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Diagnostic Accuracy of Combination of Total Leukocyte Count and CRP for Diagnosis of Early Onset Neonatal Sepsis using Blood Culture as Gold Standard

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ABSTRACT

Objective: Using blood culture as the gold standard, ascertain the diagnostic accuracy of the total leukocyte count and CRP combination for the diagnosis of early-onset newborn sepsis. **Study design and Settings:** A cross-sectional validation study was conducted at the department of Pediatrics, Allied Hospital 1, Faisalabad, for the duration of six months, from April 2024 to September 2024. **Materials and Procedures:** Participants in this study included 225 patients who met the selection criteria. Following aseptic procedures, a sterile 5-milliliter syringe was used to draw 5 milliliters of intravenous blood, which was then sent to the hospital's pathology lab for pathologist investigation. Blood was cultured for 24 hours at 37°C in a bottle of brain-heart infusion broth. Blood and MacConkey agar were used for the subculture, which was then aerobically incubated for the entire night at 37°C. In compliance with predetermined standards, organisms were identified. According to operational guidelines, a positive blood culture was regarded as the gold standard for detecting early-onset sepsis. C-reactive protein levels and total leukocyte count were combined to determine early-onset newborn sepsis. **Results:** Using blood culture results as the gold standard, the combined TLC and CRP for the diagnosis of early onset neonatal sepsis showed sensitivity of 92.04%, specificity 94.64%, PPV 94.55%, NPV 92.18%, and accuracy of 93.33%, respectively. **Conclusion:** The combination of total leukocyte count and CRP serves as an effective measure to avoid antibiotic abuse, hence inhibiting the colonization of drug-resistant microbiota in newborns.

INTRODUCTION

Infections are the primary cause of illness in newborns, with neonatal sepsis consistently emerging as a significant factor contributing to both morbidity and mortality.[1] Neonatal septicemia denotes a systemic infection in babies within the initial 28 days of life. Sepsis is a condition characterized by systemic symptoms and heightened host immune responses resulting from an infectious pathogen that has infiltrated the bloodstream.[2] Approximately 130 million babies are born each year worldwide, and 4 million of them die before they are a month old. The neonatal death rate in Pakistan is approximately 49 per 1,000 live births. Sepsis constitutes 36% of fatalities in neonates. The prevalence of newborn sepsis in Pakistan is 29.25%.[3] Neonatal sepsis continues to be a critical and life-threatening condition in neonates globally, although advancements in neonatology. Previously released statistics indicated that neonatal sepsis accounted for 15.2% of newborn fatalities globally and is a significant contributor to infant mortality. The definitive method for diagnosing newborn sepsis is blood culture.[4] Blood culture presents multiple problems, including prolonged laboratory turnaround time, insufficient blood volume, and pre-hospital antibiotic treatment. The liver produces the acute-phase protein known as C-reactive protein (CRP), which is closely related to systemic inflammation. One of the most researched and widely used laboratory tests for detecting neonatal sepsis was the measurement of CRP, with higher CRP levels being associated with an increased risk of sepsis.[5] Nonetheless, the white blood cell count (WBC) is a commonly used diagnostic test to assess sepsis, and a number of leukocyte count components have been studied for their potential predictive value in sepsis diagnosis. Previously believed to be accurate markers

of infection, leukopenia and leukocytosis—characterized by a white blood cell count of less than 5,000/mm3 or more than 20,000/mm3, respectively—are now recognized as insensitive and unclear markers.[6] Total leukocyte counts (WBC) and C-Reactive Protein (CRP) are commonly used to diagnose neonatal sepsis; however, a single test of either WBC or CRP is not enough to definitively confirm or rule out sepsis.[7] The combination of CRP and WBC was found to have a 78.5% sensitivity and an 83% specificity for screening for newborn sepsis. They found neonatal sepsis in 29.4% of cases.[6]

The objective of the current experiment is to evaluate the efficacy of WBC and CRP coupled as a diagnostic tool for neonatal infections. The objective is to identify a rapid, efficient, feasible, and logistically viable test for newborn sepsis suitable for laboratories with limited resources in developing countries such as Pakistan. Timely identification and intervention for sepsis in neonates might mitigate the disease's repercussions by enabling prompt empirical treatment and surveillance.

MATERIALS AND METHODS

The Department of Pediatrics at Allied Hospital 1, Faisalabad conducted this cross-sectional validation study from April 2024 to September 2024. The WHO calculator determined a sample size of 225 cases, based on a sensitivity of 78.5%, specificity of 83%, prevalence of 29.4%, a confidence level of 95%, and an absolute precision of 10%. Newborns of both sexes aged ≤ 6 days exhibiting two of the following three characteristics were included in this study: Hypothermia (below 36°C) or Hyperthermia (over 38.5°C), Respiratory rate exceeding 60 breaths per minute, Heart rate exceeding 190 beats per minute at rest. All neonates born before 32 weeks of pregnancy, those weighing less than 1000 grams at birth, full-term neonates who had previously been treated with antibiotics, and those with a diagnosis of hyaline membrane disease and transient neonatal tachypnea were excluded.

