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Arowosegbe AO
Ojo DA
Dedeke IOF
Shittu OB
Akinloye OA

Diagnostic value of procalcitonin in neonatal sepsis

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Arowosegbe AO (✉)
Ojo DA, Shittu OB
Department of Microbiology,
Federal University of Agriculture,
Alabata, Abeokuta, Ogun State,
Nigeria.
Email: adearowosegbe@gmail.com

Dedeke IOF
Department of Pediatrics,
Federal Medical Centre, Abeokuta,
Ogun State, Nigeria.

Akinloye OA
Department of Biochemistry,
College of Biosciences, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Abstract*: Introduction:* Neonatal sepsis is a major cause of mortality in developing countries. Accurate and quick diagnosis are difficult because clinical presentation are non-specific, bacterial cultures are time-consuming and other laboratory tests lack sensitivity and specificity. Serum procalcitonin (PCT) has been proposed as an early marker of infections in neonates.
Objectives: This study investigated the value of PCT in the diagnosis of Neonatal Sepsis.
Methods: Neonates undergoing sepsis evaluation at the Special Baby Care Unit, Federal Medical Centre, Abeokuta, Nigeria between January and April 2013 were included. Blood samples were obtained for white cell count, blood cultures, serum CRP and PCT analysis. Neonates were categorised into Proven Sepsis, Suspected Sepsis and Clinical Sepsis groups on the basis of laboratory findings and risk factors. A control group with no clinical and biological data of infection was also included. Predictive values and area under the receiver operating characteristic curve (AUC) of PCT were evaluated.
Result: Of the 85 neonates, 19 (22.4%) had positive blood culture. PCT level was significantly higher in neonates in all sepsis groups in comparison with those in the control group ($P< 0.05$). At a cut-off of 0.5 ng/ml, the negative predictive value (NPV) of PCT was 80% and the positive predictive value (PPV) 39%. There were no significant statistical difference between the AUC values of PCT in Early onset and Late onset sepsis, as well between AUC in Preterm and term cases. A higher percentage of neonates who died (96%) had elevated PCT levels compared to those who survived (46%).
Conclusion: These findings support the usefulness of the PCT in diagnosis of Neonatal sepsis.

Keywords: Neonatal Sepsis**,** Diagnosis, Procalcitonin, Receiver Operating Characteristic Curve

Introduction

Neonatal sepsis is one of the important causes of neonatal morbidity and mortality particularly in the developing countries[1]. An early diagnosis of neonatal septicaemia helps the clinician in instituting antibiotic therapy at the earliest, thereby reducing the mortality rates in the neonates. Early recognition and diagnosis of neonatal sepsis are difficult because of its variable and non-specific clinical presentation. Isolation of the causative microorganisms by using blood culture has been the gold standard method for its diagnosis. However, as pathogens in blood cultures are only detected in approximately 25% of patients, the sensitivity of blood culture is suspected to be low[2]. Besides, it is impractical to obtain blood sample for serial blood culture from infants[3]. Therefore, there is need for newer diagnostics methods to obtain a rapid indication of the infectious status of neonates with suspected sepsis. In recent years, the search for diagnostic tests for sepsis in newborn infants has turned to cytokines as well as to other substances associated with the inflammatory response, in some cases induced by cytokines, as possible indicators of infection.

Among them, serum procalcitonin (PCT) is one of the most promising[4]. PCT, a 116-amino-acid protein with a molecular weight of 13 KDa, is the precursor in the synthesis of calcitonin (CT). Firstly demonstrated to increase at the onset of bacterial infection and sepsis by

Assicot et al in 1993[5], this acute phase reactant has the characters of acute phase proteins, hormones and cytokines. Serum PCT concentration rises 2-4 hours after endotoxin injection, reaches its peak level right after 6 hours, maintains a plateau through 8 to 24 hours[6] and decreases to its normal level if the infection stimulus stops. It has been reported to be a reliable marker for severe bacterial infections and sepsis[5].
Procalcitonin levels are undetectable in healthy individuals and slightly increased in severe viral infections and non infectious inflammatory responses[7]. The results of recent studies suggest the usefulness of PCT for early diagnosis of neonatal sepsis[8, 9], although other investigators have observed lack of accuracy for this marker[10]. This study aims at evaluating procalcitonin as an early or first line marker in the diagnosis of neonatal septicemic infection.

