



## PROCALCITONIN VERSUS C-REACTIVE PROTEIN IN NEONATAL SEPSIS

<b>Dr. Arun Kumar T</b>	Postgraduate, Department of Paediatrics, Sree Balaji Medical College and Hospital
<b>Dr. Pooja Pradeep*</b>	Postgraduate, Department of Paediatrics, Sree Balaji Medical College and Hospital *Corresponding Author
<b>Dr. Sundari S</b>	Head of the Department and Professor of Paediatrics, Sree Balaji Medical College and Hospital

**ABSTRACT** **Objectives:** To compare between C-reactive protein (CRP) and Procalcitonin (PCT) regarding diagnosis and outcome of neonatal sepsis.  
**Design:** Prospective observational study.  
**Setting:** Neonatal Intensive Care Unit (NICU).  
**Participants:** Seventy newborn Infants admitted to NICU for sepsis management.  
**Results:** Of the total 70 Neonates admitted in NICU, 32.9%, 41.4% and 25.7% were categorized as proven sepsis, suspected sepsis and clinical sepsis respectively. Procalcitonin was positive in 100% compared to the CRP positivity in 63.6% of the proven sepsis cases. Mortality rate is significantly increased in proven sepsis compared to clinical sepsis.  
**Intervention:** Sepsis workup laboratory tests.  
**Main Outcome Measures:** Specific diagnostic and prognostic impact of the tests.  
**Conclusion:** Comparing with CRP, PCT is not only a good diagnostic measure of neonatal sepsis but also effective predictor of sepsis outcome.

**KEYWORDS :** Procalcitonin; CRP; Neonatal; Sepsis; UTI

### BACKGROUND

Urinary tract infection (UTI) is the most common serious bacterial infection in febrile children younger than 3 months, with reported rates ranging from 5% to 20% depending on different series. Neonates and infants up to age 2 months who have pyelonephritis usually do not have symptoms localized to the urinary tract. UTI is discovered as part of an evaluation for neonatal sepsis [1]. Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first month of life. The overall incidence of primary sepsis is 1-5/1000 live births. The incidence is much higher for very low birth weight infants (birth weight <1500gm) [2]. Since the clinical manifestations of sepsis in neonates are non-specific and associated with high morbidity and mortality, early suspicion and treatment before blood culture confirmation is crucial [3]. The mortality rate is high (13-25%) with higher rates seen in premature infants, those with early fulminant disease [2] and those with increasing severity of inflammation. The most severe infection is complicated by systemic inflammation and septic shock. However, to start therapy, an early reliable diagnosis is necessary [4]. Clinical signs and symptoms of infection often don't point towards the etiology. The diagnosis of sepsis is difficult because of non-specificity of clinical signs and symptoms and overlapping of symptoms with other noninfectious causes of systemic inflammation [5]. Elevated CRP levels are seen in infection, autoimmune disease, surgery, meconium aspiration and recent vaccination. Also, the CRP values do not rise significantly until almost 24-48 hr after the onset of infection [6,7].

Indeed, bacteriologic results need time and may be negative in newborns. Besides, it is impractical to blood sample for serial blood culture from infants [6,7]. So, a new laboratory method for early diagnosis of the diseases, evaluation of prognosis and treatment efficiency are needed. CRP is one of the acute phase proteins. Although it is a classical and sensitive marker of inflammation; it cannot be used to differentiate between bacterial and other infections. It is a disadvantage that CRP increases after Procalcitonin (PCT) [8].

Furthermore, CRP doesn't reliably differentiate between the systemic inflammatory response and sepsis [3,9]. PCT is another recently focused biomarker, and in recent years it has established its role as a candidate marker for the diagnosis of systemic inflammation, infection, and sepsis; both in children and adults [5,10,11]. In 1993, PCT was first described as a marker of the extent and course of systemic inflammatory response to bacterial and fungal infections [12].

PCT propeptide is the precursor protein of calcitonin and has no

hormonal activity. It is a glycoprotein having 116 amino acids [13]. Normally it is produced by the C cells of the thyroid gland. In healthy persons procalcitonin levels are undetectably low. But in severe bacterial, fungal, parasitic infections with systemic manifestations, a significant rise in procalcitonin levels are seen. In this condition the production site is the extra thyroid tissues [14].

