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Research Article

EVALUATION OF HAEMATOLOGICAL PROFILE IN EARLY DIAGNOSIS OF CLINICALLY SUSPECTED CASES OF NEONATAL SEPSIS

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ABSTRACT
Early diagnosis of neonatal septicemia is difficult due to lack of specific clinical and laboratory findings. Blood culture is considered gold standard for the diagnosis, but is very time consuming. Hence most of the paediatricians administer antibiotics at the least suspicion of sepsis. To overcome this, several rapid haematological tests have been evaluated for their usefulness in rapid diagnosis of neonatal sepsis. Objective behind the study was to establish the role of haematological parameters (Total Leucocyte Count, Absolute Neutrophil Count, Immature to Total Neutrophil Ratio, Platelet count, C-Reactive Protein and Micro Erythrocyte Sedimentation rate), as a reliable indicator of septicemia alone and in combinations in neonates with suspected sepsis. A prospective study of haematological parameters of 120 neonates with clinically suspected sepsis was performed. These parameters were evaluated statistically based on the standard reference values. TNR was the best individual test with the highest sensitivity, specificity, positive and negative predictive value. ANC and CRP were only beneficial to rule out the sepsis. TLC, Micro ESR and Platelet count when used alone were neither helpful for confirming sepsis nor beneficial to exclude sepsis. Results were promising when these tests were used in combinations. The combination of I: TNR+ ANC+ CRP and I: TNR+ ANC+ CRP+ ESR gave the best test results. The most useful combination was of 5 tests (I: TNR+ ANC+ CRP+ ESR+ Platelet count). It is concluded that the different permutation and combinations of haematological parameters act as a rapid adjunct to diagnose and exclude neonatal sepsis.

Key Words*: Neonatal Sepsis, Immature to Total Neutrophil Ratio, C - Reactive Protein and Micro Erythrocyte Sedimentation rate*

INTRODUCTION
Neonatal Sepsis is a clinical syndrome characterised by signs and symptoms of infection with or without accompanying bacteraemia in the first month of life (Shankar 2008). The incidence of neonatal sepsis in India is 30 per 1000 live-births (NNPD 2002-03). Early recognition and diagnosis of neonatal septicemia is difficult because of the variable and nonspecific clinical presentation of this condition (Seema, 1999). It is extremely important to make an early diagnosis of sepsis, because prompt institution of antimicrobial therapy improves the outcome. Blood culture is gold standard for definitive diagnosis of neonatal septicemia. But it has its own limitations as it requires a well equipped laboratory, has a success rate of 40%, very time consuming, and may give spurious positive results (Shankar, 2008). The early diagnosis of neonatal sepsis has been occupying the minds of paediatricians for a very long time however no reliable tests are available. Hence we have used simple laboratory tests such as Total Leucocyte Count (TLC), Absolute Neutrophil Count (ANC), Immature to Total Neutrophil Ratio (I: TNR), Platelet count, C-Reactive Protein (CRP) and Micro Erythrocyte Sedimentation (ESR) for early detection of neonatal septicemia.

MATERIALS AND METHODS
The study group comprise of 120 neonates, admitted in Padm. Dr. D. Y. Patil Medical College and Research Centre. with clinical suspicion of septicemia. Neonates presenting with two or more of the

following clinical feature were selected for sepsis evaluation: ***(A) Respiratory system:*** (1) Tachypnea, (2) Increased apnea, (3) Severe apnea, (4) Increased ventilator support and (5) Oxygen desaturation. ***(B) Cardiovascular system:*** (1) Bradycardia, (2) Pallor, (3) Decreased perfusion and (4) Hypotension. ***(C) Metabolic change:*** (1) Hypothermia, (2) Hyperthermia, (3) Feeding intolerance, (4) Glucose instability and (5) Metabolic acidosis ***(D) Neurologic changes:*** (1) Lethargy (2) Hypotonia (3) Decreased activity. Neonates who received antibiotic before collection of sample, with major congenital malformation and the one who had received blood transfusion before collection of blood sample were excluded from the study. Detailed history and clinical findings were recorded. All such neonates were subjected to a septic screen at the time of admission.

