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## DIAGNOSTIC ACCURACY OF C-REACTIVE PROTEIN IN NEONATAL SEPSIS

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### ABSTRACT

Sepsis is the most common cause of neonatal mortality and is responsible for 30-50% of total neonatal deaths each year in developing countries. The objective of the study was to determine the diagnostic accuracy of C-reactive protein (CRP) in neonatal sepsis. Fifty nine consecutive patients with risk factors and clinical features suggestive of CRP sepsis were selected as per operational definition and fulfilling the inclusion and exclusion criteria. Detailed physical examination was carried out. Blood sample for culture and CRP was taken from all the patients. Results of blood culture and CRP were noted down in the performa. Statistical analysis was performed by SPSS software version 16. Among selected patients 31/59 (52.5%) were male whereas 28/59 (47.5%) were female. Mean age of all patients was 15.47±7.26 days and mean weight was 2.94±0.63Kg. Temperature instability was present in 79.7%, Tachypnea in 69.5%, Tachycardia in 66.1%, delayed capillary refill in 64.4% and oliguria in 55.9%. Blood cultures were positive in 64.4% and raised CRP was found in 64.5%. Sensitivity, specificity, positive predictive value and negative predictive of raised CRP was found to be 97.3%, 95.2%, 97.3% and 95.2% respectively. In conclusion this study show that C-reactive protein has high sensitivity and specificity for establishing the diagnosis of neonatal sepsis which is comparable to that of blood culture results.

**Keywords:** Neonatal Sepsis, C reactive protein, blood culture

### INTRODUCTION

Neonatal sepsis is a clinical syndrome characterized by systemic signs of circulatory compromise. For example poor peripheral perfusion, pallor, hypotonia, poor responsiveness caused by invasion of the bloodstream by bacteria in the first month of life.<sup>1</sup> The incidence of neonatal sepsis is 5 per 1,000 live births, its prevalence is 39%<sup>2</sup> and mortality rate is 5%–20%.<sup>3</sup> In developing countries, the incidence reaches up to 22/1000 live births, and in Pakistan the perinatal mortality rate has been reported to be 60/1000.<sup>4</sup> The most important risk factors for neonatal sepsis are prematurity, low birth weight, invasive medical procedures and prolonged hospitalization. Neonatal sepsis presents in diverse ways. It may present with fever, poor feeding, abdominal distention, diarrhea, tachypnea, oliguria, tachycardia or bradycardia, hypotension, irritability, seizures, bulging

fontanelle or bleeding.<sup>5</sup> Definite diagnosis of neonatal sepsis depends on cultures of blood or other normally sterile body fluids. Neonatal blood cultures are found to be positive in 25-54%.<sup>2</sup> Other supportive diagnostic tests include total leukocyte count (TLC) and erythrocyte sedimentation rate (ESR). Measures of acute phase proteins, cytokines, cell surface antigens, and bacterial genomes have all been used alone or in combination to improve diagnosis of neonatal sepsis.<sup>6</sup>

Many studies have evaluated the usefulness of, procalcitonin (PCT), tumor necrosis factor alpha (TNF $\alpha$ ) and interleukins (IL-6, IL-8, and IL-10) in diagnosing sepsis. In clinical practice, CRP is the most accessible and widely used marker of infection.<sup>7</sup> CRP is an abnormal serum glycoprotein produced by the liver. It is a component of the innate immune system, an acute phase reactant, and increased levels are observed within 24 to 48 hours in response to severe bacterial infection<sup>8</sup>. CRP has

sensitivity of 97.2% and specificity of 95% in proven sepsis.<sup>9</sup> Although blood culture is gold standard for diagnosis of neonatal sepsis and allows targeted antimicrobial therapy, its result may be delayed for up to 48 hours and it may yield negative results in many cases of septic shock. Also, contamination rates are high due to the technical difficulty of obtaining a sterile sample from small babies. Considering the high mortality and potential morbidity of sepsis, it is important to start antibiotics without waiting for culture results. On the other hand, unnecessary antibiotics increase the risk of drug side effects and contribute to emergence of microbial resistance.

