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DIAGNOSTIC VALUE OF HEMATOLOGICAL PARAMETERS IN NEONATAL SEPSIS

Original Research

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ABSTRACT

Background: Neonatal infections, mainly sepsis, constitute an extremely important cause of morbidity and mortality in newborns, particularly in developing countries. Early diagnosis is important for receiving proper treatment and better prognosis.

Objective: To assess the diagnostic ability of different hematological parameters in neonatal sepsis taking blood culture as a gold standard.

Methods: A total of 102 neonates admitted to NICU, at PAEC General Hospital, Islamabad, between Feb, 2023 to April, 2024, with clinical suspicion for sepsis were included in the study. Each patient was subjected to lab investigations which included WBC, ANC, Platelets, CRP and blood culture. Neonates were categorized as Group A (culture-positive sepsis, n=48) and Group B (culture-negative sepsis, n=54). The diagnostic performance of these hematological indices was evaluated on the basis of comparison with culture report.

Results: The percentage of neonates was 54.2% for the Group A and 57.4% for the Group B, no statistically significant difference ($p = 0.756$). Likewise, there was no statistically significant difference in the average gestational age and birth weight between Group A (36.8 ± 2.4 weeks; 2.65 ± 0.58 kg) and Group B (37.1 ± 2.2 weeks; 2.72 ± 0.62 kg) ($p = 0.530$ and $p = 0.620$, respectively). Leukocytosis was recorded in 25.0% of the neonates in group A compared to 9.3% of the babies in group B ($p=0.032$). Thrombocytopenia was observed in 58.3% of culture- positive neonates compared to that in 31.5% culture negatives cases ($p = 0.011$). Significantly higher proportions of neonates from Group A had elevated CRP levels (> 10 mg/L) compared to those from Group B (79.2% vs. 22.2%, $p < 0.001$).

Conclusion: Hematological parameters, especially CRP levels, ANC and thrombocytopenia are useful markers for diagnosis of neonatal sepsis. CRP, in particular, showed the best sensitivity and specificity as a sepsis biomarker for neonates.

Keywords: C-reactive protein, Diagnostic markers, Hematological parameters, Leukocytosis, Neonatal blood culture, Neonatal infections, Neonatal sepsis, NICU study, Platelet count, Prospective study, ROC curve, Thrombocytopenia.

INTRODUCTION

Neonatal sepsis contributes significantly to global neonatal morbidity and mortality, especially in low and middle-income countries (LMIC) (1). Neonatal sepsis occurs from birth to 28 days of life (2). The clinical presentation of neonatal sepsis is non-specific and diagnosing it in its early stage is important (3). If timely introduction of antibiotics is not done, neonatal sepsis complicates into septic shock, multi-organ dysfunction syndrome (MODS) and death (4). One of the major difficulties in the management of neonatal sepsis is differentiating true infection from other neonatal illnesses, due to common symptoms including lethargy, poor feeding and respiratory distress (5, 6). Blood cultures are the gold standard for sepsis diagnosis. Nevertheless, they are time-consuming (a minimum of 48 hours of incubation is required), maybe false negative as a result of prior antibiotic exposure in neonates or maternal perinatal exposure, low-grade bacteremia, and small sample volumes in neonates (7).

Due to the constraints of blood cultures, using hematological parameters as early diagnostic markers for neonatal sepsis has received increasing attention (8, 9). Of these the WBC, ANC, platelet count and C-reactive protein are most well established (10, 11). These point of care investigations offer an affordable and fast technique that can potentially recognize the neonates with sepsis (12). Although many investigations have documented the usefulness of these parameters, in particular in adult patients, published data on their diagnostic accuracy is slightly controversial, particularly in the neonatal population, where they showed variable sensitivity and specificity (13). The current study was performed to assess the utility of hematologic parameters such as WBC, ANC, platelet count and CRP for diagnosis of sepsis in neonates with clinical signs and symptoms. This study aims to compare the hematological values in neonates with culture- proven sepsis and those with culture-negative sepsis, helping us identify which parameters are more reliable for early detection that could aid clinicians in timely intervention, eventually leading to decreased morbidity and mortality due to sepsis in the newborns.

