High pentraxin 3 level predicts septic shock and bacteremia at the onset of febrile neutropenia after intensive chemotherapy of hematologic patients

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ABSTRACT

We evaluated pentraxin 3 as a marker for complications of neutropenic fever in 100 hematologic patients receiving intensive chemotherapy. Pentraxin 3 and C-reactive protein were measured at fever onset and then daily to day 3. Bacteremia was observed in 19 patients and septic shock in 5 patients (three deaths). In comparison to C-reactive protein, pentraxin 3 achieved its maximum more rapidly. Pentraxin 3 correlated not only with the same day C-reactive protein but also with the next day C-reactive protein. High pentraxin 3 on day 0 was associated with the development of septic shock (P=0.009) and bacteremia (P=0.046). The non-survivors had constantly high pentraxin 3 levels. To conclude, pentraxin 3 is an early predictor of complications in hematologic patients with neutropenic fever. High level of pentraxin 3 predicts septic shock and bacteremia already at the onset of febrile neutropenia. *(ClinicalTrials.gov Identifier: NCT00781040.)*

Key words: febrile neutropenia, pentraxin 3, C-reactive protein, septic shock, bacteremia.

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Introduction

Sepsis remains an important cause of death among hematologic patients receiving intensive chemotherapy despite progress in supportive care and use of antimicrobial drugs.[1,2] Among patients with febrile neutropenia, early identification of those developing severe sepsis or septic shock is difficult, even with using the biomarkers available.[3] New tools are needed to predict unfavorable outcome at an early stage of the course of febrile neutropenia. Pentraxin 3 (PTX3) is a promising marker of infection and inflammation, and it correlates with disease severity.[4] Pentraxins are key components of innate immunity, which is the first line of defense against microbes. C-reactive protein (CRP) belongs to the group of short pentraxins produced principally in the liver,[5] while PTX3 is the prototype of long pentraxins produced by endothelial cells and phagocytic cells. PTX3 is released in response to early proinflammatory cytokines (tumor-necrosis factor and interleukin-1) but also after direct contact with microbial products like lipopolysaccharide and mycobacterial lipoarabinomannan.[4,6] Thus it could be a rapid marker of bacteremia and severe sepsis.[3,4] High PTX3 levels have been found to correlate with poor outcome in several acute conditions,[7,8] including severe infections.[9-13] Elevated serum PTX3 levels have been associated with dysfunction of several organ systems,[13-16] especially the cardiovascular system.[16-18] The possible beneficial or harmful biological role of PTX3 remains uncertain. Because of the difference in origin and the more rapid kinetics of PTX3 in comparison to CRP,[19] PTX3 could provide prognostic information also at onset of neutropenic fever.

Based on earlier studies, PTX3 is a promising biomarker to diagnose septic conditions more rapidly than CRP, because of both its origin and induction by proinflammatory cytokines and bacterial products.[19] Endothelial origin of PTX3 is especially interesting because the development of a life-threatening complication, septic shock, is mainly initiated in endothelium. When PTX3 was studied in patients admitted to an intensive care unit with severe meningococcal disease, PTX3 proved to be an early indicator of shock.[12]

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In this 3-year prospective study, we compared the long PTX3 with C-reactive protein as a biomarker in predicting complications (bacteremia and septic shock) among 100 hematologic patients with febrile neutropenia after intensive chemotherapy.

Design and Methods

The study population consisted of adult patients treated on the hematology ward of Kuopio University Hospital between 1st of December 2006 and 30th December 2009. Included patients had either acute myeloid leukemia or had received high-dose chemotherapy supported by autologous stem cell transplantation (ASCT). A patient was eligible for the study if the criteria for neutropenic fever were fulfilled after intensive chemotherapy (see below) and if the blood samples were available for microbiological cultures and PTX3 and CRP measurements from day 0 to day 3 after the onset of fever. The study population consisted of 100 adult patients; 61 men and 39 women. Thirty-two had acute myeloid leukemia and 68 had received high-dose chemotherapy supported by ASCT. The median age was 55 years (range 18-70 years). Only the first febrile neutropenic episode of each patient was taken into account.

