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Usefulness of Haematological Scoring System in Early Diagnosis of Neonatal Sepsis

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Abstract **Original Research Article**

Background: Neonatal sepsis is one of the major causes of morbidity and mortality of the new born, especially in developing countries. The major concerns of the clinicians are its non-specific presentation and unavailability of tests with a high predictive value. Therefore, the need is for an infallible test that can be easily performed, quick, simple and cost-effective. ***Objectives*:** To assess the reliability of Haematological scoring system in diagnosis of suspected neonatal sepsis at early stage. ***Materials and Methods:*** A total of 84 suspected cases of neonatal sepsis were included in this prospective cross-sectional study carried out in Khulna City Medical College Hospital during the period from July 2021 to June 2022. The peripheral blood smears from all 84 patients were analysed using HSS of Rowell et al by pathologists. HSS assigns a score of 1 for each of seven findings significantly associated with sepsis such as total leukocyte count, total polymorphonuclear neutrophil count, elevated immature PMN count, elevated immature: total PMN ratio, Immature: mature PMN ratio >0.3, platelet count <150000/cmm, and pronounced degenerative or toxic changes in PMNs. The HSS score of >5 was interpreted as sepsis, blood culture was done as gold standard for diagnosis of sepsis. Each haematological parameter was assessed for its individual performance and sensitivity, specificity, positive and negative predictive values were calculated. ***Results:*** Among the total 84 suspected cases, 20 cases were diagnosed neonatal sepsis, (23.8%), 31 cases had probable infection (36.9%), and 33 cases were normal (39.3%). Elevated immature: total PMN count ratio (>0.2) and Immature: Mature PMN ratio (>0.3) were the most sensitive and specific parameter for sepsis. It was also seen that with increasing score, the likelihood of sepsis also increases. ***Conclusion*:** The sensitivities and specificities of the various parameters of HSS were found to be satisfactorily significant in identifying early onset of neonatal sepsis.

Keywords: Haematological scoring system, Neonatal sepsis, Immature neutrophils, Early diagnosis.

INTRODUCTION

The new born infants are more prone to bacterial invasion than the older children or adults due to their weaker immune system particularly premature babies [1]. The infection can be contracted from the mother through transplacental route, ascending infection, during passage through an infected birth canal or exposure to infected blood at delivery [2]. Neonatal sepsis is the response to any kind of infections and it can be early or late in onset, in early onset, maximum cases are observed within 24 hours of life and smaller percentage thereafter up to 7 days [3]. The major concern of clinicians is its non-specific presentation, sometimes the rapid progression of sepsis and the unavailability of tests with a high positive predictive value. Therefore the early detection of neonatal sepsis by a reliable and convenient test is important for treatment. A definitive diagnosis is established by blood culture, but the procedure is time consuming and the facilities are not available in many laboratories and other tests like haptoglobin and counterimmunoelectrophoresis are also not easily accessible [4-6]. Various studies have revealed that haematological parameters are simple, quick, and cost-effective tools in early diagnosis of neonatal sepsis. When these were studied in a combination of tests, a significant sensitivity and specificity were observed and these were also proved as useful predictors of neonatal septicaemia helping to initiate early treatment with appropriate antibiotic [7]. We have been encouraged to evaluate the haematological scoring system for early

diagnosis of neonatal sepsis for empirical therapeutic guidelines in management of the patients.

