Abnormally High Circulation Levels of Tissue Plasminogen Activator and Plasminogen Activator Inhibitor–1 in Patients With a History of Ischemic Stroke

Maurizio Margaglione, Giovanni Di Minno, Elvira Grandone, Gennaro Vecchione, Egidio Celentano, Giuseppe Cappucci, Massimo Grilli, Pasquale Simone, Salvatore Panico, Mario Mancini

Abstract We evaluated 106 subjects with and 109 subjects without a history of ischemic stroke. All were attending a metabolic ward. The two groups were compared for major risk factors for ischemic events. A positive family history for ischemic complications of atherosclerosis was more common in subjects with a history of stroke than in those without; moreover, plasma levels of plasminogen activator inhibitor–1 (PAI-1) and tissue-type plasminogen activator (TPA) were higher in patients with documented previous events. A strong positive significant correlation was found between TPA and PAI-1 levels, and an interaction between age and TPA was observed when the sample was stratified according to ages being above or below 70 years. When the patient population was analyzed according to the number of ischemic events, it was found that 62 of the 106 subjects with a history of stroke had experienced more than one ischemic event. Under these conditions, the levels of TPA and PAI-1 still correlated with the occurrence of previous ischemic episodes. As in the whole patient sample, TPA was the strongest discriminator. We conclude that in subjects attending a metabolic ward, TPA and PAI-1 levels consistently help identify subjects with a history of cerebral ischemic episodes and that TPA is the strongest discriminator. (Arterioscler Thromb. 1994;14:1741-1745.)

Key Words • fibrinolysis • stroke • risk factors

S troke is a major thrombotic complication of atherosclerosis and a leading cause of morbidity and mortality in Western countries. Individuals who smoke, who have high plasma levels of cholesterol or glucose or high blood pressure, or who are obese are all at risk for this event.1-5 However, such established risk factors account for only about one third of the future ischemic episodes.6-8 The involvement of hemostasis in arterial thrombosis has been suspected for many years, but only recently has it been supported by clinical observations and intervention studies.9,10 Cross-sectional and prospective studies11-15 have documented that the impaired fibrinolysis of patients with angina pectoris or previous myocardial infarction is related to elevated plasma levels of the principal inhibitor of the fibrinolytic system, plasminogen activator inhibitor–1 (PAI-1). High levels of PAI-1 have also been reported in myocardial infarction,16 in the juvenile myocardial ischemia of patients with diabetes mellitus and/or hyperglycemia,17 in hyperinsulinemia,18 and in an-
factors was found between men and women. The 109 subjects without any documented episode of stroke were comparable with those with a history of stroke with respect to sex, height, obesity, smoking, social class, drug use, high blood pressure, diabetes mellitus (mostly of type II), plasma glucose (mean±SD, 113.3±54.7 mg/dL versus 114.0±50.3 mg/dL), average body weight (69.0±10.1 kg versus 72.0±14.5 kg), and plasma fibrinogen (376.6 mg/dL versus 377.4 mg/dL). The latter was measured by the Clauss clotting method.\textsuperscript{23} Mean concentrations of total, HDL, and LDL cholesterol; triglycerides; and lipoprotein(a) [LP(a)] in patients with a history of stroke were 171.2±39.5, 32.6±8.0, 110.7±33.1, 139.0±70.2, and 21.40±28.41 mg/dL, respectively; in those without such history, the values were 168.3±43.2, 32.4±8.8, 111.2±37.7, 127.7±56.2, and 15.22±17.12 mg/dL, respectively. None of these differences was significant. None of the 215 subjects had clinical evidence of cancer or acute or chronic inflammatory disease. All had been repeatedly instructed to stop smoking and drinking alcohol and to control food intake, and all were highly motivated to follow the advice. All had been on an isocaloric Mediterranean diet for at least 6 months. Medications used by two or more of them included calcium channel blockers, ACE inhibitors, digoxin, aspirin, ticlopidine, n-3 fatty acids, pentoxifylline, defibrotide, \( \beta \)-blockers (by 2 among the subjects with a history of stroke), and oral antidiabetic drugs (by 2 among the subjects with and 12 among those without a history of stroke). A complete clinical summary with emphasis on personal and family history for angina pectoris, myocardial infarction, ischemic stroke, peripheral arterial disease, and vascular risk factors was obtained from all subjects. Positive family history for ischemic complications of atherosclerosis was defined as the occurrence of stroke or myocardial infarction before the age of 55 in male and 60 in female parents and siblings.\textsuperscript{24} After approval by the local ethics committee, the studies were carried out according to the Principles of the Declaration of Helsinki; informed consent was obtained from all subjects.

Materials

From each subject after 12 to 15 hours of overnight fasting, 18 mL blood was collected between 9 and 9:30 AM without venous stasis, from the antecubital vein, via a 19-gauge scalp vein needle, into a sterile tube containing 2 mL sterile 3.8% trisodium citrate and was immediately processed. Concentrations of total cholesterol, HDL cholesterol, triglycerides, and plasma glucose were detected enzymatically\textsuperscript{25} by using commercially available reagents (Roche). Plasma fibrinogen was assayed with reagent from Boehringer Mannheim. LP(a) and anticardiolipin antibodies (ACA) of the IgG class were assayed by ELISA methods with kits from Biopool-Menarini. Immulys for PAI-1 and TPA antigens were from Biopool-Menarini as well. On the basis of our previous data, ACA IgG positivity was defined as the presence of \( >24 \) units of anti-phospholipid IgG antibodies per milliliter of the sample (GPL per milliliter). One unit is the cardiolipin binding activity of 1 \( \mu \)g/mL of an affinity-purified IgG anti-cardiolipin preparation from a standard serum. On the other hand, in agreement with the manufacturer's recommendations, normal values of TPA are 3 to 10 ng/mL, and those of PAI-1 are 4 to 43 ng/mL. Pooled normal plasma from apparently healthy drug-free volunteers used for reference was prepared and stored under the same conditions as plasma from the subject samples. In patients both with and without a history of stroke, the intra-assay and interassay coefficients of variation of all the hemostatic variables analyzed never exceeded 10%.