Measurement of CRP allows rapid identification of infected patients, does not require a sterile sample, and a normal value may help in early exclusion of infection. Serial measurements of CRP have a prognostic value, and show the effectiveness of antibiotic therapy.<sup>10</sup>

## MATERIALS AND METHODS

The objective of the study was to determine the diagnostic accuracy of CRP in neonatal sepsis. Following operational definitions explain specified terms used in this study. Neonate is newborn from first day of life to 28<sup>th</sup> day of life. Neonatal sepsis is a clinical syndrome characterized by systemic signs of circulatory compromise (e.g. poor peripheral perfusion, pallor, hypotonia, poor responsiveness) caused by invasion of the bloodstream by bacteria in the first month of life.<sup>1</sup>

This is diagnosed clinically on the presence of two or more of the following clinical features: temperature instability (<35°C or >38°C), poor skin perfusion (capillary refill >3 seconds), tachypnea (respiratory rate >60/min at rest), tachycardia (>150 beats / min at rest) and oliguria (urine output <0.5 ml/ kg/ hr). The diagnosis was confirmed as neonatal sepsis positive or refuted as neonatal sepsis negative on the basis of blood culture results

sent at the time of admission. Raised C reactive protein was defined as level of C reactive protein more than 6 mg/L in serum. Diagnostic accuracy of C- Reactive Protein taking blood culture as gold standard, accuracy was measured in terms of sensitivity and specificity, which was calculated by using True Positive (TP): If C reactive protein raised and blood culture comes positive, True Negative (TN): If C reactive protein not raised with negative blood culture result, False Positive (FP): If C reactive protein raised while the blood culture result comes negative, False Negative (FN): If C reactive protein is not raised but blood culture comes positive.

All patients (n=59) admitted in paediatric unit of Nishtar Hospital, Multan from 20-12-2011 to 20-6-2012 were included in this study. Sample size was calculated by using World Health Organization (WHO) sample size calculator and non probability consecutive sampling technique was used, cross sectional study design was adopted.

Expected sensitivity of CRP = 97.2%<sup>8</sup>; expected specificity of CRP = 95%<sup>8</sup>; expected prevalence of neonatal sepsis = 39%<sup>2</sup>; precision level 7% and confidence level 95%. Inclusion criteria was: All patients aged 0 to 28 days, patients of both genders, patients with two or more of the following clinical features: temperature instability (<35°C or >38°C), poor skin perfusion (capillary refill >3 seconds), tachypnea (respiratory rate >60/min at rest), tachycardia (>150 beats / min at rest), oliguria (urine output <0.5 ml/ kg/ hr).

Exclusion criteria was: neonates who have received antibiotics before admission, neonates with major systemic malformation, multiple congenital anomalies, underlying surgical conditions, weight less than 1000 grams. Permission was taken from the Hospital ethical committee. All patients having risk factors and clinical features suggestive of sepsis were selected as per operational definition and fulfilling the inclusion and exclusion criteria. Informed consent was taken from the parents of the neonates. A detailed history was taken and important aspects were noted like age, gender,

weight, place of delivery, gestational age, duration of symptoms and history of antibiotic administration in the primary care. Detailed physical examination of the neonate was also carried out. Weight, temperature, heart rate, respiratory rate, skin perfusion, and urine output were noted in a specifically designed performa.

The blood sample for culture and CRP was taken in all the patients at admission and sent to the Central Laboratory of Nishtar Hospital in appropriate containers. For blood culture, 2 ml blood was taken with full aseptic measures and inoculated in blood culture bottle containing brain heart infusion (BHI). For CRP estimation, 1 ml blood was taken in a 3 mL syringe. CRP was carried out by latex agglutination assay in the laboratory and a value more than 6 mg/L was considered as raised. Results of blood culture (positive/negative) and CRP (raised/not raised) were noted down in the Performa.

All the data was analyzed by SPSS, version 16. Mean and standard deviation were calculated for quantitative variables like age and weight, frequency and percentages were calculated for all qualitative variables i.e. gender, blood culture showing neonatal sepsis, raised CRP level. Confounding factors like age, gender and weight of child were controlled by stratification. Chi-square test was applied to see the effect of this on outcome. P-value < 0.05 was controlled by stratification. Blood culture result was taken as gold standard for diagnosis of neonatal sepsis. True positives, true negatives, false positive and false negative values were calculated as mentioned in the operational definitions. Sensitivity, specificity, positive predictive value and negative predictive value for CRP was calculated using following formula:

Sensitivity =  $TP / (TP + FN) \times 100$ ,  
 Specificity =  $TN / (TN + FP) \times 100$ ,  
 Positive predictive value =  $TP / (TP + FP)$ ,  
 Negative predictive value =  $TN / (TN + FN)$

## RESULTS AND DISCUSSION

Deterioration in the condition of a neonate can occur in many conditions. It is always difficult to establish a definite cause of deterioration. Of various causes bacterial infection is usually at the top. The gold standard of identifying bacterial infections in blood culture has got a low yield. Therefore the paediatricians suggest certain surrogate tests to identify neonatal sepsis. This study was performed with an aim to assess the usefulness of CRP as an indicator of neonatal sepsis.