MATERIALS AND METHODS

A total of 84 suspected cases of neonatal septicaemia were included in this prospective cross-sectional study carried out in Khulna City Medical College Hospital during the period from July 2021 to June 2022. After obtaining informed written consent from parents of each patient with consideration of inclusion and exclusion criteria, and under complete aseptic conditions, at least 2 ml of blood sample was collected by peripheral venepuncture from suspected sepsis patients within 24 hours of admission. 1 ml. was anticoagulated with tripotassium EDTA containing non-siliconized vacutainer tubes for subsequent laboratory procedures in automated cell counter. 1 ml. of blood was inoculated aseptically into conventional blood culture bottle for culture and sensitivity test. The laboratory procedures involved complete blood counts along with haematological score and culture. Total leukocyte count, the differential count, absolute neutrophil count and platelet counts were measured using Sysmex XN-1000 automated analyser. The peripheral smears were stained with Leishman's stain for examination of immature neutrophils, degenerated neutrophils and toxic changes in PMNs. Differential counts were performed on these smears by counting at least 200 cells. HSS assigns a score of 1 for each of seven findings significantly associated with sepsis: Abnormal total leukocyte count, abnormal total PMN count, elevated immature PMN count, elevated immature to total (I:T) PMN ratio, immature to mature (I:M) PMN ratio >0.3, platelet count <150000/cmm, and pronounced degenerative or toxic changes in PMNs. An abnormal total PMN count was assigned score of 2 instead of 1 if no mature PMN are seen on the peripheral smear [3]. Immature polymorphs include promyelocyte, myelocyte, metamyelocyte, and band forms. Degenerative changes include vacuolization, toxic granules, and Dohle bodies. All the smears were examined and analysed by pathologists using HSS of Rodwell *et al*., [8].

Table-1: Haematological scoring system

Criteria	Abnormality	score
Total WBC count	>25000/cmm at birth <5000/cmm at birth >30000/cmm after 24 hrs >21000/cmm after 2 days.	1
Total PMN count	1800-5400 No mature PMN Increased/decreased	0 2 1
Immature PMN count	600 increased	0 1
I:T PMN ratio (>0.2)	0:120 Increased	0 1
I:M PMN ratio (>0.3)	<0.3 >0.3	0 1
Changes in PMN	Degeneration, Toxic granules or cytoplasmic vacuoles	1
Platelet count	<150000/cmm	1

WBC-White Blood Cell, PMN-Polymorphonuclear Neutrophil, I: T PMN ratio- Immature: Total PMN ratio, I:M ratio- Immature: Mature PMN ratio.

The study comprises three categories; category (A): Infants with sepsis and having positive blood culture, category (B): Infants with probable infection but negative blood culture, category (C): Infants without evidence of sepsis. Other investigations such as C-reactive protein was done and recorded and compared with haematological score. All the suspected cases were verified by positive blood culture as "gold standard". Score of <2 was interpreted as sepsis unlikely, score 3-4 as possibility of sepsis, and the score of >5 as sepsis is very likely [3]. For the purpose of present study, to assess the reliability of HSS score, more than 5 was considered positive and compared to CRP as positive when it is more than 10mg/L. in calculation of the specificity, sensitivity, positive predictive value and negative predictive value. The individual parameters of HSS score were also assessed for all the suspected cases of neonatal sepsis. All the data and observations were recorded and presented in tables and charts and analysed by using a computer generated software "SPSS" 'Statistical package for the social sciences (SPSS Inc; Chicago, IL, USA)'.

RESULTS

All of 84 cases were classified into three categories; sepsis (20/23.8%), probable infection (31/36.9%), and normal (33/39.3%) on the basis of diagnoses by clinical findings and laboratory tests.

Table-2: Distribution of the patients in different categories, (N=84)

Category	Character	Number of cases	Percentage
A (Sepsis)	Blood culture: +ve=18 & clinically diagnosed=2	20	23.8%
B (Probable infection)	Blood Culture: -ve	31	36.9%
C (Normal)	Blood culture: -ve	33	39.3%
Total		84	100%

Among the 84 cases, 20 cases were diagnosed sepsis by positive blood culture and strong clinical features (20/23.8%), 31(36.9%) cases had probable infection and 33(39.3%) cases were normal infants, (Table-2).

Table-3: Score of each of the categories

Category	Score 0-2 (%)	Score 3-4 (%)	Score >5 (%)
Sepsis(N=20)	-	4(20%)	16(80%)
Probable infection(N=31)	05(16.1%)	19(61.2%)	08(25.8%)
Normal(N=33)	17(51.5%)	12(36.3%)	03(9%)
Total(84)	22(26.1%)	35(41.7%)	27(32.1%)

HSS score (0-2) were in 22(26.1%) cases, score (3-4) were in 35(41.7%) cases and score >5 were in 27(32.1%) cases. 80% of the cases diagnosed sepsis had score of >5, and 61.2% of the cases having probable infection had score of 3-4, (Table-3).

