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Research Article

The Clinical Insignificance of Procalcitonin as an Acute Phase Reactant in Neonatal Sepsis inNigeria

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Abstract

Introduction: Neonatal sepsis is an important cause of morbidity and mortality in developing countries. Early diagnosis and treatment is necessary in order to mitigate this trend. Procalcitonin is one of the acute phase reactants meant for this purpose. The objective of the study was to determine the usefulness of procalcitonin as an early marker in the diagnosis of neonatal sepsis in University of Calabar Teaching Hospital, Calabar, Nigeria. **Methods:** This was a prospective case-control hospital-based study conducted from September, 2017 to April, 2018 among 60 neonates with suspected sepsis admitted into the newborn unit as well as healthy 60 age and sex-matched controls. Socio-demographic data, presenting complaints and risk factors for sepsis among others, were obtained using the pre-tested questionnaire. The history suggestive of sepsis included a history of prolonged rupture of membranes, peripartum pyrexia, prolonged labour, fever, fast breathing, convulsions among others. Physical examination was done with focus on signs suggestive of sepsis such as temperature instability, tachypnoea, dyspnoea, seizures, abdominal distension. Samples for procalcitonin test were collected along with samples for routine sepsis workup and blood smear for malaria parasite at presentation. Blood culture was done for anaerobic and aerobic organisms. The serum samples were pooled and analyzed for procalcitonin using quantitative ELISA test. Data was analyzed using SPSS version 20 and $p< 0.05$ was taken as statistically significant. **Result:** Out of the 60 subjects, 19 (31.7%) had blood culture positive sepsis, 36 (60.0%) had blood culture negative sepsis and 5 (8.3%) had neonatal malaria. The gram positive organisms accounted for 13 (68.4%) of the 19 bacteria isolated. Of the 36 neonates with blood culture negative sepsis, 31(86.1%) neonates had clinical signs and/or haematological parameters suggestive of sepsis. In addition, 3(8.3%) neonates had bronchopneumonia while 2(5.6%) had ophthalmia neonatorium. The median procalcitonin (PCT) level in the subjects compared with the controls was 0.98ng/ml and 0.40ng/ml respectively (p value = 0.000). The mean PCT level was 5.97±7.36 ng/ml in the subjects and 1.58±3.64 ng/ml in the controls. Among the subjects, the median PCT was 1.28ng/ml in blood culture negative sepsis and 0.93ng/ml in blood culture positive sepsis (p value = 0.000). In addition, the mean PCT level was also higher in neonates with blood culture negative sepsis (7.46 ± 8.07ng/ml) when compared with blood culture positive sepsis (4.60±5.99ng/ml). Based on the receiver operating characteristics curve, PCT levels ≥ 0.5ng/ml was suggestive of bacterial infection. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the curve at PCT level of 0.5ng/ml were 68.4%, 29.3%, 30.0%, 66.7% and 0.51 respectively. **Conclusion:** These findings show that although serum PCT levels were higher in neonates with suspected sepsis when compared to the healthy babies, they were nevertheless lower in culture proven sepsis (septicaemia). PCT was insignificant as an acute phase reactant because of its low specificity and PPV in our environment.

Keywords: Neonatal sepsis; Procalcitonin; Diagnostic marker; Acute phase reactant; Blood culture

Introduction

Neonatal sepsis continues to be a major cause of morbidity and mortality despite great advances towards ending preventable deaths in the newborn period [1]. Sepsis accounts for 15% of all neonatal deaths globally, with mortality rates highest in Sub-Saharan Africa [2]. Nigeria has one of the highest hospital-based incidence rates of neonatal sepsis globally even though there have been some improvements in the figures from 54.9 per 1,000 live births [3] in 2008 to 18.2 per 1000 live births [4] in 2017.

The gold standard for diagnosis of neonatal sepsis currently is blood culture [3]. The newer blood culture systems which have the advantages of earlier detection of microorganisms, decreased detection of pseudo bacterium and increased sensitivity of blood culture results are unfortunately not readily available in developing countries like Nigeria because they are costly, have limited medium types and require large-sized instruments. Therefore, there is still a need for newer diagnostic markers to obtain a rapid indication of the infectious status of neonates with suspected sepsis [5].This has directed attention on acute phase reactants like Procalcitonin and C-reactive protein as early markers of infection [6].

