ROLE OF SERUM PROCALCITONIN IN EARLY DIAGNOSIS OF NEONATAL SEPSIS

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Abstract

Introduction: Neonatal sepsis is a major cause of neonatal mortality and morbidity throughout the world. The clinical findings of sepsis are uncertain in newborn infants and these findings may be associated with multiple conditions besides infection. The difficulty in making early diagnosis of neonatal sepsis, despite improved bacteriologic techniques, is attested to by recent reviews. Therefore a group of tests were studied to assess the usefulness of procalcitonin either singly or in combination in predicting neonatal sepsis. The aim of the present study is to evaluate the role of PCT as an early marker in diagnosis of neonatal sepsis.

Objective: The present study was undertaken to assess the role of serum procalcitonin in making early diagnosis of neonatal sepsis.

Methods: 100 cases of each confirmed neonatal sepsis and possible infection were included in the study and 100 age matched healthy neonates served as control. Serum Procalcitonin was determined by specific immunoluminometric assay (LUMItest®, Brahms Diagnostica GmbH, Berlin Germany).

Results: PCT differed significantly among infection categories (p<0.0001) and correlated significantly with C-reactive protein at presentation (correlation coefficient 0.408,p<0.001). PCT predicted 90.1% of definite infection. Receiver operating characterstic (ROC) analysis for PCT to predict definite infection showed oddsratio (OR) 1.168 (95% CI: 1.06-1.26). PCT had a negative predictive value of 0.98 (95%CI: 0.917-0.989) for definite infection.

Conclusion: Our findings suggest that PCT is of more value in ruling out neonatal sepsis on presentation in comparison to making the confirm diagnosis of neonatal sepsis, but it would be useful as part of a full sepsis evaluation.

Key words:PCT-Procalcitonin, CRP-C-reactive protein, ROC-Receiver operating characterstic, OR-Odds ratio, CI-Confidence interval

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Introduction:

The importance of early treatment with antibiotics in newborns and infants with sepsis has been demonstrated by the sepsis home-treatment study performed by Bang and colleagues in rural India[1,2]. But as the first sign of this disease is very nonspecific and there is no single reliable laboratory marker available early rapid diagnosis is very problematic. Several leukocyte indices and acute-phase protein levels have been evaluated for the diagnosis of sepsis, and more recently, measurement of multiple plasma cytokines [3] and leukocyte activation markers [4] have showed promising results. However, to date, no single laboratory test hasprovided rapid and reliable

identification of early infected neonates. The best prediction is obtained using a combination of inflammatory markers including interleukin 6 (IL6) andinterleukin 8 (IL8) and C-reactive protein (CRP) .This inability has led to a search for new diagnostic markers[5,6]. Serum procalcitonin (PCT) is one of many inflammatory markers and is now available as a routine laboratory investigation. PCT a prohormone of calcitonin occurs in very low concentration in in the serum of normal individuals. PCT is preferentially induced in bacterial sepsis, especially in severe sepsis and septic shock[7,8]. PCT can therefore be used to discriminate systemic inflammation due to bacterial sepsis from other causes and can also be used to monitor the progress and prognosis of patients with sepsis[7,9]. The aim of the present study is to evaluate the role of PCT as an early marker in diagnosis of neonatal sepsis.

Material methods:
This prospective study was conducted on one hundred cases of neonatal sepsis conformed by positive blood culture, abnormal CRP, platelet count and WBC count and one hundred cases of possible infection having negative blood culture with abnormal CRP or a combination of at least two of the following abnormal platelet count, white cell count and CRP admitted in pediatrics department of Major S.D.Singh Medical College, Farrukhabad ,India, from January 2012 to October 2013 were selected for the present study. Infants born to mothers with gestational diabetes were also excluded (because available data indicate that PCT values are higher for neonates born to diabetic mothers[10]). One hundred healthy neonates having negative blood culture, normal CRP, platelet count and white cell count were selected to serve as normal control. Blood samples were centrifuged within 30 minutes of collection. Serum was stored at -20º before analysis. All patients were entered in the study after written informed consent was obtained from the parent or guardian and the study was approved by the ethical committee of the Major S.D.Singh Medical College Farrukhabad.

Estimation of PCT
PCT was measured by a specific immunoluminometric assay (LUMItest®, Brahms Diagnostica GmbH, Berlin Germany), requiring

20μl of serum and 2hr to complete. The detection limit of this immunoluminometric assay is .08 ng/ml. Luminescence was measured automatically on a Lumat LB 9507tube luminometer (Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany).

Statistical analysis
Continuous variable and proportions for categorical variables includes mean and standard deviation. Kruskal-Wallis one - way analysis of variance was done to determine whether any variable differed significantly among infection categories. Spearman's rank correlation and logistical regression were also done to determine the best predictors of infections. Receiver operating characteristics (ROC) analysis was done to evaluate the application of PCT as a diagnostic test for neonatal infection.

