**Original article**

# A study of haematological parameters in neonatal septicemia

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**Abstract:**

**Introduction:** Neonatal septicemia is a leading cause of mortality and morbidity during the neonatal period. By definition, neonatal sepsis is a clinical syndrome characterized by systemic signs and symptoms of infection and accompanied by bacteremia in the first four weeks of life. Of late, various laboratory studies have been evaluated in India for early diagnosis of neonatal infection. But less number of such study are being done in this part of our country i.e. Assam. So, keeping this factor in mind the present study was undertaken with the following aims and objectives in view-

to carry out simple, easy to perform haematological tests for detection of neonatal sepsis.

**Materials and Methods :** The study was undertaken in the Department of Pathology, Assam Medical College and Hospital,Dibrugarh(Assam) for a period of one year **.** The study group consisted of 60 neonates of 0-28 days old with clinical evidences of sepsis and 60 age matched healthy controls..

**Results and Observations:** In our study among 3750 life birth babies, 60 cases of clinically suspected septicemia were observed giving an incidence of 16/1000 live births. Blood cultures were positive in 33 cases giving an incidence of proven septicemia to be 8.8/1000 live births.

**Conclusion:** Neonatal sepsis remains one of the most important causes of neonatal morbidity and mortality in our country at large and Assam in particular. Despite considerable progress in hygiene, introduction of new antimicrobial agents and advancement in all spheres of medical world, clinicians are handicapped to an extent when it comes to combating infections, especially in neonates and infants

## Introduction:

Neonatal septicemia is a leading cause of mortality and morbidity during the neonatal period. By definition, neonatal sepsis is a clinical syndrome characterized by systemic signs and symptoms of infection and accompanied by bacteremia in the first four weeks of life. 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[1].Existing published data have suggested that sepsis causes about 10% of all maternal and 26% of all neonatal deaths[2]. In the developed countries mortality due to sepsis has increased by approximately 13.7% each year over the past 2 decades[3]. But in developing countries, like India, it is difficult to access the true incidence because data from community setting is not available and even in hospitals the diagnosis is often not based on studies but on clinical grounds alone. Nonetheless, the reported incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD) is 30 per 1000 live births. The NNPD network comprising of 18 tertiary care neonatal units across India found sepsis to be one of the commonest causes of neonatal mortality contributing to 19% of all neonatal deaths[4]. According to the National



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Neonatology forum, neonatal sepsis can be of two type [5].

*Proven sepsis: newborn with clinical picture suggestive of sepsis and isolation of pathogen from blood,CSF(cerebrospinal fluid),urine or other body fluids or autopsy evidence of sepsis.

* Probable sepsis: newborn with clinical picture suggestive of sepsis with one or more of the following criteria:

a) Predisposing factors like maternal fever, foul smelling liquor or prolonged rupture of membrane>12hrs, gastric polymorph count of more than 6/HPF.

b) Positive sepsis screen with two of the following four parameters

---Total leukocyte count < 5000/cumm

---Band cell to total neutrophil ratio of ≥ 0.2

---CRP ≥ 0.6 mg/dl

---Micro-ESR ≥ 15 mm AEFH (at the end of first hour)

c) Radiological evidence of pneumonia.

During the last few decades advances in neonatal intensive care have led to an impressive decrease of neonatal mortality and morbidity. But even with all these developments, neonatal septicemia often presents a diagnostic problem. The "gold standard" for the diagnosis of septicemia has been the isolation of micro-organisms from blood culture. The culture procedure takes at least 48 hours to confirm the diagnosis, a delay, which a neonate cannot afford for initiation of appropriate therapy. To meet this end, various indirect haematological parameters are evaluated in terms of efficacy for early diagnosis of neonatal septicemia when the clinical picture may not have fully evolved. The present study was conducted to evaluate a sepsis screen consisting of –Total leukocyte count, Ratio between band cell count and neutrophil count, Micro –ESR and C-reactive protein in their efficacy in predicting neonatal sepsis. Though viruses, fungi and bacteria equally share the infective role in neonatal infection, but in our study the neonatal infection of only bacterial origin was taken up. Only those babies born in the Department of Obstetrics and Gynae., Assam Medical College & Hospital, Dibrugarh was taken up for the study.