Methods

In this prospective cohort study, all neonates undergoing evaluation for sepsis at the Federal Medical Centre, Abeokuta between January and April 2013 were eligible for inclusion. Written consent was obtained from the parents/guardians of all the investigated neonates. Ethical clearance was obtained from the Research and Ethical Clearance Committee of the hospital. For each baby, a written informed consent was also obtained from the parent(s) or guardian. Neonates suspected on clinical grounds to have sepsis were included in the study at the point of admission or while on admission in the hospital. Exclusion criteria were obvious congenital anomalies or prior antibiotic therapy. The clinical criteria for the evaluation of sepsis were: Maternal risk factor such as fever, prolonged rupture of amniotic membrane >24hr; Neonatal history: low birth weight (< 2500 grams), preterm birth (<37 weeks); Signs and symptoms of sepsis: Respiratory distress, refusal to feed, convulsion, poor cry, abdominal distension, high pitched cry, irritability, apnea, and palor.

A sepsis work-up which included white cell count (WCC), blood culture and PCT and CRP level determination was done for all neonates enrolled. Procalcitonin level was determined using the BRAHMS PCT-Q test kit, a semi-quantitative detection of PCT. At a PCT concentration ≥ 0.5 ng/ml, this sandwich complex can be seen as a reddish band. The colour intensity of the band of a PCT Q test kit is directly proportional to the PCT concentration of the sample and is related to the following PCT concentration ranges:< 0.5 ng/ml (At PCT < 0.5 ng/ml - Systemic infection/sepsis is not likely[11]), ≥ 0.5 ng/ml (At PCT > 0.5 and < 2 ng/ml- Moderate risk for progression to severe systemic infection[11]), ≥ 2 ng/ml, (PCT> 2 and < 10 ng/ml- High risk for progression to severe systemic infection[11]), ≥ 10 ng/ml. (PCT > 10 ng/ml-High likelihood of severe sepsis or septic shock[11])
According to clinical symptoms of sepsis, microbiologic and laboratory results, neonates classified in to different categories of infection (similar to previous studies by Magudumana *et al*., 2000[13],White*et al*., 2007[21]and Ballot *et al*., 2004[12]) as follows: (a) Group I (proven sepsis): Clinical signs and symptoms with positive blood culture. (b) Group II (suspected sepsis): Clinical signs and symptoms with negative bacterial culture but with positive screening test (CRP, WCC). (c) Group III (clinical sepsis): Clinical signs and symptoms with negative bacterial culture and negative screening test. Patients were placed into 3 groups rather than just present and absent infection as it is acknowledged that some babies with sepsis will have negative blood cultures. Excluding the patients with possible infection would result in the potential exclusion of some patients with actual infection[12]. A control group consisting of 12 healthy neonates with no clinical and biological data of infection in an immunization clinic prior to immunization was also included in the study.

Data was analyzed using SPSS for windows version 17.0. Statistical test between variables was done using Chi-squared test ($\chi 2$). Where the numbers in a cell was less than five, a Fisher's exact test was used. Differences between groups were assessed by z-test. A p value <0.05 was considered significant for all statistical tests. Diagnostic efficiency was defined by sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). In line with Ballot et al., 2004[12], Group I and Group II patients were regarded as positive, whereas Group III group was regarded as negative. Receiver operating characteristic (ROC) curves were also constructed. . The closer the ROC curve is to the upper left corner, the higher the overall accuracy of the test[14].If the p-value of an ROC curve is less than 0.05 (P<0.05), there is evidence that the laboratory test does have an ability to distinguish between the two groups.

