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Role of Procalcitonin in Diagnosis of Neonatal Sepsis

KEYWORDS: neonatal sepsis, early diagnostic markers, Procalcitonin, CRP, Blood cultures

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ABSTRACT *Background:*
Sepsis is a leading cause of neonatal mortality. Lack of reliable diagnostic tests is a major obstacle in reducing sepsis related mortality and morbidity. Elevated serum Procalcitonin appears to have a better diagnostic accuracy compared to CRP. In this study, we propose to evaluate the efficacy of PCT in the early diagnosis of sepsis in comparison to that of CRP.
Setting:
Neonatal intensive care unit at Gandhi Medical College, Secunderabad, Telangana, India
Subjects and methods:
This is a prospective study involving neonates admitted for suspected sepsis and those who were admitted for non-infective conditions and later developed clinical features of sepsis. Blood samples from all the neonates were drawn before administering antibiotics and estimation of serum levels of CRP, PCT, blood culture and antibiotic sensitivity and other necessary investigations were carried out.
Results: Reports from 85 samples drawn from the 85 recruited neonates were analysed. Blood cultures were positive in 29 cases, PCT was positive in 38 and CRP was positive in 31 cases. Correlation of positive CRP and PCT with positive blood cultures was assessed.
Conclusion:
This study establishes the usefulness of PCT in predicting sepsis early in the course of illness and earlier than CRP. Including PCT in the sepsis work up will enhance the accuracy of diagnosis of sepsis.

MANUSCRIPT

Background:

With a neonatal mortality of 29 in India, about 0.76 million neonates die every year contributing to more than half of under-five child deaths(1).It is estimated that 32% of these deaths are due to neonatal infections.(2). Despite the major advances in new born care, lack of an ideal diagnostic test to detect sepsis makes optimal management of neonatal sepsis a big challenge for clinicians. The high mortality and morbidity associated with delay in diagnosing sepsis compels clinicians to start antibiotic therapy on the slightest suspicion of sepsis. Such an approach results in treatment of about 18-38 uninfected infants for every one infant who is infected. (3). Needless to say this means a huge financial burden apart from the risk of development of resistance to the commonly used broad spectrum antibiotics. This emphasises the need for a reliable test with a rapid turnover time to help in the timely management. Unfortunately, to date there is no single ideal predictor of sepsis. To circumvent this limitation, clinicians depend on `sepsis screen'- a set of tests with rapid turnover time to decide on antibiotic therapy. Estimation of the acute phase proteins is one important component of sepsis screen. C reactive protein (CRP) is an acute phase protein that is most widely estimated in the diagnosis of sepsis. Several studies reported that Procalcitonin (PCT) is a better marker compared to CRP owing to its biophysical profile and better sensitivity and specificity in the diagnosis of neonatal sepsis (4,5,6) This study is aimed at evaluating the utility of PCT as an early diagnostic marker of neonatal sepsis

Aims and Objectives:

To evaluate Procalcitonin as an early diagnostic marker of neonatal sepsis.

To compare efficacy of Procalcitonin with that of CRP in diagnosing sepsis using positive blood cultures as reference.

STUDY DESIGN AND SETTINGS

This is a prospective study conducted on neonates who were admitted to the neonatal intensive care unit (NICU) at Gandhi Medical College Hospital, Secunderabad, Telangana over a 12 month period (August 2012 to August 2013). This study was approved by the Hospital Ethical Committee, and written informed consents were obtained from the parents of the babies recruited in the study.

INCLUSION CRITERIA

The inclusion criteria were infants who were admitted to this NICU with signs suggestive of sepsis, or those who developed signs of sepsis while they were in the ward.

EXCLUSION CRITERIA

Neonates who were on antibiotics prior to hospitalization and those who had birth asphyxia, aspiration pneumonia, congenital anomalies and those who had laboratory findings suggestive of inborn errors of metabolism. Babies whose mothers received antibiotics within 24 hours prior to delivery were excluded.

INVESTIGATIONS

Prior the commencement of the antibiotics, blood was drawn for investigations which included micro erythrocyte sedimentation rate, total leukocyte count, the absolute neutrophil count (ANC), the immature neutrophils to total neutrophil count ratio (I/T ratio), degenerative changes in the neutrophils, blood culture and antibiotic sensitivity, PCT and C-reactive protein (CRP) estimation**.** Other investigations like CSF analysis, chest x ray, and urine culture were performed wherever indicated.