Procalcitonin (PCT) is an early marker of bacterial infection in critically ill patients including newborns [6,7]. It is the peptide precursor of the hormone calcitonin and is composed of 116 amino acids [5,6]. In healthy neonates, plasma PCT levels increase gradually after birth, reach peak levels after 24 hours of age (mean 1.5±2.5 ng/ml, range 0.1-20 ng/ml) and then decrease to normal levels below 0.5ng/ml by 48-72 hours of age. A number of studies [5,7] in children and neonates after 72 hours of age, demonstrated that PCT levels less than 0.5 ng/ ml seem to be normal. Increases in the range of 0.5-2 ng/ml is found in non-infectious inflammation, malarial infections, viral or focal bacterial infections while PCT levels above 2-2.5 ng/ml occur in bacterial or fungal systemic infections [5]. Procalcitonin is said to have an added advantage of better monitoring of disease progression when compared to traditional laboratory markers [8].

Acute phase reactants have been suggested as a guide in ensuring the rational use of antibiotics in health institutions. Some studies [9,10] have challenged the clinical usefulness of PCT as a diagnostic marker of sepsis in the newborn period. This study was therefore conducted to answer the research question "What is the significance of procalcitonin in the diagnosis of neonatal sepsis in our subregion" in relationship to blood culture. The answer to this question would help neonatologists and other health practitioners in preventing inappropriate antibiotic use. In addition, it would ultimately prevent the development of multi-drug resistance in our clinical setting.

Methods

Study Area

This prospective case-control study was conducted in the newborn unit of the University of Calabar Teaching Hospital, Calabar. The hospital is a 600- bed capacity tertiary hospital in the South- South region of Nigeria. The neonatal unit as earlier described by Anah et al. [3] and Enyuma et al. [11] is a 50-bed capacity and is divided into two sections; Special Care Baby Unit (SCBU) for neonates delivered in the hospital and Sick Babies Unit (SBU) for neonates delivered outside the hospital. The aim of separating into these two sections is to avoid spread of infection from outborn babies to inborn babies.

Recruitment of Participants

Sixty neonates undergoing evaluation for sepsis at the newborn unit of the hospital between September 2017 to April 2018 as well as 60 age- and sex-matched controls were consecutively recruited until the desired sample size was met. Term babies aged 0-28 days with any of the following: risk factors for sepsis, temperature <36.5°C or > 37.4°C [12] and/or symptoms and signs of sepsis were included in the study. The history suggestive of sepsis included a history of prolonged rupture of membranes, peripartum pyrexia, prolonged labour, fever, fast breathing, convulsions etc [12]. Exclusion criteria were preterm babies, obvious congenital anomalies, infant of diabetic mothers, prior antibiotic therapy and babies whose mothers took antibiotics within 72 hours of delivery. Socio-demographic data, presenting complaints and risk factors for sepsis, among others, were obtained using the pre-tested questionnaire. Physical examination was done with focus on signs suggestive of sepsis such as on temperature instability, jaundice, pallor and signs of respiratory distress [12].

Sample Collection

As a departmental policy, all subjects suspected of having sepsis had full sepsis workup and blood film for malaria parasite done within one hour of admission [11]. Samples for PCT estimation were also collected at the time of presentation with initial sepsis workup. About 5.5mls of blood was collected once from an appropriate peripheral vein of the subject and shared out as follows: 0.5mls for Full blood count, 3mls for blood culture test and 2mls for PCT test. All samples for laboratory investigations were taken before the commencement of antibiotics. Healthy controls were those in the lying-in ward/immunization centre that met the inclusion criteria. Two milliliters of blood for PCT test were collected from the well babies.

Laboratory methods

Full Blood Count (FBC)

About 0.5mls of venous blood was collected into an

appropriately labeled EDTA bottle and sent to the Haematology laboratory within 30 minutes of collection. Where this was not possible, the sample was refrigerated at 4-8ºC for a period not greater than 12 hours. Analysis was done using automated analyzer (Full automatic blood cell counter, Model PCE-210N, ERMA Inc, Manufactured 05/2012). The total white cell count and differentials, haemoglobin level, packed cell volume and platelet count obtained in the print-out were recorded. Blood smear for morphology of the cells was also carried out by the laboratory scientist following standard procedures [13]. Haematological parameters suggestive of sepsis such as left shift of neutrophils and neutrophil degenerative changes (vacuolization, toxic granulations and Dohle bodies) were documented.