As it is a well-known fact that sepsis is a medical emergency and failure to diagnose will be lethal and to initiate antibiotic therapy in non-septic neonate will be a financial loss to the family. Thus it is important to use a screening test that it ideally recognizes all infected neonates (high sensitive) so that disease can be excluded with negative results (high negative predictive value). Non-infectious conditions by giving positive results reduce the specificity and positive predictive value. But the loss of specificity and positive predictive value is acceptable when cost of treatment is much less than missing an infected case with potentially life threatening infection.

Since late 70's and early 80's various screening methods are being used for rapid diagnosis of neonatal sepsis. The most commonly used methods are micro ESR, CRP, absolute neutrophil count, band neutrophil ratio etc. Recent studies have used some newer techniques like nitro blue tetrazolium (NBT) acridine orange test. In our study there were 59 patients in total. Males were slightly more (52.5%) than the females (47.5%) elaborating the fact that male sex has a predisposition for neonatal sepsis.<sup>10</sup> Mean age of the patients was  $15.47 \pm 7.26$  days and mean weight of the patients was  $2.94 \pm 0.63$  kg which are comparable to those observed in other studies<sup>11</sup> (Table 1).

**Table 1: Quantitative variables in the study population**

Quantitative Variable	Mean + Standard deviation
Age (days)	15.47 ± 7.26
Weight (Kg)	2.94 ± 0.63

Temperature instability with pyrexia or hypothermia was the most frequently noted symptom in patients suspected to have neonatal sepsis and was present in as many as 79.70% of the patients. Tachypnea was the second most frequently encountered symptom seen in 69.50% of the patients followed by tachycardia observed in 66.10% of the patients. Capillary refill was found to be delayed in 64.40% of the patients and the least encountered presentation was with decreased urine output observed in 55.90% of the patients. Blood culture results and CRP were found to be elevated in 64.40% of the cases.

Despite having the same percentage when the relationship of raised CRP levels with blood culture results was evaluated further by cross tabulation, it was found that among 38 patients who had raised CRP levels, 37 (97.30%) patients were blood culture positive and thus were true positive (TP) whereas 1 (2.6%) had a negative blood culture and thus was false positive (FP). When the relationship of negative CRP was evaluated against blood culture results, it was noted that among 21 patients with normal CRP value 20 (95.2%) had negative blood culture results and thus were true negative (TN), either due to patient presentation to tertiary hospital after taking broad spectrum antibiotics or systemic inflammatory response syndrome (SIRS). Whereas 1 (4.7%) had positive blood cultures and were false negatives (FN). Sensitivity of CRP (TP/TP+ve FN) was found out to be 97.3% whereas specificity (TN/TN+ve FP) of the test was found out to be 95.2 %. Positive predictive value (TP/all CRP+ve cases) was found out to be 97.3% whereas negative predictive value was found out

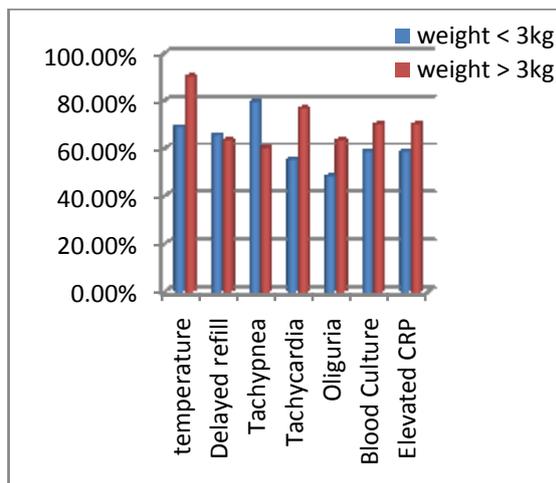
to be (TN/all CRP–ve cases) 95.2%.

These results suggested that the sensitivity of the CRP is slightly higher than the specificity. These results are in line with other studies which have shown a high sensitivity, specificity, positive predictive value and negative predictive value of CRP when compared against blood culture results. In one series of 76 newborns suspected to have neonatal sepsis<sup>12</sup>, sensitivity of the test was found to be 100%, specificity of 94%, positive predictive value of 91.6% and a negative predictive value of 100%. The slight difference observation in our study may probably be attributable to a smaller sample size due to limitation of our local resources.