In healthy individuals, production of PCT and subsequently calcitonin is restricted to the thyroid C-cells. Bacterial infections selectively induce an increase in the concentration of PCT; because both endotoxins released from the bacterial cell wall as well as the host responses to infection activate the production of PCT mainly in parenchymal tissues. This results in accumulation of PCT, because unlike neuroendocrine cells, parenchymal cells lack the ability to cleave PCT into its mature form calcitonin [15,16]. As such, the release of PCT into the circulation in large amounts in various disease states is not accompanied by significant rise in calcitonin levels.

### METHODS

This prospective study was conducted on neonates admitted for sepsis workup in Neonatal Intensive Care Unit, at, Sree Balaji Medical College and Hospital from June, 2018 to March, 2019. A total of 70 neonates with suspected sepsis who required sepsis evaluation on clinical basis were considered.

### Inclusion criteria

Any suspected case of neonatal sepsis with maternal risk factors for sepsis e.g. prolonged labor, premature rupture of membrane (PROM) or prolonged PROM >18 hours, maternal intrapartum fever, urinary tract infection, chorioamnionitis and sepsis related clinical signs: (temperature instability, apnea, need for supplemental oxygen, bradycardia, tachycardia, hypotension /hypoperfusion, feeding intolerance, abdominal distension). Initially, Apgar score was used to indicate the infant condition in the first and fifth minutes after birth that includes: (appearance, heart rate, muscle tone, respiratory effort). Nosocomial infection is an infection whose development is favored by a hospital environment.

### Exclusion criteria

There were administration of antibiotic therapy prior to admission, birth asphyxia, hyaline membrane disease, documented Necrotizing Enterocolitis (NEC), aspiration syndromes, laboratory finding suggestive of inborn error of metabolism, infant of diabetic mother, and congenital anomalies including congenital heart disease. Before starting antimicrobial therapy, blood samples (Sample 1) for, CBC,

CRP, ESR, PCT and culture were collected. This procedure was repeated at day 5 after treatment with antibiotics (Sample 2). CSF, urine, tracheal and gastric aspirate cultures were obtained. Serum PCT level was measured using quantization immuno-luminometry by lumitest kit (Brahms Diagnostic, Berlin, Germany). The assay employs two antigen specific monoclonal antibodies, one directed at the calcitonin region which carries the luminescence label and the other at the katacalcin region. It is rapid and sensitive laboratory-based assay, providing results in 20 min.

The detection limit for the assay is 0.1 ng/mL and the coefficient of variation between 1-1,000 ng/mL is 5-10%. The assay is also free of interference from the antibiotics, sedatives, and vasoactive agents that are commonly used in ICU. PCT is present at very low levels, usually <0.05 ng/mL and may be undetectable by some assays in healthy individual [17]. In this assay a PCT level of  $\geq 0.5$  ng/ml was accepted as pathological. PCT level 0.5-2 ng/ml, 2-10 ng/ml and >10 ng/ml considered as weak positive, positive, and strong positive respectively. In contrast to bacterial and parasitic infections, only modest elevations of PCT are seen in viral infections. Consequently, serum PCT levels have been proposed as a marker to differentiate between viral and bacterial sepsis [18]. The study protocol was approved by the ethical committee Board and Informed parental consent for all infants. We used SPSS for statistical analysis. Pearson correlation between variables and statistical differences were analyzed using Fisher exact, ANOVA and Wilcoxon tests. P values of <0.05 were considered to be significant.

Criteria employed for defining the sepsis score are:

1. Group I, proven sepsis (22 cases): Clinical signs plus a positive bacterial culture.
2. Group II, suspected sepsis (30 cases): Clinical signs with negative bacterial culture but at least with 2 positive screening tests
3. Group III, Clinical sepsis (18 cases): Clinical signs with negative bacterial culture and negative screening tests.

## RESULTS

A total of 70 newborns who met the inclusion criteria were eligible for the study. These neonates are classified into three groups; proven sepsis (23 cases), suspected sepsis (29 cases) and clinical sepsis (18 cases) according to the study protocol. Early onset sepsis was confirmed in 37 (52.8%), late onset sepsis 20 (28.6%) and nosocomial sepsis 13 (18.6%). Blood culture was positive in 14 cases (Pathogens were *Staphylococcus aureus*, Group B *Streptococcus*, *E. coli*, and *Klebsiella*). Urine culture was positive in 4 (*E. coli*, *Staphylococcus epidermidis*), positive CSF culture in 2 patients, (Group B *Streptococcus*, *E. coli*) and positive tip of chest tube in 2 patients, (*Pseudomonas*, *Klebsiella*).

The demographic data of the proven, suspected and clinical septic groups are shown in Table 1. A statistically significant difference is observed between the mean birth weight and gestational age in proven and suspected septic groups in comparison with clinical septic group ( $P < 0.05$ ). Mortality rate is significantly increased in proven sepsis compared to clinical sepsis.

