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Original Article

Procalcitonin as a Marker of Neonatal Sepsis in Intensive Care Units

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Abstract

Background: The appropriateness of using serum levels of procalcotonin (PCT) for early diagnosis of newborn sepsis is still controversial. Therefore, the objective of the present study was to compare the usefulness of PCT with those of serum levels of C-reactive protein (CRP) and white blood cell (WBC) counts in the diagnosis and response to treatment of neonatal sepsis.

Methods: A total of 47 neonates (1-30 days old) were assigned to two control (n=22) and sepsis (n=25) groups. Blood samples were obtained at the outset and after 7 days of treatment for blood culture, measurement of serum levels of PCT and CRP as well as WBC counts. Data were analyzed using within and between group comparisons.

Results: Serum levels of PCT were significantly higher in sepsis group (14.1±18.7 ng/ml) than that in the control group (0.38±0.43 ng/ml). In addition, after 7 days of treatment neonates who had achieved clinical recovery had a significantly lower serum PCT levels (0.26±0.37 ng/ml) than that of the same group at the beginning of the study. At a cut-off value of ≥ 0.8 ng/ml, the sensitivity, specificity, positive predictive value of, and negative predictive value of PCT were 84%, 86%, 86% and 84%, respectively.

Conclusion: The findings of the present study suggest that serum levels of PCT might be a more reliable marker of infection than serum levels of CRP, or WBC counts in the early diagnosis and responses to antibiotic therapy of neonatal sepsis.
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Keywords ● Procalcitonin ● sepsis ● neonatal ● C-reactive protein ● white blood cell count

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Introduction

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection accompanied by bacteriemia in the first month of life.[1] Despite major advances in neonatology in the past few decades, bacterial sepsis is still one of the most important causes of morbidity and mortality in newborns, particularly preterm infants.[2,3] The incidence of sepsis is estimated to be 10 in 1,000 live births, and the risk increases by 3-4 times for newborns weighing less than 1,500 g.[3,4]

Early diagnosis of neonatal sepsis is complicated because the First signs of the disease may be minimal, and are similar to those of various noninfectious processes. Furthermore, blood

culture results are not usually available until at least 48-72 hours after the specimen reaches the laboratory, and the laboratory tests such as leukocyte count, immature / total neutrophil (I/T) ratio, and C- reactive protein are unable to provide a definitive early diagnosis.[5-8] Therefore, the availability of a laboratory test that provides accurate and rapid diagnosis of the disease would be of paramount importance in improving the outcome of this challenging problem.

Procalcitonin (PCT) is produced by the C cells of the thyroid gland, and is the propeptide of calcitonin hormone. Moreover, it is composed of 116 amino acids with a molecular mass of 14.5 kDa,[9] and has no hormonal activity. It was initially described as a potential marker of bacterial disease by Assicot et al.[10] It was shown in healthy volunteers that PCT was detectible in the plasma two hours after the injection of a small amounts of bacterial endotoxins, increasing to a plateau in 6-8 hours, and then decreasing to normal levels after 24 hours.[11,12]

Procalcitonin is degraded by specific protease into three peptides namely, katacalcin, calcitonin and a N-terminal fragment by a half-life of 25 and 30 hrs. In severe bacterial infections and sepsis, macrophages and monocytic cells of various organs, such as the liver, are believed to be involved in the synthesis and release of PCT in response to bacterial infections.[10,12]

In the neonatal period, PCT values rise soon after the birth, peak at 21/24 hrs, and then fall again. Gestational diabetes appears to significantly increase PCT values however, the reason for such changes is not clear at present. Prematurity does not appear to have any effect on the plasma levels of PCT.[6]

The findings of some of the recent studies suggest the usefulness of PCT for early diagnosis of neonatal sepsis,[7,13,14] while some others conclude that PCT is less useful than CRP,[15,16] or is of little value.[17] Such negative findings, in addition to likely publication biases, indicate that the case for PCT is not yet conclusively settled.[6] The objective of the present study was to compare the usefulness of PCT in the diagnosis of neonatal sepsis, and to evaluate the clinical response to antibiotic therapy with those of plasma levels of CRP and white blood cell (WBC) counts.

