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Original Research Article

# Biomarkers and gold standard: Assessing CRP, Procalcitonin, and blood culture in the diagnosis of neonatal sepsis

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## Abstract

**Background:** Neonatal septicemia is a leading cause of morbidity and mortality among newborns worldwide, posing a significant public health concern. The incidence of neonatal sepsis ranges from 1 to 5 cases per 1000 live births, with an overall mortality rate of 24.4%. The present study was conducted to compare biomarkers, such as CRP and PCT, with blood culture for the diagnosis of neonatal sepsis.
**Materials and Methods:** The present study was conducted at a rural-based tertiary care hospital. All blood culture samples were processed in the BD BACTEC FX 40 automated blood culture system. Qualitative analysis of CRP was done by the latex agglutination test, and further quantitative analysis of positive samples was done using the MISPA-i3. Quantitative procalcitonin analysis was performed using the Finecare fluorescence immunoassay test
**Results:** In the present study, among the 330 sepsis cases, there were 243 (73.63%) cases of early-onset septicemia and 87 (26.36%) cases of late-onset septicemia. The blood culture results were positive in 175 patients (53.03%) out of 330 neonates. Among blood culture-positive samples, the highest prevalence was of *Staphylococcus aureus* (21.71%). The sensitivity and specificity of CRP in correlation with positive blood culture are 88.80% and 40.65%, respectively. The sensitivity and specificity of PCT in correlation with positive blood culture were 93.71% and 72.90%, respectively. The specificity of PCT is higher compared to CRP; thus, it is a more useful marker for diagnosing neonatal sepsis.
**Conclusion:** Procalcitonin (PCT) is an effective marker for diagnosing early-onset sepsis and can be utilized to monitor the response to antibiotic treatment.

**Keywords:** Neonatal septicemia, *Staphylococcus aureus*, Acute phase reactant, C-reactive protein, Procalcitonin.

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## 1. Introduction

Neonatal septicemia is a leading cause of morbidity and mortality among newborns worldwide, posing a significant public health concern.[1] The incidence of neonatal sepsis ranges from 1 to 5 cases per 1000 live births, with an overall mortality rate of 24.4%. The mortality rate increases with decreasing gestational age of newborns, can be as high as 30% in neonates with gestational age of 25 to 28 weeks, and can increase up to 50% in neonates with gestational age of 22 to 24 weeks.[2,3] Neonatal septicemia is further classified into early-onset sepsis (develops within 72 hours) and late-onset sepsis (develops after 72 hours).[4]

Various risk factors associated with neonatal sepsis include maternal factors such as preterm pregnancy, chorioamnionitis, premature rupture of membranes, multiple pregnancies, and maternal fever. Various neonatal risk factors predisposing to sepsis include low birth weight, respiratory illness, preterm birth, and low APGAR score.[5,6]

The clinical manifestations include poor feeding, respiratory distress, cold extremities, temperature instability (hypothermia or hyperthermia), hypotonia, bulged fontanelles, convulsions, jaundice, and disseminated intravascular coagulation (DIC).[7,8] Neonatal septicemia may lead to several complications such as encephalopathies,

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convulsions, ventriculomegaly, hydrocephalus, brain infarction, and neurodevelopmental delay.[9,10]

Blood cultures are considered the gold standard for diagnosing neonatal sepsis. A major drawback of microbial culture is that it may yield false-negative results due to prior antibiotic administration and may also produce false-positive results from contamination by normal commensals.[3,11,12] Additionally, microbial identification and antibiotic susceptibility testing can take 2 to 3 days to provide final results. Therefore, biomarkers can be used alongside microbiological culture for rapid and timely diagnosis of sepsis. Biomarkers will also help rule out other non-infectious etiologies and guide empirical treatment.[13]

In day-to-day clinical practice, accurate and timely diagnosis of neonatal septicemia is challenging. An ideal serological marker should possess high sensitivity, high specificity, strong positive predictive value, and negative predictive value. The biomarkers are classified into acute-phase proteins, cell surface markers, cytokines, chemokines, and soluble adhesion molecules.[4,14,15] C-reactive protein (CRP), procalcitonin, and serum amyloid A are routinely used for the rapid diagnosis of neonatal sepsis. The cytokines and chemokines implicated in newborn sepsis include Interleukin IL-1, IL-6, IL-8, tumor necrosis factor (TNF), and TNF receptors.[2,3]

