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Predictive Value of Procalcitonin and C-Reactive Protein in the Diagnosis of Neonatal Sepsis at LAUTECH Teaching Hospital, Ogbomoso

Mayowa Mary Adetoye[1], Adetola Adedamola Ayankola[1], Victor Idowu Joel-Medewase[1*], Odunayo Oluseun Afolayan[1], Olusegun Joseph Adebami[2], Olusola Adetunji Oyedeji[1]

[1]Department of Pediatrics and Child Health, Ladoke Akintola University of Technology Teaching Hospital, Ogbomoso, Nigeria
[2]Department of Pediatrics and Child Health, Osun State University, Osogbo, Nigeria
Email: *vicmedjoel@yahoo.com, *vijoel-medewase@lautech.edu.ng

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Abstract

Background: Neonatal sepsis is a generalized infection of the neonate's blood and is an important cause of morbidity and mortality globally, with variable and non-specific clinical presentation. Even though blood culture is the gold standard for diagnosis, the yield is low and it takes about 5 to 7 days before the result is obtained and may cause a delay in the commencement of appropriate treatment. Therefore, this study evaluated the diagnostic value of procalcitonin (PCT) and C-reactive protein (CRP) as a rapid diagnostic test for neonatal sepsis. **Methods and Materials:** This prospective study was carried out on 174 neonates admitted with risk factors and features suspected of sepsis into the Special Care Baby Unit (SCBU) at LAUTECH Teaching Hospital, Ogbomoso, Oyo State, Nigeria, from 1st August, 2023 to 31st July 2024. Babies were evaluated and blood samples were obtained for blood culture, serum PCT, CRP levels and complete blood count from each neonate prior to commencement of antibiotics. Data was analyzed on the IBM SPSS version 25.0. **Results:** Of the 174 babies, 74 (42.5%) had positive blood culture (proven sepsis). The optimal cut-off value for procalcitonin was 540 pg/ml, and C-reactive protein was 2.6 µg/ml. The sensitivity of PCT and CRP for predicting sepsis was 85.1% and 81.1% respectively; its specificity was 78.0%, its positive predictive value was 74.1% and its negative predictive value was 87.6%. The sensitivity, specificity, positive predictive value and negative predictive value of CRP for predicting sepsis were 81.08%, 52.0%, 55.6% and 78.8%, respectively. The sensitivity and specificity of PCT and CRP combined were 89.20 % and 50.0 %, respectively. **Conclusions:** Both markers identified 66 (89.2 %) of the

74 culture-positive babies. Both biomarkers are therefore useful as a screening method individually and combined for neonatal sepsis, while giving immediate results.

Keywords

Procalcitonin, C-Reactive Protein, Neonatal Sepsis, Nigeria

1. Introduction

Neonatal sepsis is a generalized bacterial infection that occurs in the first 28 days of life [1]. It is a common and important cause of neonatal morbidity and mortality throughout the world, most especially in developing countries, accounting for 30% - 50% of neonatal deaths [2]-[4]. It continues to be a common and significant healthcare burden, especially among low birth weight (LBW) infants, even with advancement in neonatal care [5]. Neonatal sepsis can be early-onset, manifesting within the first 72 hours of birth or late-onset after the first 72 hours of birth [6] [7].

The gold standard for diagnosing neonatal sepsis is blood culture, which has been the convention [8]-[11]. However, pathogens in the blood are detectable in about 25% - 55% of cases [1] [3] [4] [7], because of the small amount of blood (inoculum) and prenatal maternal antibiotic use. The blood culture results may not even be obtained till 5 to 7 days after sample collection. These factors (low sensitivity and delay in obtaining results) limit blood culture's clinical utility. Hence, there is need for reliable diagnostic tests of sepsis early in its course among the highly vulnerable neonatal population, which will allow prompt antibiotic therapy to reduce morbidity and mortality, thus improving the outcome. Currently, there is no single reliable test for the early definitive diagnosis of neonatal sepsis, therefore, the search for new infection markers persists along with continuous validation of the already identified markers in order to attain optimal sensitivity, reliability, and positive predictability.