Results:
300 neonates were evaluated within the first 72 hours of life. The mean birth weight was 1985g (SD 897g) and gestational age 34.7 weeks (SD 4.3weeks).One-way analysis of variance (Kruskal-Wallis) showed that birth weight (p=0.001) gestational age (p=0.0005) and PCT (p<0.0001) differed significantly among healthy neonates and neonates with sepsis. Logistical regression and ROC analysis was performed among 'no infection' versus 'any infection' and 'no infection' versus 'definite infection'. PCT alone correctly predicted 73.65 of any infection and 90.1% of definite infection. If birth weight, gestational age and platlets were included, the prediction improved to 82.7% and 93.1% respectively.The predictive values for PCT using a cut off of 0.5ng/ml are shown in table 2. ROC analysis using PCT to predict 'no infection' versus 'any infection' gave an odds ratio (OR) of 1.136 (95% CIs 1.07- 1.26). The OR for the prediction of PCT for 'no infection versus 'definite infection' was 1.168 (95% CI;1.06-1.26).

Table No. 1: Procalcitonin levels among the different categories

Category	Number of subjects	PCT level (median ng/dl)	Range	IQR
Healthy Neonate	100	0.47	32.6	0.67
Neonate with sepsis	100	8.21	35.8	12.85

Table No. 2: Predictive values for PCT < 0.5 NG/ML (95% CI)

category	Sensitivity	Specificity	Positive predictive value	Negative predictive value
None versus any infection	0.81 (0.724-0.847)	0.6 (0.431-0.573)	0.53 (0.402-0.541)	0.9 (0.748-0.868)
None versus definite infection	0.772 (0.708-0.784)	0.56 (0.416-0.587)	0.15 (0.087-0.217)	0.98 (0.917-0.989)

Discussion

Neonatal sepsis is a major cause of neonatal mortality and morbidity throughout the world. The clinical findings of sepsis are uncertain in newborn infants and these findings may be associated with multiple conditions besides infection. Acute phase reactants have been used frequently as an earlymarker of bacterial sepsis. Previous studies have shown CRP to be a useful marker of bacterial sepsis in the neonate. There is no single reliable test for the early confirmation of definite neonatal sepsis. Therefore, there is a continuing search for a new infection marker, including investigation of PCT.

In 1993, a study identified elevated level of PCT in patients with bacterial infection[11]. Since then, PCT has become the most widely studied and reportedputative biomarker for sepsis in children[12-14].While circulating levels of PCT in healthy subjects are below the limit of detection, production of PCT during inflammation correlates with both the presence of bacterial endotoxin and inflammatory cytokines[11]. PCT has been reported to:

- Differentiate between SIRS and sepsis[15].
- Serve as a marker for sepsis in neonates[16,17].
- Identify children at high risk of death from sepsis after bone marrow transplant[18].
- Have a better correlation with sepsis than CRP or WBC count in patients admitted to apediatric intensive care unit [19].
- Correlate with poor outcome in pediatric sepsis[20].
- Differentiate between fever of viral and bacterial etiology with more specificity than and similar sensitivity to CRP[21].

However, other studies have reported that PCT is nonspecificand/or insensitive in the diagnosis of invasive fungal infections[22], sepsis in burns patients[23], meningococcemia[24], and neonatal sepsis[25].

PCT is a 116 amino acid peptide divided into 3 sections: amino terminus of PCT, immature calcitonin and katacalcin (CCP-1). It is encoded by CALC-1 gene located on chromosome [11].

It is expressed in tissue-specific manner (produced predominantly by monocytes and hepatocytes). PCT is synthesized to smaller peptides and mature calcitonin which is stored in secretory granulesand secreted in the blood to regulate calcium concentration.

In the present study it is found that though PCT has a predictive role in neonatal sepsis but it is not sufficient alone as a sole marker of sepsis because specific cut-off values of PCT may be required in the first 48 hours of life, as various maternal and perinatal factors affect the PCT levels[26]. The explanation for this difference in the PCT levels between two groups may be due the fact that in absence of infection CALC-1 gene transcription in non-neuroendocrine tissue is suppressed except lfor thyroid gland C cells producing the precursor of calcitonin in healthy and non-infected individuals.In presence of infection, non-neuroendocrine tissue (parenchymal tissue and differentiated cell types)express the CALC-1 gene to produce increased levels of PCT; function of this increase is currentlyunknown.

The negative predictive value for definite infection in our study is 98%. This is in agreement with Guibourdencheet al[27]who have suggested that a

negative PCT on presentation may be useful to rule out sepsis in neonates.

Conclusion:

From above study we reach to the conclusion that PCT alone is of moderate diagnostic value for the detection of neonatal sepsis. PCT alone is insufficient in confirming the diagnosis of neonatal sepsis, but would be useful as part of a sepsis evaluation. A negative PCT at presentation may be useful to rule out sepsis, but this should be evaluated further.

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