Results

Patients'Characteristics

There were 180 neonates admitted during the study period and 105 of them met the inclusion criteria. Twenty of these 105 neonates were excluded from the study. The study group included 85 neonates with suspected (presumed and probable) neonatal sepsis (Table 1). Of these 85 neonates, 45 were females (52.1%) and 40 were males (47.9%). The mean age of the participants at enrolment was 3.4±0.5 days and the mean weight was 2.5± 0.8 Kg. Blood culture was positive in 19 (22.4%) of the neonates. The sepsis grading and the differences between sepsis groups are shown in Table 1.

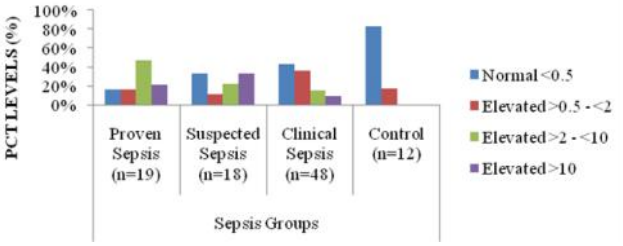
Table 1: Sepsis Grading and Characteristics of Study Population

Characteristics	Total (n= 85)	Group I (n= 19)	Group II (n= 18)	Group III (n= 48)
Age				
<3 Days	55	14 (25.5)	08 (14.5)	33 (60)
> 3 Days	30	5 (16.7)	10 (30.3)	15 (50)
EGA				
Preterm	32	11 (34.3)	03 (09.4)	18 (56.3)
Term	53	08 (15.1)	15 (28.3)	30 (56.6)
Birth weight / g				
Low	39	10 (25.6)	08 (20.5)	21 (53.8)
Normal	46	09 (19.6)	10 (21.7)	27 (58.7)

Procalcitonin Results

Fifty five (64.7%) of the patients had elevated PCT values (>0.5ng/ml). Elevated PCT levels were recorded in 84.2%, 66.7%, 57.4% and 16.7% of neonates in the proven, suspected, clinical and control groups respectively.

Fig 1: Procalcitonin Levels In Sepsis Groups



PCT level was significantly higher (PCT >0.5) in infants in all sepsis groups (Proven, Suspected or Clinical) compared with the control group (p-value < 0.05) More than two-third of patients with proven sepsis and more than half of patients with suspected sepsis had PCT values indicating a high risk for progression to severe systemic infection or septic shock. Majority of patients with clinically suspected sepsis had PCT values indicating a low or moderate risk for progression to severe systemic infection.

Sensitivity, Specificity, PPV and NPV

As cut off values increased, Specificity, PPV, and FN increased while Sensitivity and FP decreased. Overall, using a cut-off of 0.5 ng/ml, the NPV of PCT was 69% and the PPV 50%. (Table 2).

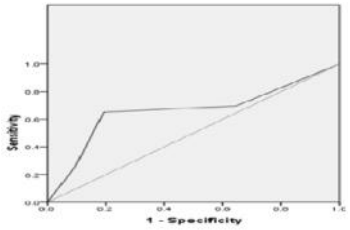
Table 2: Predictive Values For Various Cut-Offs of PCT

	Cut off Values 0.5ng/mL	2.0ng/Ml	10ng/Ml
Sensitivity	75.7	62.2	27.0
Specificity	41.7	77.1	91.7
PPV	50.0	67.7	71.4
NPV	69.0	72.6	62.0
FP	58.3	22.9	8.3
FN	24.3	37.8	73.0

Receiver operating characteristics (ROC) analysis

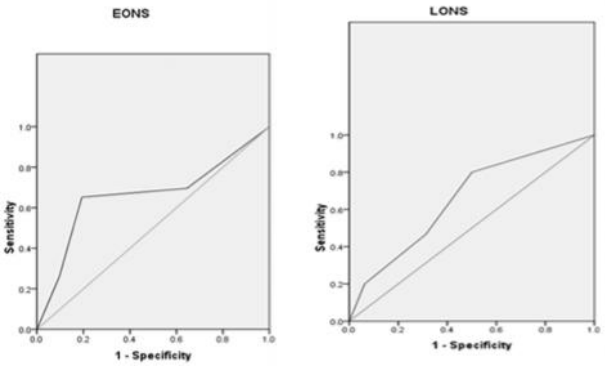
The ROC AUC for the whole group was 0.686 (Fig: 2). No significant statistical difference was found between the AUC values of PCT in Preterm or Term cases.

