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REGULAR ARTICLE

Measurement of Prethrombotic Markers in the Assessment of Acquired Hypercoagulable States

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Abstract

Hypercoagulable states can be detected by measuring activation peptides, enzyme-inhibitor complexes, and fibrin/fibrinogen degradation products, which are markers of hemostatic activation. A series of these prethrombotic markers has been evaluated in the elderly, pregnancy, diabetes and acute myocardial infarction patients (n=30 in each group) as well as in hematologic malignancies (n=42). The parameters assayed were: prothrombin fragment 1+2 (F1+2), thrombin-antithrombin III complexes (TAT), fibrinopeptide A (FPA), plasmin-α_2 antiplasmin complexes (PAP) and D-Dimer. Results were compared with those obtained in a group of 30 healthy subjects.

We found a significant increase of F1+2, TAT and FPA in elderly ($p<0.05$), acute myocardial infarction (AMI) ($p<0.01$), hematologic malignancies ($p<0.01$), and pregnancy ($p<0.0001$), indicating a marked clotting activation. Diabetic patients under strict metabolic control only presented a moderate increase of TAT ($p<0.05$), suggesting a slight activation. We also observed a highly significant elevation of PAP and D-Dimer in elderly ($p<0.001$), AMI ($p<0.0001$), and malignancy ($p<0.0001$), indicating an activation of the fibrinolytic system. The combination of selected fibrinolytic and coagulation measurements is useful for the detection of a hypercoagulable state in conditions characterized by a risk of thrombosis.

Key Words: Hypercoagulability; Elderly; Pregnancy; Diabetes; Myocardial infarction; Malignancy

A prethrombotic state may be defined as a condition characterized by an imbalance in hemostasis with a tendency to hypercoagulability, due to pathological activation of the enzymes of the coagulation cascade, but without clinical signs of thrombosis or evidence of fibrin deposition [1]. Hypercoagulable states can be classified into two broad categories: congenital and acquired. The former are generally inherited abnormalities of hemostasis clearly identified, while acquired hypercoagulable states include clinical conditions associated with an increased risk of thrombosis in whom the exact pathophysiologic mechanism remains unclear [2,3].

The importance of detecting these prethrombotic states deals with the possibility to diminish the thrombotic tendency by treating or eliminating the responsible factors. Advances in our knowledge of the biochemistry of the hemostatic mechanism have allowed the development of sensitive and specific assays to detect activation of coagulation and fibrinolysis in vivo [4,5]. Among them the measurement of plasma levels of activation peptides, released from the zymogen molecules

Abbreviations: F1+2, prothrombin fragment 1+2; TAT, thrombin-antithrombin complex; PAP, plasmin-antiplasmin complex; FPA, fibrinopeptide A; AMI, acute myocardial infarction.

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when activated to their corresponding enzymes, the fibrinogen/fibrin degradation products, released by the action of plasmin, and the enzyme-inhibitor complexes, which have a longer half-life in contrast to their precursor molecules, emerge as sensitive prethrombotic markers [6].

Previous studies analyzing the integrity of the hemostatic system in prethrombotic states do not offer a complete perspective since, in general, only individual components have been assessed. The aim of our study was to include a complete set of prethrombotic markers in a series of clinical conditions characterized by a high thrombotic tendency.

1. Patients and Methods

1.1. Patients

The study included 162 patients divided into the following categories: advanced age (n=30), pregnancy (n=30), diabetes (n=30), acute myocardial infarction (n=30), and hematologic malignancies (n=42). A control group consisted of 30 healthy subjects.