Blood sampling was done under all aseptic precautions in the NICU. Soon after admission two ml blood sample was taken in EDTA vacutainer and was processed for TLC, PBS, DLC, ANC, I: TNR, Micro – ESR and Platelet count. Another 2 ml blood sample was taken for conventional blood culture. Also 1 ml blood sample was taken for estimation of a qualitative CRP result.

Auto hematology analyzer (3 parts) was used to analyze the sample and was counter checked. TLC was done by direct counting of leucocytes in a Improved Neubauer`s Chamber. The count and $\leq$ 5000 or $\geq$ 20000 / cumm was considered abnormal. I: TNR was calculated on PBS stained by Leishman`s stain and the value > 2 was considered abnormal. ANC was calculated on PBS stained by Leishman`s stain and the value > 1750 / cumm were considered abnormal. Micro – ESR was performed using standard micro haematocrit tubes. Value of Micro ESR $\geq$ 15 mm at the end of one hour was considered abnormal. Direct counting of platelets in an improved Neubauer`s Chamber was done. The count < 1.5 Lack / cumm were considered abnormal. CRP in serum was estimated by CRP Latex Kit. Test showed positivity when CRP value was $\geq$ 0.6 mg / dl. Blood culture bottle containing Brain Heart Infusion Broth was used and colony growth was observed

RESULTS AND DISCUSSION

In the present study out of 120 neonates, 100 (83.3%) neonates were $\leq$7 days of age, 97 (80.8%) neonates were low birth weight, 88.3% neonates were preterm. Male to female ratio was 1.8: 1. Respiratory distress and refusal to feed were the most frequent symptoms associated with septicemia. Blood culture was positive in 54.17% cases. Klebsiella Pneumonae (27.7%) was the most common organism isolated followed by E. coli and S. Aureus. On application of Chi- Square test association between age of onset and gestational age, age of onset and birth weight, age of onset and outcome, birth weight and outcome were statistically insignificant(P value > 0.05) whereas gestational age and outcome were statistically significant(P value < 0.05).

The results of individual diagnostic test are shown in Table 1. As an individual test only I: TNR was helpful for confirming sepsis and was beneficial to rule out sepsis. ANC and CRP as an individual test were only helpful to exclude sepsis. Whereas individually TLC, Micro ESR and Platelet count were neither helpful for confirming sepsis nor beneficial to rule out sepsis. The results of combination of three diagnostic tests for neonatal sepsis are shown in Table: 2. Best test result in three test combination was observed in I: TNR+ ANC+ CRP (sensitivity-87% and negative predictive value-85.19%). The results of combination of four diagnostic tests are shown in Table: 3. The results of combination of five diagnostic tests for neonatal sepsis are shown in Table: 4. The combination of I: TNR+ ANC+ CRP+ ESR+ Platelet count was found to be the best to predict the diagnosis of neonatal sepsis.

Neonates are uniquely susceptible to overwhelming bacterial infections (Ho 1992). It is very essential to diagnose the sepsis in early phase and also it is equally important to rule out neonatal sepsis (Shankar 2008).

Table 1: Individual Diagnostic Test for Neonatal Sepsis

Tests	Sensitivity (%)	Specificity (%)	Positive Predictive value (PPV) (%)	Negative Predictive value (NPV) (%)
TLC	50.77	63.64	62.26	52.24
ANC	66.15	90.91	89.58	69.44
I: TNR	89.23	70.91	78.38	84.78
Platelet Count	46.15	83.64	76.92	56.79
M- ESR	63.08	72.73	73.21	62.50
CRP	68.46	73.64	71.83	71.43