In recent years, the search for newer methods for detecting neonatal sepsis has turned to cytokines and other substances associated with the inflammatory response [1]-[3] [5] [12]. Ultimately, this should reduce the indiscriminate use of antibiotics and lessen antibiotic resistance, and economic cost [1] [2] [5]. Among these markers, procalcitonin (PCT) and C-reactive protein (CRP) are the most commonly used for early diagnosis and follow-up [2] [12]-[14]. Procalcitonin begins to rise as early as 4 hours after infection or exposure to a bacterial toxin, peaking at 6 - 8 hours and correlates with the severity of the illness [2] [3] [12] [15] [16], while C-reactive protein begins to rise after 6 hours, peaks at 24 - 48 hours and then declines as the inflammatory process decreases.

The fear of missing cases of neonatal sepsis frequently leads to indiscriminate use of antibiotics, and prescription programme optimization is suggested for reducing

this inappropriate usage. While different authors have studied how to reduce antibiotic over-prescription in the case of early-onset sepsis episodes, with different approaches being available, less is known about late-onset sepsis episodes. Biomarkers (such as PCT, CRP, interleukin-6 and 8, and presepsin) can play a crucial role in the prompt diagnosis of late-onset sepsis, but their role in antibiotic stewardship should be further studied, given that different factors can influence their levels and newborns can be subjected to prolonged therapy if their levels are expected to return to zero. There is good evidence that procalcitonin performs well in this sense [17] [18], but more studies and protocols for biomarker-guided antibiotic stewardship are needed.

Thus, this study was conducted to evaluate the diagnostic value of PCT and CRP as early diagnostic markers for the detection of neonatal sepsis among neonates at a Nigerian tertiary hospital in comparison to that of blood culture.

2. Materials and Methods

2.1. Study Design

This was a descriptive cross-sectional study on the newborns admitted into the Special Care Baby Unit (SCBU) of the department of Paediatrics and Child Health of LAUTECH Teaching Hospital, Ogbomoso over a one-year period (August 2023 to July 2024).

The study was approved by the Institutional Scientific and Ethical Committee (ID No: LTH/OGB/EC/2021/263, Dated 10th January, 2022) and written informed consents were obtained from the parents/caregivers of the babies.

2.2. Inclusion Criteria

The inclusion criteria were neonates who were admitted to the SCBU of this hospital with risk factors for sepsis, such as maternal peripartum fever, prolonged rupture of membranes (PROM), prematurity, and low birth weight. Also, neonates who developed clinical signs suggestive of sepsis, such as fever/hypothermia, respiratory distress, refusal of feed, vomiting, diarrhoea, poor cry, abdominal distention, and apnea while they were on the ward.

2.3. Exclusion Criteria

The exclusion criteria were neonates who were on antibiotics or those who developed the signs of sepsis within 72 hours of discontinuation of the antibiotics and those who had perinatal asphyxia, meconium aspiration syndrome or other infections like retroviral disease, congenital anomalies, and inborn errors of metabolism.

2.4. Sample Size Determination

The total number of babies enrolled in the study was calculated by the formula [19]:

$$n = \frac{z^2 pq}{d^2}$$

Since the estimated population size is less than 10,000, our minimum sample size, N_f, was derived by the formula given below:

$$N_f = \frac{n}{\left(1 + \frac{(n)}{N}\right)}$$

The total sample size obtained was 148, and further 10% of non-responders were added to make an approximate minimum sample size of 163. For this study, 174 neonates were recruited and samples were taken from all of them.

2.5. Sampling Procedure

Purposive non-probability sampling technique was used. Recruitment into the study was done consecutively. Questionnaires were prepared and administered to the consenting parents/caregivers of recruited patients to obtain the parents' and caregivers' personal details and history.

A general examination of each baby to elicit physical signs like pallor, fever, jaundice, respiratory distress, and other features of sepsis was initially carried out. Systemic examinations of the respiratory, cardiovascular, nervous, and digestive systems were also carried out.

