Serial procalcitonin responses in infection of children with secondary immunodeficiency

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Abstract

Purpose: Procalcitonin has proven to be a sensitive inflammatory marker in non-neutropenic patients. The aim of this study was to determine and compare Procalcitonin with other inflammatory markers in the serum of immunosuppressed children with haematological malignancies; and to assess the predictive value of these mediators in distinguishing between bacterial and non-bacterial infection.

Methods & Results: The study included 37 children with acute lymphoblastic leukaemia undergoing intensive chemotherapy. They were divided into 3 groups, A, B and C. Group A consisted of 29 neutropenic children with 94 febrile episodes, group B of 20 neutropenic children with 56 afebrile episodes and group C of 13 non-neutropenic children with 58 afebrile episodes. Serial serum levels of PCT, C-Reactive Protein, Neopterin, Interleukin-6 and NO₂/NO₃ were all determined on a day-to-day basis for 7 consecutive days. In serum the concentrations of CRP was determined by nephelometry, of PCT by immunoluminescence and of Neopterin, IL-6 and NO₂/NO₃ by ELISA method.

Conclusions: According to our results the Procalcitonin concentration increased rapidly in patients with microbial infection; the response was detectable within 24 hs of the onset of fever due to microbial infections. Procalcitonin is a specific and sensitive marker of microbial infection in patients with neutropenic fever. The markers, C-Reactive Protein, Interleukin-6 and NO₂/NO₃ may not help to identify infections and distinguish the etiology of infection in neutropenic febrile children with acute lymphoblastic leukaemia.

Leukemia and/or intensive chemotherapy may cause neutropenia and an impaired host defence to infection.¹ Fever is a common sign in these infections but not always. Also, the febrile neutropenia may be attributed to an underlying infection, or disorder or to the administration of drugs or blood products.² Along with the findings concerning more effective antileukemic chemotherapies, the knowledge and methods for the diagnosis of severe treatment-related infections have also improved and led to generally accepted guidelines for diagnosis and empirical antimicrobial therapy of neutropenic leukemic patients.³ Various traditional serological markers of infection have been applied, in order to early diagnose the bacterial or fungal infections in neutropenic febrile patients with ALL.⁴

C-reactive protein (CRP) is the most widely analysed acute phase protein in clinical practice.⁵ The determination of CRP has been used for years in the diagnosis of infection, as well as to monitor the outcome of infection treatment in patients with haematological and paediatric malignancies and profound neutropenia.⁶⁻⁷

Neopterin is a pteridine intermediate metabolite in the biopterin synthetic pathway and it is synthesized and secreted by activated monocytes and macrophages.⁸ Increased neopterin concentrations

have been found in a large variety of conditions in which the cell-mediated immune system is activated, such as organ and bone marrow transplantation, rheumatoid arthritis, systemic lupus erythematosus, autoimmune diseases, inflammatory bowel diseases, multiple sclerosis, sarcoidosis, diabetes, congestive heart failure and infectious diseases.⁹

The IL-6 cytokine, along with others is a proinflammatory cytokine.¹⁰⁻¹⁷ It is postulated to play a major role in the pathogenesis of infections.⁶ The presence of circulating IL-6 has been found in patients with documented bacteremia or with signs of sepsis, often correlating with the severity of the infection, thus suggesting an important role for the IL-6 cytokine in the development of infection.⁷

Serum levels of nitrite and nitrate, the stable oxidative end-products of NO, are used as an indirect measure of in vivo whole body NO production in sepsis.¹⁸⁻²² The overproduction of nitric oxide (NO), a potent vasodilator formerly known as endothelium-derived relaxing factor, has recently been held responsible for the hemodynamic and metabolic consequences of sepsis and endotoxemia. High levels of nitrite and nitrate, the stable end-products of NO metabolism, are found in patients with severe sepsis and these levels may correlate with vasodilatation.¹⁸ Several in vitro studies have shown that NO itself may have both pro- and anti-inflammatory effects and plays a role in modulating the immune response.²³⁻²⁷

