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Original Research Article

# Prospective Observational Assessment of the Efficacy of Role of C-Reactive Protein and Gastric Aspirate Polymorphs in Early Onset Neonatal Sepsis

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**Abstract**

**Aim:** The aim of the present study was to evaluate the role of C-reactive protein and gastric aspirate polymorphs in early onset neonatal sepsis.

**Methods:** This was a prospective observational study conducted in the Department of Pediatrics, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India. study period from February 2022 to January 2023. 70 babies who had clinical symptoms and signs of suspected neonatal sepsis/high risk factors for developing the sepsis, were included in the study.

**Results:** Only 15 patients had TLC more than 25000 /dl. Maximum TLC value in the study was 42500 /dl. 55 patients showed positive CRP values. 45 patients had polymorphs in the GA more than 5 per high power field. By combination of any CRP and TLC specificity increased to 81%. While sensitivity approached to 100% when TLC with GA polymorphs and CRP with GA polymorphs were combined with significant p values of 0.001 and 0.015 respectively. When all the three parameters were combined together, both the sensitivity and specificity increased to 100% and 92.68% respectively with p values of 0.001.

**Conclusion:** CRP showed high sensitivity while GA polymorphs showed high specificity. GA cytology as a screening tool for neonatal sepsis with intermediate sensitivity, specificity, positive predictive value and negative predictive values serves as good tool, added to a detailed antenatal history and clinical examination of the neonate.

**Keywords:** CRP, Gastric Aspirate, Sepsis



## Introduction

Neonatal septicemia remains a significant cause of morbidity and mortality in newborn infants. [1] Neonatal sepsis is a clinical syndrome resulting from the pathophysiological effects of severe bacterial infection in the first month of life. Worldwide many neonates with sepsis die due to lack of early diagnosis. [2,3] Annually five million neonates die mostly in Asia and Africa, out of which 1.6 million (20%) are due to NS. [4] The incidence of neonatal sepsis varies between 11.0 and 24.5/1000 live births in India. [1] Neonatal sepsis is classified depending on the hours of presentation into early onset: within first 72 hours of life; late onset: occurring after 72 hours of life. Although the onset of illness is often inconspicuous, the clinical course may be alarmingly fulminant, leading to septicemic shock, disseminated intravascular coagulation, and death within hours of the onset of clinical manifestations. [5]

Early onset neonatal sepsis is often due to organisms present in the maternal vaginal flora. [2] In contrast to bacteremia (bacteria in blood), septicemia usually consists of bacteriemia plus a constellation of signs

and symptoms caused by microorganisms or their toxic products in circulation. There may be progression of bacteriemia to septicemia depending on clinical manifestations. [5] The clinical signs of early-onset sepsis are usually apparent in the first hours of life; 90% of infants are symptomatic by 24 hrs of age. Respiratory distress is the most common presenting symptom. Respiratory symptoms can range in severity from mild tachypnea and grunting with or without supplemental oxygen requirement to respiratory failure. Other less-specific signs of sepsis include irritability, lethargy, temperature instability, poor perfusion, and hypotension. Gastrointestinal symptoms can include poor feeding, vomiting, and ileus. Meningitis may present with seizure activity, apnea, and/or depressed sensorium. So, the early onset disease can manifest as asymptomatic bacteremia, generalized sepsis, pneumonia, and/or meningitis. [6]

The clinical diagnosis of neonatal sepsis is difficult because the signs and symptoms are not always specific. There is no laboratory test with 100% sensitivity and specificity.' [7,8] A definitive diagnosis based on culture of blood, cerebrospinal fluid (CSF), or urine is usually reached only after a delay of a day or two. Initiation of antibiotic therapy before diagnostic results are available is recommended for neonates with clinical signs or epidemiologic factors associated with NS. However, some patients with bacterial infection may have negative blood cultures (clinical infection), and other approaches to identification of infection are required. [9]