**Aims and Objectives**:

Of late, various laboratory studies have been evaluated in India for early diagnosis of neonatal infection. But less number of such study are being done in this part of our country i.e. Assam. So, keeping this factor in mind the present study was undertaken with the following aims and objectives in view-

1. To carry out simple, easy to perform haematological tests for detection of neonatal sepsis.
2. To evaluate the validity of single and combined haematological parameters in case of proven septicemia.
3. To compare the utility of C - reactive protein versus haematological indices in neonatal sepsis.
4. To find out the relationship between haematological parameters and bacteriologically proven sepsis.

**Materials and Methods**

**Place of study**:- The study was undertaken in the Department of Pathology, Assam Medical College and Hospital,Dibrugarh(Assam) for a period of one year

The study group consisted of 60 neonates of 0-28 days old with clinical evidences of sepsis and 60 age matched healthy controls..

**Inclusion criteria**: - Only the hospital born babies with clinical signs and symptoms of septicemia was included in the study.

**Exclusion criteria: -** Babies with respiratory distress syndrome, extreme prematurity (<30weeks), gross congenital anomalies or any

previous antibiotic therapy was excluded from the study.

In all neonates blood samples were obtained from peripheral venipuncture and the following investigations were done:

1. **TOTAL LEUKOCYTE COUNTS (TLC):** In this study a count of<5000/cu.mm was considered as leukopenia.
2. **DIFFERENTIAL LEUKOCYTE COUNT (DLC):**
3. **BAND CELL COUNT AND BAND**/NEUTROPHIL (B/N) RATIO: A band cell count of more than 20% and B/N ratio of equal to or more than 0.2 was considered as abnormal.
4. **MICRO-ESR (mESR):** This was obtained by collecting capillary blood in a standard micro haematocrit tube (75mm length, internal diameter of 1.1mm and outer diameter of 1.5mm) and reading the fall of erythrocyte column after one hour. During the neonatal period a value of more than 15mm was considered as suggestive of infection.
5. **DETERMINATION OF C-REACTIVE PROTEIN:** This was done by latex agglutination slide test. The cut off value of 0.6 mg/dl as recommended by the manufacturer (Tulip diagnostics (p) Ltd, India) was considered as evidence of infection.
6. **BLOOD CULTURE:** This was done in all the cases before starting antibiotics. Those having positive blood culture were taken as 'proved' sepsis and the remaining as 'probable' sepsis

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

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of TLC, B/N ratio, mESR and CRP were determined to define the diagnostic significance of each test using cut-off values.

Blood culture was used as the gold standard to consider a neonate positive for sepsis. The student's paired t-test was done wherever required. The tests with optimum sensitivity, specificity and predictive accuracy were used to formulate a sepsis screen.

**Results and Observations:**

In our study among 3750 life birth babies, 60 cases of clinically suspected septicemia were observed giving an incidence of 16/1000 live births. Blood cultures were positive in 33 cases giving an incidence of proven septicemia to be 8.8/1000 live births as shown in Table-1.

33 (55.0%) out of the sixty suspected cases showed positive blood culture, 10(30.3%) of which died. The mortality was 14.8% in cases with negative blood culture as shown in Table -2 and Fig-1.Among the organisms isolated from blood culture E.coli was the most common organism followed by Klebsiella (Table -3, Fig-2).The Band/Neutrophil ratio of ≥ 0.2 was observed in 72 .7% of proven septicemia and 37.0% of probable septicemia cases. The overall positivity of the test was 56.7% in clinically suspected septicemia cases (Table-4).Similarly leucopenia (<5000/cumm) was found in 54.5% of proven septicemia and 37% of probable septicemia cases. The overall positivity for leucopenia was 46.7% in clinically suspected septicemia cases (Table-5). The m-ESR was more than or equal to 15mm at one hour in 60.6 % of proven septicemia cases and 37% in clinically suspected septicemia cases. The overall positivity of the test was 50% in clinically suspected cases (Table-6).

The latex agglutination test for CRP was positive (≥0.6mg/dl) in 84.8% of proven septicemia and 48.1% of probable septicemia cases.The overall positivity in clinically suspected septicemia cases was 68.3%(Table-6).When the four most useful tests were considered individually, latex –CRP was



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found to be most sensitive (84.8%) but less specific (51.9%) in identifying septicemia. The other tests were found to be less sensitive (Table-7, Fig-3).When two different combination of tests was considered, the combination of CRP and leucopenia was found to be most sensitive (48.5%) and specific (40.7%) in detection of septicemia followed by CRP and band/neutrophil ratio with a sensitivity of 48.5% and specificity of 33.3% (Table-8,Fig-4).The sepsis screen was positive in 87.9% and 51.9% of proven and probable septicemia respectively(Table-9,Fig5).

