

INTERPRETATION OF HAEMATOLOGICAL PARAMETERS IN NEONATES AT RISK FOR SEPSIS

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ABSTRACT

BACKGROUND

Neonates are uniquely susceptible to overwhelming bacterial infections. It is very essential to diagnose the sepsis in early phase and it is also important to rule out sepsis to prevent irrational use of antibiotics. Though blood culture is considered as gold standard, it is time consuming with limitation in preterm and has high false negative rates. For the same reason, several rapid haematological tests are done as part of sepsis screen for early diagnosis of neonatal sepsis.

OBJECTIVE

The study was done to establish the role of haematological parameters like Total Leucocyte Count (TLC), Absolute Neutrophil Count (ANC), Immature to Total Neutrophil ratio (I/T ratio), Platelet count, C – Reactive Protein (CRP), Micro-Erythrocyte Sedimentation Rate (m-ESR), either alone or in combination as reliable indicators of septicaemia in clinically suspected neonates.

METHODS

A prospective study of 100 neonates clinically suspected as having sepsis, who were admitted to NICU, Navodaya Medical College, Hospital and Research Centre, Raichur were included. The above haematological parameters were evaluated and statistically analysed.

RESULTS

Among 100 neonates, only 42 cases were positive for blood culture and were considered true positives. Haematological parameters like TLC, I/T ratio, CRP showed high sensitivity, specificity, positive predictive value and negative predictive value as individually or in combination. ANC and micro-ESR showed low sensitivity but high specificity. Results were promising when tests were done in combination using haematological scoring system.

CONCLUSION

Irrespective of blood culture-positivity, evaluation of haematological parameters aid in early diagnosis and provide a rational basis for therapy and management of neonatal sepsis.

KEYWORDS

Neonatal Sepsis, C-Reactive Protein, Total Leucocyte Count, Micro-Erythrocyte Sedimentation Rate, Immature to Total Neutrophil Ratio.

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INTRODUCTION: Neonatal sepsis is the single most important cause of neonatal deaths, with incidence rate of 30 per 1000 live births.^{1,2} Neonatal sepsis is defined as a clinical syndrome of bacteraemia with systemic signs and symptoms of infection in the first 4 weeks of life. The often nonspecific early symptoms and the potential rapid deterioration necessitate early identification of patients at risk.¹

Neonatal sepsis can be classified into subtypes depending upon whether the onset of symptoms is before 72 hours of life (early onset) or later (late onset). Early-onset infections (EOS) are caused by organisms prevalent in the

maternal genital tract or in the delivery area.² However, the potentially life threatening character of EOS is reflected by high mortality rates reaching up to 37% in preterm infants. Late-onset septicaemia (LOS) is caused by the organisms thriving in the external environment either nosocomial or community acquired and neonates present with septicaemia, pneumonia or meningitis.³

Blood culture is considered as the gold standard for diagnosis of sepsis, has limitations especially in preterm.⁴ However, the majority (>98%) of these neonates have negative blood cultures, resulting in unnecessary exposure to antimicrobials.⁵

Despite a myriad number of laboratory investigations, risk scores and clinical features in neonatal sepsis, still no single parameter is accepted to define the diagnosis of sepsis in its early course with 100% accuracy.^{6,7} A positive "sepsis screen" takes into account two or more positive tests such as: Leukopenia (Total Leucocyte Count<5000/cmm),

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Neutropenia (Absolute Neutrophil Count < 1500/cmm), Immature to Total Neutrophil (I/T) ratio (> 0.2), Micro Erythrocyte Sedimentation Rate (> 15 mm 1st hour), C-Reactive Protein (> 6 mg/dL).⁸

Presence of two abnormal parameters in a screen is associated with a sensitivity of 93-100%, specificity of 83%, positive and negative predictive values of 27% and 100% respectively in detecting sepsis.⁸ So this study has been taken up for early, timely diagnosis of neonatal sepsis by performing septic screen on neonates at risk, thereby reducing sepsis related mortality.

