

A prospective study on reliability and effectiveness of different hematological parameters for early diagnosis of neonatal sepsis in a tertiary care hospital of sub-Himalayan region



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ABSTRACT

Background: Neonatal sepsis is a major cause of morbidity and mortality in newborn. Early diagnosis of neonatal sepsis is the top priority to all clinicians for early initiation of appropriate treatment which will reduce the morbidity and mortality in neonates and minimizes the unnecessary antibiotic therapy which will lead to overwhelming threat of multidrug-resistant organism. **Aims and Objectives:** The recent study was undertaken to study the reliability and effectiveness of hematological parameters and Rodwell's hematological score for early diagnosis of sepsis in neonates. **Materials and Methods:** In this study, 212 neonates were studied out of which 88 were blood culture proven sepsis and 39 were culture negative but hematological parameters positive probable sepsis. The rest 85 were both culture negative and hematological parameter negative and were considered normal baby and taken as control group in this study. Hematological parameters were studied in all cases included in the study which include total leukocyte count, absolute neutrophil count, immature/total neutrophil ratio, platelet count, C-reactive protein (CRP), and other components of Rodwell's scoring criteria. Recent statistical analysis of all the components and hematological scoring system (HSS) done to find out the reliability and effectiveness. **Results:** Analysis of hematological parameters showed that absolute neutrophil count, immature to total neutrophil ratio and platelet count have a better sensitivity and specificity in relation to other parameters. CRP and total leukocyte count have better specificity but sensitivity was very low. In the HSS, it has been shown that this scoring system was highly sensitive and specific with a high positive predictive value and negative predictive value. Among the different parameters studied, association of total leukocyte count ($P < 0.0001$) and reduced platelet count ($P < 0.0001$) is the most consistent indicator found in this study but association with CRP was found to be statistically insignificant ($P = 0.0703$). **Conclusion:** HSS system, total leukocyte count, absolute neutrophil count and platelet count are very reliable indicator for early diagnosis of neonatal sepsis which is cheap, simple, less time consuming, and cost effective.

Key words: Sepsis; Neonate; Hematological parameter; TLC; Platelet count

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INTRODUCTION

Neonatal sepsis is one of the common causes of neonatal mortality contributing to 23% of all neonatal deaths.¹ It is the most common clinical disease category in neonates

with an incidence of 24/1000 live birth. According to data from National Neonatal Perinatal Database 2000 in India, the incidence of neonatal sepsis has been reported to be 38/1000 intramural live birth in tertiary care institution.¹ It is the most common cause of neonatal mortality and is

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probably responsible for 30–50% of total neonatal deaths each year in the developing countries.^{2,3} Neonatal sepsis is clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the 1st month of life which affects multiple systems and set out a systemic inflammatory response and multiple organ damage. The initial clinical presentation is very subtle and needs high index of suspicion for diagnosis. There is no particular hematological test or clinical sign to confirm a neonate has sepsis. Early diagnosis and initiation of treatment is the mainstay to reduce the morbidity and mortality. This forces every clinician to initiate early antimicrobials in neonates before diagnosis which sometimes leads to the development of multidrug resistance. Although blood culture is the gold standard for diagnosis of sepsis, it yields positive results only in 35–75% of cases.⁴ Moreover it requires 36–72 h time to become positive, low inoculum in the sample is not suitable for culture, inability of the laboratory to identify all organisms and culture become negative in some spurious organisms.⁵ In recent years, there are some sophisticated new biomarkers very useful for diagnosis such as serum amyloid A, cytokines, and chemokines, but these are very costly and out of reach to most of the institutions.⁶ Hence, it is a both sided problem. Late initiation of treatment after blood culture positivity will raise case fatality and early administration of antimicrobials in undiagnosed cases leads to serious antibiotic resistance in some cases, seeking an effective, cheap, rapid, and reliable diagnostic screening tool which will direct clinicians to initiate treatment early.⁷ There are different hematological parameters which will used in diagnosis of culture negative sepsis such as total leukocyte count, different neutrophil parameters, platelet count, and C-reactive protein (CRP). However, these parameters in single do not have much clinical significance. Rodwell et al., studied individual parameters and developed hematological scoring system (HSS) which combined different components of hematological parameters.⁸ Individually each parameter does not have much significance but a combination of them has excellent sensitivity and specificity.^{7,9} Different studies observed that diagnostic ability of neutropenia to be higher than leukocytosis in neonatal sepsis, so that neutropenia has been given importance and given more scoring accordingly to the scoring system.^{10,11} Rodwell's HSS is the helpful tool which will help neonatologists to initiate early treatment.

Aims and objectives

The aim of the study is to find out the effectiveness and rationality of different hematological parameters and Rodwell's HSS which is quite rapid and can be done at bed side/side laboratory so that early initiation of treatment can be possible.