2.6. Specimens and Tests Which Were Performed

The specimens of blood were obtained from each neonate before the commencement of antibiotic therapy. The tests performed on each subject included:

Full blood count (FBC): Total WBC and differentials, platelet count, micro erythrocyte sedimentation rate, blood culture and antibiotic sensitivity, PCT and CRP estimations.

2.7. Blood Sample Collection and Analysis

From each recruited baby, 5 ml of blood was collected:

1.5 ml of blood was added to each of the two paediatric BACTEC culture bottles for aerobic and anaerobic blood culture. Blood culture samples were incubated for at least 8 hours at 37˚C and sub-cultured on blood agar, chocolate agar and MacConkey agar. The agars were incubated for 18 - 24 hours at 37˚C. The chocolate agars were incubated anaerobically in a candle jar, while the other two agars were incubated aerobically in the incubator. Gram staining was done on isolated colonies. This was followed by a biochemical test to determine the species of the bacteria's growth. Afterward, an antibiotic susceptibility test (AST) was done to determine the sensitivity pattern.

2 ml of blood was dispensed into plain vacutainer serum gel separator bottle for PCT and CRP evaluations. The serum for PCT and CRP investigations was extracted and frozen at −20˚C till analysis was done.

2.8. Serum PCT

The serum PCT level was measured by immunoassay technique using enzyme linked immunosorbent assay kit (ElabscienceHuman PCT ELISA KIT) according to the manufacturer's instructions. The mean intra-assay precision (103.1 pg/ml - 921 pg/ml), while the optimal cut-off value for procalcitonin was 540 pg/ml. This is the point on the ROC that optimizes balance between sensitivity and specificity.

2.9. Serum CRP

The serum CRP level was measured by immunoassay technique using high-sensitivity CRP test system (Accu-Bind ELISA Microwells). In this assay, CRP < 1.0 μg/ml was considered low risk, normal for 1 - 3 μg/ml and high risk for >3.0 μg/ml. The optimal cut-off value for CRP was 2.6 μg/ml. This is the point on the ROC that optimizes balance between sensitivity and specificity.

After blood sample collection, babies were treated empirically with antibiotics according to the standard operating procedure of the hospital SCBU for sepsis prior to the availability of blood culture results.

2.10. Data Analysis

Data obtained was analyzed on the IBM SPSS version 25.0. A descriptive analysis of the study population was done. Data was summarized using means, ranges and standard deviations for normally distributed numerical data. Categorical data was summarized using frequencies and percentages. Data was presented using tables, figures and charts as appropriate. t-test was used to compare the mean procalcitonin and C-reactive protein levels among culture-positive and culture-negative neonates. A receiver operating characteristic (ROC) analysis was plotted to determine the best cut-off value to predict the outcome. By using the blood culture results as the gold standard, the sensitivity, specificity, positive predictive values and the negative predictive values of the PCT and CRP for diagnosing sepsis were calculated. The sensitivity of a test was defined as the proportion of babies with sepsis and this was correctly identified by the test. The specificity of the test was defined as the proportion of babies without sepsis and this was correctly identified by the test. The positive predictive value (PPV) of a test was defined as the proportion of babies with positive test results and who had sepsis. The negative predictive value (NPV) of a test was defined as the proportion of babies with negative test results and who did not have sepsis. A p-value of <0.05 was considered statistically significant for all tests.

3. Results

A total of two hundred and ninety-four neonates were studied over 12 months, of which 174 were presumed with sepsis. Of the 174 neonates, 91 (52.3%) were females and 83 (47.7%) were males, giving a female-to-male ratio of 1.1:1. 115 (66.1%) were preterm, while 59 (33.9%) were term. The admission weights of the neonates ranged

from 0.76 kg to 3.90 kg, with a mean weight of 2.28 ± 0.78 kg.

The majority of the neonates were delivered spontaneously, vaginally at government-owned hospitals, while home delivery was the least common place of delivery. Lack of antenatal care (41.4%) and PROM (27.6%) were the most maternal frequent risk factors for sepsis, while prolonged labor (5.7%) and Maternal UTI (5.7%) were the least commonly associated maternal risk factors.

