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Original Research Article

Neonatal septicemia: Diagnostic challenges and the role of CRP and blood culture

Saurabh Chhotalal Norris[1], **Rachana Dhirubhai Patel**[1], **Himani Bhardwaj Pandya**[1], **Binda Prakashbhai Pipaliya**[1]*, **Tanuja Bakul Javadekar**[1]

[1]*Dept. of Microbiology, Smt. B. K. Shah Medical Institute & Research Centre, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, India*

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ABSTRACT

Background: Neonatal septicemia, a serious blood infection occurring within the first four weeks of life, is a significant cause of neonatal deaths. Its diagnosis is challenging due to the non-specific nature of its signs and symptoms. C-reactive protein (CRP) serves as a valuable biomarker for early detection, facilitating timely treatment and improving survival rates as a rapid adjunct to the standard blood culture process. This study explores the relationship between CRP levels and blood culture findings in diagnosing Neonatal septicemia.

Aim and Objectives: The objectives were to determine common organisms causing neonatal septicemia, to evaluate CRP as a diagnostic tool for Neonatal septicemia compared to blood culture results.

Materials and Methods: The study examined the correlation between CRP levels and blood culture outcomes in neonates suspected of having sepsis. Blood samples were aseptically collected and inoculated in 1 set of BD BACTECTM Peds Plus/F blood culture bottles, incubated for five days using a BD BACTEC automated machine. The bacteria isolated from positive blood culture were processed using Vitek-2. CRP levels were determined using the latex agglutination card test, with positive samples further analyzed via quantitative nephelometry.

Results: Of the 321 samples, 28.97% (93) tested positive for bacterial sepsis via blood culture. Among these, 33.3% (31 cases) were *Staphylococcus epidermidis*, 18.3% (17 cases) were *Staphylococcus haemolyticus*, and 11.8% (11 cases) were *Staphylococcus capitis*, with coagulase-negative staphylococci (CONS) being the most common group overall. Among the Gram-negative organisms, *Klebsiella pneumoniae* (8.6%) and *E. coli* (6.5%) were significant contributors to neonatal septicemia. CRP was positive in 82 cases with blood culture-positive samples and in 105 cases with blood culture-negative samples, underscoring its potential as a diagnostic marker for Neonatal septicemia.

Conclusion: While CRP is a valuable biomarker for detection of Neonatal septicemia, it should not be used as a sole diagnostic tool due to its lack of specificity. CRP testing provides a presumptive diagnosis that can guide early antibiotic therapy, emphasizing its significance in neonatal care.

1. Introduction

Neonatal septicemia remains a leading cause of mortality in newborns, particularly in developing countries, where it accounts for 30-50% of all neonatal deaths each year.[1] In 2019, an estimated 2.4 million newborns died within their first month, with over 6,700 dying each day. Nearly one-third of these deaths happened within the first 24 hours, and over three-quarters within the first week. Most neonatal

* *Corresponding author*.
E-mail address: bindapipaliya@gmail.com (B. P. Pipaliya).

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deaths occur in low- and middle-income countries, where neonatal sepsis is a primary cause of infant mortality and morbidity.[2] The National Perinatal Database (NNPD) study in India, conducted from 2002 to 2003, identified the rate of neonatal septicemia as 30 cases per 1,000 live births.[3]

1.1. Classification of neonatal septicemia

Neonatal septicemia is classified into two types based on the time of onset:

1. Early-Onset Sepsis (EOS): This type occurs within the first 72 hours of life and is primarily linked to bacteria acquired from the mother during delivery or from the hospital environment.[4]
2. Late-Onset Sepsis (LOS): This type occurs between 72 hours and 28 days after birth, often associated with organisms acquired after delivery.

Gram-positive and Gram-negative microorganisms can lead to Neonatal septicemia.[5] The increasing occurrence of antibiotic-resistant pathogens presents a significant challenge in effectively managing this condition.[6] Understanding the distribution of these organisms and their resistance patterns is critical for selecting suitable antimicrobial therapy for patients with septicemia. Timely identification and management are essential to reduce complications and fatalities associated with Neonatal septicemia.[7]

1.2. Diagnosis of neonatal septicemia

The established standard for identifying Neonatal septicemia is blood culture. However, this technique has several drawbacks: it is labor-intensive, requires advanced laboratory facilities, and may produce misleading results due to limited blood sample sizes and previous antibiotic administration in the mother.[8]

1.3. C-reactive protein (CRP) as a diagnostic marker

C-reactive protein (CRP), initially characterized by Tillett and Francis in 1930, is an acute-phase protein produced by the liver in response to inflammatory cytokines such as IL-1, IL-6, and TNF-α.[9,10] CRP plays a crucial role in the innate immune response of the body. This study intends to investigate CRP as an indicator of Neonatal septicemia and to analyze its relationship with blood culture findings.

