Original Article

Peripheral Blood Smear Can Be a Reliable and Inexpensive Alternative to Blood Culture in Early Diagnosis of Neonatal Sepsis

Arijit Majumdar[1], Soumali Biswas[2], Angshuman Jana[3]

[1]Department of Pathology, DMGMC & H, Purulia, West Bengal, [2]Department of Anatomy, Institute of Post-Graduate Medical Education and Research, Kolkata 700020, West Bengal, [3]Department of Microbiology, Bankura Sammilani Medical College, Bankura, West Bengal, India

Abstract

Background: Neonatal sepsis is one of the major causes of morbidity and mortality in newborns, more so in the developing countries. **Objectives:** The aim of this article is to study the role of peripheral smear, to evaluate the usefulness of white blood cell (WBC) parameters, and to establish the sensitivity and specificity of each parameter for early diagnosis of neonatal septicemia. **Materials and Methods:** The prospective study is of 150 neonates admitted and fulfilling the inclusion criteria, over the period of June 2019 to December 2020, and tests were performed at the Department of Pathology in a tertiary medical college and hospital. Peripheral blood sample was obtained by venipuncture and the sepsis work-up included routine blood counts and blood culture. Total leucocytes counts were counted on an automated hematology analyzer. Leishman-stained peripheral blood smears were used for the study. **Results:** Based on the clinical findings and laboratory data, neonates were classified into three categories: sepsis (n = 70), probable infection (n = 50), and no infection (n = 30). The blood culture reports of confirmed sepsis cases reveal the commonest organism as *Escherichia coli*, followed by *Klebsiella*, *Pseudomonas*, and beta-hemolytic streptococci. Abnormal *I:T* ratio (≥0.2) shows highest sensitivity (90%) and specificity (95%). **Conclusion:** The peripheral smear study is found to be very useful in the early diagnosis of neonatal septicemia as it can be performed even in a small laboratory without any special equipment and within a short period of time. Among WBC parameters, the *I:T* ratio shows highest sensitivity and specificity.

Keywords: Hematological scoring, neonatal septicemia, peripheral smear

Introduction

The peripheral blood smear (PBS) is a laboratory investigation to study the peripheral blood cells. It is a basic yet invaluable tool in screening, diagnosis, and monitoring of disease progression and therapeutic response. The diagnostic relevance of PBS is not limited to hematologic disorders, but encompasses some primarily non-hematologic conditions such as neonatal sepsis. Sepsis is a leading cause of morbidity and mortality in children. Neonatal sepsis is a clinical manifestation of a systemic infection during the first 28 days of life.[1]

Early diagnosis of neonatal septicemia is a problem yet to be solved properly because of its non-specific and/or subtle clinical picture. Sensitivity and positive predictive value (PPV) of biomarkers at the onset of symptoms are suboptimal. Thus fatal septicemia may occur with little warning. Hence, the timely diagnosis of sepsis in neonates is critical as the illness can be rapidly progressive and in some instances fatal. Blood culture positivity, though, is the yardstick for declaring septicemia in newborns and adults; it takes 48–72 h and demands a well-equipped laboratory which is unavailable everywhere in developing countries like ours. Further, yield of blood culture is 30–70%, so some neonates may be missed.[2] Clinical

Access this article online

Quick Response Code:

Website: www.medjbabylon.org

DOI: 10.4103/MJBL.MJBL_56_21

Address for correspondence: Dr. Arijit Majumdar, C/o Sanat Kumar Majumdar, Opposite Duilya Panchayat Office, Charaktala, Mourigram, Andul, Howrah 711302, West Bengal, India. E-mail: arijitmajumdar23@gmail.com

Submission: 25-July-2021 **Accepted:** 06-Oct-2021 **Published:** 17-Dec-2021

How to cite this article: Majumdar A, Biswas S, Jana A. Peripheral blood smear can be a reliable and inexpensive alternative to blood culture in early diagnosis of neonatal sepsis. Med J Babylon 2021;18:371-6.

suspicion therefore frequently leads to empirical antibiotic therapy in uninfected infants which ultimately results in antibiotic resistance. Novel markers such as interleukin-6, interlukin-8, and plasma-elastase are more sensitive in early diagnosis but are expensive and not routinely available.[3] So, the significance of various screening tests, either singly or in combination, is observed.