Low birth weight (44.8%) and prematurity (41.4%) were the most common risk factors among the neonates studied. Of the 174 neonates clinically diagnosed with sepsis, bacterial isolates were obtained from the blood samples of 74 (42.5%) neonates, and the remaining 100 (57.5%) had negative blood culture.

The most common organism that was found in blood culture was *Staphylococcus aureus* (30, 40.5%), followed by coagulase-negative *Staphylococcus aureus* (18, 24.3%). Other organisms included *Streptococcus agalactiae* (10, 13.5%), *Escherichia coli* (10, 13.5%), multi-drug-resistant *Staphylococcus aureus* (4, 5.4%), and *Pseudomonas aeroginosa* (2, 2.7%) (Figure 1).

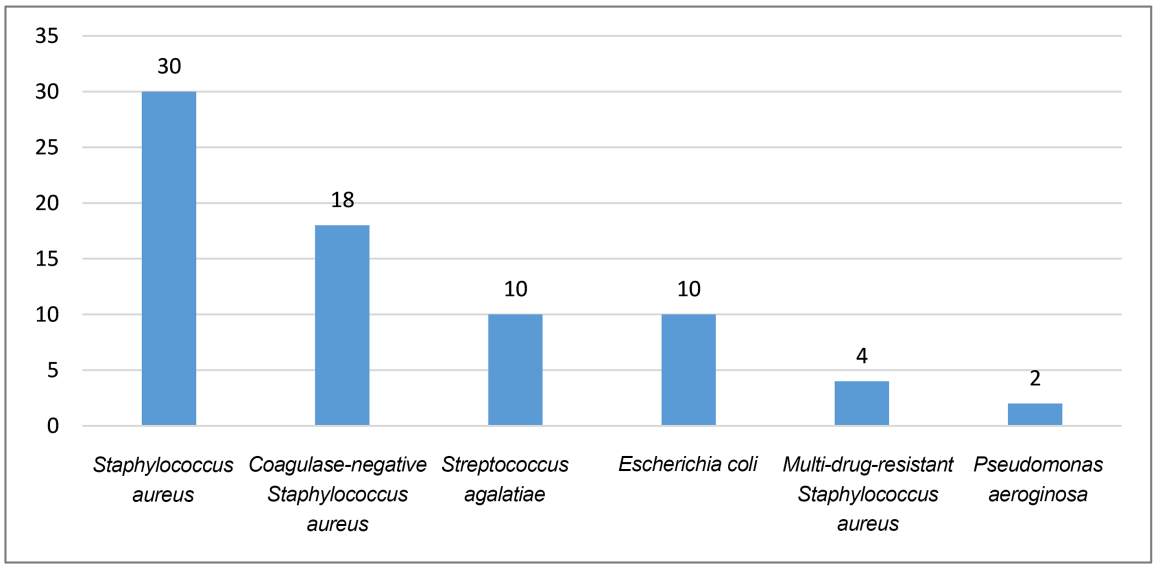


Figure 1. Organisms isolated from blood culture.

Procalcitonin (PCT) levels in the studied neonates ranged from 130 pg/ml to 2000 pg/ml, with a mean PCT level of 819.22 pg/ml ± 690.28 pg/ml. C-reactive protein (CRP) levels ranged from 0.60 μg/ml to 30.00 μg/ml, with a mean level of 7.55 μg/ml ± 9.27 μg/ml.

The means of procalcitonin and C-Reactive protein levels in the culture-negative and confirmed sepsis groups were both significantly higher in culture-positive neonates (at least $p = 0.042$) (Table 1).

The levels of procalcitonin and C-reactive protein were slightly elevated in early onset neonatal sepsis (865.17 pg/ml ± 678.53 and 8.15 μg/ml ± 9.25, than late onset neonatal sepsis 742.15 pg/ml ± 708.13 and 6.56 μg/ml ± 9.28, respectively; though the differences were not statistically significant.

