

## Original Research Article

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## Role of Procalcitonin in Diagnosis of Early Onset Neonatal Sepsis in a North Indian Tertiary Care Centre

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### ABSTRACT

Neonatal sepsis is a major cause of mortality in developing countries. Accurate and quick diagnosis is difficult because of non-specific signs and symptoms, cultures being time-consuming and other laboratory tests lack sensitivity and specificity. This study was designed to determine the sensitivity and specificity of Procalcitonin (PCT) levels, as a diagnostic marker of early onset neonatal sepsis (EONS). 150 neonates admitted to neonatal ward with signs suggestive of neonatal sepsis were recruited into the study. Prior to commencement of antibiotics, the following investigations were carried out on neonates: blood culture, PCT and C-reactive protein (CRP) estimation. The PCT levels were measured by human procalcitonin ELISA kit. The neonates were categorized into proven, probable and clinical sepsis on the basis of laboratory findings. Predictive values and area under the ROC curve of PCT were evaluated. Out of 150 neonates, 44 had positive blood cultures. PCT level was significantly higher in neonates with proven sepsis ( $p < 0.05$ ). At a cut-off of 1.32ng/ml, the sensitivity and specificity, PPV and NPV of PCT in early onset neonatal sepsis was found to be 86.3 %, 44.34 %, 39.58 % and 88.68 % respectively for proven sepsis. These findings support usefulness of the PCT in diagnosis of EONS.

#### Keywords

ELISA, Neonatal sepsis, Procalcitonin, ROC curve, Sensitivity, Specificity

#### Article Info

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### Introduction

Neonatal sepsis is defined as infection with evidence of systemic inflammatory process. (Ebrahim, 2011) It is a clinical syndrome characterized by systemic signs and symptoms of infection in the first month of life. (Aggarwal *et al.*, 2001) The incidence of neonatal sepsis in the various studies has been reported to vary from <1 to 8.1 cases per 1000 live births. (Nizet and Klein, 2011) Neonatal

sepsis is classified into early-onset neonatal sepsis (EONS <7 days of birth) and late-onset neonatal sepsis (LONS >7 days). (Abdel Mohsen and Ahmed Kamel, 2015) The source of infection in these two categories varies. (Nizet and Klein, 2011; Abdel Mohsen and Ahmed Kamel, 2015) Numerous factors such as; lack of antenatal care, unhygienic and unsafe delivery practices, prematurity and low birth weight contribute to the high morbidity and mortality. (Viswanathan *et al.*, 2011)

The diagnosis of neonatal sepsis remains a challenge. The gold standard in the diagnosis of neonatal sepsis is the isolation of pathogen from sterile sites such as blood, CSF and ascitic fluid (Labib *et al.*, 2013). Isolation of bacteria from blood is a standard and most specific method used to diagnose neonatal sepsis. Positive cultures range from 8% to 73% in the diagnosis of potential neonatal sepsis (Tripathi and Malik, 2010). The main disadvantages of many current microbiological diagnostic methods are diagnostic delays especially with culture-based methods, suboptimal sensitivity and low specificity because of contamination; in addition, some sample types are not amenable to routine diagnostics because of their invasive nature (Tripathi and Malik, 2010; Nasir *et al.*, 2015).

It is therefore imperative to identify other markers that can help in early diagnosis. Several serum biomarkers have been identified in the recent years that have the potential to help diagnose local and systemic infections, differentiate bacterial and fungal infections from viral syndromes or noninfectious conditions, prognosticate, and ultimately guide management, particularly of antibiotic therapy. In pediatrics, the most frequently employed biomarker to differentiate sepsis from non-infectious systemic inflammatory response syndrome (SIRS) is the C-reactive protein (CRP), which, however, is highly non-specific and has an unfavorable kinetics (Póvoa *et al.*, 2005). Among the different molecules investigated as biomarkers of sepsis, procalcitonin (PCT) seems to be one of the most promising and most extensively studied biomarker (Pacífico *et al.*, 2013; Rowland *et al.*, 2015).

