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Fibrinogen and antithrombin in hematological patients with neutropenic fever

Research Article

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Abstract: The value of plasma fibrinogen and antithrombin as predictors of severe sepsis was investigated in 69 adult hematological patients, who had altogether 93 periods of neutropenic fever. Patients had either acute myeloid leukemia or had received a high dose of chemotherapy supported by autologous stem cell transplantation. In febrile periods with severe sepsis, the median fibrinogen concentration at the start of the fever was significantly higher (5.0 g/L) than that without severe sepsis (4.5 g/L) (p=0.009). Normal plasma fibrinogen could rule out a group of patients with severe sepsis at the beginning of the fever. The antithrombin activity decreased, both in fever periods with severe sepsis and in those without. The decrease in antithrombin activity was found to be greater in fever periods characterized by severe sepsis. In conclusion, elevated plasma fibrinogen and constantly decreasing antithrombin were shown to be linked to the development of severe sepsis.

Keywords: Fibrinogen • Antithrombin • Severe sepsis • Neutropenic fever • Hematological patients

1. Introduction

Chemotherapy-induced neutropenia is frequently associated with febrile episodes in patients with hematological malignancies [1,2]. Neutropenic sepsis is an important cause of morbidity and mortality in these patients [3-5]. The activation of coagulation as a response to severe infection is evident; it is suspected to result from tissue factor-mediated thrombin generation, downregulation of physiological anticoagulant mechanisms, and inhibition of fibrinolysis [6-8].

During coagulation activation in severe infections, antithrombin (AT) is consumed as a result of accelerated thrombin generation [9]. This leads to the decreased activity of AT that was found to be an indicator of an unfavorable prognosis during severe sepsis and septic shock in neutropenic patients [9].

Fibrinogen is one of the proteins in the coagulation cascade [10]; moreover, it is also one of the acute phase proteins [6]. Fibrinogen concentrations have been studied as a part of hemodynamic status in septic patients [11] or during induction chemotherapy for acute lymphoblastic leukemia [12]. However, the predictive value of fibrinogen in assessing the risk of severe sepsis in neutropenic patients with hematological malignancies is unknown.

We evaluated the use of plasma fibrinogen and plasma AT activity to distinguish which patients were at risk of severe sepsis at the beginning of the

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neutropenic fever in adult patients with a hematological malignancy. Furthermore, we investigated the kinetics of these laboratory parameters and evaluated how they correlated with serum C-reactive protein (CRP).

2. Material and Methods

2.1. Patients and Treatment

The study population consisted of adult patients treated in the hematology ward of Kuopio University Hospital between 1st of December 2006 and 30th November 2008. Those patients were eligible if they had neutropenic fever (see below) after the intensive treatment. All clinical data were collected prospectively. This study was approved by the Ethical Committee of Kuopio University Hospital. All patients gave their formal written consent before the study entry. The study was performed in accordance with the principles in the Declaration of Helsinki.

The study entry criteria were fulfilled by 77 consecutive patients, of whom 69 patients (90%) were enrolled in the study (44 men and 25 women). Seven patients, who did not get fever, were excluded. One of the patients had a different AT profile compared with all the other patients and was therefore excluded. This patient was a 57-years old female suffering from a relapse of follicular lymphoma who had received autologous stem cell transplantation (ASCT) after second-line chemoimmunotherapy. She had neutropenic fever after ASCT, complicated by severe sepsis. Her AT activity was 124% at the beginning of the fever, and it increased to 132%, 143%, 128%, and 146% during the follow up on days 1 to 4, unlike the AT activity in the other patients.

There were 19 patients with acute myelogenous leukaemia (AML). Fifty patients had received ASCT: non-Hodgkin lymphoma (n=24), multiple myeloma (n=14), Hodgkin lymphoma (n=9), chronic lymphocytic leukemia (n=2), and amyloidosis (n=1). The chemotherapy agents used with these patients were as follows: carmustine, etoposide, cytarabine and cyclophosphamide (BEAC, n=14); carmustine, etoposide, cytarabine and melphalan (BEAM, n=20); high-dose melphalan (n=16); idarubicin, cytarabine and thioguanine (n=8); high-dose cytarabine and idarubicin (n=6); mitoxantrone and high-dose cytarabine (n=2); and high-dose cytarabine and idarubicin (n=3).

During each neutropenic fever period, the body temperature, the blood pressure, oxygen saturation, fluid intake, and the amount of diuresis were monitored bedside. The daily fluid intake (intravenous and oral) and urine output were registered every 12 hours during the first three days of neutropenic fever by the hematology ward nursing staff. Patients received empirical antibiotic treatment after blood cultures were obtained (a combination of a beta-lactam and an aminoglycoside). All ASCT recipients received a granulocyte-colony stimulating factor after stem cell infusion (filgrastim or a single injection of pegfilgrastim).

