

## Haematological Parameters in Neonatal Sepsis in a Tertiary Care Centre Study

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### ABSTRACT

#### BACKGROUND

Neonatal septicaemia is a bacterial infection with positive blood culture in first four weeks of life. The early sepsis screen is vital as it detects earlier and benefits the clinician to treat the infection reducing neonatal mortality and morbidity. We wanted to evaluate various haematological screening parameters and C-reactive protein (CRP) in blood culture positive neonates in sepsis.

#### METHODS

This prospective study was conducted for a period of one year. Blood samples from hundred clinically suspected neonatal septicaemia cases were subjected to aerobic culture and sepsis screen tests like C-reactive protein, erythrocyte sedimentation ratio (ESR), total WBC count, absolute neutrophil count, immature / total neutrophil count (I / T) ratio and platelet count. The culture results were correlated with the sepsis screen tests.

#### RESULTS

Of the hundred cases studied, 18 % were blood culture positive and 66 % were males. Early onset septicaemia was more common, seen in 64 % of cases than late onset septicaemia (26 %) cases. *Staphylococcus aureus* was the commonest organism isolated in 38.46 % of cases followed by *Klebsiella pneumoniae* & *E. Coli*. Among the haematological parameters, the positivity was best with C-reactive protein (94.44 %) followed by immature & mature neutrophil ratio (I / M) (94.44 %), I / T ratio (88.88 %) and the least with absolute neutrophil count (66.0 %). Any two or more parameters were positive in 94.44 % of the subjects.

#### CONCLUSIONS

Sepsis screen has good sensitivity, specificity and is a valuable aid for early diagnosis of neonatal septicaemia. Sepsis screen is simple, cost effective, less time consuming and easy to perform. As an individual test C-reactive protein has shown highest sensitivity, specificity and is a sensitive and responsive indicator of neonatal sepsis.

#### KEYWORDS

Blood Culture, Neonatal Septicaemia, Sepsis Screen

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## BACKGROUND

Neonatal septicaemia is a leading cause of mortality and morbidity in the neonatal period. By definition, neonatal sepsis is a clinical syndrome consisting of symptoms and signs with bacterial infection in the first four weeks of life. Neonatal septicaemia shows systemic infections of the new-born such as meningitis, septicaemia, pneumonia, arthritis osteomyelitis & urinary tract infections.<sup>1</sup> Early diagnosis of neonatal sepsis is difficult due to its non-specific clinical presentation. The reported incidence of neonatal sepsis in the developed countries varies from 1 to 10 per 1000 live births<sup>2</sup>. Rational antibiotic therapy with the help of certain indirect markers such as leucopenia, toxic granules, immature neutrophil to total neutrophil ratio, micro-ESR and C-reactive protein, collectively known as sepsis screen helps in early diagnosis. For early diagnosis of neonatal septicaemia, a hematologic scoring system (HSS) of Rodwell<sup>3</sup> [includes total & differential leukocyte count, total neutrophil count, immature & total neutrophil ratio (IT ratio), immature & mature neutrophil ratio (IM ratio), total immature polymorphonuclear cell (PMNs) count & platelet count] is preferable because it includes all parameters. We wanted to evaluate the various haematological screening parameters and CRP in blood culture positive neonates in sepsis.

## METHODS

This prospective study was conducted in the Department of Pathology, S. Nijalingappa Medical College Bagalkot, Karnataka for a period of one year (December 2018 to December 2019). The study group consisted of hundred neonates of 0 - 28 days old with clinical evidences of sepsis.

### Inclusion Criteria

Suspected sepsis neonates admitted to neonatal intensive care unit (NICU) and only the hospital born babies with clinical signs and symptoms of septicaemia was included in the study and informed consent was taken.

### Exclusion Criteria

Neonates with respiratory distress syndrome, extreme prematurity (< thirty weeks), gross congenital anomalies or any previous antibiotic therapy was excluded from the study.

In all neonates' blood samples were obtained from peripheral venipuncture and under aseptic precautions blood was drawn for blood culture from clinically suspected neonates with sepsis and also processed for blood cell count with differential count & quantitative CRP & micro ESR.

All neonates admitted in NICU were investigated as follows -

1. Sepsis screen – cell counts with the help of CBC analyser
2. C-Reactive protein
3. Culture reports were compared
4. Blood smears were studied after Leishman stain for morphological features which were looked under 40 X and oil immersion using cedar wood oil –

RBCs morphology

WBCs = differential count for 100 cells

Neutrophils = hyper segmented, band form. Absolute neutrophil count was calculated, toxic granules, premature cells also noted

Platelet count was done.