Blood Film for Malaria Parasite

A pin prick of capillary blood was used to prepare both thick and thin blood films following standard protocols [14]. Asexual forms of the parasite (trophozoites or ring forms) and the sexual forms (gametocytes) were counted in each field and reported. Presence of malaria pigment in white blood cells (especially neutrophils) was also reported. A slide was declared negative if after examining a minimum of 200 fields, for at least 15 minutes and no malaria parasite was found according to standardized protocols [14]. The species of the malaria parasite was determined on a thin film using parameters developed by the World Health Organization [14].

Blood culture

Three milliliters of venous blood was taken under strict aseptic conditions and inoculated into appropriately labeled brain heart infusion broth (1.5mls) and thioglycolate broth (1.5mls) for culturing of aerobic and anaerobic organisms respectively. The samples were transported to the Microbiology laboratory within 30 minutes of collection. When this was not possible, samples were stored in a Microbiology incubator, located in the newborn unit, at a temperature of 37ºC for a period not greater than 12 hours.

The blood culture technique used in this study was the conventional manual detection system. Once there was microbial growth, full identification of organism was done using gram stain, biochemical methods and Microbact 12EA identification system [15]. Antibiotic sensitivity pattern of bacterial isolates was determined by disc diffusion susceptibility testing using antimicrobial disc [15]. Antibiotic discs used were ceftriaxone, ceftazidime, cefuroxime, gentamicin, amikacin, levofloxacin, ciprofloxacin, clindamycin, and imipenem. The zone of inhibition was directly related to the susceptibility of the organism. It was measured carefully and compared with the interpretative charts published by the National Committee for Clinical Laboratory Standards [15].

Procalcitonin test

The serum procalcitonin concentration was determined using a sandwich enzyme-linked immunosorbent assay (ELISA) produced by Elabscience (Elabscience Biotechnology, Germany) [16]. Interpretation of the results was guided by the procalcitonin ranges : <0.5ng/ml (systemic infection/sepsis is not likely), > 0.5 and < 2.0ng/ml (moderate risk for progression to severe systemic infection), >2 and < 10ng/ml (high risk for progression to severe systemic infection) and ≥ 10ng/ml (high likelihood of severe sepsis or septic shock) [17].

Definitive Diagnosis

According to risk factors and/or clinical symptoms of sepsis, microbiologic and laboratory results, neonates were classified into different categories of infection (similar to previous studies by Phua et al., 2013 [18]. and Klingenberg et al., 2018 [19]. as follows: (a) Blood culture positive sepsis: A clinical syndrome in a neonate manifested by systemic signs of infection and isolation of a pathogen, usually bacteria, from the blood stream. (b) Blood culture negative sepsis: This applies to situations where an infant has signs and symptoms of SIRS ascribed to a bacterial aetiology, but in whom an organism was not isolated from blood culture. (c) Malaria: The newborn had asexual forms of Plasmodium species detected in their peripheral blood.

Data Analysis

The data were analyzed using the Statistical package for Social Sciences version 20 software package (SPSS Inc. Chicago IL, USA). Statistical tests between categorical variables were done using Chi-square test. Where the expected count was less than five, a Fisher's exact test was used. Differences between three or more normally distributed continuous variables were assessed using One-way analysis of Variance (ANOVA). Where needed, Bonferroni test was used when ANOVA was significant. The Mann-Whitney test was used to compare the median (interquartile range) of non-normally distributed continuous variables. Diagnostic efficiency was defined by sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) [20]. Correlation between blood culture results and PCT was done. Receiver Operating Characteristics (ROC) curve was done to determine the best cut-off value of PCT indicating neonatal sepsis. The closer the ROC curve is to the upper left corner, the higher the accuracy of the test [16]. P values <0.05 were considered statistically significant at 95% confidence interval.