2.2. Definitions
2.2.1. Neutropenic fever
We used the criteria employed by the Infectious Diseases Society of America (IDSA) (Centers for Disease Control) for the definition of neutropenia and neutropenic fever [13]. Neutropenia was defined as a neutrophil count of <0.5 x 10^9/L, or a count of <1 x 10^9/L with a predicted decrease to <0.5 x 10^9/L. Fever was defined as a single oral temperature of ≥38.3°C or a temperature of ≥38.0°C for ≥1 h.

Sepsis is a clinical syndrome in which a systemic inflammatory response is present with infection [14,15].

2.2.2. Severe sepsis
Was present if sepsis was complicated by organ dysfunction, hypoperfusion or hypotension (systolic arterial pressure <90 mmHg, a mean arterial pressure <60 mmHg or a reduction in systolic blood pressure of >40 mmHg from the baseline, despite adequate volume resuscitation, in the absence of other causes of hypotension) [14,15].

2.3. Blood Cultures
Blood cultures were processed using the automated blood culture system Bactec 9240 (Becton Dickinson, Sparks, USA). The incubation period for both aerobic and anaerobic bottles was 7 days and for MYCO F/Lytic bottles 42 days.

A single positive blood culture was considered significant if the microbe was a clinically relevant cause of infection. Common skin contaminants (e.g. coagulase-negative staphylococci) were considered significant only if they were found in two consecutive blood cultures or if there was a concurrent skin or catheter infection.

2.4. Blood Samples
The first blood samples for plasma fibrinogen, plasma AT activity, and serum CRP measurement were collected at the beginning of the onset of neutropenic fever (day 0). The other samples were collected on the next morning and then every 24 hours during up to four days. Plasma and serum were separated and stored at -70°C until analyzed.

2.5. Plasma fibrinogen

Plasma fibrinogen was measured by a clotting rate assay, using the Fibri-Prest®Automate 2 thrombin reagent (Diagnostica Stago, France) and Sysmex CA-1500 CP coagulation analyser (Sysmex Corporation, Kobe, Japan). The method was calibrated against Unicalibrator Calibration Plasma (Stago, France) (2nd Int Std for Fibrinogen 98/612, 1999). The reference range of plasma fibrinogen was 1.7-4.0 g/L.

2.6. Plasma antithrombin

Plasma AT was measured by a chromogenic method, using a Stachrom AT reagent (Stago, France) and Sysmex CA-1500 CP coagulation analyser (Sysmex Corporation, Kobe, Japan). The method was calibrated against Unicalibrator Calibration Plasma for Coagulation tests (Stago, France) (2nd Int Std for AT 93/768, 1994). The reference range of plasma AT activity was 80-130%.

2.7. Serum CRP

The concentration of serum CRP was measured with a Konelab60i Clinical Chemistry Analyzer (Lab systems CLD, Konelab, Helsinki, Finland). The reference value of serum CRP in healthy persons was <10 mg/L.

2.8. Statistical Methods

Statistical analysis was performed with an SPSS version 14.0 for Windows (SPSS, Inc., Chicago, IL, USA). Continuous variables are expressed as means with standard deviations, as medians with interquartile ranges, or as ranges from minimum to maximum. The Mann-Whitney U test was used for comparing continuous variables. Spearman's correlation was used to study the correlation between continuous variables. Categorical variables were expressed as absolute numbers or percentages, and their association was studied by a Chi-square test.

ANOVA for repeated measurements was used to assess the impact of severe vs. non-severe sepsis (defined as between-subjects factor) on plasma fibrinogen concentrations, while the within-subjects factor was defined as a concentration on day 0, 1, 2, 3 and 4. The change of AT concentration from d0 to days 1, 2, 3 and 4 was also compared with repeated measures ANOVA, with severe sepsis vs. non-severe sepsis defined as a between subjects factor and the repeated measurements defined as a within-subjects factor. A p-value <0.05 was considered statistically significant. An interaction with a p-value <0.10 was considered statistically significant.

The receiver-operating characteristic (ROC) curve was used to determine the diagnostic accuracy and cut-off value of plasma fibrinogen.

3. Results

The study included 69 patients (44 men and 25 women), the median age being 55 years (range 18–70 years). These patients experienced altogether 93 periods of neutropenic fever. Fifteen patients with AML had more than one episode of neutropenic fever during the study period: ten patients had two, two patients three, two patients four, and one patient five episodes following intensive chemotherapy courses. All these episodes of neutropenic fever were included in the study. Four patients had therapeutic antithrombotic medication (enoxaparin) at the study entry.