The mean of CRP and PCT in studied groups are shown; A significant difference between the mean of CRP and PCT levels in clinical and proven septic infants is noted ( $P < 0.05$ ). In addition, significant difference between proven sepsis and suspected sepsis, ( $P < 0.05$ ) is evident (Table 1).

**Table 1: Characteristics of the studied groups including, CRP and PCT serum levels.**

Character	Proven sepsis	Suspected sepsis	Clinical sepsis	P - value
Number	23	29	18	
Birth weight (g)	2070 $\pm$ 50	2320 $\pm$ 80	2730 $\pm$ 40	0.02
GA (week)	31.9 $\pm$ 4	33.9 $\pm$ 3.6	36.2 $\pm$ 8.2	0.04
Apgar score 1	6.3 $\pm$ 2.3	6.7 $\pm$ 2.7	8.1 $\pm$ 1.3	0.189
Apgar score 5	7.8 $\pm$ 1.5	8.4 $\pm$ 2	9.2 $\pm$ 1	0.157
Mortality	7	6	3	0.002
CRP(Mean $\pm$ SD), mg/l	23.17 $\pm$ 32.7	9.51 $\pm$ 13.80	4.82 $\pm$ 2.76	$P < 0.05$
PCT(Mean $\pm$ SD), ng/ml	5.80 $\pm$ 8.77	3.06 $\pm$ 5.84	0.60 $\pm$ 0.51	$P < 0.05$

In Table 1, the demographic data of the proven, suspected and clinical septic groups are shown; A statistically significant difference is observed between the mean birth weight and gestational age in proven and suspected septic groups in comparison with clinical septic group ( $P < 0.05$ ). Mortality rate is significantly increased in proven sepsis compared to clinical sepsis. There is a significant difference between the mean of CRP and PCT levels in clinical and proven septic infants ( $P < 0.05$ ).

Comparison of serum PCT level before and after treatment reveals significant changes in clinical sepsis ( $P = 0.001$ ) and proven sepsis ( $P = 0.003$ ) groups (Table 2). The relation between elevated procalcitonin levels and normal sepsis screening tests is represented in Table 3. In cases with proven sepsis, in spite of the negative result for sepsis screening tests, the result of PCT was positive. This result was seen also in some patients with clinical sepsis. The value of R is -0.3277. Although statistically Pearson correlation test shows negative value, the relationship between variables is only weak (the nearer the value is to zero, the weaker the relationship). P Value is 0.673. The result is not significant at  $p < 0.05$ .

**Table 2: Serum procalcitonin level (ng/ml) among studied groups before and after treatment.**

Group	Proven sepsis		Suspected sepsis		Clinical sepsis	
	Before	After	Before	After	Before	After
PCT test						
Negative (PCT<0.5 ng/ml)	0	0	7	22	4	16
Weak Positive (PCT=0.5-2 ng/ml)	0	0	2	0	3	1
Positive (2-10 ng/ml)	2	1	15	7	10	1
Strong Positive (PCT>10 ng/ml)	20	2	6	1	1	0

Table 2 shows the comparison of serum PCT level before and after treatment reveals significant changes in clinical sepsis ( $P = 0.001$ ) and proven sepsis ( $P = 0.003$ ) groups.

**Table 3: Relation between elevated pro calcitonin levels and normal sepsis screening tests**

Sepsis Group	Clinical sepsis N=18	Suspected Sepsis N=30	Proven sepsis N=22
Normal ESR	7	8	10
Normal CRP	7	4	8
Normal WBCs	17	10	12
Normal Chest X-Ray	12	11	16

WBC: White Blood Cell Count; CRP: C Reactive protein; ESR: Erythrocyte sedimentation rate.

According to Table 3, In cases with proven sepsis, in spite of the negative result for sepsis screening test, the result of PCT was positive. This result was seen also in some patients with clinical sepsis. The value of R is -0.3277. Although statistically there is a negative correlation, the relationship between variables is only weak the nearer the value is to zero, the weaker the relationship. P Value is 0.673. The result is not significant at  $p < 0.05$ .

