ORIGINAL ARTICLE

Evaluation of procalcitonin and neopterin level in serum of patients with acute bacterial infection

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ABSTRACT

Background: Fever as a common presenting complaint in pediatric patients can be due to various causes. Differentiating bacterial infection from other causes is important because the prompt use of antibiotics is critical in bacterial infection. Traditional markers of infection such as BT and WBC count may be unspecific and culture may be late or absent. CRP and Procalcitonin (PCT) have been considered to evaluate the evolution of infections and sepsis in patients presenting with SIRS. Neopterin has also been proposed to aid in the diagnosis of bacterial infection. In this study, we compared the value of the serum PCT, neopterin level, and WBC count for predicting bacterial infection and outcome in children with fever. **Methods**: 158 pediatric (2-120-month-old) patients suspected to have acute bacterial infection, based on clinical judgment in which other causes of SIRS were ruled out were included in the study. WBC count with differential was determined and PCT and neopterin levels were measured. **Results**: PCT level was higher in bacterial infection and patients who were complicated or expired. **Conclusion**: Rapid PCT test is superior to neopterin and WBC count for anticipating bacterial infection, especially in ED where prompt decision making is critical. **Abbreviations**: BT, body temperature; WBC, white blood cell; PCT, procalcitonin; CRP, C-reactive protein; SIRS, systemic inflammatory response syndrome; ED, emergency department.

Keywords: procalcitonin, neopterin, SIRS.

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INTRODUCTION

Fever is a common presenting complaint in pediatric emergency department (ED). In most children, a benign infection is diagnosed after a good history and a careful examination. In rare instances, especially in infants, infection is manifested only by fever and vague or nonspecific signs and symptoms, and no focus is evidenced after clinical examination.[1,2] Conversely, not all patients who appear septic demonstrate an infection, and the widespread administration of antibiotics to all these patients carries problems of antibiotic resistance, drug toxicity, and increased medical costs.[3] Also, differences in body temperature (BT), heart rate, white blood cell (WBC) count, and respiratory rate often are small. As a consequence, diagnosis of infection can be difficult: positive bacteriological samples may be late or absent, the clinical interpretation of local colonization may be ambiguous, and traditional markers of infection such as BT and WBC count may be unspecific.[4] Other parameters such as C-reactive protein (CRP) and procalcitonin (PCT) have been considered to evaluate the evolution of infections and sepsis in patients presenting with systemic inflammatory response syndrome (SIRS).[1-3,5]

PCT is a 116 amino acid peptide with a sequence identical to that of the pro-hormone of calcitonin,[2] normally synthesized by the C-cells of thyroid gland, and its level in blood of healthy people is less than 0.01 ng/mL. In bacterial infection and sepsis, however, its level increases to 20-200 ng/mL and is related to the severity of sepsis.[3,6-8] As the level of PCT rises in patients who have been underwent to thyroidectomy, it seems that in bacterial infections PCT must be synthesized somewhere other than the thyroid gland, most probably in the liver or monocytes, in response to cytokines such as IL-6 and TNF-α.[9,10] PCT increases in blood 6 hours after a stimulus, reaches a plateau between 12 and 48 hours, and then decreases if the stimulus stops. CRP increases later than PCT.[1]

Neopterin has also been proposed to aid in the diagnosis of bacterial infection.[11] Neopterin is a pyrimidine derivative, found to be raised in pa-

tients suffering from infections, neoplastic and inflammatory diseases.[12] Human monocytes/macrophages produce neopterin when stimulated by interferon-γ released from activated T cells. Other cell types do not produce measurable amounts of neopterin following various stimuli. Therefore, neopterin production appears to be closely associated with activation of the cellular immune system.[13] The biological function of neopterin is not completely clear: it is associated with nitric oxide synthesis and formation of reactive metabolites of oxygen, and it may be toxic to microorganisms. Increased concentrations are related to endothelial damage and risk for septic complications.[14,15] The upper limit of the normal range is approximately 10 nmol/L serum (= 2.5 ng/mL).[13] In contrast to PCT, neopterin also increases in viral infections.[16]

In this study, we aimed to compare the value of serum PCT, neopterin level, and WBC count for predicting bacterial infection and outcome in children with fever.