PCT is the precursor of calcitonin that does not demonstrate hormonal activity. It is a 116 amino acid peptide with a molecular weight of 14.5 kDa. PCT is synthesized in the C cells of

the thyroid gland of healthy individuals. In healthy people, PCT levels are very low. In systemic infections, including sepsis, PCT levels are generally at least 0.5 to 2 ng/mL, and often reach levels of greater than 10 ng/mL. Higher levels correlate with the greater severity of illness and poorer prognosis (Nasir *et al.*, 2015). This makes PCT is a potential diagnostic variable for the diagnosis of bacterial infection.

Diagnosis of sepsis is however, far too complex to be reduced to a single cut off of PCT marker. However, its levels may be influenced by antimicrobial pre-treatment and non-infectious conditions like severe trauma and some autoimmune disorders (Nasir *et al.*, 2015). In spite of these limitations; PCT has the potential to guide initiation of appropriate therapy at the earliest and reduce the emergence and spread of antibiotic resistance. There is a need to extensively evaluate and possibly consider serum PCT as an adjunctive marker to be combined to other commonly used approaches to enhance prompt and rational clinical management, before blood culture results become available (Nasir *et al.*, 2015). The present study was aimed to assess the usefulness of serum PCT as a diagnostic marker of neonatal sepsis in our setting and its correlation with blood culture positivity.

## **Materials and Methods**

The prospective study was conducted on neonates admitted for sepsis workup in the Neonatal ICU of Kalawati Saran Children Hospital (KSCH), New Delhi from November 2016 to March 2018. This study was conducted in the Department of Microbiology in collaboration with Department of Neonatology at LHMC, New Delhi. This study was approved by the Institutional Scientific and Ethical Committee, and written informed consents were obtained from the parents.

A total of 150 neonates with suspected sepsis who required sepsis evaluation were considered. The inclusion criteria were neonates who were admitted to the NICU with clinical signs suggestive of sepsis.

The exclusion criteria were infants who were on antibiotics or those who had congenital anomalies. The clinical criteria for the evaluation of sepsis were: Maternal risk factors; such as fever, prolonged rupture of amniotic membrane >24hr; Neonatal history such as low birth weight (< 2500 grams), preterm birth (<37 weeks); Signs and symptoms of sepsis like respiratory distress, refusal to feed, convulsion, poor cry, abdominal distension, high pitched cry, irritability, apnea, and palor.

### **Specimens and tests performed**

Before starting antibiotic therapy conventional sepsis workup was carried out in all cases including: CBC counts, chest x-ray, urine culture, CSF culture and 0.5 mL blood sample for blood culture.

### **Sepsis screen**

It was determined for all the neonates enrolled in the study. Positive sepsis screen was considered if any two of the following parameters were present: TLC (<5000/mm<sup>3</sup>), Immature neutrophils to TLC ratio >0.2, CRP positive, micro ESR>10 mm-first hour, Chest X-ray (suggestive of pneumonia)

### **Blood culture**

Blood culture was performed by automated blood culture system in all the cases. Approximately 0.5 ml of blood was inoculated aseptically into BacT/Alert pediatric blood culture bottle. BacT/Alert bottles were incubated in BacT/Alert 3D system for 7 days. Growth obtained was identified by standard

microbiological techniques. Identification of the organisms was based on cultural characteristics, results of various tests and biochemicals. Samples from other sites; as CSF, urine & other body fluids were also processed for diagnosing neonatal sepsis, wherever necessary (Nizet and Klein, 2011).

### **PCT assay**

Blood samples were centrifuged within 30 minutes of collection. Serum was stored at -20 degree Celsius before analysis. PCT levels were estimated by a quantitative ELISA kit (BioVendor Human Procalcitonin ELISA) in serum. The test was performed and interpreted as per the manufacturer's instructions.

According to clinical symptoms of sepsis, microbiologic and laboratory results, neonates were classified into different categories of infection as follows: (a) Group I (proven sepsis): Clinical signs and symptoms with positive blood culture. (b) Group II (probable sepsis): Clinical signs and symptoms with negative bacterial culture but with positive septic screen. (c) Group III (clinical sepsis): Clinical signs and symptoms suggestive of systemic inflammatory response (SIRS) with negative bacterial culture and negative screening test.

### **Data collection and management**

Self-designed, pre tested proforma was used to collect demographic data, clinical presentation, associated risk factors (maternal & neonatal), results of the laboratory investigation generated during the admission.