## DISCUSSION

In recent years measurement of procalcitonin and other inflammatory mediators have been reported as a sensitive parameters for the early diagnosis of neonatal sepsis and evaluating its outcome [19]. Proinflammatory cytokines plays a role in the pathogenesis of bacterial sepsis. Production of these cytokines seems to trigger procalcitonin secretion from target cells. The increase in the serum concentration of CRP is rather slow during the first 24-48 hr of infection and this may negatively affect the sensitivity of the test. In addition, increase CRP concentrations in non-clinical conditions in such as meconium aspiration, prolonged rupture of membranes are thought to affect the specificity of the test [20]

PCT levels more than 0.5 mg/dl are useful to determine the type of infectious process (bacterial or not) [20]. In our study all the patients that presented positive cultures had PCT levels greater than 0.5 mg/dl and in most of them were greater than 2mg/dl. The correlation of PCT with culture was highly significant ( $p = 0.004$ ) and the relative risk was

much greater than with CRP values. This is fundamental because by having this clear determination it is feasible to initiate early and efficient therapy prior to the infectious process being evident or having a positive culture. Thus, the sepsis related morbidity and mortality could be greatly reduced.

Our findings demonstrated that the mortality rate is significantly increased in proven sepsis, particularly if PCT levels greater than 10ng/ml, so it is possible to predict a prognosis. The potential mortality related to other confounder in our study such as decreased gestational age, is unlikely to be anticipated as the decreased age per se (31-37 weeks gestation) could not affect the mortality. However, the prematurity related complications are those which are potentially responsible for mortality. Similarly, Brunkhorst et al. [21], have even demonstrated that very high PCT values are related to septic shock and death with considering a bad prognostic values greater than 10ng/dl. Nonetheless more studies should be made to demonstrate that early therapy with higher PCT and CRP values can reduce mortality.

A study in Denmark clarified that patients with septic process present less mortality risk with PCT levels less than 1mg/dl, and increments in this value of more than, 1mg/dl per day are associated with an increment in mortality [22]. The results reported that the correlation of the PCT levels with those of CRP was not statistically significant. The relative risk of mortality with CRP was significant with values greater than 128mg/dl. When these values are compared with current literature it is possible to affirm that patients with CRP levels greater than 128mg/dl have a greater risk of death.

In an Australian study a cohort value for the CRP values was obtained, mentioning a 7% risk of death with levels greater than 150mg/dl and up to 21% when levels are greater than 300mg/dl [23]. With regards to the utility of CRP as a prognostic marker, the majority of studies failed to demonstrate any correlation between CRP concentration and the survival [24].

Nonetheless, Vincent et al. (1996) [25] confirmed the relationship between CRP concentrations, severity of multiple organ dysfunction and mortality. PCT has been intensively investigated for its diagnostic role in neonatal sepsis. It has been reported that high concentration of plasma PCT was found in infants with severe infection, while PCT levels were very low in those without infections [26]. Many authors found that procalcitonin are a promising marker for the diagnosis of neonatal sepsis [9,27,28].

In these studies, PCT sensitivity in the early diagnosis of neonatal sepsis was found to be 83-100% while the specificity was 70- 100% [9,27,28] but some investigators questioned the accuracy of PCT in detecting neonatal sepsis. In these studies, it was reported that serum levels had also increased in non-infected neonates with perinatal asphyxia, intracranial hemorrhage, pneumothorax, or after resuscitation, and these conditions had negatively affected the specificity of PCT [29,30].

However, the protocol of our study implemented exclusive criteria that omit the influence of noninfectious confounders. Blommendahl et al. [31] reported that although the PCT test appeared to be useful for the diagnosis of neonatal sepsis in his small study, it didn't offer any significant advantages over traditional test like CRP for the diagnosis of sepsis, and this is consistent with other study conducted by Naher BS et al. [8] which concluded that PCT test can be done as an additional test with CRP for the diagnosis of neonatal sepsis, but the small sample size of those studies may limit drawing any firm conclusions. Thus, it is clear that the apparent discrepancy between the various studies performed so far could be explained by the geographical differences in infection patterns within the population, the possible interference of other confounders or variations in the study design and bias.

It is postulated that cytokines are prognostic for the development of severe disease, sepsis and death in hospitalized patients. The elevated concentrations of cytokines and markers reflect the complex changes in the immune response to microorganisms that are associated with alterations of the neuroendocrinal and vascular systems [32]. In short, both endotoxins released from the bacterial cell wall as well as the host responses to infection activate the production of PCT in parenchymal tissues. Accordingly, the more severe infection is the more production of cytokines and PCT, the more alterations of the neuroendocrine-vascular axis, and consequently the increased level of mortality would

be the outcome.

## CONCLUSIONS

Comparing with CRP, Procalcitonin is not only a good diagnostic measure of neonatal sepsis but also effective predictor of sepsis outcome.

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