ORIGINAL ARTICLE

PROCALCITONIN AS A DIAGNOSTIC MARKER OF NEONATAL SEPSIS

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HOW TO CITE THIS ARTICLE:
Rajeev D, Sujatha R. "Procalcitonin as a Diagnostic Marker of Neonatal Sepsis". Journal of Evidence based Medicine and Healthcare; Volume 1, Issue 16, December 22, 2014; Page: 1973-1980.

ABSTRACT: BACKGROUND: Neonatal sepsis is difficult to diagnose because it is associated with nonspecific signs and symptoms and a high index of suspicion is required for early diagnosis. Several acute phase reactants help to indicate the presence of infection in a neonate, of which Procalcitonin (PCT) and C-reactive protein (CRP) are important. **OBJECTIVES**: The current study was conducted to assess PCT levels and the suitability of this assessment in diagnosis of early onset sepsis, and to compare PCT with CRP levels. **METHODS**: The blood samples from 100 neonates whose mothers had any one of the risk factors including premature rupture of membranes (PROM) > 12 hours, more than 3 vaginal examinations after rupture of membranes, intrapartum fever, foul smelling liquor, meconium stained liquor, maternal UTI within 2 weeks prior to delivery and prolonged and difficult delivery with instrumentation, were collected. Blood culture and sensitivity, PCT and CRP levels, total count, absolute neutrophil count and band width were done. **RESULTS**: A total of 100 neonates were included in this study. Of the risk factors, meconium stained liquor was the most common, present in 60% of the cases. Of the 100 neonates, procalcitonin was positive in 34 (34%; 90% CI 26.7-42.1), CRP in 22 (22%; 90% CI 15.9-29.5) and blood culture in 9 (9%; CI 5.3-14.9). Of all the maternal risk factors, a statistically significant association was observed only in case of foul smelling liquor for both procalcitonin as well as CRP positivity. Procalcitonin when compared with CRP had a sensitivity of 100%, specificity of 84.6%, positive predictive value (PPV) of 64.7% and negative predictive value of 100%. **CONCLUSION**: Assessing PCT and CRP levels are relatively accurate and rapid diagnostic methods in diagnosing neonatal sepsis and also help in guiding antibiotic therapy. Although testing for PCT is expensive, it seems to be a better marker than CRP, especially in diagnosing early onset sepsis.

INTRODUCTION: Sepsis is a common cause of morbidity and mortality in new born infants. The incidence of neonatal sepsis in various regions is 7.1-38 per 1000 live births in Asia, 6.5-23 per 1000 live births in Africa, 3.5-8.9 per 1000 live births in South America and Caribbean and 6-9 per 1000 live births in UK and Australia.[1]

According to the World Health Organization, there were about 5 million deaths in 1995 and 98% occurred in developing countries. The number of deaths decreased to 4 million in 2005, but the percentage of deaths was same in the developing countries.[2]

There are two patterns of neonatal sepsis: early-onset (<7 days of birth) and late-onset (>7 days).[3] In severe cases, the neonate may be symptomatic in utero or within a few hours after birth,[4] while late onset sepsis is acquired from the environment.

The pathogenesis of neonatal septicemia involves interplay of host factors like gestational age and birth weight of neonate, environmental factors such as intra partum risk factors and agent factors such as virulence and load of organism causing sepsis.

Neonatal sepsis is difficult to diagnose because it is associated with nonspecific signs and symptoms and a high index of suspicion is required for early diagnosis.[1] Some of the important clinical features suggestive of neonatal septicemia are alteration in behavior, change in established feeding pattern, lethargy, temperature instability (hypothermia /fever), pallor, cyanosis, cold clammy skin, bradycardia/tachycardia, poor capillary filling and hypotension, apnea, dyspnoea, tachypnea with chest retraction, cyanosis, grunting and flaring, irritability, high pitched cry, hypotonia, abnormal reflexes, seizures, tremors and bulging anterior fontanel, vomiting, diarrhea, abdominal distension and hepatosplenomegaly, oliguria, jaundice, pallor, splenomegaly, petechiae, purpura and mucosal bleeding, multiple pustules, abscesses, scleroderma, mottling, umbilical redness and discharge.

Any of these clinical features along with laboratory tests like leukocytosis and C-reactive protein (CRP) are used to diagnose sepsis.[5] Once a diagnosis has been made, treatment should be started immediately because any delay in treating severe bacterial infection can cause significant morbidity and mortality.[3]

Several acute phase reactants help to indicate the presence of infection in a neonate. Those which increase with inflammation are Procalcitonin (PCT) and CRP.