MATERIALS AND METHODS

This study was a prospective study done in SNCU and NICU of the Department of Pediatrics, North Bengal Medical College and Hospital, Darjeeling, India, for 9 months from January 2021 to September 2021 after taking the institutional ethical clearance. A total of 212 neonates fulfilling the inclusion criteria were included in the study.

Inclusion criteria

The study included all the neonates having features of sepsis and those neonates who had predisposing factors for occurrence of sepsis such as maternal fever in intrapartum period, gross prematurity, premature rupture of membrane, foul smelling liquor, and multiple internal examinations.

Exclusion criteria

The following babies were excluded from the study:

- Major congenital anomaly
- Pathological jaundice
- Suspected inborn errors of metabolism or metabolic disorder
- Respiratory distress syndrome (surfactant deficiency)
- Who received antibiotics before taking samples for blood culture
- Not given consent for the study.

Informed consent was taken from the parents of all neonates included in the study. About 3.5–4 mL of blood was drawn taking aseptic measures from all neonates as early as possible after admission. One mL of blood was anticoagulated with EDTA. Another 1 mL of blood was collected in red vacuum container and allowed to rest for ½ h to become coagulated. The rest 1.5–2 mL of sample was inoculated in the blood culture bottle and all the samples were sent to the department of pathology and microbiology accordingly. The analysis of the samples findings was done by pathologists and microbiologists blinded to the infection status of the neonates. The report of hematological parameters was available within 6–12 h after sending the samples. Results were checked by inter departmental digital platform. Blood culture report was found at least 48–72 h after sending the samples.

The hematological work up includes complete blood count, total leukocyte count, total neutrophil count, and platelet count. Absolute neutrophil count was calculated from observed values of the neonatal hematological parameters of Monroe et al.,¹² who were used as the standard values. Repeat sampling was done on day 3 for total leukocyte count, platelet count, and CRP of those neonates who found to be blood culture positive immediately after getting culture report to determine

significance of these parameters. The parameters were applied on hematological score as demonstrated by Rodwell et al (Tables 1 and 2).

Sensitivity, specificity, and positive predictive value of each hematological parameters and HSS were calculated using statistical analysis. For statistical analysis, data were entered into a Microsoft Excel spreadsheet and then analyzed by SPSS 24.0 and GraphPad Prism version 5. Data had been summarized as mean and standard deviation for numerical variables and percentages for categorical variables. Hematological scoring system was calculated for every neonates included in the study.

RESULTS

Among the admitted neonates in the SNCU and NICU in the study period, only 212 neonates were selected for the study and were analyzed. Out of the 212 neonates, 133 were preterm. Blood culture-positive sepsis comes out to be positive in 88 patients. Culture negative but sepsis screen positive in 39 neonates (probable sepsis) and the rest 85 neonates were both culture negative and screen negative and were taken as control group. A significant finding in this study was preterm babies were predominant in both culture-positive (69%) and culture-negative sepsis (56%).

Analysis of hematological parameters showed (Table 3) that absolute neutrophil count, immature to total neutrophil

ratio, and platelet count have a better sensitivity and specificity in relation to other parameters. CRP and total leukocyte count have better specificity but sensitivity was very low.

Among the 88 culture-positive neonates, 75(85%) had hematological score ≥ 5 and 13(15%) had score 3–4. Hence, all patients were HSS positive. In the probable sepsis group (n=39), 14(36%) had score ≥ 5 and 15(38%) had scores 3–4. Hence, out of 39 neonates, 29(74%) had HSS positive. Hence, in the HSS, it has been shown that it is highly sensitive and specific with a high positive predictive value and negative predictive value (Table 5).

At day 1, the mean TLC (mean \pm SD) of patients was 8051.1364 ± 2217.1740 /cu.mm with range 2000.0000–14,000.0000/cu. mm and the median was 7800.0000/cu.mm. At day 3, the mean TLC (mean \pm SD) of patients was 5270.4545 ± 1548.7599 /cu.mm with range 2000.0000–10,000.0000/cu.mm and the median was 5400.0000/cu.mm (Table 6). Association of TLC in D1 and D3 for culture-positive (Table 1) neonates was statistically significant ($P < 0.0001$).

At day 1, the mean platelets (mean \pm SD) of patients were 2.7590 ± 1.0137 lacs/cu.mm with range 0.4100–4.2000 lacs/cu.mm and the median was 3.1000 lacs/cu.mm. At day 3, the mean platelets (mean \pm SD) of patients were 1.2710 ± 0.6879 lacs/cu.mm with range 0.3400–4.0000 lacs/cu.mm and the median was 1.1000 lacs/cu.mm (Table 7). Association of platelets in D1 and D3 for culture-positive neonates was statistically significant ($P < 0.0001$).

