Original Research

Correlation of blood culture with C-reactive protein in the diagnosis of neonatal sepsis: A prospective study

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Received: 19 February, 2024 Accepted: 22 March, 2024

Abstract
Background and Objective: Sepsis in neonates is difficult to diagnose clinically. When diagnosing neonatal sepsis, physicians frequently utilise biomarkers such as c-reactive protein (CRP). The aim of this study was to investigate the connection between blood culture and CRP levels as a potential early marker of sepsis in neonates.
Material and method: The study involved 500 newborns over a seven-month period who were suspected of having neonatal sepsis. Each patient had a blood culture and had their CRP qualitatively assessed. For the individuals who tested positive for CRP, CRP semi-quantification was performed.
Result: We found that the culture result for newborn sepsis was positive in 34.6% of cases. The results showed that CRP was a good predictor of sepsis, with sensitivity, specificity, PPV, NPV, and diagnostic accuracy of 69%, 48%, 41%, 74%, and 55%, respectively.
Conclusion: Neonatal sepsis can be diagnosed quickly with the use of CRP estimate, a quick test with good sensitivity.
Key words: Blood culture, CRP, neonatal sepsis

Introduction

One of the leading causes of illness and mortality among newborns is still neonatal septicaemia. In India, the frequency of neonatal sepsis ranges from 11.0 to 24.5/1000 live births [1]. Due to limited resources, diagnosing bacterial illnesses can be difficult in the majority of developing nations. Identifying the cause of sepsis is typically accomplished through blood culture testing. Prenatal antibiotic exposure causes low yield because the culture reports would only be positive in 25% to 45% of cases [2].Antibiotics are administered inappropriately to newborns suspected of sepsis when culture reports are delayed. However, the key to preventing infant mortality from sepsis is early diagnosis. In order to improve therapeutic outcomes and prevent the emergence of antibiotic resistance, culture-independent diagnostics are urgently needed [3].Neonatal sepsis rapid biomarker screening is urgently needed, rather than waiting for a long-time blood culture-sensitivity result.Among the non-specific indicators for sepsis include procalcitonin, micro-ESR, CRP, and absolute neutrophil count etc. [4].Acute-phase proteins are primarily produced by the liver in response to an infection or tissue damage. The most widely used and studied acute phase reactant is C-reactive protein (CRP), which has been shown to have higher sensitivity and specificity as a

diagnostic marker in neonates than both total neutrophil count and I/T ratio. The sensitivity of CRP measurements taken 24 and 48 hours after the onset of illness is significantly improved (82.0% and 84.0%, respectively), and these measurements also showed very high predictive values for the diagnosis of neonatal sepsis and were superior to those of leucocyte indices of CBC [5].

Because of its extremely short half-life (T½ of 19 hours), CRP levels decrease rapidly when antigenic stimuli are removed. In light of this, CRP can be used as a measure to determine when antibiotic treatment can be safely stopped in cases ofsuspected newborn septicaemia.While persistent rises suggest insufficient treatment or a potential consequence, a quick return of CRP to normal mayindicate a favourable response to antibiotics [6].

The purpose of this research was to determine the relationship between blood culture and CRP levels in the blood as an early indicator of newborn sepsis.

Material and methods

Study Centre: Sick Newborn Care Unit, Department of Paediatrics, and Department of Microbiology, Gajra Raja Medical College and J. A. Group of Hospitals, Gwalior, Madhya Pradesh.

Ethical Approval: Ethical approval was obtained from the Institutional Ethical Committee.

Study Subject: Neonates with probable sepsis or neonates with PSBI (possible serious bacterial infection) who were admitted to the Sick Newborn Care Unit (SNCU) at Gajra Raja Medical College and J. A. Group of Hospitals, Gwalior.

Study Design and Period: A prospective study of neonates with probable sepsis, or PSBI, was conducted for a period of seven months from August 2019 to February 2020.