C-reactive protein (CRP) is a positive acute-phase protein, produced by hepatocytes, and its synthesis is regulated by interleukin-6 and IL-1. The half-life of CRP is 24 to 48 hours, and its level rises after 10 to 12 hours of sepsis.[16,20] Thus, CRP is not a useful marker for the early detection of neonatal sepsis. CRP can be used as a prognostic marker to decide the course of antibiotic treatment. CRP levels are elevated in bacterial and viral infections, acute rheumatic fever, rheumatoid arthritis, collagen disease, myocardial infarction, and several types of malignancies.[18,21]

Procalcitonin is a calcitonin prohormone produced by macrophages and hepatocytes. It is a positive acute-phase reactant with a half-life of 24-30 hours.[7,11] After the initiation of infection with the release of bacterial endotoxin PCT level rises within 2 to 4 hours, reaching its peak in 6 to 8 hours.[14,15]

The major drawback is that the PCT level also rises in non-septic conditions such as severe traumatic injuries, thyroid carcinoma, and following major surgeries.[17]

In the present study, we compared CRP and PCT levels for the diagnosis of neonatal septicemia and their correlation with positive blood culture. The study also aimed to study the bacteriological profile of positive blood culture.

## 2. Materials and Methods

This cross-sectional study was conducted over one year (January 2024 – December 2024) at a rural-based tertiary care hospital. It was conducted after obtaining approval from the ethical committee, and informed consent was obtained from the parents of neonates. (Ethical Approval No.: SVIEC/ON/MEDI/RP/OCT/2310)

### *2.1. Inclusion criteria*

The study included neonates admitted to our neonatal intensive care unit who presented with signs and symptoms of sepsis. The detailed history of all enrolled neonates was taken along with clinical findings and laboratory investigations.

### *2.2. Exclusion criteria*

Neonates referred from other hospitals and with incomplete clinical data were excluded.

### *2.3. Sample collection procedure*

About 1 ml of blood was collected from each neonate with complete aseptic precautions and immediately inoculated into a paediatric blood culture bottle (BD BACTEC). Preferably, two blood samples were collected from each neonate from different sites to reduce the risk of contamination by normal flora.

All blood culture samples were processed in the BD BACTEC FX 40 automated blood culture system. The positive blood culture samples were streaked on Blood agar and MacConkey agar plates and incubated at 37 °C for 24 hours. The further identification of bacterial species and antibiotic sensitivity was done by Vitek 2 Compact bioMérieux.

### *2.4. C-reactive protein (CRP) Test*

Qualitative analysis of CRP was performed by the latex agglutination test. The polystyrene latex particles are coated with anti-CRP that binds with CRP present in test serum and gives a visible agglutination reaction (**Figure 2**). The quality control was done by performing tests using the internal positive and negative controls provided in the kit. Further quantitative analysis of positive samples was done using the MISPA-i3 instrument based on a latex-enhanced turbidimetric immunoassay. CRP in the sample binds with specific anti-CRP antibodies (rabbit polyclonal antibody), which have been adsorbed to a latex particle. The quality control of the instrument was carried out using Agappe protein control (51614007).CRP values more than 10mg/L were considered significant and indicative of septicemia.

### *2.5. Procalcitonin (PCT) estimation*

Quantitative procalcitonin analysis was performed using the Finecare fluorescence immunoassay test based on the sandwich immunodetection method. The normal reference value was set at 0.5 ng/ml. Values above 0.5 ng/ml suggested potential sepsis. The system is cartridge-based and coated with fluorescence-labelled antibodies. When test samples are added to the cartridge well, these labelled antibodies bind to PCT antigens in the blood sample, forming immune

complexes. These complexes migrate on a nitrocellulose matrix and become immobilized. The fluorescence signal emitted by the labelled antibody correlates with the amount of PCT antigen in the sample. Each test included an internal control, and if the internal control produced invalid results, the test was repeated.