Results

Patients' Characteristics

A total of 120 neonates participated in the study. Of these study population; 96 neonates (80%) were 48 hours old. The

median age for the study population was 24 hours. Seventy-four (61.7%) were males and 46 (38.3%) were females with a Male: Female ratio of 1.6:1.

During the study period of six-months, 311 neonates were admitted into the newborn unit. Of these, 60 neonates met the inclusion criteria. Twenty-four (40%) of the subjects were inborns while 36 (60%) were outborns. Twenty-three (95.8%) of the inborns were 48 hours old when compared to 25(69.4%) of the outborns.

Clinical characteristics

Prolonged rupture of membranes (PROM) was the commonest risk factor for sepsis; it was seen in 25 (41.7%) of the subjects. Among the clinical signs noted in the subjects, tachypnoea was seen in 33(55%) neonates, dyspnoea in 30 (50%) neonates while fever was seen in 26 (43.3%) neonates. (Table I)

Variable	Present (%)	Absent (%)
History		
PROM*	25(41.7)	35(58.3)
Foul smelling liquor	9(15.0)	51(85.0)
Maternal fever	8(13.3)	52(86.7)
Prolonged labour	13(21.7)	47(78.3)
Physical examination		
Fever	26(43.3)	34(56.7)
Subnormal temperature	10(16.7)	50(83.3)
Tachypnoea	33(55.0)	27(45.0)
Dyspnoea	30(50.0)	30(50.0)
Poor suck	18(30.0)	42(70.0)
Convulsions	5(8.3)	55(91.7)

Table 1: Clinical characteristics of the study population;*PROM = Prolonged rupture of membranes;**Neonates with suspected sepsis had one or more symptoms and/or signs suggestive of sepsis.

Hematological parameters

Leucocytosis was the commonest abnormal hematological test result parameter; it was seen in 24(40%) neonates. It was followed by neutrophilia which was seen in 20(33.3%) neonates (Table 2).

	Study Subjects		
	Present	Absent	Total
Leucocytosis	24(40.0)	36(60.0)	60(100.0)
Leucopenia	3(5.0)	57(55.0)	60(100.0)
Neutrophilia	20(33.3)	40(66.7)	60(100.0)
Neutropenia	14(23.3)	46(76.7)	60(100.0)
Thrombocytopenia	10(16.7)	50(83.3)	60(100.0)
Abnormal PBF*	15(25.0)	45(75.0)	60(100.0)

Table 2: Hematological parameters among the study subjects; PBF* = Peripheral blood film

Definitive diagnosis

Of the 60 neonates recruited into the study, 19 (31.7%) had blood culture proven sepsis, 36 (60.0%) had blood culture negative sepsis and 5 (8.3%) had uncomplicated malaria. Of the 19 culture positive results, 17(89.5%) were early onset sepsis and 13(68.4%) were gram positive organisms. Among the gram positive organisms, 12(63.1%) were Staphylococcus aureus; of which 8(42.1%) were Methicillin susceptible strains. No anaerobe was cultured (Figure 1).

