

Factor XIII and Fibronectin

**New clinical and
biological approaches**

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Editors

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Contents

Main Lectures

- P. Henriksson, J. McDonagh:*
Factor XIII activation and interactions 1 ○
- D. F. Mosher:*
Fibronectin as a substrate for factor XIIIa 15 ○

Factor XIII Session

I. Clinic of Factor XIII Deficiency

- H. Rasche:*
Acquired diminution of plasma fibrin stabilizing factor
(factor XIII) 23
- M. Gramse, R. Egbring, K. Havemann:*
Detection of granulocyte elastase (ELP) and
chymotrypsin (CLP) in acute leucemia in relation
to factor XIII deficiency 27
- K. J. A. Miloszewski, M. S. Losowsky:*
The clinical consequences of inherited
and acquired factor XIII deficiency 31
- A. Kröniger, R. Egbring:*
Long-term treatment (12 years) with factor XIII
concentrate in a patient with congenital
factor XIII deficiency 41

II. Factor XIII Test Method and Preparation

- G. Carlebjörk:*
A precise routine method for determination
of factor XIII activity in plasma 45
- J. K. Smith, L. Winkelman:*
A concentrate of factor XIII from human plasma 51

III. Biology of Factor XIII Subunits

- T. Matsuda:*
Changes of concentrations of subunits A and B
and activities of plasma factor XIII in patients
with various diseases 57

<i>D. Gulba, W. Heller, H.-E. Hoffmeister, Chr. Huth:</i> Studies on the fibronectin level under extracorporeal circulation conditions using a turbidimetric immunoassay	61
<i>P. G. Board, S. Castle:</i> Electrophoretic studies of coagulation factor XIII and fibronectin	69
<i>P. Kreckel, P. Kühnl, I. Scharrer:</i> Formal genetics and population data of the A and B subunits of the fibrin stabilizing factor (factor XIII)	81
<i>R. Egbring, H.-G. Klingemann, M. Gramse, K. Havemann:</i> Factor XIII deficiency in patients with septicemia	91
<i>M. Jochum, K.-H. Duswald, E. Hiller, H. Fritz:</i> Plasma levels of neutrophil elastase- α_1 -proteinase inhibitor complexes and factor XIII in septicemia and leucemia	107
<i>IV. New Aspects on the Action of Factor XIII</i>	
<i>D. Rider Wierzbicki, D. F. Mosher:</i> Enhancement of the action of plasma factor XIIIa on α_2 -macroglobulin by zinc ion	117
<i>M. Barthels, I. Siedentopf</i> Influence of various thrombin formation rates in haemophilia and in phenprocoumon treated patients on fibrin cross-linking	125
<i>H. E. Karges:</i> Inactivation of F XIII by α -thrombin and during coagulation	133
<i>V. Factor XIII and Wound Healing</i>	
<i>S. I. Chung, S. K. Chang, S. Y. Lee:</i> The effect of factor XIII-deficiency on wound- healing and disseminated intravascular coagulation (DIC) in an experimental animal model	141
<i>A. Sugitachi, I. Sakamoto, K. Takagi:</i> New materials enhancing wound healing	143
<i>VI. Factor XIII and Platelets</i>	
<i>T. Tsukada:</i> Factor XIII and transglutaminase activities in human blood platelets	147

<i>D. Rider Wierzbicki:</i> Plasma factor XIII levels in thrombocytopenic patients	159
<i>H.-G. Klingemann, Th. Krause, R. Egbring:</i> Factor XIII activity in thrombocytopenic patients	163

Fibronectin session

I. Structure of Fibronectin

<i>T. Vartio, A. Vaheri:</i> Structure-function relationships of plasma fibronectin and its proteolytic fragments	165
<i>H. Hörmann, F. Jilek, M. Seidl, H. Richter:</i> Location of transamidase sensitive sites and of fibrin binding regions within the domain structure of fibronectin	175
<i>K. Skorstengaard, H. C. Thøgersen, K. Vibe-Pedersen, P. Sahl, T. E. Petersen:</i> Partial primary structure of bovine plasma fibro- nectin evidence for three different types of internal homology	185

