

## Original Article

### Sensitivity and Specificity of Procalcitonin in Diagnosis of Neonatal Sepsis

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#### ABSTRACT

**Background and Objective:** According to the fact that neonatal infection is a challenging diagnosis field, several studies have tried to test sensitivity and specificity of diagnostic tests. This study was conducted to evaluate the sensitivity and specificity of procalcitonin (PCT) as a single early marker of neonatal sepsis.

**Materials and Methods:** In this study, 150 neonates admitted to NICU and neonatal ward in Shahrekord Hajar hospital were enrolled. A full workup including blood culture and other tests and PCT was conducted. Sensitivity, specificity, positive and negative predictive values for PCT was determined.

**Results:** It was found out that 8 patients had definite infection, 15 patients had possible infection, and 127 patients had no infection. Although PCT was not able to significantly differentiate between those with definite and possible infections ( $p>0.05$ ), but there was a significant difference for frequency of abnormal PCT between non-infectious patients and other patients. Sensitivity, specificity, positive and negative predictive values of PCT was 87.5%, 87.4%, 30.4%, 99.1%, and 87.41% respectively.

**Conclusion:** Beside the limitation of the sample size, the satisfactory diagnostic characteristics of PCT highlight it as a good measure for diagnosis of neonatal sepsis. Further studies are essential to be carried out.

**Key words:** Neonate, Sepsis, Procalcitonin

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## Introduction

Neonatal sepsis has been one of the major diagnostic problems for physicians due to non-specificity of its symptoms and the absence of a reliable Para clinical marker. This condition is clinically important since it is one of the main causes of mortality in infants, especially those with a low at-birth weight (1). Mortality rate for neonatal sepsis has been reported as 10-50% (2). Therefore, it's fast and on time diagnosis could lower mortality rate in infants (3). Diagnosis of neonatal sepsis using clinical symptoms is not possible (1). Although isolating its responsible microorganisms using blood culture has been the golden standard method for its diagnosis (4), but its result is ready 24-72 h after sampling and during this time period it is necessary to treat suspicious infants for sepsis with antibiotics according to clinical symptoms and risk factors. It is also possible that a pseudo-negative result is obtained in some cases (1;4). The present trend applied for infants suspicious of neonatal sepsis may lead to unnecessary increased antibiotic consumption, a higher incidence of side-effects due to their use, increased resistance to antibiotics, a long hospitalization, and separation of infants from their mothers and increased health costs (5;6).

Therefore, using fast diagnostic methods including laboratory markers could be beneficial in diagnosis of neonatal sepsis. In addition to blood culture, other tests that are usually used for diagnosis of neonatal sepsis include complete blood count and acute phase protein (CRP). Unfortunately, these tests do not have a high sensitivity and specificity. Meanwhile, some studies have shown that CRP has a limited use in diagnosis of neonatal infections (3;7).

Recently, serum procalcitonin (PCT) has been reported as a measurable laboratory marker in inflammatory response to infection in some studies. This factor is the preformed of calcitonin that although is not measurable in serum of normal individuals, but its titer increases during microbial, fungal, and parasitic infections (8). The goal of this study was to evaluate PCT level in infants with sepsis and to assess its diagnostic value.

## Materials and Methods

This study had a descriptive-analytical strategy and was conducted on suspicious infants to have sepsis and hospitalized in infants ward and intensive care

unit of Hajar hospital (Shahrekord) from 2006 to 2007. Those infants treated for sepsis were hospitalized with 2-3 symptoms of sepsis in addition to one of the maternal and infantile risk factors.

Clinical criteria for diagnosis of sepsis were as follows: 1) maternal risk factors including fever, use of alcoholic beverages, premature rupture of water sac (PROM) greater than 24 h, chorioamnionitis, and urinary tract infection (9), 2) neonatal risk factors including low birth-time weight (less than 2500 g) and still birth (less than 37 weeks) (9), and 3) clinical symptoms and signs of sepsis including loss of appetite, changes in body temperature (fever and hypothermia), jaundice, apnea, respiratory distress, tachycardia, tachypnea, cyanosis, and vomiting (9).

Before initiating venous antibiotic treatment, blood sampling was ordered by responsible physician for routine tests to diagnose neonatal sepsis. Simultaneously, 2 ml of clotted blood was sent to laboratory for determination of PCT level. After centrifugalization and its serum isolation, it was kept at  $-70^{\circ}\text{C}$ .