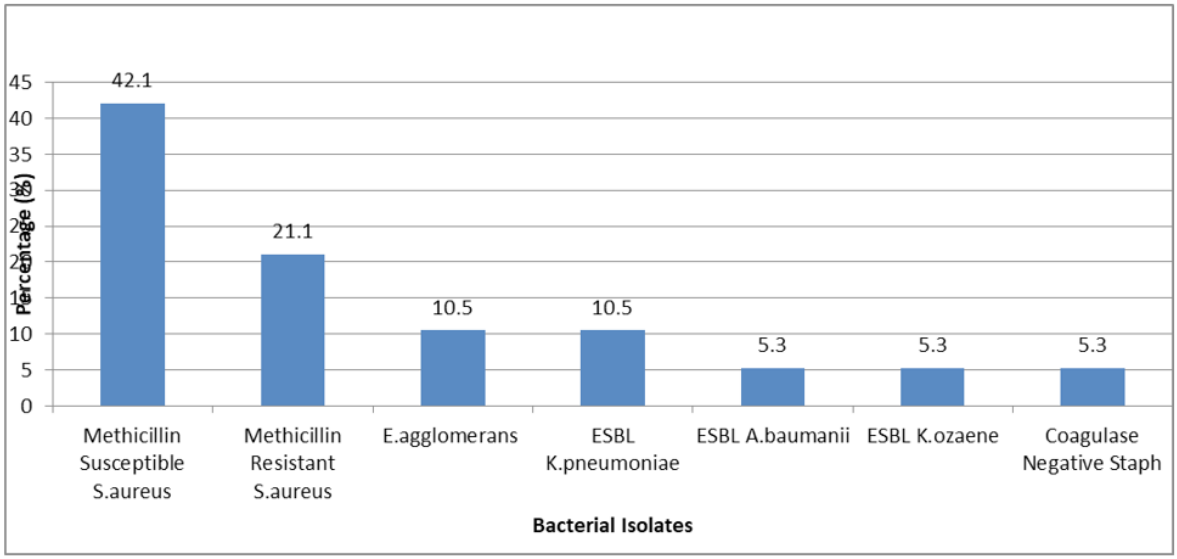

Figure 1: Bacteria isolated from subjects with positive blood culture results; *ESBL= Extended Spectrum Beta Lactamase.

Overall, 18(94.7%) of the organisms were sensitive to Imipenem, 16(84.2%) to Levofloxacin, 12(73.6%) to Amikacin, 13(68.4%) to Gentamicin and 12(63.2%) to Ciprofloxacin. Multiple antibiotic resistance patterns were reported in 8(42.1%) of the bacteria isolated. Out of the 36 neonates with blood culture negative sepsis, 31 (86.1%) had clinical signs and/or hematological parameters suggestive of sepsis, 3(8.3%) had bronchopneumonia and 2(5.6%) had ophthalmia neonatorum. All the malaria parasites detected were of the Plasmodium falciparum species.

Procalcitonin results in the study population

The median PCT level in the subjects and controls was 0.98ng/ml and 0.40ng/ml respectively. The difference was statistically significant (p=0.000). Mean procalcitonin level was higher among subjects compared with controls (5.97 ±7.36ng/ml versus 1.58 ± 3.64ng/ml) (Table 3).

Serum Procalcitonin levels (ng/ml)					
	Subjects(n=60)	**Controls(n=60)**	**Total(N=120)**	**U-test**	**p-value**
Median	0.98	0.40	0.73	4.134	0.000*
Range	0.03 – 22.57	0.03 – 16.18	0.03 – 22.57		
Q_1,Q_3	0.38, 12.73	0.03, 0.84	0.04, 5.64		
Mean ± SD	5.97 ±7.36	1.58 ± 3.64	3.78 ± 6.18		

Table 3: Comparison of serum levels of procalcitonin in neonates with suspected sepsis and control group; *=Statistically significant.

Comparison of serum procalcitonin levels among study subjects' diagnostic categories

Out of the 60 subjects, 19 (31.7%) had blood culture proven sepsis, 36 (60.0%) had blood culture negative sepsis and 5 (8.3%) had uncomplicated malaria. The gram positive organisms accounted for 13(68.4%) of the 19 isolates while the gram-negative organisms represented 6(31.6%). Out of the 36 neonates with blood culture negative sepsis, 31 (86.1%) had clinical signs and/or hematological parameters suggestive of sepsis, 3(8.3%) had bronchopneumonia and 2(5.6%) had ophthalmia neonatorum (Table 4).

Serum Procalcitonin levels (ng/ml)					
Variable	**N**	**Median**	**Q_1,Q_3**	**Range**	**Mean ± SD**
Blood culturepositive sepsis	19	0.73	0.31,11.11	0.03-17.79	4.60 ± 5.99
Bronchopneumonia	3	1.00	0.90,1.00	0.90-12.58	5.12 ± 7.24
Ophthalmia neonatorium	2	0.86	0.14,0.85	0.14-1.43	0.86 ± 1.01
Malaria	5	0.30	0.15,0.78	0.03-0.93	0.43 ± 3.50

Table 4: Definitive diagnoses among the study subjects.