Elderly people, with an age over 65 years, who presented no other known risk factors were included in the advanced age group (mean age= 70.7 ±4.4 years, male/female=20:10). The pregnancy group included 30 women in the third trimester of uncomplicated gestation (mean age= 34.4±3.3 years). Diabetic patients were under metabolic control and presented no signs of retinopathy, neuropathy or nephropathy (mean age= 54.3±15.2 years, male/female: 12:18). Patients with myocardial infarction (AMI) were studied during the first week from the onset of diagnosis (mean age= 61.3±10.6 years, male/female=18:12); all patients were receiving aspirin (250 mg/d) and heparin (7500 IU) subcutaneously twice daily. A group of 42 patients with hematologic malignancies were studied during the active phase of the disease, before chemotherapy (mean age=47.3±17.6 years; male/female=25:17). Malignancies included: Hodgkin's disease (n=11), non Hodgkin's lymphoma (n=8), myeloma (n=11), and acute leukemia (n=12). Subjects included in the control group (mean age=45.3±9.15 years; male/female=19:11) did not present any known thrombotic risk factor and had not taken neither anti-inflammatory nor analgesic drugs during the week before the inclusion in the study.

1.2. Blood Collection

Blood samples were collected in the morning, with patients at rest, in siliconized vacutainer tubes containing 1:10 of 0.13 M trisodium citrate. Samples were kept on ice until centrifugation at 3000g for 15 minutes, performed within the first hour after collection. Aliquots of platelet-poor plasma were stored at −70°C.

1.3. Assays

Prothrombin fragment 1+2 (F1+2), was assayed using the commercial Enzygnost$^{(R)}$ F1+2 Kit (Behringwerke AG, Germany) [7]. Thrombin-antithrombin III complexes (TAT) were measured using the ELISA Kit Enzygnost$^{(R)}$ TAT (Behringwerke AG, Germany) [8]. Fibrinopeptide A (FPA) was measured with the ELISA FPA Kit (Hemodiagnostica-Stago, Asnieres, France), which is a competitive enzyme immunoassay [9]. Plasmin-α_2-antiplasmin complexes (PAP) were measured with the commercial ELISA Kit EIA APP micro (Berhingwerke AG, Marburg, Germany) [10]. D-Dimer levels were measured with the FibrinostiKa FbDP Kit (Organon Teknika, Turnhout, Belgium), which incorporates a specific monoclonal antibody anti–D-Dimer [11].

1.4. Statistical Analysis

Data was statistically described using mean values, standard deviation (SD) and standard error of the mean (SEM). Values were tested for the type of distribution using Kolmogorov-Smirnov test. As the parameters followed a not normal distribution the median was also assessed. Statistical analysis was done with the two-tail Mann-Whitney U test for comparison of median of impaired data. Correlations were calculated using Spearman's rank test. A value of $p<0.05$ was considered to be significant.

2. Results

The plasma levels of selected coagulation and fibrinolysis markers were measured in 162 patients,

with a variety of clinical conditions known to be associated with an increased thrombotic tendency. Individual distribution of data, according to different patients categories, is shown in Figures 1 and 2, whereas the mean values are reported in Table 1.

2.1. Elderly People

The study included 30 healthy subjects over the age of 65. As shown in Table 1, the levels of F1+2 ($p<0.0001$), TAT ($p<0.05$), and FPA ($p<0.01$) were elevated in relation to values observed in the control group (Figure 1 and Table 1). A correlation of FPA with F1+2 (r=0.42; $p<0.05$) and TAT (r=0.55; $p<0.01$) was found.

A marked increase of PAP complexes ($p<0.0001$) and D-Dimer generation ($p<0.01$) was also observed (Figure 2). Besides, D-Dimer levels correlated with F1+2 (r=0.49; $p<0.01$), TAT (r=0.46; $p<0.05$), and FPA (r=0.45; $p<0.05$).

2.2. Pregnancy

Thirty healthy pregnant women in the third gestational trimester were included in the study. As shown in Figure 1 and Table 1, we found a significant increase of F1+2, TAT, and FPA in relation to the levels obtained in the control group ($p<0.0001$). A correlation between F1+2 and TAT (r=0.51; $p<0.01$) was observed. TAT complexes also correlated with D-Dimer levels (r=0.61; $p<0.001$).

Among the fibrinolytic parameters, D-Dimer levels were significantly increased ($p<0.0001$) and PAP complexes ($p<0.05$) were lower than in the control group (Figure 2). A significant correlation between PAP and D-Dimer (r=0.41; $p<0.05$) was observed.