AIMS AND OBJECTIVES:

1. To study the prevalence of neonatal sepsis in tertiary care hospital.
2. To evaluate the Total Leucocyte Count (TLC) and Absolute Neutrophil Count (ANC) with special reference to Immature to Total Neutrophil ratio (I/T) as a marker for detecting neonatal sepsis.
3. To evaluate the importance of C - Reactive Protein (CRP) levels and Micro ESR as indicators of sepsis or as a serial study during infection to assess the response to antibiotics.
4. To evaluate the sensitivity and specificity of haematological parameters in relation to positive bacterial culture.

MATERIALS AND METHODS: The present prospective study comprises of 100 neonates at risk of sepsis that were admitted to NICU of Navodaya Medical College, Hospital and Research Centre, Raichur from January 2015 to January 2016.

Inclusion Criteria: All clinically suspected neonatal cases for early and late onset sepsis which were admitted to NICU.

Exclusion Criteria: Neonates who received antibiotics or blood transfusion before collection of sample and those with major congenital anomalies were excluded from the study group.

After taking written consent and clinical data, venous blood was collected and following laboratory investigations were done in Central Laboratory of Navodaya Medical College Hospital and Research centre (NMCHRC), Raichur.

1. Total leucocyte count (TLC).
2. Differential count with Absolute Neutrophil Count and Platelet count (DC, ANC and PC).
3. Immature to Total Neutrophil ratio (I/T).
4. Micro Erythrocyte Sedimentation Rate (Micro ESR).
5. C - Reactive protein (CRP).
6. Blood culture.

Haematological investigations were obtained from 5-part Haematology Analyser from Horiba Pentra ES 60 and I/T ratio were calculated on peripheral blood smear examinations which were stained with Leishman stain. Micro ESR was estimated with standard 75 mm heparinised micro-haematocrit tubes. Quantitative determinations of CRP levels

were done by Fully Automated Biochemistry Analyzer- A15 Biosystems by using ERBA CRP kit. Blood cultures were done by Brain Heart Infusion Broth by HIMEDIA.

Peripheral blood smears of all 100 neonates were analysed using the haematological scoring system of Rodwell et al by assigning a score for each of the seven criteria found to be significantly associated with sepsis.⁹

Parameter	Finding	Score
Total WBC Count	< 5000/mm ³	1
	> 25000/mm ³ at birth > 30000/mm ³ at 12-24 hours > 21000/mm ³ at day 2 onwards	1
Total PMN Count (T)	No mature PMN seen on smear	2
	Increased/Decreased	1
Immature PMN Count (I)	Increased	1
I/T Ratio	Increased	1
I/M Ratio (Immature to Mature Neutrophil)	> 0.3	1
Degenerative changes in PMN	Toxic granules, cytoplasmic vacuoles	1
Platelet Count	< 1,50,000/mm ³	1
Table 1: Haematological Scoring System⁹		

Interpretation: Minimum score: 0 Maximum score: 8.

Score	Interpretation
< 2	Sepsis is very unlikely
3 or 4	Sepsis is possible
> 5	Sepsis/infection is very likely

STATISTICAL METHODS: Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of TLC, ANC, I/T Ratio, Platelet count, Micro ESR and CRP were determined to define the diagnostic significance of each test. Positive blood culture was used as the standard to consider the neonate positive for sepsis. A p-value of < 0.05 was considered as significant.

RESULTS: A total of 100 neonates who were clinically suspected to have neonatal sepsis were included and the following haematological parameters were evaluated in them.

Based on the age of the baby, cases were further categorised into early onset sepsis (EOS) in neonates who were < 3 days of age and late onset sepsis (LOS) for those who were > 3 days of age but < 28 days. There were 64 cases of EOS and 36 cases of LOS.

Age of Onset	Term (%)	Preterm (%)	Total
Early onset sepsis	25 (62.5%)	39 (66%)	64
Late onset sepsis	15 (37.5%)	21 (35%)	36
	P<0.04	P<0.0001	
Table 2: Distribution of Cases According to Maturity			

Among these 100 neonates, 60 were preterm and 40 were term babies. The mean age for preterm neonates is 34.60 + 1.12 (29-36 weeks) and similarly for term is 38 + 0.36 (37-40 weeks). Sepsis was seen to be common among preterm neonates.