MATERIAL AND METHODS

During a 12-month period, 200 pediatric (2-120-month-old) patients admitted to ED and Infectious Disease ward of a tertiary university hospital, suspected to have acute bacterial infection based on clinical judgment, were included in the study. Patients with other causes of SIRS (trauma, inflammation and burn) that could explain their signs and symptoms were excluded. Thus, 158 patients were selected and demographic data were collected. Blood samples were taken from all patients. WBC count with differential was determined and PCT and neopterin levels were measured. The serum PCT level was measured by a rapid semiquantitative immunochromatographic test (Brahms PCT-Q, Hennigsdorf, Germany). Briefly, the patient plasma was applied onto the test strip. PCT in the sample is bound by mouse anti-catacalcin antibodies conjugated with colloidal gold to form a complex. This complex moves by means of capillarity through an area containing fixed anti calcitonin antibodies to form a sandwich complex that can be seen as a reddish band. The color intensity of the band is directly proportional to the PCT concentration of the sample. The results were reported as following ranges: < 0.5 ng/mL, ≥ 0.5 and < 2 ng/mL, ≥ 2 and < 10 ng/mL, ≥ 10 ng/mL. The serum neopterin level was measured by enzyme-linked immunoassay (ELISA).

Bacterial infection was defined as positive blood, urine or CSF culture or CXR compatible with pneumonia or positive culture of synovial or peritoneal fluid. The outcome was classified as: 1) cured; 2) complicated or expired.

STATISTICAL ANALYSIS

The Kolmogorov–Smirnov test was used to assess sample distributions. To compare two independent samples, we used an unpaired t-test or the Mann–Whitney U-test (data non-normally distributed). To compare three independent groups, we used analysis of variance or the Kruskal–Wallis test (data non-normally distributed). We used the chi-square test to compare proportions; $p < 0.05$ was considered significant. Statistical calculations were performed with SPSS statistical software (version 16.0; SPSS Inc., Chicago, IL, USA).

RESULTS

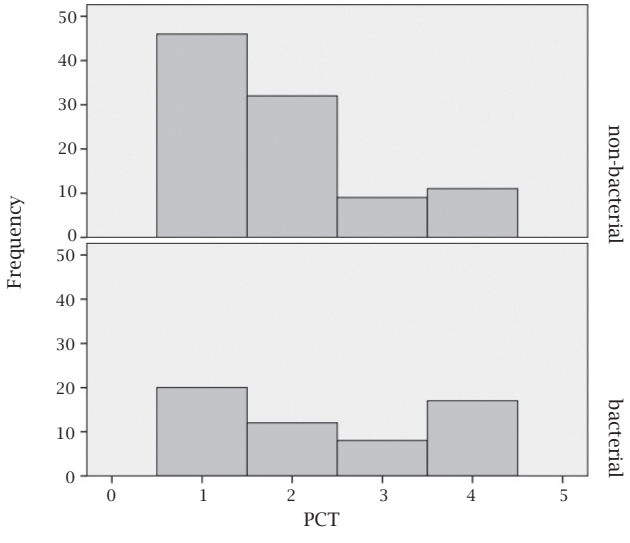
200 pediatric patients were included in this study and 42 were excluded because they were supposed to suffer from other causes of SIRS (trauma, inflammation, burn) except infection. Therefore, data of 158 cases were analyzed. Patient population included 82 (51.9%) male and 76 (48.1%) female. Mean age of patients was 42.8 ± 3.5 months. The commonest site of infection was urinary tract, followed by lungs. In 13 (8.2%) cases no site of infection was found (FUO). Mean WBC count and neopterin level in patients with bacterial and non-bacterial infection is shown in Table 1.

WBC count was significantly higher in bacterial infection ($p = 0.009$). WBC count ≥ 1,2000/mm^3 was suggestive of bacterial infection with sensitivity of 51.9% and specificity of 70%. Serum PCT level increased in bacterial infection (Figure 1); 35.1% of patients suffering from bacterial infection

Table 1. Mean WBC count neopterin level in patients with bacterial and non bacterial infection

Type of infection	WBC/mm^3	neopterin (nmol/L)
Bacterial	10,486.21	44.43
Non-bacterial	7,244.33	42.93

Figure 1: PCT level in bacterial and non-bacterial infection. 1 = PCT < 0.5, 2 = PCT ≥ 0.5 and < 2, 3 = PCT ≥ 2 and < 10, 4 = PCT ≥ 10 ng/mL.



had PCT < 0.5 ng/mL and 29.8% had PCT ≥ 10 ng/mL. In non-bacterial infection group, PCT was less than 0.5 ng/mL in 46.9% and 10 ng/mL or more in 11.2% (p = 0.014). Considering PCT level ≥ 10 ng/mL as strongly positive PCT test, the difference was more significant: 29.3% with bacterial infection and 11.2% with non-bacterial infection had PCT ≥ 10 ng/mL (p = 0.006). Sensitivity and specificity of PCT test with cut-off point of 10 ng/mL for predicting bacterial infection was 29.3% and 88.7%, respectively. Mean neopterin level was 44.43 nmol/L in bacterial infection and 42.93 nmol/L in non-bacterial (viral or fungal) infections which was not statistically significant (p = 0.88).

