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A study of hematological parameters in neontal sepsis

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Abstract
Introduction: Neonatal septicemia is major cause of morbidity and mortality in newborns, which requires early diagnosis and prompt treatment to reduce case fatality rate with help of various hematological parameters, till results of blood culture become available.
Aims: The present study was undertaken to analyse the efficacy of various hematological parameters in comparison with blood culture in neonates presented with septicemia.
Methods and Material: Blood samples were received in central diagnostic laboratory, Shree Krishna Hospital, Karamsad and processed in respective sectional laboratory. Efficacy of all the parameters were assessed by measuring sensitivity, specificity, PPV and NPV.
Results: Out of 100 cases, culture positivity was seen in 76% of cases with male preponderance (59%). Toxic granulation (TG) was the most sensitive test (86.84%). The most specific test was TLC (91.66%).
Conclusions: The hematologic parameters are simple and cost effective tests, reports of which are obtained within few hours which helps in early initiation of treatment.

Keywords: septicemia, hematological parameter, blood culture, TLC, PPV, NPV

1. Introduction
Neonatal sepsis is the most important cause of mortality and morbidity, especially among low birth weight and preterm babies in developing countries like India. Neonatal septicaemia can be defined as generalized bacterial infection in infants during the 1st month of life. The incidence of neonatal sepsis is around 30 per 1000 live births according to pooled hospital data based on National Neonatal Perinatal Database (NNPD) [1].
It accounts for about 30 – 50% total neonatal death in developing countries. Early diagnosis of neonatal septicaemia is a great challenge despite advances in diagnostic modalities due to its non-specific subtle manifestation. Blood culture provides definite diagnosis, however it takes about 48 – 72 hour [2].
Neonatal septicaemia includes various systemic infections of the newborn such as septicaemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections.
Clinical diagnosis of neonatal septicaemia is a difficult chore. Clinical signs and symptoms of neonatal sepsis are vague and nonspecific when compared to older children and adults. Early warning signs and symptoms of neonatal sepsis are often minimal and subtle. Respiratory symptoms include tachypnea and grunting with or without supplemental oxygen requirement and respiratory failure. Other vague signs of sepsis include irritability, lethargy, temperature instability, poor perfusion and hypotension. Disseminated intravascular coagulation with purpura and petechiae can occur in more severe septic shock. Gastrointestinal symptoms can include poor feeding, vomiting and ileus. Meningitis may present with seizure activity, apnea and depressed sensorium.
Neonatal sepsis may take a fulminant course, leading to septicaemic shock, disseminated intravascular coagulation and death within hours of the onset of clinical manifestations. Neonatologists have a critical need for laboratory tests that aid in the early diagnosis of neonatal sepsis, firstly since the clinical status of neonates can worsen quickly and lead to rapid deterioration and secondly to avoid unnecessary instillation of antibiotics and prevent the development of resistance.
Depending upon the onset of symptoms whether it is during the first 72 hours of life or later, neonatal sepsis can be divided into two subtypes: Early onset neonatal sepsis and late onset neonatal sepsis.

After birth, the baby is exposed to the environment contaminated with microorganisms, which start settling or colonizing at various places. The organisms colonizes the body through the portals like umbilicus, skin or mucosa. Due to weak immunological defense of the new born, even locally prevalent infections tend to become global. Infections are more commonly encountered with preterm and low birth weight babies.

Various studies have shown that haematological parameters are simple, consume less time and are cost effective in the early diagnosis of neonatal sepsis. With haematology reports being made available within an hour or two, precious time can be saved while awaiting culture results. This study aims to evaluate the various haematological parameters that can be utilized for making early diagnosis of neonatal sepsis and hence enable early initiation of empirical treatment.

2. Material and Methods

The present study was a prospective study of 100 cases of neonatal sepsis admitted to Neonatal Intensive Care Unit (NICU), Shree Krishna Hospital, Karamsad with clinical evidences of septicaemia from December 2019 to July 2021.

