HAEMATOLOGICAL CHANGES IN NEONATAL SEPSIS- A STUDY IN A TERTIARY CARE HOSPITAL

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ABSTRACT

BACKGROUND

Sepsis is the commonest cause of neonatal mortality. Early recognition of neonatal sepsis is difficult as the clinical signs and symptoms are non-specific and the failure or delay in treatment may result in significant mortality and morbidity.

MATERIALS AND METHODS

Blood samples were taken from neonates attending Regional Institute of Medical Sciences, Imphal, Manipur, India with clinical features of sepsis. Various haematological tests were performed including haemoglobin level, total WBC count, total PMN count, immature PMN count, I:T PMN ratio, I:M PMN ratio, degenerative changes in PMN and platelet count. Haematological scoring and blood culture were done for each case. Correlation of the various haematological parameters was done with the blood culture.

RESULTS

A total of 101 neonates with clinical features of neonatal sepsis were included in the study. Out of all the cases, 59 (58.4%) cases were ≤7 days of age. Early onset sepsis was present in 58.4% of cases and late onset sepsis was present in 41.6% of the cases. Bacterial culture was positive in 27 (26.7%) cases. Among the organisms grown, coagulase negative staphylococci (CoNS) was the most common organism accounting to about 55.6%. Abnormal total PMN count had the highest sensitivity of 96.2% but the lowest specificity of 28.4%, among all the individual parameters. 47 cases had a haematological score ≤2, 38 cases had a score of 3-4 and 16 cases had a score ≥5. The sensitivity, specificity, positive predictive value and negative predictive values of the tests increased as the score increased. Also, the specificity of the two-test combination was either similar to or higher than the individual test at the cost of sensitivity.

CONCLUSION

None of the haematological parameters studied can be used alone for a reliable diagnosis of neonatal sepsis. However, a combination of tests and a haematological scoring system is a very useful diagnostic aid to the clinicians.

KEYWORDS

Neonatal Sepsis, Haematological Scoring System, Blood Culture.

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BACKGROUND

Neonatal sepsis is defined as a clinical syndrome characterized by signs of systemic infection and documented by a positive blood culture in the first four weeks of life.[1] The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live births.[2] Early recognition of sepsis in neonates is difficult as the clinical signs and symptoms are non-specific and the failure or delay in treatment may result in significant mortality and morbidity.[3] Infected infants must, therefore, be promptly identified and differentiated from non-infected patients, and antibiotics started without delay. Blood culture is considered to be the 'gold standard' for diagnosis of septicaemia; however, it is a time-consuming procedure with spurious positive results and demands a well-equipped laboratory. In addition, antimicrobial treatment based solely on risk factors and clinical grounds is likely to result in overtreatment. Therefore, a test that is cheap, reliable, easily performed with quick availability of reports is required. Various changes in different haematological parameters are seen in neonatal sepsis. Present study evaluates the haematological scoring system and the following 7 parameters individually and in combination: i.e. leucocyte count, neutrophil count (PMN), immature PMN count, immature to total PMN ratio (I:T), immature to mature PMN ratio (I:M), platelet count and degenerative changes in neutrophils, in early diagnosis of neonatal sepsis.

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Aims and Objectives

The objective of the study was to assess the significance of the various haematological parameters and the haematological scoring system in early diagnosis of neonatal sepsis.

MATERIALS AND METHODS

The study was conducted in the Department of Pathology, Regional institute of Medical sciences (RIMS), Imphal, Manipur from October 2012 to September 2014. All neonates below the age of 28 days attending the Department of Paediatrics, RIMS with clinical features of neonatal sepsis were included in the study. Excluded from the study were neonates who had received antibiotics, blood transfusion before collection of sample and neonates with inborn errors of metabolism. A careful detailed history of the neonates was recorded in the predesigned proforma. Blood sampling was done in the Neonatal Intensive Care Unit (NICU). With all aseptic precautions, blood sample was drawn within 24 hours of admission. For haematological examination, 1 ml of blood sample was taken in an ethylene diamine tetra acetic acid (EDTA) container and received in the Pathology Department. For blood culture, 0.5 ml of blood was put in 5 ml of brain heart infusion broth.[4] The neonates were investigated as follows:

Blood samples of neonates with clinical suspicion of sepsis received in the Pathology laboratory were taken to study haematological parameters. Total WBC count, total PMN count, immature PMN count, I:T PMN ratio, I:M PMN ratio and platelet count were done. Immature neutrophils include promyelocyte, myelocyte, metamyelocytes and, band form. I:T ratio was calculated by dividing the total immature count by the total neutrophil count (including both the mature and the immature neutrophil count). Degenerative changes in neutrophils including vacuolization, toxic granulations, and Dohle bodies were assessed in Leishman's stained smear. Data was collected in the predesigned proforma. The smears were examined using light microscope. The haematological findings were analysed according to the hematologic scoring system (HSS) of Rodwell et al.[5]