Various studies have shown that hematological parameters are simple, quick, and cost-effective tools in the early diagnosis of neonatal sepsis, thus helping to initiate early treatment with appropriate antibiotics.[4] When these were studied together as combination of tests, sensitivity and specificity both increased. Rodwell *et al.*[5] developed a hematological scoring system (HSS) based on the white blood cell (WBC) count, degenerative changes in neutrophils, total and immature neutrophil counts and ratios, and thrombocytopenia. In that system, sensitivity was 96% and negative predictive value (NPV) was 99%. They concluded that the HSS should improve the diagnostic accuracy so that it can be used as a screening test for sepsis and could make interpretation of test easy and simple. In the present study, we evaluated the utility of the peripheral smear, a HSS along with certain tests like C-reactive protein (CRP) aids in early diagnosis of neonatal sepsis in a cost-effective manner that can be useful, particularly in developing countries such as India.

Materials and Methods

The prospective study is of 150 neonates admitted and fulfilling the inclusion criteria (having suspicious of sepsis clinically with other tests being negative), during the period of June 2019 to December 2020, and tests were performed at the Department of Pathology, Dr B. C. Roy PGIPS, Kolkata, West Bengal, India. Neonates were divided into three groups mainly, Group 1 (proven sepsis): neonates with sepsis (with positive blood culture), Group 2 (probable sepsis): neonates with probable infection (with strong clinical history and negative blood culture), Group 3 (no sepsis): normal neonates (with minimal or no signs of sepsis). Peripheral blood sample was obtained by venipuncture and the sepsis work-up included routine blood counts and blood culture. The total leucocyte counts were counted on an automated hematology analyzer (Sysmex KX-21). For every sample, a peripheral smear was prepared [Figure 1] and was stained with Leishman's stain [Figure 2]. The WBC count was corrected for nucleated red cells, and a differential count was performed manually. The polymorphonuclear (PMN) leucocytes were also examined for degenerative morphological changes such as toxic granulation, toxic vacuolization [Figure 3], ring neutrophils [Figure 4], etc.

The hematological findings were analyzed according to the HSS of Rodwell *et al.*[5]

Hematologic scoring system (HSS)

Criteria	Abnormality	Score
I:*T* >0.2	Increase	1
Total PMN count	Increase/decrease	1
I:*M*	≥0.3	1
Immature PMN count	Increase	1
Total WBC count	Increase/decrease (≥5000/cmm or 25,000, 30,000, and 21,000/cmm at birth, 12–24 h, and day 2 onwards, respectively)	1
Degenerative changes in PMN	≥0.3+	1
Platelet count	≤100,000/cmm	1

Interpretation

Score	Interpretation
≤2	Sepsis is very unlikely
3/4	Sepsis is suspected
≥	Sepsis is very likely

CRP levels were also recorded. This test was done in the immunology laboratory by the latex agglutination method. Blood culture and CRP results of the same patient were compared with the hematological parameters. Culture positivity was taken as the criterion for definitive diagnosis.

The data collected were statistically analyzed. Sensitivity, specificity, PPVs, and NPVs were calculated for each parameter. The research work was approved by the Institutional Ethical Committee, and the informed consent was also obtained from the parents of all the neonates.

