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Research Article

Blood culture

Correlation of Blood culture with C-Reactive Protein, WBC count in the diagnoses of Bacterial Neonatal Sepsis

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Introduction: The clinical diagnosis of sepsis in neonates is challenging. White blood cell counts, C reactive protein (CRP) are the widely used biomarkers in neonatal sepsis screening by clinicians. **Aim:** To assess the value of C-reactive protein alone and in combination with total White Blood Cell (WBC) count in the screening of neonatal sepsis. **Materials and Methods:** The Retrospective data regarding neonatal sepsis, collected from March 2018 to November 2018 from NICU, R L Jalappa Hospital& Research Centre, attached to Sri Devaraj Urs Medical College, Kolar, Karnataka, India. Statistical software, SPSS version 22.0, was used for further analysis. Statistical analysis was performed using the Chi-square tests. Cut-offs were defined by plotting receiver operator characteristic curves. **Results:** A total of 127 neonatal sepsis cases were analyzed. Proven sepsis cases by blood culture were positive in 28 (22.04 %) neonates. CRP was positive in 20 (71.42%) blood culture-positive cases. Culture negative sepsis was seen in 99 cases. WBC had a sensitivity and specificity of 21.43% and 78.79%. The sensitivity, specificity of CRP were 71.4%, 69.9%. On the combination of positive CRP levels with WBC counts, sensitivity and specificity raised to 78.57% and 81.81%. **Conclusion:** In resource-limited settings for blood cultures, the positive CRP assay in combination with increased WBC counts in neonates with clinical features of sepsis should be considered for immediate management of neonatal sepsis to reduce morbidity and mortality.

Keywords: Neonatal sepsis, CRP, WBC count.

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Introduction

Annually, more than one million neonates die due to sepsis[1]. India accounts for 17,000 neonatal sepsis cases / 1,00,000 live births [2]. Despite recent advances in the medical field, the prevention of neonatal deaths due to sepsis is challenging. The clinical presentation of sepsis is subtle and varied, sometimes mimicking non-infectious causes [3].There is no consensus case definition for neonatal sepsis [4]. Blood culture is a standard test for naming the causative agent of sepsis. The culture reports would be positive in only 25 % to 45% of cases, resulting in low yield following antenatal antibiotic exposure [5]. Delay in culture reports leads to inappropriate usage of antibiotics among the neonates suspected of sepsis [6]. On the other hand, early diagnosis is the key to the prevents neonatal mortality due to sepsis. Culture-independent diagnostics are the need of the hour to have a better clinical outcome to avoid the emergence of antibiotic resistance[6].For diagnosis of neonatal sepsis, to guide on the antibiotic administration, identification of biomarkers is essential [7]. CRP, PCT, IL-6, IL-8, IFN-g, TNF-a, CD64, and s ICAM are the predominant biomarkers known for diagnosing neonatal sepsis. [8]. Important factors in determining the suitability of a diagnostic marker for clinical application are cost, availability of specimens at the appropriate time, the complexity of the assay methods, laboratory turnover time, reliability of the tests [9]. None of the biomarkers has sufficient sensitivity or specificity to employ in routine clinical practice [10]. The laboratory parameters, white blood cell count, and CRP are widely employed in routine clinical practice by clinicians. On combining biomarkers with clinical assessment increases the possibility of correct diagnosis and prompt initiate of antimicrobial therapy [11]. This study is undertaken to know the diagnostic value of WBC count, CRP levels, and its combination in the management of neonatal sepsis.

Materials & Methods

Study design & Setting: The Retrospective data regarding neonatal sepsis, collected from March 2018 to November 2018 from NICU, R L Jalappa Hospital& Research centre, attached to Sri Devaraj Urs Medical College, Kolar, Karnataka, India.

Inclusion Criteria

Newborn babies with the clinical diagnosis of sepsis based on two of the risk factors and clinical features of bacterial infections based on WHO-AIIMS 2014 [8] protocol

-Birth weight < 2.2 kgs

-Gestational age < 36 weeks

-Foul-smelling / meconium-stained vaginal discharge

- Presence of maternal fever within two weeks before delivery

-Suspected chorioamnionitis

-Rupture of membranes >18 hours

-Prolonged labour

-Perinatal asphyxia (Apgar score < 4 at 1 minute)

-Clinical signs of sepsis – poor reflexes, lethargy, respiratory distress, bradycardia, apnea, fever, convulsions, abdominal distention, bleeding.

Exclusion Criteria: Neonates referred in from other health care setups. Neonatal sepsis data collection, based on the AIIMS protocol.

Laboratory data: From the Microbiology section, the Laboratory data of Blood culture, CRP were collected from neonates with clinical suspicion of sepsis.

Blood culture done by automated blood culture system, CRP by latex agglutination method using a kit by Arkary Healthcare Pvt Ltd. WBC count done by Sysmex 500.

Total WBC count </ = 5,000 /mm3or >/= 20,000 / mm3 and C-reactive protein 6 mg / L or more was considered for correlation with the blood culture reports.