Procalcitonin (PCT) is a 116 amino-acid propeptide of calcitonin, has been proposed as a new diagnostic marker of severe infections.²⁸ PCT is presumably synthesized in tissues other than thyroid C-cells, which are the source of calcitonin in normal physiology.²⁹ According to recent studies the major source of PCT seemed to be the liver, and PCT may thus be considered as an acute phase protein. Procalcitonin (PCT) is significantly elevated in the serum of immunocompromised patients with bacterial infections, such as pneumonia and sepsis, but remains low in viral infections and inflammatory diseases.³⁰⁻³¹ This change in concentration is rapid and the molecule is stable and easy to determine, making it a potentially useful marker. The reason for increased PCT secretion

in patients with severe infections has remained unknown.³²⁻³⁴

The aim of this study was to determine whether PCT is a sensitive diagnostic parameter in febrile neutropenia and to compare PCT with other inflammatory markers in the serum of immunosuppressed children with haematological malignancies; and to assess the predictive value of these mediators in distinguishing between bacterial and non-bacterial infection.

Methods

The study included 37 children with acute leukemia undergoing intensive chemotherapy. They were divided into 3 groups, A, B and C. Group A consisted of 29 neutropenic children with 94 febrile episodes, aged 1-14 yr (5.8 ± 2.9). This group was divided into two subgroups A1 and A2 according the aetiology of infection. Subgroup A1 included 18 children with 60 febrile episodes with microbial infection and A2 of 11 children with 34 febrile episodes with virus infection or with fever of unknown origin.

Group B consisted of 20 children, aged 2-14 yr (6.8 ± 3.1), with 56 neutropenic afebrile episodes and group C of 13 non-neutropenic children with 58 afebrile episodes, aged 1-14 yr (5.9 ± 2.1). Neutropenia was defined as an absolute neutrophil count (ANC) $< 0.5 \times 10^9$ or absolute leukocyte count $< 1 \times 10^9$.³⁴ Fever was defined as an axillary temperature of > 38.0 °C over a 6-h observation period or > 38.5 °C once.³⁵

According to the classification of the International Immunocompromised Host Society (ICHS)³⁰, febrile episodes in neutropenic cancer patients are (a) microbiologically documented infection, (b) clinically documented infection and fever of unknown origin (FUO). The site of infection for group A is shown in Table 1. The diagnosis of microbiological infections was based on positive cultures of blood, urine, faeces and throat swabs. The diagnosis of urinary tract infection required both symptoms and growth of a single microorganism $> 10^5$ cfu/ml in urine culture. Therefore, the urine cultures were quantitative. Bacteraemia was defined as fever with positive blood cultures for bacteria (peripheral blood or from the central venous indwelling catheter), with or without septic symptoms

and with no other clinical signs of infection. Clinically documented infection was defined as the fever in connection with unambiguous diagnosis signs of localized infection, e.g. pneumonia or skin/soft tissue inflammation. FUO was defined as fever $>38^{\circ}\text{C}$ over at least 1 hr or twice within 12 hr without evident cause or fever without clinical or microbiological evidence. Fever was regarded as non-microbial if there was FUO or it was an association with blood products transfusion and/or chemotherapy with high-dose cytosine arabinose for the duration of fever with no other sign of infection or documented virus infection.

In groups A, B and C blood samples were collected upon admission and before the start of any antimicrobial treatment and then for 7 days on a day-to-day basis. For all children the same blood tests and cultures were performed. The blood samples were used for culture, serological tests, virus antibodies, leukocyte count and for analysis of levels of CRP, PCT, Neopterin, IL-6 and NO_2/NO_3 . The concentration of CRP in serum was determined by nephelometry. PCT concentrations were determined by immunoluminescence assay using a diagnostic Kit (LUMitest Pro-Calcitonin, BRAHMS Diagnostica, Berlin Germany). This assay, which is specific for procalcitonin, requires 20 μl of serum and can be completed within 1 hr. The detection limit is 0.1 ng/ml.

Serum neopterin levels were measured as duplicated by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Neopterin ELISA, IBL, Hamburg, Germany) according to the manufacturer's protocol. Serum concentrations of IL-6 and NO_2/NO_3 were determined as duplicated by ELISA method using commercially available ELISA kits (R&D Systems) according to the manufacturer's guidelines.

For IL-6 the minimum detectable dose is typically less than 0.094 pg/ml and for NO_2/NO_3 the sensitivity less than 0.22 $\mu\text{mol/L}$.