2.3. Diabetes Mellitus

Thirty patients with diabetes mellitus under strict metabolic control were included. Whereas there was a trend for a TAT increase ($p<0.05$), F1+2 and FPA levels showed no significant differences in relation to control (Table 1). We also found a significant increase of PAP complexes ($p<0.01$), while the concentration of D-Dimer was within the normal range (Figure 2).

2.4. Acute Myocardial Infarction (AMI)

Plasma samples from 30 patients who had suffered a myocardial infarction were drawn within a week after the acute episode. The levels of all clotting markers were significantly elevated as compared to the control group (Table 1 and Figure 1). TAT correlated significantly with F1+2 (r=0.81; $p<0.0001$) and with FPA levels (r=0.48; $p<0.05$). F1+2 also correlated with FPA (r=0.39; $p<0.05$).

Among the fibrinolytic parameters, PAP complexes and D-Dimer were significantly increased with respect to controls ($p<0.0001$) (Table 1 and Figure 2). A positive correlation between PAP and D-Dimer (r=0.73; $p<0.001$) was observed. Finally, D-Dimer correlated significantly with both F1+2 (r=0.66; $p<0.01$) and TAT (r=0.61; $p<0.01$), and PAP levels also correlated with F1+2 (r=0.63; $p<0.01$) and TAT (r=0.49; $p<0.05$).

2.5. Hematologic Malignancies

Forty-two patients with different hematologic malignancies were included. As shown in Figure 1, the levels of all clotting markers, mainly TAT, increased significantly in relation to controls ($p<0.0001$) and a correlation between TAT and F1+2 (r=0.60; $p<0.01$) was observed.

The levels of PAP complexes and D-Dimer were also very significantly increased in relation to the values observed in the control group ($p<0.0001$). A positive correlation between PAP and D-Dimer (r=0.72; $p<0.0001$) was observed. D-Dimer levels also correlated with TAT (r=0.64; $p<0.0001$) and F1+2 (r=0.55; $p<0.01$), while PAP correlated only with TAT levels (r=0.55; $p<0.01$).

The hemostatic activation was significantly higher in patients with acute leukemia as compared to lymphoproliferative disorders: F1+2 (3.05±0.71 vs. 1.50±0.11), TAT (19.37±5.49 vs. 6.35±1.15), FPA (3.42±0.66 vs. 1.85±0.26), PAP (1924.17± 478.48 vs. 736.00±65.46), and D-Dimer (6439.00± 2547.05 vs. 1381.00±200.50), though significance ($p<0.05$) was only reached for TAT, FPA, and PAP levels due to the wide data dispersion.

3. Discussion

The assessment of prethrombotic markers may be useful to elucidate the role of clotting activation