Under strict aseptic precautions, venous blood was collected from the vein after cleaning the venepuncture site. With a sterile needle and syringe, 0.5 ml of blood was drawn and injected directly into 5 ml of the blood culture medium (brain heart infusion broth) in the ratio 1:10. The bottle was shaken immediately to prevent blood clot. The sample was sent immediately to the Microbiology Department where it was incubated at 37^0 C overnight and subculture was done on blood agar, nutrient agar and MacConkey agar. The inoculated plates were incubated at 37^0 C overnight. If there was growth in the media, the colonies were identified by Gram stain, hanging drop and biochemical tests. If no growth was obtained on second day, the broth was further re-incubated for another 5 days for further subculture with culture media mentioned above.[4,6] Sensitivity, specificity, positive predictive value and negative predictive value were calculated for the parameters and results analysed using SPSS.

RESULTS

A total of 101 neonates with clinical features of neonatal sepsis were included in the study. 59 (58.4%) cases were males and 42 (41.6%) cases were females. Out of all the cases, 59 (58.4%) cases were ≤ 7 days of age. Early onset sepsis was present in 58.4% of cases and late onset sepsis was present in 41.6% of the cases. Bacterial culture was positive in 27 (26.7%) cases. Among the organisms grown, Coagulase negative staphylococci (CoNS) was the most common organism accounting to about 55.6%. Escherichia coli (22.2%), Klebsiella (14.8%) and Staphylococcus aureus (3.7%) were the other organisms isolated. Abnormal total PMN count had the highest sensitivity of 96.2%. Haematological score≥ 5 had the highest specificity 98.6% and the highest positive predictive value of 93.8%. The highest negative predictive value of 96.2% was shown by I:T ratio ≥0.12 (Table 1)

39 cases had both elevated I:T ratio and total PMN count, out of which 25 were culture positive and 14 were culture negative (Table 2). The sensitivity, specificity, positive predictive value and negative predictive value of this combination were 92.5%, 81.1%, 64.1% and 96.7% respectively.

17 cases had elevated I:T ratio and also showed degenerative changes in PMN, out of which 12 were culture positive and 5 were culture negative (Table 3). The sensitivity, specificity, positive predictive value and negative predictive value of this combination were 44.4%, 93.2%, 70.5% and 82.1% respectively.

Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Abnormal WBC Count	48.1%	91.9%	68.4%	82.9%
Abnormal Total PMN Count	96.2%	28.4%	32.9%	95.4%
Immature PMN ≥600/µl	92.6%	48.6%	39.7%	94.7%
I: T Ratio ≥ 0.12	92.6%	68.9%	52.1%	96.2%
I: M Ratio ≥30	33.3%	95.9%	75.0%	79.8%
Degenerative Changes in PMN	59.3%	94.6%	80.0%	86.4%
Platelet Count <1.5 Lakhs	25.9%	89.2%	46.7%	76.7%
Haematological Score ≤ 2	11.2%	6.3%	6.4%	55.6%
Haematological Score 3, 4	33.3%	60.8%	23.7%	71.42%
Haematological Score ≥ 5	55.6%	98.6%	93.8%	85.8%

Table 1. Comparison of Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of Each Test

I:T Ratio + Total PMN Count	Culture		
	Bacteriologically Positive	Bacteriologically Negative	Total
Positive	25	14	39
Negative	02	60	62
Total	27	74	101

Table 2. Combination of Two Tests I:T Ratio and Total PMN Count

I: T ratio + Degenerative Changes in PMN	Culture		
	Bacteriologically Positive	Bacteriologically Negative	Total
Positive	12	05	17
Negative	15	69	84
Total	27	74	101

Table 3. Combination of Two Tests I:T Ratio and Degenerative Changes in PMN

DISCUSSION

The present study showed wide variation in values of WBC count and revealed that abnormal WBC count had sensitivity, specificity, PPV and NPV of 48.1%, 91.9%, 68.4% and 82.9% respectively. Khair KB et al[7] studied 100 neonates admitted at neonatal ICU, BSMMU, Dhaka and found that total WBC count had a sensitivity of 50% and specificity of 91%, PPV of 43% and NPV of 93%. Similar findings were seen by Philip AGS and Hewitt JR[8] and Namdeo UK et al.[9] The positive predictive value of abnormal WBC count is poor. This is not surprising since many non-infectious conditions can be associated with an abnormal WBC count. In the past, changes in the WBC count were regarded least useful for the diagnosis of sepsis as these values were thought to be too erratic which also holds true in the present study.