Results

Based on clinical findings and laboratory data, infants were classified into three categories: sepsis (n = 70), probable infection (n = 50), and no infection (n = 30)

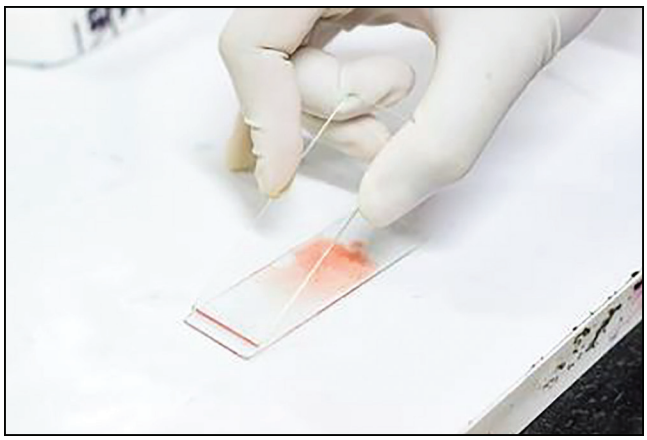

Figure 1: Drawing PBS

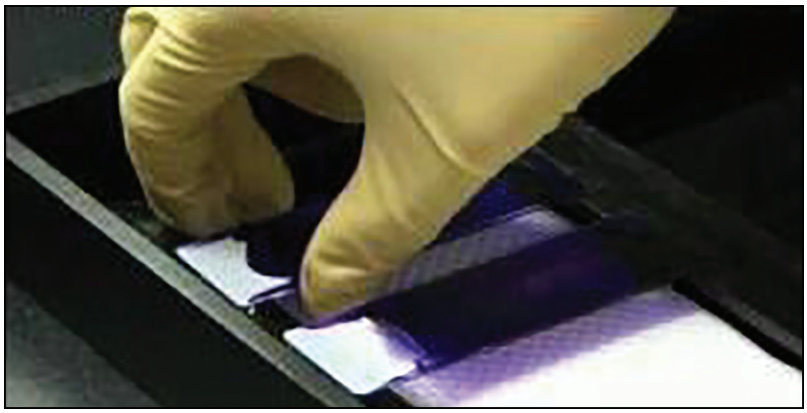

Figure 2: Staining of PBS

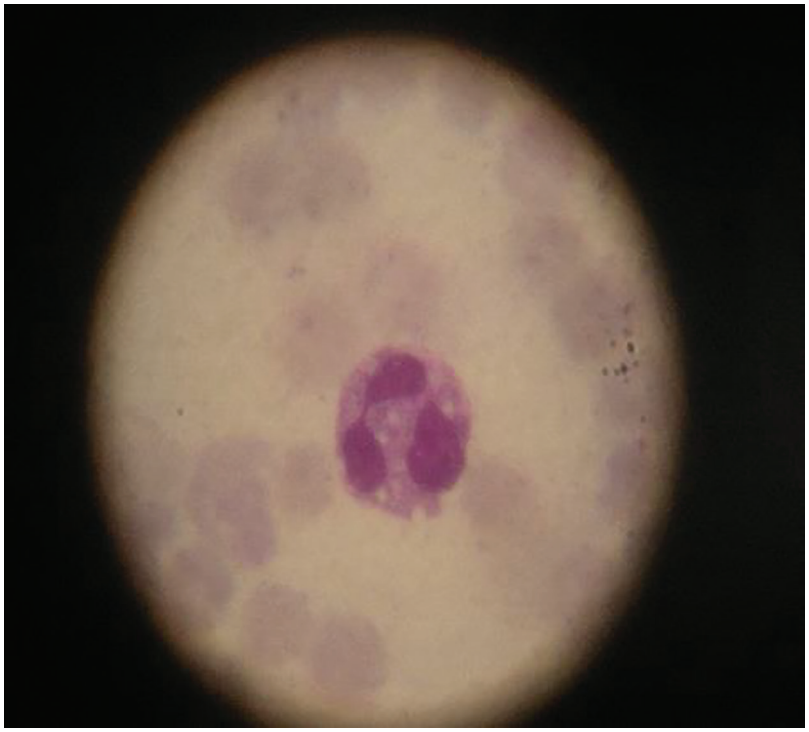

Figure 3: Cytoplasmic vacuolation: Leishman-stained smear under oil immersion objective (×1000)

[Table 1]. The diagnosis of sepsis was made when there were positive findings on blood culture. Infants were classified as probable infection when the blood culture was negative but there were obvious clinical courses of neonatal septicemia. Infants were considered to be uninfected when the blood culture was negative and there was no clinical evidence of infection. In our study, the male–female ratio was *M*:*F*=1:1 but among the children with sepsis (n = 20) the ratio was *M*:*F*=2:1.