Of 93 periods with neutropenic fever, the criteria of severe sepsis were fulfilled in 12 periods (13%). The median time for the fulfillment of criteria for severe sepsis was 1 day (range 1–7 days) from the start of the fever. The median number of days with fever was 3 (range 1–15 days). Blood cultures were positive in 23 periods (25%) with neutropenic fever. Two patients were treated for severe sepsis in the intensive care unit; both died due to multiorgan failure. Thus, the fatality rate of severe sepsis was 17% of the periods with severe sepsis (2.8% of the patients).
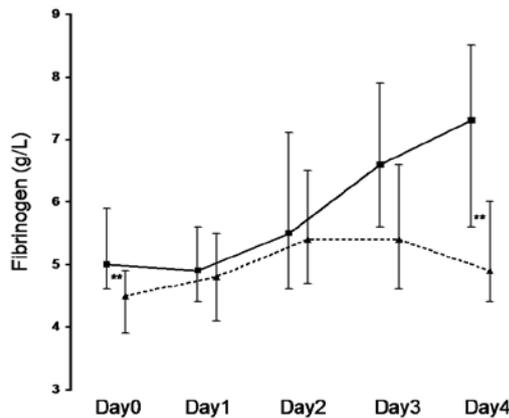
3.1. Fibrinogen

In repeated measures ANOVA there was a significant difference in the level of fibrinogen between fever periods with and those without severe sepsis with a p-value 0.019 when including all five measurements. In periods with severe sepsis, the median fibrinogen concentration on day 0 was significantly higher (5.0 g/L) than that without severe sepsis (4.5 g/L) (p=0.009). The respective interquartile ranges were 4.6–5.9 g/L in fever periods with severe sepsis and 3.9–4.9 g/L in fever periods without severe sepsis (Figure 1). During the 4-day study period, significant differences in fibrinogen concentrations between fever periods with severe sepsis and those without severe sepsis were not found until day 4 (Table 1) (Figure 1 and Figure 2).

In the ROC curve analysis, the AUC was 0.73 (95% CI, 0.61–0.86, p=0.009) for plasma fibrinogen to discriminate between severe and non-severe sepsis among neutropenic fever periods at the start of the fever. At the fibrinogen concentration of ≥4 g/L on day 0, sensitivity and specificity were 100%, and 26%, respectively. Using ROC curve analysis, the ideal

Table 1. Comparison of plasma fibrinogen and plasma antithrombin activity on days 0 to 4 in neutropenic fever periods with (n=12) and without (n=80) severe sepsis. Blood samples available for tests at different time points are shown in the table. The data are expressed as medians (range).

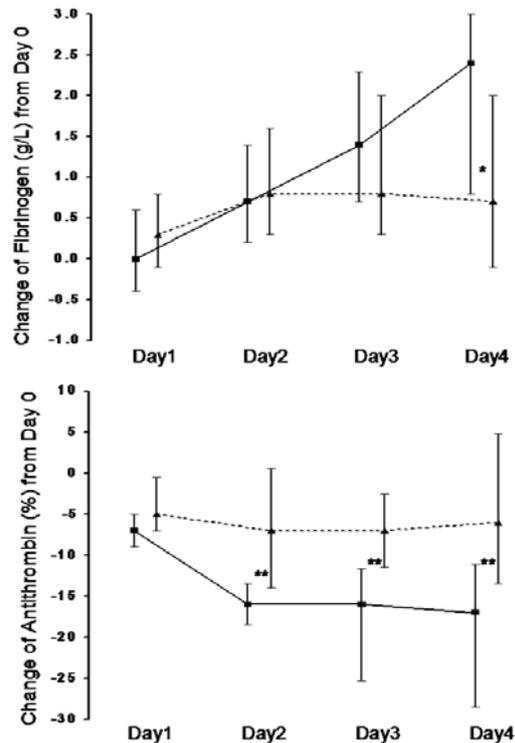| Time point	n	With severe sepsis	n	Without severe sepsis	p-value
		Fibrinogen (g/L)		Fibrinogen (g/L)	
d0	12	5.0 (4.4-7.9)	80	4.5 (2.2-8.5)	0.009
d1	11	4.9 (4.2-9.0)	74	4.8 (2.2-8.6)	0.424
d2	9	5.5 (4.5-9.5)	75	5.4 (3.0-8.9)	0.422
d3	8	6.6 (4.4-10.0)	73	5.4 (3.1-9.0)	0.056
d4	8	7.3 (5.2-10.0)	51	4.9 (2.6-9.0)	0.003
		Antithrombin III (%)		Antithrombin III (%)	
d0	12	92 (63-106)	80	95 (58-125)	0.830
d1	11	86 (82-96)	77	90 (54-135)	0.480
d2	9	81 (69-90)	74	87 (53-133)	0.076
d3	8	81 (59-86)	73	86 (53-128)	0.069
d4	8	78 (55-91)	51	87 (49-127)	0.049
		CRP (mg/L)		CRP (mg/L)	
d0	12	41 (16-253)	79	34 (5-239)	0.373
d1	12	78 (28-218)	79	80 (9-272)	0.921
d2	11	90 (57-251)	78	110 (7-333)	0.069
d3	11	162 (59-340)	77	86 (7-320)	0.031
d4	9	191 (44-452)	60	79 (8-239)	0.013