Isolation of the infecting organism from blood provides the definitive diagnosis and is considered as the gold standard. However, this culture procedure takes at least 48 hours to confirm the diagnosis. Therapy cannot wait this long in a critically sick neonate. Hence, certain indirect early markers of neonatal infections have been identified. [3] Serum concentrations of many acute phase reactants rise in response to infection which can be used as non-specific indicators of bacterial sepsis including C-reactive protein (CRP), multiple leucocyte activation markers, interleukin 6, interleukin 8, tumour necrosis factor-alpha and procalcitonin in the diagnosis of NS. [10-13] CRP is a non-specific, acute-phase protein that rises in response to inflammatory processes. Sufficient evidence exists to support the use of CRP measurements in conjunction with other established diagnostic tests, such as a total and differential leukocyte count (TLC and DLC) and blood culture to establish or exclude the diagnosis of sepsis in full-term or near-term infants. [10]

The aim of the present study was to evaluate the role of C-reactive protein and gastric aspirate polymorphs in early onset neonatal sepsis.

## Materials and Methods

This was a prospective observational study conducted in the Department of Pediatrics, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India. study period from February 2022 to January 2023. 70 babies who had clinical symptoms and signs of suspected neonatal sepsis/high risk factors for developing the sepsis, were included in the study.

Blood samples were taken for complete blood count, CRP (quantitative) and investigated as per the protocol. An informed written consent was taken from the parents/attendants of the admitted neonates. The inclusion criteria were babies with age less than 7 days of life, inborn or out born with suspected sepsis and with high risk factors (antenatal, natal, postnatal). The high-risk factors included preterm neonates, with history of fetal distress, maternal history of leaking P/V (more than 18 hours), maternal fever, history of any maternal infection like urinary tract infection, chorio-amnionitis, multiple obstetrical procedures or difficult labour .Babies with age more than 7 days of life, having septic shock patients or rapidly deteriorating clinical condition, weighing <1500 gms, with history of severe perinatal asphyxia, any congenital malformations/chromosomal anomalies/congenital metabolic defects or babies with family history of any immunodeficiency syndrome were excluded from the study.

Each patient was subjected to detailed history and physical examination. Blood samples were taken at admission and subjected to TLC and CRP. The blood sample for blood culture and sensitivity was collected at the same time. Following this the decision to start antibiotic therapy was based on combination of clinical signs, obstetric risk factors and sepsis screen. Furthermore, sepsis screen was repeated whenever new clinical signs of infection developed. The samples were collected in EDTA vial for TLC and in the plain vial for CRP. Under strict aseptic measures, samples for blood culture and sensitivity were collected. Gastric aspiration was sent for cytology in plain sterilized tubes. TLC was measured by manual method using Neubauer chamber as well as using an electronic cell counter. TLC report on coulter machine was verified by manual method. RHELAX CRP reagent was used to detect CRP concentrations greater than 0.6 mg/dl. Blood culture sample was collected from venipuncture under aseptic measures, cleaning the skin with spirit- betadine-spirit and collected in a 2ml syringe and then transferred to BacT/ALERT PF bottle (20 ml) using another sterile needle. The BacT/ALERT microbial detection system was used to determine microorganisms present in blood that provide both a microbial detection system and culture media. An inoculated bottle was placed into the instrument for incubation and monitoring to

detect the growth of any microorganisms. Positive or negative results are determined by software contained in the BacT/ALERT microbial detection system. GA was obtained by infant feeding tube within 12 hours of life in a neonate and put in plain vial. One drop of GA was mixed with one drop of methylene blue on a slide and covered with a cover slip. Slide was seen under microscope for polymorphs/HPF.

Significant values for screening tests were taken as TLC of >25,000/<5000 and CRP positive (0.6 mg/dl) and GA polymorphs >5/HPF. Sepsis screen positive was two or more positive tests. The babies were started on IV antibiotics, while blood culture reports were awaited. Blood culture was used as gold standard and the decision to continue antibiotics was taken depending upon the blood culture report. The statistical analysis was done using SPSS 21.0.