Neonates with blood culture negative sepsis had the highest mean procalcitonin level (7.46 ± 8.07ng/dl) followed by those with blood culture positive sepsis (4.60±5.99ng/dl) then controls (1.58±3.64ng/dl). The difference in mean procalcitonin values between diagnostic categories was statistically significant (p=0.000). The median PCT levels in neonates with blood culture negative sepsis and blood culture positive sepsis were 1.28ng/ml and 0.93ng/ml respectively. (Table 5a)

Serum Procalcitonin levels (ng/ml)						
Variable	**Controls (n=60)**	**Culture positive sepsis (n=19)**	**Culture negative sepsis (n=36)**	**Total (n=120)**	**ANOVA**	**p-value**
Mean ± SD	1.58 ± 3.64	4.60 ± 5.99	7.46 ± 8.07	3.78 ± 6.18	11.883	0.000*
Median	0.40	0.93	1.28	0.73		
Range	0.03 – 16.18	0.03 – 17.79	0.03 – 22.57	0.03-22.57		
Q_1,Q_3	0.03, 0.84	0.31, 11.11	0.52, 15.72	0.04, 5.64		

Table 5a: Comparison of serum procalcitonin levels among study subjects' diagnosis category.

A post-hoc test (Bonferroni test) was performed to detect the particular test groups that differ significantly (Table 5b). The mean difference in procalcitonin level between controls and those with blood culture negative sepsis was statistically significant (mean difference = 5.870).

Diagnosis category	**Controls**	**Culture positivesepsis**	**Culture negative sepsis**
Controls	1	3.011	5.870*
Culture positivesepsis		1	2.859
Culture negativesepsis			1

Table 5b: Comparison of procalcitonin of various test groups using the Bonferroni correction for multiple tests; *=Statistically significant mean difference at $p<0.05$ (p value = 0.000)

Subset analysis of procalcitonin levels among study participants' diagnosis category

A subset analysis of the procalcitonin levels among study participants' diagnostic categories was done to aid in interpretation of the results. Thirty-one (51.7%) of the well babies (controls) had procalcitonin levels less than 0.5ng/ml. Out of the 5 neonates with positive blood culture results and PCT levels >10ng/ml, 1(20%) neonate was aged less than 48 hours old. Of the 13 neonates with negative blood culture results and PCT levels > 10ng/ml, 4(30.8%) were aged less than 48 hours. (Table 6)

		Diagnosis Category	
Procalcitonin (ng/ml)	**ControlsN (%)**	**Culture positive sepsisN (%)**	**Culture negative sepsisN (%)**
<0.5	31 (51.7)	6 (31.6)	9 (25.0)
0.5- 2.0	22 (36.7)	6 (31.6)	10 (27.8)
2.0- 10.0	4 (6.6)	2 (10.5)	4 (11.1)
>10.0	3 (5.0)	5 (26.3)	13 (36.1)
Total	**60 (100.0)**	**19 (100.0)**	**36 (100.0)**

Table 6: Subset analysis of procalcitonin levels among study participants' diagnosis category.

Diagnostic value of Procalcitonin in Predicting Bacterial Infections

A receiver operating characteristics curve was used to determine the diagnostic value of procalcitonin. The area under the ROC curve was 0.51 (Figure 2). From the curve, the cut off level of PCT using the Pythogoras rule corresponded to 0.49ng/ml. Thus, PCT levels ≥0.5ng/ml is suggestive of bacterial infection.

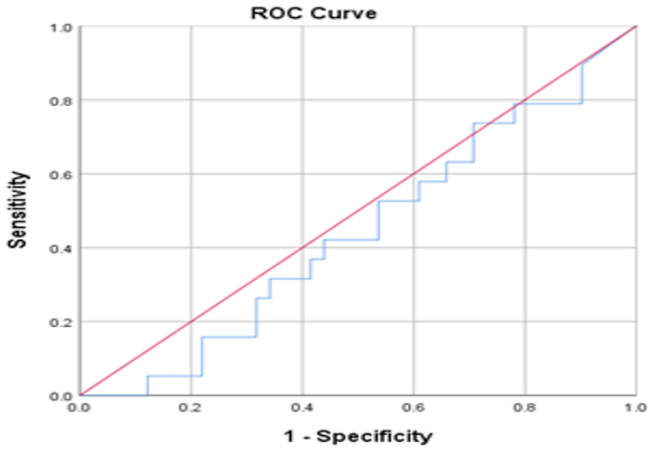

Figure 2: Receiver operating characteristics (ROC) curve to compare the diagnostic value of procalcitonin test and blood culture results

