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CLOTTING ACTIVATION AND IMPAIRMENT OF
FIBRINOLYSIS IN MALIGNANCY

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ABSTRACT

Different coagulation and fibrinolysis parameters were investigated in 149 patients with metastatic and non-metastatic tumours and results were compared with those obtained in a healthy population. Results showed a significant increase of thrombin-anti-thrombin complexes, fibrinopeptide A (FPA) and fibrin monomers in the group of patients ($p < 0.001$). There was also a significant prolongation of euglobulin lysis time ($p < 0.005$) and an increase of plasminogen activator inhibitor activity ($p < 0.0001$), fibrinogen degradation products ($p < 0.001$), and D-dimer ($p < 0.05$) in the group of patients as compared to controls; FPA levels were also increased in patients with metastases ($p < 0.005$). This study demonstrates clotting activation, at the level of fibrinogen to fibrin conversion, and impairment of fibrinolysis in patients with malignancy.

INTRODUCTION

The association of deep venous thrombosis with malignancy (Trousseau syndrome) is poorly understood and but the underlying mechanisms responsible for alterations in the clotting system are far from being established (1-3). Tumours may release substances into the circulation that directly or indirectly activate the coagulation mechanism and contribute to

Key Words: Malignancy; Thrombosis; Clotting activation; Fibrinolysis.

fibrin deposits as demonstrated by histological and immunological methods (4,5). To which extent the fibrinolytic system is clearing these deposits, is uncertain. A relation between the fibrinolytic activity of tumour cells and their invasive potential has been suggested and there are many examples of increased plasminogen activator activity in malignant tissues as compared to their normal counterparts (6,7). Some authors have found an increase in plasminogen activators in the blood of patients with carcinomas (8,9) whereas others have shown reduced blood fibrinolytic activity (10-13).

The aim of our work was to analyze the hypercoagulable state present in malignancy by studying the mechanism of thrombin formation, fibrinogen to fibrin conversion and the possible role of the main components of the fibrinolytic system.

PATIENTS AND METHODS

The study was carried out in 149 patients with malignancy: 93 males and 56 females with a mean age of 57±13 years (range 17-81 years) of which 65 patients (44,8%) presented with local disease and 84 (55,2%) had metastases. Tumour localization was as follows: 61 gastro-intestinal (40.9%), 17 pulmonary (12%), 24 urological (16.1%), 16 gynaecological (10.7%) and 30 others (20.3%). No anti-inflammatory drugs were given during the week preceding the study. Forty-four age-sex matched healthy subjects served as control group.

Blood from the antecubital vein was collected into 0.1 vol trisodium citrate (final concentration 0.011 M). Platelet-poor plasma was obtained by centrifugation for 15 min at 2.500 g and 4° C. Plasma samples for the fibrin plate assay and euglobulin lysis time were tested immediately whereas other samples were stored at -70°C until use. For the determination of fibrinopeptide A samples were collected on anticoagulant containing sodium citrate, heparin and aprotinin.

The assay of the thrombin-antithrombin (TAT) complex was performed by using an enzyme-immunoassay (Enzygnost TAT, Behring Institute, W. Germany), of fibrinopeptide A (FPA) with an enzyme-immunoassay (ELISA FPA, Boehringer Mannheim, W. Germany) and of soluble fibrin in plasma was determined by a quantitative spectrophotometric assay according to Wiman and Ranby (14). Euglobulin lysis time (ELT) was performed according to von Kaulla et al (15). Fibrinolytic activity of the euglobulin fraction (EFA) was assessed on fibrin plates (16). Tissue-type plasminogen activator (t-PA) activity was determined by a spectrophotometric assay (17) and t-PA antigen (t-PA Ag) using a t-PA ELISA kit (Biopool AB, Umea, Sweden). Plasminogen activator inhibitor (PAI) activity was measured as previously described (18). For the plasminogen and α_2 -antiplasmin (α_2 -AP) assay the chromogenic substrate (Kabi Diagnostica, Sweden) was used. Fibrinogen degradation products (FDP) and fibrin degradation products (D-dimer) were both measured according to a microelisa system using specific monoclonal antibodies FDP-Y18 (Fibrinostika FGDP) and FDP-DD13 (Fibrinostika FBDP) respectively (Organon Teknika, the Netherlands).

