Selected Topics in Clinical Enzymology

Proceedings (selected) of the
Third International Congress of
Clinical Enzymology
Salzburg, Austria,
September 6 – 9, 1981

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Walter de Gruyter · Berlin · New York 1983

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PLASMA LEVELS OF HUMAN GRANULOCYTIC ELASTASE-α_1-PROTEINASE INHIBITOR COMPLEX (E-α_1PI) IN PATIENTS WITH SEPTICEMIA AND ACUTE LEUKEMIA

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Abstract

Polymorphonuclear granulocytes release neutral proteinases such as elastase (E) and cathepsin G in the course of septicemia or acute leukemia. These proteinases may inactivate plasma proteins by nonspecific degradation before they are eliminated via complex formation with endogenous inhibitors, e.g. the α_1-proteinase inhibitor (α_1PI). In this study, plasma levels of the E-α_1PI complex were evaluated in order to find a correlation to the severity of postoperative infections or to clotting factor depletion in acute leukemia. Using a newly developed, sensitive enzyme-linked immunoassay we determined the plasma levels of E-α_1PI in patients who had undergone major abdominal surgery. Up to three-fold of the normal level (83.9 ± 24.7 ng/ml) was observed in patients without postoperative infections and up to 10- to 20-fold of normal was seen in patients suffering from septicemia. In patients who recovered, the E-α_1PI levels returned to normal, whereas in patients with persistent septicemia high levels of E-α_1PI were measured until death.

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Studying various kinds of leukemia, only patients with acute myelogenous leukemia (AML) and chronic myelogenous leukemia with blastic transformation (CML) showed moderate-to-high levels of E-α_1PI (2- to 20-fold of normal). However, clotting factor depletion observed in different kinds of acute leukemia seemed to be independent of elastase liberation.

The results obtained indicate that E-α_1PI plasma levels may be a suitable marker for the severity of postoperative infections as well as a helpful tool in discriminating acute leukemia in myelogenous and lymphatic or undifferentiated forms.

Introduction

Bleeding in both gram-negative sepsis and acute leukemia is thought to be due not only to thrombocytopenia, but also to the depletion of clotting factors. It is well known that activators released from leukocytes, platelets and endothelial cells may induce specific activation of clotting, fibrinolysis, and complement by limited proteolysis resulting in the elimination of activated proteinases via complex formation with inhibitors. In addition to such *specific* consumption, *nonspecific* proteolytic degradation of plasma proteins by neutral granulocytic proteinases (e.g. elastase or cathepsin G) has also been postulated (1,2). These proteinases may be released from their cells into the circulation by the action of endotoxins or other factors such as opsonized particles, immune complexes, and aggregated IgG. Liberated elastase may destroy blood proteins, e.g. factor XIII (F XIII) or antithrombin III (AT III), before inhibition occurs by the plasma proteinase inhibitors, α_1-proteinase inhibitor (α_1-PI, formerly α_1-antitrypsin) and α_2-macroglobulin (α_2M). Due to the presence of these potent inhibitors, direct measurement of the granulocytic proteinase activities

in plasma or serum is not possible. However, increased levels of the elastase-α_1PI complex (E-α_1PI) would be an indirect but clear indication of elastase liberation in the course of the above mentioned diseases. Indeed, Egbring et al (1) have demonstrated such complexes (by means of one- and two-dimensional Laurell electrophoresis) in the plasma of patients suffering from acute leukemia or septicemia.

To establish whether a relationship exists between the levels of E-α_1PI and the severity of postoperative infections, we studied the degree of F XIII and AT III depletion. These clotting factors are known to be very sensitive substrates for elastase *in vitro* (1,3). Similar studies were performed with patients suffering from various kinds of leukemia. Quantitative estimation of the E-α_1PI plasma levels of these patients was carried out for the first time with a sensitive enzyme-linked immunoassay (4).

Materials and Methods

Patients

Patients suffering from septicemia after abdominal surgery fulfilled the following criteria: clearly defined primary site of infection with positive culture of the invading organisms; body temperature > 38.5°C; leukocytes > 15,000 or < 5,000/mm^3; platelets < 100,000/mm^3 or platelet drop < 30% below the preoperative value; positive blood culture.

Classification of the type of leukemia (AML = acute myelogenous leukemia; APL = acute promyelocytic leukemia; CML = chronic myelogenous leukemia with blastic transformation; ALL = acute lymphatic leukemia) was based on the morphology of the bone marrow and peripheral blood smears in May-

Grünwald stained films. In some cases, cytochemical reactions were also performed.