After determination of results for complete cell count, CRP, and blood culture, the infants were grouped within the following three groups: group (1) those infants with positive blood culture and CRP and more than two clinical symptoms (8 cases), group (2) those cases with negative blood culture, positive CRP, and more than two clinical symptoms (15 cases), and group (3) infants with negative blood culture and CRP and more than two clinical symptoms (127 cases). CRP measurement was done according to latex agglutination method. In addition, PCT measurement was performed after collection of 150 serum samples. For this purpose, PCT-G kit (Brahms, Germany) was used. The related samples for PCT measurement were exposed to room temperature (immunology laboratory) 30 min before assay. The basis for this assay was an immunochromatographic one that is considered a semi-quantitative method for measurement of PCT. The kits were removed from their packages immediately before use and 200-microliter sampler was used for sample taking and to pour it on labeled round part of the kit chromatographic paper. This paper had also two other parts including control and test bands. The latter band turned pink or red if any PCT exist in case serum and its color intensity was closer to control band if level of PCT was higher. Each kit had also a reference card for recording test results. Meanwhile, three colors as weak pink, intense pink and red were indicative as different concentration of

PCT in serum. In this respect, red, intense, and weak pink colors were indicative of a PCT level equal or greater than 10 ng/ml, greater than 2 ng/ml, and 0.5 ng/ml respectively.

The results of studies on normal infants have shown that during the first 48 h of lifetime, a PCT level of 2 ng/ml is normal (10). In our study, a PCT level higher than 2 ng/ml during the first 48 h and a PCT level greater than 0.5 ng/ml after that time considered abnormal (11;12).

For blood culture, a biphasic medium was used. In this regard, 1-3 ml of blood was added to culture medium and incubated for 5 days at 25 °C. Culture media were daily evaluated with respect to turbidity and bacteria growth and in condition of such growth; special media was used for passage to determine its type and sensitivity to antibiotics.

For inclusion in the study, a written consent was taken from their mothers. In addition, infants with a low blood calcium or glucose level during the study were excluded. For data analysis, SPSS software

was used. Meanwhile, blood culture was regarded as the golden standard method and for other tests their diagnostic attributes were compared to the standard one. In this way, specificity, and positive and negative predictive values for PCT as a marker for neonatal sepsis was reported.

## Results

In this study, 150 infants suspected of sepsis were evaluated. In this respect, 93 cases (62%) were boy. Time of first clinical symptoms in 60 cases (40%) was within the first 48 h after birth and in others it was after this period. Weight average of infants was  $2670 \pm 637$  g. In addition, 49 infants (32.7%) were premature. Meanwhile, 41 (27.3%), 62 (41.3%) and 47 (31.3%) out of cases had a good, intermediate, and weak infantile reflex responses respectively. Data for range, average, and standard deviation of blood glucose and calcium levels, WBC, neutrophil, and lymphocyte counts have been presented in Table 1

**Table 1: Laboratory findings in neonates suspicious to neonatal sepsis**

Laboratory findings	range	Mean $\pm$ SD
Serum glucose (mg/dl)	40 – 397	87.8 $\pm$ 50.5
Serum calcium (mg/dl)	7.5 – 13	1.2 $\pm$ 9.6
White blood cell count(Number/ $\mu$ l)	4 – 73.4	9.4 $\pm$ 13
Neutrophil %	10 - 81	16.1 $\pm$ 41.5
Lymphocyte %	3.1 – 85.5	50 $\pm$ 16.8

Furthermore, CRP measurement was done at the time of hospitalization before treatment. In this regard, it was positive in 20 cases. The most prevalent pathogen in blood culture was Klebsiella. Serum level of PCT in group 1 (those infants with positive blood culture and CRP and more than two clinical symptoms) was positive in 87.5% of cases. Serum level of PCT in group 2 (those cases with negative blood culture, positive CRP, and more than two clinical symptoms) was positive in 12.6% of cases. Serum level of PCT in group 3 (infants with negative blood culture and CRP and more than two clinical

symptoms) was positive in 20% of cases. In addition, there was a statistically significant difference between group 3 and the other two groups, i.e. PCT in group 3 was lower as compared to groups 1 and 2 ( $p < 0.05$ ). Meanwhile, with regard to blood culture as a golden standard method, sensitivity, specificity, and positive and negative predictive values of PCT level for neonatal sepsis was 87.5%, 87.4%, 30.4%, and 99.1% respectively. Table 2 shows distribution frequencies for positive and negative PCT in different groups and their diagnostic attributes.

