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Hematology

CORRELATION OF HEMATOLOGICAL PARAMETERS WITH BLOOD CULTURE IN THE DIAGNOSIS OF NEONATAL SEPSIS- A TERTIARY CARE HOSPITAL-BASED STUDY

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ABSTRACT **Introduction:** Neonatal sepsis is a leading cause of morbidity and mortality in neonates, particularly in developing countries. As the early manifestations of sepsis are vague and ambiguous, a high index of suspicion is required. Aims and objectives: 1. To evaluate the hematological parameters in neonates, clinically suspected of sepsis. 2. To correlate the hematological parameters with blood culture. Materials and **Methods:** Prospective study was done on 150 clinically suspected neonates. Hematological parameters were assessed and scored as per Rodwell's hematological scoring system (HSS). Blood culture was taken as the criterion for definitive diagnosis. **Results:** 29.3% were culture positive and 62% were low birth weight neonates. Klebsiella was the commonest organism. A raised Immature: Total PMN ratio was highly sensitive (95.45%) while raised Immature: Mature PMN ratio was highly specific (87.74%). The HSS score of ≥3 showed 100% sensitivity. **Conclusion:** The HSS is a useful tool to distinguish the infected from the non-infected and a cut-off score of 3 may guide antibiotic therapy decisions.

KEYWORDS : Hematological scoring system, Neonatal sepsis, blood culture

INTRODUCTION

Neonatal sepsis is a clinical syndrome caused by the pathophysiologic effects of local and systemic infection during the first month of life.1 Neonatal sepsis is a leading cause of morbidity and mortality in newborns, particularly in developing countries like India.2 The current neonatal mortality rate of 17 death per 1000 live births globally and 20.3 death per 1000 live births in India.3 Early detection of sepsis can facilitate early treatment and avoid life-threatening complications associated with it. However, blood culture still remains the "Gold Standard" for diagnosing sepsis but it has limitations, including being time-consuming (48–72 hours), a low yield of positive results (8–73 percent), false-positive results, and negative blood cultures findings even in fatal generalized bacterial infections, and is not easily accessible in rural health care facilities.4 With the advent of newer technology, some highly sensitive and specific inflammatory markers have been evaluated for diagnosis, but they are sophisticated and expensive and are not easily available in a resource-poor setup. The Hematological Scoring System (HSS) formulated by Rodwell RL et al..5 in 1988 is a quick and reliable method for early diagnosis of neonatal sepsis as it includes all parameters which are considered to be significantly associated with sepsis.6 Thus, keeping this in mind our study we planned this study to evaluate the utility of the HSS in the early.

AIMS AND OBJECTIVES

1. To evaluate the hematological parameters in neonates, clinically suspected of sepsis.
2. To correlate the hematological parameters with blood culture.

MATERIALS AND METHODS

This prospective hospital-based cross-sectional study was conducted over a period of 1 year, from September 2021 to August 2022 in the Department of Pathology, Fakruddin Ali Ahmed Medical College and Hospital (FAAMCH). A total of 150 neonates with clinical suspicion of septicemia were included in this study. Neonates with major congenital anomalies, administration of antibiotics prior to admission, and neonates of mothers with pregnancy-induced hypertension and asphyxia were excluded from the study. Ethical clearance for the study was obtained from the Institutional Ethics Committee, and informed written consent was taken from the legal guardians before the commencement of the study.

Relevant maternal and neonatal histories were recorded in a predesigned and pretested proforma. Under all antiseptic precautions, 1 mL of blood was inoculated in a brain heart infusion broth for blood culture. The collected blood sample was then sent to the Department of Microbiology, FAAMCH for culture and sensitivity. A 1ml blood sample was collected in vacutainers anticoagulated with K3-EDTA to study hematologic parameters. Complete blood counts along with hematological score and blood culture together form the sepsis workup. Total leukocyte and differential counts, total polymorphonuclear (PMN) count, and platelet counts were measured using Sysmex XS-1000i automated hematology analyzer.

Peripheral blood smears stained with Leishman's stain were examined for nucleated RBC, differential WBC count, platelet count, absolute neutrophil count, immature neutrophils, band forms, and degenerative changes in neutrophils. The peripheral smears were examined by a pathologist blinded to the infant's infection status. The total nucleated red blood cells, differential counts, and immature PMN count including band forms were analyzed by counting at least 200 cells, and corrected total white blood cells were obtained.