**Results**

**Table 1: Comparing TLC with blood culture positive neonates**

| TLC | N | Blood culture | | Sensitivity | Specificity | NPV | PPV | Accuracy | P value |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | | | |
| **<25000** | 55 | 30 | 25 | | | | | | |
| **>25000** | 15 | 11 | 4 | 77.83 | 47.83 | 29.51 | 87.00 | 52.38 | 0.130 |
| **Total** | 70 | 41 | 29 | | | | | | |

Only 15 patients had TLC more than 25000 /dl. Maximum TLC value in the study was 42500 /dl.

**Table 2: Comparing CRP with blood culture positive neonates**

| CRP | N | Blood culture | | Sensitivity | Specificity | NPV | PPV | Accuracy | P value |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | | | |
| **<6.0** | 15 | 2 | 13 | | | | | | |
| **>6.0** | 55 | 40 | 15 | 88.12 | 49.00 | 91.29 | 71.36 | 75.65 | 0.001 |
| **Total** | 70 | 42 | 28 | | | | | | |

55 patients showed positive CRP values.

**Table 3: Comparing gastric aspirate polymorphs with blood culture positive neonates**

| GA | N | Blood culture | | Sensitivity | Specificity | NPV | PPV | Accuracy | P value |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | | | |
| **<5.0** | 25 | 0 | 25 | | | | | | |
| **>5.0** | 45 | 38 | 7 | 100.00 | 81.00 | 100.00 | 88.52 | 92.68 | 0.001 |
| **Total** | 70 | 38 | 32 | | | | | | |

45 patients had polymorphs in the GA more than 5 per high power field.

**Table 4: Comparing TLC and CRP with blood culture positive neonates**

| TLC+CRP | N | Blood culture | | Sensitivity | Specificity | NPV | PPV | Accuracy | P value |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | | | |
| **Positive** | 15 | 11 | 4 | | | | | | |
| **Negative** | 15 | 2 | 13 | 91.92 | 81.00 | 93.34 | 75.95 | 83.67 | 0.001 |
| **Total** | 30 | 13 | 17 | | | | | | |

By combination of CRP and TLC specificity increased to 81%.

**Table 5: Comparing TLC and gastric aspirate polymorphs with blood culture positive neonates**

| TLC+GA | N | Blood culture | | Sensitivity | Specificity | NPV | PPV | Accuracy | P value |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | | | |
| **Positive** | 12 | 10 | 2 | | | | | | |
| **Negative** | 3 | 0 | 3 | 100.00 | 65.69 | 100.00 | 90.91 | 93.34 | 0.001 |
| **Total** | 15 | 10 | 5 | | | | | | |

**Table 6: Comparing CRP and gastric aspirate polymorphs with blood culture positive neonates**

| CRP+GA | N | Blood culture | | Sensitivity | Specificity | NPV | PPV | Accuracy | P value |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | | | |
| **Positive** | 42 | 36 | 6 | | | | | | |
| **Negative** | 14 | 0 | 14 | 100.00 | 74.36 | 100.00 | 88.42 | 92.85 | 0.015 |
| **Total** | 56 | 36 | 20 | | | | | | |

While sensitivity approached to 100% when TLC with GA polymorphs and CRP with GA polymorphs were combined with significant p values of 0.001 and 0.015 respectively (Tables 5 and 6).

**Table 7: Comparing TLC, CRP and gastric aspirate polymorphs with blood culture positive neonates**

| TLC+CRP +GA | N | Blood culture | | Sensitivity | Specificity | NPV | PPV | Accuracy | P value |
|---|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | | | | | | |
| **Positive** | 13 | 11 | 2 | | | | | | |
| **Negative** | 13 | 0 | 13 | 100.00 | 92.68 | 100.00 | 90.91 | 96.44 | 0.001 |
| **Total** | 26 | 11 | 15 | | | | | | |

When all the three parameters were combined together, both the sensitivity and specificity increased to 100% and 92.68% respectively with p values of 0.001.

