Increased Fibrin Turnover and High PAI-1 Activity as Predictors of Ischemic Events in Atherosclerotic Patients

A Case-Control Study


A case-control comparison within the framework of the prospective, multidisciplinary PLAT Study was performed to assess whether altered baseline fibrinolytic variables were associated with an elevated risk of ischemic thrombotic events in patients with documented coronary, cerebral, and/or peripheral atherosclerotic disease. Fibrinogen, D-dimer, tissue plasminogen activator (t-PA) antigen, and fibrinolytic activity before and after venous stasis (Δ=difference between the two values), t-PA inhibitor, and lipid levels in 60 atherosclerotic patients with a thrombotic event during the first year of follow-up were compared with those in 94 atherosclerotic patients without such events, who were matched for age, sex, and diagnosis at enrollment. Events were associated with a higher release of Δ t-PA antigen (P=.047), higher D-dimer (P=.024), and higher t-PA inhibitor (P=.001) levels. Δ Fibrinolytic activity was correlated inversely with t-PA inhibitor (P<.01) and triglycerides (P<.05). D-Dimer was also correlated with systolic blood pressure (P<.01). Atherosclerotic patients at higher risk of thrombotic ischemic events are characterized by increased fibrin turnover and impaired fibrinolytic activity due to high t-PA inhibitor levels. This hemostatic disequilibrium may participate with conventional risk factors such as elevated triglyceride levels and systolic blood pressure in the multifactorial mechanism of ischemic sequelae in patients with preexisting vascular atherothrombotic disease. (Arterioscler Thromb. 1993;13:1412-1417.)

KEY WORDS  • atherothrombosis • ischemic events • fibrinolytic variables • plasminogen activator inhibitor • D-dimer

Recent clinical studies have reported decreases in fibrinolytic activity in patients with coronary artery disease.1-5 The major mechanisms involved in the type of fibrinolytic impairment seen in these patients are an increase in plasma tissue-type plasminogen activator (t-PA) inhibitor, particularly in subjects with hypertriglyceridemia, and a selective depression of t-PA activity in euglobulins. The t-PA inhibitor (PAI-1) has also been shown to be an independent risk factor for reinfarction in young survivors of myocardial infarction (MI).6

The prospective, multidisciplinary PLAT Study, which was performed to investigate the associations between hemostatic variables and ischemic events in 953 patients with documented atherosclerotic disease,7-9 afforded the opportunity of carrying out a nested case-control study, with the aim of assessing whether altered baseline fibrinolytic variables characterize subjects at a higher risk of ischemic sequelae, and we now report the results. Fibrinogen, fibrinolytic activity, and t-PA antigen before and after venous stasis and D-dimer and PAI-1 plasma levels of 60 atherosclerotic disease patients who subsequently developed an arterial ischemic event were compared with those of 94 control patients, matched for age, sex, diagnosis at enrollment, and recruitment center, who remained free of vascular events during the first 12 months of follow-up.

Methods

Study Design

From March 1986 to December 1987, 953 patients with documented coronary, cerebral, or peripheral atherosclerotic disease were enrolled in the PLAT Study; its design, organization, and preliminary and principal results have been described elsewhere.7-9 Briefly, 4 diagnostic subgroups of patients were enrolled at the following times: (1) 335 patients who survived an acute MI (AMI), 3 months after the acute index event; (2) 123 patients with stable angina, within 7 days of the coronary angiography that defined the pathology; (3) 160 patients with documented atherothrombotic transient ischemic attack (TIA), 3 to 12 months after their last ischemic event; and (4) 335 patients with documented atherothrombotic transient ischemic attack (TIA), 3 to 12 months after their last ischemic event; and (4) 335 patients with peripheral vascular disease (PVD) at Fontaine stages IIA (intermittent claudication, >1 block), IIB (intermittent claudication, <1 block), or III (rest pain), at least 3 months after any surgical procedures (endarterectomy or by-
Evaluation of Risk Factors

The risk factors that were evaluated in this study included body mass index (BMI, calculated by dividing the weight in kilograms by the square of the height in meters); plasma concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides; a family history of ischemic vascular disease (defined as positive if a first-degree relative had a coronary, cerebral, or peripheral atherothrombotic event); and smoking status (defined at the time of the ischemic event in the MI group and at study entry in the other patients; patients who had not smoked for the preceding 3 months were not considered "current smokers").