The hematological scoring system (HSS) formulated by Rodwell et al.5 is based on normal values, as defined by Manroe et al..7 The HSS of Rodwell et al..5 assigns a score of 1 for each of the seven parameters if they were abnormal. An abnormal total leukocyte count, abnormal total PMN count, elevated immature PMN count >600, elevated immature to total (I: T) PMN ratio ≥0.2, immature to mature (I: M) PMN ratio ≥0.3, platelet count ≤150,000/mm3, and pronounced degenerative or toxic changes in PMNs were scored as 1. If no mature polymorphs are seen on the peripheral smear, an abnormal total PMN count is assigned a score of two instead of one to compensate for the low I: M PMN ratio [Table 1]. Immature polymorphs include promyelocytes, myelocytes, metamyelocytes, and band forms. A band cell (Figure 1) is described as a PMN when there was no nuclear segmentation or when the width of the nucleus at any constriction was not less than one-third the width at its widest portion. Degenerative changes (Figure 2) include vacuolization, toxic granulations, and Dohle bodies. A minimum score of 0 and a maximum score of 8 can be obtained. A Score of ≤ 2 will be interpreted as sepsis unlikely while a score of 3-4 as sepsis possible and a score of ≥5 as Sepsis or Infection is very likely. (Table 2)

Table 1: Hematological scoring system[5]

CRITERIA	ABNORMALITY	SCORE
Total WBC count	≤ 5000/µl ≥ 25,000 at birth ≥ 30,000 In 12-24hrs ≥ 21,000 after day 2	1 1 1 1
Total PMN count	No mature PMN seen Increased/Decreased	2 1
Immature PMN count	Increased >600/mm3	1
Immature to total PMN ratio	Increased ≥0.2	1

Immature to mature PMN ratio	≥0.3	1
Degenerative changes in PMN	Toxic granules/ cytoplasmic vacuoles/ Dohle bodies	1
Platelet count	≤ 1.5 lakhs/mm^3	1

Table 2: Interpretation of HSS

Score	Interpretation
≤ 2	sepsis unlikely
3-4	sepsis possible
≥5	Sepsis/Infection very likely.

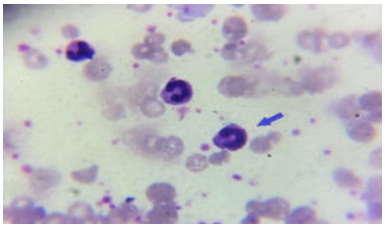

Figure 1: Band cell (Leishman's stain 100x)

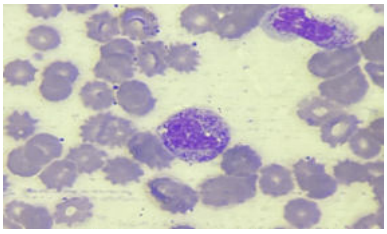

Figure 2: Degenerative changes in PMN showing toxic granulations and cytoplasmic vacuolization (Leishman's stain 100x)

STATISTICALANALYSIS

The data obtained were tabulated on a Microsoft Excel spreadsheet and analyzed using IBM-SPSS version 26 software. The sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were calculated for each parameter. The p-value was also calculated for different parameters.

RESULTS AND OBSERVATION

In our study, out of the 150 neonates, 94 (62.7%) neonates were male and 56 (37.3%) neonates were female with male to female ratio of 1.68:1. The study had 86 (57.3%) preterm neonates while 64 (42.7%) were term neonates. The commonest age group was 0-72 hours with 116 (77.3%) neonates and 34 (22.7%) neonates being more than 72 hours of age. The mean age of the neonates was 3.73±4.5 days. In this study, 93 (62%) neonates had a birth weight of <2.5kg and 57 (38%) cases had a birth weight of ≥2.5kg. The mean birth weight of the neonates was found to be 2.23kg.

On evaluating the HSS of Rodwell et al.,5 neonates were categorized as sepsis unlikely (Score 0-2) in 72 (48%) cases, possible sepsis (Score 3-4) in 29 (19.3%) cases, and sepsis/infection very likely (Score of ≥5) in 49 (32.7%) neonates (Table 3). Blood culture was positive in 44 (29.3%) and negative in 106 (70.7%) neonates (Table 4). Among the culture-positive neonates, the majority were gram-negative infections (65.9%) while the rest were gram-positive infections (34.1%). Klebsiella pneumoniae was found to be the most common organism followed by Staphylococcus aureus. Among the 44 culture-positive neonates all 44 (100%) neonates had a hematological score of ≥3 while among the 106 culture-negative neonates, 72 (67.9%) neonates had a score of <3. HSS score of ≥5 was seen in 35 (79.5%) neonates among the culture-positive neonates, and 93 (87.7%) neonates had scores of <5 among the culture-negative neonates. The sensitivity, specificity, PPV, and NPV of HSS with a cut-off score of ≥3 in predicting sepsis were 100%, 67.92%, 56.41%, and 100% respectively. Similarly, the sensitivity, specificity, PPV, and NPV of HSS with a cut-off score of ≥5 in predicting sepsis were 79.55%, 87.74%, 72.92%, and 91.18% respectively.

The individual hematological findings (Table 5) were significantly associated with sepsis. Total WBC count had a sensitivity of 68.18% and specificity of 81.13%, PPV 60% and NPV 86%. The total PMN count had a sensitivity of 84.09%, specificity of 67.92%, PPV of 52.11%, and NPV of 91.14%. Immature PMN count had a sensitivity of 93.18%, specificity of 81.13%, PPV of 67.21%, and NPV of 96.63%. An I:T PMN ratio had a sensitivity of 95.45%, specificity of 76.42%, PPV of 62.69%, and NPV of 97.59%. The I:M ratio had 86.64% sensitivity, 87.74% specificity, 75% PPV and NPV of 94.90%. Platelet count showed 70.45% sensitivity, 70.75% specificity, 50% PPV, and 85.23% NPV. Degenerative changes in PMNs had 77.27% sensitivity, 69.81% specificity, 51.52% PPV and 88.10% NPV. Among the hematological parameters, the I:M PMN ratio showed the highest accuracy of 88%.