Table-4: Performances of different tests for diagnosis of neonatal sepsis

No. of cases in different Test categories	HSS Positive (>5)	CRP Positive (>10 mg/L)	Blood culture Positive
14	Positive	Positive	Positive
35	Negative	Negative	Negative
07	Positive	Positive	Negative
01	Positive	Negative	Positive
05	Positive	Negative	Negative
02	Negative	Positive	Positive
19	Negative	Positive	Negative
01	Negative	Negative	Positive
Total(84)	27(+ve/32.1%)	42(+ve/50%)	18(+ve/21.4%)

Out of 84 cases, HSS score were >5 in 27 cases (32.1%), CRP were more than 10mg/L in 42 cases (50%), and blood culture were positive in 18 cases (21.4%), (Table-4). 2 cases were diagnosed sepsis without blood culture positive result on the basis of strong clinical manifestations and possibly negative culture result were due to faulty laboratory techniques and misinterpretation.

Table-5: Specificity, sensitivity, positive and negative predictive values of HSS and CRP, (N=84)

Tests	Sensitivity	Specificity	Positive predictive value	Negative predictive value
HSS score	83.33%	81.8%	55.5%	94.7%
C-reactive protein	88.88%	60.6%	38.1%	95.2%
Combined	77.77%	89.4%	66.6%	93.6%

For all 84 cases, HSS score >5 showing high sensitivity (83.33%), specificity of 81.8%, and negative predictive value of 94.7%, but low positive predictive value of 55.5%. The combination of these two tests shows high specificity (89.4%) and negative predictive value of 93.6%, (Table-5).

Table-6: The performance of individual haematological parameter in diagnosis of neonatal sepsis, (N=84)

Hematologic Tests	Specificity %	Sensitivity %	PPV %	NPV %	Accuracy
Total WBC count	77.35	51.6	57.14	71.92	67.85
Total PMN count	73.33	47.82	68.75	45.45	85
Immature PMN count	72.72	58.82	76.92	53.33	90
Imm: Total PMN ratio	93.84	84.21	80	95.31	91.66
Imm: Mature PMN ratio	92.06	80.95	77.27	93.54	89.28
Degenerative changes	62.5	57.14	43.24	74.46	60.71
Platelet count	72.72	44.82	46.42	71.42	63.09

Among the individual haematological parameters, imm: total PMN ratio (>0.2) shows specificity of 93.84%, sensitivity of 84.21%, PPV of 80%, NPV of 95.31% and accuracy of 91.88%. Imm: mature PMN ratio (>0.3) shows specificity of 92.06%, sensitivity of 80.95%, PPV of 77.27%, NPV of 93.54% and accuracy of 89.28%, (Table-6).

DISCUSSION

Aim of this study was to evaluate the reliability of haematological scoring system as a test for early diagnosis of neonatal sepsis. Nowadays, clinicians are careful in prophylactic use of antibiotics due to development of drug resistance, cost of unnecessary therapy and problems of drug toxicity. Therefore, we need a quick diagnostic test with high sensitivity and specificity as a screening test [4]. The limitations in the diagnosis of neonatal sepsis are frustrating for clinicians and at present, there is no single reliable test for its diagnosis [9]. This scoring system is significant regarding its easy availability, accessibility, low cost, less time consuming and practically possible in all the laboratories. Manroe et al devised a criteria that used three parameters of total PMN count, immature PMN count and I:T ratio as a diagnostic tool [10]. In the present study, specificity, sensitivity, positive predictive value and negative predictive value of individual parameters of HSS were calculated. Elevated I:T ratio of PMN was found to be the most reliable indicator of sepsis and also in various studies like those done by Ghosh *et al.*, [4] and Narsimha *et al*., [11]. Immature PMN count was also a very sensitive indicator. Degenerative changes in PMNs made no significant contribution in diagnosis but presence of toxic granules indicates unusual PMNs production and they are not present in normal patients [10, 11].