Sampling procedure

Plasma samples from normal donors (n = 10), from patients after abdominal surgery (n = 41), and from patients with various kinds of leukemia (n = 17) were obtained by withdrawal of 4.5 ml venous blood into plastic syringes containing 0.5 ml of sodium citrate (2.2 g/100 ml distilled water) to prevent clot formation. After centrifugation at 1,500 x g, the plasma samples were stored at -70°C until assayed.

Principle of the enzyme-linked immunoassay

Plasma samples were incubated in plastic tubes coated with antibodies against elastase. After washing with buffer, the surface fixed E-α_1PI complex molecules reacted with alkaline phosphatase (AP) labelled antibodies directed against α_1PI. Under the conditions used, the activity of AP towards p-nitrophenylphosphate was proportional to the concentration of E-α_1PI in the sample. For details of this assay, see Neumann et al (4).

Determination of clotting factors

Antithrombin III (AT III) was determined using the thrombin-specific chromogenic peptide substrate S-2238 (Deutsche Kabi Munich). The biological activity of the fibrin-stabilizing factor (F XIII) was measured with a commercial test system (Factor XIII-Schnell test, Behringwerke AG, Marburg). These assays were performed as previously described (5). Plasma levels of F XIII subunits A and S were tested with monospecific antisera from Behringwerke AG Marburg (Clotimmun-Faktor XIII-A, Clotimmun-Factor XIII-S), according to Laurell (6).

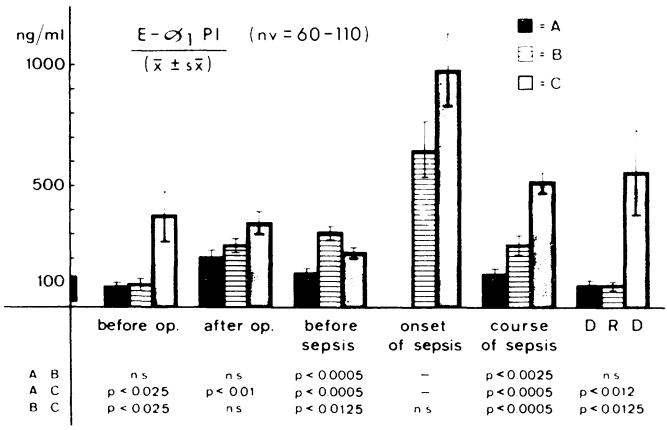

Fig. 1. Elastase-α_1-proteinase inhibitor complex (E-α_1PI) levels in plasma of patients with major abdominal surgery.

A = patients without postoperative infection
B = patients who survived postoperative infection
C = patients who died due to postoperative infection

The E-α_1PI levels are given as mean values for the day before operation, the day after operation, the period before onset of sepsis and the course of septicemia. The last determination was done on the day of discharge (D) in group A, on the day of recovery (R) in group B, and before death (D) in group C. ($s\bar{x}$ = SEM)

Results

Elastase-α_1PI levels in patients after major abdominal surgery

Thirty patients suffering from postoperative septicemia were investigated. Of these patients, 14 survived the infection (group B), whereas the other 16 died as a direct result of the septicemia (Group C). Additionally, 11 patients without infection after abdominal surgery served as controls (Group A).

E-α_1PI plasma levels are given as mean values for the day be-

fore operation, the day after operation, the period before onset of sepsis, and through the course of septicemia. The last determination was done on day of discharge in group A, on day of recovery in group B, and before death in group C (Figure 1).

In patients without preoperative infection (groups A and B), the operative trauma was followed by an increase of the E-α_1PI levels up to three-fold of the normal value. Patients suffering from preoperative infections (six out of 16 in group C), showed significantly elevated preoperative E-α_1PI levels. Immediately after surgery, a slight decrease was observed, probably due to elimination of the infection focus. Before onset of sepsis, the E-α_1PI concentrations of groups B and C showed a moderate elevation but no significant changes compared to the postoperative levels. However, at the beginning of septicemia a highly significant increase of the E-α_1PI levels could be detected, up to six-fold in group B and up to 10-fold in group C. Peak levels were more than 2,500 ng/ml in both groups. The E-α_1PI levels of septic patients who recovered showed a clear tendency towards normal values. In patients with persisting septicemia, high levels of E-α_1PI were measured until death.

As shown in Figure 2, in infected patients the activity of AT III, the most important inhibitor of the clotting system, was already below the critical concentration of about 75% of the standard mean value (7) measured before onset of septicemia. This low value normalized in all patients overcoming the infection, whereas a further significant decrease (up to 45% of the norm) was found in group C with lethal outcome. Remarkably, the AT III activity in plasma of patients of group A was in the normal range during the whole observation period.