The blood culture reports of 70 confirmed sepsis cases reveal that the commonest organism is *Escherichia coli* followed by *Klebsiella*, *Pseudomonas*, and β-hemolytic streptococci.

Table 2 shows the performance of individual hematological findings in 70 proven cases of sepsis. This reveals each of an *I*:*T* ratio>0.2 and an *I*:*M* ratio >0.3 is having sensitivity 86% and 90%, respectively. Platelet count <100,000/cmm is of specificity 85% and NPV 92%. The degenerative changes of neutrophils had no significant association with sepsis. By comparison of culture result with CRP, it was found that association was highly statistically significant ($P < 0.01$).

Out of the 70 cases with culture-proven sepsis, 58 (83.34%) infants had score ≥5 and 9 (13.33%) infants had scores 3–4. Fifteen (30%) cases with probable infection had scores 3–4 and 25 (50%) had score ≥5. Three (10%) of the normal infants had score ≥5 suggesting the presence of sepsis and three (10%) had scores 3–4, suggesting the possibility of sepsis in these cases. Twenty-four (80%) of the normal infants and 10 (20%) with probable infection had score ≤2, which implies that sepsis was unlikely in these cases [Table 3].

The comparison of hematological score and CRP is demonstrated in Table 4. Patients with higher hematological score show higher CRP values.

Discussion

Sepsis is the commonest cause of neonatal mortality. It is responsible for about 30–50% of the total neonatal deaths in developing countries. It is estimated that up to 20% of the neonates develop sepsis and approximately 1% die of sepsis-related causes.[4] The limitations in the diagnosis of neonatal sepsis are frustrating for clinicians; at present, there is no single test which meets the criteria of an ideal diagnostic test.[6] Although blood culture is the most definitive test for the diagnosis of neonatal sepsis, it has low sensitivity and leads to delay in the diagnosis.

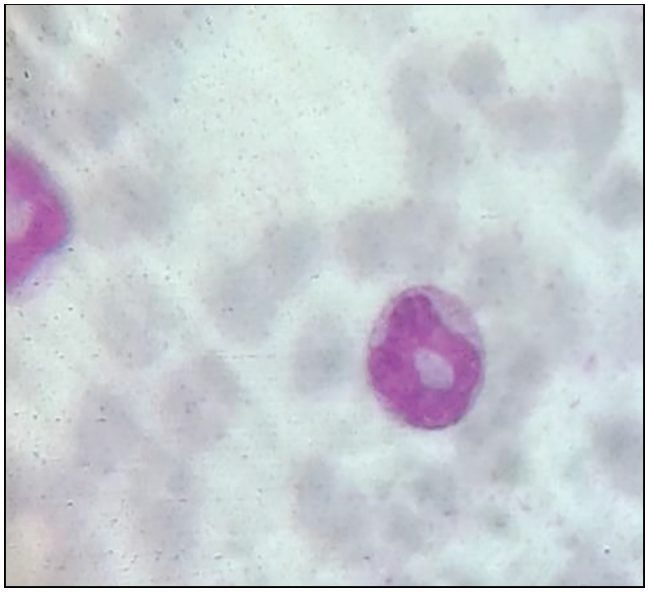

Figure 4: Ring form of neutrophil: Leishman-stained smear under oil immersion objective (×1000)

Table 1: Group distribution of cases

Group	No. of cases
Sepsis	70
Probable infection	50
Normal infants	30

Therefore, quick diagnostic tests with greater sensitivity are desirable, and we need a useful screening protocol in which a balance must be achieved between sensitivity and specificity.[7] Examination of PBS is an inexpensive but powerful diagnostic tool. Hematological values in neonates differ significantly from those in older children and adults. The present study is conducted to see the role of PBS and to analyze the diagnostic utility of HSS and its correlation with CRP and blood culture in neonatal sepsis. This is a simple and cost-effective test which can be done quickly before giving antibiotic therapy to the neonate. Hematological changes in neonatal sepsis include leucocytosis or leucopoenia, increase or decrease in absolute neutrophil count, increase in immature neutrophils, and change in immature-to-mature PMN ratio. However, individual hematological parameters lack enough sensitivity or specificity. HSS of Rodwell combines various hematological changes and increases sensitivity and specificity.