Figure 1. Medians of plasma fibrinogen (g/L) in neutropenic fever periods without severe sepsis (dotted lines) and with severe sepsis (solid lines) on days 0 to 4, with interquartile ranges. The *p* values denote the comparison of fever periods without severe sepsis with fever periods with severe sepsis, * *p*<0.05, ** *p*<0.01.

Figure 2. Medians of increase in plasma fibrinogen (g/L) (top) and decrease of plasma AT activity (%) (bottom) from day 0 in neutropenic fever periods without severe sepsis (dotted lines) and with severe sepsis (solid lines) on days 1 to 4 with interquartile ranges. The *p* values denote the comparison of fever periods without severe sepsis with fever periods with severe sepsis, * *p*<0.05, ** *p*<0.01.

threshold of the plasma fibrinogen level necessary to predict severe sepsis was determined to be 4.35 g/L with a sensitivity of 100%, a specificity of 43%, a positive predictive value of 21%, and a negative predictive value of 100%.

The incidence of severe sepsis in fever periods with abnormal plasma fibrinogen concentration (≥4.0 g/L) at the start of the fever was 17% (12/71) (Table 2). Of note, no severe sepsis developed in fever periods with normal plasma fibrinogen. The positive and negative predictive

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Table 2. The incidence of severe sepsis in patients having normal and abnormal plasma fibrinogen concentration at the start of the neutropenic fever period (n=92).

	Severe sepsis	
	Present	Absent
Plasma fibrinogen ≥ 4.0 g/L	12	59
Plasma fibrinogen < 4.0 g/L	0	21

Figure 3. Medians of plasma antithrombin activity (%) in neutropenic fever periods without severe sepsis (dotted lines) and with severe sepsis (solid lines) on days 0 to 4 with interquartile ranges. The outlier values are shown as solid circles. The p values denote the comparison of fever periods without severe sepsis with fever periods with severe sepsis, * $p<0.05$, ** $p<0.01$.
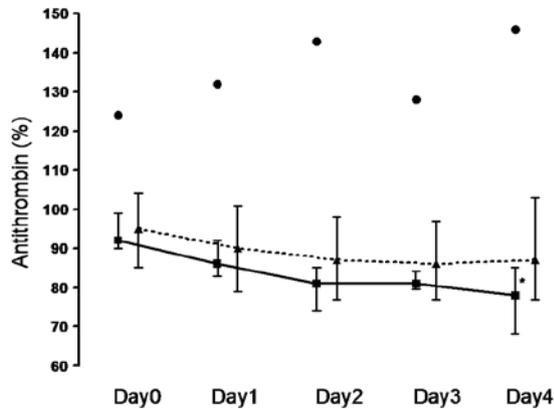

values of fibrinogen ≥4 g/L on day 0 for the development of severe sepsis were 17%, and 100%, respectively. There were significant correlations between plasma fibrinogen and serum CRP concentrations during the study period (day 0: $r=0.521$, $p<0.001$; day 1: $r=0.645$, $p<0.001$; day 2: $r= 0.524$, $p<0.001$; day 3: $r=0.592$, $p<0.001$; day 4: $r= 0.547$, $p<0.001$).

3.2. Antithrombin

In repeated measures ANOVA, no significant difference in the level of AT activity was found between fever periods with and without severe sepsis ($p=0.156$). However, there was a correlation between the severity of sepsis and the change of AT ($p=0.016$ for the interaction term), with a more prominent decrease in those with severe sepsis. The decrease in AT activity was found to be significantly greater in fever periods associated with severe sepsis than those without on days 2 to 4 (Figure 2). In periods with severe sepsis, the median AT activity on day 4 was significantly lower (78%) than that without severe sepsis (87%) ($p=0.049$)(Table 1, Figure 3). There were slightly negative correlations between plasma AT activity and serum CRP on day 3 ($r= - 0.323$, $p=0.004$) and on day 4 ($r= - 0.304$, $p=0.024$).