The data were statistically analyzed on the basis of mean values and standard deviations (SD). Student's-t-test was used for comparison of

means between patients and controls and between patients with local disease and those with metastases. Analysis of variance was used for comparison of results according to tumour localization.

RESULTS

One hundred and forty nine patients with different malignancies were studied. There was a significant increase ($p < 0.001$) of TAT complexes (Fig 1) in patients (22.3 ± 27.4 ug/l) as compared to controls (3.6 ± 2.6 ug/ml). Fig 1 also shows a significant increase ($p < 0.001$) of fibrinopeptide A and fibrin monomers in patients (13.7 ± 14.3 ng/ml and 45.4 ± 55.7 nmol/l respectively) as compared to controls (4.4 ± 4.2 ng/ml and 8.1 ± 13.1 nmol/l respectively).

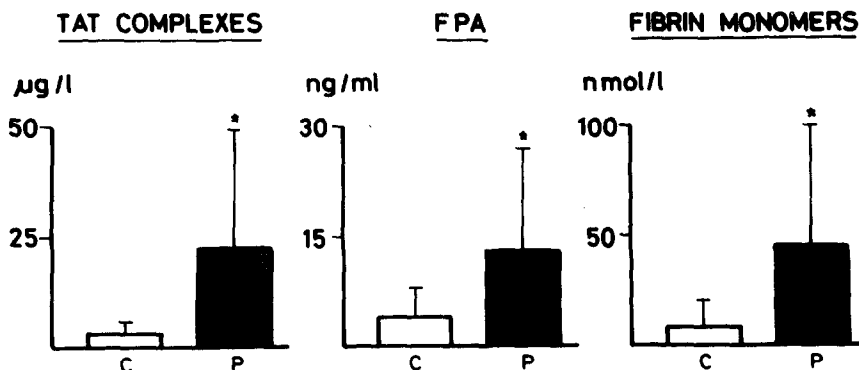


Fig. 1. Mean (SD) concentrations of TAT complexes, FPA and fibrin monomers in patients (P) and controls (C). (* $p < 0.001$ with respect to control group).

Figures 2 and 3 show the distribution of the fibrinolytic parameters analyzed. A significant prolongation of ELT ($p < 0.005$) was found in patients (286.2 ± 111.6 min) as compared to controls (231.8 ± 58.7 min). There was a significant increase in PAI activity ($p < 0.0001$) in patients (2.1 ± 1.5 U/ml) when compared to controls (0.9 ± 0.9 U/ml). FDP concentrations were significantly higher ($p < 0.001$) in patients (1.1 ± 2.3 ug/ml) than in controls (0.06 ± 0.07 ug/ml). There was a slightly significant increase in D-dimer concentrations in patients (1.2 ± 2.5 ug/ml) as compared to controls (0.1 ± 0.1 ug/ml) ($p < 0.05$). EPA, t-PA activity and antigen, plasminogen, and α_2 -AP did not differ significantly between patients and controls.

No correlation was found between overall fibrinolytic activity and t-PA activity, nor between t-PA and PAI.