If more than two criteria were positive out of the following parameters, it was considered positive for sepsis screen:

- a. Elevated micro ESR i.e. > [age + 3] mm in first hour in less than three days old neonate or > 15 mm in first hour at any age.
  - b. Leukopenia i.e. total leucocyte count less than 5000 cells / cu mm
  - c. Neutropenia i.e. absolute neutrophil count less than 1500 cells / cu mm
  - d. Elevated ratio of immature neutrophils (I) [band cells] to the sum of all total neutrophils (T), granulocytes [i.e. I / T ratio > 0.20.]
  - e. Thrombocytopenia: Decreased platelet counts less than 1.5 lakhs / cumm.
  - f. Presence of toxic granules in immature neutrophils [toxic granules in cytoplasm of neutrophil], cytoplasmic vacuolation & Dohle's bodies [aggregates of rough endoplasmic reticulum which stains light blue on Giemsa stain] on the smear are suggestive of sepsis.
  - g. Elevated C-reactive protein [more than 6 mg / lit. on day one and day two of life or more than 10 mg / lit. on subsequent days of life]
- Positive blood culture

### Statistical Analysis

Among the haematological parameters, the positivity was best with C-reactive protein (94.44 %) followed by I / M (94.44 %), I / T ratio (88.88 %) and the least with absolute neutrophil count (66.0 %). Any two or more parameters were positive in 94.44 % of the subjects. All data obtained was calculated in an Excel sheet and statistical analysis was done using the Open Epi software version 2.3.1.

Sensitivity formula –

True positive / true positive + false negative and specificity formula: True negative / true negative + false positive

## RESULTS

Clinically suspected cases of neonatal sepsis within the first forty-eight hours of life, based on clinical features, were

subjected to various haematological screening parameters and blood cultures. Our study of hundred cases showed early onset sepsis cases were more than late onset sepsis cases. 66 % were male and 34 % were female with a ratio of 1.94:1. Blood culture was positive in eighteen cases, amongst which sixteen cases were of early onset sepsis & only two cases of late onset sepsis.

Leucopenia was found in more cases than leucocytosis. Immature / total ratio, immature / mature ratio, platelet count and C-reactive protein (CRP) were positive in higher proportion of culture positive cases in percent of 88.88, 94.44, 88.88 & 94.44 respectively.

Raised micro erythrocyte sedimentation ratio (ESR) and CRP showed higher number of false positive cases in respect to culture positivity.

All screening parameters were found to be significant with respect to culture proven sepsis.

Sl. No.	Parameters	Culture Positive N = 18 Percent	Culture Negative N = 82 Percent	Total Cases %
1	Raised micro ESR	72.21	60.97	63
2	Leucopenia	83.33	24.34	35
3	Neutropenia	66.00	39.00	44
4	Elevated ratio of immature to total neutrophils	88.88	24.30	36
5	Elevated ratio of immature to mature neutrophils	94.44	26.82	37
6	Thrombocytopenia	88.88	34.14	45
7	Immature neutrophils with toxic granules	83.33	24.35	35
8	Raised CRP	94.44	87.00	89
9	More than any two parameters positive	94.44	14.10	29

**Table 1. Various Sepsis Screen Parameters in Relation with Blood Culture Positive & Negative Cases**

High sensitivity is shown by CRP (94.44 %), I / M ratio (94.44 %) followed by I / T ratio (88.88 %) and low platelet count (88.88 %).

High specificity is shown by I / T ratio, I / M and presence of toxic granules in neutrophils (75.60 %) respectively. If two or more of the above tests were positive, sensitivity of the screening tool increased above 90 percent & specificity above 80 percent.

Sl. No.	Screening Parameters	Sensitivity (%)	Specificity (%)
1	Raised micro ESR	72.21	39.02
2	Leucopenia	83.33	73.17
3	Neutropenia	66.00	70.73
4	Elevated ratio of immature to total neutrophils	88.88	75.60
5	Elevated ratio of immature to mature neutrophils	94.44	75.60
6	Thrombocytopenia	88.88	65.85
7	Immature neutrophils with toxic granules	83.33	75.60
8	Raised CRP	94.44	12.10
9	More than any two parameters positive	94.44	85.36

**Table 2. Sensitivity & Specificity Pattern of Various Sepsis Screen Parameters & Their Combination**

**DISCUSSION**

In the present study maximum neonatal sepsis cases (75 %) were found to be in less than three days old (early onset septicaemia) as compared to neonates aged more than three days (late onset septicaemia) (25 %), similar observations were seen in the study done by National Neonatal Perinatal Database<sup>4</sup> & Sriram,<sup>5</sup> J N Mishra<sup>6</sup>

observed that early onset was 71.7 % in his study. T. Vesikari et al.<sup>7</sup> reported early onset in most of the patients with neonatal sepsis. In 410 cases studied on set less than seven days was found in 370 cases.