The pitfall of this study is that a considerable number of patients showed HSS of 3-4 which indicate the possibility of infection and two cases were diagnosed sepsis without positive blood culture report and also there is no facilities for detection of microbial organism such as DNA probe, automated blood culture system, fluorometric detection system and PCR laboratory.

A study on 100 neonates, considering HSS as significantly positive of score >3, observations were the sensitivity of 100%, specificity of 21%, PPV of 15%, and NPV of 100% ($p<0.05$), and using HSS of >4 as significantly positive, they found sensitivity of 100%, specificity of 60%, PPV of 26% and NPV of 100% in which blood culture report were positive only in 12 cases ($p<0.001$) [12]. In the present study, we considered HSS positive when it is >5 and found sensitivity of 83.33%, specificity of 81.8%, negative predictive value of 94.7% but low PPV of 55.5%, and overall performance of HSS in our study is satisfactory.

The individual haematological parameters of scoring system were significantly associated with sepsis ($P<0.05$) and their performances were evaluated (Table-6). The total leukocyte count (TLC) is of little clinical use in the diagnosis of neonatal infection as it has wide variation in values. In our study, an elevated leukocyte count showed sensitivity of 51.6%, specificity of 77.35%, PPV of 57.14% and NPV of 71.92% which were comparable to the findings of Philip AGS and Hewitt JR [13]. In few cases, neutropenia were found in association with sepsis, probably because of increased adherence to altered endothelial surface and utilization at the site of infection [14].

A shift to the left in differential white cell count with a raised immature neutrophil count such as appearance of band form has been documented in patients with bacterial infection [15]. In the present study, Immature: total PMN ratio and Immature: mature PMN ratio appeared the most important parameters, as immature: total PMN ratio showed specificity of 93.84%, sensitivity of 84.21%, PPV of 80%, NPV of 95.31% and accuracy of 91.66%. Immature: mature PMN ratio showed specificity of 92.06%, sensitivity of 80.95%, PPV of 77.27%, NPV of 93.54% and accuracy of 89.28%. These findings are comparable with other studies. The total PMN count showed specificity of 73.33%, sensitivity of 47.82%, PPV 0f 68.75%, and NPV of 45.45% and these findings are comparable with the observations of study done by Akenzua *et al.*, [16] in which it was observed that total PMN count were normal but band form were elevated and rise of total count was late and inconsistent.

We compared sensitivity, specificity, PPV and NPV of HSS to that of C-reactive protein considering its cut-off value of >10mg/L in our laboratory and its cut-off value was considered lower in study by Suppawat Boonkasideche *et al.*, [17] and we found HSS positive (>5) with sensitivity of 83.33%, specificity of 81.8%, PPV of 55.5% and NPV of 94.7% which are comparable to that of CRP except specificity and PPV which were higher in HSS than those with CRP. The combination of these two tests giving slight higher value of specificity (Table-5).

Thrombocytopenia was frequently associated with sepsis and this is due to increased platelet destruction, sequestration resulting from infection and failure in platelet production as a consequence of reduced megakaryocyte or damaging effects of endotoxin [18]. In the present study, thrombocytopenia was associated with sepsis showing specificity of 72.72%, sensitivity of 44.82%, PPV of 46.42%, NPV of 71.42% and accuracy of 63.09%, which were comparable to other study [19].

As no single individual haematological parameter is superior in comparison with another in prediction of neonatal sepsis, so a combination of these parameters in the form of HSS has been recommended.

CONCLUSION

HSS is simple, quick and cost-effective tool which has considerable sensitivity and specificity for early diagnosis of suspected neonatal sepsis and it can be performed easily at primary health care centres as routine screening test and thus it helps to provide an effective guidelines for management of neonatal sepsis.

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