Statistics: Statistical software, SPSS version 22.0, was used for further analysis. Sensitivity, Specificity, Positive predictive value, Negative predictive value were used to determine the validity, reliability of CRP, WBC taking blood culture as the gold standard. Summaries of measures were presented as tables, figures, and percentages. Comparisons between categorical data were conducted with Chi-square test The combination of CRP, WBC counts with the blood culture was analyzed by the receiver–operator characteristic curve (ROC).

Results

One hundred twenty-seven neonates with clinical suspicion of sepsis were considered for the study. Among the 127 neonates, blood culture-proven cases were 28 (22.04 %), while 99 (77.9%) had negative blood culture. CRP was positive in 20 (71.42%) blood culture-positive cases. Out of 99 culture-negative cases, WBC changes were noted with 30 (30.30%) cases **(Table 1).**

On the other hand, sensitivity and specificity of WBC were 21.43% and 78.79%, with PPV and NPV of 22.22% and 78.5%, respectively. When CRP was combined with WBC, the sensitivity and specificity raised to 78.57% and 81.81%, respectively.

The sensitivity, specificity of CRP were 71.4%, 69.9%, respectively, with a positive predictive value (PPV) of 36.6% and negative predictive value (NPV) of 88.89%.

Table 1: Comparison of CRP, Total WBC count using blood culture as the gold standard.

Parameter	Blood culture positive 28	Blood culture-negative 99	Total 127
CRP	20	35	55
WBC	8	30	38

Figure 1. Receiver operating characteristics (ROC) of a combination of two tests (Total CRP & WBC count) and blood culture show the performance in detecting neonatal sepsis. A variety of two tests (Total CRP & WBC count) has a sensitivity of 78.57%, specificity of 81.81%.

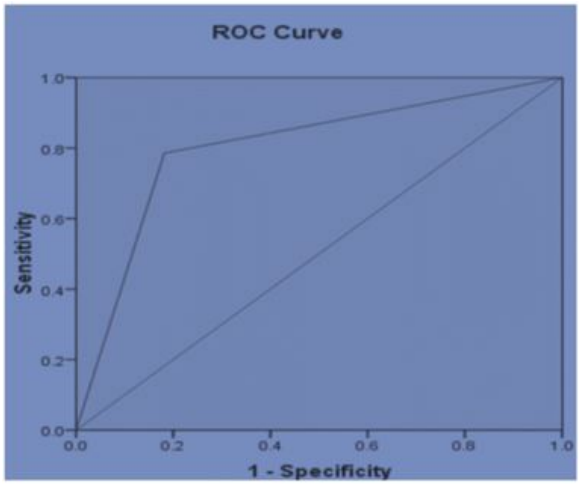

Table 2: Sensitivity, specificity, positive predictive value (PPV), Negative predictive value (NPV), Area under the curve (AUC) of laboratory markers.

Parameter	Sensitivity	Specificity	PPV	NPV	p value	Are under curve (AUC)
CRP	71.4%	69.9%	36.6%	88.89 %	0.001	0.626
WBC	21.43%	78.79%	22.22 %	78%	0.79	0.578
CRP +WBC	78.57%	81.81%	55%	93.10 %	0.000	0.802

Discussion

Neonatal sepsis defines the systemic condition associated with hemodynamic changes due to either bacterial, viral or fungal agents [12]. The highly variable, non -specific nature of the signs and symptoms of sepsis makes the diagnosis of sepsis difficult [10].

Based on the onset, neonatal sepsis is classified as early-onset (0-6 days) or late-onset (7-90 days). [13]. Early-onset sepsis, due to the vertical acquisition of the microorganisms from mother [13]. Chorioamnionitis is the risk factor for early-onset sepsis. Late-onset sepsis is due to horizontal transmission from individuals responsible for neonatal care or environmental sources [12].

Neonatal sepsis can present with groaning, contraction of the accessory muscles of respiration, nasal wing breathing, apnea, cyanosis, tachypnea in the respiratory system; bradycardia/tachycardia, peripheral circulatory disturbance, hypotension, prolonged capillary refill time in the cardiovascular system; nutritional intolerance, difficulty sucking, vomiting, diarrhea, abdominal distention, hepato-splenomegaly, jaundice in the digestive system; sclerema, cutis marmaratus, pustule, abscess, petechiae, purpura in the skin; and lethargy, hypotonicity, sleepiness, weak or high-pitched crying, bulging fontanelle, irritability, convulsion, hypoactivity, body temperature regulation problems and difficulty sucking in the central nervous system [14].

Bacteria such as *Streptococcus, L. monocytogenes, E. faecalis, E. faecium*, group D *Streptococci*, α-hemolytic *Streptococci*, and *Staphylococci, S. pneumonia, H. influenzae* type B are common causative agents of early-onset sepsis. *E.coli*, *Staphylococcus aureus,*

Is a known agent to cause late-onset sepsis [15].