Out of hundred cases, sixty-six cases were males & thirty-four were females with a high prevalence in the age group of 24 - 48 hours. Present study showed male preponderance with a ratio of 1.97:1. Garg et al.<sup>8</sup> & Bhat & Rao<sup>9</sup> observed male, female ratio as 2.57:1. & 1.08:1 respectively. Others like Piyush Gupta et al.<sup>10</sup> N. Somu, et al.<sup>11</sup> Khatau, et al.<sup>12</sup> observed male predominance in their study.

In the present study, the blood culture was positive in 18 %. This was similar to the 28.6 - 42.2 % yield which was obtained by many authors.<sup>13</sup>

The most common organism isolated in present study was *Staph. aureus* (44 %) and *klebsiella* (22 %), while in other studies commonest are *klebsiella* (31.8 %) & *E. Coli* (22.7 %). Vandana<sup>14</sup> observed *E. coli* were commonest.

The sepsis work up included various haematological parameters and CRP. In the present study, the sensitivity of the haematological screening parameters and CRP varied from 66.0 - 94.44 %.

The total leukocyte count and the B: N ratios have been correlated with an increased risk of bacterial infections in neonates. However, they had a wide range of sensitivity (17 - 90 %).<sup>9</sup> In our study, the sensitivity was 83.33 % for TLC and 94.44 % for B: N ratio. Neutropenia was observed in 66.0 % of the culture proven sepsis cases.

During the bacterial infections increased number of neutrophils is released from bone marrow into the blood stream providing neutrophils to migrate at the infected site. This increase in neutrophils appear essential for the host resistant to bacterial infection. As more neutrophils are released, more & more immature cell reaches the circulation, a process called as "shift to left." This finding has been found valuable in early diagnosis of bacterial infection.

In our study toxic granulation had 83.33 % sensitivity, 75.60 % specificity. Our studies are consistent with other studies. Vandana et al.<sup>14</sup> observed that toxic granulation had 80 % sensitivity, 70 % specificity. Zipursky et al.<sup>15</sup> showed a very close relationship between the presence of vacuolated neutrophils and bacterial infections. Xanthou<sup>16</sup> in her study of neonatal infection, described toxic granulation as an important feature. She felt that toxic granulation was invariably present during sepsis a change never seen in healthy newborn babies.

The micro-ESR is an inexpensive, easy bedside screening test. Its sensitivity ranges from 30 % to 73 % in proven sepsis cases.<sup>9</sup> In the present study, sensitivity of micro-ESR was 72.21 %.

Our study shows platelet count had sensitivity (88.88 %), specificity (65.85 %). Our observations are consistent with other studies. Khursid S et al.<sup>17</sup> observed in their study out of fifty neonates suspected of sepsis twenty-two neonates had thrombocytopenia. Out of fifty neonates twenty-one neonates had positive blood culture, eleven neonates had thrombocytopenia. In their study

thrombocytopenia had sensitivity of 52 %, specificity of 62 %.

Other relevant investigations like chest x-ray and lumbar puncture were done as and when required.

Benitz et al.<sup>18</sup> found that the sensitivity of C-reactive protein in culture proven early onset sepsis rose from 35 % at the initial evaluation to 78.9 % in next twenty-four hours. Our study shows sensitivity of C-reactive protein in culture proven cases as 94.44 %.

Combination of any two or more positive tests in culture has shown 80 % sensitivity in early onset septicaemia reported earlier from Indian studies.<sup>9</sup> Our studies showed sensitivity of 94.44 % by the combination of any two or more parameters. M Singh et al.<sup>19</sup> also found that when two or more tests were combined the specificity was increased than the individual test.

The diagnosis of neonatal septicaemia is difficult due to its non-specific clinical presentation. The major problem in neonatal septicaemia is the identification of the infected infant. Often overlooked is the equally important task of identifying the non-infected infant. It is desirable to administer appropriate therapy as early as possible to the affected infant for favourable outcome and to avoid such therapy in others to prevent emergence of resistant strains of organisms.

## CONCLUSIONS

Neonatal sepsis remains one of the most important causes of neonatal morbidity and mortality in our country. The sepsis screen suggested in this study was based on haematological parameters which can be easily performed in the side laboratory of a peripheral health centre.

The sensitivity of a combination of any two or more parameters is more satisfactory. Blood culture is still the "Gold standard" for the diagnosis of septicaemia in neonates and should be done in all cases of suspected septicaemia. Prevention of sepsis has to be given its due importance. Prevention of factors which predispose to neonatal septicaemia like prematurity and low birth weight infants, aseptic techniques in delivery rooms and wards are crucial. Further, newborns who develop sepsis often deteriorate rapidly. Rational antibiotic policy and proper protocols in utilisation of anti-microbials will reduce the development of resistance which is emerging as a global threat. Because of failure or delay in treatment is likely to result in significant mortality and morbidity, early and efficient diagnosis is challenging to the clinician. Blood culture not only takes time, but is also complicated, with a low yield.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

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