Hypertension, diabetes mellitus, and hyperlipidemia were defined as a history of the disorder (>160 mm Hg systolic and/or ≥95 mm Hg diastolic blood pressure in the case of hypertension) and/or current use of antihypertensives; current use of oral hypoglycemic agents or insulin; and current use of lipid-lowering drugs, respectively. We excluded blood pressure values and plasma glucose and lipid levels from statistical comparison because no restrictions were placed on pharmacological and/or dietary interventions in our PLAT Study, and these values could have been altered by treatment.

Evaluation of Vascular Status

Clinical cardiologic status was evaluated on the basis of history, ECG evidence of previous AMI or angina, and/or a positive exercise ECG. The exercise ECG was defined as positive either when the ST-segment depression was >2 mm or the upsloping ST segment was >1 mm; nondiagnostic results (ie, those that were close to the cutoff point between abnormal and normal, or exercise ST segments that were normal but for which the target level of cardiac stress was not attained) were considered negative.

Cerebrovascular status was determined by Doppler examination, ie, scanning of right and left periorbital, common, internal and external carotid, subclavian, retromastoid, and vertebral arteries. Doppler examination of the supra-aortic trunk was defined as abnormal when stenosis or occlusion was detected in at least one of the aforementioned vessels.

Peripheral vascular status was defined by the ankle/arm systolic blood pressure index.

Blood Sampling and Venous Stasis

For hemostatic tests, blood was collected without venous stasis through a 20-gauge needle from the antecubital vein directly into polyethylene tubes containing 3.8% sodium citrate (9 volumes of blood to 1 volume of trisodium citrate solution) in the morning from fasting patients who had not smoked during the last 10 hours and after a 30-minutes rest in the supine position. Venous stasis was then produced at the contralateral arm by applying a sphygmomanometer cuff to the upper arm for 10 minutes at a pressure between the patient's systolic and diastolic arterial pressures, and blood was obtained before deflating the cuff. Blood samples were immediately centrifuged at 1250g for 15 minutes at room temperature; then the plasma samples were divided into aliquots, snap-frozen, and stored at −80°C until assayed. At the time of assay the frozen samples were transferred to a water bath at 37°C and then handled at room temperature. For lipid assays blood samples were collected into disposable tubes, and after coagulation the serum was separated and stored at −80°C.

Blood Analyses

Plasma fibrinolytic activity was expressed as the lysed area (in square millimeters) produced by the eguglobulin fraction on a fibrin film. Euglobulins were prepared by acidification at pH 5.9 of diluted plasma (1:1, vol/vol with distilled water) with 0.25% glacial acetic acid at 4°C. The resulting precipitate was resuspended in an EDTA-gelatin-barbital buffer (pH 7.8); 30-μl aliquots were placed on the surface of two different fibrin plates, and the diameter of the lysed area was measured after incubation at 37°C for 18 hours.

Plasma levels of t-PA antigen were determined by an enzyme-linked immnosorbent assay (Imubind-5, American Diagnostica, Greenwich, Conn) following the manufacturer's recommendations. This assay detects both free t-PA and t-PA complexed with inhibitors and includes the use of quenching and irrelevant antibodies to exclude false-positive results. Values are expressed as nanograms per milliliter.

Plasma fibrinolytic activity and t-PA antigen levels were measured after and before venous stasis, and the difference between values before and after venous stasis was expressed as Δ (values before venous stasis are referred to as "basal").
Functional PAI-1 activity was assayed by a two-stage indirect enzymatic test (Coaset PAI, Ortho Diagnostic Systems, Milan, Italy). Briefly, t-PA was added in excess (40 and 30 IU/mL) to undiluted and diluted (1:2, vol/vol) plasma, and after 10 minutes of incubation at room temperature the residual t-PA activity was measured with the chromogenic substrate S-2251 in the presence of plasminogen and cyanogen bromide fibrinogen fragments. The results are expressed in arbitrary units per milliliter.