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The serum PCT level was measured using a semi quantitative immunochromatographic PCT-Q kit (BRAHMS Diagnostic, Berlin, Germany). The PCT-Q test uses a monoclonal mouse antikatacalcin antibody conjugated with colloidal gold (tracer) and a polyclonal sheep anticalcitonin antibody (solid phase). The test requires only 200 microliter of plasma. As the sample is applied to the test strip, PCT in the sample gets tagged to the tracer and flows to the test band region containing anticalcitonin antibodies. A positive test read at 30 minutes shows a reddish band - the colour intensity of which is directly proportional to the PCT concentration of the sample. In this assay, a PCT level of ≥0.5 ng/ml was considered as pathological. PCT levels of 0.5-2 ng/ml, 2-10 ng/ml and >10 ng/ml were taken as weakly positive, positive and strongly positive respectively**.**

The serum CRP level was measured by using the A-15 CRP Kit (Bio-system, Costa Brava, Barcelona, Spain) .which is an immunoturbidimetric method. A value of more than 6 mg/L was taken as positive.

Blood samples for cultures were collected from peripheral vein with all aseptic precautions. 0.5 ml-1ml of blood was collected in 5 ml of glucose broth. Three subcultures were observed after 24, 48 and 120 hrs. If no growth was observed after five days, the culture was reported as negative. If growth was observed, the isolate was further analysed for specific organisms and their antibiograms.

Complete blood counts were done using SYSMEX KX-21, an automatic multi-parameter blood cell counter. Peripheral blood smear using Leishman`s staining was studied to determine absolute neutrophil count, band cell count and immature to total neutrophil ratio. An I/T ratio of more than 0.2 was considered abnormal.

Observations and results:
The study was conducted on 85 neonates below the age of 28 days with clinical suspicion of neonatal sepsis. Out of the total 85 babies 40 were female and 45 were male babies; 24 were preterm and 61 were term babies. 57 (67%) of the studied babies had birth weight >2.5 kg and 28 (33%) had birth weight <2.5kg. The age of onset of the sepsis ranged from day 1 to day 26 with EOS and LOS almost equal in number - 42 (49.4%) and 43(50.6%) respectively. Common clinical manifestations of neonatal sepsis were refusal of feeds (56%), temperature abnormality (46%), sclerema (44%), jaundice (42%), pallor (36%), rash (20%) and convulsions (16%).

Blood Cultures were bacteriologically positive in 29cases (34.1%) and negative in 56 cases (65.9%) with the commonest organism isolated being Klebsiella *pneumoniae*(31%) *, followed by* E.*coli*(20.6%), Acinetobacter(13.8%) and Staphylococcus *aureus*(13.8%) , CONS(10.4%) , Citrobacter *koseri* (6.9%) and Pseudomonas *aeruginosa* (3.5%).

Analysis of the frequency of positive tests with various markers showed that PCT scores over other markers in picking up sepsis. A comparison of PCT with other sepsis markers (table 1) shows that PCT was positive in most of the culture positive cases (89.6%) followed by CRP (38.4%), WBC (31%), Micro ESR (27.6%) and I: T ratio (17.2%).

STATISTICAL ANALYSIS
The correlation of serum PCT, CRP levels with positive blood cultures for the diagnosis of neonatal sepsis was compared statistically and the results were analysed by chi-square test. P values of <0.05 were considered to be significant. By using the blood culture results as the gold standard, the sensitivity, specificity, positive predictive values and the negative predictive values of the PCT and CRP for diagnosing sepsis were calculated (Fig.2). The sensitivity of PCT in detecting sepsis was 89.6 %, specificity was 78.57 %, positive predictive value (PPV) was 65% % and negative predictive value (NPV) was 93.6 %. The sensitivity of CRP for predicting sepsis was 34.4 %, its specificity was 62.5 %, its PPV was 32.25 % and its NPV was 77.78 %. P value for CRP was 0.7603. P value for PCT was less than 0.001.

DISCUSSION
Blood culture is the gold standard diagnostic test for sepsis, but has a high false negative yield and is not useful in deciding on starting antibiotic therapy. Sepsis screen is commonly used to overcome this problem though there is no agreement on what constitutes an ideal sepsis screen(7) and the existing sepsis screens are not useful in making an early diagnosis.(8) The C-reactive protein is the most important component of sepsis screen. The rise of CRP after the onset of sepsis is comparatively slower **(**9) and has low sensitivity early in sepsis (10). PCT has a better profile with a quick rise following exposure to bacterial endotoxin making an earlier diagnosis possible.

PCT is a116 amino acid peptide prohormone of calcitonin. .In the absence of infection, PCT is produced only by the C cells of thyroid gland and is barely detectable in circulation. In the presence of bacterial sepsis, PCT is believed to be produced in a large quantity by the neuroendocrine cells of the lungs and intestines and gets released into circulation (11). The physiological role of PCT in sepsis is not clear as of now. The reference titres of PCT at birth are less than 0.08ng/ml and rise to a peak of 0.6ng/ml owing to translocation of endotoxins from the gut wall following bacterial colonisation of the gut. The levels fall back to normal by 48 hours (12). This is considered physiological. However, with the onset of sepsis PCT levels increase significantly beyond the physiological limits.