Detailed clinical history was taken and thorough clinical examination was done for every neonate admitted in NICU.

Appropriate samples were taken from each neonates and received to Central Diagnostic Laboratory, Shree Krishna Hospital, Karamsad and processed respective sections accordingly.

EDTA samples were processed on Automated hematology analyzer and TLC and Platelet counts were obtained.

Differential leukocyte count, Immature granulocytes, Degenerative neutrophilic changes and Nucleated RBCs were noted by peripheral smear examination. Low platelet counts were confirmed on smear stained with Field stain or Giemsa stain.

The indices were calculated as follows:

- **I/T ratio** = Total number of immature neutrophils/ Total number of neutrophils
- **I/M ratio** = Total number of immature neutrophils/ Total number of mature neutrophils

The plain sample were used for CRP level estimation.

Culture results were observed after 48 hours.

2.1 Stastical analysis

Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive value (NPV) were calculated for each parameter.

3. Results

A total of 100 cases who were admitted in Neonatal Intensive Care Unit (NICU), Shree Krishna Hospital, Karamsad with clinical suspicion of sepsis during the period from December 2019 to July 2021 were included in the study and various laboratory parameters were evaluated in them.

An initial demographic patient profile was rendered based on age and gender distribution.

The present study showed a male preponderance with the Male: Female ratio of 1.43:1. Majority of neonates are of age group less than 7 days (79%), followed by age group of 7-14 days (13%) and 15-28 days (8%) and significant number of neonates presented with sepsis were full term (52%), followed by 31% cases being pre term and 17% cases of late pre term.

Majority of babies neonates in present study were having low birth weight (54%), while 5% cases were presented with very low birth weight and 41% are of normal birth weight.

Out of 100 neonates, 44 had onset of sepsis within first 72 hours of life, while remaining 56 had late onset of symptoms (>72 hours).

Out of 100 cases, 76 case were culture positive while remaining 24 cases were culture negative. Out of 76 culture positive cases, 44 were males and remaining 32 were females.

Nearly half of the neonates born by LSCS (49%), followed by normal vaginal birth (47%), and remaining four cases by forceps and vaccum assisted delivery respectively (2% for each). Most of the septic neonates were born to primigravida mothers in this study (57%), while very few were born to fourth gravida mothers (5%). Remaining babies were born to second gravida (31% cases) and third gravida (7% cases).

Out of 100 neonates, about 38% were born to the healthy mother, followed by groups of babies born to mothers with PROM and obstructed labour respectively (09% for both). Mothers had placenta previa in 7% of cases.

Most common organism isolated was coagulase negative staphylococcus (28.94%), followed by Klebsiella pneumonia and Enterobacteriaceae (14.47% for each), and next followed by Bacillus sp. and Non-Enterobacteriaceae (10.52% for each). Least common organisms isolated were Staphylococcus epidermidis, candida and Enterococcus hirae (1.31% for each).

Following tables show distribution of neonates according to various test results and culture report.

Table 1: Total Leukocyte Count (N = 100)

Total leukocyte count (/cumm)	Culture		Total
	Positive	Negative	
Abnormal <5000 or >25000	15	02	17
Normal	61	22	83
Total	76	24	100

Table 2: Absolute Neutrophil Count (N = 100)

Absolute neutrophil count (/cumm)	Culture		Total
	Positive	Negative	
Abnormal <1800 or >5400	53	17	70
Normal	23	07	30
Total	76	24	100

Table 3: Absolute Immature Neutrophil Count (N = 100)

Absolute immature neutrophil count (/cumm)	Culture		Total
	Positive	Negative	
Abnormal >600	42	15	57
Normal	34	09	43
Total	76	24	100

Table 4: Immature to Total Neutrophil Count Ratio (N = 100)