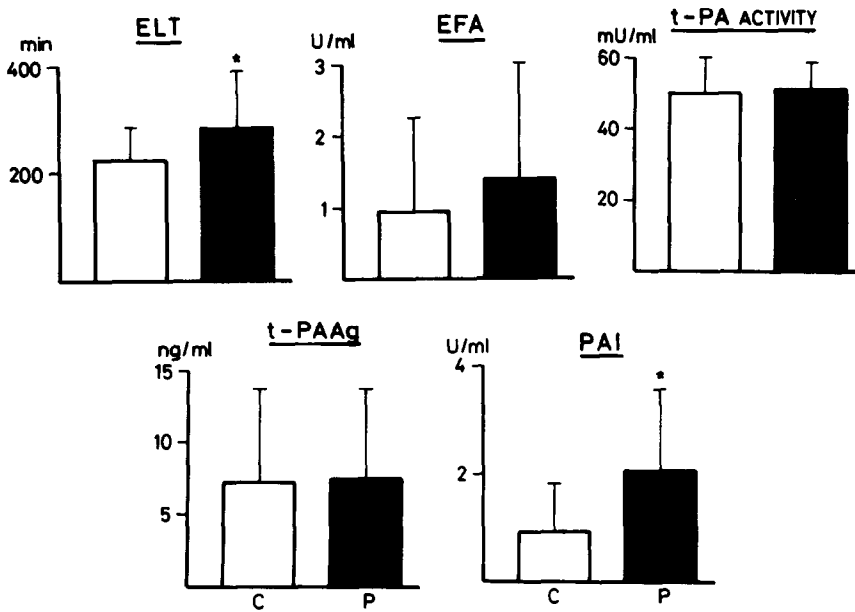


Fig. 2. ELT, EFA, t-PA activity, t-PA Ag and PAI in patients (P) and controls (C). Mean \pm SD is reported. (* $p < 0.005$ and ** $p < 0.0001$ with respect to control group).

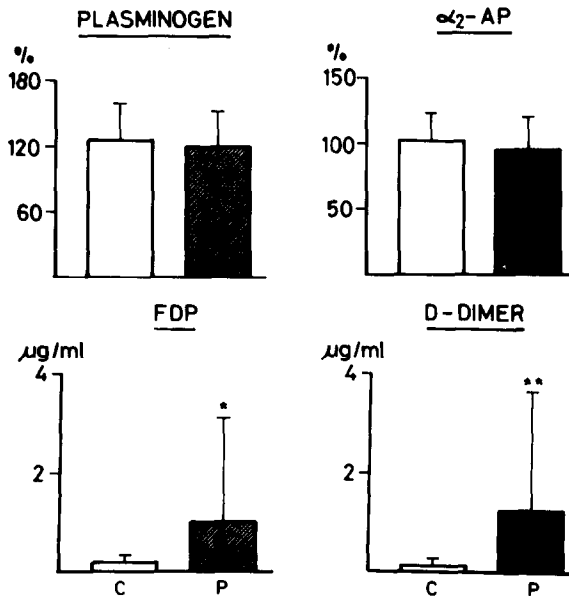


Fig. 3. Mean (SD) concentrations of plasminogen, α_2 -AP, FDP and D-dimer in patients (P) and controls (C). (* $p < 0.001$ and ** $p < 0.05$ with respect to control group).

Sixty-five patients presented with metastases and 84 with local disease. The values of the coagulation and fibrinolysis parameters analyzed between patients with and without metastases is shown in Table 1. Only the levels of FPA were significantly higher ($p < 0.005$) in patients with metastases as compared to those with local disease.

TABLE 1

Parameters analyzed in patient groups with and without metastases. Mean \pm SD is reported.

PARAMETER	Local disease (n= 84)	Metastases (n= 65)
TAT complex (ug/l)	19.7 \pm 23.7	26.1 \pm 31.8
FPA (ng/ml)	10.8 \pm 11.3	17.5 \pm 16.9*
Fibrin monomers (nml/l)	44.3 \pm 57.8	47.1 \pm 52.9
ELT (min)	278.9 \pm 91.3	295.5 \pm 132.9
EFA (U/ml)	1.5 \pm 1.9	1.3 \pm 2.3
t-PA activity (mIU/ml)	52.2 \pm 8.1	49.9 \pm 6.7
t-PA Ag (ng/ml)	7.7 \pm 6.9	7.1 \pm 6.1
PAI (U/ml)	1.9 \pm 1.2	2.2 \pm 1.9
Plasminogen (%)	121.6 \pm 31.1	120.6 \pm 35.7
α_2 -AP (%)	96.7 \pm 27.2	92.1 \pm 22.2
FDP (ug/ml)	1.1 \pm 2.2	1.3 \pm 2.6
D-dimer (ug/ml)	0.9 \pm 2.6	1.6 \pm 2.4

* $p < 0.005$ with respect to local disease

There was no difference in the coagulation and fibrinolysis parameters according to tumour localization (not shown).