Fibrinogen levels were determined in citrated plasma by the Clauss thrombin clotting method and a Fibrinogen Reagent (Boehringer Mannheim) and expressed in milligrams per deciliter. Day-to-day quality control was done using Preciclot I and II (Boehringer Mannheim).

D-Dimer, the terminal degradation fragment of cross-linked fibrin by plasmin, was measured by a commercial enzyme immunoassay sandwich test (Dimertest EIA kit, Ortho Diagnostic Systems). The results are expressed in nanograms per milliliter.

All values are the means of duplicate measurements. Reagents from a single batch were used to avoid batch-to-batch variability. A pool of plasma obtained from 150 healthy blood donors, snap-frozen and stored in liquid nitrogen, was used as an internal standard.

Statistical Analysis

For statistical analysis, cases were grouped independent of the arterial location of the ischemic event. Clinical characteristics of the cases and controls were compared by the Mann-Whitney test.

The four diagnoses at the time of recruitment (AMI, angina pectoris, TIA, and PVD) were used to subdivide the cases and controls. A 4x2 factorial structure was therefore obtained, allowing us to test not only the main effects (diagnosis and events) but also their interaction.

A series of least-squares analyses of variance was carried out on the ranks of the seven fibrinolytic variables, a procedure that is useful when original values are not distributed normally. No further comparisons were performed when the preliminary analysis of variance did not show any significant differences among the means of the eight subgroups. When the preliminary analysis indicated the presence of significant differences and no significant interaction was detected, case-control comparisons were done on the total sample. In the presence of a significant interaction, a series of comparisons between cases and controls was performed in each diagnostic group by using Bonferroni’s procedure.

All computations were carried out using the Rank and Glm procedures of SAS version 6.04 for the personal computer.

Results

The 60 events observed during the first year of follow-up are reported in Table 1 according to the 4 diagnostic subgroups. They were distributed as follows: 1 fatal and 7 nonfatal AMIs, 2 sudden cardiac deaths, 1 fatal and 1 nonfatal stroke, and 3 TIAs in AMI survivors; 7 nonfatal AMIs, 2 sudden cardiac deaths, 1 nonfatal stroke, and 1 TIA in the angina pectoris subgroup; 3 sudden cardiac deaths, 1 fatal stroke, 12 TIAs, and 1 acute peripheral ischemia in patients enrolled in the TIA group; and 1 fatal and 3 nonfatal AMIs, 1 sudden cardiac death, 1 fatal and 2 nonfatal strokes, 3 peripheral bypass occlusions, and 6 acute peripheral ischemic events in the PVD subgroup.

Clinical and Risk Factors at Entry

The demographic and clinical features of the case and control groups are reported in Table 2. Because case and control patients were matched for age and sex, the distribution of these variables was identical. The proportion of patients with a family history of atherosclerosis was higher in the case group, in which a greater mean BMI was also recorded. The other generally recognized risk factors for ischemic disease such as smoking; arterial hypertension; diabetes; hyperlipidemia; and plasma concentrations of total cholesterol, HDL cholesterol, and triglycerides showed nonsignificant differences between the two groups. Similarly, no differences were found between case and control patients in terms of coronary, cerebral, and peripheral vascular involvement.

Fibrinolytic Function

Compared with controls, cases had significantly higher plasma concentrations of D-dimer and Δ t-PA antigen (Table 3). The mean values of the whole series were increased in comparison with the reference values (45.7±0.73 and 5.1±0.8 ng/mL, respectively).

Basal fibrinolytic activity, which was reduced in comparison with the reference value (171±18.8 mm²), and Δ fibrinolytic activity (fibrinolytic capacity) were similar in the two groups.

The differences in PAI-1 activity between cases and controls varied in the 4 diagnostic subgroups; PAI-1 values are therefore reported separately in Table 4. Although the values were greater in cases for all diagnostic subgroups, a significant difference was found only in PVD.

Considering cases and controls together, fibrinolytic capacity was correlated positively with Δ t-PA antigen (r=0.73, P<.01) and inversely with triglycerides (r=−0.20, P<.05). PAI-1 was correlated positively with basal t-PA antigen (r=0.21, P<.05) and inversely with fibrinolytic capacity (r=−0.35, P<.01); this last correlation was stronger (z=1.89, P<.05) in cases (r=−0.56, P<.02) than controls (r=−0.26, P<.05). D-Dimer was correlated with systolic blood pressure (r=0.24, P<.01).