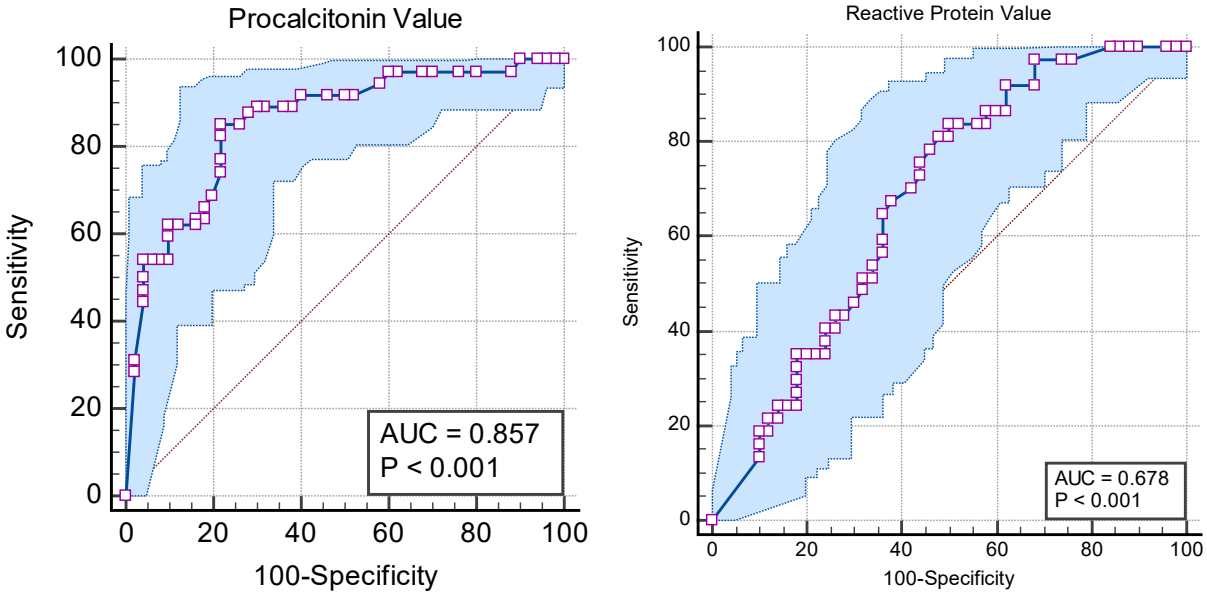
Table 1. Comparison of the means of procalcitonin and C-reactive protein levels in admitted neonates with suspected sepsis and confirmed sepsis.

Variables	Mean (SD)		p-value*
	Culture Negative	Culture Positive	
Procalcitonin (pg/ml)	453.44 (± 410.34)	1313.51 (± 685.49)	0.000
C-reactive Protein (µg/ml)	6.33 (± 8.75)	9.21 (± 9.74)	0.042

*t-test.

Procalcitonin has an area under curve (AUC) of 0.857, while C-reactive protein shows an AUC of 0.678.

The p-values associated with procalcitonin and C-reactive protein are 0.0001 and 0.001, respectively. For procalcitonin, the 95% confidence interval for the AUC spans from 0.796 - 0.905, while for C-reactive protein, it spans from 0.603 - 0.747 (Figure 2(a) & Figure 2(b)).



(a) (b)

Figure 2. (a) ROC curve denoting sensitivity and specificity for procalcitonin; (b) ROC curve denoting sensitivity and specificity for C-reactive protein.

The optimal cut-off value for procalcitonin was 540 pg/ml, whereas, for C-reactive protein, it was 2.6 µg/ml. Based on their biomarker levels, these cut-off values were utilized to categorize neonates as sepsis-positive or sepsis-negative (Table 2).

Procalcitonin had a sensitivity of 85.14%, specificity of 78.0%, positive predictive value (PPV) of 74.1%, and negative predictive value (NPV) of 87.6%, while CRP had a sensitivity, specificity, PPV, and NPV of 81.08%, 52.0%, 55.6%, and 78.8%, respectively (Table 3).

Table 2. Sensitivity and specificity of C-reactive protein and procalcitonin in predicting neonatal sepsis.