Statistical Analysis

The data of this study were evaluated with descriptive statistical methods (as mean values $\pm$ SE) and used student's *t* test. In all statistical tests, value of $p < 0.05$

were considered significant. One-way analysis of variance (ANOVA) was used for multiple comparisons of repeated measurement data. The Kruskal-Wallis test was used to compare the distribution of CRP, PCT and cytokines between groups. The correlation analysis was carried out with Pearson's correlation coefficient.

To evaluate whether the investigated parameters are able to reliably discriminate between cases with bacterial infections and those without, receiver-operating characteristic (ROC) curves were generated (Statistical Program for Social Science version 11, SPSS). The area under the ROC curve (AUC) is proportional to the probability of a correct distinction. Differences of continuous variable were assessed with the Mann-Whitney and U-Wilcoxon Test.

Results

Admission diagnosis of the children of group A are presented in Table 1. The mean values of leukocytes in all groups during the seven days is shown in Figure 1. In comparison with the two groups A and B, the majority of both febrile patients and afebrile neutropenic patients showed marked leucopenia and neutropenia ($P < 0.005$). In febrile patients, the median duration of neutropenia following admission was 2.8 days. The degree of cytopenia, i.e. neutropenia, lymphopenia and monocytopenia, respectively, was more severe in febrile patients than in afebrile immunosuppressed patients. The mean levels of CRP, PCT, IL-6, Neopterin and NO_2/NO_3 in all groups for the consecutive 7 days are shown in figures 2, 3, 4, 5 and 6 respectively. After the onset of fever, the PCT concentration was higher in group A than B and C ($P < 0.001$ and $P < 0.001$ respectively). Also after the onset of fever, the PCT concentration was higher in subgroup A1 (microbial infected patients) than in subgroup A2 (non-microbial infected patients) through the study period ($P < 0.001$). On the second day after the start of antimicrobial therapy there was a decrease in the value of PCT by 30-44% in subgroups A1 and A2. PCT levels discriminated best between more severe infections (pneumonia, gram-positive and gram-negative bacteraemia) and mild infections (FUO and

TABLE 1. Diagnosis of the patients of group A

Subgroup A1	Number of episodes	%
Urinary tract infection	16	26.7
Pneumonia, empyema and lung abscess	13	21.7
Bacteremia	14	23.3
Perianal abscess	9	15
Soft tissue abscess	5	8.3
Dental abscess	3	5
Total	60	100
Subgroup A2		
Virus infection	10	29.4
FUO	15	44.1
Drug fever, blood products	9	26.5
Total	34	100

FIGURE 1. The mean values of leukocytes in all groups during all days.

FIGURE 2. The mean values of CRP in all groups during all days.

localized infections), both at admission and in the assessment of the febrile course. The neopterin concentration changed during the study period ($P<0.05$), and the pattern of change in this concentration differed between groups ($P<0.05$).

Although IL-6 concentrations varied widely they were higher in febrile neutropenic patients ($P<0.05$). An increase of IL-6 serum levels was noted in patients during febrile episodes. This rise in serum concentra-

tions was detectable at the onset of fever. However, no differences were demonstrated in afebrile patients between those with severe leukopenia and those without leukopenia. After the onset of fever, the CRP increased similarly in patients with documented infection and FUO.

The discriminatory power of the two markers for infection in patients was evaluated in terms of the area under ROC curves. To evaluate the sensitivity and

FIGURE 3. The mean values of PCT in all groups during all days.

FIGURE 4. The mean values of IL-6 in all groups during all days.

FIGURE 5. The mean values of Neopterin in all groups during all days.

FIGURE 6. The mean values of NO₂/NO₃ in all groups during all days.

specificity of PCT, CRP, IL-6, Neopterin and NO₂/NO₃ measurements in the given setting, ROC curves were calculated (Figures 7, 8 and 9). PCT was a good marker to discriminate bacterial from non-bacterial infections. A moderate correlation was documented for the PCT and CRP peak levels ($r=0.52$).