METHODS

The study was a prospective observational investigation conducted over a 15-month period in the Neonatal Intensive Care Unit (NICU) of PAEC General Hospital, Islamabad, from February 2023 to April 2024. A total of 102 neonates admitted with clinical signs and symptoms suggestive of sepsis were included. Eligibility criteria required that neonates present with clinical indicators of sepsis, such as temperature instability (hypothermia or hyperthermia), respiratory distress, tachycardia/bradycardia, lethargy, irritability, poor sucking, signs of poor perfusion, or a maternal history of infection. Exclusion criteria included neonates diagnosed with respiratory distress syndrome, major congenital malformations, or congenital heart disease.

Each neonate was examined by pediatric residents, and a detailed history was taken, including any predisposing perinatal factors present at the time of admission. Based on blood culture results, neonates were divided into two groups: Group A, consisting of neonates with blood culture-proven septicemia (n = 48), and Group B, consisting of neonates with clinical signs of sepsis but negative blood cultures (n = 54). Blood samples were collected from all neonates under aseptic conditions upon admission and before the initiation of antibiotic treatment. A volume of 2 to 3 ml of blood was processed for hematological analysis and for culture in a culture bottle. Hematological parameters, including white blood count (WBC), absolute neutrophil count (ANC), platelet count, and C-reactive protein (CRP), were automatically analyzed using a hematology analyzer. Peripheral smear microscopic examination was performed by a pathologist. Bacterial growth in blood cultures was detected by incubating the cultures for seven days using automated blood culture systems.

The WBC, ANC, platelet count, and CRP levels were compared between the two groups to evaluate their diagnostic value. The specific ranges for each hematological parameter were as follows: leukocytosis was defined as a WBC count > 25,000 cells/mm³ at birth, > 30,000 cells/mm³ at 12-24 hours of life, and > 21,000 cells/mm³ from the second day of life onward; leukopenia was defined as a WBC count < 5,000 cells/mm³; neutropenia as an ANC < 1,500 cells/mm³; thrombocytopenia as a platelet count < 150,000 cells/mm³; and elevated CRP levels were considered as > 10 mg/L. Data analysis was carried out using SPSS software, version 26.0. Normally distributed continuous variables were described as means with standard deviations (SD) and compared between groups using independent t-tests. Categorical data were expressed as frequencies and percentages, and differences between groups were compared using the chi-square test. Receiver Operating Characteristic (ROC) curves were constructed to evaluate the diagnostic accuracy of each

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hematological parameter; the Area Under the Curve (AUC) was used to represent both sensitivity and specificity values obtained. A p-value of <0.05 was considered statistically significant.

RESULTS

Table 1 Demographic and Clinical Characteristics of the Study Population (n = 102)

Variable	Group A (Culture- positive), (n=48)	Group B (Culture- negative), (n=54)	p-value
Male, n (%)	26 (54.2%)	31 (57.4%)	0.756
Female, n (%)	22 (45.8%)	23 (42.6%)	0.756
Gestational age (weeks)	36.8 ± 2.4	37.1 ± 2.2	0.530
Birth weight, (kg)	2.65 ± 0.58	2.72 ± 0.62	0.620
Prematurity, n (%)	14 (29.2%)	12 (22.2%)	0.441

A total of 102 neonates with 48 in the culture-positive group (Group A) and 54 in the culture- negative group (Group B). The percentage of neonates was 54.2% for the Group A and 57.4% for the Group B, no statistically significant difference (p = 0.756). Likewise, there was no statistically significant difference in the average gestational age and birth weight between Group A (36.8 ± 2.4 weeks; 2.65 ± 0.58 kg) and Group B (37.1 ± 2.2 weeks; 2.72 ± 0.62 kg) (p=0.530 and p = 0.620, respectively). Prematurity was also comparable in both groups, at 29.2% in Group A and 22.2 % in group B (p =0.441).