Table -1 Incidence of neonatal septicemia

| | |
|---|---|
| Total live births | 3750 |
| Clinically suspected septicemia | 60 |
| Proven septicemia | 33 |
| Incidence of proven septicemia/1000 live births | 8.8 |
| Incidence of clinically suspected septicemia/1000 live births | 16 |

Table -2 Blood Culture & Mortality in Septicaemia

| **Blood Culture** | **Positive** | **Negative** | **Total** |
|---|---|---|---|
| No Of Patients | 33 ( 55.0 % ) | 27 (45.0 %) | 60 |
| Died | 10 (30.3% ) | 4 ( 14.8 % ) | 14 (23.3% ) |

Fig-1 **Blood Culture & Mortality in Septicaemia**

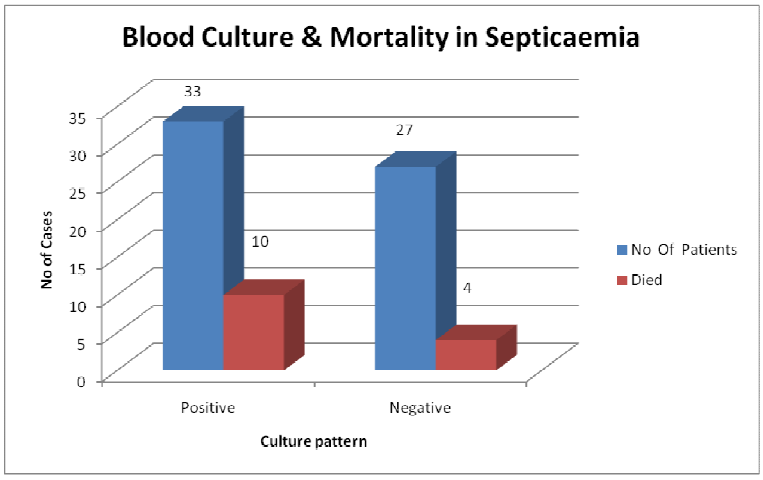




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Table -3 Individual organisms isolated from blood culture:

| **Type of organisms** | **Percentages** |
|---|---|
| E. Coli | 34% |
| Klebsiella | 27 % |
| Staph aureus | 24 % |
| Pseudomonas | 9% |
| Proteus | 3% |
| Beta- hemolytic strepto cocci | 3% |

Fig-2

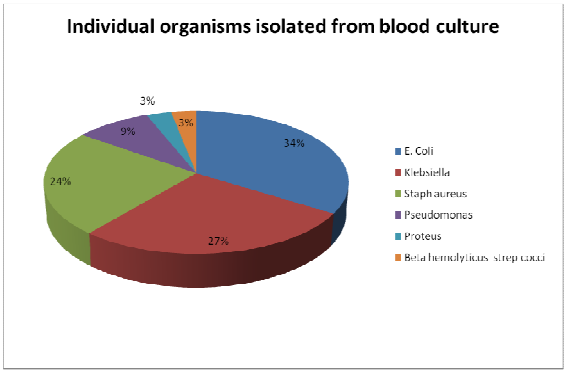


Table -4 Bands / Neutrophil count in septicaemia

| **Band/ Neutrophil Ratio** | **Proven sepsis (n=33)** | | **Probable sepsis (n=27)** | | **Total no (n=60)** | | | |
|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % |
| ≥ 0.2 | 24 | 72.7 | 10 | 37.0 | 34 | 56.7 | 3 | 5 |
| ‹ 0.2 | 9 | 27.3 | 17 | 63.0 | 26 | 43.3 | 57 | 95 |

Table -5 Leukocyte count profile in septicaemia

| **Leukocyte count (per cu mm )** | **Proven septicemia (n= 33)** | | **Probable spticemia (n= 27)** | | **Total (n=60)** | | **Control (n=60)** | |
|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % |
| ‹ 5000 | 18 | 54.5 | 10 | 37 | 28 | 46.7 | 2 | 3.3 |
| ≥5000 | 15 | 45.5 | 17 | 63 | 32 | 53.3 | 58 | 96.6 |



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Table -6 Micro –ESR Profile in neonatal septicaemia

| **Micro – ESR At one hour** | **Proven sepsis (n=33)** | **Probable sepsis (n=27)** | **Total (n=60)** | **Control (n=60)** |
|---|---|---|---|---|
| | No(%) | No (%) | No (%) | No (%) |
| ≥15 mm | 20 (60.6%) | 10 (37.0) | 30 (50 % ) | 3 ( 5 % ) |
| <15 mm | 13 (39.4% ) | 17 ( 63.0 ) | 30 ( 50% ) | 57 (95 % ) |