CRP has been considered a good marker for diagnosing neonatal sepsis. Its levels are increased in various conditions like infection, autoimmune disease, surgery, meconium aspiration and recent vaccination. However, a significant increase in CRP levels is observed only 14-48 h after the onset of infection.[3] Since an early diagnosis of neonatal sepsis is quintessential in its management, in order to improve outcomes, there is a need for new laboratory methods for early diagnosis, evaluation of prognosis and treatment efficiency.

PCT acts as a marker of bacterial sepsis in critically ill patients. PCT is a precursor of calcitonin and a 116 amino acids protein.[3] However, these two are different from each other. While calcitonin is exclusively produced by C cells of thyroid gland, PCT is secreted by several cell types and many organs in response to proinflammatory stimuli, especially bacterial products.[6] In healthy persons, PCT is hardly detectable.[7] Although the exact sites of production of PCT in sepsis is not clear, monocytes and hepatic cells are considered to be potential sources.[3] Calcitonin that has a short half-life of 10 min but PCT has a longer half-life of 25-30 hr.[7]

PCT release into the systemic circulation is induced by bacterial lipopolysaccharide (LPS) and the concentration of PCT begins to increase from 3-4 h after an endotoxin challenge, reaches a peak at about 6 h, and remains elevated for more than 24 h.[3]

The objectives of the current study were to assess PCT levels and the suitability of this assessment in diagnosis of early onset sepsis, and to compare PCT levels with CRP levels.

MATERIALS AND METHODS: This was a prospective study conducted in the Neonatology division of the Department of Pediatrics of a teaching hospital over a period of 1 year.

The inclusion criteria were neonates born to mothers with at least one of the following risk factors: (i) premature rupture of membranes (PROM) >12 hours, (ii) more than 3 vaginal

examinations after rupture of membranes, (iii) intrapartum fever (>38^{o} C), (iv) foul smelling liquor, (v) meconium stained liquor, (vi) maternal UTI within 2 weeks prior to delivery and (vii) prolonged and difficult delivery with instrumentation.

Exclusion criteria were (i) new born babies with gestational age < 28 weeks, (ii) neonates with birth weight less than <1000gm, (iii) neonates with lethal congenital anomalies, (iv) still born and fetal deaths and (v) postdated neonates.

Informed oral and written consent were taken from the parents. The blood samples from 100 babies meeting the inclusion and exclusion criteria was collected and sent for various tests including blood culture and sensitivity, PCT and CRP levels, total count, absolute neutrophil count and band width.

For blood culture, about 1ml of blood was drawn aseptically and inoculated into a blood culture bottle containing 10 ml of Brain Heart Infusion broth, thus making a dilution of 1 in 10 to nullify the natural bacteriostatic/bacteriocidal activity of blood. After inoculation, the blood culture bottles were incubated at 37 degree centigrade under aerobic conditions in the incubator for 7 days. The first subculture was done after 24 hours of incubation, the second on the third day and final on the seventh day. Subcultures were done on to chocolate agar, 5% sheep blood agar and MacConkey agar plates. The inoculated plates were incubating aerobically in the incubator for 37 degree centigrade and the plates were observed for growth. The growth was identified by colony characteristics, grams stain and biochemical tests. Cultures which did not yield any growth following three subcultures were reported negative at the end of 7 days. Procalcitonin level analysis was done using enzyme linked immunoflourescence assay by using VIDAS BRAHMS PCT KIT manufactured by BIOMERIUX INDIA (P) LTD. CRP analysis was done using Immunoturbidometry Method which is a quantitative method of analyzing CRP level. A drop of EDTA blood was taken on a clean dry slide and a thin tongue shaped smear was made, air dried and stained with Leishman stain. The Total count, absolute neutrophil count and band cell ratio were calculated as per standard hematological methods.

Detailed birth events, Apgar score, sex of the baby, weight of baby was recorded on the pre coded proforma made available. Gestational age was assessed by using modified Ballard scoring system.

Neonates were followed up for up to 72 h from the time of birth for the development of any symptoms and signs suggestive of neonatal sepsis and if present were recorded.