Sample Size: A total of 500 samples of neonates with probable sepsis or PSBI admitted to the J. A. Group of Hospitals.

Inclusion criteria: All intramural and extramural neonates with probable sepsis or PSBI during the study period whose mothers or caretaker′s consent to be part of the study.

Exclusion criteria: 1. Neonates with no clinical suspicion of sepsis. 2. The patient's age is >28 days of life. 3. All neonates who have had prior antibiotic administration. 4. Neonates with<30 weeks of gestational age. 5. Neonates with birth weight <1000gm. 6. Neonates with gross congenital malformation. 7. Neonates for whom mother or caregivers are not willing to give consent.

Procedure: Estimation of C-reactive Protein: Qualitative and semi-quantitative analysis of CRP were done by using CRP Latex kit (Beacon Diagnostics Pvt. Ltd.)

The latex reagent, positive and negative controls and serum sample were brought to the room temperature. Fifty microliter of positive control, serum sample and negative control were placed in separate cells on the slide. After adding 1 drop of latex reagent to all 3 cells, the fluid in the cell was mixed and spread to cover the entire area using separate plastic sticks. The slide was then gently rotated back and forth for 2 minutes. At the end of 2 minutes the results were read under bright light. Serum with CRP content of more than 6 mg/l showed distinct agglutination.

Interpretation of the results: Distinct coarse agglutination: Positive&Smooth suspension/No clumps: Negative

Semi-quantitative analysis: Serum samples showing positive results in the screening tests were proceeded with the semi-quantitative test. Fifty microliters of 0.9 % saline solution were placed in all cells of the slide. Fifty microliter of serum was placed in the first cell and mixed well. Fifty microliter of this mixture was taken and added to the next cell and mixed. The procedure was repeated through the remaining cells. This procedure of doubling dilution of the serum used for performing semiquantitative analysis. One drop of latex reagent was added to all the cells and the test was performed as previously said.

The titre was calculated by taking the highest dilution which gave visible agglutination and it was multiplied with the conversion factor 6 to get the CRP values in mg/l. eg. If the titre was 1:16, the CRP concentration was calculated as 16X6= 96 mg/l.

Statistical analysis: The statistical analysis was performed using statistical software SPSS (2.1). The data was represented as percentages and proportions. Two or more set of variables were compared by using Chi-square test and Z-test. If the p-value was less than 0.05, it was considered significant.

Result

This prospective study was conducted in the Department of Microbiology and Paediatrics, Gajra Raja Medical College and J. A. Group of Hospitals were studied over the period of 7 months from August 2019 to February 2020. In study period 500 neonates with probable sepsis or PSBI admitted in Neonatal intensive care unit, J. A. Group of Hospital were analysed.

Table: 1 Correlation Between Cr p And Blood Culture

CRP	Culture		Total
	Positive	Negative	
Elevated	119(69%)	171(52%)	290(58%)
Normal	54(31%)	156(48%)	210(42%)
Total	173(100%)	327(100%)	500(100%)

The serum samples of the neonates tested for C-reactive protein using latex agglutination test were positive in 290 neonates (58%) of which 119 (69%) neonates had culture proven sepsis. The sensitivity of CRP was 69% and the specificity was 48%. The positive and negative predictive values were found to be 41 % and 74 % respectively.

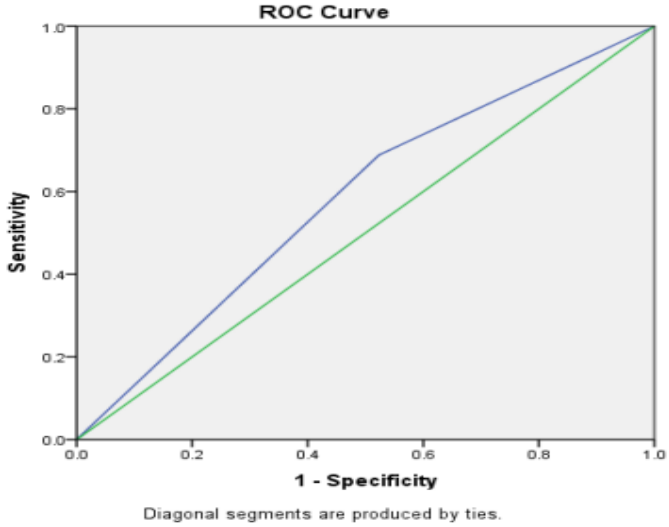
Table 2: Statistical analysis of CRP (sepsis marker)