Each patient was examined daily for clinical signs and sources of infections. Body temperature, weight, blood pressure, peripheral blood oxygen, daily fluid intake and urine output were monitored at the bedside and chest X-rays were taken. No antibacterial prophylaxis was used until January 2008 when ciprofloxacin-prophylaxis was included for lymphoma patients receiving ASCT. Empirical antibiotics were initiated after blood cultures with a combination of a betalactam and an aminoglycoside. Antibacterial treatment was adjusted according to blood culture results and radiological findings. If fever persisted over 3-5 days, blood cultures were drawn again and antifungal therapy was started. All ASCT recipients received granulocyte-colony stimulating factor after stem cell infusion.

Study end points

End points in this study were bacteremia and septic shock. Fatal outcome within the same hospital stay was registered.

Definitions

Neutropenic fever was defined using the criteria from the Infectious Diseases Society of America.[20] Neutropenia was defined as a neutrophil count less than 0.5×10^9/L or a count less than 1×10^9/L with a predicted decrease to less than 0.5×10^9/L. Fever was defined as a single oral temperature of 38.3°C or over, or a temperature of 38.0°C or over for 1 h or more.

Septic shock was defined according to the American College of Chest Physicians Consensus[21] as a subset of severe sepsis: hypotension despite adequate fluid resuscitation, along with the presence of hypoperfusion abnormalities or organ dysfunction.

Blood cultures

Blood cultures were processed using the automated blood culture system Bactec 9240 (Becton Dickinson, Sparks, USA). The incubation period was seven days for both aerobic and anaerobic bottles, and 42 days for MYCO F/Lytic bottles. A single positive blood culture was considered significant if the microbe was a clinically relevant cause of infection. Common skin contaminants were considered significant only if they were found in two consecutive blood cultures or if there was concurrent skin or catheter infection.

Sample collection and laboratory analysis

Plasma samples for PTX3 and CRP analyses were taken at the onset of neutropenic fever on day 0 (d0) and further samples were collected next morning (d1) and then every 24 h up to three days (d2-d3).

The concentration of plasma PTX3 level was measured with a sandwich-type ELISA (R&D Systems, Minneapolis, MN, USA). The minimum detectable dose of PTX3 in this assay is 0.03 μg/L. The samples were analyzed in batches.

The concentration of serum CRP was measured with a Konelab60i Clinical Chemistry Analyzer (Lab systems CLD, Konelab, Helsinki, Finland) or Cobas 6000-analyzer (Hitachi, Tokyo, Japan). The between-run variations were 2.3-4.3%. The upper reference limit of serum or plasma CRP of a healthy reference population is 10 mg/L.

Statistical analysis

The statistical analyses were performed with SPSS version 14.0 for Windows (SPSS, Inc., Chicago, IL, USA). The continuous variables were expressed as medians with ranges. Mann-Whitney U-test was used to detect differences between groups in continuous variables. The association of categorical variables was studied by χ^2 test or linear-by-linear association in case of more than two classes. Joncheere-Terpstra test was used to evaluate the significance of differences in continuous variables between ordered classes. Spearman's correlations were examined to evaluate the relationship between PTX3 and CRP. Parametric tests were performed after log-transformation of original values to correct the log-normal distribution of PTX3. General linear model for repeated measurements was applied to evaluate differences between groups in repeated measurements of PTX3 and CRP from day 0 to day 3. The difference between the groups was considered as a between-subject factor and the difference from day to day as a within-subject factor. Receiver characteristics curve analysis (ROC) was performed to compare and describe the diagnostic ability between CRP and PTX3. A *P* value less than 0.05 was considered significant.

Ethics

This study was approved by the Ethical Committee at Kuopio University Hospital. Written informed consent was obtained from all patients.

Results and Discussion

Bacteremia was observed in 19 patients with neutropenic fever (19%), gram-negative bacteremia in 6 patients (6%), and septic shock in 5 patients (5%). Three out of 5 patients with septic shock had bacteremia. Altogether 3 patients died: 2 patients due to septic shock and multiorgan failure and one due to influenza A (pandemic H1N1 2009) with respiratory failure.