2. Materials and Methods

2.1. Objectives

To analyze the correlation between CRP levels and the presence of positive blood culture results in neonates suspected of sepsis.

2.2. Inclusion criteria

Neonates admitted to the NICU of a tertiary care facility in Vadodara, Gujarat, who were clinically evaluated for septicemia and underwent both CRP and blood culture testing. Data recorded in the microbiology department register from January 2020 to December 2020.

2.3. Exclusion criteria

Neonates with incomplete data were excluded from the study.

2.4. Procedure

In neonatal blood culture collection, if a central line (CL) is absent, two blood samples are aseptically collected from different venipuncture (VP) sites to reduce the risk of contamination. However, if a CL is present, one sample is drawn from a VP site and another directly from the CL to help determine if the infection is related to the central line. With informed consent obtained from the neonate's guardians, these samples are then inoculated into one set of BD BACTECTM Peds Plus/F blood culture bottles.

1. Blood culture analysis
   (a) Inoculation: Samples were inoculated into one set of sterile BD BACTECTM Peds Plus/F blood culture bottles containing enriched soybean casein digest broth to support aerobic bacterial growth.
   (b) Processing: All samples were processed using the BD BACTEC automated culture system, in accordance with established microbiological procedures.
   (c) Incubation conditions: Positive blood culture bottles were subsequently subculture onto sheep blood agar and MacConkey agar and incubated at 37°C for 24 hours.
   (d) Bacterial identification and antibiotic susceptibility test were done using Vitek-2 compact system
2. C-reactive protein (CRP) estimation
   (a) Initial test: CRP was estimated using a latex agglutination card test.
   (b) Criteria for positivity: Samples that tested positive underwent further quantitative analysis using nephelometry.
   (c) Cut-off value: CRP values >10 mg/L were considered indicative of sepsis.
3. For differentiation of pathogenic CONS from normal flora: Neonatal septicemia cases were assessed by evaluating risk factors (e.g., low birth weight, indwelling catheters), conducting quantitative blood

cultures (≥100,000 cfu/ml), and performing multiple blood cultures to confirm CONS bacteremia. Elevated C-reactive protein (CRP) levels alongside CONS isolation indicated pathogenicity, and antibiotic susceptibility tests helped identify multidrug-resistant hospital-acquired strains. This approach differentiated pathogenic CONS from normal flora.

3. Results

In this study, a total of 321 neonatal blood samples were collected for culture, revealing bacteriological sepsis in 28.97% (93 of 321) of the subjects. Among the 93 sepsis cases, 67 (72.04%) were classified as early-onset septicemia (EOS), occurring within the first 72 hours of life, while 26 (27.96%) were categorized as late-onset septicemia (LOS), occurring after 72 hours. The distribution of primary causative agents of septicemia is summarized in Figure 1.

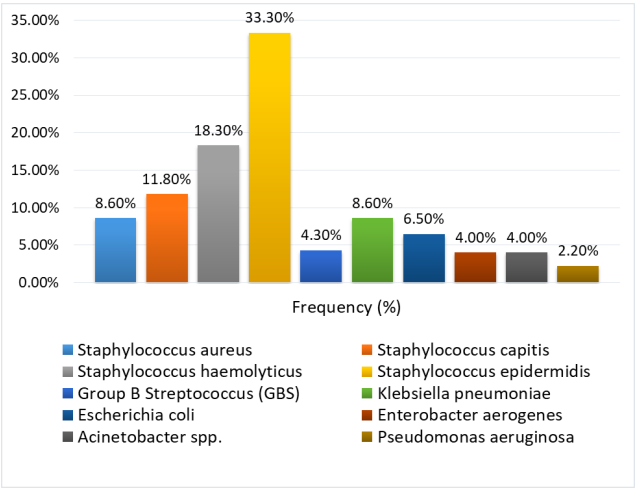

Figure 1: Primary causative agents of neonatal septicemia

Figure 1 summarizes the distribution of primary causative agents identified in the blood cultures of neonates diagnosed with septicemia. Of the 93 sepsis cases, a significant majority (76.3%) were caused by Gram-positive organisms, highlighting their prominence in early and late-onset septicemia.