Measures	CRP
Sensitivity	69
Specificity	48
PPV	41
NPV	74
FPR	52
FNR	31
Accuracy	55

Table: 3 Diagnostic accuracy of CRP for sepsis as compared to blood culture

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.582	0.026	0.002	0.531	0.634

Graph: ROC curve showing comparison between CRP and blood culture



Discussion

Blood culture not only takes time, it is also a complicated process with low yield. The readily available complete blood counts and the leukocyte differential assays have relatively low specificity for diagnosis and is widely used as an adjunct to culture. There is no single reliable test for definitive diagnosis as of date and there is a continuing search for a new marker. During an inflammatory reaction, C-reactive protein functions as a scavenger by opsonizing bacteria and activating the complement system, which facilitates phagocytosis. Of all the measures, the CRP test has been the most analysed for years and has a higher chance of predicting sepsis.There were 34.6% (n=173) blood culture-proven instances of neonatal sepsis in our study. Neonatal sepsis cases confirmed by blood culture are rare because it might be challenging to obtain enough blood for the culture, blood culture facilities are expensive, and these factors are present only in remote places [7].In the present study, out of 500 cases CRP was positive in 290 (58%) cases. Out of total 173 culture positive cases, 119 were CRP positive which shows significant correlation of CRP and blood culture positivity with a significant p value. The sensitivity of CRP was 69% and specificity was 48%. This finding correlates with the studies done by Sakha et al (2008) and Vazzalwar et al (2009) with sensitivity of 70.4% and 72%

respectively [8, 9].Boo N.Y et al (2008), Garland Suzanne et al (2003) and Sucilathangam G et al (2012) revealed lower sensitivity patterns (less than 55%) in their studies [10, 11, 12]. But higher sensitivity and specificity have also been reported by Zeeshan et al (2005) with 82.7% & 95.9% and Jan AZ et al (2013) with 88.36% & 89.13% respectively [13, 14]. An intriguing 100% sensitivity has also been recorded in a study done by Nuntnarumit P et al [15]. In this study, we found 48% specificity of CRP, which is similar to the study done by Bhatia S et al. (42.86%) [16].The variations in these studies might arise from changes in the timing of the CRP assay following the clinical onset of infection, in addition to the fact that each study used a different cut-off point for quantitative CRP measurement. Since there is only a gradual rise in CRP within the first 48 hours of infection, its sensitivity is nullified. It is also discovered that illnesses other than sepsis, such as PROM and meconium aspiration, have further raised CRP values that impact its specificity. In the present study, CRP was positive in 171 (52%) cases, even if a blood culture was found to be negative. It may be because of a variety of non-infectious conditions like MAS, traumatic or ischemic tissue injury, haemolysis, or histologic chorioamnionitis. For predicting sepsis on the basis of CRP as compared to blood culture (gold standard). In current study ROC curve indicates the area under the curve is 0.582 (0.531 – 0.634) with p value 0.002 (highly significant).

Conclusion

We have concluded that the CRP estimation is a rapid and sensitive test for early diagnosis and management of neonatal sepsis.CRP is a good diagnostic and therapeutic tool, so that antibiotic can be started in culture negative and asymptomatic cases. This reduces the delay administrating antibiotic therapy. In this study we found significant correlation of CRP and blood culture positivity.

Source of funding: none

Conflicts of interest: none declared

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