The blood culture finding was positive in 19 patients, gram-positive in 13 (68%) patients and gram-negative in 6 (32%) patients. The gram-positive findings included *Staphylococcus epidermidis* (n=6), *Staphylococcus haemolyticus* (n=1), *Staphylococcus capitis* (n=1), *Streptococcus viridans* (n=1), *Streptococcus mitis* (n=1), *Streptococcus oralis* (n=1), *Streptococcus pneumoniae* (n=1), and *Enterococcus faecium* (n=1). The gram-negative findings included *Escherichia coli* (n=4), *Enterobacter cloacae* (n=1), and *Klebsiella pneumoniae* (n=1).

The median serum pentraxin 3 concentration (minimum, maximum) was 9.0 μg/L (0.3, 2000 μg/L), 11.8 μg/L

(0.3, 247 μg/L), 15.1 μg/L (0.3, 779 μg/L), and 12.5 μg/L (0.4, 2000 μg/L), on days 0, 1, 2, and 3, respectively. Median of CRP (minimum, maximum) was 35 mg/L (5, 253 mg/L), 77 mg/L (9, 307 mg/L), 101 mg/L (6, 357 mg/L), and 97 mg/L (7, 410 mg/L) on days 0, 1, 2, and 3, respectively. There was a statistically significant increase in the level of PTX3 concentration from day 0 to day 1 after which it remained stable. The level of CRP continued to increase to day 2. Age, sex or co-morbidities had no statistically significant association with PTX3 on day 0 (*Online Supplementary Table S1*).

Maximal PTX3 level was achieved slightly earlier than maximal CRP (during days 0 to 3 *P* value for linear trend < 0.001). Maximal PTX3 was achieved on day 0 in 14% of patients, on day 1 in 31 patients (31%), on day 2 in 28 patients (28%) and on day 3 in 27 patients (27%).

Table 1. Pentraxin 3 (PTX3) and C-reactive protein (CRP) concentrations from day 0 to day 3 from the beginning of neutropenic fever in the study patients (n=100) with non-complicated neutropenic fever, with bacteremia but no septic shock, and with septic shock. There were 3 patients with both bacteremia and septic shock, and they are classified only to the category of 'septic shock'. The data are expressed as medians (range). The *P* values stand for association of PTX and CRP with the severity of complications of febrile neutropenia according to the Joncheere-Terpstra test.

	Non-complicated neutropenic fever (n=79)	Bacteremia but no septic shock (n=16)	Septic shock (n=5)	*P* value
PTX3 (μg/L)				
day 0	7.7 (0.3-163.1)	13.9 (1.5-93.3)	33.9 (25.2-2000)	0.006
day 1	10.9 (0.3-214.1)	33.4 (1.9-247.2)	44.6 (32.9-51.1)	0.035
day 2	12.1 (0.3-289.7)	19.0 (1.5-779.0)	46.5 (27.2-157.0)	0.122
day 3	12.0 (0.4-630.4)	13.7 (0.9-444.4)	59.7 (9.9-2000)	0.106
CRP (mg/L)				
day 0	31 (5-212)	48 (10-194)	128 (57-253)	0.004
day 1	66(9-234)	104 (31-300)	205 (87-307)	0.010
day 2	99 (6-333)	132 (27-357)	237 (29-281)	0.016
day 3	91(7-344)	98 (24-325)	205 (12-410)	0.240

PTX3: pentraxin 3; P *values are results of the non-parametric Joncheere-Terpstra test.*