In our study, we correlated the sensitivity, specificity, PPV, and NPV of the various parameters with different groups. Clinical use of total leukocyte count (TLC) in the diagnosis of neonatal septicemia is not significant because of wide variation in values due to variation in blood sampling time, the severity of infection, and reduced sensitivity of this test after the first week of life. The sensitivity of TLC is 25%, specificity 85%, PPV 40%, and NPV 87%, which were consistent with other studies such as those done by Makkar *et al.*,[8] Khair *et al.*,[9]

Table 2: Performance of HSS and CRP in 70 proven cases of sepsis

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Total WBC count	25	85	40	87
Total PMN count	90	40	20	97
Immature PMN count	80	40	15	82
I:*T* ratio > 0.2	90	95	85	100
I:*M* ratio ≥ 0.3	86	80	12	100
Platelet count (<100,000/cmm)	75	85	40	92
Degenerative changes in PMN	50	60	55	50
CRP (%)	85	70	75	80

Table 3: Score of each group of the cases (n = 150)

Groups	Score (0–2%)	Score (3–4%)	Score (≥5%)
Sepsis	3 (3.33%)	9 (13.33%)	58 (83.34%)
Probable infection	10 (20%)	15 (30%)	25 (50%)
Normal	24 (80%)	3 (10%)	3 (10%)

Table 4: Hematological score in comparison with CRP value (n = 150)

HSS	CRP (>6 mg/L)	CRP (<6 mg/L)
0	5	5
1	17	25
2	12	25
3	16	7
4	10	3
5	5	5
6	10	5

and Gerdes.[10] Elevated *I*:*T* ratio was found to be the most reliable indicator of sepsis in our study and also in various other studies such as those done by Ghosh *et al.*[7] and Narasimha *et al.*[11] Immature PMN count and *I*:*T* PMN ratio were also a very sensitive indicator of neonatal sepsis. Degenerative changes in the PMNs made no significant contribution in the diagnosis, in this study. They are never seen in healthy babies. Their presence invariably indicates sepsis, but their count is not always increased.[12] Also, in our study, the total PMN count had a limited role in sepsis screening. This finding correlated well with the study done by Akenzua *et al.*,[13] who inferred that the patients in their study had normal PMN count but the band forms are raised, and the elevation was often very late and inconsistent. Thrombocytopenia was frequently associated with sepsis and indicated poor prognosis. This is thought to be due to increased platelet destruction, sequestration secondary to infections, failure in platelet production due to reduced megakaryocytes, or damaging effects of endotoxin.[14] This correlated well with various other studies done by Speer *et al.*,[15] Rodwell *et al.*, Philip and Hewitt,[16] and Basu *et al.*[17]

We also found out that higher the score, more are the chances of sepsis and vice versa. The HSS improves the efficiency of the complete blood count as a screening test for sepsis and permits an objective assessment of hematological changes. CRP had a sensitivity of 85% and specificity of 70%. PPV and NPV were 75% and 80%, respectively. Other studies have also shown similar sensitivity and specificity ranging from 81% to 90% and from 50% to 84%, respectively.[18-20] Most of the cases with HSS ≥3, in our study, show CRP level more than 6 mg%.