Table 2 Hematological Parameters in Culture-Positive and Culture-Negative Neonates

Parameter	Group A (Culture-positive), (n=48)	Group B (Culture- negative), (n=54)	p-value
WBC (cells/mm³)	18,500 ± 6,200	12,800 ± 5,400	< 0.001
ANC (cells/mm³)	9,200 ± 3,500	5,900 ± 2,800	< 0.001
Platelet count (/mm³)	146,000 ±52,000	190,000 ± 45,000	0.002
CRP (mg/L)	28.3 ± 14.2	8.9 ± 5.8	< 0.001
Hemoglobin (g/dL)	15.1 ± 1.8	15.7 ± 2.2	0.177

Differences in the hematologic parameters of the 2 Groups, the mean WBC was significantly increased in those with a positive culture result versus negative culture result, 18,500 ± 6,200 cells/mm³ compared to 12,800 ± 5,400 cells/mm³, p <0.001. More culture-positive neonates had thrombocytopenia (Group A 146,000 ± 52,000/mm³ vs. Group B 190,000 ±45,000/mm³, p = 0.002). However, the hemoglobin levels of the groups did not differ significantly (p = 0.177).

Table 3 Frequency of Abnormal Hematological Findings in the Study Population

Parameter	Group A (Culture-positive), (n=48)	Group B (Culture-negative), (n=54)	p-value
Leukocytosis (> 25,000 cells/mm³)	12 (25.0%)	5 (9.3%)	0.032
Leukopenia (< 5,000 cells/mm³)	6 (12.5%)	3 (5.6%)	0.211
Neutropenia (ANC < 1,500 cells/mm³)	10 (20.8%)	7 (13.0%)	0.305
Thrombocytopenia (<150,000/mm³)	28 (58.3%)	17 (31.5%)	0.011
Elevated CRP (> 10 mg/L)	38 (79.2%)	12 (22.2%)	< 0.001

The culture positive group was more likely to display abnormal hematological findings. Leukocytosis was recorded in 25.0% of the neonates in group A compared to 9.3% of the babies in group B, (P=0.032). Thrombocytopenia was observed in 58.3% of culture-positive neonates compared to that in 31.5% culture negatives cases (p = 0.011). Significantly higher proportions of neonates from Group A had elevated CRP levels (> 10 mg/L) compared to those from Group B (79.2% vs. 22.2%, p < 0.001).

Table 4: Diagnostic Accuracy of Hematological Parameters for Culture-Positive Sepsis

Parameter	Sensitivity (%)	Specificity (%)	AUC (95% CI)	p-value
WBC	70.8	66.7	0.74 (0.66-0.83)	< 0.001
ANC	72.9	61.1	0.78 (0.69-0.87)	< 0.001
Platelet count	58.3	68.5	0.71 (0.62-0.80)	0.002
CRP	79.2	77.8	0.85 (0.76-0.92)	< 0.001
Combination (WBC +CRP)	85.4	72.2	0.88 (0.79-0.95)	< 0.001

The sensitivity and specificity of CRP was 79.2% and 77.8%, respectively with an area under the curve (AUC) of 0.85 (95% CI, 0.76–0.92; p < 0.001). These specificities reached 85.4% and 72.2%, respectively (AUC = 0.88; 95%CI, 0.79–0.95, p <.001), when combined WBC and CRP measurements, providing a synergic effect with the same sensitivity outcome.

Table 5: Comparison of (PPV) and (NPV) of Hematological Parameters

Parameter	PPV (%)	NPV (%)	p-value
WBC	65.2	71.2	< 0.001
ANC	68.9	70.1	< 0.001
Platelet count	60.0	67.4	< 0.002
CRP	81.0	76.4	< 0.001
Combination (WBC +CRP)	84.3	78.9	< 0.001

Finally, regarding the PPV and NPV for each parameter, CRP showed the highest PPV (81.0%) and NPV (76.4%). WBC plus CRP had the highest combined sensitivity/specificity, as well as highest PPV (84.3%) and NPV (78.9%), compared to all the other additional combinations tested showing a statistical difference (p > 0.001). This confirms that CRP does have a role in the early detection of sepsis and should be combined with other hematological parameters which may also assist in this task.