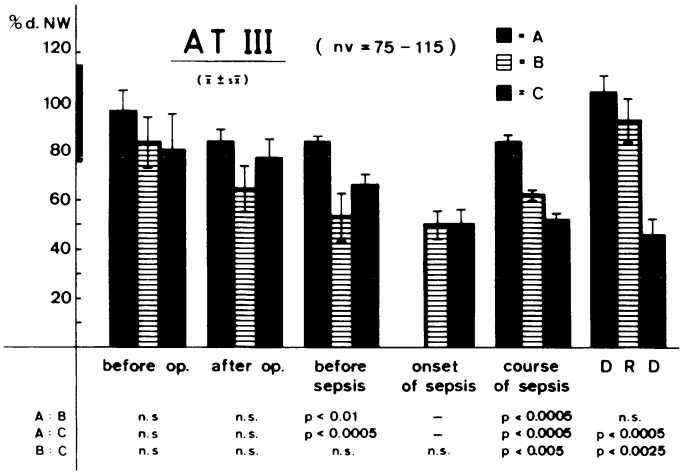

Fig. 2. Plasma levels of antithrombin III in patients with major abdominal surgery. For details see legend to Figure 1. ($s_{\bar{x}}$ = SEM)

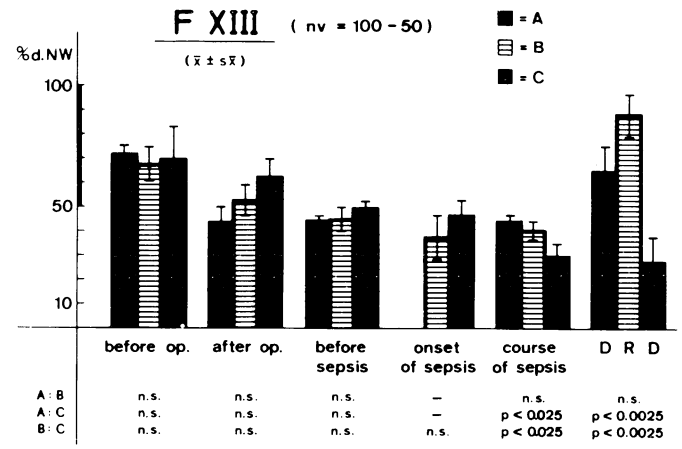

Fig. 3. Plasma levels of factor XIII fibrin-stabilizing activity in patients with major abdominal surgery For details, see legend to Figure 1. ($s_{\bar{x}}$ = SEM)

Similar results were obtained for F XIII, the fibrin-stabilizing coagulation factor (Figure 3). In plasma of patients who did not survive septicemia, the F XIII activity decreased up to 28% of the standard mean value. As measured by immunoelectrophoresis, these patients also had very low concentrations of both subunit A, comprising the active enzyme, and subunit S, the carrier protein (data not shown). In contrast, group A patients with uncomplicated postoperative course showed normal or only slightly decreased concentrations of subunit S, although subunit A and fibrin-stabilizing activities were often significantly reduced.

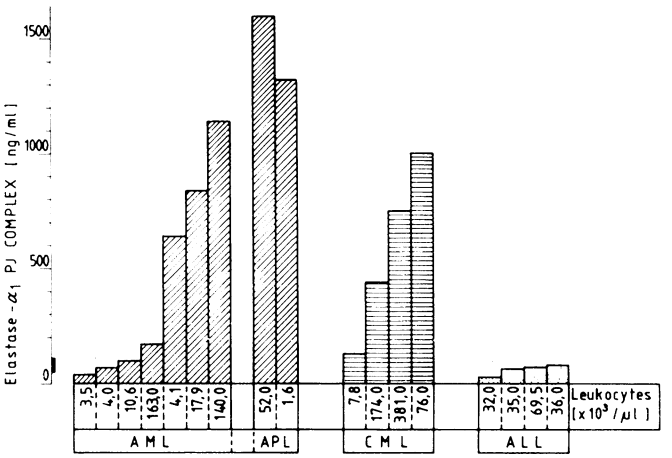

Fig. 4. Elastase-α_1-proteinase inhibitor complex (E-α_1PI) levels in plasma of patients with various kinds of leukemia, AML = acute myelogenous leukemia, APL = acute promyelocytic leukemia, CML = chronic myelogenous leukemia with blastic transformation, ALL = acute lymphatic leukemia.