For the purpose of the study neonates were divided in 3 groups:

- **Definite sepsis:** Neonate with signs and symptoms suggestive of sepsis with a positive blood culture.
- **Probable sepsis:** Neonates with two or more signs suggestive of sepsis with at least one abnormal laboratory parameters; or one or more signs suggestive of sepsis with two or more abnormal laboratory parameters.
- **No Sepsis:** No signs of sepsis or abnormal lab parameter.

STATISTICAL ANALYSIS: Descriptive statistical analysis was carried out in the present study. Results on continuous measurements are presented as Mean ± SD (Min-Max) and on categorical

measurements are presented in Number (%). Significance was assessed at 5% level of significance. Chi-square and Fisher Exact test has been used to find the significance of study parameters on categorical scale between two groups. Diagnostic statistics viz. Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Accuracy, and 90% confidence interval were computed in the study.

SIGNIFICANCE VALUES ARE: (i) suggestive significance (P value: $0.05<P<0.10$), (ii) moderately significant (P value: $0.01<P \leq 0.05$) and (iii) strongly significant (P value: $P \leq 0.01$).

RESULTS: The blood samples from 100 babies meeting the inclusion criteria constituted the material for study. Among the 100 babies, there were 55 (55%) males and 45 (45%) females. Twenty eight (28%) had a birth weight <2.5 kg (90% CI - 21.3-35.9) and 72 (72.%) a birth weight of >2.5 kg (90% CI 64.1-78.7). Twenty three (23%) were born at a gestational age of <37 weeks (90% CI 16.8-30.6) and 77(77%) at a gestational age of >37 weeks (90% CI 69.4-83.2). Of the 100 cases, 67 had no sepsis, 24 had probable sepsis and 9 had definite sepsis.

The distribution of cases according to maternal risk factors is shown in table 1. Meconium stained liquor was the most common factor, present in 60% of the cases.

Maternal risk factors	No of cases (n=100)	%	90% CI
Meconium stained liquor	60	60.0	51.8-61.7
PROM	25	25.0	18.6-32.7
Prolonged or Instrumental Delivery	12	12.0	7.6-18.4
Maternal UTI	5	5.0	2.5-9.9
> 3 Vaginal examination	5	5.0	2.5-9.9
Foul smelling liquor	2	2.0	0.7-5.9
Intrapartum fever(>38*C)	2	2.0	0.7-5.9
Maternal infections	0	0.0	-

Table 1: Shows distribution of cases according to risk factors (n = 100)

Among 100 babies who developed signs of sepsis, 53 (53%) had developed respiratory problems with (90% CI 44.8-61.1), 27 (27%) had developed general signs with (90% CI 20.4-34.8), 25 (25%) developed gastrointestinal tract related problems with (90% CI 18.6-32.7), 13 (13%) babies had CNS related problems with (90% CI 8.4-19.5), 7 (7%) babies had cardiovascular problems with (90% CI 3.8-12.4) and 4 babies had hematological problems with (90% CI 1.8-8.6).

Of the 100 neonates, PCT was positive in 34 (34%; 90% CI 26.7-42.1), CRP in 22 (22%; 90% CI 15.9-29.5) and blood culture in 9 (9%; CI 5.3-14.9).

The association of gestational age at the time of birth and PCT vs. CRP positivity is shown in table 2. There was no statistically significant association observed.

Gestational age in weeks	No of cases (n=100)	PCT positive cases (%)	P value	CRP positive cases (%)	P value
<37 weeks	23	10 (43.5)	0.336	8 (34.8)	0.138
>37 weeks	77	24 (31.2)	0.604	14 (18.2)	0.421

Table 2: Association of Gestational age and PCT vs. CRP positivity

The association of maternal characteristics and PCT vs. CRP positivity is described in table 3. A statistically significant association was observed only in case of foul smelling liquor for both PCT as well as CRP positivity.

Risk factors	No of cases (n=100)	PCT positive cases (%)	P value	CRP positive cases (%)	P value
Foul smelling liquor	2	2 (100)	0.049	2 (100)	0.008
> 3 Vaginal examination	5	3 (60)	0.219	2 (40)	0.331
PROM	25	9 (36)	0.832	5 (20)	0.809
Prolonged or Instrumental delivery	12	4 (33.3)	0.957	2 (16.7)	0.657
Meconium stained liquor	60	20 (33.3)	0.908	13 (21.7)	0.955
Maternal UTI	5	1 (20)	0.509	0 (0)	-
Intrapartum fever	2	0 (0)	0.544	0 (0)	-

Table 3: Association of Maternal characteristics and PCT vs. CRP positivity

With respect to the WBC counts, total count was >5000 in 90 (90%) neonates; absolute neutrophil count >1000 in all 100 cases and a band cell ratio <20% was noted in 96 (96%) of cases.