After the ethics committee gave its approval, patients who met the study's selection requirements were enrolled, and each participant gave their informed consent. Following aseptic procedures, a 5 mL syringe was used to draw 5 mL of intravenous blood, which was then sent to the hospital's pathology lab for pathologist investigation. The distribution looked like this: A qualitative method of C-reactive protein by slide latex agglutination and a three-part automated hematology analyzer (Councell-23 Plus) were used to calculate the total blood count. It also included one milliliter of ethylenediamine tetra-acetic acid (EDTA) and three milliliters of brain heart infusion broth. A 24-hour culture was conducted on blood in a bottle of brain-heart infusion broth at 37°C. The subculture was cultivated aerobically for the entire night at 37°C with the use of blood and MacConkey agar. Organisms met the set criteria for recognition. A positive blood culture was the most effective method of detecting early-onset sepsis, according to the operational criteria. The operational criteria required the use of both C-reactive protein (CRP) and total leukocyte count (TLC) to detect early onset neonatal sepsis. All of the data was collected and entered into the pre-structured form that is attached.

SPSS version 25 was used to enter and analyze all of the data. Age, weight, TLC count, and CRP were among the quantitative variables for which the mean and standard deviation were calculated. For both gender and true positives, frequency and percentage were calculated. A 2x2 contingency table was created in order to calculate the following metrics: sensitivity, specificity, negative predictive value, positive predictive value, and accuracy.

		Blood culture	
Combination of TLC and CRP	Yes	Yes	No
		A (TP)	B (FP)
	No	C (FN)	D (TN)

The likelihood ratio was determined. Using stratification, effect modifiers such as gender, age, and weight were managed. The accuracy of post-stratification diagnosis was computed. A P-value of less than 0.05 was considered significant.

RESULTS

The average age of the participants in the study was 3.59 ± 1.41 years. There were 44.44% females and 55.56% men, giving a male to female ratio of 1.25:1. A mean weight of 2238.63 ± 156.20 kg was recorded. Of the individuals who tested positive for both TLC and CRP, 104 had true positive results and 06 had false positive results. Nine were false negatives and 106 were real negatives out of the 115 patients who had both TLC and CRP negative results. Overall sensitivity, specificity, PPV, NPV, and accuracy of TLC and CRP combination for early onset neonatal sepsis diagnosis were 92.04%, 94.64%, 94.55%, 92.18%, and 93.33%, respectively, using blood culture results as the gold standard. ROC curve is shown in Figure 1.

Table 1

Diagnostic Accuracy of Combination of Total Leukocyte Count and CRP for Diagnosis of Early Onset Neonatal Sepsis.

	Positive result on blood culture	Negative result on blood culture	P-value
Positive result on TLC and CRP	104	06	0.0001
Negative result on TLC and CRP	09	106	

Sensitivity: 92.04%, **Specificity:** 94.64%, **Positive Predictive Value (PPV):** 94.55%, **Negative Predictive Value (NPV):** 92.18% **Liklihood ratio for positive test result:** 17.18, **Liklihood ratio for negative test result:** 0.08, **Diagnostic Accuracy:** 93.33%

Figure 1

ROC curve

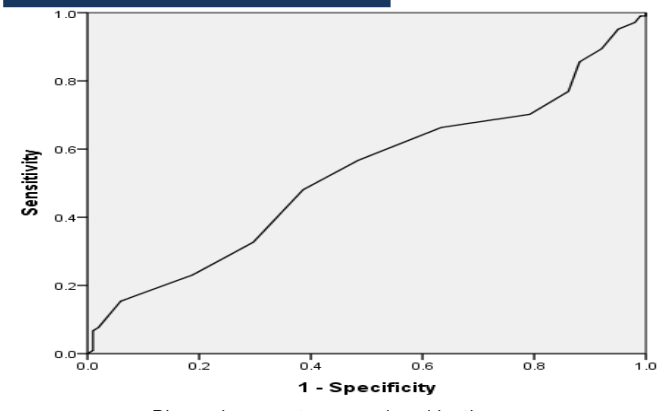

Table 2

Stratification of Diagnostic Accuracy with Respect to Age, Gender and Weight

Variables		Sensitivity	Specificity	PPV	NPV	Accuracy	P-value
Age (days)	≤3	90.0%	95.45%	96.43%	87.50%	92.31%	0.001
	4-6	94.34%	94.12%	92.59%	95.52%	94.21%	0.001
Gender	Male	89.39%	91.53%	92.19%	88.52%	90.40%	0.001
	Female	95.74%	98.11%	97.83%	96.30%	97.0%	0.001
Weight (gms)	≤2500	92.42%	93.85%	93.85%	92.42%	93.13%	0.001
	>2500	91.49%	95.74%	95.56%	91.84%	93.62%	0.001