II. Function of Fibronectin

<i>A. Vaheri, T. Vartio, E.-M. Salonen, K. Hedman, G. De Petro, S. Barlati:</i> Conditional interactions of fibronectin: C-reactive protein, sulfated proteoglycans, and malignant transformation	195
<i>N. Aoki, T. Tamaki, Y. Sakata, A. Ichinose:</i> Cross-linking of α_2 -plasmin inhibitor to fibrin. Its comparison with fibronectin cross-linking and its physiological significance	205
<i>E. Williams, R. Johnson, P. Janmey, J. Ferry, D. F. Mosher:</i> The effect of disulfide bond reduction on the aggregation and factor XIIIa-mediated cross-linking of human plasma fibronectin	213
<i>D. F. Mosher, P. J. McKeown-Longo:</i> Factor XIIIa-mediated cross-linking of ^{125}I -fibro- nectin bound reversibly and irreversibly to cell layers of cultured human fibroblasts	223

<i>H. D. Bruhn, K. H. Zurborn:</i> Factor XIII and fibronectin as regulators of cell proliferation	229
<i>E. G. Hahn, M. Colombo, V. Gauss-Müller, H. Hörmann:</i> Fibronectin in normal, regenerating and malignant human hepatocytes	237
<i>III. Fibronectin Preparation</i>	
<i>L. Winkelman, J. K. Smith, E. Norman:</i> Fibronectin as a by-product of large- scale factor VIII production	241
<i>IV. Clinical Significance of Fibronectin</i>	
<i>J. Soria, C. Soria, P. Hourdille, R. Sanchez, P. Lafay, M. Boisseau:</i> Fibronectin level in burnst patients – confrontation with the level of other factors of coagulation – clinical application	245
<i>P. Lundsgaard-Hansen, E. Rubli:</i> Fibronectin in intensive care patients	255
<i>J. Lohmann, G. Pott:</i> Qualitative and quantitative alterations of plasma fibronectin in patients suffering from shock and septicemia	257
<i>H. P. Schwarz, P. Aiginger, R. Kuzmits:</i> Fibronectin plasma concentrations in patients with malignant diseases	263
<i>H. Höfeler, H.-G. Klingemann, R. Egbring:</i> Fibronectin and factor VIII R:AG in acute and chronic hepatic failure	269
<i>H.-G. Klingemann, H. Höfeler, M. Kosukavak, K. Havemann:</i> Fibronectin in acute leucemia	277

Plasma levels of neutrophil elastase- α_1 -proteinase inhibitor complexes and factor XIII (including subunits A & S) in septicemia and leucemia

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Introduction

During the inflammatory response various or local tissue cells are activated thereby releasing internal, mostly lysosomal enzymes. Due to the liberation of these enzymes depletion of clotting factors in septicemia or acute leucemia may occur via two major routes (fig. 1):

- *System-specific* proteinases, such as thrombokinases and plasminogen activators, trigger the activation of the clotting,

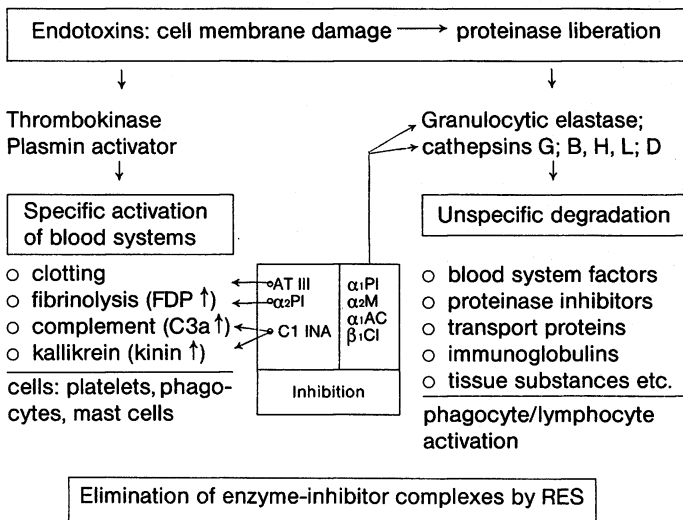


Fig. 1. Consumption of plasma factors during inflammation. For details see text. Abbreviations: Antithrombin III (AT III), α_2 -plasmin inhibitor (α_2 PI), C1 activator (C1 INA), α_1 -proteinase inhibitor (α_1 PI, formerly α_1 -antitrypsin), α_2 -macroglobulin (α_2 M), α_1 -antichymotrypsin (α_1 AC), β_1 -collagenase inhibitor (β_1 CI), fibrin(-ogen) degradation products (FDP), complement factor (C3a), reticuloendothelial system (RES).