Early diagnosis of sepsis in neonates is crucial to prevent mortality and morbidity [16]. In the management of neonatal sepsis, clinical evaluation in correlation with the laboratory parameters impacts the appropriate usage of antimicrobial agents [17]. Blood culture is considered the standard in the clinical diagnosis of neonatal sepsis [18]. Worldwide records show that the isolation rates on blood cultures vary from 6.7% to 55.4% [19]. In our study, blood culture-proven neonatal sepsis cases were 28 (22.04 %). The study reports, 26.2% of blood cultures have proven neonatal sepsis [20]. Blood culture-proven neonatal sepsis cases are low as there is difficulty in drawing a sufficient amount of blood for culture, cost factor, in the availability of blood culture facilities in remote areas [21]. Infants with culture-negative sepsis are treated with more antibiotics [6]. Excessive usage of antibiotics may disturb the early life microbiota leading to colonization with resistant bacteria [6].

In this scenario, biomarkers in combination with clinical features are helpful in the management of sepsis [6]. Biomarkers like WBC count, CRP, Procalcitonin has widely used biomarkers. An increase or decrease in the WBC count is extensively evaluated concerning neonatal sepsis [6]. Increased band form neutrophils, represented by the high immature-to-total neutrophil ratio (IT-ratio), are often suggested to indicate sepsis [6].

In our study, in 8 positive blood cultures cases, high WBC counts were noted. WBC counts with blood culture as the gold standard test shows specificity 78.79% and sensitivity 21.43% with PPV 22.22%, NPV 78%. Our study is in concordance with a similar survey done by Tushar Priyanka [22]. Large intra- and inter-laboratory variations in interpretation limit its use [6]. C reactive protein is an acute-phase protein synthesized in the liver [12]. Cytokines like IL-6 [12] upregulate its secretion. It binds to the phospholipid component of microorganisms and facilitates their removal by macrophages [23]. Compared to other acute-phase reactants, CRP rises more significantly during acute inflammation [23]. It is half-life is between 24 -48 hours. Serial CRP measurements have been shown to increase the sensitivity in the diagnosis of sepsis within 24 -48 hours of symptoms onset [24]. We observed a rise in CRP levels in 20 out of 28 blood culture-positive cases in our study. Considering Blood culture as the gold standard, sensitivity and specificity of CRP was 71.4% and 69.9%, respectively. Our study is in correlation with a similar study done by Abebe Sorsa [25].

CRP is also raised in other non-infectious conditions like premature rupture of the membranes, maternal fever, fetal - distress, difficult birth, and perinatal asphyxia [12]. CRP is a late-rising diagnostic marker that has a limited role in the initial decision to start an antibiotic [26]. CRP is valid as a negative predictor of sepsis, but the specificity in the diagnosis of neonatal sepsis is low [12].

CRP, in combination with the early-rising biomarkers in the infection course like WBC indices, are helpful to rule out sepsis [26]. Combining the cut-off values of CRP and WBC count increases the specificity, positive predictive value, negative predictive value [27]. On combination of the increased WBC counts and positive CRP values, the sensitivity and specificity of the tests have risen to 81% from 66.14%. Abebe Sorsa [25] reports that on the combination of two abnormal parameters, the sepsis screening yielded a sensitivity of 78%, specificity of 83%, which is in concordance with our study. CRP, WBC count, is affordable and available parameters in all clinical settings. The combination of CRP levels, WBC counts are used as a triage instrument in combination with clinical history in the daily clinical practice [27].

Conclusion

Early diagnosis of neonatal sepsis is the key to reducing neonatal mortality and morbidity due to sepsis. In culture-negative neonatal sepsis, the combination of CRP levels, WBC count with clinical history can become a valuable biomarker in arriving at the diagnosis. The combination works as an effective parameter in preventing the misuse of antibiotics, thereby preventing the colonization of drug-resistant microbiota in neonates.

What new this study adds to existing knowledge

In the management of neonatal sepsis, blood culture is considered as the standard diagnostic test for confirmation. But in culture negative sepsis management, decision regarding the antibiotic withdrawal or continuation is difficult. WBC counts, CRP are the parameters done on routine clinical work up of sepsis case. We found among 127 cases

Studied, blood culture positivity was seen in only 28 (22.04%) cases. 99 (77.9%) neonatal sepsis cases were culture negative.

Among culture negative cases, CRP and Change in WBC count was noted in 35 (35.35%), 30 (30.30%) cases respectively. The sensitivity, specificity of CRP were 71.4%, 69.9%. whereas the sensitivity and specificity of WBC were 21.43% and 78.79%,respectively

When these parameters were used in combination, the sensitivity and specificity of the parameters was increased *ie,* 78.57% and 81.81% respectively.

The combination of routine diagnostic parameters in combination with clinical features can help even in resource limited settings.

Authors contribution

Dr Bharathi R, Concept and design, Data Collection, Manuscript Preparation. Dr Beena PM, Manuscript editing. Dr Mamata K , Data analysis and Statistical Analysis. Dr Parimala S Manuscript editing.

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