Immature to total neutrophil count ratio	Culture		Total
	Positive	Negative	
Increased (>0.2)	08	03	11
Normal (≤0.2)	68	21	89
Total	76	24	100

Table 5: Degenerative Neutrophil Changes (N = 100)

Degenerative neutrophil changes		Culture		Total
		Positive	Negative	
Toxic granules	Present	66	17	83
	Absent	10	07	17
	Total	76	24	100
Cytoplasmic vacuoles	Present	20	04	24
	Absent	56	20	76
	Total	76	24	100

Table 6: Nucleated RBCS (N = 100)

Nucleated RBCs	Culture		Total
	Positive	Negative	
Present	21	07	28
Absent	55	17	72
Total	76	24	100

Table 7: Platelet Count (N = 100)

Platelet count (/cumm)	Culture		Total
	Positive	Negative	
Abnormal (<150x10^3)	30	09	39
Normal	46	15	61
Total	76	24	100

Table 8: C-Reactive Protein Level (N = 100)

C-reactive protein	Culture		Total
	Positive	Negative	
Increased (>3 mg/L)	64	23	87
Normal	12	1	13
Total	76	24	100

Table 9: Diagnostic Utility of Various Laboratory Parameter

Tests	Sensitivity	Specificity	PPV	NPV
TLC	19.73%	91.66%	88.23%	26.50%
ANC	69.73%	29.16%	75.71%	23.33%
AIMNC	55.26%	37.50%	73.68%	20.93%
I/T ratio	10.52%	87.50%	72.7%	23.59%
Toxic granules	86.84%	19.16%	79.51%	41.20%
Cytoplasmic vacuolation	26.31%	83.33%	83.33%	26.31%
Nucleated RBCs	27.63%	70.23%	75%	23.61%
Platelet count	39.47%	62.50%	76.92%	24.59%
CRP level	84.21%	4.16%	73.56%	7.69%
ANC and I/T ratio	9.21%	91.66%	77.7%	24.17%
ANC and CRP	57.89%	29.16%	72.13%	17.94%
CRP and I/T ratio	7.89%	87.5%	66.66%	23.07%

Above table shows sensitivity and specificity of various tests out of which toxic granules (TG) showed highest sensitivity, closely followed by CRP levels. Most specific parameter was TLC, followed by cytoplasmic vacuoles.

4. Discussion

The accurate diagnosis of sepsis is particularly challenging in the neonate as blood cultures and clinical presentation are less informative. False negative blood culture is possible due to low sample volume, low bacterial density, culture contamination or suppression of bacterial growth due to antibiotic administration. This variability can lead to drastic underdiagnosis of neonatal sepsis.

Several studies have suggested various laboratory parameters to be individually and collectively helpful in detecting bacteremia. The present study was undertaken with an objective of evaluating the diagnostic performance of the various available haematological parameters.

The present study showed male predominance (59%), which is comparable with studies done by Khair *et al.* [3], Darnifayanti D *et al.* [4], Vinay BS *et al.* [5] and Piyush Gupta *et al.* [6]. Newborn males are more vulnerable to infections and death than females.

The present study stated that incidence of septicaemia is more in early age of life (<7 days) (79%) compared to delayed age at presentation (21%). Vinay BS *et al.* also reported similar findings in their study (90%). [5] Khair *et al.* [3], Piyush Gupta *et al.* [6] and Aggarwal *et al.* [7] too had similar findings.

Khatua *et al.* [8], Anand *et al.* [9] and Vamshi *et al* [10] in their studies documented that preterm babies are most commonly affected by sepsis, however present study showed contrary results with majority of babies being full term (52%) followed by pre term babies (31%) and late pre term babies (17%).

Low birth weight babies are more prone to infections. Present study stated that 54% babies had low birth weight while 5% had very low birth weight (<1.5 kg). Anand *et al.* [9], Barbara J Stall *et al.* [11] and Sinha *et al.* [12] reported low birth weight in 68%, 81.3% and 64.9% of cases respectively. Present study was in concordance with other studies for birth weight.