Among the 321 samples tested for both blood culture and CRP, 82 (88.17%) were positive for both blood culture and CRP, indicating a significant correlation between positive culture results and elevated CRP levels. Additionally, 105 samples that were negative for blood culture tested positive for CRP, as detailed in Figure 2. This finding highlights the potential for CRP to be a useful marker for infection in the neonatal population, despite negative culture results. However, the presence of 105 blood culture-negative but CRP-positive cases highlights the need for careful clinical evaluation and potentially further diagnostic investigation in neonates with elevated CRP levels.

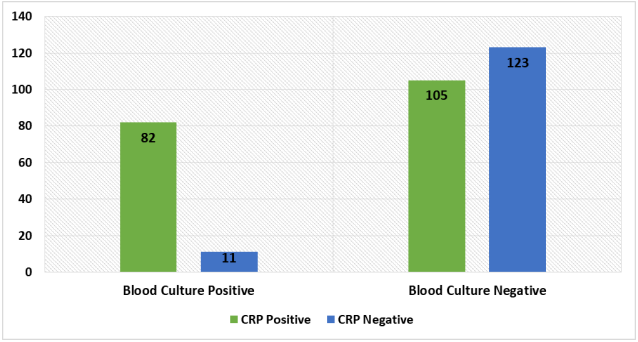

Figure 2: Association between CRP levels and blood culture outcomes in neonatal septicemia

4. Discussion

This study assessed the relationship between CRP levels and blood culture findings in Neonatal septicemia. Out of 321 blood samples collected, 28.97% (93 samples) were confirmed positive for bacterial sepsis. Among these, 72.04% (67 cases) were early-onset septicemia (EOS), while 27.96% (26 cases) were late-onset septicemia (LOS). Gram-positive bacteria were the most frequently detected, with *Staphylococcus epidermidis* (33.3%), *Staphylococcus haemolyticus* (18.3%), and *Staphylococcus capitis* (11.8%) being the leading pathogens in the coagulase-negative staphylococci (CONS) category. Among the Gram-negative bacteria, significant contributors to neonatal septicemia included *Klebsiella pneumoniae* (8.6%), *E. coli* (6.5%), *Enterobacter aerogenes* (4%), and *Acinetobacter spp.* (4%).

4.1. Diagnostic limitations[11,12]

CRP was positive in 82 samples with positive blood cultures and 105 samples with negative blood cultures, highlighting its role as a valuable biomarker for early intervention in Neonatal septicemia. While C-reactive protein (CRP) has proven to be a useful biomarker in identifying neonatal sepsis, there are several limitations that should be considered when interpreting CRP levels.

1. False positives: CRP levels may rise due to non-infectious conditions, such as inflammatory responses triggered by trauma, surgery, or autoimmune disorders. In neonates, perinatal stress or birth-related hypoxia can also elevate CRP levels, potentially leading to false positives.
2. False negatives: In the early stages of sepsis, particularly within the first 12 to 24 hours of infection, CRP levels may not rise sufficiently to be detected. This can lead to a delay in diagnosis, especially in neonates with immature immune responses, resulting in false-negative results.
3. Non-specificity: CRP is a general marker of inflammation and does not specifically distinguish

between bacterial, viral, or fungal infections. As such, while elevated CRP levels suggest an inflammatory response, further diagnostic tests (e.g., blood cultures, microbiological analysis) are crucial for confirming the causative pathogen.

4. Transient bacteremia: In cases of transient bacteremia or self-limiting infections, CRP may be elevated without confirming sustained infection, leading to potential over-diagnosis.

CRP, though useful for detecting Neonatal septicemia, lacks specificity as elevated levels can also result from non-infectious conditions, leading to potential false positives. In our study, 105 blood culture-negative cases showed elevated CRP levels, indicating possible over-diagnosis.

4.2. Blood culture as the gold standard

Blood culture is considered the gold standard for diagnosing sepsis due to its ability to directly identify the presence of pathogens in the bloodstream. It provides definitive microbiological confirmation of infection and allows for the characterization of the specific organism responsible for the sepsis. Furthermore, blood cultures can guide appropriate antibiotic therapy based on susceptibility testing, which is critical for effective management of the infection. However, it has limitations, including sensitivity issues due to factors such as small sample volumes, prior antibiotic use, and contamination, which can complicate interpretation and lead to delayed results.[13–15]

A combined approach of clinical evaluation, CRP testing, and blood culture is recommended for a more accurate diagnosis of Neonatal septicemia.