*2.6. Statistical methods*

The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of CRP and PCT tests were calculated using MedCalc statistical software.

## 3. Results

In the present study, 330 neonates showing signs and symptoms of septicemia were included. In the present study, among the 330 sepsis cases, there were 243 (73.63%) cases of early-onset septicemia and 87 (26.36%) cases of late-onset septicemia. The blood culture results were positive in 175 patients (53.03%) out of 330 neonates. Out of 175 blood culture-positive, gram-positive cocci were isolated in 76 samples, and gram-negative bacilli were isolated in 99 samples. Among gram-negative bacilli, the highest prevalence was of *Klebsiella pneumoniae* (20%). (**Figure 1**)

Of 175 positive blood cultures, 154 were positive for CRP, and the remaining 21 were negative for CRP. Among 155 negative blood cultures, CRP was positive in 92 samples and negative in 63 samples. (**Table 1**)

The sensitivity and specificity of CRP in correlation with positive blood culture are 88.80% and 40.65%, respectively. (**Table 2**) As the specificity of CRP is low, it must be combined with other biomarkers for the accurate diagnosis of neonatal septicemia.

**Table 1:** Association between CRP levels and blood culture results in neonatal septicemia

| Total blood culture received (n=330) | Positive blood culture (n=175) | Negative blood culture (n=155) |
|---|---|---|
| CRP level >10 mg/dl (Positive) | 154 | 92 |
| CRP level <10 mg/dl (Negative) | 21 | 63 |

**Table 2:** Sensitivity, specificity, PPV and NPV of CRP in correlation with positive blood culture

| CRP | | |
|---|---|---|
| | **Value** | **95% CI** |
| Sensitivity | 88.00% | 82.24% to 92.42% |
| Specificity | 40.65% | 32.84% to 48.82% |
| Positive Likelihood Ratio | 1.48 | 1.29 to 1.71 |
| Negative Likelihood Ratio | 0.30 | 0.19 to 0.46 |
| Positive Predictive Value | 62.60% | 59.24% to 65.85% |
| Negative Predictive Value | 75.0% | 65.80% to 82.39% |
| Accuracy | 65.76% | 60.36% to 70.87% |

**Table 3:** Association between PCT levels and blood culture in neonatal septicaemia

| Total blood culture received (n=330) | Positive blood culture (n=175) | Negative blood culture (n=155) |
|---|---|---|
| PCT >0.5 ng/ml (Positive) | 164 | 42 |
| PCT <0.5 ng/ml (Negative) | 11 | 113 |

**Table 4:** Sensitivity, specificity, PPV, and NPV of PCT in correlation with positive blood culture

| PCT | | |
|---|---|---|
| | **Value** | **95% CI** |
| Sensitivity | 93.71% | 89.03% to 96.82% |
| Specificity | 72.90% | 65.19% to 79.72% |
| Positive Likelihood Ratio | 3.46 | 2.66 to 4.49 |
| Negative Likelihood Ratio | 0.09 | 0.05 to 0.15 |
| Positive Predictive Value | 79.61% | 75.05% to 83.52% |
| Negative Predictive Value | 91.13% | 85.19% to 94.83% |
| Accuracy | 83.94% | 79.52% to 87.73% |

**Table 5:** Comparison of sensitivity, specificity, PPV, NPV, and Accuracy of CRP among various studies

| CRP Cut off >10 mg/dl | Present study | Emad A. Morad et al.[14] | In Ho Park et al.[15] |
|---|---|---|---|
| Sensitivity | 88% | 89.5% | 100% |
| Specificity | 40.65% | 66.7% | 85.66% |
| PPV | 62.60% | 92.5% | 33.3% |
| NPV | 75% | 60% | 100% |
| Accuracy | 65.76% | 86% | - |