## Discussion

Neonatal sepsis is an inflammatory response to bacteremia occurring during the first month of life and it remains a big problem in developing countries. [14] Septicaemia in newborns is a systemic inflammatory reaction to local infection that may lead to the development of more serious conditions. [15] Sepsis often presents a diagnostic challenge in the resource poor setting of most developing countries. Successful treatment depends on early initiation of antibiotics, but early diagnosis of neonatal infections is difficult because clinical signs are non-specific and may initially be subtle. Respiratory distress, apneic spells, episodes of bradycardia, feeding intolerance, lethargy, and the clinical signs of early onset sepsis are usually apparent in the first hours of life; 90% infants are symptomatic by 24 hours of age. Respiratory distress is the most common presenting symptom. Temperature instability, as well as minor changes on physical examination or in clinical status is some of the conditions that suggest a possible neonatal infection and needs sepsis evaluation. [16]

Only 15 patients had TLC more than 25000 /dl. Maximum TLC value in the study was 42500 /dl. 55 patients showed positive CRP values. 45 patients had polymorphs in the GA more than 5 per high power field. By combination of any CRP and TLC specificity increased to 81%. While sensitivity approached to 100% when TLC with GA polymorphs and CRP with GA polymorphs were combined with significant p values of 0.001 and 0.015 respectively. When all the three parameters were combined together, both the sensitivity and specificity increased to 100% and 92.68% respectively with p values of 0.001. Studies have shown presence of polymorphs in GA to represent a fetal intra-amniotic inflammatory response. [17,18] GA cytology is simple and can be done without specially trained staff even in rural hospital settings. This is of great importance in a developing country like ours with a high infection rate and limited resources. [19] In the present study, we evaluated the utility of GA cytology as a screening tool for neonatal sepsis.

Chatterjee et al studied the role of raised IL-6 and CRP in neonatal sepsis. The concluded that the IL-6 is the highly sensitive marker and CRP is the more specific marker for the identification of neonatal sepsis. The combination of IL-6 and CRP has the high sensitivity and negative predictive value when compared to other markers. Therefore, a combination of markers, IL-6 and CRP would be the better predictors of neonatal sepsis. [20] Similar results were obtained in our study where combination of various parameters showed high sensitivity and specificity. Of the rapid diagnostic tests, CRP was found to be most useful when taken singly. Its elevation and returning to normal levels once the infection is controlled occurs in a matter of a few hours. Kite et al have reported elevated CRP levels in 80% of cases of neonatal sepsis. [21] They further added that evaluation of sepsis screen markers is important in the diagnosis of neonatal septicemia, especially in areas where adequate micro-biological facilities are lacking.

Gyllensvärd et al studied the role of CRP and clinical symptoms guided strategy in term neonates with early- onset sepsis. They concluded that CRP and clinical symptoms guided decision-making for early onset neonatal sepsis significantly decreased the duration of antibiotic therapy and hospital stay and hence reduced healthcare cost. [22] GA cellularity correlates directly with the occurrence of clinical infection with sensitivity of 75% and specificity of 70%. CRP with GA was found to be the best combination with sensitivity of 80% and specificity of 70%. [19,23] GA polymorphs also showed high sensitivity and specificity in the present study and

also with the combination of GA polymorphs and CRP. Kaur et al studied the role of CRP and immature to total neutrophil ratio in early onset neonatal sepsis and concluded that CRP showed high sensitivity while I/T ratio was found to be highly specific. The combination of CRP with I/T ratio showed significant association with blood culture (p=0.016). [24] Combination of various parameters in our study showed high sensitivity and specificity.

## Conclusion

CRP showed high sensitivity while GA polymorphs showed high specificity. GA cytology as a screening tool for neonatal sepsis with intermediate sensitivity, specificity, positive predictive value and negative predictive values serves as good tool, added to a detailed antenatal history and clinical examination of the neonate. GA cytology with its relatively high specificity and negative predictive values serves as a good screening tool to rule out neonates unaffected by sepsis and prevent unnecessary antimicrobial usage.

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