Discussion

Neutropenic infections in leukemic patients have been investigated for decades.¹ Compared with neutropenic infections, which are mainly treated empirically ac-

cording to the generally accepted guidelines, the management of non-neutropenic infections is not so systematically guided, and the indications for antibiotic treatment are individually determined, mostly based on the origin and focus of the infection. In addition, the severity of these infections is not generally recognized.² The severity of infection, the patient's general condition and the state of the immune system as a result of long-lasting, repeated treatments and their combinations often determine the decision of how to treat these very complicated patients and overall very heterogeneous.³

FIGURE 7. The serum PCT assay is compared with circulating CRP in patients with bacterial infection on admission. They are displayed in a receiver operating curve (ROC) analysis. Area under curve was 0.869 for PCT and 0.728 for CRP ($P<0.01$).

Before the start of fever the PCT values remain within normal levels in patients with hematological malignancies with or without neutropenia (PCT mean values of 0.26 ng/ml and 0.27 ng/ml respectively). In the present study the start of fever is followed by a rapid increase of the PCT levels in neutropenic patients with proven bacterial infection. Based on a PCT

cutoff value of 2ng/ml for the diagnosis of bacterial infection, the sensitivity and the specificity of PCT in the A1 Group are 96.5% and 97% respectively. The PCT mean value in the group of children with fever due to bacterial infection was significantly higher in comparison to the group of children with fever but with no bacterial infection ($P<0.001$). The results of the study show that PCT is a rapidly increasing parameter with very good sensitivity and highlighted text removespecificity for the diagnosis of bacterial infection in immunosuppressed patients.

In immunosuppressed children with viral infections the PCT concentration reached 1.94 ng/ml. In viral infections for PCT levels ≤ 2 ng/ml the specificity nears 100%. The mean PCT value on day 1 in the children with clinically proven bacterial infection was 8.68 ng/ml, while the corresponding mean value on day 1 in the children with viral infection was 1.58ng/ml. The difference was statistically significant. These data may be considered indicative of the usefulness of PCT on the first day of a febrile disease for the differential diagnosis between bacterial and viral infections.

In order to assess the potential value of PCT to predict microbial infection serum was collected and PCT level was determined on admission, prior to the

FIGURE 8. Receiver operation characteristic (ROC) curve analysis for the assessment of PCT and IL-6 in predicting the severity of bacterial febrile neutropenic attacks on admission. Area under curve was 0.833 for PCT and 0.723 for IL-6 ($P<0.001$).

FIGURE 9. Receiver operation characteristic (ROC) curve analysis for the assessment of PCT, Neopterin and NO₂/NO₃ in predicting the severity of bacterial febrile neutropenic attacks on admission. Area under curve was 0.913 for PCT, 0.761 for Neopterin ($P<0.001$) and 0.562 for NO₂/NO₃ ($P<0.001$).

beginning of antimicrobial therapy. Elevated PCT concentrations were found exclusively in neutropenic patients with microbial infection. Thus, our results corroborate previous findings demonstrating the association of elevated PCT with microbial, not viral or other aetiology febrile episodes in immunocompromised patients.³¹

The mechanisms responsible for the marked increase of serum PCT during microbial infections and the exact site of its production remain enigmatic.³⁷⁻⁴⁰ It has been suggested that the stimulation of PCT is closely related to the induction of pro-inflammatory cytokines.³⁰). In microbial infections, the proinflammatory effect of cytokines is controlled by anti-inflammatory cytokines. Anti-inflammatory cytokines while potentially beneficial, may in certain situations impair the host's inflammatory response.^{30,31} Several factors, including the variability in microbial species and age of a patient, may influence the proinflammatory and anti-inflammatory cytokines differently,⁴¹⁻⁴⁸ which may result in different serum PCT levels.

At admission, PCT offered the best discrimination of all parameters between mild and serious microbial infections.^{31, 40} During treatment, decreasing PCT levels reflected defervescence, rapid or gradual clinical improvement and successful anti-microbial therapy, irrespective of the kind of bacterial infection.⁴⁹⁻⁵⁵

The PCT concentration was higher in microbial infected than in non-microbial infected patients through the study period ($P < 0.001$). The admission values of CRP were higher in the neutropenic leukemic patients with infection than in leukemic patients without infection.⁵⁶⁻⁵⁹ However, CRP was unable to differentiate infections from other origins of fever. Thus, CRP is also an unreliable infection marker in neutropenic patients with suspected infection.