Table 1: Hematological scoring system as defined by Rodwell et al.⁸

Criteria	Parameters	Score
Total leukocyte count	≤ 5000 /ul	1
	$\geq 25,000$ at birth	1
	$\geq 30,000$ after 12–24 h	
Total neutrophil count (normal value 1800–5400)	Immature PMN	2
	Increased/decreased	1
Immature PMN count (normal value 600)	Increased	1
I: T PMN ratio (normal value 0.12)	Increased	1
I: M PMN ratio	≥ 0.3	1
Degenerative changes in PMN	Toxic granules/cytoplasmic vacuole present	1
Platelet count	$\leq 150,000$	1

Table 2: Interpretation

Score	Interpretation
≤ 2	Sepsis unlikely
3 or 4	Probable sepsis
≥ 5	Sepsis very likely

Table 3: Sensitivity and specificity of hematological parameters of babies with suspected sepsis (n=212)

Hematological tests	Sensitivity (%)	Specificity (%)
Total leukocyte count increased/decreased	65	84.4
Absolute neutrophil count increased/decreased	89	76
I: T ratio	90	85
Platelet count $150,000 \text{ mm}^3$	90	82.6
CRP	26	86

Normal values are defined by reference range of Monroe et al.¹²

Table 4: Distribution of cases according to sepsis score (n=212)

No of Cases	Scores 0–2	Scores 3–4	Score ≥ 5
Sepsis (88)	0	13 (15%)	75 (85%)
Probable sepsis (39)	10 (26%)	15 (38%)	14 (36%)
Normal (85)	56 (64.7%)	15 (17.6%)	14 (16.4%)
Total (212)	66	43	103

At day 1, the mean CRP (mean±SD) of patients was 10.8784±7.6322 mg/L with range 3.2000–34.0000 mg/L and the median was 6.9000 mg/L. At day 3, the mean CRP (mean±SD) of patients was 13.0193±7.9607 mg/L with range 4.5000–35.4000 mg/L and the median was 6.4000 mg/L (Table 8). Association of CRP in D1 and D3 for culture-positive neonates was not statistically significant (P=0.0703).

DISCUSSION

Blood culture is the gold standard for diagnosis of neonatal sepsis. However, it is positive in less number of cases and is a time-consuming procedure. This study finds out the usefulness of total leukocyte count, absolute neutrophil count, immature to total neutrophil ratio and platelet count as early indicator of neonatal sepsis. Rodwell's HSS also found to be statistically significant in this study. These hematological tests are cheap, simple, cost effective and much less time consuming which is readily available in all laboratory setup. Although various tests are useful as a diagnostic tool for neonatal sepsis,

the complete blood count with differentials is widely used either singly or in conjunction with other tests for diagnosis. The criteria of Manroe et al., are the most reliable of the published criteria evaluated which will help to identify most of the cases of neonatal sepsis.¹² Among the different parameters studied, association of total leukocyte count (P<0.0001) and reduced platelet count (P<0.0001) are the most consistent indicator found in this study but association with CRP was found to be statistically insignificant (P=0.0703). Choudhary et al., found similar results in their study regarding importance of low platelet count for diagnosis of neonatal sepsis.¹³ CRP usually getting rise after 6 h of infection and serial monitoring after 24–48 h after onset of sepsis improves sensitivity (by 82% and 84%, respectively).⁶ This finding is contradictory to the finding of this study. The advantages of HSS in this study are that it has a high sensitivity, specificity, positive predictive value and negative predictive value which will add the accuracy for diagnosis in case of culture-negative sepsis. This findings is supportive of different studies about HSS.^{14,15,16} Hence, we can easily make a criteria using these parameters and HSS in the future to make early diagnosis of sepsis in neonates to initiate judicious early treatment.

Table 5: Distribution of cases according to sepsis on blood culture and sepsis on hematological score

No fo Cases	Positive culture	Negative culture	Total
Positive HSS	75 (85.2%)	28 (22.5%)	103
Negative HSS	13 (14.7%)	96 (77.4%)	109
Total	88	124	212

Sensitivity of HSS 85.2%, Specificity of HSS 77.4%, Positive predictive value 72.8%, Negative predictive value 88%, HSS: Hematological scoring system

Limitations of the study

Some limitations are also there in this study. Only the cases admitted in a tertiary care hospital are evaluated, so the large district level hospital and community scenario may be different. This study should be followed up and should be performed on a larger cohort to find out reliability and effectiveness of different haematological parameters and HSS.