fibrinolysis, and complement cascades (summarized as »blood systems«) by *substrate-specific* proteolysis of proenzymes or cofactors. Due to the subsequent inhibition and rapid elimination of the enzyme/inhibitor complexes via the reticuloendothelial system (RES) not only the proteinases but also their specific inhibitors (e. g. antithrombin III, α_2 -antiplasmin, C1-inactivator) are consumed. For a long time the given sequence of reactions was assumed to be exclusively responsible for the development of disseminated intravascular coagulation.

- Results obtained recently^{1, 2, 3, 4} indicate that an additional reaction pathway contributes considerably to the consumption of plasma factors during severe inflammations. This implies inactivation of plasma proteins by *substrate-unspecific* proteolysis due to liberated lysosomal proteinases such as elastase and cathepsin G from polymorphonuclear granulocytes (neutrophils).

Especially elastase may destroy blood proteins before inhibition occurs by the plasma proteinase inhibitors, α_1 -proteinase inhibitor (α_1 PI, formerly α_1 -antitrypsin) and α_2 -macroglobulin. On account of these potent inhibitors, direct measurement of the granulocytic proteinase activities in plasma is not possible. However, increased levels of the elastase- α_1 PI complex (E- α_1 PI) would be an indirect but clear indication of elastase liberation. Indeed Egbring and coworkers have demonstrated such complexes by means of one- and two-dimensional Laurell electrophoresis in plasma of patients suffering from acute leucemia or septicemia¹.

In the studies performed in our laboratory we were especially interested to see whether a relationship exists between the levels of E- α_1 PI complex and the severity of postoperative infections after major abdominal surgery, or clotting factor depletions in acute leucemia.

To achieve this purpose, besides other factors, the levels of factor XIII (measured by a modified F XIII-Schnelltest from Behringwerke Marburg and by rocket-immunoelectrophoresis) and antithrombin III (determined by the coatest antithrombin from Deutsche Kabi, München) were continuously monitored, because both clotting factors are known to be easily degraded by neutrophil elastase *in vitro*^{5, 6}.

Quantitative estimation of the plasma levels of the elastase- α_1 -proteinase inhibitor complex was carried out with a *highly sensitive enzyme-linked immunoassay*. This assay has been

developed recently by Neumann and coworkers⁷ and was adapted to clinical conditions in our laboratory.

Briefly, the E- α_1 PI complex in the plasma sample is bound to surface-fixed antibodies directed against neutrophil elastase. After washing with buffer, a second alkaline phosphatase-labelled antibody directed against α_1 PI is fixed to the complex. Under suitable conditions, the activity of the bound alkaline phosphatase

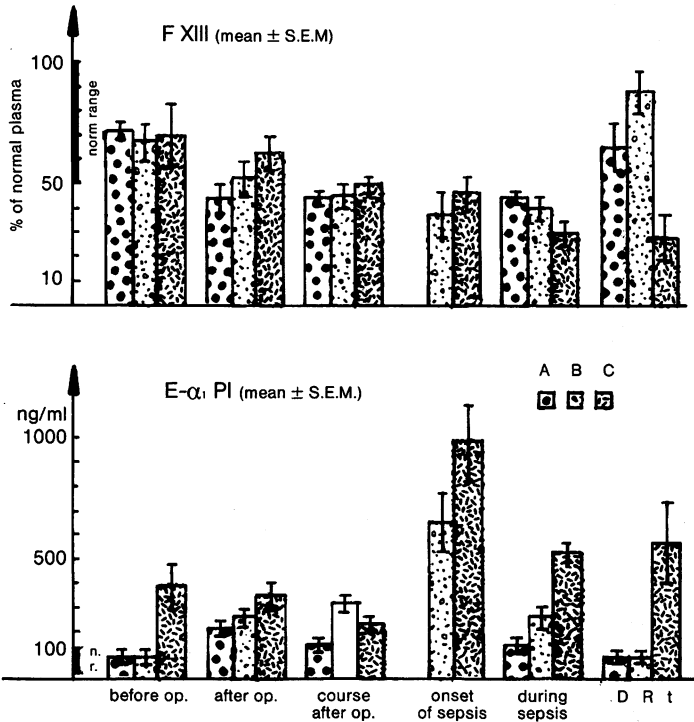


Fig. 2. Plasma levels of elastase- α_1 -proteinase inhibitor complex (E- α_1 PI) and fibrin stabilizing activity of factor XIII (F XIII) in patients subjected to major abdominal surgery.