When the effect of weight was noted on the various characteristics, it was found that in weight group < 3 kg there were more females (55.17%) than males (44.9%) whereas in those with weight ≥ 3kg there were more males (60%) than females (40%). This suggests the fact that in patients with a lower birth weight females are more prone to develop neonatal sepsis whereas in those with normal birth weight male gender is a risk factor for development of neonatal sepsis. Similar results were observed in neonates with low birth weight.<sup>13</sup>

Temperature instability was found in 90% of patients in age group ≥ 3kg as compared to only 68.9% of patients in weight group < 3kg and the difference was statistically significant (p-value 0.045). This suggests the fact that neonatal sepsis in low birth weight babies may not manifest essentially with pyrexia due to not fully mature hypothalamic temperature control mechanisms and sepsis should be suspected in neonates in the absence of pyrexia if other features suggestive of sepsis are present, this especially holds true in patients with a low birth weight. In weight group < 3 kg capillary refill was delayed in 65.5% patients as compared to 63.34% patients weighing ≥ 3kg, but the difference was statistically non significant (p-value=0.861). In patients weighing < 3kg tachypnea was present in 79.3% patients whereas in those with weight ≥ 3kg tachypnea was identified in 60% patients, again the

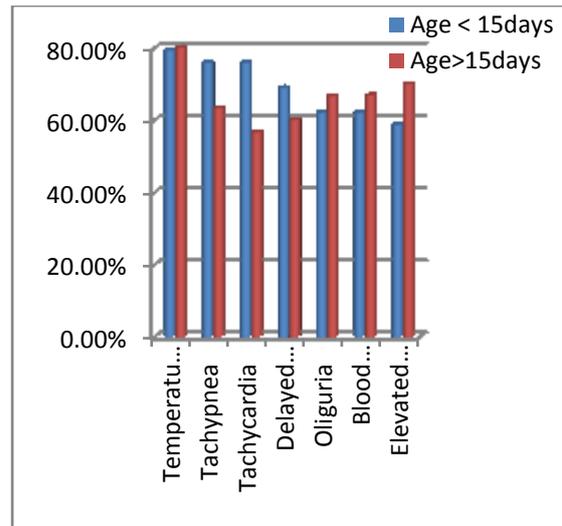
difference was statistically non significant (p-value = 0.107). Tachycardia was recorded more frequently in age group > 3kg as compared to those with weight < 3kg with a statistically non-significant difference (p-value=0.81). In those with weight <3kg oliguria was present in 48.2% patients whereas in those with weight ≥ 3kg it was present in 63.3% patients with a p-value=0.244. Blood cultures were found to be positive more frequently in those with age ≥ 3kg (70%) patients as compared to those with weight < 3kg (58.6%), however the difference was statistically non significant (p-value=0.361). Similarly CRP was found to be elevated more in age group > 3kg (70%) as compared to 58.6% in those with weight < 3kg with a statistically non-significant difference (p-value=0.361) (Fig 1).



**Fig 1. Effect of weight on various characteristics in the patient population.**

When the effect of age was noted on the various characteristics of study population it was noted that in age group ≤15 days there were more females (44.8%) than males (55.17%) while in age group > 15 days there were almost equal number of males and females (50%). Temperature instability was found almost equally in both age groups (80% for age group > 15 days as compared to 79.3% for age group ≤ 15 days) (p-value=0.948). Tachypnea, tachycardia, delayed capillary refill and oliguria were present more frequently in age group ≤ 15 days (75.8%, 75.8%, 68.9% & 62% respectively) as compared to those in age > 15 days (63.3%, 56%, 60% &

50% respectively). But all of these had p-value > 0.05 and thus were clinically insignificant. Blood cultures and CRP turned out to be more positive in age group > 15 days (66.6% & 70% respectively) as compared to those in age group < 15 days (62% & 58.6%) but again the difference was not statistically significant as the p value was more than 0.05 (Fig 2).



**Fig 2. Effect of age on various characteristics in the study population.**

## CONCLUSION

C-reactive protein has high sensitivity and specificity for establishing the diagnosis of neonatal sepsis which is comparable to that of blood culture results. With the added benefit of early test result availability, it is highly recommendable that it should be used routinely in the evaluation of neonates with any features suggestive of sepsis to reliably include or exclude the diagnosis of neonatal sepsis.

Although proinflammatory cytokines (IL-2, IL-6, interferon gamma, and tissue necrotic factor alpha) and anti-inflammatory cytokines (IL-4 and IL-10) are increased in infected infants compared to those without infections. However, these cytokines are not routinely measured because of their high cost of testing and because no single biomarker or panel of tests is sufficiently sensitive to reliably detect neonatal sepsis.<sup>14</sup>

Several observational studies have also suggested that procalcitonin may be a useful marker to detect serious bacterial infections in young febrile infants.<sup>15</sup> Limited data in preterm infants report that elevated procalcitonin (greater than 0.5 ng/mL) is equivalent or better than CRP in detecting bacterial infection. Although procalcitonin is a promising marker, it appears not to be sufficiently reliable as the sole or main diagnostic indicator for neonatal sepsis, and, at this time, it is not routinely available in hospital laboratories. Further research, which better understands the neonatal inflammatory response to sepsis, may result in the identification of sensitive and specific markers of inflammation or the development of pathogen-specific rapid diagnostic tests for early detection of neonatal sepsis.

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