Thus, Present study denoted that low birth weight male babies were more prone to get infection.

Khatua *et al.* [8] and Supreetha *et al.* [13] reported incidence of early onset of sepsis in 70% and 74.5% of cases respectively. The present study had slight increased incidence of late onset of sepsis (56%).

In present study, majority of mothers were healthy (38%), followed by presented with PROM and obstructed labour (9% for both). It is next followed by placenta previa (7%) and PIH (6%). Mehrotra *et al.* [14] Anand *et al.* [9] showed PROM as a major cause for infection.

Other findings of our study are: Majority of babies were born to primigravida mothers (57%) and through LSCS (49%). 48% babies were born through normal vaginal delivery.

The culture positivity rate in present study was 76%. Vinay BS *et al.* [5] documented 80% culture positivity in their study. Darnifayanti *et al.* [4], Supreetha *et al.* [13] and Rodwell *et al.* [15] had 45.6%, 21% and 9% culture positive cases respectively.

The blood culture positivity rate reported by different researchers varies widely. Some studies have taken into account the multiple cultures of same cases while others have considered a single blood culture report only.

In the present study, the most common organism isolated was coagulase negative staphylococcus (28.94%), followed by Klebsiella pneumonia and Enterobacteriaceae (14.47% for both). In Enterobacteriaceae, most common organism isolated was Escherichia coli (45.45%).

Staphylococcus epidermidis followed by Pseudomonas were the most common organism isolated by Darnifayanti *et al.* [4] Klebsiella was the second most common organism isolated in the study by Khair KB *et al.* [3] and Misra RN *et al.* [16] which was similar to the present study.

There is wide variability in the organisms isolated, which can be attributed to the fact that distribution of isolates in blood culture and the spectrum of organisms that can be isolated is subjected to geographical location.

With all the limitations, there is no single ideal test available which can be used alone to diagnose sepsis in the newborn. A diagnostic test or procedure is used in clinical practice to determine whether a patient is likely to have a particular disease or condition.

A diagnostic test is used in preference to a definitive gold standard test when the definitive test is invasive, expensive, time consuming and so impractical for use in routine clinical practice for early diagnosis.

In present study, TLC was found to be highly specific (91.66%), however sensitivity was low. These findings are comparable to study conducted by Rodwell RL *et al* [15] , Khair *et al*. [3] , Makkar *et al*. [17], Supreetha *et al*. [13] and Manucha *et al*. [18] Misra *et al* showed high sensitivity and low specificity. [119]

The present study stated that ANC has high sensitivity (69.73%) and high positive predictive value or accuracy (75.71%). Rodwell RL *et al*. [15] documented 96% sensitivity, 61% specificity, 20% PPV and 99% NPV. Vinay BS *et al*. [5] and Supreetha *et al*. [13] had similar finding compared to present study, while Manucha V *et al*. [18] had low PPV.

The present study documented high sensitivity (69.73%) and positive predictive value or accuracy (75.21%) for AIMNC, which is comparable with study conducted by Senthilnayagam B *et al* [19] and Amrita Duhan *et al*. [20] However, Nigro *et al*. reported low sensitivity (33%) and high specificity (88%) for AIMNC. [21]

Supreetha *et al*. [13], Rodwell RL *et al* [15], Misra *et al*. [16], Makkar *et al*. [17] and Baig *et al*. [22] showed I/T ratio being most sensitive and specific marker for sepsis.

However present study didn't report similar results, rather reported I/T ratio not sensitive but specific. Manucha *et al*. [18] and Singh A *et al*. [23] also documented high specificity.

Present study showed concordance in specificity (87.50%) and positive predictive value (72.7%) for I/M Ratio with two similar studies conducted by Makkar *et al*. [17] and Supreetha *et al*.[13] Sensitivity was in concordance with study done by Manucha *et al*. [18] Rodwell RD *et al*. [15] also has high specificity (81%), but also had high sensitivity (91%).