Patients and Methods

The study was performed prospectively recruiting 55 preterm and term neonates admitted to the Neonatal Intensive Care Unit (NICU) of the Department of Pediatrics, University Hospital, Aleppo, Syria, from November 2008 to May 2009. The study was approved by the hospital ethics committee, and written informed consents were obtained from the parents.

Exclusion criteria were congenital malformations, exchange transfusion for neonatal hyperbilirubinemia, and death during follow up. Eight neonates were excluded due to death during follow up (n=4), congenital malformations (n=3) and exchange transfusion because of hyperbilirubinemia (n=1). The remaining neonates were assigned to the following groups.

Control group comprising 22 non-infected newborns, who were born healthy and followed in neonatal care units because of their perinatal risk factors such as twin pregnancy, preterm birth and fetal distress, or being born from diabetic mothers. Infections were ruled out on the basis of the absence of clinical signs of sepsis and the presence negative blood cultures. Single blood samples were obtained from each subject of this group at the beginning of the study, and were used for the measurement of serum levels of PCT and CRP as well as WBC counts.

Case group comprised of 25 infected newborns, whose diagnosis was based on *a* positive blood cultures associated with more than two of the following criteria: septic status, respiratory distress syndrome, neutropenia and raised serum CRP levels (>20 mg/l). Blood samples were obtained from the subject of this group at the beginning of the study and after 7 days of treatment, and were used for the measurement of serum levels of PCT, CRP as well as WBC counts.

Gestational age, birth weight and gender of subjects of both groups were recorded. Prior to starting the antimicrobial therapy, blood samples were obtained for routine laboratory measurements including blood culture, WBC counts, and measurement of serum levels of PCT and CRP. Blood samples for PCT were allowed to clot for 30 minutes, followed by separation of serums, which were stored at -70°C, and thawed once at the time of the analysis.

Laboratory Measurements

White blood cell counts were performed by the hospital hematology laboratory. Serum levels of CRP were determined by a turbidimety method using BioSystm BTS 330 device (BioSystem S.A, Barcelona, Spain). The normal limit was up to 0.5 mg/dl based on the manufacturer's instruction. Serum levels of PCT were measured by an Enzym-Linked Fluorescent

Assay kit (VIDAS Brahms PCT Kit, BioMérieux, France). The assay requires 200 µl of serum and 20 minutes to complete. The detection limit of this assay is 0.05 ng/ml. Fluorescent was measured automatically by a miniVIDAS instrument (BioMérieux, France), and the results of the test were calculated using software built into the analyzer.

Statistical Analysis

The data, presented as mean ± SD, were analyzed using Statistical Package for Social Sciences (SPSS) version 17 (SPSS Inc., Chicago, IL, USA). The categorical variables were analyzed using Chi-Square test. Student t-test or Mann-Whitney U-test was used for between-group comparisons considering the normality of distribution of the data. The data obtained at the beginning and after 7 days of treatment from the case group were compare using paired-t test. The data from blood cultures were used as the gold standard to evaluate the optimum sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the Receiver Operative Characteristic (ROC) curves. A P value of ≤0.05 was considered statistically significant.

Results

There was no significant difference between the two groups with respect to age (P=0.68), birth weight (P=0.10), gestational age (0.33) or gender (P=0.28) (table 1).

The causative pathogens of confirmed sepsis included *Coagulase Negative Streptococcus* (11 cases), *Klebsiella pneumonia* (4 cases), *group B streptococcus* (3 cases), *Staphylococcus Aureus* (3 cases), *Escherichia coli* (2 cases), *Pseudomonas aeruginosa* (1 case), and *Candida albicans* (1 case).

Baseline serum PCT concentration were significantly (P=0.001, Student t test) higher in the case group than that in the control group. Moreover, serum levels of CRP as well as WBC counts from the case group were significantly higher than those of the control group (P=0.001, P=0.02 for CRP and WBC, respectively). After 7 days of treatment, when the patients had recovered from the disease and blood cultures were negative, serum levels of PCT in the case group were 0.26±0.37 ng/ml, which were significantly lower that the levels obtained at the beginning of the treatment (P=0.001, paired –t test) (table 2).