Fig 2: ROC Curve for all neonates (Area under the curve = 0.686)



Similarly, there was no statistically significant difference between the AUC values of PCT in EONS or LONS cases.

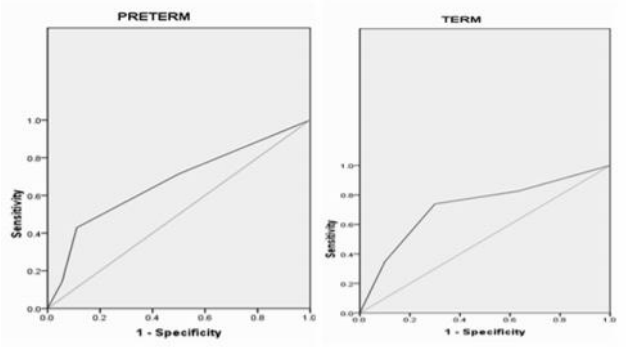
Fig 3a and b: ROC curves comparing the sensitivity and specificity of serum PCT in neonates with EONS and LONS (Area under the curves = 0.662 and 0.658 for EONS and LONS respectively).



(a) (b)

A higher proportion of neonates with respiratory distress and convulsion compared to neonates without these sign/symptom had elevated PCT levels. These differences were statistically significant. (p<0.05) Also, a higher percentage of neonates who died compared to neonates who were discharged had elevated PCT levels and the difference was statistically significant. (p<0.000)

Fig 4: ROC curves comparing the sensitivity and specificity of serum PCT in Preterm and Term neonates (Area under the curves = 0.671 and 0.722 respectively).



(a) (b)

Table 3: Procalcitonin Levels in relation to Clinical Findings and Outcome of Management

Clinical Signs/ Symptom and Outcome of Management	Variables	Total	Normal (n=30)	Elevated (n=55)	P value
Respiratory distress	Present	39	09 (23.1%)	30 (66.9%)	0.030
	Absent	46	21 (45.7%)	25 (54.3%)	
Poor Cry	Present	11	02 (18.2%)	09 (81.8%)	0.350
	Absent	74	28 (37.8%)	46 (62.2%)	
Palor	Present	03	00 (0.0%)	03 (100%)	0.492
	Absent	82	30 (36.6%)	52 (63.4%)	
Abdominal distension	Present	04	01 (25%)	03 (75%)	
	Absent	81	29 (35.8%)	52 (64.2%)	
Fever	Present	21	05 (23.8%)	16 (76.2%)	0.204
	Absent	64	25 (39.1%)	39 (60.1%)	
High Pitched Cry/ Irritability	Present	04	03 (75%)	01 (25%)	0.243
	Absent	71	27 (38%)	54 (62%)	
Apnea	Present	04	00 (0.0%)	04 (100%)	0.328
	Absent	81	30 (37%)	51 (63%)	
Convulsion	Present	21	03 (14.3%)	18 (85.7%)	0.031
	Absent	64	27 (42.2%)	37 (57.8%)	
Refusal to Feed	Present	23	06 (26.1%)	17 (73.9%)	0.279
	Absent	62	24 (38.7%)	38 (61.3%)	
*Outcome of Management	Discharge	48	26 (54.2%)	22 (45.8%)	0.000
	Death	27	01 (3.7%)	26 (96.3%)	

*Ten of the neonates were discharged against medical advice and were excluded from this analysis