### **Statistical analysis**

All the statistical data analysis was done with the help of the SPSS version 22. For qualitative and quantitative data, Chi square test and Fisher exact test were done to analyze

the data. p value less than or equal to 0.05 was considered to be statistically significant.

## Results and Discussion

There were total of 150 neonates with suspicion of sepsis admitted during the study period. Majority (84.6%) presented with respiratory distress followed by tachycardia (42%), fever (31%), hypothermia (22%), lethargy (15%). Other signs and symptoms were poor feeding, anterior fontanelle bulging, anemia of prematurity, delayed cry, abdominal distension, jaundice etc. Males (70.40 %) were predominantly affected than females (29.50%). However, this difference was statistically not significant ( $p=0.07$ ).

Blood culture was positive in 44 (29.3%) of the neonates. Of the 44 blood culture isolates obtained, 30(68%) were gram negative bacteria followed by gram positive bacteria 14(32%). No organism was isolated from CSF or other sterile fluids. Most common organism isolated was *Klebsiella* spp. (36.6%) to be followed by *Staphylococcus aureus* (22.7%), *Acinetobacter* spp. (20.4%), *Enterococcus* spp. (9%), *Escherichia coli* (9%) and *Pseudomonas aeruginosa* (2%). The sepsis grading and the differences between sepsis groups are shown in Table 1. Out of 150 neonates, PCT was raised in 97 neonates (64.6%). The mean value of PCT was significantly higher ( $p$  value  $<0.0001$ ) in proven cases ( $3.39\pm 2.55$ ) ng/ml than probable ( $2.07\pm 2.89$ ) ng/ml and suspected cases ( $1.92\pm 2.91$ ) ng/ml. This was analysed by Kruskal Wallis test (Figure 1).

The analysis of the receiver operating characteristic (ROC) curves is depicted in figure 2 and it revealed that the best cut-off value for PCT in our study population was 1.32 ng/ml. The area under the ROC curve (95% CI) was 0.79(0.71-0.87). PCT was found to be positive in higher proportion (86.36%) of

culture positive cases and we found a highly significant association between PCT levels and cultures. ( $p=0.0003$ ) In this study, at a cut-off point of 1.32 ng/ml, the sensitivity and specificity, PPV and NPV of PCT in early onset neonatal sepsis was found to be 86.3 %, 44.34 %, 39.58 %, 88.68 % respectively for proven sepsis (Table 2).

In the present study, regarding the clinical profile of the 150 clinically suspected neonates, majority (84.6%) presented with respiratory distress followed by tachycardia (42%), fever (31%), hypothermia (22%) and lethargy (15%). This is consistent according to the National Neonatal Perinatal Database (NNPD) 2000 report (National Neonatology Forum, 2000) and the study by Chacko *et al.*, (2005).

The culture positivity rate in the current study was 29.3 %. The rate reported in other studies varied from 21% by Naher *et al.*, (2013), 23% by Kuruvilla *et al.*, (1998) and 18.2 % by Nwadioha *et al.*, (2010). However some studies have reported a higher rate of culture positivity (Zakariya *et al.*, 2011; Tallur *et al.*, 2000; Mishra *et al.*, 2006). This difference could be because of differences in the blood volume used for culture, blood culture techniques applied, administration of antimicrobials to the mother or neonate before the collection of sample. Although bacteria are the most common agents implicated in neonatal sepsis, it can also be caused by organisms other than bacteria like viruses and fungi. Therefore, only a proportion of the blood culture from cases with clinical sepsis will be positive for pathogenic organisms.

The detection of microorganisms in the blood of a neonate with sepsis has a great therapeutic and prognostic significance. In view of this, timely detection of blood borne pathogens is one of the most important functions of a microbiology laboratory.

