation, while subjective variation in calculating the immature to total neutrophil (I/T) ratio through peripheral blood smear (PBS) examination introduces inconsistency, affecting diagnostic accuracy. Addressing these limitations calls for larger, multicenter studies integrating diverse biomarkers and standardized methodologies to bolster diagnostic reliability and applicability.

Conclusion

In conclusion, while numerous markers for diagnosing neonatal sepsis exist, the quest for the ideal one continues. This study identified three tests—CRP, I/T ratio, and toxic granulations in neutrophils—as useful tools for promptly diagnosing neonatal sepsis cases. Specifically, the I/T ratio proved most beneficial for early-onset sepsis, while CRP was more effective for late-onset sepsis. Overall, CRP emerged as the most reliable single marker for neonatal sepsis and can be utilized either in combination (for early-onset) or independently (for late-onset) with satisfactory efficacy. Moreover, significant disparities in ferritin levels were observed between septic and non-septic groups, indicating a need for further research to ascertain its diagnostic value in neonatal sepsis.

Recommendations: Clinicians should integrate C-reactive protein (CRP) testing for late-onset sepsis (LOS) diagnosis, while utilizing immature to total neutrophil (I/T) ratio and toxic granules analysis for early-onset sepsis (EOS) detection. Combining CRP, I/T ratio, and toxic granules analysis can provide a comprehensive approach to sepsis diagnosis, particularly in cases with ambiguous clinical presentations. Rapid and accurate diagnosis of neonatal sepsis can facilitate appropriate antibiotic treatment, thereby mitigating antibiotic resistance and overuse. Clinicians should adhere to antibiotic stewardship principles and utilize diagnostic tools judiciously to minimize unnecessary antibiotic exposure in neonates.

Ethical Approval: Approved by IEC via letter no. BLDE(DU)/IEC/680/2022-23, dated 30-08-2022.

Acknowledgement: I would like to thank Dr. Prakash M. Patil, Professor, Department of Pathology, and Dr. Siddu Charki, Assistant Professor, Department of Paediatrics, for their immense support and valuable guidance throughout the study. I would also like to thank Dr. Vijaya M. Sorganvi, Associate Professor in Statistics, for helping me in calculating statistics in this study. Last but not least, I would like to thank my Head of Department, Dr. Surekha B. Hippargi, and all the laboratory technicians for their support and assistance in completing this study.

Funding: *None*

Competing Interests: *There are no conflicts of interest in this study.*

References

1. Saboohi E, Saeed F, Khan RN, Khan MA. Immature to total neutrophil ratio as an early indicator of early neonatal sepsis. Pak J Med Sci Q [Internet]. 2019;35(1):241–6.
2. Abdelmoktader A, Hussein S, Ali D, Eisa O. Diagnostic value of eosinopenia and neutrophil to lymphocyte ratio in early onset neonatal sepsis. Fayoum Univ Med J [Internet]. 2020;7(1):161–71.
3. Rohil A, Dutta S, Varma N, Sachdev MS, Bansal A, Kumar P. Cell-surface biomarkers, C-reactive protein and hematological parameters for diagnosing late-onset sepsis in pre-term neonates. J Trop Pediatr. 2021;67(1).

4. Li X, Li T, Wang J, Feng Y, Ren C, Xu Z, et al. Clinical value of C-reactive protein/platelet ratio in neonatal sepsis: A cross-sectional study. J Inflamm Res [Internet]. 2021;14:5123–9.
5. Jethani S, Bhutani N, Yadav A. Diagnostic utility of combined immature and total neutrophil counts along with C-reactive protein in early detection of neonatal sepsis: A cross-sectional study. Ann Med Surg (Lond) [Internet]. 2022;77:103589.
6. Worku M, Aynalem M, Biset S, Woldu B, Adane T, Tigabu A. Role of complete blood cell count parameters in the diagnosis of neonatal sepsis. BMC Pediatr [Internet]. 2022;22(1):411.
7. Mannan MA, Shahidullah M, Noor MK, Islam F, Alo D, Begum NA. Utility of CRP and haematological parameters in detection of neonatal sepsis. Mymensingh Med J. 2010;19(2):259–63.
8. Shyamala K, Subbalakshmi NK, Raghuveera K. Role of platelet count and CRP level in Gram negative versus Gram positive bacterial sepsis in low birth weight neonates. J Chin Clin Med. 2010;5(8):474–9.
9. Alexejew B, Handrick W, Braun B, Domule M, Winiecki P. [Paediatr Grenzgeb. 1990;26(6):443–54. German].
10. Godula-Stuglik U, Skałba P, Witalis J. Evaluation of serum ferritin levels in newborn infants with septicemia and pneumonia. Pediatr Pol. 1986;61(12):798–802.