DISCUSSION

Patients with malignancies show different alterations of the clotting and fibrinolytic systems. Although these abnormalities most often cause no symptoms, up to 10-15% of patients present clinically evident thrombosis, which is a common cause of death in hospitalized cancer patients (2, 19, 20).

This study clearly demonstrates that activation of the coagulation system is common in patients with malignant disease. There was a significant increase of TAT complexes, FPA and fibrin monomers in the group of patients, suggesting thrombin generation followed by an increase of fibrinogen to fibrin conversion.

Generation of thrombin represents a central event within the coagulation cascade. Thrombin acts on different physiological substrates and is inhibited by antithrombin III, thereby forming an inactive proteinase/inhibitor complex (21, 22). Determination of the TAT complex may be important in the diagnosis of thrombosis. Patients with risk factors for thrombosis and disseminated intravascular coagulation are found to have increased levels of TAT (23, 24). An increase of TAT complexes in patients with malignancy has also been shown (25).

Fibrinogen to fibrin conversion was also enhanced in these patients as indicated by the raised FPA and fibrin monomers levels, suggesting an action of thrombin on its natural substrate with release of FPA and fibrin monomer formation. It is known that tumours can produce coagulation activators such as tissue factor or activators of factor X. Monocytes from cancer patients are more likely to produce tissue factor, the amount of which can be correlated with FPA levels (26,27). We have also found increased FPA levels in patients with metastases indicating its possible role as a marker of tumoral activity. Similar results have been previously reported as related to tumour progression (28, 29). Not to be overlooked, however, is the possibility that FPA may be generated at an extravascular site related to an enzyme that is not inhibited by heparin since it has been found that heparin does not reduce FPA levels in cancer patients (2, 29).

The present study also demonstrates an impairment of the fibrinolytic system in cancer, which agrees with a previous study (13). It seems reasonable to assume that the alteration of fibrinolysis may represent an additional risk factor to be taken into consideration in the pathogenesis of thrombotic phenomena.

Only limited data are available on plasminogen concentrations, plasminogen activators and PAI activity in the blood of patients suffering from oncologic disorders (30). We have found a significant prolongation of ELT and an increase of PAI activity. In contrast, t-PA concentrations were normal, as shown both by functional and immunologic methods. The observation that overall fibrinolytic activity does not correlate with t-PA activity could be explained by the fact that the euglobulin fraction contains other plasminogen activators, distinct from t-PA, which may contribute to the total activity. The increased PAI concentrations found in the patients studied are similar to those reported by other authors (11-13). No correlation was found between t-PA and PAI which is not surprising since their release from the vascular endothelial cells, is independently regulated (31, 32). Moreover, a low concentration of plasminogen activator and increased PAI has been found to be present in patients with venous thrombosis (33). Increased levels of PAI in the patient group cannot be attributed to an acute-phase reaction as only a few of the patients showed a weak inflammatory protein pattern and no differences between PAI levels and tumoral activity were observed. It can be concluded that the enhancement of PAI activity may contribute to the impairment of blood fibrinolysis and thus represents an additional risk factor to be taken into account for explaining the thrombotic tendency of malignant disease.

In spite of the reduced fibrinolytic activity there was an increase of FDP and D-dimer suggesting that the fibrinolytic system remains active. However, this increase, although statistically significant, does not appear to be biologically very relevant. On the other hand, the increased FDP and D-dimer levels does not necessarily derive from changes in the plasma fibrinolytic activity, but may be due to other causes, such as, breakdown of extravascular fibrin deposits or activity of enzymes different from plasmin which may be produced by tumor cells (34-36).

No differences in any of the fibrinolytic parameters analyzed were found either between patients with local disease and those with metasta-

ses or related to tumour localization. This would suggest that the observed changes are not totally specific of malignant disease.

In conclusion, we have found significant abnormalities in coagulation and fibrinolysis in patients with cancer. Thrombin generation, fibrinogen to fibrin conversion and impairment of fibrinolysis suggest a hypercoagulable state which may contribute to the thrombotic complications in these patients.

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