Table -6 CRP Profile ( Latex Agglutination Method)

| **Latex CRP Test** | **Proven septicemia (n=33)** | | **Probable septicemia ( n =27 )** | | **Control (n= 60)** | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| Positive ( ≥o.6 mg/dl) | 28 | 84.8 | 13 | 48.1 | 2 | 3.3 |
| Negative ( < 0.6 mg/dl ) | 5 | 15.2 | 14 | 51.9 | 58 | 96.6 |

Table-7 Sensitivity & Specificity of four most useful individual test

| **Test** | **Sensitivity (%)** | **Specificity (%)** |
|---|---|---|
| Latex CRP ( ≥0.6 mg /dl ) | 84.8 | 51.9 |
| Band /Neutrophil (≥0.2) | 72.7 | 63.0 |
| Micro ESR ( ≥ 15 mm at one hour ) | 60.6 | 63.0 |
| Leukopenia ( ‹ 5000/cu. mm ) | 54.5 | 63.0 |

Fig-3 Sensitivity & Specificity of four most useful individual tests

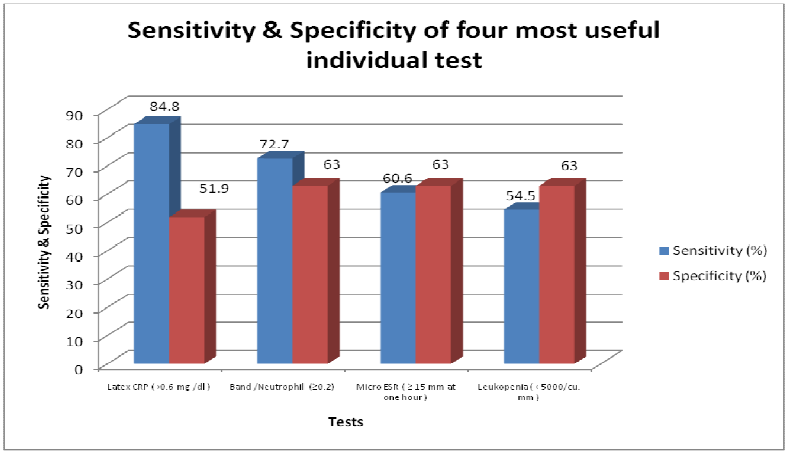


Table -8 Sensitivity & specificity of two different combinations of tests

| **Combination of test** | **Sensitivity (%)** | **Specificity (%)** |
|---|---|---|
| CRP + m- ESR | 45.5 | 37.0 |
| CRP + band /neutrophil | 48.5 | 33.3 |
| CRP + Leukopenia | 48.5 | 40.7 |
| Leukopenia + band/ neutrophil | 33.3 | 37.0 |
| Leukopenia + m-ESR | 33.3 | 33.3 |
| m-ESR + band/neutrophil | 33.3 | 29.6 |

Fig -4

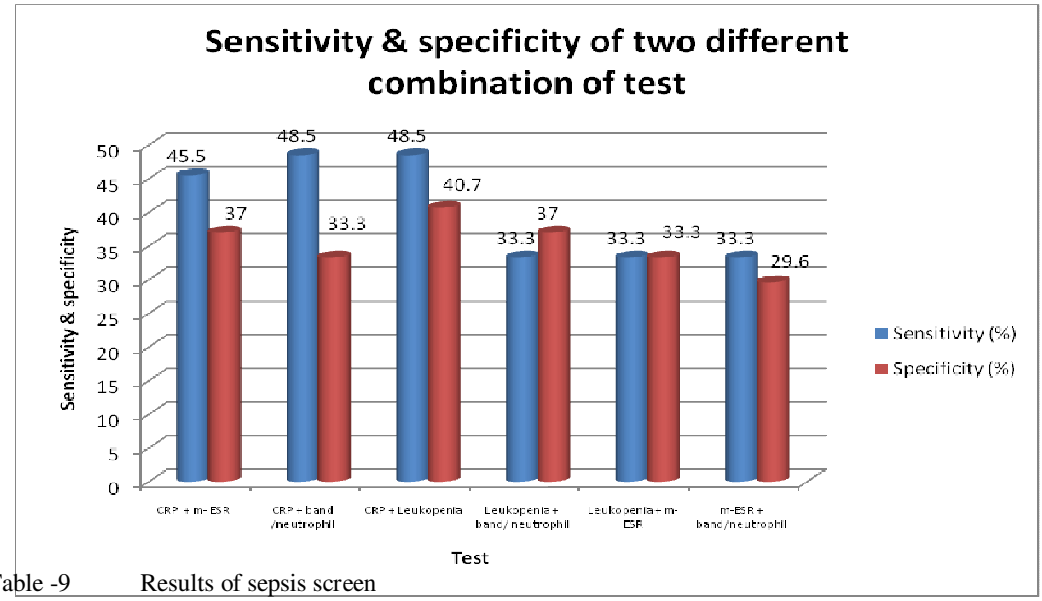
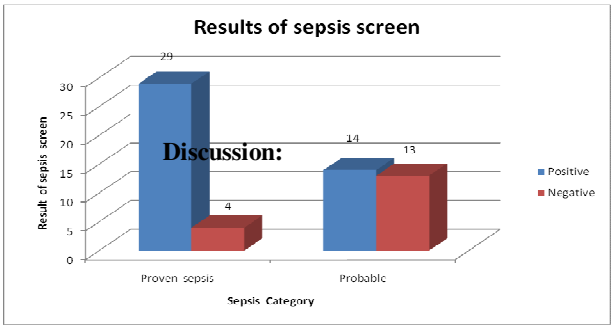