4.3. Differentiation of pathogenic CONS from normal flora

Coagulase-negative staphylococci (CONS) are common skin and mucosal commensals, often present as part of the normal flora in healthy individuals. However, differentiating pathogenic CONS from normal flora in Neonatal septicemia cases can be challenging. Several factors can help indicate pathogenicity:

1. Clinical context: Pathogenic CONS are more likely to cause sepsis in neonates with risk factors such as indwelling catheters, low birth weight, or prolonged hospitalization. Clinical signs of infection, including fever, hypotension, or respiratory distress, often accompany true CONS bacteremia.[16]
2. Quantitative blood culture: To perform quantitative blood cultures, a specified blood volume is collected aseptically and inoculated into blood culture bottles, then incubated in automated systems like BD BACTEC to monitor for bacterial growth. Once growth is detected, the bacterial load, measured in colony-forming units per milliliter (cfu/mL), is estimated by plating serial dilutions of the culture onto agar plates. These plates are incubated to allow visible colonies to form, which are then counted. The colony count is multiplied by the dilution factor to determine the cfu/mL of the original sample. A result of ≥100,000 cfu/mL suggests a high bacterial load and a significant infection, distinguishing it from contamination by normal flora.[17]
3. Multiple positive cultures: Repeated isolation of the same CONS species from multiple blood culture samples enhances the likelihood of true bacteremia rather than contamination.[18,19]
4. CRP levels: Elevated CRP levels in conjunction with positive CONS blood cultures provide further evidence that the CONS isolate is pathogenic rather than part of the normal flora.[18]
5. Antibiotic resistance patterns: Pathogenic CONS often show resistance to multiple antibiotics, especially in hospital-acquired infections, which can differentiate them from typical commensal CONS strains.[20]

4.4. Comparison of study data with different studies

Table 1: Comparison of frequency of blood culture positivity in clinically diagnosed neonatal sepsis with different studies

Study	This study	Muley VA et al.[21]	Pavan Kumar DV et al.[22]	Thakur S et al.[23]
Frequency	28.97%	26.6%	26.2%	42%

Table 2: Comparison of most common isolated organisms from blood cultures of neonatal septicemia

Organisms	This study	Jyothi P. et al.[24]	Yadav NS et al.[25]
CONS	46.67%	27.5%	10.2%
Klebsiella pneumoniae	8.6%	30.5%	15.3%
Staphylococcus aureus	8.6%	10.6%	35.6%

Table 2 indicates that *Klebsiella pneumoniae*, CONS, and *Staphylococcus aureus* are commonly isolated in neonatal septicemia across different studies.

1. The high isolation rates of coagulase-negative staphylococci (CONS) (46.67%) highlight the evolving microbial landscape in neonatal care, especially concerning device-related infections.
2. Notably, the study contrasts with others regarding the prevalence of *Klebsiella pneumoniae* and *Staphylococcus aureus*, reflecting local microbiological patterns and the need for tailored empirical treatment guidelines.

These comparisons stress the necessity for ongoing surveillance of microbial patterns in neonatal units to adjust treatment protocols effectively.

Table 3: Correlation of CRP in blood clture positive neonates: A comparison with other studies

Study	CRP Positive	CRP Negative
This study	82 (88.17%)	11 (11.83%)
Bharathi R et al.[26]	35 (35.35%)	15 (28.57%)
Maurya A et al.[27]	17 (43.59%)	19 (37.25%)

Table 3 provides a comparative analysis of the correlation between CRP levels and blood culture results across different studies. The findings indicate that this study had a higher percentage of CRP positivity in blood culture-positive cases compared to other studies, suggesting improved detection or possibly differing thresholds for CRP positivity.

5. Conclusion

Our findings reaffirm that while blood culture remains the definitive method for diagnosing Neonatal septicemia, CRP serves as a useful initial screening tool.

However, it's important to understand that CRP, on its own, lacks the specificity to be used as a single diagnostic test. Instead, CRP measurement can aid in making an early presumptive diagnosis, allowing for the prompt initiation of antibiotics while awaiting blood culture confirmation. This integrated strategy—leveraging CRP for early identification and blood culture for conclusive diagnosis—may lead to improved clinical outcomes by enabling quicker intervention and more efficient management of Neonatal septicemia.

6. Ethical Approval

This study was done after taking approval from Institute Ethical approval committee. (SVIEC/ON/Medi/RP/Aug/24/9).

7. Source of Funding

None.

8. Conflict of Interest

None.

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Author's biography

Saurabh Chhotalal Norris, Associate Professor https://orcid.org/0000-0002-9813-4132

Rachana Dhirubhai Patel, Associate Professor https://orcid.org/0000-0002-2514-6634

Himani Bhardwaj Pandya, Associate Professor https://orcid.org/0000-0001-9444-9279

Binda Prakashbhai Pipaliya, Associate Professor https://orcid.org/0009-0000-2681-6698

Tanuja Bakul Javadekar, Professor

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