Mean WBC count was significantly lower in cured patients (5,184.21/mm^3) in comparison to uncured group (11,412.63/mm^3) (p < 0.001). Five out of 49 (10.2%) patients with PCT level < 0.5 ng/mL was complicated or expired and 44 (89.8%) cured, while among 24 patients with PCT ≥ 10 ng/mL, 9 (37.5%) were complicated or expired and 15(62.5%) cured (p = 0.03). 88.2% of patients with PCT < 10 ng/mL cured, while the cure rate was 62.5% for patients with PCT ≥ 10 ng/mL (p = 0.006). Neopterin level was not significantly different in patients who cured and those complicated or expired (p = 0.59). Table 2 shows the cure rate in different PCT groups.

Table 2. The cure rate in different PCT groups

PCT level (ng/mL)	< 0.5	≥ 0.5 & < 2	≥ 2 & < 10	≥ 10
Cure rate	91.2%	80.0%	85.7%	40.0%

DISCUSSION

The diagnosis of bacterial infection in pediatric patients is not always straightforward. Clinical manifestations of bacterial and non-bacterial infections are the same and reflect the host immune response. Routine laboratory tests in patients presenting with SIRS frequently lack both sensitivity and specificity in differentiating which patients should receive antibiotics, and culture that confirms the diagnosis are not immediately available.[16,17] Early institution of an appropriate antimicrobial regimen in infected patients is associated with a better outcome.[3,4] On the other hand, inappropriate use of antibiotics can cause resistance to these widely prescribed, useful drugs. Assicot *et al.*[18] first described high serum PCT concentrations in children with severe bacterial infections. Suprin *et al.* compared PCT and CRP in ICU patients with positive SIRS and suggested CRP as a more specific and sensitive sepsis marker than PCT.[19] But at the same time, Muller *et al.*[6] investigated 101 patients admitted to a medical ICU and suggested PCT as a more reliable marker of sepsis than CRP, IL-6 and lactate levels. Selberg *et al.*[20] study suggested PCT, IL-6 and C3a concentrations more reliable parameters for differentiating between septic and SIRS patients than CRP and elastase. Balcl *et al.*[21] compared PCT with CRP, IL-2, IL-6, IL-8, and tumor necrosis factor-α as an early sepsis marker. These data indicated PCT as a useful early marker for discriminating between SIRS and sepsis CRP and PCT have different kinetics and profiles. The kinetics of CRP is slower than that of PCT, and CRP levels may not further increase during more severe stages of sepsis. On the contrary, PCT rises in proportion to the severity of sepsis and reaches its highest levels in septic shock. PCT tends to be higher in non-survivor than in survivor. Therefore, PCT demonstrated a closer correlation with the severity of sepsis and outcome than CRP.[22] Neopterin (D-erythro-1',2',3'-trihydroxypropylpterin) was first isolated from human urine in 1965.[23] The discovery of neopterin as a marker for T-cell activation dates back to the 1980s.[24] Neopterin is formed preferentially in response to Th1-type cytokine interferon-γ, and, because of its ability to amplify deleterious effects of radicals in various cellular systems, neopterin seems part of the pro-inflammatory and cytocidal armature of the activated human macrophage.[25] Neopterin concentrations are among the best predictors of the future disease course in patients with cardiovascular disorders, after multiple traumas and with several types of cancer.[26] In patients with HIV infection, neopterin concentrations are even more closely related with survival than virus load. Monitoring of neopterin concentrations also allows early detection of immunological complications in allograft recipients. Because of its high sensitivity to detect acute virus infections early, neopterin screening is nationwide in use to improve virus safety in blood donation in Austria.[27] PCT and neopterin are novel markers introduced for predicting infection. Various recently published studies indicated that there is a significant relationship of PCT not only to infection and systemic inflammation, but also to the outcome of the disease. In our study, the serum PCT level was significantly higher in bacterial infection and in patients with more severe infection who were complicated or expired. Currently, PCT was related to the severity of the disease. Neopterin level had no relationship to bacterial infection. As neopterin level rises in viral infections as well as bacterial infections, it was expected not to detect any difference in neopterin level of these two groups. PCT is a rapid test that can predict bacterial infection and outcome of disease. PCT level of 10 ng/mL or more is suggestive of bacterial infection and associated with poor prognosis, while normal PCT level (less than 0.5 ng/mL) indicates better prognosis and low probability of bacterial infection. Applying this test in ED will lead to better decision making for starting antibiotics.

CONCLUSION

Rapid PCT test is superior to neopterin and WBC count for anticipating bacterial infection, especially in ED where prompt decision making is critical. With regards to test expenses, measurement of neopterin concentration, which does not reveal any additional information, to aid diagnosis is not worthwhile.

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