DISCUSSION

The prevalence of newborn sepsis in underdeveloped nations such as Pakistan exceeds that in affluent countries. According to a study conducted at Lady Reading Hospital in Peshawar, 115 cases of blood culture-confirmed baby sepsis over a 10-month period were found, with early-onset sepsis accounting for 29.57% and late-onset sepsis for 70.43%.[8] C-reactive protein has an 84% sensitivity and a 65% specificity in identifying newborn sepsis.[9] According to a meta-analysis, CRP has a sensitivity of 69% and specificity of 77% for diagnosis.[10]

The combined TLC and CRP sensitivity, specificity, PPV, NPV, and accuracy for the diagnosis of early onset newborn sepsis, using blood culture data as the gold standard, were 92.04%, 94.64%, 94.55%, 92.18%, and 93.33%, respectively. The combination of CRP and WBC was found to have a 78.5% sensitivity and an 83% specificity for screening for newborn sepsis. 29.4 percent of cases had neonatal sepsis.[6] The specificity, sensitivity, PPV, and NPV of PC, TLC, and CRP in combination were 92.5%, 75.3%, 94.6%, and 67.9%, respectively.[11]

C-reactive protein (CRP), a recognized indicator of inflammation, demonstrated a sensitivity of 92.04%, consistent with results from analogous research.[12,13] Nonetheless, its specificity in this study was diminished (51%), corroborating studies that suggests CRP alone may be inadequate for a conclusive sepsis diagnosis.[14] This highlights the necessity of utilizing CRP alongside other biomarkers to enhance diagnostic precision.[15]

In a study[16], the mean age of the newborns was 5.72 days ± 3.86. Among the patients, 55.1% were male and 44.9% were female. Blood culture confirmed newborn sepsis in 43 neonates (29.25%), while 104 neonates (70.75%) tested negative for the disease. In the identification of acute neonatal sepsis, CRP exhibited a sensitivity of 76.92% and a specificity of 53.49%. The negative predictive value was 48.94%, whereas the positive predictive value was 80%. The total diagnostic accuracy of CRP for diagnosing newborn sepsis was 70.07%.[16]

While Shabbir et al.[18] found that C-reactive protein had sensitivity of 74% and specificity of 76%, Ahmed Z et al.[17] showed that CRP's sensitivity, specificity, PPV, and NPV were 85.5%, 95.0%, 82.7%, and 95.9%, respectively. According to Khashabi J et al.[19], C-reactive protein (CRP) can be a useful marker for deciding when to stop antibiotic treatment, allowing for early discharge and significantly lowering expenses, treatment issues, antibiotic abuse, and family anxiety.

The total leukocyte count (TLC) demonstrated moderate diagnostic utility, exhibiting a sensitivity of 62% and a specificity of 76%, consistent with previous research indicating comparable moderate diagnostic value.[20,21] The dependability of TLC as a singular indicator has been challenged in other research, as leukopenia or leukocytosis may arise from diverse non-septic reasons.[22] In the current study, as well as in previous research conducted by Singh et al., Karthikeyan et al., and Ahmed et al., it was observed that CRP levels are elevated in many non-infectious circumstances, including premature rupture of membranes, maternal fever, fetal distress, complicated delivery, and perinatal asphyxia.[23]

CRP is a delayed diagnostic measure with a restricted function in the preliminary choice to initiate antibiotic therapy. CRP serves as a valid negative predictor for sepsis; however, its specificity in diagnosing newborn sepsis is limited. CRP, together with early-elevated indicators such as WBC indices throughout the illness progression, is beneficial for excluding sepsis.[24] Specificity, positive predictive value, and negative predictive value are all improved by combining the CRP and WBC count cut-off values.[25] Increased WBC counts and positive CRP results have improved the tests' sensitivity and specificity from 66.14% to 81%. According to Abebe Sorsa[6], combining two abnormal readings produced a 78% sensitivity and 83% specificity for sepsis screening, which is consistent with our results. White blood cell (WBC) count and C-reactive protein (CRP) are accessible and reasonably priced metrics in all clinical settings. In standard clinical practice, the combination of WBC counts and CRP levels functions as a triage tool in conjunction with clinical history.[25]

CONCLUSION

Early detection of newborn sepsis is essential for reducing the condition's related neonatal mortality and morbidity. The combination of CRP levels, WBC count, and clinical history can be a valuable indicator for diagnosis in culture-negative neonatal sepsis. By preventing antibiotic overuse, the combination effectively prevents drug-resistant microbiota from colonizing infants.

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