Conclusion

Neonatal sepsis is one of the most dreadful conditions increasing the pediatric morbidity and mortality. Inability to adequately diagnose the neonatal sepsis can result in unnecessary and prolonged exposure to antibiotics to uninfected newborns. Thus laboratory tests that assist the clinician in the diagnosis of infection in neonates have considerable relevance. The peripheral smear study is found to be very useful in early diagnosis of neonatal septicemia as it can be performed even in a small laboratory without any special equipment and within a short period of time. The HSS improves the efficiency of the PBS as a screening test for sepsis and permits an objective assessment of hematological changes. Though there are several methods for rapid detection of microorganisms in blood cultures of newborn infants using automated blood culture system, DNA probe, and fluorometric detection systems, they are highly expensive and need a well-equipped laboratory, which is not available in most of the community hospitals in our country. Instead, a simple peripheral smear-based test like HSS, supplemented with CRP which can distinguish between infected and uninfected neonates with little laboratory backup, is indeed a boon for peripheral pathologists and neonatologists.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Chaurasia S, Sivanandan S, Agarwal R, Ellis S, Sharland M, Sankar MJ. Neonatal sepsis in South Asia: Huge burden and spiralling antimicrobial resistance. Br Med J 2019;364:k5314.
2. Gengaimuthu K, Karthikeyan V. Towards an ideal neonatal sepsis screen panel—A review. Indian J. Child Health 2017;4:614-8.
3. Makadia KD, Pragnesh S. A study of haematological changes in neonatal sepsis. Ann Pathol Lab Med 2020;7:236-9.
4. Haider S, Riaz S, Tahir R. Role of hematological profile in early diagnosis of neonatal sepsis. Ann Pak Inst Med Sci 2010;6: 152-6
5. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr 1988;112:761-7.
6. Singh M, Narang A, Bhakoo ON. Evaluation of a sepsis screen in the diagnosis of neonatal sepsis. Indian Pediatr 1987;24:39-43.
7. Ghosh S, Mittal M, Jaganathan G. Early diagnosis of neonatal sepsis using a hematological scoring system. Indian J Med Sci 2001;55:495-500.
8. Makkar M, Gupta C, Pathak R, Garg S, Mahajan NC. Performance evaluation of hematologic scoring system in early diagnosis of neonatal sepsis. J Clin Neonatol 2013;2:25-9.
9. Khair KB, Rahman MA, Sultana T, Roy CK, Rahman MQ, Shahidullah M, *et al*. Role of hematologic scoring system in early diagnosis of neonatal septicemia. BSMMU J 2010;3:62-7.
10. Gerdes JS. Clinicopathologic approach to the diagnosis of neonatal sepsis. Clin Perinatol 1991;18:361-81.
11. Narasimha A, Harendra Kumar ML. Significance of hematological scoring system (HSS) in early diagnosis of neonatal sepsis. Indian J Hematol Blood Transfus 2011;27:14-7.
12. Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. I. Reference values for neutrophilic cells. J Pediatr 1979;95:89-98.
13. Akenzua GI, Hui YT, Milner R, Zipursky A. Neutrophil and band counts in the diagnosis of neonatal infections. Pediatrics 1974;54:38-42.

14. Zipursky A, Palko J, Milner R, Akenzua GI. The hematology of bacterial infections in premature infants. Pediatrics 1976;57:839-53.
15. Speer CP, Gahr M, Schröter W. Early diagnosis of neonatal infection. Monatsschr Kinderheilkd 1985;133:665-8.
16. Philip AG, Hewitt JR. Early diagnosis of neonatal sepsis. Pediatrics 1980;65:1036-41.
17. Basu S, Guruprasad NA, Garewal G. Diagnosis of sepsis in the high risk neonate using a hematologic scoring system. Indian J Hematol Blood Transf 1999;17:32-4
18. Ms S, Alva SR, Vs S, Tm K. Research article evaluation of neonatal septicaemia using hematological parantelers. Int J Recent Sci Res 2015;6:2775-8.
19. Bs V, Girish GN, Adhikari S, Hugara S. Evaluation of septic screen as a diagnostic tool for neonatal sepsis in a tertiary hospital at Mysore. Sch J Appl Med Sci 2015;3:1005-10.
20. Bhale CP, Kale AV, Kale SS, Mahajan M, Smulay S. Utility of sepsis screen in the early diagnosis of neonatal sepsis. Indian J Neonatal Med Res 2016;4:1-7