Discussion

Our study was undertaken to investigate whether, in a group of patients with vascular ischemic disease in different beds (coronary, cerebral, and/or peripheral), subgroups with vascular event recurrences in the follow-
ing year could be characterized on the basis of fibrinolytic indexes. High plasma values of PAI-1 activity, when measured in a clinically and metabolically stable stage of the disease, were significantly associated with vascular events, providing further support for the hypothesis that PAI-1 activity in plasma might contribute to the development of acute ischemic vascular disease. A supposedly causal relation between PAI-1 elevation and the risk of recurrent MI has already been demonstrated in longitudinal studies of young and elderly MI survivors. Moreover, plasma PAI-1 levels have been found to be significantly higher in patients with unstable angina at rest compared with a stable-angina group and in patients with chronic angina pectoris with angiographic evidence of coronary sclerosis compared with those without. The association between high PAI-1 levels and ischemic events was particularly strong in PVD patients, who also had higher mean PAI-1 values in comparison with other atherosclerotic disease patients. This finding could be explained by the defective fibrinolytic capacity in peripheral atherosclerosis that was recently described by ourselves and the stronger inverse relation between PAI-1 and fibrinolytic capacity observed in cases in comparison with controls.

Basal t-PA antigen levels were increased in our atherosclerotic disease patients, a finding that concurs with other previous reports of patients with angina pectoris and AMI survivors, and t-PA antigen has recently been proposed as a marker for preclinical atherosclerosis and has been reported to be predictive of AMI in apparently healthy individuals. In our patients, however, basal t-PA antigen, although posi-

### Table 2. Clinical and Conventional Risk Factors in Case and Control Groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n=60)</th>
<th>Controls (n=94)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, % of males</td>
<td>93.3</td>
<td>91.5</td>
<td>MV</td>
</tr>
<tr>
<td>Age, y (mean±SD)</td>
<td>57.8±8.5</td>
<td>57.7±8.2</td>
<td>MV</td>
</tr>
<tr>
<td>Family history, % positive</td>
<td>78.3</td>
<td>55.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smoking, % current smokers</td>
<td>66.7</td>
<td>62.8</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, % present</td>
<td>38.3</td>
<td>29.8</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidemia, % present</td>
<td>29.3</td>
<td>20.0</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes, % present</td>
<td>15.0</td>
<td>6.4</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, mean±SD</td>
<td>26.0±3.0</td>
<td>24.3±2.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L (mean±SD)</td>
<td>5.71±0.98</td>
<td>5.86±1.18</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L (mean±SD)</td>
<td>0.96±0.26</td>
<td>1.02±0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L (mean±SD)</td>
<td>1.66±0.79</td>
<td>1.67±0.86</td>
<td>NS</td>
</tr>
<tr>
<td>Previous AMI, % positive</td>
<td>25.0</td>
<td>17.0</td>
<td>NS</td>
</tr>
<tr>
<td>Exercise ECG, % positive</td>
<td>32.4</td>
<td>32.2</td>
<td>NS</td>
</tr>
<tr>
<td>Supra-aortic trunk Doppler examination, % abnormal</td>
<td>28.3</td>
<td>25.5</td>
<td>NS</td>
</tr>
<tr>
<td>Ankle/arm pressure index, mean±SD</td>
<td>94.1±19.0</td>
<td>97±49.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; HDL, high-density lipoprotein; AMI, acute myocardial infarction; ECG, electrocardiogram; NS, not significant (>.05); MV, matching variable.