**Table.1** Sepsis grading and characteristics of neonatal sepsis (n=150)

Characteristics	Total (n=150)	Group I (n=44) Proven sepsis	Group II (n=62) Probable sepsis	Group III (n=44) Clinical sepsis
Age				
< 3 days	128 (85.3%)	33 (25.7%)	55 (43%)	40 (31.3%)
> 3 days	22 (14.6%)	11 (50%)	7 (31.8%)	4 (18.2%)
EGA*				
Preterm	119 (79.3%)	38 (32%)	54 (45.3%)	27 (22.7%)
Term	31 (20.7%)	6 (19.3%)	8 (25.8%)	17 (54.9%)
Birth weight/grams				
Low	127 (84.6%)	41 (32.2%)	53 (41.7%)	33 (26%)
Normal	23 (15.4%)	3 (13%)	9 (39.1%)	11 (47.9%)

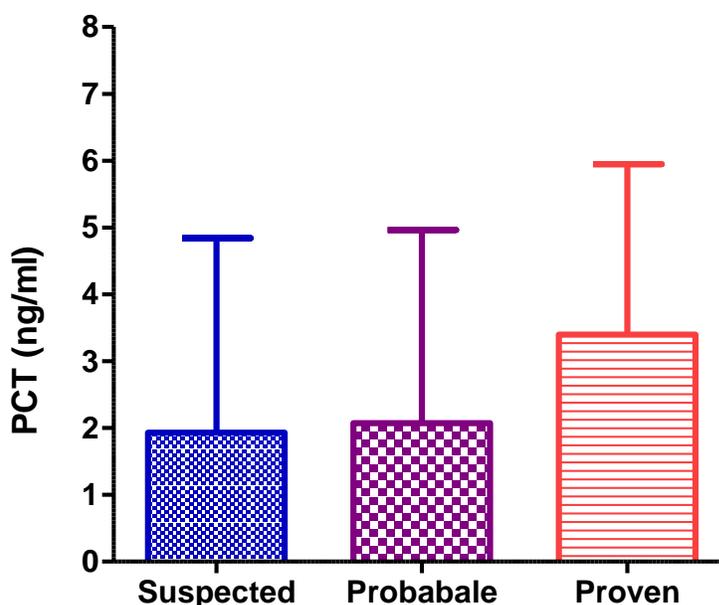
\*EGA: Estimated gestational age

**Table.2** Sensitivity and specificity of procalcitonin levels as a diagnostic marker of early onset neonatal sepsis

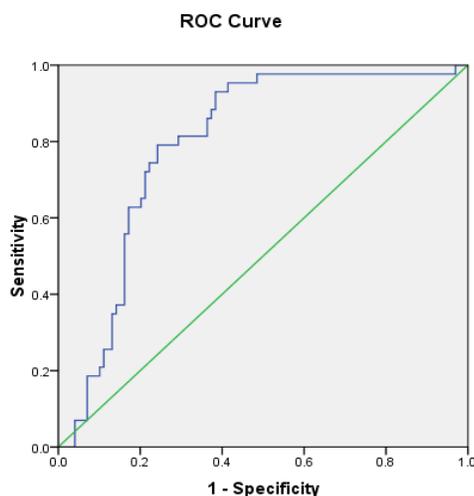
Blood Culture	PCT >1.32 ng/ml	PCT <1.32 ng/ml	Sensitivity (95% CI)	Specificity(95% CI)	PPV	NPV
Positive	38	6	86.36%	44.34 %	39.58%	88.68%
Negative	59	47	(69.93% -93.36%)	(31.60% - 51.76%)		

\*CI= Confidence Interval, PPV= Positive Predictive Value, NPV= Negative Predictive Value

**Fig.1** Procalcitonin profile in all cases included in the study (n=150)



**Fig.2** Figure of ROC curve



In the current study, using BacT/ Alert system, majority (72%) of clinically significant pathogens were recovered within the first 12 hours of incubation and all pathogens were recovered within 24 hours. These findings are similar to those of Gopi *et al.*, (2014) and Pal *et al.*, (2009) using the BACTEC system.

In the present study gram negative organisms (68%) were more common than gram positive organisms (32%) as etiological agent which is in accordance with many other studies (Viswanathan *et al.*, 2011; Kuruvilla *et al.*, 1998; Nwadioha *et al.*, 2010; Zakariya *et al.*, 2011; Tallur *et al.*, 2000).