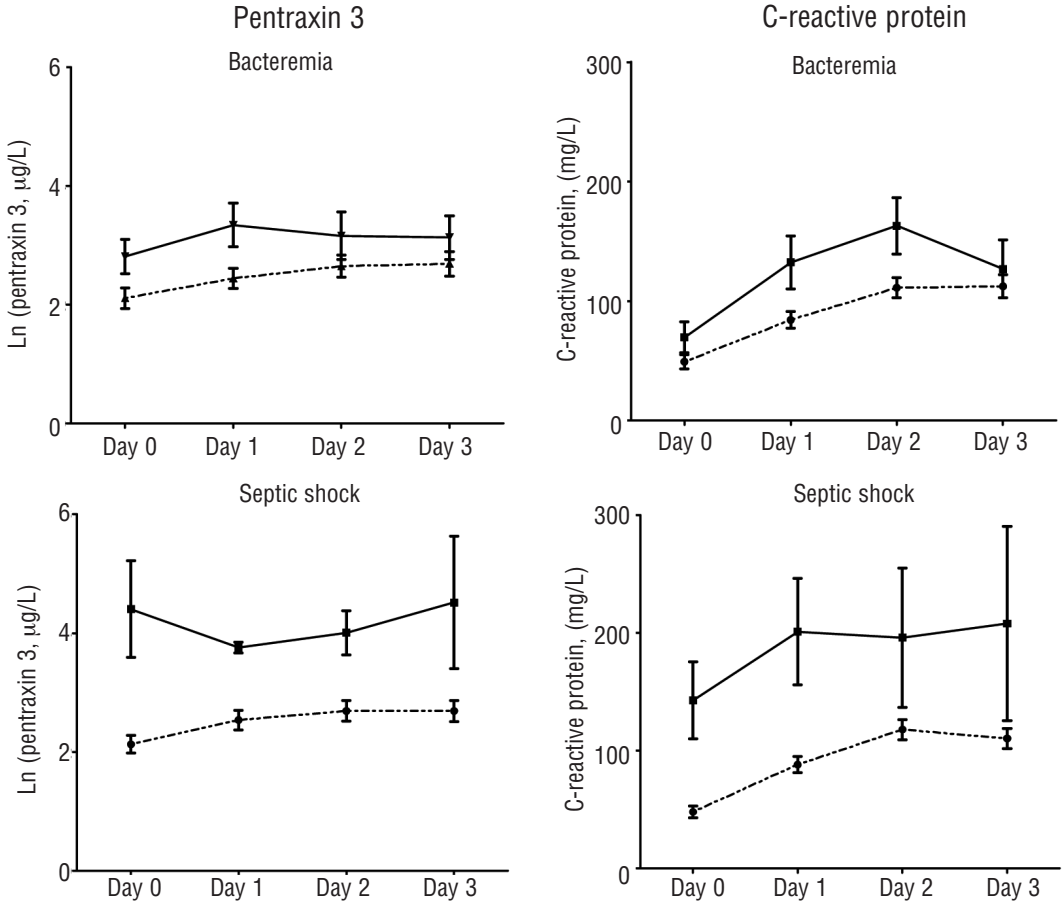

Figure 1. Pentraxin 3 and C-reactive protein (CRP) means with SEs from day 0 to day 3 according to the presence of bacteremia (upper panel) and septic shock (lower panel). Patient groups with bacteremia and septic shock are marked with continuous lines, and those with no bacteremia and no septic shock are marked with dash lines.