Among the 85 cases, blood cultures were positive in 29 cases, an elevated PCT level was detected in 38 cases, an elevated CRP level in 31 cases, abnormal total WBC count in 24 cases, an elevated micro ESR level in 27 cases and abnormal I/T ratio in 15 cases.

Out of the 29 culture positive cases, 12 babies had early onset sepsis and 17 had late-onset sepsis; 09 were preterm and 20 were term babies; 16 were males and 13 were females. Of all the diagnostic markers, PCT level had the highest correlation with culture positive cases with elevations in 26 (89.65%) cases of the 29 culture positive cases. This was followed by an elevated CRP level in 10 (34.4 %) cases, abnormal WBC in 09 (31.0%), an elevated micro ESR in 08 (27.6%).and an abnormal I/T ratio in 05 cases (17.2%).

Since blood culture is considered gold standard test for sepsis, it is logical to assess efficacy of CRP and PCT by correlating with blood culture reports. Observations from the present study suggest that PCT is more sensitive in picking up sepsis earlier when compared to CRP. Similar observations were made by Sucilathangam et al (13). Benitz et al also stated that a single CRP estimation early in the course of sepsis has low sensitivity. (10) In practice, a positive CRP or a positive sepsis screen triggers the decision of drawing blood for culture. Since CRP rises later than PCT after the onset of sepsis, depending on the tim-

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ing of the sampling we may get a negative CRP result thus delaying the diagnosis. Mohsen et al in their study demonstrated that PCT had high sensitivity, specificity, a high PPV, and a high NPV (80%, 85.7%, 84.8%, and 81.1% respectively) and concluded that PCT is a sensitive, independent, and useful biomarker in comparison to CRP in early diagnosis of neonatal sepsis (14).Our study also shows similar results with PCT showing better sensitivity, specificity, PPV and NPV than CRP.

Since PCT has a higher sensitivity, it is a better predictor of sepsis than CRP and should guide the management plan of a neonate with suspected sepsis. The higher specificity and higher NPV are helpful in ruling out sepsis (15) and thus can give clinicians confidence in withholding antibiotics in a case with a low probability of sepsis. Ballot et al in their study concluded that PCT has good correlation with sepsis and can be useful in ruling out sepsis on presentation (16).

Conclusions:

Neonatal sepsis is a significant contributor for mortality and morbidity. To reduce the mortality and morbidity, there is a need for a reliable diagnostic test for early detection. The evidence at present does not support PCT as a sole predictor of sepsis. Since PCT has a better correlation with proven sepsis, it can be recommended to be made a part of full sepsis evaluation.

MARKER	No. of positive cases (Out of n = 85)	Blood culture positive (29 cases out of 85)
PCT	38	26 (89.6%)
CRP	31	10 (38.4%)
TOTAL WBC	24	09 (31.0%)
MICRO ESR	27	08 (27.6%)
I:T RATIO	15	05 (17.2%)

Table 1. Various diagnostic markers in neonates with culture positive sepsis.

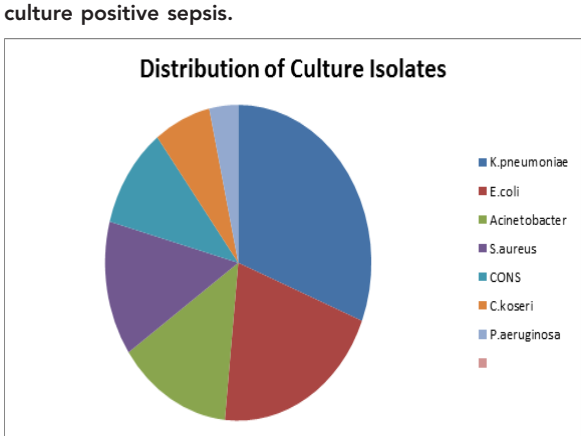

Fig.1: Distribution of bacteria in culture positive cases

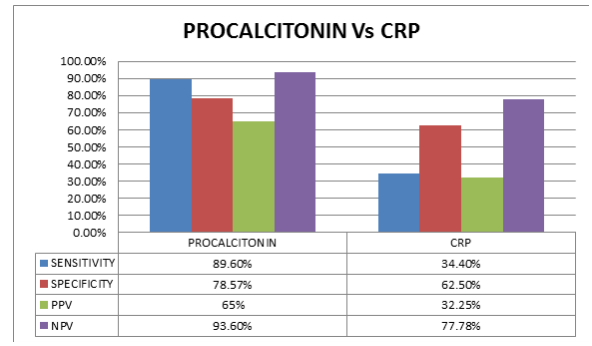

Fig 2. Chart showing comparison of PCT with CRP

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