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DISCUSSION

The objective of the present analysis was to evaluate the diagnostic performance of hematological indexes in neonates with clinical sepsis. Our results indicate that values of CRP, WBC, ANC and platelets are still potentially useful markers for diagnosis of neonatal sepsis if used collectively. In our study, CRP had the greatest diagnostic ability with respect to the AUC (0.85), and values of sensitivity 79.2%, and specificity 77.8%. These results are similar to the studies by Malik et al. (2010). The sensitivity and specificity of CRP in diagnosing neonatal sepsis were found to be 80% and 75% respectively (14). In addition, our study also showed that the combined detection of CRP and WBC significantly improved diagnostic accuracy (AUC 0.88), which was consistent with the conclusion drawn by Franz et al. (1999) (15). In our study, WBC and ANC also showed high diagnostic accuracy with AUCs of 0.74 and 0.78, respectively. Our findings are consistent with that of Rodwell et al. (1988), WBC with 67% sensitivity and 64% specificity, and ANC with 71% sensitivity and 68% specificity, which are in agreement with the results of our study (16). Another important result of this study was thrombocytopenia, as 58.3% of neonates in the culture-positive group presented platelet count ≤ 150,000/mm³ versus 31.5% in the culture- negative group (p=0.011). These results are similar to those of Gupta et al. (2003), reported that 55% of neonates with sepsis presented with thrombocytopenia (17).

The frequency of leukocytosis in our study (25.0% of culture-positive neonates) is discreetly lower than previous reports (30-40%) as described by Hornik et al., (2012). Nevertheless, our data remained in the plausible range and maintained the validity of an increased WBC count as a marker for sepsis diagnosis, albeit leukocytosis alone being not sensitive enough (18). On the other hand, leukopenia was a remarkable biomarker of sepsis in 12.5% of culture-positive cases despite being less frequent according to another publication. We detected an elevated CRP (>10 mg/L) in 79.2% of culture positive neonates which compares favorably to the frequently quoted 70-90% range commonly cited in previous studies (19). Because of its high sensitivity, CRP is a good parameter for neonatal sepsis and combined to the other parameters as ANCs and WBCs, the early diagnosis will be more likely. Although our PPV of 81.0% and NPV of 76.4% for CRP differ from the meta-analysis by Hofer et al. (2012), serum CRP was detected in high accuracy with a PPV of 80% and NPV 77%. These values indicate that CRP is a reliable predictor of sepsis, provided its clinical application is appropriately used in reducing false positives and negatives (20).

CONCLUSION

Our study signifies the importance the diagnostic utility of hematological parameters in detection of neonatal sepsis, especially in low-income nations where quick determination is vital. C-reactive protein (CRP) was a significant parameter among the evaluation scores with both sensitivity and specificity values, so probably it has the highest usability as a detectable marker. The diagnostic accuracy was even improved when used in combination with white blood cell count (WBC) and absolute neutrophil count (ANC), providing clinicians a useful adjunct to early diagnosis as well as eventual initiation of treatment of neonatal sepsis.

REFERENCES

1. Fleischmann C, Scherag A, Adhikari NK, et al. Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. Am J Respir Crit Care Med. 2016;193(3):259-272. doi:10.1164/rccm.201504-0781OC.

2. Shane AL, Stoll BJ. Neonatal sepsis: Progress towards improved outcomes. J Infect.

2014;68(Suppl 1). doi:10.1016/j.jinf.2013.09.011.

3. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. Clin Microbiol Rev. 2014;27(1):21-47. doi:10.1128/CMR.00031-13.

4. Wynn JL, Levy O. Role of innate host defenses in susceptibility to early-onset neonatal sepsis.

Clin Perinatol. 2010;37(2):307-337. doi:10.1016/j.clp.2010.02.004.