Out of 100 neonates, 65 were males and 35 were females with male to female ratio being 1.3:1. The most common presentations were respiratory distress, refusal of feeds followed by lethargy, jaundice etc.

Investigations	Term (Cases)	Preterm (Cases)	Total (Cases)
Total Leucocyte count (<5000/mm ³)	3	7	10
Total Leucocyte count (>20000/mm ³)	15	23	38
Absolute Neutrophil count (<1500/mm ³)	8	13	21
I/T Ratio > 0.2	22	38	60
I/M Ratio > 0.3	18	28	46
Toxic Granules	30	34	64
Platelet Count (<1.5 lakhs/mm ³)	13	25	38
Blood Culture	14	28	42
CRP (>6 mg/L)	26	29	55
Micro ESR (>15 mm/hr.)	22	19	41
Table 3: Summary of Investigations			

Among the haematological parameters, TLC was decreased, normal and increased in 10(10%), 52(52%) and 38(38%) cases. Leucocytosis to predict sepsis was seen only in 38 cases among which 23 cases were preterm neonates, which are less sensitive to total count in predicting the sepsis.

Absolute Neutrophil count > 1500 cells/mm³ was common in both term and preterm neonates. Only 21% neonates showed ANC < 1500 cells/mm³, among which 8% were term and 13% preterm cases.

I/T ratio > 0.2, present in 60% of cases with being more common in preterm neonates (38%) than term (22%).

I/M ratio > 0.3, present in 46% cases with suspected sepsis and common among preterm compared to term neonates (28% vs. 18%).

In this study, 64% of neonates showed toxic granules, more commonly seen in term neonates compared to preterm (34% vs. 30%).

Thrombocytopenia (< 1.5 lakh/mm³) was seen in 38 % cases of suspected sepsis, among which 25% of cases were preterm neonates.

CRP > 6 mg/L was observed in 55% of cases of which 29% were preterm and Micro ESR was elevated in 41% of cases, of which 22% were term and 19% were preterm.

Blood culture was positive in 42% of cases among which 14% were term and 28% were preterm neonates, suggesting susceptibility of preterm neonates to septicaemia.

In this study, Klebsiella Pneumoniae was predominate isolate (38%), followed by CONS (28%). Gram-negative organisms formed the majority of the isolates compared to Gram-positive organisms (60% vs. 40%) respectively.

Clinical diagnosis		Interpretation of HSS	Score	No. of cases (%)	Total (%)
Group 1	42	Sepsis Likely	> 5	Term 22 (37%)	60
				Preterm 38 (63%)	
Group 2	53	Probable Sepsis	3 or 4	Term 13 (39%)	33
				Preterm 20 (61%)	
Group 3	5	Sepsis Unlikely	< 2	Term 3 (43%)	7
				Preterm 4 (57%)	
Table 4: Haematological Scoring System - Distribution of Cases					

By observing above table, among 53 cases of group 2 culture-negative probable infection cases, 18 were grouped higher as sepsis likely and 33 cases were grouped as probable sepsis and both started with antibiotic treatment.

Tests	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
TLC	100	90.62	87.5	100
ANC	66.6	100	100	73.41
I/T Ratio	100	76.3	70	100

Platelet count	91.3	100	100	93.5
CRP	100	81.6	76.36	100
Micro ESR	97.6	100	100	98.3
Table 5: Correlation of Sepsis Screen Parameters with Positive Blood Culture				

The most useful individual tests which showed high sensitivity and PPV were TLC, I/T ratio, Platelet count, CRP and Micro ESR. The tests which showed high specificity were ANC, Platelet count and Micro ESR.

DISCUSSION: A missed diagnosis of neonatal sepsis can be catastrophic as majority of these have negative blood cultures, resulting in widespread exposure to antimicrobials, need for intravenous accesses and admission to NICU.