Table -9 Results of sepsis screen

Fig-5




## Discussion:

| Result of sepsis screen | Proven sepsis (n=33) | | Probable sepsis (n=27) | |
|---|---|---|---|---|
| | No | % | No | % |
| Positive | 29 | 87.9 | 14 | 51.9 |
| Negative | 4 | 12.1 | 13 | 48.1 |

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.

At present the early diagnosis of neonatal septicaemia is primarily based on clinical evaluation. Many babies are treated with several days of antibiotics because of possible infection while waiting for blood culture report. This results in a disproportionate high number of babies without sepsis being treated with antibiotics. A battery of indirect markers of infection when collectively studied is an extremely useful and reliable index for early diagnosis of neonatal septicaemia[6,7]. The predictive ability of these parameters has been found to vary widely in different literatures[8,9,10,11,12,13,14]. In the present study, four most useful tests (Leukopenia<5000/cu.mm, band/neutrophil ratio≥0.2, m-ESR≥15mm at first hour and positive latex CRP≥0.6mg/dl) which can be performed easily and rapidly were selected to devise a sepsis screen. Sensitivity (diagnosing infection when it is present) and specificity (not diagnosing infection when it is not present) of these tests singly or in different combinations were calculated based on definitions by Feinstein (1975) in detecting septicaemia cases. It was found that when single test was considered, positive latex CRP (≥0.6mg/dl) alone could predict the diagnosis of septicaemia with a sensitivity and specificity of 84.8 percent and 51.9 percent respectively. This was consistent with the study by Varsha, Usha et al[15]. When two positive tests combinations were analysed for detection of sepsis, it was found that the best combination was that of positive CRP and leukopenia which had a sensitivity and specificity



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of 48.5 percent and 40.7 percent respectively followed by band/neutrophil ratio and positive CRP.A study by Philip AGS, Hewitt JR et al found leukopenia and increased band/neutrophil ratio to be correlated with an increased risk of bacterial infection in neonates[9]. Hence, the sepsis screen which evolved during this study comprised of positive latex-CRP and leukopenia. By the application of sepsis screen this study could diagnose 43 out of 60 cases of clinically suspicious septicemia, 33 of which were proved by blood culture. Sepsis screen was negative in 17 cases and 13 of these were proved by blood culture. The sepsis screen was positive in 87.9 percent cases of proven sepsis and could give result within an hour making it very useful for diagnosis of neonatal sepsis.

**Conclusion**

Neonatal sepsis remains one of the most important causes of neonatal morbidity and mortality in our country at large and Assam in particular. Despite considerable progress in hygiene, introduction of new antimicrobial agents and advancement in all spheres of medical world, clinicians are handicapped to an extent when it comes to combating infections, especially in neonates and infants.

It can be concluded from this study that a sepsis screen is a valuable adjunct for detection of sepsis in neonates. Moreover 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.

Prevention of sepsis has to be given its due importance. Prevention of factors which predisposes to neonatal septicemia like prematurity and low birth weight infants, aseptic techniques in delivery rooms and wards are crucial. Rationale antibiotic policy and proper protocols in utilization of anti-microbials will reduce the development of resistance which is emerging as a global threat.

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