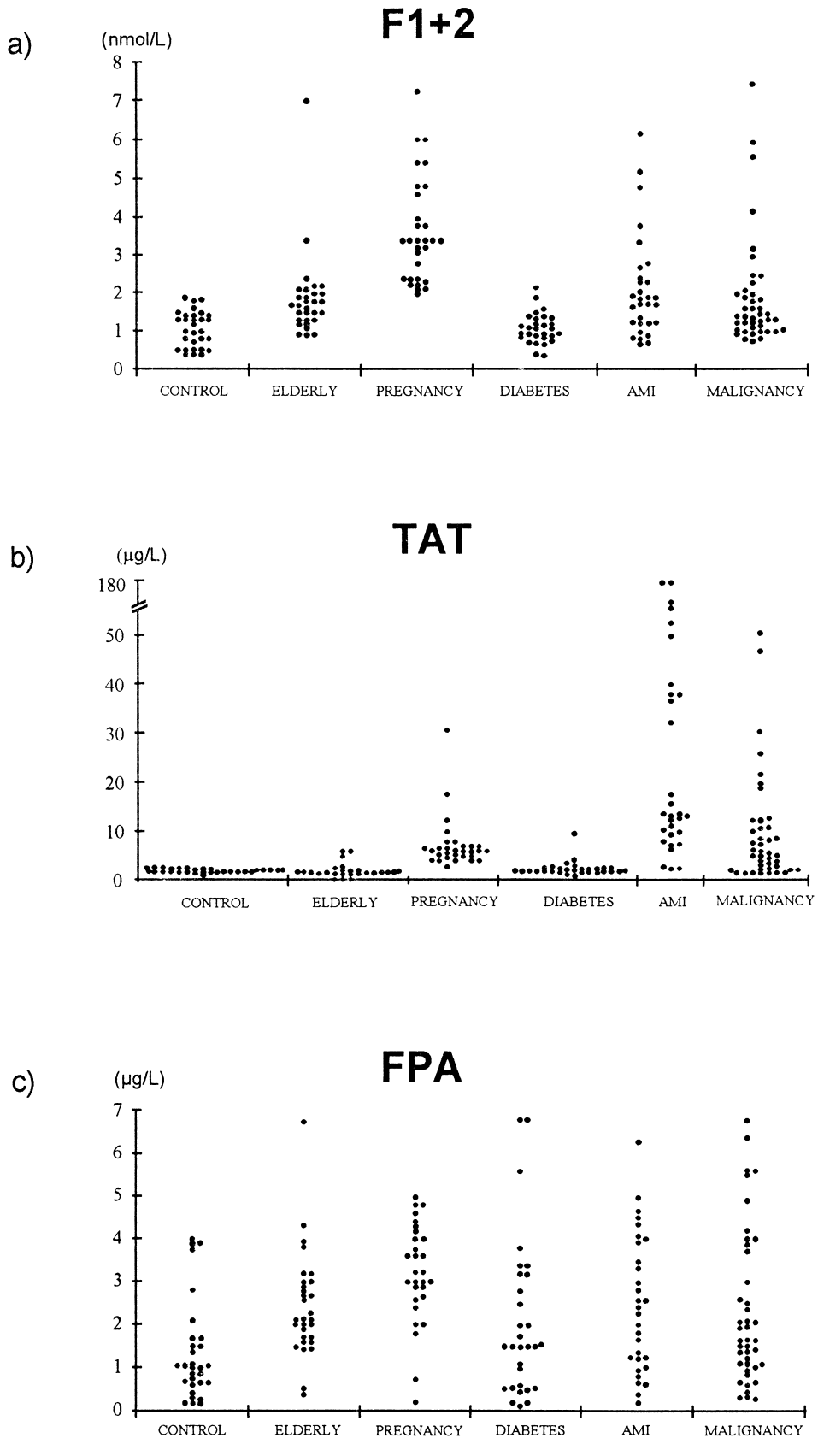
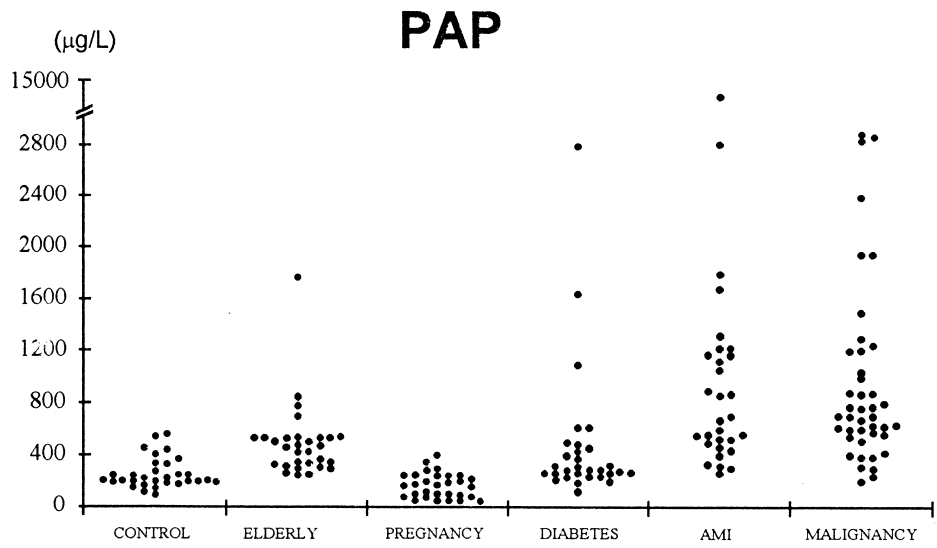