In this study, incidence of sepsis was more in males (65%) than female neonates, suggesting that in developing country like India, high male-to-female birth ratio and neglected female neonates add to high rate of sepsis in male child. Several other workers have reported similar findings like Antoniette et al and A.C. Buch et al.^{10,11}

Preterm neonates (60%) had higher incidence of sepsis compared to term, and risk factors seem to be associated are low birth weight, meconium stained liquor, prolonged labour and perinatal asphyxia. Similar observations were seen in study done by Himayun et al.¹²

Clinical features of septicaemia were nonspecific and most frequent were respiratory distress and refusal to feed and was comparable with study done by Waseem and Buch et al.^{11,13}

Bacteriological culture-positivity (42%) was higher among early onset sepsis, these cases were attributed to poor antenatal facilities, low birth weight, both preterm and small for gestational age (SGA) because they have low maternal acquired IgG and inherent susceptibility to infection and these findings were comparable with study done by Hussein and Lee.^{14,15}

Abnormal TLC with high sensitivity, proved as predictor of bacterial infection. In this age group, using a normal range with upper limits for WBC count and ANC will lead to newborns with high WBC count and ANC being labelled as having abnormal results, when in fact only leukopenia and low ANC are associated with increased likelihood of infection. Leukopenia has high specificity and NPV, the differences in result of this parameter shown by different studies may be due to variation in blood sampling time, severity of infection, age of neonates and the reduced sensitivity of this test in first week of life.^{11,16}

The sensitivity of ANC (66%) was low like that of study conducted by Christopher P et al but specificity and PPV were high.¹⁷

Our study showed high sensitivity (100%) and NPV (100%) for I/T ratio, which is still used by many as single most useful test for diagnosing neonatal sepsis, as shown by Bhandari and Buch et al.^{11, 18} According to Christensen et al, higher the degree of elevated I/T ratio was, the higher was the risk of bone marrow depletion and death from sepsis.¹⁹ Our study showed 55% positivity for CRP with sensitivity (100%) and NPV (100%). Value above 6 mg/L allows rapid identification of infected patients and serial measurements

of CRP have a prognostic value and show effectiveness of antibiotic therapy as shown by Younis et al.²⁰

Micro ESR levels with 97.6% sensitivity were comparable to study done by Okolo et al and NPV of 98.3% implies that neonates with normal micro ESR have 98.3% probability that sepsis is absent.²¹

The multitude of published data on the scoring systems and varying laboratory tests and their various combinations have shown that no single test is at present accurate or reliable in sepsis according to Hiew and Buch et al.^{11,22}

Hence, we have used haematological tests along with haematological scoring system and the best results were obtained in the form of sensitivity, specificity, PPV and NPV. According to HSS, 42% of cases belonged to group 1 as sepsis likely, 53% belonged to probable sepsis and 5% were categorised as group 3 as sepsis unlikely and these results were comparable with Aparna Narsimha et al.²³

Study conducted by neonatology division in AIIMS, which used sepsis screening consisting of panel of 5 tests i.e. TLC, ANC, I/T ratio, Micro ESR and CRP. They found sensitivity of 93% to 100%, specificity of 83%, PPV and NPV of 27% and 100% respectively. Observations done by various studies reveal serial investigations of tests increases NPV and are helpful to exclude sepsis.⁸ Our study is comparable with them and study done by Tripathi and Buch et al.^{11,24}

CONCLUSION: As clinical signs in preterm and term infants with severe bacterial infection are often nonspecific and scarce, results of blood culture can perhaps most importantly provide more information about the risk of sepsis after first few hours of birth.

Complete blood count with reduced Total Leucocyte Count (TLC) and Absolute Neutrophil Count (ANC) with high Immature to Total Neutrophil ratio (I/T), raised C – Reactive Protein (CRP) and Micro ESR levels can be of early diagnostic utility as first screening procedure followed by blood culture. Serial determination of laboratory parameters will help in assessing the disease course and prognosis.

Many times when cultures are negative even in a symptomatic neonate, haematological scoring system can accurately predict the presence or absence of sepsis. Combination of tests increases the specificity and positive predictive value. They aid in diagnosis of sepsis in neonates and to institute early and proper antibiotic therapy. Early recognition or diagnosis by simple laboratory parameters reduces the disease burden by early intervention, thereby reducing the neonatal mortality rate.

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