Elastase-α_1PI levels in acute leukemia

Figure 4 shows the E-α_1PI plasma levels of patients suffering from various kinds of leukemia. Prior to chemotherapy, six of nine patients with myelogenous leukemia had moderate to high levels of E-α_1PI (170 - 1,440 ng/ml), the highest

levels being found in the two patients with APL (up to 1,600 ng/ml). This type of acute leukemia is frequently associated with disseminated intravascular coagulation. Elevated E-α_1PI levels (130 - 1,000 ng/ml) were also measured in the plasma of four patients with blastic transformation (CML). In contrast, none of the four patients suffering from ALL had increased levels of E-α_1PI. The same holds true for the four patients with malignant lymphoma (data not shown).

Table 1 summarizes the results of leukocyte counts as well as of plasma levels of E-α_1PI and the coagulation factors, F XIII and AT III, for each leukemia group. In plasma of the AML patients the fibrin-stabilizing activity of F XIII was significantly reduced. We expected, therefore, increased levels of E-α_1PI in these plasma samples. However, we did not find such a reverse correlation. The extremely low F XIII activity in patients suffering from ALL was especially striking in view of their normal E-α_1PI levels. Moreover CML patients with blastic transformation and increased E-α_1PI levels showed normal or even elevated F XIII activity.

The AT III activity was in the normal range except for three patients. Their low AT III level, however, was not correlated to a high E-α_1PI concentration. The individual leukocyte counts showed no relationship to the plasma levels of E-α_1PI or clotting factors studied, neither within one group nor between groups (Figure 4).

During remission induction chemotherapy (high-dose adriamycine, vincristine, and cytosine arabinoside) of AML patients having highly elevated E-α_1PI levels, a dramatic drop towards normal values occurred concurrently with the number of leukocytes (Table 2). AT III activity showed a marked decline under chemotherapy, followed by a complete restoration to normal values after full bone marrow remission (Table 2).

TABLE 1. Leukocyte counts and plasma levels of the elastase-α_1-proteinase inhibitor complex (E-α_1PI), factor XIII (F XIII), and antithrombin III (AT III) in patients with acute myelogenous leukemia with blastic transformation (CML), or acute lymphatic leukemia (ALL), respectively. For experimental details, see text

Patients	Diagnosis	Leukocytes (x $10^3/\mu l$)	E-α_1PI (ng/ml)	F XIII activity (% of the norm)	AT III activity (% of the norm)
Kh	AML	3.5	27	87.5	87
Re	AML	4.0	66	62.5	98
Ec	AML	10.6	96	37.5	66
Schö	AML	163.0	170	50.0	70
Hi	AML	4.1	640	62.5	83
Bo	AML	17.9	840	50.0	88
Ha	AML	140.0	1,140	50.0	98
We	APL	1.6	1,320	87.5	104
Si	APL	52.0	1,600	n.d.	n.d.
Me	CML	7.8	132	120	109
Bo	CML	174.0	336	75.0	109
Ri	CML	381.0	750	75.0	110
Ay	CML	76.0	1,000	120	107
He	ALL	32.0	30	37.5	85
Sa	ALL	35.0	64	25.0	70
Ri	ALL	69.5	68	25.0	100
Schi	ALL	36.0	76	37.5	105

TABLE 2. Blood cell counts and plasma levels of the elastase-α_1-proteinase inhibitor complex (E-α_1PI), factor XIII (F XIII) and antithrombin III (AT III) in a patient with acute myelogenous leukemia (AML) during remission induction chemotherapy

Day[a]	Leukocytes (X $10^3/\mu l$)	Thrombocytes (X $10^3/\mu l$)	E-α_1PI (ng/ml)	F XIII activity (% of norm)	F XIII subunits (% of norm)		AT III activity (% of norm)
					A	B	
1	17.9	32	840	50.0	38.5	74.5	88
9	9.8	11	492	37.5	33.5	69.5	66
11	2.4	15	186	37.5	40.0	72.5	54
16	1.0	28	200	37.5	33.5	50.5	52
21	0.7	27	106	37.5	41.5	76.0	57
30	2.1	185	88	50.0	46.0	83.5	71
35	2.5	184	44	37.5	34.5	57.0	76

[a] Chemotherapy with adriamycine (ADM), vincristine (VCR), and cytosine-arabinoside (ARA-C) was performed from the fourth to the ninth day after admission. Full remission of bone marrow occurred on the 28th day.

For further details, see text.

However, the very low F XIII activity and subunit A level, as well as the only moderately decreased subunit S level did not change throughout the observation period (Table 2). In patients with originally low levels of the complex, no alterations in E-α_1PI levels of clotting factor activities were observed during chemotherapy.