Fig. 1. Individual distribution of F1+2 (A), TAT (B), and FPA (C) in patients and controls.

and fibrinolysis impairment before the development of clinical thrombosis. However, previous studies on hemostasis alterations in hypercoagulable states offer a partial view on the pathophysiological role of coagulation and fibrinolysis mechanisms [reviewed in references 1–6]. In this report, we have included a wide set of prethrombotic markers to have a more complete information on the hemostatic impairment in different clinical conditions characterized by an increased tendency to thrombosis.

In view of the known association between vascular complications and advanced age [12,13], we have analyzed several hemostatic factors in a group of apparently healthy elderly subjects. We found an activation of the coagulation system as indicated



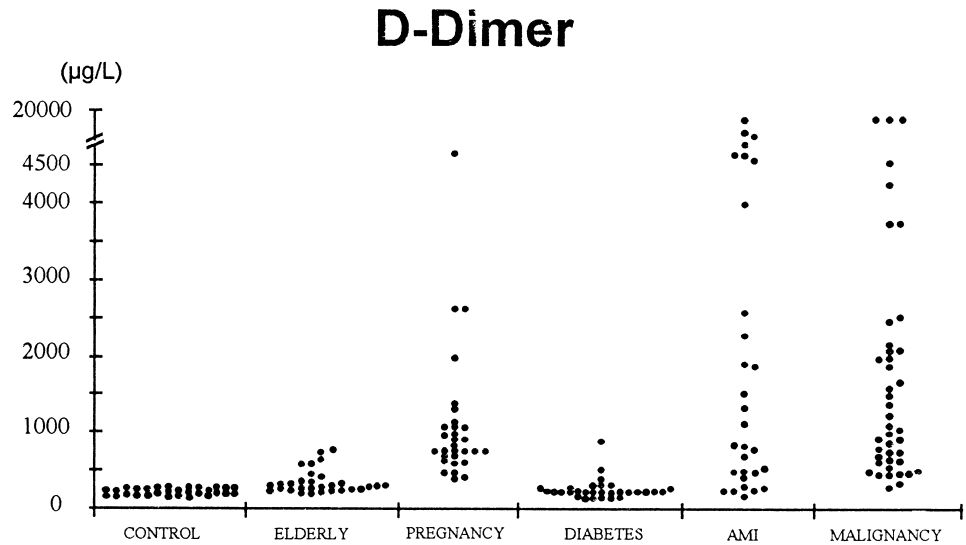


Fig. 2. Individual distribution of PAP (top) and D-Dimer (bottom) in patients and controls.

Table 1. Markers of hypercoagulability in the different groups of patients and controls.

	Control	Elderly	Pregnancy	Diabetes	AMI	Hematologic Malignancy
F1+2 (nmol/L)	1.06±0.09 (1.10)	1.86±0.20[a] (1.70)	3.62±.025[a] (3.40)	1.09±0.07 (1.09)	2.12±0.25[b] (1.80)	1.92±0.22[a] (1.40)
TAT (μg/L)	1.66±0.05 (1.60)	1.78±0.28[c] (1.60)	7.28±0.94[a] (6.00)	2.25±0.29[c] (1.70)	38.33±9.21[a] (13.75)	9.93±1.87[a] (5.40)
FPA (μg/L)	1.37±0.21 (1.02)	2.44±0.22[b] (2.10)	3.22±0.21[a] (3.30)	2.07±0.33 (1.50)	2.47±0.29[b] (2.32)	2.34±0.27[b] (1.65)
PAP (μg/L)	260.67±22.40 (205.00)	496.00±51.82[a] (465.00)	167.83±17.89[c] (162.50)	478.33±96.95[b] (290.00)	1,228.63±395.21[a] (670.00)	1,075.48±163.53[a] (700.00)
D-Dimer (μg/L)	240.67±7.78 (260.00)	355.17±29.91[b] (300.00)	1,237.33±261.42[a] (820.00)	267.10±25.80 (230.00)	3,443.83±892.31[a] (1,000.00)	2,645.71±688.44[a] (1,080.00)