Figure 4. Medians of serum C-reactive protein (mg/L) in neutropenic fever periods without severe sepsis (dotted lines) and with severe sepsis (solid lines) on days 0 to 4 with interquartile ranges. The p values denote the comparison of fever periods without severe sepsis with fever periods with severe sepsis, * $p<0.05$, ** $p<0.01$.
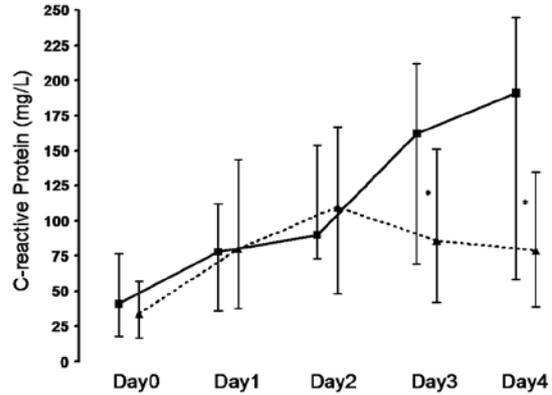
3.3. CRP

In repeated measures ANOVA, no significant difference in the level of serum CRP was found between fever periods with and without severe sepsis ($p=0.307$). However, there was a relationship between the severity of sepsis and the change of CRP in repeated measures, with a continuing rise in CRP on days 3 and 4 in severe sepsis (Figure 4). There were no differences found in the median CRP concentration between the periods characterized by severe sepsis and those without at the beginning of the fever (day 0) or during the following two days. Not until on day 3 was the serum CRP significantly higher in periods with severe sepsis, compared with those without (Figure 4).

4. Discussion

Severe infections, especially sepsis, are associated with both a systemic inflammatory response characterized as acute phase reaction [16] and activation of the coagulation system [6]. In our patients, right from the start of the fever episodes, the median fibrinogen concentration was elevated in periods characterized by severe sepsis. Normal plasma fibrinogen at the start of fever was found to rule out severe sepsis in this group of patients. However, as many false positive cases lead to poor specificity and low positive predictive value, plasma fibrinogen cannot be considered to be an ideal predictive indicator of severe sepsis.

We expected that plasma fibrinogen might show a decreasing tendency with the consumption of fibrinogen due to the activation of coagulation, which might predict a risk of disseminated intravascular coagulation (DIC) in patients with severe sepsis. However, plasma fibrinogen

concentrations continued to increase in patients with severe sepsis during the follow-up, indicating a strong acute phase response, as positive correlations between plasma fibrinogen and serum CRP concentrations were found at each time point.

AT measurement could not either pinpoint the patients at risk of severe sepsis at the beginning of the fever episode. However, a decrease in plasma AT activity was stronger in periods with severe sepsis than in those without. A negative correlation between plasma AT activity and serum CRP may indicate an increased consumption of AT because of the activation of coagulation during infection in this study. Still, we cannot rule out a reduced protein synthesis or impaired AT activity in the course of sepsis [7,9,10].

Mesters and his co-workers observed significantly lower plasma AT activity in septic shock patients at the onset of the fever, compared with patients with severe sepsis. However, they did not find decreasing plasma AT activity in patients with either septic shock or severe sepsis [12], which differs from our findings. A decrease in plasma AT has been considered a marker of poor prognosis in septic shock with DIC [7,8,12]. Most of our patients still had AT values within a reference range, but it must be borne in mind that the majority of our patients

with severe sepsis recovered and were not treated in an intensive care unit.

Decreasing physiological anticoagulation may predispose patients to thrombotic complications, and elevated fibrinogen concentration has also been shown to contribute to the hypercoagulopathy in septic patients [17]. However, the pathophysiology of hypercoagulopathy in cancer patients is complex [18], and AT replacement therapy was not found to reduce hypercoagulability in septic patients in a recent study [17]. AT activity decreased to <60% in some of our patients, but none of our patients received AT supplementation or experienced thrombotic complications.

In conclusion, at the beginning of the neutropenic fever, normal plasma fibrinogen indicated patients who would not develop severe sepsis, and in a group of patients at the beginning of the fever, both elevated plasma fibrinogen at the start of the fever period and constantly decreasing AT were linked to the development of severe sepsis. However, because of its low positive predictive value, fibrinogen cannot be considered an ideal indicator in predicting the development of severe sepsis among hematological patients with neutropenic fever.

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