**Table 2: Positive and negative PCT in different groups**

Groups	No infection	Suspicious to infection	Definite infection	Total
Negative	111	12	1	124
Positive	16	3	7	26
Total	127	15	8	150

## Discussion

The results of the present study showed that sensitivity, specificity, and positive and negative predictive values of PCT level for neonatal sepsis was 87.5%, 87.4%, 30.4%, and 99.1% respectively. With regard to the turning point of 2 ng/dl, this level of sensitivity and specificity is appropriate.

According to other studies, PCT is suitable for diagnosis and evaluation of response to treatment in neonatal infection (10;13). Some studies have reported a higher sensitivity for this marker in early stages of neonatal infection in comparison with acute phase protein (CRP) (14). In another study, a negative predictive value of 95% has been obtained for PCT (15). Monneret et al conducted a study in Stockholm on 94 infants hospitalized in ICU and found that PCT level increases more rapidly in neonatal infection than acute phase C protein (CRP) and its level also decreases more rapidly after successful treatment. PCT level increases 3-4 h after exposure to bacterial agents and within 6 h, it reaches to maximum level, while CRP increase requires 12-18 h (15). In a study by Chiesa et al in Italy on 120 infants hospitalized in neonatal ICU and 83 normal neonates, the cases were divided into non-infectious (control), suspected of infection with clinical symptoms, and infectious with a positive blood culture groups. In this regard, sensitivity and specificity for increased serum level of PCT during the first 48 h after birth were determined as 99.6% and 97.5% in infectious groups respectively and they were 100% in control group (11). In another study by Chiesa et al, sensitivity for PCT in diagnosis of neonatal sepsis in early measurement was obtained as 85.7% (3). PCT also completely predicted infection in 89.5% of cases (16).

In this study, 34.7% of suspected patients for infection were diagnosed as having infection following a positive blood culture. This figure was 10% in Vali-Asr hospital (Tehran) in 1999 (17), 11% in Mofid hospital (Tehran) from 1997 to 1998 (18), 41% in Ali-Asghar hospital in 1997 (19), and 16.8% in Ghaem hospital (Hamedan) from 1997 to 1998 (20;21). The results of our study showed that incidence of true septic infection in suspected infants is low and many of them with no signs of infection are treated with antibiotics. In addition, although the most prevalent pathogen responsible for neonatal infection in our study was *Klebsiella*, in other studies conducted in our country it has been *Staphylococcus* coagulase positive and negative and *Klebsiella* (22),

*Pseudomonas* and *Klebsiella* (23), Gram-negative and non-fermentative Bacilli and *Klebsiella* (15), *Staphylococcus epidermis* and *Enterobacter* (19).

Since type of prevalent pathogen differs in different studies and with regard to the fact that PCT positivity is lower in infections with low virulence microorganisms (14), therefore, sensitivity and specificity will be different in various studies and under different conditions. It is noteworthy that in some studies the turning point has been regarded as 0.5 ng/dl (24) and it has been as 2 ng/dl (10) in other studies. In our study, all cases of group 1 with abnormal PCT showed a lower PCT after antibiotic treatment up to the extent that was unmeasurable. In Chiesa et al study in 2003, PCT was used as an index for treatment follow up and duration (12).

In the present study, only cases with a definite infection (blood culture positive) were included for determination of PCT sensitivity and specificity and hence sample size was relatively small, but since there have been limited studies in this field in our country, it was necessary to report these data. Since PCT was not capable to differentiate suspected cases of infection from control cases, it is not of much value in diagnosis of possible infection without the presence of a positive blood culture. However, its sensitivity in diagnosis of definite and possible cases of infection is 56.5% and its negative predictive value in these groups is 90%.

## Conclusion

With regard to the results of the present study and high negative predictive value of PCT, it is recommended that PCT test is conducted at the time of or before hospitalization to evaluate possible sepsis. In PCT-negative cases, there is certainly a negative blood culture that could lower hospitalization period and in this way to compensate for its high cost. However, cost-benefit studies are warranted to be conducted. Its high cost is still a negative factor for its clinical and routine application.

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