Table 2: Combination of 3 diagnostic tests for neonatal sepsis

Combination of 3 Tests	Sensitivity (%)	Specificity (%)	Positive Predictive value (PPV) (%)	Negative Predictive value (NPV) (%)
I: TNR+ANC+CRP	87.69	83.64	86.36	85.19
I: TNR+TLC+CRP	83.08	70.90	77.14	78.00
I: TNR+ Platelet Count +CRP	80.00	87.27	88.14	78.69
TLC+ Platelet Count +CRP	64.62	80.00	79.24	65.67
I: TNR+ANC+TLC	76.92	78.18	80.64	74.14
I: TNR+M-ESR+CRP	86.15	76.36	81.16	82.36
I: TNR+ANC+M-ESR	78.46	90.91	91.01	78.12
M-ESR+ANC+CRP	80.00	83.33	88.14	78.69

Table 3: Combination of 4 diagnostic tests for neonatal sepsis

Combination of 4 Tests	Sensitivity (%)	Specificity (%)	Positive Predictive value (PPV) (%)	Negative Predictive value (NPV) (%)
I: TNR+ANC+CRP+M-ESR	73.85	94.55	94.12	75.36
I: TNR+TLC+CRP+M-ESR	67.69	81.82	81.48	68.18

Table 4: Combination of 5 diagnostic tests for neonatal sepsis

Combination of 5 Tests	Sensitivity (%)	Specificity (%)	Positive Predictive value (PPV) (%)	Negative Predictive value (NPV) (%)
I: TNR+ANC+CRP+ Platelet Count +M-ESR	81.54	94.55	94.64	81.25
I: TNR+TLC+CRP+M-ESR+ANC	80.00	90.91	91.23	93.36

In our study the incidence of neonatal sepsis was higher in male (60%) than female neonates. Several other workers have reported similar finding (Chandra 1988 and Antoniette 2005). This is probably due to the fact that the factors regulating the synthesis of gamma globulin are situated on the X- chromosome and male has only one X-chromosome (Chandra 1988). In Indian scenario we think high male: female birth ratio and neglected female neonates add to high rate of neonatal sepsis in male child. In our study the proportion of culture positive septicemia cases was higher among the early onset septicemia (n=100). This is in agreement with NNPD (2002-03). They found 67% of cases developing septicemia in less than seven days. Neonates may acquire the pathogens presumably transmitted perinatally from the mother (Claudio 2004). The cellular and humoral immune system is immature at birth, hence neonates are more

vulnerable to infections (Antoniette 5005). In developing countries like ours, we think poor antenatal facilities and poverty also plays important role in early onset septicemia. It was found that the incidence of septicemia was higher in preterm neonates (83.33%). Several other studies have reported similar finding (Hussein 2007 and Himayun 2010). Waseem 2005) observed septicemia more common in low birth weight and preterm babies. Higher incidence of sepsis in low birth weight, both Preterm and small for gestational (SGA) is because they have low maternal acquired IgG and inherent susceptibility to infection. Placental transport of IgG from maternal to fetal circulation increases with maturity, this transport is hampered in SGA neonates who are often the products of placental insufficiency (Bhakoo 1988). Clinical features of septicemia are varied and nonspecific. We observed respiratory distress and refusal to feed were the most frequent symptoms associated with septicemia. The study was comparable with the study done by Waseem (2005).

Hussein (2007), Sharma (1993) and Lee (1997) had lower culture positivity 42%, 20%, and 41.2% respectively, present study has relatively higher culture positive (54.17%) cases. Klebsiella Pneumoniae (n=18) was the most predominate isolate, followed by S. Aureus (n=16) and E. coli (n=15).

High mortality was associated with early onset septicemia, preterm and low birth weight neonates. According to Gerdes (1998), neonatal sepsis is a ‘‘low incidence, high risk’’ disease and babies die rapidly (approximately 25%). We found the mortality rate of 24.61 % in culture positive cases and 18.18 % in culture negative cases. NNPD (2002-03) has reported a neonatal mortality of 18.6 % due to infection. The sepsis related mortality rate has been steadily decreased. This can be explained, due to early detection, good antibiotic treatment and overall improvement in the health care facility.