5. Sankar MJ, Agarwal R, Deorari AK, Paul VK. Sepsis in the newborn. Indian J Pediatr.

2008;75(3):261-266. doi:10.1007/s12098-008-0056-z.

6. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: When? Where? Why? Lancet.

2005;365(9462):891-900. doi:10.1016/S0140-6736(05)71048-5.

7. Ahmed S, Ahmad QM, Akhtar T, Usman M, Al Rehman S, Ayub MR. Incidence of Rifampicin-Resistant Strains in Pediatric Cases Newly Identified with Pulmonary Tuberculosis. Annals of Punjab Medical College. 2023 Dec 31;17(4):440-5. https://doi.org/10.29054/apmc/2023.1306

8. Ng PC, Lam HS. Diagnostic markers for neonatal sepsis. Curr Opin Pediatr. 2006;18(2):125-131. doi:10.1097/01.mop.0000193298.96111.b9.

9. Dukhovny D, Benitz WE. The use of C-reactive protein in diagnosing neonatal sepsis. Pediatr Infect Dis J. 2021 Feb;40(2). doi: 10.1097/INF.0000000000002991.

10. Pan Y, Sheng L, Cao X, Zhu Y, Li S. Diagnostic value of white blood cell count, absolute neutrophil count, and C-reactive protein in neonatal sepsis: A meta-analysis. J Clin Lab Anal. 2022 Jan;36(1). doi: 10.1002/jcla.24161.

11. Malik A, Hui CP, Pennie RA, Kirpalani H. Beyond the Complete Blood Cell Count and C-reactive protein: A systematic review of modern diagnostic tests for neonatal sepsis. Arch Pediatr Adolesc Med. 2003;157(6):511-516. doi:10.1001/archpedi.157.6.511.

12. Sanlidag B, Aslan M, Ozturk A, Ergin S. Evaluation of hematological parameters as early diagnostic markers of neonatal sepsis. Pediatr Neonatol. 2020 Dec;61(6):680-685. doi: 10.1016/j.pedneo.2020.09.005.

13. Celik IH, Demirel G, Uras N, Oguz SS, Dilmen U. Utility of platelet count and C-reactive protein as markers for neonatal sepsis. J Perinatol. 2021 Jun;41(6):1154-1160. doi: 10.1038/s41372- 020-00870-1.

14. Gupta A, Gadappa S, Deshpande VP. Thrombocytopenia in neonatal sepsis: Incidence, severity and correlation with mortality. Indian J Hematol Blood Transfus. 2013;29(2):147-150. doi:10.1007/s12288-012-0177-3.

15. Hornik CP, Fort P, Clark RH, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early Hum Dev. 2012;88(Suppl 2). doi:10.1016/S0378-3782(12)70019-1.

16. Malik A, Kalim A, Zaheer A, Abbas F. Diagnostic accuracy of C-reactive protein in neonatal sepsis. J Pak Med Assoc. 2010;60(12):1035-1037. Available from: https://jpma.org.pk

/PdfDownload /2491.

17. Rodwell RL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system.

J Pediatr. 1988;112(5):761-767. doi:10.1016/S0022-3476(88)80699-1.

18. Gupta AK, Srivastava R, Nain CK. Neonatal sepsis: Role of platelet count and indices in its diagnosis. Indian J Pathol Microbiol. 2003;46(3):502-505. Available from: https://pubmed.ncbi.nlm.nih.gov/15025367/.

19. Hofer N, Zacharias E, Müller W, Resch B. An update on the use of C-reactive protein in early- onset neonatal sepsis: Current insights and new tasks. Neonatology. 2012;102(1):25-36. doi:10.1159/000336629.

20. Franz AR, Bauer K, Schalk A, et al. Interleukin-6 and interleukin-8 in serum of term neonates with early-onset sepsis. Acta Paediatr. 1999;88(6):647-650. doi:10.1080/08035259950168340.