A = patients (n = 11) being without postoperative infection

B = patients (n = 14) surviving postoperative septicemia

C = patients (n = 16) dying as a result of septicemia.

The E- α_1 PI levels are given as mean values (\pm SEM) for the day before operation, the day after operation as well as for the postoperative phase before sepsis, at onset of sepsis and during septicemia. Last determinations were done on day of discharge (D) for group A, on day of recovery (R) for group B, and before death (+) for group C. nr = normal range

towards p-nitrophenylphosphate is proportional to the concentration of the E- α_1 PI complex in the sample.

Results and Discussion

a) Levels of elastase- α_1 PI complex and factor XIII in patients after major surgery

In the prospective clinical study⁸ more than 120 patients were included. Thirty of them fulfilled defined septic criteria during the postoperative course. Of these patients fourteen survived the infection (group B), whereas sixteen died as a direct result of septicemia (group C). Eleven patients, being without infection after abdominal surgery, served as controls (group A).

With the enzyme-linked immunoassay normal levels of complexed elastase were found between 40 and 150 ng/ml in 153 healthy individuals (mean value: 86.5 ± 25.5 ng/ml).

As shown in fig. 2, in patients without preoperative infection (group A and B), the operative trauma was followed by an increase of the E- α_1 PI level up to 3-fold of the normal value. Patients suffering from preoperative infections showed already clearly elevated preoperative complexed elastase levels. Immediately after surgery a slight decrease was observed, probably due to elimination of the infection focus.

At the beginning of septicemia a highly significant increase of the complexed elastase could be detected: up to 6-fold in group B and up to 10-fold in group C. Peak levels were found above 2.500 ng/ml in both groups. The E- α_1 PI levels of septic patients who recovered showed a clear tendency towards normal values. In patients with persisting septicemia, high levels of complexed elastase were measured until death.

In comparison to the E- α_1 PI changes throughout the postoperative phase, the factor XIII activity showed an inverse pattern (fig. 2). A decrease in F XIII activity far below the normal value could be observed in all three groups during the early postoperative course. At onset of sepsis no statistically significant difference, either within the infected groups or between these groups and the control, could be demonstrated. However, throughout the course of septicemia, the factor XIII activity in plasma of patients who did not survive decreased further up to 28% of the standard mean value. Last determinations showed a clear tendency towards normal in patients of the control group and of the group overcoming the infection. In

group C patients the mean value was still below 30% of the norm concomitantly to the highly elevated levels of E- α_1 PI.

These patients also had very low concentrations of both: subunit A comprising the active enzyme, and subunit S representing the carrier protein (data not shown). In control patients, however, only the F XIII activity and subunit A exhibited low values during the early postoperative phase, whereas subunit S was diminished insignificantly.

Interestingly, during clotting, only subunit A is consumed but not subunit S. Elastase, however, is able to degrade both subunits to a similar degree as demonstrated clearly by Egbring and coworkers^{1, 5}. These data and the results presented in our clinical trial suggest that in the patients suffering from septicemia, unspecific proteolytic degradation by neutrophil elastase and/or other lysosomal proteinases is involved to a significant degree in the depletion of F XIII.

The changes of the most important plasma factors after major surgery are summarized in fig. 3: Simultaneously to the rise of E- α_1 PI complex a marked decrease of antithrombin III, factor XIII and α_2 -macroglobulin as well as an increase of the acute phase reactant C-reactive protein was measured during sepsis. These plasma factors all normalized in patients who overcame the

Parameter	Sepsis	Survival	Non-survival
E- α_1 PI complex	↑↑	n	↑↑
Antithrombin III	↓↓	n	↓↓
Factor XIII	↓↓	n	↓↓
α_2 M concent.	↓↓	n	↓↓
α_2 M activity	↓↓	n→	↓↓
C-reactive protein	↑↑	n	↑