Although CRP turned out to be a poor infection marker on admission, decreasing CRP values differentiated infections from non infections fever and the follow-up CRP level in patients with non-infection fever remained unchanged. This is in harmony with some earlier studies on febrile neutropenia as well as many other conditions.^{5, 36}

Based on a CRP cutoff value of 5 mg/ml for the diagnosis of bacterial infection, the sensitivity and the

specificity of CRP in the A1 Group are 90% and 78% respectively. According to our results, there was an increase of CRP on the second day from 10-25% in all groups of children with fever and in the sequel a gradual decrease during the following days. This increase does not allow us to use CRP as an indicator for monitoring the course of the infection during the second day after the start of the antibacterial therapy, as it happens with PCT.

Neopterin which is synthesized and released in excess by monocytes/macrophages upon stimulation with interferon- γ has become a valuable diagnostic marker in a number of conditions associated with increased activity of the cellular immune response.^{37, 60-61} Therefore, activation of macrophages with the subsequent release of neopterin is a common finding in several diseases characterized by an activated cellular immune response.³⁸ According to our results the Neopterin mean value on day 1 was higher in groups with fever and infection and especially in the children with viral infections. On day 2 there was an increase in the Neopterin mean value from 23-50% in the groups of children with fever and in the sequel a gradual decrease during the following days. A greater increase was observed in the children with proven viral infections. Therefore, the determination of neopterin represents a useful tool for clinical monitoring especially of viral infections and especially when combined with a determination of PCT levels.

In the present study, the highest IL-6 values were found in the groups with fever. The mean IL-6 values presented an increase on day 2 from 13-30% and a gradual decrease in the sequel. The IL-6 levels were elevated in patients with bacterial infections compared to those with fever of non-bacterial or unknown etiology. The small specificity regarding the microbial infections and the increase of IL-6 values on day 2 of the antibacterial infections imply that IL-6 during the first 2 day does not constitute a useful indicator for the diagnosis, etiology, prognosis and monitoring of the infection. These results are verified by the ROC curves, which compare the PCT values to those of IL-6, proving once more the superiority of PCT versus IL-6.

In this study, although infection is associated with release of IL-6, the results do not suggest a major role for the assessment of IL-6 in clinical practice. These results reflect the variable experience with cytokine measurements in other indications. Some groups reported that there was a correlation to the clinical course in neutropenic children; others failed to demonstrate such a relationship in similar patient populations. There are several possible explanations for these unsatisfactory results, such as inflammatory cytokines frequently display two-sided activities in different models of infectious diseases.⁶ It seems that the quantity and the type of infections determine whether IL-6 protects from or is involved in tissue damage during infection.⁷ Clinical outcome is probably related to the virulence of the microbes and the sensitivity of the pathogen to the therapy applied. Also, the duration of neutropenia and concomitant disease greatly influence the outcome of a neutropenic febrile episode.⁶²⁻⁶³

There was evidence of increased NO₂/NO₃ concentrations in the group of bacterial infection patients only. Our study's comparison of NO₂/NO₃ concentrations between patients with bacterial infections and other groups of patients is an important addition to the literature.⁶⁴⁻⁶⁵ An increased concentration of NO₂/NO₃ in bacterial infection was not correlated with renal failure in our study. Increase in NO₂/NO₃ concentrations appears to be highly correlated with bacterial infections, since patients with other conditions in our study had about normal values. Concentrations of NO₂/NO₃ remained increased throughout the entire course of the bacterial infection, and further studies would be required to understand the reasons for this sustained production of NO₂/NO₃ (18).

In conclusion, we found that PCT was a better marker than CRP, neopterin, NO₂/NO₃ and IL-6 for distinguishing fever due to microbial infection from fever of other aetiology in immunosuppressed children. It was also a useful indicator of the severity of microbial infection. Our results further suggest that PCT is a rapid, sensitive and specific marker of microbial infection in these patients. The positive predictive values of serum PCT were about 100%, while the negative predictive value of serum PCT was even higher than those found for other parameters routinely

used in clinical practice. Also, PCT is a marker for the prognosis of infection as well as for monitoring the course of the disease.

This study showed also, that the activity of the underlying acute leukaemia itself, the chemotherapy-induced tissue damage (such as severe mucositis) and the severity of neutropenia, lymphopenia or monocytopenia did not cause considerable increases in PCT serum levels.

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