**Table 6:** Comparison of sensitivity, specificity, PPV, NPV, and Accuracy of PCT among various studies

| PCT Cut off >0.5 ng/ml | Present study | Emad A. Morad et al.[14] | M. Adib et al.[11] | In Ho Park et al.[15] | Haniya Jafar et al.[16] |
|---|---|---|---|---|---|
| Sensitivity | 93.71% | 97.6% | 75% | 88.89% | 73.8% |
| Specificity | 72.90% | 89% | 80% | 58.17% | 47.1% |
| PPV | 79.61% | 97% | 80% | 13.2% | 38% |
| NPV | 91.13% | 88.9% | 75% | 98.6% | 70% |
| Accuracy | 83.94% | 96% | - | - | 55.3% |

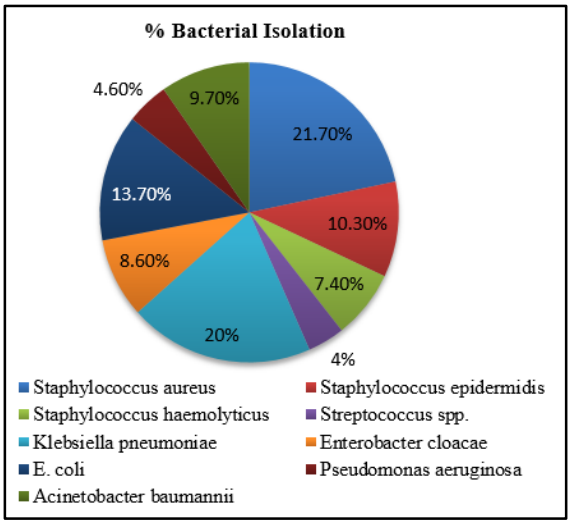


**Figure 1:** % Isolation of bacteria causing neonatal septicaemia


**Figure 2:** CRP Latex agglutination test

Out of 175 positive blood culture samples, 164 were positive for PCT and 11 samples were negative for PCT. And out of 155 negative blood cultures, 42 were positive for PCT and 113 were negative for PCT (**Table 3**).

The sensitivity and specificity of PCT in correlation with positive blood culture was 93.71% and 72.90%, respectively. (**Table 4**)

The specificity of PCT is higher compared to CRP thus it is more precise marker for neonatal sepsis.

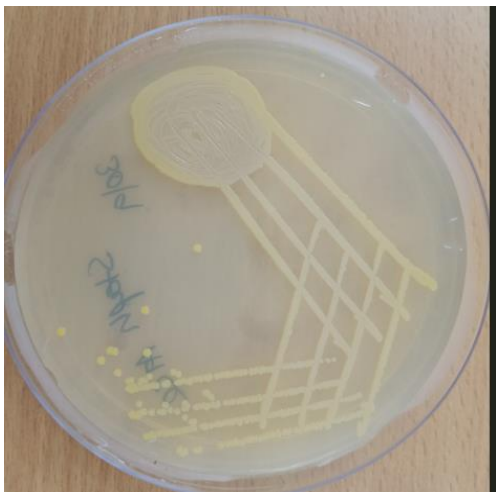

**Figure 3:** Golden-yellow pigmented colonies of *Staphylococcus aureus* on nutrient agar

## 4. Discussion

In the present study, among the 330 sepsis cases, there were 243 (73.63%) cases of early-onset septicemia and 87 (26.36%) cases of late-onset septicemia. The blood culture results were positive in 175 patients (53.03%) out of 330 neonates. Among blood culture-positive samples, the highest prevalence was of *Staphylococcus aureus* (21.71%) (**Table 3**), followed by *Klebsiella pneumoniae* (20%) and *E. coli* (13.7%). Yadav et al[19] also reported the highest prevalence of *Staphylococcus aureus*, 35.6%, among neonatal sepsis cases.

*4.1. Blood culture as gold standard test*

Microbial blood culture is regarded as the gold standard for diagnosing neonatal septicemia. It aids in guiding appropriate antibiotic treatment based on susceptibility testing. However, certain factors influence the sensitivity and specificity of the blood culture test, which may complicate interpretation and delay diagnosis.