DISCUSSION

Neonatal sepsis is a leading cause of morbidity and mortality in newborns, particularly in developing countries like India.2 Sepsis can be treated and life-threatening consequences can be avoided if detected early. As neonates are unable to fully organize the minimal inflammatory response, they are more vulnerable to infections than adults, and the dangers are significantly higher in preterm and low birth weight babies.[8,9]

In our study, among the 150 suspected neonates of sepsis, male 62.7% were found predominant and similar data was seen in the study done by Khair *et al.*[10] (58.33%), Ahirrao B *et al.*[11] (62%) and Sonawane *et al.*[12] (64.82%) in their study. Male predominance was observed in a few studies, which could be due to the location of factors regulating the production of globulin, which is located on the X chromosome, making males less immunologically protected than females.

Due to wide variations in the total WBC count it is of little clinical use in the diagnosis of neonatal infection. The sensitivity, specificity, PPV, and NPV were consistent with others studies.[13,14] Neutropenia has been more common in association with sepsis, compared with neutrophilia, probably because of increased adherence to altered endothelial cells and utilization at the site of infection.[10] In this study total PMN count showed good sensitivity of 84.09% and PPV of 91.14% which were similar to studies by Rodwell *et al.* and Sawadkar *et al*.[5,15] The total PMNs count was associated with low positive predictive value and low specificity. Therefore, it should not be used in isolation as a predictor of sepsis.

In the present study, I:T ratio >0.2 had a sensitivity, specificity, PPV, and NPV of 95.45%, 76.42%, 62.69%, and 97.59% respectively. The specificity and positive predictive value were lower probably due to false positive results though these results for an elevated I/T ratio were consistent with other studies.[14,16] The I:M ratio (>0.3) showed 86.64% sensitivity, 87.74% specificity, 75% PPV and NPV of 94.90%. Dutta N *et al*.[14] found similar results.

In our study degenerative changes in PMNs had 77.27% sensitivity, 69.81% specificity, 51.52% PPV and 88.10% NPV which was similar to the study by Sawadkar *et al*.[15] Neonates with sepsis develop thrombocytopenia due to increased platelet destruction and sequestration. It can also be secondary to infections and ineffective platelet production resulting from decreased megakaryocytes in the bone marrow and/or damaging effects of endotoxin in the blood vessel. A sensitivity of 70.45%, specificity of 70.75%, PPV of 50%, and NPV of 85.2361% were found which was consistent with the other studies.[13,17]

Among the individual hematological parameters, the I:M PMN ratio showed the highest accuracy of 88%. In this study, HSS score ≥3 was highly significant (P<0.0001), with 100% sensitivity, 67.92% specificity, 56.41% PPV, and NPV of 100%. These results were consistent with other studies.93,92. The sensitivity, specificity, PPV, and NPV of HSS with a score ≥5 in predicting sepsis were 79.55%, 87.74%, 72.92%, and 91.18% respectively and these results were similar to other studies.[10,14]

CONCLUSION

The Hematological Scoring System of Rodwell (HSS) is a quick, simple, cost-effective, and reliable method for early diagnosis of neonatal sepsis as it includes multiple parameters which are considered to be significantly associated with sepsis parameters. The HSS is a useful tool to distinguish the infected from the non-infected which would aid in the early detection of neonatal sepsis. HSS with a cut-off score of 3 may provide clinicians with a guideline to make decisions regarding the judicious use of antibiotic therapy, thereby reducing morbidity and mortality as well as minimizing the risk of the emergence of resistant organisms due to misuse of antibiotics.

Table 3: Distribution of cases according to HSS

HSS	Number of cases	Percentage
Score 0-2	72	48
Score 3-4	30	20
Score ≥5	48	32
Total	150	100

Table 4: Blood Culture Results

Blood culture results	Number of Cases	Percentage
Positive	44	29.3
Negative	106	70.7
Total	150	100

Table 5: Performance of individual hematological parameters

	Sensitivity	Specificity	PPV	NPV	Accuracy
Total WBC count	68.18%	81.13%	60%	86%	77.33%
Total PMN count	84.09%	67.92%	52.11%	91.14%	72.67%
Immature PMN count	93.18%	81.13%	67.21%	96.63%	84.67%
I:T PMN ratio	95.45%	76.42%	62.69%	97.59%	82%
I:M PMN ratio	86.64%	87.74%	75%	94.90%	88%
Degenerative changes	77.27%	69.81%	51.52%	88.10%	72%
Platelet count	70.45%	70.75%	50%	85.23%	70.67%

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