Table 6: Distribution of mean TLC in D1 and D3 for blood culture-positive neonates

TLC on No of Day	Number of cases	Mean	SD	Minimum	Maximum	Median	P-value
Day 1	88	8051.1364	2217.1740	2000.0000	14000.0000	7800.0000	<0.0001
Day 3	88	5270.4545	1548.7599	2000.0000	10000.0000	5400.0000	

Table 7: Distribution of mean platelets in D1 and D3 for blood culture-positive neonates

Platelets on No of Day	Number	Mean	SD	Minimum	Maximum	Median	P-value
Day 1	88	2.7590	1.0137	0.4100	4.2000	3.1000	<0.0001
Day 3	88	1.2710	0.6879	0.3400	3.2000	1.1000	

Table 8: Distribution of mean CRP in D1 and D3 for blood culture-positive neonates

CRP on No of Day	Number	Mean	SD	Minimum	Maximum	Median	P-value
Day 1	88	10.8784	7.6322	3.2000	34.0000	6.9000	0.0703
Day 3	88	13.0193	7.9607	4.5000	35.4000	9.6500	

CRP: C-reactive protein

CONCLUSION

As there are no single haematological investigations to diagnose neonatal sepsis very early, it can be given in conclusion that HSS with reduced platelet count can be a good parameter for early diagnosis of sepsis in neonates to reduce morbidity and case fatality.

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REFERENCES

1. Report of the National Neonatal Perinatal Database. Delhi: National Neonatology Forum; 2000.
2. Bang AT, Bang RA, Bactule SB, Reddy HM and Deshmukh MD. Effect of homemade neonatal care and management of sepsis on neonatal morbidities: Field trial in rural India. *Lancet*. 1999;354(9194):1955-1961. [https://doi.org/10.1016/S0140-6736\(99\)03046-9](https://doi.org/10.1016/S0140-6736(99)03046-9)
3. Stoll BJ. The global impact of neonatal infection. *Clin Perinatol*. 1997;24(1):1-21.
4. Hornik CP, Benjamin DK, Becker KC, Benjamin DK Jr., Li J, Clark RH, et al. Use of complete blood cell count in the late-onset sepsis. *Pediatr Infect Dis J*. 2012;31(8):803-807. <https://doi.org/10.1097/INF.0b013e31825691e491e4>
5. Michel M, Brown LS and Rosenfeld CR. Serial neutrophil values facilitate predicting the absence of neonatal early-onset sepsis. *J Pediatr*. 2014;164(3):522-528.e1-3.
6. Bhandari V. Effective biomarkers for diagnosis of neonatal sepsis. *J Pediatr Infect Dis Soc*. 2014;3(3):234-245. <https://doi.org/10.1093/jpids/piu063>
7. Narasimha A and Kumar MH. Significance of haematological scoring system(HSS) in early diagnosis of neonatal sepsis. *Indian J Hematol Blood Transfus*. 2011;27(1):14-17. <https://doi.org/10.1007/s12288-010-0050-2>
8. Rodwell RL, Taylor KM, Tudehope DI and Gray PH. Hematologic scoring system in early diagnosis of sepsis in neutropenic newborns. *Pediatr Infect Dis J*. 1993;12(5):372-376. <https://doi.org/10.1097/00006454-199305000-00004>
9. Bhat YR and Rao A. The performance of haematological screening parameters and CRP in early onset neonatal infections. *J Clin Diagn Res*. 2010;4(6):3331-3336.
10. Chiesa C, Panero A, Osborn JF, Simonetti AF and Pacifico L. Diagnosis of neonatal sepsis: A clinical and laboratory challenge. *Clin Chem*. 2004;50(2):279-287. <https://doi.org/10.1373/clinchem.2003.025171>
11. Paolucci M, Landini MP and Sambri V. How can the microbiologist help in diagnosing neonatal sepsis? *Inter J Pediatr*. 2012;2012:120139. <https://doi.org/10.1155/2012/120139>
12. Manroe BL, Weinberg AG, Rosenfeld CR and Browne R. The neonatal blood count in health and disease.I. Reference values for neutrophilic cells. *J Pediatr*. 1979;95(1):89-98. [https://doi.org/10.1016/s0022-3476\(79\)80096-7](https://doi.org/10.1016/s0022-3476(79)80096-7)
13. Choudhary DK, Tiwari AK, Narang S and Chhabra J. Correlation of platelet count and plate indices with neonatal sepsis-diagnostic and prognostic indicator. *J Pediatr Res*. 2017;4(8):511-518.
14. Saleem M, Shah KI, Cheema SM and Alam M. Hematological scoring system for early diagnosis of neonatal sepsis. *J Rawalpindi Med Coll*. 2014;18(1):68-72.
15. Manucha V, Rusia U, Faridi M and Madan N. Utility of haematological parameters and C-reactive protein in the detection of neonatal sepsis. *J Paediatr Child Health*. 2002;38(5):459-464. <https://doi.org/10.1046/j.1440-1754.2002.00018.x>
16. Gandhi P and Kondekar S. A review of the different haematological parameters and biomarkers used for diagnosis of neonatal sepsis. *EMJ Hematol*. 2019;7(1):85-92.

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