There was no sepsis observed in 67 (67%) of cases, probable sepsis in 24 (24%) and definite sepsis in 9(9%).

When compared with blood culture, PCT was true positive in 5 cases, false positive in 29, false negative in 4 and true negative in 62. CRP was true positive in 5, false positive in 17, false negative in 4 and true negative in 74 cases (see Table 4).

	True positive	False Positive	False Negative	True negative	Total
PCT (>0.5 mg/dL)	5	29	4	62	100
CRP (>5 mg/dL)	5	17	4	74	100

Table 4: Correlation of PCT, CRP and Total count in relation to blood culture

Procalciton in when compared with CRP had a sensitivity of 100%, specificity of 84.6%, positive predictive value (PPV) of 64.7% and negative predictive value of 100%. CRP when

compared with PCT, had a sensitivity of 64.7%, specificity of 100%, PPV of 100% and NPV of 84.6%. Therefore, PCT, when compared to CRP, showed better sensitivity and negative predictive value.

DISCUSSION: Neonatal sepsis with its high mortality rate, still remains a diagnostic and therapeutic challenge for neonatal health care providers, especially in developing countries where the incidence and mortality rates are very high. Early diagnosis of neonatal septicemia helps the clinician in instituting antibiotics therapy at the earliest thereby reducing mortality in neonates.
In the present study, we have attempted to document the effects of intrapartum risk factors for early onset sepsis on PCT and CRP levels in neonates and to assess the suitability of the test in the diagnosis of neonatal sepsis.

According to recent reports, measurement of PCT and other inflammatory mediators like CRP are considered to be sensitive parameters for the early diagnosis of neonatal sepsis and evaluating its outcome.[7,8] Procalcitonin and CRP are both acute phase reactants which are increased in case of inflammation. Measurement of CRP is an established method for the diagnosis of acute inflammation and infections. However, the increase in the serum concentration of CRP is slow during the first 24-48 hr of infection and this may negatively affect the sensitivity of the test. Additionally, CRP levels are increased in non-infective conditions such as meconium aspiration and prolong rupture of membranes, which can affect the specificity of the test.[3]

There have been several studies on the diagnostic role of PCT in neonatal sepsis.[7,9,10] While the PCT levels are significantly elevated in infants with severe infections, they are very low in those with no infections.[11] Studies have shown that PCT sensitivity in the early diagnosis of neonatal sepsis was 75-100% while the specificity was 59-100%.[9,10,12,13] Chin Yi-Ling et al. demonstrated a sensitivity of 69.5% and specificity of 64.5% for PCT, compared to 67.25% of sensitivity and 93.9% of specificity for CRP.[14] In their study, Chiesa et al. reported that a serum PCT rise caused by prenatal events other than infection was smaller than the PCT response against infection.[11] Adib et al. demonstrated 75% sensitivity, 80% specificity 80%, PPV and 75% NPV for PCT as a marker for the early diagnosis of neonatal sepsis.[3] In a study by Zahed Pasha et al., it was observed that PCT levels were remarkably high in neonates with proven sepsis and these levels dropped dramatically after treatment with antibiotics.[15,16] In our study, when compared with CRP, PCT had a sensitivity of 100%, specificity of 84.6%, positive predictive value (PPV) of 64.7% and negative predictive value of 100%.

CONCLUSION: Neonatal septicemia is a leading cause of mortality and mortality of neonates in our country. Early diagnosis with a reasonable degree of accuracy will help the clinician to decide on the usage of proper antibiotic which will help in reducing the morbidity and mortality. A positive blood culture is the only definitive and fool proof method of confirming a case of septicemia, but this takes a minimum of 48 hours, which is a precious time in making a decision in the treatment of sepsis in the newborn. Assessing PCT and CRP levels are relatively accurate and rapid diagnostic methods in diagnosing neonatal sepsis and also guide antibiotic therapy. Overuse of antibiotics in cases of conditions stimulating sepsis can thus be avoided. Although test

for PCT is expensive, studies have shown that it is a better marker than CRP especially in diagnosing early onset sepsis.

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Date of Submission: 24/11/2014.
Date of Peer Review: 25/11/2014.
Date of Acceptance: 02/12/2014.
Date of Publishing: 16/12/2014.