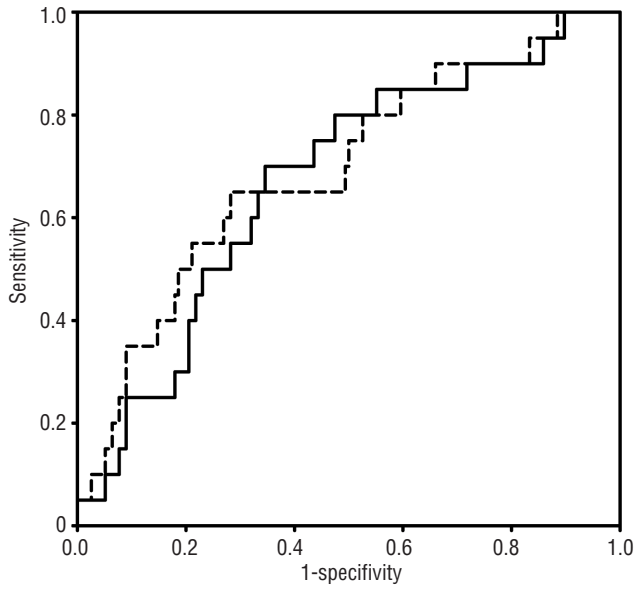

Figure 2. ROC-curve analysis comparing pentraxin 3 (PTX3) (continuous line) and C-reactive protein (CRP) (dash line) predicting the development of bacteremia or septic shock on day 0. For PTX3 the area under the curve (AUC) was 0.68 (95% CI 0.56-0.81) with SD 0.06 and *P* value 0.011 to differentiate septic shock or bacteremia. For CRP the AUC was 0.69 (0.56-0.82) with SD of 0.07 and *P* value 0.008 to differentiate septic shock or bacteremia.

Respectively, the maximal CRP was achieved on day 0 in 7% of patients and on day 1 in 21 patients (21%), on day 2 in 40 patients (40%), and on day 3 in 32 patients (32%). On each day PTX3 correlated well with the same and following day CRP (all *P* values < 0.001).

The two non-survivors due to septic shock had constantly high PTX3 levels. One of these patients had PTX3 2000 μg/L on day 0. The other non-survivor had rising PTX3: 25.2 μg/L - 32.9 μg/L - 157.0 μg/L - 2000 μg/L on days 0 to 3, respectively. The third non-survivor with influenza A (pandemic H1N1 2009) and respiratory failure had intermediately elevated PTX3 level: 31.1 μg/L - 43.4 μg/L - 48.1 μg/L - 55.9 μg/L on days 0 to 3, respectively. When comparing PTX3 and CRP levels in groups by increasing severity of complications, differences were equal (Table 1).

Figure 1 illustrates the kinetics of PTX3 and CRP according to the presence of bacteremia or septic shock. Elevated PTX3 on day 0 predicted not only septic shock (*P*=0.009) but also bacteremia (*P*=0.046). Elevated CRP was predictive only for septic shock (*P*=0.003). In repeated measure analyses, based on serial measurements during days 0 to 3, the patients with septic shock or bacteremia had higher levels of PTX3 (*P*=0.046), but also higher levels of CRP (*P*=0.02) than those without septic shock or bacteremia.

By the results of ROC curve analysis, both PTX3 and CRP on day 0 predicted septic shock and the combination of septic shock or bacteremia (Figure 2). Additionally, PTX3 on day 0 predicted bacteremia. The area under the curve (AUC) was 0.85 (95% CI 0.75-0.95), with SD of 0.05 and *P* value of 0.009, for PTX3 on day 0 to predict septic shock.

In accordance with our results, PTX3 was recently observed to have better prognostic value than CRP during the first days after diagnosis of bacteremia; CRP remained equally high both among non-survivors and survivors.[22] Discrepancies in PTX3 and CRP responses in various studies likely result from the difference in the timing of sampling perceiving the dissimilarity of the kinetics of PTX3 (early) and CRP (late).[10] In our study, sampling was early, at the onset of fever, unlike in most previous studies.

The strengths of our study are the homogeneity of the study population, the prospective study design over three years and the systematic timing of sampling. Also the end points were reliable with accurate definitions for bacteremia and septic shock. Our study population was restricted to neutropenic patients. However, these results may also be useful in the context of an unselected population as the endothelial cell function should not be markedly affected by neutropenia. The main limitation of the study was the small number of patients with septic shock.

In this 3 year prospective study, we observed that high levels of PTX3 were associated, already at the onset of fever, with the development of septic shock and bacteremia in neutropenic hematologic patients receiving intensive chemotherapy. PTX3 was not superior to CRP as a biomarker predicting a complicated course of neutropenic fever. Furthermore, the clinical value of PTX3 is limited because this value is often not available. However, we could demonstrate that PTX3 achieved its maximum slightly earlier than CRP and that the non-survivors had constantly high PTX3 levels. As an additional tool, PTX3 could offer a possibility of selecting high-risk patients, but this requires further analysis to establish optimal cut-off values.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

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