	Procalcitonin	C-Reactive Protein
Area under Curve	0.857	0.678
p-value	0.0001	0.001
Confidence Interval (Lower Bound - Upper Bound)	0.796 - 0.905	0.603 - 0.747
Cut-off Value	540 pg/ml	2.6 µg/ml
Sensitivity (%)	85.14	81.08
Specificity (%)	78.00	52.00
PPV (%)	74.1	55.6
NPV (%)	87.6	78.8

Procalcitonin demonstrated a sensitivity of 85.14%, while C-reactive protein exhibited a sensitivity of 81.08%. The combined use of PCT and CRP resulted in a higher sensitivity of 89.20% (Table 3).

Table 3. Comparison of sensitivity, specificity, negative predictive values and positive predictive values of procalcitonin, C-reactive protein and combined C-reactive protein and procalcitonin in the diagnosis of neonatal sepsis.

	Procalcitonin	C-Reactive Protein	Combined
Sensitivity	85.14	81.08	89.20
Specificity	78.00	52.00	50.00
PPV	74.1	55.6	56.90
NPV	87.6	78.8	86.20

Procalcitonin was positive in 63 (85.14%) out of 74 culture-positive cases and negative in 78 (78.0%) out of 100 culture-negative cases. This association is statistically significant (p = 0.000). C-Reactive protein was positive in 60 (81.1%) out of the 74 culture-positive cases and 52 (52.0%) of the 100 culture-negative patients were predicted correctly, with a p-value of 0.000 (Table 4).

Table 4. Comparison of procalcitonin and C-reactive protein with blood culture in the diagnosis of sepsis.

	Blood Culture		Total	p-value*
	Positive	Negative		
Procalcitonin				
Present	63	22	85	0.000
Absent	11	78	89	
Total	74	100	174	
C-Reactive Protein				
Present	60	48	108	0.000
Absent	14	52	66	
Total	74	100	174	

*Chi-square.

A comparison of combined procalcitonin and CRP prediction of neonatal sepsis against blood culture is depicted in Table 5.

Table 5. Comparison of combined procalcitonin and CRP with blood culture.

PCT & CRP	Blood Culture		Total	p-value*
	Positive	Negative		
Present	66	50	116	0.000
Absent	8	50	58	
Total	74	100	174	

*Chi-square.

Low levels of procalcitonin were found among neonates who survived, and the highest mean value was recorded among neonates who died. However, the difference in the PCT and CRP mean values amongst the neonates who died and those who survived was not statistically significant, p = 0.056, for both biomarkers (Table 6).

Table 6. Comparison of means of procalcitonin and CRP with outcome.

Variable	Procalcitonin	CRP
Alive	764.41 (666.18)	7.74 (9.62)
Discharged against Medical Advice (DAMA)	1095.00 (929.53)	7.30 (2.31)
Died	1106.67 (744.96)	6.43 (7.79)
p-value*	0.056	0.814

*ANOVA.

4. Discussion

Neonatal sepsis, with its high morbidity and mortality rates throughout the world, remains a diagnostic challenge for the health care providers due to lack of reliable tests for the early confirmation of the disease, and its non-specific clinical presentation [20]-[24]. A high index of suspicion and early initiation of therapy are of great importance [5] [10] [25]-[27].

In the present study, lack of antenatal care and premature rupture of membranes (PROM) were the prevailing maternal risk factors for neonatal sepsis identified among the mothers of the studied neonates. This is similar to the findings of Osrin *et al*. [28] [29], who reported 87.5% in Delhi, India. Similarly, Yadav *et al*. [2] reported a high incidence of PROM, and it has been reported to be a significant factor for neonatal sepsis [28]. Neonates generally have immature, defective protective mechanisms.

The blood culture results from this study showed *Staphylococcus aureus* to be the commonest cause of neonatal sepsis. This is similar to the finding of Osrin *et al*. [29] in the United Kingdom. Also, in a meta-analytic study by Meduge *et al*. [30],

Staphylococcus aureus was reported as the most prevalent isolate. Ako-Nai *et al*. [31] also reported similar findings.