Sensitivity, Specificity, Positive Predictive Values and Negative Predictive Values of Procalcitonin

Using PCT level of 0.5ng/ml which most researchers [22-23] used, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 68.4%, 29.3%, 31.0% and 66.7%. Also, the sensitivity, specificity, PPV and NPV were calculated using cut-off values of 1.1ng/ml, 2.0ng/ml and 10.0ng/ml suggested by Adib et al [24] as shown in (Table 7).

PCT values (ng/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (AUC)
0.5	68.4	29.3	31.0	66.7	0.51
1.1	57.9	43.9	32.4	69.4	0.51
2.0	36.8	58.5	29.2	66.7	0.52
10.0	26.3	68.3	27.8	66.7	0.53

Table 7: Diagnostic value of procalcitonin in predicting bacterial infections at various cut- off.

Outcome

Six neonates among the study population died giving a case fatality rate 10.0% Of the 6 neonates that died, 3(50.0%) died from complications of sepsis, 2(33.3%) from perinatal asphyxia and 1(16.7%) from meconium aspiration syndrome. The mean procalcitonin levels among the neonates discharged or died were 5.78 ± 7.39ng/ml and 7.06 ± 8.10ng/ml respectively. There was no statistically significant difference when the PCT levels of neonates that were discharged were compared with neonates that died (p value = 0.695). The mean duration of hospital stay was 8 days (range of 1-22 days).

Discussion

The blood culture positivity rate in this study was 31.7% with majority (89.5%) being early onset sepsis (EONS). This rate was higher than the prevalence rates reported in an earlier study in Calabar [7] (27%) and Abakaliki [25] (23.8%) but lower than the prevalence rates in Jos [26] (34.4%) and Lagos (41%) [27]. The figure obtained in this study may be due to the fact that most (60%) of the participants were outborns where antenatal and delivery practices may have been suboptimal. Furthermore, this study was prospective in nature and mothers and babies who had prior antibiotics intake were excluded, unlike the studies done earlier in Calabar [7] and Abakaliki [25].

Gram positive organisms were the predominant bacteria isolated, with Methicillin- susceptible Staphylococcus aureus being the commonest. The observation of S. aureus being the prevalent bacteria isolate in this study agrees with reports of two previous studies [3,28] carried out at the University of Calabar Teaching Hospital, Calabar. This is also similar to the findings documented by Ambe et al. [29] in Maiduguri but in contrast to the findings of West and Peterside [30] in Port harcourt and Onyedibe et al. [26] in Jos. Interestingly, Acinetobacter baumannii was isolated for the first time in a hospital-based study in Calabar. The technique of bacterial isolation and identification has improved from previous studies [3,28] in Calabar. In the past, majority of the gram-negative organisms were grouped as Unclassified Coliforms [3,28]. Surprisingly, the organism Escherichia coli was not isolated in this study. It is difficult to adduce a reason for the constant occurrence of S. aureus over the years as the commonest cause of neonatal sepsis in Calabar.

The serum procalcitonin level was elevated in neonates with suspected sepsis when compared with the controls. This is similar to the reports of another study in Nigeria [7] as well as in Rome [5] and Iran [24]. The high serum PCT level seen in the subjects is likely due to systemic inflammatory response to bacterial infection. In-addition, the optimum cut-off level in this study for diagnosis of neonatal sepsis was 0.5ng/ml. A cut-off level of 0.5ng/ml was also used by Arowosegbe et al. [7] and Zahedpasha et al. [23]. However, Adib et al. [24] used subjects in the same age range as this study but documented a cut-off value of 1.1ng/ml. To the best of the author's knowledge, there is no known internationally validated cut-off value, since the cut-off value is dependent on the sample population and the test kit used in the PCT analysis. Majority (80.0%) of the study population was 48 hours old, and during this period, physiological increase in serum PCT levels in healthy newborns is expected. This may have played a role in the cut off value obtained in this study. This underscores the need to develop different reference ranges for the first 72 hours of life applicable to newborn infants at different hours of age as a potential solution to this grey area.