Figure 1 shows the ROC curves illustrating the sensitivity and specificity of serum levels of PCT, CRP, and WBC counts for the diagnosis of neonatal sepsis. The area under the ROC for PCT [Median: 0.926, 95% confidence interval (CI): 0.855 to 0.998] was significantly more than those for CRP [Median: 0.851, 95% CI: 0.7443 to 0.958, P=0.0001] and WBC counts (median: 0.670, CI: 0.513 to 0.827, P=0.046)

The sensitivity, specificity, PPV and NPV of serum and WBC counts for neonatal sepsis are presented in table 3. Diagnostic cut off levels with the optimum sensitivity and specificity derived from the ROC curve for PCT and CRP were found to be ≥0.8 ng/ml and ≥20 mg/l, respectively.

Discussion

There is no a single reliable test for the early definite diagnosis of neonatal sepsis, therefore, there is a continuing search for a new infection marker. Previous studies have shown CRP to be a use-

Table 1: Demographic Characteristics of Patients participating in the study and levels of statistical significance between control and case groups

	Control group (n=22)	Case group (n=25)	P value
Age (days)	9.6±8.3	8.6±7.8	0.68
Birth weight (grams)	3081±739	2719±1172	0.10
Gestational age (weeks)	37.3±2.4	36.4±4.3	0.33
Gender (boys/girls)	8/14	13/12	0.28

Data are shown as mean±SD

Table 2: Serum levels of procalcitonin (PCT) and C reactive protein (CRP), and white blood cell (WBC) counts of control and case groups at the beginning of the study.

	Control group (n=22) Mean±SD (Max-Min)	Case group (n=25) Mean±SD (Max-Min)	P value
PCT (ng/ml)	0.38±0.43 (0.05-1.45)	14.1±18.7 0.1-80.21	0.001
CRP(mg/l)	18.6±23.7 (1-77)	60.2±32.4 3-99	0.001
WBC (C/mm^3)	7.5±2.8 (3.7-14) 10^3	11±6.5 (1.8-27.8) 10^3	0.02

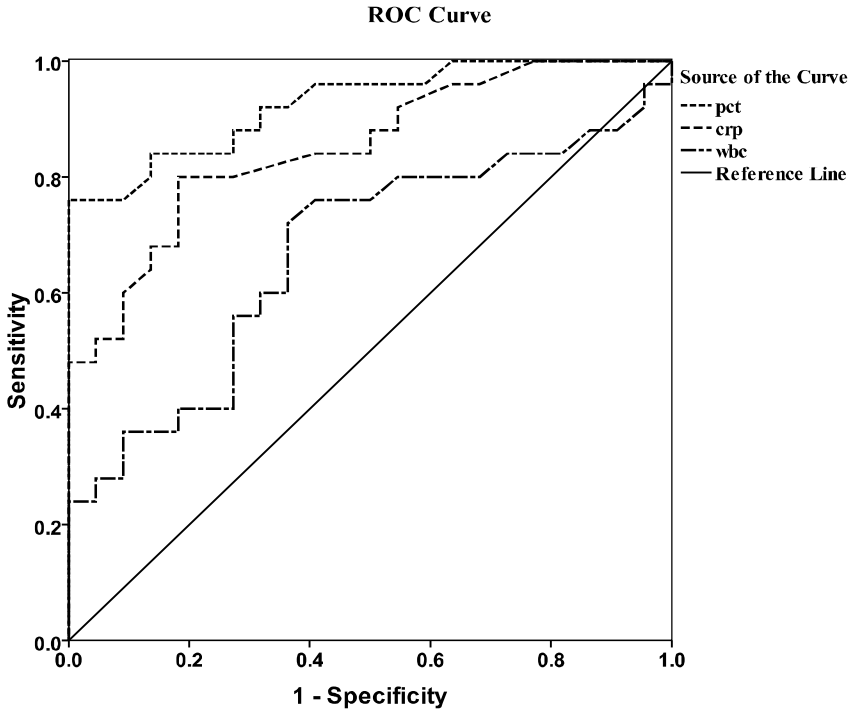

Figure 1: Receiver operating characteristic (ROC) curves of procalcitonin (PCT), C- reactive protein (CRP) and white blood cell (WBC) counts using the data from all participants (n=47). The area under the curves for PCT, CRP and WBC were 0.926, 0.851, 0.670, respectively.