Mean values±SEM (median).
[c] $p<0.05$
[b] $p<0.01$
[a] $p<0.0001$

by the increase of F1+2, TAT, and FPA. Bauer et al. demonstrated that the increased values of F1+2 in elderly patients were due to excessive production and not to a diminished clearance of the fragment [14]. We also found that FPA levels correlated positively with F1+2 and TAT, suggesting that thrombin formation is accompanied by an increase in fibrin generation [15]. The correlations between D-Dimer levels and clotting markers suggest that fibrinolysis could compensate for the hypercoagulable state in the elderly group.

We found a significant increase of F1+2, TAT and FPA in pregnancy, indicating a marked activation of the coagulation system during the third trimester of gestation [16]. Some studies have demonstrated that the increase of TAT levels may be a marker for detecting pregnancies complicated with hypertension or pre-eclampsia [17,18]. The observed correlations of TAT levels with F1+2 and D-Dimer in our study suggest that increased thrombin formation associated with fibrin generation is also present in uncomplicated pregnancies even though PAP complexes decreased. An increase in plasminogen activator inhibitors during pregnancy [19–21] could account for a reduction in PAP complex formation.

Diabetes mellitus is often associated with microangiopathy, atherosclerosis, and an impairment of the hemostatic system [22]. We found a slight increase of TAT complexes with normal levels of F1+2 and FPA in the diabetic group, indicating a moderate coagulation activation. Previous studies have suggested that the clotting impairment in diabetes depends on the presence of vascular complications and on the degree of metabolic regulation, mainly the glycemic control [23–25]. Our study supports these observations, since our patients were under strict metabolic control and had no vascular complications. On the other hand, despite the significant elevation of PAP levels, no D-Dimer increase occurred, and no correlation between both parameters could be demonstrated, suggesting that no significant amounts of fibrin have been generated.

The hemostatic mechanism is known to be activated in the acute phase of myocardial infarction [26]. In our study, a significant increase of all clotting activation markers and a positive correlation among them was present in patients who had suffered an AMI within 7 days after the onset of symp-

toms, even though they were under antithrombotic therapy [27,28]. Our results also showed that, despite the well-known PAI-1 enhancement [29], a marked activation of the fibrinolytic system takes place as a response to the thrombotic occlusion of coronary arteries in AMI [30].

Hypercoagulability coexisted with hyperfibrinolysis in recently diagnosed hematologic malignancy patients [31,32]. A marked clotting activation was demonstrated in the series analyzed, being TAT levels those more significantly elevated. Interestingly, TAT, FPA, and PAP levels were significantly higher in patients with acute leukemia in relation to those observed in patients with lymphoproliferative syndromes, indicating a stronger hemostatic activation in the former group [33,34]. As regards the fibrinolytic parameters, PAP and D-Dimer were strongly elevated in more than 80% of malignant patients, indicating an important degree of fibrinolysis activation [35]. The observed correlations with the coagulation markers suggest a fibrinolytic response to clotting activation in this particular group of patients [36].

Our results emphasize the usefulness of an association of selected fibrinolytic and coagulation measurements for the detection of a hypercoagulable state in different groups of subjects in which dissimilar triggering mechanisms result in a risk of thrombotic events. Testing of the plasma levels of TAT, F1+2, PAP and D-Dimer in future studies, in larger patients subgroups, seems to be warranted to examine their value for risk stratification in patients with hypercoagulable states.

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