Discussion

Based on *in vitro* and *in vivo* studies, Egbring et al (1) postulated that, besides disseminated intravascular coagulation, direct proteolysis by granulocytic proteinases contributes to the consumption of clotting factors in patients with septicemia or acute leukemia. They found granulocytic elastase-α_1PI complexes in plasma samples of patients having reduced levels of F XIII (both subunits) and increased concentrations of fibrinogen degradation products (FDP). They used a standard curve from 12.5 to 100 ng of elastase per assay in the Laurell technique, which would correspond to 2.5 - 20 µg/ml plasma. It is not evident how elastase values as low as 0.5 ng per assay, i.e. 100 ng/ml plasma, could be quantitated by this assay. Moreover, less than 300 ng/ml protein is rarely detectable by the staining technique applied (8).

Normal E-α_1PI plasma levels can be accurately measured with the newly developed and sensitive enzyme-linked immunoassay. The detection range is between 0.2 and 3.2 ng elastase in the E-α_1PI complex per assay, i.e. 20 - 320 ng/ml plasma.

Patients suffering from septicemia showed highly elevated E-α_1PI levels during infection, although peak levels of 25 - 51 µg/ml plasma as reported by Egbring et al (1) were never observed. A significant correlation was found between the increase in E-α_1PI levels and the decrease in F XIII subunit

S in patients of group B and C, though the absolute degree of the carrier protein consumption did not correspond in each case to the amount of E-α_1PI present. On the other hand, patients without infection (group A) and normal or only slightly elevated E-α_1PI levels had F XIII-S concentrations in the normal range, but often clearly reduced fibrin-stabilizing activity and F XIII-A concentration as well. As demonstrated by Egbring et al (1) and Ikematsu et al (9), reduction of both subunits of F XIII cannot be due to activation of the clotting cascade alone. During clotting, i.e. by the action of thrombin, only subunit A is consumed together with the F XIII activity but not subunit S. These data and the results presented in our clinical trial suggest that in the patients suffering from septicemia nonspecific proteolytic degradation by granulocytic elastase is significantly involved in the depletion of F XIII.

Moreover, the extremely low AT III activity in patients having permanently elevated E-α_1PI levels may also be due to degradation by elastase. This conclusion is based on recent *in vitro* studies showing that purified AT III is rapidly inactivated by purified granulocytic elastase (3).

Summarizing the data of the septicemia study, we suggest that E-α_1PI plasma levels are a suitable marker for the severity of postoperative infections after major surgery. Furthermore, the correlation existing between elevated E-α_1PI levels and the depletion of clotting factors possibly indicates an overstressed regulatory inhibitor system of the organism. As postulated previously (2), application of suitable exogenous proteinase inhibitors during and after major surgery might assist the natural defense mechanisms for maintaining the physiological proteinase inhibitor balance.

In our preliminary study of E-α_1PI levels in acute leukemia,

we found elevated E-α_1PI concentrations in patients with acute myelogenous leukemia or CML with blastic transformation, but not in those with acute lymphatic leukemia. A decrease in F XIII activity was observed in the patients with AML, however, no correlations could be found between the levels of E-α_1PI and F XIII or the number of leukocytes at the time of admission. In AML patients having highly elevated E-α_1PI levels prior to chemotherapy, the concentration of the complex decreased towards normal levels concurrently with chemotherapy, whereas the low F XIII activity and the only slightly decreased subunit S level of these patients did not change even after full remission of bone marrow. This observation is in contrast to that of Egbring et al (1). These authors found increased levels of E-α_1PI, accompanied by corresponding changes of the F XIII and FDP levels after chemotherapy. In their patients, the reduced activity of the coagulation factors returned to normal levels with complete remission, corresponding to the disappearance of the E-α_1PI complex.

Surprisingly, in four patients with ALL the fibrin-stabilizing activity as well as subunit A and S levels of F XIII were much lower than in the patients with AML, although the E-α_1PI levels were in the normal range in the ALL patients. Moreover, the significantly elevated E-α_1PI concentration did not correspond to the normal levels of F XIII and AT III in CML patients with blastic transformation. These preliminary data suggest that the mechanism of F XIII depletion in acute leukemia seems to be not directly dependent on elastase liberation and, should be therefore, different from that postulated by Egbring et al (1). The measurement of E-α_1PI levels may be helpful, however, in discriminating acute leukemia in myelogenous and lymphatic or undifferentiated forms. However, further studies have to be performed before final conclusions can be drawn.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 51 (B 30), München.

We thank Drs. S. Neumann and H. Lang, Biochemical Research Institute of E. Merck Darmstadt, for the E-α_1PI enzyme-linked immunoassay reagents and Mrs. U. Hof and Mrs. C. Seidl for technical assistance.

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