### Table 3. Fibrinolytic Variables Expressed as Mean±SE for the Number of Observations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases</th>
<th>Controls</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>306.2±8.5</td>
<td>295.3±6.7</td>
<td>300.7±5.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=60</td>
<td>n=94</td>
<td>n=154</td>
<td>NS</td>
</tr>
<tr>
<td>D-Dimer, ng/dL</td>
<td>121.7±11.9</td>
<td>99.1±9.8</td>
<td>[110.4±7.3]</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>n=58</td>
<td>n=91</td>
<td>n=149</td>
<td></td>
</tr>
<tr>
<td>t-PA antigen, ng/mL</td>
<td>12.1±0.8</td>
<td>12.5±0.6</td>
<td>12.3±0.5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>n=47</td>
<td>n=84</td>
<td>n=131</td>
<td></td>
</tr>
<tr>
<td>∆ t-PA antigen, ng/mL</td>
<td>17.4±2.3</td>
<td>10.6±1.8</td>
<td>[14.0±1.4]</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>n=47</td>
<td>n=84</td>
<td>n=131</td>
<td></td>
</tr>
<tr>
<td>Fibrinolytic activity, mm²</td>
<td>68.8±9.0</td>
<td>66.1±7.0</td>
<td>67.4±5.4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>n=46</td>
<td>n=82</td>
<td>n=128</td>
<td></td>
</tr>
<tr>
<td>∆ Fibrinolytic activity, mm²</td>
<td>227.0±25.0</td>
<td>219.2±19.6</td>
<td>223.1±15.0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>n=46</td>
<td>n=81</td>
<td>n=127</td>
<td></td>
</tr>
</tbody>
</table>

Values in square brackets are means obtained from the combination of noncomparable groups of patients. t-PA indicates tissue-type plasminogen activator; NS, not significant.
tively correlated with PAI-1, did not predict the occurrence of ischemic events, whereas increased t-PA antigen release after venous stasis was found to be associated with ischemic sequelae. This increased release of t-PA antigen may be a compensatory mechanism to overcome PAI-1 antifibrinolytic potential. The increase in t-PA antigen release, however, did not coincide with a corresponding increase in fibrinolytic capacity, as would have been expected from the positive correlation between these two variables, probably due to higher levels of PAI-1. Indeed, in our study there was a negative correlation (stronger in cases) between plasma PAI-1 levels and fibrinolytic capacity. In another follow-up study of postinfarction patients, the high-risk group for recurrences was characterized by higher t-PA antigen release and lower t-PA activity.

D-Dimer, a sensitive marker for detecting coagulation and fibrinolytic system activation in hypercoagulable states and thrombotic disorders, determined in a stable phase of ischemic disease, was increased in our atherosclerotic disease patients, particularly in those who later developed vascular events. The apparent discrepancy between increased levels of the specific degradation products of cross-linked fibrin and the finding of reduced basal fibrinolytic activity may be explained by the very high sensitivity of the D-dimer assay. This marked elevation of D-dimer may be compatible with enhanced fibrin degradation at local injury sites, where plasmin generation escapes PAI-1 inhibitory control, or it could reflect a more extensive thrombotic process, with other vascular beds participating in the fibrinolytic response in addition to the index-event arterial area. In the latter case, increased D-dimer levels would be due to numerous but minimal amounts of fibrin degradation products resulting from chronic, extensive, intravascular fibrin formation and degradation, as recently described in patients with peripheral atherosclerosis.

In view of its correlation with systolic blood pressure, D-dimer may be a useful index in the study of the multifactorial mechanism of atherothrombotic phenomena in patients with ischemic vascular disease.

The poor response of t-PA antigen to venous stasis in both cases and controls (it hardly doubled in comparison with the basal value) is in apparent contrast with the good response of fibrinolytic activity (which showed a more than fourfold enhancement). However, in the presence of an altered t-PA-to-PAI-1 balance, as in patients with increased PAI-1 activity, the response to venous stasis may vary when specific tests and global tests are run in parallel, and increased activity by other endogenous fibrinolytic systems can compensate for the net decrease in t-PA activity.

Serum triglycerides correlated inversely with Δ fibrinolytic activity. This finding, together with the positive strong correlation between triglycerides and PAI-1 reported in the literature, suggests that hypertriglyceridemia, a controversial risk factor for ischemic vascular disease, may be associated with a predisposition to thrombosis because of reduced fibrinolytic activity.

In conclusion, atherosclerotic disease patients at higher risk of thrombotic ischemic events are characterized by increased fibrin turnover and impaired fibrinolytic activity due to high PAI-1 levels. This is the first prospective study to indicate that high D-dimer levels are predictive of ischemic events in patients with different chronic atherosclerotic diseases and high PAI-1 activity in PVD patients. Other prospective studies are required to validate our findings.

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Appendix

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References


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