The most useful individual test for confirming and excluding neonatal sepsis was I: TNR. A raised immature neutrophil count has been demonstrated in patients with bacterial infections. This proves the use of I: TNR as a predictor of bacterial infections. This ratio is still believed by many as a single most helpful test available for diagnosing neonatal sepsis (Polin 2005, Bhandari 2010 and Hiew 1992). ANC and CRP had lower sensitivity, higher specificity and NPV compared to I: TNR, hence were useful to exclude the sepsis. Several studies considering ANC (Polin 2005 and Varsha 2003) and CRP (Himayun 2010 and William 1998) have published similar results. Relatively poor sensitivity of ANC and CRP could be due to non infective conditions affecting their results. Abnormal TLC, Platelet count and Micro-ESR as an individual tests were not significant in the diagnosis of neonatal septicemia as against the results of Hiew (1992). These variations in the results shown by different studies may be due to differences in blood sampling time, severity of infections, the age of neonates and reduced sensitivity of these tests in the first week of life.

The multitude of published data on the many laboratory tests and their varying combinations into a ‘scoring’ method or sepsis screen is a clear testimony to the fact that no single test is at present sufficiently accurate or reliable in identifying an infected infant (Chandra 1988 and Hiew 1992). Hence, we have used different permutation and combination of various tests. Analysis of the three test combination results revealed an increase in the sensitivity and positive predictive value, as compared to single test. The best result was observed in negative predictive value, which was high enough to exclude sepsis. Out of all combination which we tried, the best test result was observed in I: TNR+ANC+CRP (sensitivity-87% and negative predictive value-85.19%). The single test which did not gave satisfactory result was TLC, platelet and CRP, as their sensitivity (64.62%) and negative predictive value (65.67%) were quite low and cannot be used as a sepsis screen.

Gerdes (1998) observed in his study that leucocyte indices (TLC, ANC and I: TNR) are not accurate enough for early diagnosis of septicemia and their observation was in accordance with our finding.

Gerdes and Polin performed two septic screens 12 to 24 hours apart; the screen consisted of four test combinations i.e. I: TNR, TLC, CRP and Micro ESR. This method identified 100% of infected infants and had a negative predictive accuracy of 100% (Polin 2005). We used the same septic screen once and the results were not as promising as observed by them. Study done by Somu (1976) and Aulia (2003) using the same combinations once had comparable results with ours. We think that the significant

observational difference between our study and Gerdes and Polin's study was due to their serial septic screen.

In a study conducted in AIIMS by neonatology division of Paediatrics, screening panel of 5 test combination consisting of TLC, ANC, I: TNR, Micro ESR and CRP was used on all the patients who were suspected to have sepsis. If the screen was negative but clinical suspicion persists, it was repeated within 12 hours. If the results are still negative, sepsis was excluded with reasonable certainty. They found sensitivity of 93 to 100%, specificity of 83%, positive and negative predictive value of 27% and 100% respectively (Shankar 2008). Their results were comparable with our study and study done by Tripathi (2010). However same combination used by Nandy (2007) was not of much use in sepsis.

By reviving the various studies, it was observed that serial investigations of different tests combinations increases negative predictive values to greater extent (100%) than sensitivity. Hence are more helpful to exclude sepsis.

In present study, we found that the combinations of tests are more helpful in diagnosing as well as excluding sepsis than single test, but none of the combinations were able to achieve 100% sensitivity and positive predictive value. We also found that where ever both TLC and Platelet count were used in the combination of tests, the results were not very exciting. Our results revealed that combination of I: TNR, ANC, CRP, Platelet count, ESR was able to give the best outcome in diagnosing neonatal sepsis.

After analysing different permutation and combination of tests we conclude that, haematological profile may be used as a simple, easy, cheap and quick / rapid adjunct for the diagnosis of clinically suspected cases of neonatal sepsis.

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