In the present study, among the individual parameters, an abnormal total PMN count showed the highest sensitivity and the lowest specificity. We observed a sensitivity of 96.2%, a specificity of 28.4% along with a PPV of 32.9% and NPV of 95.4% which is consistent with the findings reported by Khair KB et al[7] and Majumder A et al.[10] Inspite of the high sensitivity, total PMN alone cannot be used for diagnosis of neonatal sepsis because of its very low specificity. However, Buch AC et al[11] observed that total PMN as an individual test was helpful to rule out sepsis since it had lower sensitivity (66%), higher specificity (90.91%) and NPV (69.44%). Shirazi H et al[12] also observed a low sensitivity (35%) and a high specificity (74%). These variations in the results shown by different studies may be due to differences in blood sampling time, severity of infections, and the age of the neonates.

A shift to the left in differential white cell count with a raised immature neutrophil count has been documented in patients with bacterial infections.[7] In the present study immature PMN count had a sensitivity of 92.6%, specificity of 48.6%, PPV of 39.7% and NPV of 94.7%. Majumder A et al[10] observed similar results. Despite a significant rise in immature neutrophil count in infants with suspected infection, abnormal immature PMN count gave low specificity due to a large number of false positive result. Therefore, this parameter alone should not be evaluated for diagnostic purposes.

The present study revealed that an elevated I:T ratio had a sensitivity of 92.6%, specificity of 68.9%, PPV of 52.1% and NPV of 96.2%. The high sensitivity and NPV were consistent with the studies by Khair KB et al[7] and Buch AC et al.[11] Considering high mortality and morbidity associated with sepsis, tests with high sensitivity and NPV are most desirable because all infants with sepsis have to be identified. A study by Makkar M et al[13] and Ghosh S et al[14] found elevated I:T ratio to be the most reliable indicator of sepsis.

I:M ratio in the present study revealed sensitivity, specificity, PPV and NPV 0f 33.3%, 95.9%, 75.0%, 79.8% respectively. Rodwell RL et al[5] used I:M ratio as a predictor of infection and observed a sensitivity of 93%, specificity of 81%, PPV 32% and NPV of 99%. Ghosh S et al[14] found similar results. Khair KB et al[7] observed that the sensitivity, specificity, PPV and NPV of I:M ratio are respectively 100%, 07%, 11% and 100%. The wide differences in the values of I:M ratio in different studies may be partly attributed to their subjective measurement and inter observer variability in assessment of immature PMN.

In the present study, degenerative changes in PMN had a sensitivity, specificity, PPV and NPV of 59.3%, 94.6%, 80.0%, 86.4%. Zieve PD et al[15] showed a very close relationship between the presence of vacuolated neutrophils and bacterial infections. Xanthou M[16] observed that toxic granulation was invariably present during sepsis, a change never seen in healthy newborn babies. Neonates with sepsis develop thrombocytopenia, possibly because of disseminated intravascular coagulation (DIC) and the damaging effects of toxins on platelets. Zaki MES et al[17] evaluated newborn infants with clinical diagnosis of neonatal sepsis where thrombocytopenia had a sensitivity of 41% specificity of 87%, PPV of 50% and NPV 0f 52%. This study shows thrombocytopenia with a sensitivity of 25.9% specificity of 89.2%, PPV of 46.7% and NPV of 76.7%. Buch AC et al[11] had similar findings on thrombocytopenia except for a higher sensitivity of 46.15%. The low sensitivity of platelet count in the present study renders it a less ideal test as a single parameter for screening of neonatal sepsis. The specificity was, however, high and therefore this parameter if combined with other tests can be used to exclude sepsis with some confidence.

As no single haematological parameter is superior in comparison to another in predicting neonatal sepsis, a combination of these parameters in the form of HSS is a very useful screening tool. Manucha V et al[18] observed that majority of neonates with a haematological score of ≥ 3 had a sensitivity of 86% and NPV of 96%. Khair KB et al[7] concluded that scores ≥4 are more specific and increases the likelihood of sepsis in relation to other scores. Makkar M et al[13] evaluated the HSS of Rodwell RL et al[5] in 110 neonates for early detection of sepsis in high risk infants and observed that higher the score, more the chances of sepsis and vice versa. The present study revealed that the sensitivity, specificity, PPV and NPV increases as the score increases.

Buch AC et al[11] observed that the combination of five parameters I:T ratio + absolute neutrophil count + CRP + ESR + Platelet count to be the best to predict the diagnosis of neonatal sepsis Mishra PK et al[19] observed that the positive predictive value and the specificity of two test combination was higher than the individual test at the cost of sensitivity. In the present study, it was observed that when total PMN and I:T ratio were combined, the specificity and positive predictive value of the combination was higher than that of the individual tests, while sensitivity was lower than the individual tests. Similarly, the combination of I:T ratio and degenerative changes in PMN revealed a lower sensitivity than the individual test. Our observations are, therefore, consistent with other studies.

CONCLUSION

No single haematological parameter is superior to another in predicting neonatal sepsis. However, the combination of tests and HSS are helpful in diagnosing as well as excluding sepsis and can provide an effective guideline to make decisions regarding judicious use of antibiotic therapy which will be lifesaving, provide early cure, as well as minimize the risk of emergence of resistant organism due to misuse of antibiotics.

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