E: Elastase; α_2 M: α_2 -Macroglobulin

Fig. 3. Correlation of the increase of elastase- α_1 -proteinase inhibitor complex (E- α_1 PI) and the decrease of antithrombin III (AT III), factor XIII (F XIII) and α_2 -macroglobulin (α_2 M) as well as the increase of the C-reactive protein (CRP) in plasma of patients suffering from septicemia after major abdominal surgery.
n = normal value

Patients	Diagnosis	Leukocytes (x 10 ⁹ /μl)	E-α ₁ PI (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	1140	50.0	98
We	APL	1.6	1320	87.5	104
Si	APL	52.0	1600	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	1000	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 1. Leucocyte counts and plasma levels of elastase- α_1 -proteinase inhibitor complex (E- α_1 PI), factor XIII, and antithrombin III in patients with acute myelogenous leukemia (AML), acute promyelocytic leukemia (APL), chronic myelogenous leucemia with blastic transformation (CML), or acute lymphatic leucemia (ALL), respectively. For details see text.

infection disease, whereas they showed pathological values in patients with fatal outcome. We take this as a clear indication that especially neutrophil proteinases contribute significantly to the inflammatory response of the organism.

b) Levels of elastase- α_1 PI complex and factor XIII in acute leucemia

In our preliminary study of elastase release throughout leucemia⁹, seventeen patients suffering from various kinds of leucemia were included (table I).

Prior to chemotherapy, six out of nine patients with acute myelogenous leucemia (AML) had moderate to high levels of E- α_1 PI (170–1.440 ng/ml), the highest levels being found in the two patients with acute promyelocytic leucemia (APL; up to 1.600 ng/ml). This type of acute leucemia is frequently associated with disseminated intravascular coagulation. Elevated E- α_1 PI levels

(130–1.000 ng/ml) were also measured in the plasma of four patients suffering from chronic myelogenous leucemia with blastic transformation (CML). In contrast, none of the four patients with acute lymphatic leucemia (ALL) had increased levels of E- α_1 PI.

Although the fibrin stabilizing activity of F XIII was frequently reduced in AML patients no clear correlation could be found between the absolute amount of elevated levels of E- α_1 PI and the decrease in F XIII activity.

Surprisingly, the ALL patients showed extremely low F XIII activity although their E- α_1 PI levels were in the normal range. In contrast, CML patients with blastic transformation and increased E- α_1 PI levels showed normal or even elevated F XIII activity. Additionally, in contrast to Egbring and coworkers¹, no clear correlation between elevation of E- α_1 PI complex and decrease of subunit S could be demonstrated (data not shown).

The AT III activity was in the normal range, except for three patients. Their low AT III level, however, was also not correlated to a high E- α_1 PI concentration.

These preliminary data suggest that the mechanism of F XIII depletion in acute leucemia seems to be not directly dependent on elastase liberation, and should be therefore, different from that postulated for septicemia. Unfortunately, there is no explanation for this discrepancy at the moment.

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Discussion

Rasche:

You told us that elastase inhibitor complexes are elevated in patients with acute leucemia. I would be interested in what stage of acute leucemia has blood samples been taken. I am especially interested if you have some data of those patients, who had a drug induced bone marrow depression.

Jochum:

Unfortunately I cannot tell you something about the stage of acute leucemia, because we have received the samples from Dr. Hiller from Munich and there was not enough time to get some information about the disease. But we also had samples after chemotherapy in acute myelocytic leucemia and the starting values of the complexes were very high and they came down to normal during chemotherapy and also the factor XIII activity came up to normal value at the end of the chemotherapy.

Rasche:

I think the question seems very important to me, because what is with the elastase-inhibitor complexes during the phase of hypoplastic bone marrow?

Jochum:

We have in the meantime only one patient after chemotherapy, so I cannot answer your question sufficiently.

Discussant:

Several of the last speakers have alluded to possible connection between raised levels of elastase and lower levels of factor XIII. We all know that DIC and septicemia are very complex disease states and I do not really feel convinced that elastase is the only factor responsible for the factor XIII decline.

Egbring:

We did not say that it is the only reaction. I have, in the first slide, showed that three mechanisms are responsible for degradation of patients with septicemia, including DIC, possibly including hyperfibrinolysis. And another point is that factor XIII may be degraded by bacterial proteinases. That is a hypothesis, but we will attempt to prove it.