Discussion

Neonatal sepsis still remains a diagnostic and treatment challenge for the neonatal health care providers. This challenge leads to the over treatment of large number of neonates who present with clinical suspicion of sepsis[15]. In recent years measurement of procalcitonin and other inflammatory mediators have been reported as sensitive parameters for the early diagnosis of neonatal sepsis and evaluating its outcome[16]. The aim of this study was to evaluate PCT as diagnostic marker for neonatal sepsis.
The incidence of culture-proven sepsis was low (22%). This is consistent with the reports of incidence of 20% and 25.7% by Adeleke and Belonwu, 2006[17], and Naher and Khamel, 2013[18] respectively. This corroborates previous reports on the low sensitivity of blood culture in neonatal sepsis[2]. PCT levels were high in the neonates with proven and suspected sepsis cases. This finding was similar with reports of some studies[19, 20]. Three neonates in the proven sepsis group had PCT lower than 0.5ng/ml. These neonates were preterm and two had very low birth weights. Two of the 12 neonates in the control group had procalcitonin higher than 0.5 ng/ml. This may be due to physiological increase of procalcitonin, reported up to 21-48 hr postpartum, even in the absence of infection. The postnatal increase of PCT observed in the healthy neonate with peak values at 24 h of age most likely represents endogenous synthesis[21].

In this study, at a cut-off point of 0.5ng/ml, the sensitivity and specificity, PPV and NPV of PCT in neonatal sepsis was found to be 89% and 23%, 84% and 83% respectively for proven infection. This high sensitivity and NPV of PCT is consistent with the reports of Ballot *et al*., 2004[12]in South Africa and Sucilathangam, *et al*., in 2012[20] in India. White, *et al*., in 2007[22]in South Africa recorded similar report of NPV of 80%, but a lower sensitivity of 48%. NPV and Sensitivity increased to 100% and specificity 56% as cut off values increased to 10 while PPV dropped to 21%.In the study of White, *et al*., in 2007[22], increased cut-off value (10.1 ng/ml) had no effect on the NPV, worsened the sensitivity (98% v. 22%, respectively), but improved the PPV (78% v. 79%, respectively), and the specificity (74%v.98%).
ROC analysis for PCT had an area under the curve (AUC) of 0.686 which is similar to reports of White, *et al*., 2007[22], where ROC analysis had an area of 0.631. Boraey, *et al*., 2012[23] reported an AUC value of 0.92 for PCT at a cut off value of 1.3ng/ml. AUC values were 0.662 and 0.658 for Preterm and Term neonates respectively without any significant statistical difference. This is in agreement with the reports of White *et al*., 2007[22].Also, no significant statistical difference was found between the AUC values of PCT in EONS and LONS cases. This suggests that the PCT seems to be equally accurate for the diagnosis of neonatal sepsis in preterm and term neonates; as well as in EONS and LONS cases.

The result from this study suggests PCT as a good predictor of mortality as almost all neonates who died (96.3%) had elevated PCT. This is in agreement with Adib, *et al*., 2012[24].

Limitation

The overall diagnostic value of PCT for neonatal sepsis could probably be improved if PCT was measured more precisely with an ultrasensitive PCT assay instead of the semiquantitative assay used in this study. Another limitation of our study may be the modest prevalence culture proven sepsis in this study, which, of course, directly influences the values of PCT as a biomarker for neonatal sepsis. With the use of more sophisticated diagnostic techniques like PCR, a causative bacterial microorganism might have been demonstrated in a subset of neonates allocated to the group "suspected sepsis." Also, a better comparism would have been achieved using a sufficient population of control. The population of control in this study was limited.

Conclusion

These findings support the usefulness of the PCT to support an early diagnosis of neonatal sepsis. Results from this study suggest that any increase in PCT in an ill neonate suggests the possibility of a septicemic infection. However, PCT is not sufficiently reliable to be the sole marker of neonatal sepsis and would be useful as part of a full sepsis evaluation. A negative PCT on presentation is not exclusively sufficient to rule out sepsis, but needs to be evaluated further. PCT is also of great advantages where prediction of severity and mortality is concerned.

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