Table 3: The values of cut-off, sensitivity, specificity, and positive predictive values (PPV) and negative predictive values (NPV) of serum levels of procalcitonin (PCT) and C reactive protein (CRP), and white blood cell (WBC) counts.

	Cut-off value	Sensitivity%	Specificity %	PPV%	NPV%
PCT (n=47)	0.8 ng/ml≥	84	86	86	84
CRP (n=47)	20 mg/l≥	80	77	77	90
WBC counts	<4000 (C/mm^3) >12000(C/mm^3)	72	63	66	69

ful marker of bacterial sepsis in neonates.[18,19] However, in the early course of the disease the serum levels of CRP may be normal, showing a long duration between the invasion by infectious agents and the rise in serum CRP concentrations.[20] Moreover, CRP levels may not rise further, if the disease condition becomes more severe.[5] White blood cell counts and ratios may be helpful in diagnosing sepsis, however, normal WBC counts may be observed in as many as 50% of culture-proven sepsis cases, and neonates who are not infected may also have abnormally high WBC counts as a result of the stress of delivery.[21] More recently PCT has been proposed as an early marker of bacterial sepsis in neonates and children.[22-24] Procalcitonin, which is low or undetectable in the serums of healthy subjects, infants or adults, reaches high concentrations in patients with severe bacterial infections, septicemia or meningitis, and decreases rapidly after appropriate antibiotic therapy. Moreover, in patients with acute viral infections or with inflammatory diseases, PCT levels are low.[25] In contrast, several conditions in which serum PCT concentration rise independent of sepsis and infection have been described. These conditions include surgery, polytrauma, heat shock, burn injuries, prolonged cardiogenic shock and severe systemic inflammation such as the one secondary to multiple organ dysfunction syndrome (MODS).[26] Increased PCT production has also been reported to occur in newborns during the first days after delivery.[22]

Several studies suggested a positive correlation between the serum PCT levels and the severity of neonatal sepsis or recovery from the disease after appropriate treatment.[10,13,27] In the present study, the PCT levels were remarkably high in neonates with proven sepsis, and dropped dramatically after appropriate treatment with antibiotics. The findings show

that serum concentrations of PCT, CRP, and WBC were much higher in neonates with bacterial sepsis than in those without the disease. Moreover, they show that the sensitivity, specificity and area under the ROC curve of PCT were more than those of CRP or WBC counts. Such findings are in agreement with those obtained by Hatherill et al.[28]

Bonac and colleagues,[29] compared the serum levels of PCT and CRP, in the diagnosis of neonatal sepsis in 58 newborns. Using a cut-off value of 0.99 ng/ml, they found that sensitivity, specificity, PPV and NPV of PCT were 59%, 82%, 36% and 96%, respectively. They also reported that, using a cut-off value of 14 mg/l, sensitivity, specificity, PPV and NPV of CRP at the time of diagnosis were 36%, 92%, 43% and 89%, respectively. In another study, a cut-off value of 0.7 ng/ml for PCT led to an improved sensitivity for PCT (99%) compared to that of CRP.[30] Other studies have reported sensitivities ranging from 60% to 100% and specificities from 79% to 100%.[31-33]

Interpretation of the literature about procalcitonin is complicated by variations in the choice of the abnormal cut off values, by the diverse age range, and the nature of the study populations. The findings of the present study support the views of some authors,[28,34] that WBC counts is of little value in the diagnosis of neonatal sepsis.

The findings of the present study should be interpreted in the light of a number of limitations. First, the sample size was relatively small, which was due to strict selection criteria. The second limitation was the lack of measurement of serum concentrations of PCT at the end of the second day of treatment, which was due to limited resources. The final limitation was the inclusion of neonates with both early-onset and late-onset sepsis.

Conclusion

The findings of the present study confirm those of others that serum levels of PCT is a more reliable marker than serum levels of CRP or WBC counts in the early diagnosis of neonatal sepsis and evaluation of the response of the disease to antibiotic therapy. The benefits of measuring serum PCT routinely in the diagnosis and follow-up of neonatal sepsis is to reduce the hospital cost. Such benefit might support a wider acceptance of the test in routine practice.

Conflict of Interest: None declared

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