Assicot *et al.*, (1993) were the first to describe procalcitonin as a potential biomarker for sepsis and infection in 1993, and since then many studies on PCT have been carried out. The cut-off of PCT used in our study was determined by the ROC curve. The range of physiological concentrations in neonates is wide and depends on the day of life (Chiesa *et al.*, 2000). At the same time, the sensitivity and specificity for the diagnosis of infection, depend on the subjectively presumed cut-off

point (Chiesa *et al.*, 2000; Ugarte *et al.*, 1999). In this study, at a cut-off point of 1.32 ng/ml, the sensitivity and specificity, PPV and NPV of PCT in early onset neonatal sepsis was found to be 86.3 %, 44.34 %, 39.58 %, 88.68 % respectively for proven sepsis. This high sensitivity and NPV of PCT is consistent with the studies of Ballot *et al.*, (2004) in South Africa and Sucilathangam *et al.*, (2012) in India and Zahedpasha *et al.*, (2009) in Iran. Thus it can be inferred that PCT assay is an effective tool to help to diagnose sepsis.

PCT values in cases of neonatal sepsis with gram negative organisms were higher (mean value 3.633 ng/ml) than those with gram positive organisms (mean value 2.941 ng/ml). This finding is similar to Yu *et al.*, (2015).

Cases with a very high PCT value suggest more likelihood of gram negative bacterial blood infection. Yu *et al.*, (2015) this may be explained by the fact that gram-negative bacteremia induces a greater inflammatory response than gram-positive bacteremia and hence the higher PCT levels in gram-negative bacteremia (Reinhart *et al.*, 2012; Brodská *et al.*, 2013).

In the present study, the specificity of PCT was 64.5% at cut-off of >1.32 ng/ml. In the study by Kordek *et al.*, (2008), the cut-off point for PCT in cord blood was 1.22 ng/mL, sensitivity of 80.43%, specificity of 71.67%, PPV of 35.24% and NPV of 95.03%. Chan Y-L *et al.*, (2004) reported sensitivity of 69.5% and specificity of 64.5% for PCT, using a cut-off of 0.6ng/ml. Janota *et al.*, (2001) indicated the sensitivity and specificity of 75% and 59% for procalcitonin respectively, at a cut-off of 2ng/ml.

The high proportion of false positive results (and therefore low specificity) could be partially explained by the normal progressive rise of PCT during the first 48 hours of life for both term and preterm neonates. The physiological peak of serum PCT concentrations in healthy neonates has been previously reported (Chiesa *et al.*) and increased PCT concentrations have also been found in neonates with very low probability of infection (Monneret *et al.*, 1997). This postnatal surge of PCT might be attributed to direct stress on the baby during the perinatal period. The normal birth process and the extrauterine adaptation stimulates an acute phase reaction in the newborn infant with a release of C-reactive protein (CRP), interleukin-6 (IL-6), and serum amyloid A (Chiesa *et al.*, 2003).

Another reason for low specificity in our study is that, majority of neonates presented with respiratory distress (84.6%), this is also consistent with previous studies, in which higher concentrations of PCT observed in uninfected neonates with respiratory disorders and hemodynamic failure compared with asymptomatic neonates (Lapillonne *et al.*, 1998). Monneret *et al.*, (1997) demonstrated elevated PCT values in uninfected newborns who presented with respiratory distress syndrome (RDS) and suggested that hypoxemia could be responsible for these

increased PCT values, providing further support for the hypothesis of pulmonary PCT synthesis. (Monneret *et al.*, 1997)

In neonates, an elevated PCT level may help in predicting sepsis; furthermore, low PCT levels were helpful in ruling out sepsis as a diagnosis. In our study, NPV was 88.68 %. The good negative predictive value found suggested that PCT can be tested rapidly and is a highly discriminating means to rule out bacteremia. Therefore, PCT assessment could help physicians limit the number of unnecessary prescriptions for antibiotics.

Rapid diagnosis by using Procalcitonin gives a reasonable degree of accuracy in early diagnosis of neonatal sepsis which will also help to initiate early antibiotic therapy. Limitations of our study were that it was single centric study, small sample size and serial sampling was not possible.

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### **Ethical approval**

The study was conducted after obtaining ethical approval from the institutional ethical committee.

### **Conflicts of interest**

The authors declare no personal or financial conflict of interest.

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