Present study denoted that, sensitivity was high for toxic granules (86.84%), which is in concordance with studies conducted by Bhuvanamha Devi *et al*. [2], Abhilasha G *et al*.[24] and Panwar C *et al*. [25] However, Ghafoor *et al*. [26] had contrary results with specificity being high (90%). Positive predictive value (79.51%) is comparable with results of Abhilasha *et al*. [24]

In present study, specificity is high (83.33%) for cytoplasmic vacuolation compared to sensitivity (26.31%), which is comparable with studies done by Ghafoor *et al* [26], Supreetha *et al*. [13], Amrita Duhan *et al*. [20] and Gautam C *et al*. [27] Positive predictive value or accuracy was in concordance with study done by Ghafoor *et al*. [26]

Very few studies have evaluated the utility of NRBC count in neonatal sepsis. Present study showed high specificity (70.23%) and positive predictive value or accuracy (75%) for NRBCs. Results of specificity and PPV are in concordance with Kulandaivel M *et al*. [28], Rathi *et al*. [29] and Dr N. Muthukumaran *et al*. [30] Abhishek MG *et al*. [31] reported low sensitivity (35%), which was similar with present study (27.63%). Though majority of studies had shown high sensitivity, present study reported low sensitivity (27.63%) for NRBCs.

Present study showed 39.47% sensitivity and 62.50% specificity for platelet count, which is in concordance with results of Manucha *et al*. [18] Study done by Supreetha *et al*. [13], Rodwell *et al*. [15] and Makkar *et al*. [17] and showed very high sensitivity compared to sensitivity. PPV (76.92%) of present study was in concordance with Rodwell *et al*. [14]

Present study showed high sensitivity (84.21%) for CRP levels in culture proven babies. This findings are in concordance with Ghafoor *et al*. [26] and Supreetha *et al*. [13] Results of Amrita Duhan *et al*. [20], Gautam C *et al*. [27] and Patel U *et al*. [32] showed high specificity compared to sensitivity. In present study, specificity of CRP is extremely low (4.16%) in comparison of other studies.

The present study also evaluated diagnostic utility of certain combination of tests, like ANC with CRP, ANC with I/T Ratio and CRP with I/T ratio.

The present study showed sensitivity, specificity, PPV and NPV of 57.89%, 29.16%, 72.13% and 17.94% respectively for combination of ANC and CRP, while Ghafoor *et al*. reported sensitivity, specificity, PPV and NPV of 94.4%, 86%, 82.9% and 95.6% respectively for same combination. [26]

For combined CRP and I/T Ratio sensitivity, specificity, PPV and NPV were 7.89%, 87.50%, 66.66% and 23.7% respectively in present study. Patel U *et al*. [32] showed sensitivity, specificity, PPV and NPV of 77.6%, 100%, 100% and 50% respectively, while Maysaa Sayed *et al*. reported sensitivity, specificity, PPV and NPV of 65%, 100%, 100% and 74% respectively. [33]

However, this did not yield much significance from their individual utility, except for few combination, like combination of ANC and I/T Ratio achieved more specificity than ANC alone. Specificity and NPV of CRP significantly increased after its combination with I/T Ratio.

5. Conclusions

Degenerative neutrophil change like toxic granulation was found to be the most useful parameter in detecting sepsis early. CRP also had high sensitivity. NRBC count, I/T Ratio and I/M Ratio were not found to be very useful tests.

In developing countries like ours, where the cost of a diagnostic test is a limiting factor, these tests can be employed for routine screening of all clinically suspected cases of sepsis. These tests do not require sophisticated equipment and the results can be obtained within few hours. Hence these parameters aid in early clinical decision making; without having to wait for the blood culture reports for initiation of treatment.

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