*4.1.1. False positive*

Blood cultures may produce false-positive results due to contamination by normal flora, which can enter during the blood collection process because of inadequate skin antisepsis. Inappropriate storage or delay in transportation may also result in overgrowth of commensal bacteria in the blood culture. Certain commensals, such as Coagulase-negative staphylococci (CONS) and bacillus spp. can be mistaken as pathogens. To differentiate commensal bacteria from pathogenic ones, two sets of blood cultures should be collected from two different sites, and the results of blood culture must be evaluated along with the proper clinical history of the patient and should be used in conjunction with biomarkers such as CRP or procalcitonin.

*4.1.2. False negative*

Blood culture may produce false-negative results due to prior antibiotic treatment, low bacterial load, especially in low birth weight or preterm neonates, insufficient volume of blood collected, improper timing of blood culture collection, and bacteremia due to fastidious bacteria.

*4.2. CRP as a diagnostic marker for neonatal septicemia*

Of 175 positive blood cultures, 154 were positive for CRP, and the remaining 21 were negative for CRP. Among 155 negative blood cultures, CRP was positive in 92 samples and negative in 63 samples. In our study, the sensitivity and specificity of CRP in correlation with blood culture are 88.80% and 40.65%, respectively. The comparison of sensitivity, specificity, PPV, NPV, and Accuracy of CRP among various studies is shown in **Table 5**.

*4.2.1. False positive*

CRP level may also rise in certain inflammatory conditions or following trauma and post-surgery. CRP may show false-positive results in certain prenatal circumstances, like fetal distress, meconium aspiration syndrome, prolonged rupture of membrane, intraventricular hemorrhage, and maternal fever.

*4.2.2. False negative*

In the initial stages of neonatal sepsis, CRP level may not be detectable, as it rises slowly during the first 24-48 hours following infection, which produces false-negative results.[4,5,18,20]

*4.2.3. Limitation*

The specificity of CRP is low as it produces false-positive results in non-infectious conditions also. CRP can be used as a diagnostic marker, but in conjunction with other serum biomarkers. Another disadvantage of CRP as a biomarker is that it lacks age-specific reference values, and several factors, such as birth weight and gestational age, affect CRP kinetics.[2,3] It does not help in differentiation between bacterial and viral etiology.

*4.3. PCT as a diagnostic marker for neonatal septicemia*

Out of 175 positive blood culture samples, 164 were positive for PCT and 11 samples were negative for PCT. And out of 155 negative blood cultures, 42 were positive for PCT and 113 were negative for PCT. The sensitivity and specificity of PCT in correlation with positive blood culture were 93.71% and 72.90%, respectively. Several studies reported that PCT is a more accurate marker for neonatal septicemia, with sensitivity ranging between 83% to 100% and specificity ranging from 70% to 100%.[12,16]

*4.3.1. False positive*

PCT levels may show false-positive results in newborns in certain instances, such as hypoxemia, prolonged rupture of membrane, birth asphyxia, maternal GBS colonization, antibiotic misuse, and administration of surfactant.[17]

*4.3.2. False negative*

In the early phase of infection, the PCT result may be negative as the newborn's immune system is relatively immature and does not mount sufficient PCT production.PCT is a more sensitive marker in systemic infections as compared to localized bacteremia.

*4.3.3. Advantages of PCT as a diagnostic marker*

PCT maintains a high serum concentration in comparison to other serum biomarkers such as tumor necrosis factor and interleukin-6; thus, it is a more useful sepsis marker. The main advantage of PCT is that the birth weight and gestational age of newborns do not affect PCT levels.[2,14,15]

CRP is routinely used as a marker for acute infection, but its level rises more slowly compared to the PCT level. Thus, PCT is a more useful marker for early-onset sepsis in neonates.[6,7,11]

## 5. Conclusion

Procalcitonin (PCT) is a good diagnostic marker of early-onset sepsis. The PCT level decreases rapidly after the initiation of antibiotic treatment, so it can be used to monitor treatment response. PCT also helps to differentiate between infections caused by actual pathogens and those caused by commensal bacteria. However, it is comparatively expensive to use as a routine test, which is a major limitation in developing countries.

## 6. Conflict of Interest

None.

## 7. Source of Funding

None.

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