C-reactive protein as an inflammatory marker is unreliable in distinguishing between systemic inflammatory response and bacterial infection. Procalcitonin (PCT), as a precursor of calcitonin, is markedly elevated in bacterial neonatal sepsis. This study found that procalcitonin levels were highly elevated among the neonates who died. This is similar to the report of Bharti *et al*. [27]. Yadav *et al*. [2] and Adib *et al*. [25]. Procalcitonin appears to be a good prognostic marker.

Procalcitonin has an area under curve (AUC) of 0.857, while C-reactive protein shows an AUC of 0.678. These values reflect the discriminatory capacity of each biomarker to distinguish between neonates with and without sepsis, with a higher AUC indicating superior discriminatory ability.

This is comparable to the findings of Arowosegbe *et al*. [3] and suggests that PCT and CRP levels show discriminatory properties related to the duration of neonatal sepsis.

In this study, C-reactive protein identified 60 of the total 74 blood cultures as positive with a positive predictive value (PPV) of 55.6%, while procalcitonin identified 63 of the 74 positive blood cultures with a PPV of 74.1%. Furthermore, it was observed that CRP has a sensitivity of 81.08% and specificity of 52.00%, while procalcitonin has a higher sensitivity of 85.14% and specificity of 78%. This implies that while a positive PCT or CRP will correctly diagnose 8 out of 10 neonates with suspected sepsis, procalcitonin has a better performance. Thus, PCT and CRP are reliable markers that aid early diagnosis of neonatal sepsis and have about equal diagnostic accuracy [32] and each can independently diagnose bacterial neonatal sepsis. This can help clinicians to initiate empirical antibiotic therapy for neonates suspected of sepsis. These observations are consistent with Bunduki *et al*. [33] in the Democratic Republic of the Congo (DRC), who reported a relatively higher CRP sensitivity and a predictive value. Similarly, Morad *et al*. [1] reported a sensitivity of 89.5% with CRP, with a PPV of 92.5%.

In contrast, Chaurasiya *et al*. [16] observed in their study that CRP had a low sensitivity of 30% and specificity of 90% with a PPV of 92.3%. This disparity may be due to different cut-off levels and laboratory methods used. The results from the present study suggest that PCT shows a better relationship to neonatal sepsis. Chaurasiya *et al*. [16], Ashraf *et al*. [34] and Morad *et al*. [1] recorded PCT sensitivity values of 96.25%, 87.5% and 97.6%, respectively, all in keeping with our findings.

Procalcitonin has been shown to discriminate between true neonatal sepsis and contaminated blood culture results. Therefore, it is a useful rapid diagnostic tool in diagnosing neonatal sepsis [10] [26] [35].

The combination of PCT and CRP increased the probability of detecting neonatal sepsis. This observation confirmed the findings of previous investigators. This may be of immense clinical utility in low-income countries where neonatal sepsis is common but blood culture facilities are scarce.

5. Conclusion

Procalcitonin and CRP levels were significantly higher in blood culture-positive neonates. Both PCT and CRP are useful diagnostic biomarkers for evaluating neonatal sepsis. Procalcitonin and C-reactive protein each showed comparable high sensitivity for detecting neonatal sepsis, but PCT had a much better specificity and predictive values. Combining PCT and CRP as diagnostic markers improves the sensitivity, specificity, positive and negative predictive values of neonatal sepsis diagnosis. The early use of these biomarkers in neonatal infection may definitely help us limit the number of neonates that are put on antibiotics due to suspected risk of sepsis.

6. Limitations of the Study

Lack of follow-up repeat PCT and CRP levels and blood culture-negative results, even in clinically proven sepsis, to monitor response to treatment.

The purposive sampling technique employed was a non-probability sampling method, implying that all suspected cases of sepsis were included in the study. These may not affect the outcome, generalizability or external validity of findings from the study because it is a single-institutional study. However, a multi-centered study, which will give room for probability sampling, is recommended for similar future research. The purposive sampling selection is not randomized and this gives room for some elements of bias.

Further clinical study is required to determine the cut-off levels of PCT and CRP with shifting neonatal age, early-onset neonatal sepsis, and late-onset neonatal sepsis.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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