Interestingly, the serum PCT levels of neonates with blood culture negative sepsis was higher than the serum PCT levels of neonates with blood culture positive sepsis. One would have expected neonates with blood culture positive sepsis to have higher levels of PCT. The finding of this study is similar to the report of Zahedpasha et al [23]. The reason for this reversal is unclear. It is known that PCT increases with inflammatory reaction which neonatal sepsis is a cause, but surprisingly culture positive sepsis did not elaborate high PCT as envisaged. Possibly, birth stressors like perinatal asphyxia and meconium aspiration syndrome may have contributed to the high levels of serum PCT among the neonates with blood culture negative sepsis. In addition, the reduced response to inflammatory markers seen in overwhelming sepsis cannot be excluded until further studies are undertaken. This makes PCT insignificant in clinical practice since it cannot discriminate discretely septicaemia. Its use in the hospital will not support rational antibiotic use which is one of the goals of this study.

The few patients with neonatal malaria showed low values of PCT. This is not surprising since uncomplicated malaria produces low systemic inflammatory reaction [19]. The study also emphasizes that not all neonates being evaluated for suspected sepsis eventually have a definitive diagnosis of sepsis. Some of these neonates have isolated malaria infection. This underlines the reason why blood smear for malaria parasite is now a routine investigation in our neonatal unit. There was no co-existence of neonatal sepsis with malaria in this study.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) using a cut off of 0.5ng/ml were 68.4%, 29.3%, 31.0% and 66.7% respectively. The sensitivity reported is similar to the finding in Pakistan [30] but lower than the sensitivity reported in an earlier study in Nigeria [7] as well as in Rome [5] and Malaysia [31]. However, the specificity derived from this study is higher than the finding reported in Nigeria [7] and Iran [18] though, other studies [5,22] have reported higher specificities when compared to the finding of this study. Procalcitonin was not accurate as a sole diagnostic marker of neonatal sepsis in this study because of its lack of specificity. This agreed with earlier studies [30,32] that PCT was not a reliable sole diagnostic marker rather it should be used in combination with other acute phase reactants like C-reactive protein. The differences in the sensitivity, specificity, PPV and NPV in this study when compared with other published studies [22,24] may be due to heterogeneity of the study population, different sampling techniques as well as different assay methods used. It is possible the results may have been different if the sample size was higher than 60.

The case fatality rate in this study was 10.0%. This rate is lower than 19% and 19.3% documented in previous studies [3,28] in Calabar and 23.8% in Maiduguri [29] respectively. The relatively high case fatality rate despite advancement in neonatal care highlights the role of co-morbidities such as birth asphyxia and meconium aspiration syndrome in contributing to neonatal deaths.

Limitations

Analysis of PCT was not repeated after treatment of patients for comparison. Another limitation of the study was that only one acute phase reactant was assessed.

Conclusion

The blood culture positivity rate in the University of Calabar Teaching Hospital is 31.7% with 89.5% of the sepsis being early onset sepsis. The commonest organism isolated was Methicillin-susceptible Staphylococcus aureus. The serum procalcitonin level was elevated in neonates with suspected sepsis when compared with the controls. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) using a cut off of 0.5ng/ml were 68.4%, 29.3%, 31.0% and 66.7% respectively. Procalcitonin could not discretely differentiate blood culture positive sepsis from blood culture negative sepsis due to the high procalcitonin levels in neonates with blood culture negative sepsis. Thus, the use of PCT in the diagnosis of neonatal sepsis in this subregion is clinically insignificant.

Data Availability Statement

The datasets generated for this study are available on request to the corresponding author.

Ethics Statement

The study was reviewed and approved by the Health Research Ethics Committee of the University of Calabar Teaching Hospital, Calabar. For each baby, a written informed consent was also obtained from the parent or guardian before recruitment and collection of samples.

Author Contributions

EN designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. OH, OS, EE designed the data collection instruments, reviewed and revised the manuscript. OH, OS and AM designed the study, coordinated and supervised data collection. EN and EE were involved in data collection. AM critically reviewed the manuscript for intellectual content. All authors approved the final manuscript and agree to be accountable for all aspects of the work.

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Conflict Of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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