Statistical Analysis

All analyses were performed according to the SPSS/PC V2.0 statistical package following the recommended procedures.\textsuperscript{26} The Kolmogorov-Smirnov test, a nonparametric method, and the median test were used to compare the distributions of the variables. Pearson's \( \chi^2 \) statistics were employed to evaluate the independent nature of the clinical condition with respect to categorical variables. For continuous variables, the values of quintiles were employed, and Spearman's correlation analysis helped evaluate the association between the variables and previous ischemic event(s). Odds ratios (ORs) were calculated to evaluate the interaction between variables, and the Mantel-Haenszel \( \chi^2 \) was used to evaluate confidence intervals. Appropriate models were also set up to evaluate in a logistic analysis the independent contribution of each variable to the ischemic event. In the models, an enter method was employed, and the log-likelihood and the \( \chi^2 \) models are presented. In each case and for each variable, estimated coefficients (B) and Wald statistics as well as the significance are given. For all the tests, significance was established at a value of \( P < .05 \).

Results

Although subjects with and without a history of stroke were comparable for gender and established risk factors for thrombotic complications of atherosclerosis, there was a difference in the mean age of the two groups (\( \text{mean±SD}, 66.5 \) years; range, 38 to 86 years in subjects with a history of stroke versus 61.3 years; range, 31 to 86 years in the other subjects) (Table 1). Among the whole population analyzed, the number of subjects with a positive family history was significantly higher in those with a history of stroke than in those without (41 versus 27). The number of subjects positive for ACA IgG was also different (Table 1), but the difference was not statistically significant (OR, 2.24; 95% confidence interval [CI], 0.92 to 5.49).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Stroke - n (%)</th>
<th>Stroke + n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>109</td>
<td>106</td>
</tr>
<tr>
<td>Men</td>
<td>57 (52.3)</td>
<td>55 (51.9)</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>66.5</td>
<td>61.3*</td>
</tr>
<tr>
<td>Age &gt;70 y</td>
<td>28 (26.4)</td>
<td>39 (36.8)</td>
</tr>
<tr>
<td>Previous ischemic episodes</td>
<td>-</td>
<td>61 (59.8)</td>
</tr>
<tr>
<td>Positive family history</td>
<td>27 (24.8)</td>
<td>41 (39.6)†</td>
</tr>
<tr>
<td>Cigarette smokers</td>
<td>40 (36.7)</td>
<td>34 (33.3)</td>
</tr>
<tr>
<td>Alcohol consumers</td>
<td>36 (33.0)</td>
<td>23 (22.5)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>59 (54.1)</td>
<td>56 (53.8)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>36 (33.0)</td>
<td>29 (27.4)</td>
</tr>
<tr>
<td>LDL cholesterol &gt;135 mg/dL</td>
<td>27 (24.8)</td>
<td>24 (22.6)</td>
</tr>
<tr>
<td>TPA &gt;10 ng/mL</td>
<td>21 (19.4)</td>
<td>53 (50.0)‡</td>
</tr>
<tr>
<td>PAI-1 &gt;43 ng/mL</td>
<td>15 (13.8)</td>
<td>31 (29.2)§</td>
</tr>
<tr>
<td>ACA IgG &gt;24 GPL</td>
<td>8 (7.3)</td>
<td>16 (15.1)</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; TPA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor type 1; ACA, anticardiolipin antibodies; and GPL, units for these antibodies (see text). \*P<.007; †P<.019; odds ratio (OR), 2.01; 95% confidence intervals (CI), 1.12-3.61; §P<.006; OR, 2.59; 95% CI, 1.30-5.15; and ‡P<.0001; OR, 4.14; 95% CI, 2.25-7.62.
the other group, \( P < .001 \). The number of subjects with plasma levels of TPA > 10 ng/mL and of PAI-1 > 43 ng/mL was different in the two settings as well (Table 1). No difference was found in the levels of the fibrinolytic variables in men compared with women in both subjects with and without a history of stroke.

Spearman’s coefficients revealed that plasma TPA values significantly correlated with PAI-1 levels (\( r = .405, P < .0001 \)). On the other hand, according to previous data,\(^{29}\) TPA levels were significantly correlated with age (\( r = .155, P < .024 \)). This information was further analyzed. In a logistic regression model in which several variables including age were considered, TPA appeared to be the strongest discriminator of subjects with and without a history of stroke (Table 2). On the other hand, TPA significantly interacted with age (when 70 years was used as a cutoff point) but not with PAI-1, familial risk, or ACA IgG in accounting for the clinical condition (Table 3).

The clinical summary revealed that 43 of the 106 patients had experienced one ischemic episode, while 62 of them survived more than one event. In view of this, we have analyzed the distribution of the variables in the patient population according to the number of ischemic episodes. Under these conditions, the familial risk no longer discriminated between subjects with and without a history of stroke (\( P = .052 \)). By contrast, plasma concentrations of TPA were still significantly different in subjects with or without a stroke history (9.75 ± 3.63 ng/mL in patients with a history of one event, 10.69 ± 4.76 ng/mL in those with more than one event, and 7.28 ± 3.98 ng/mL in subjects without a history of stroke, \( P < .0001 \)). Plasma levels of PAI-1 (\( > 43 \) ng/mL) was detected in 12 of the subjects (23.0% of those with one ischemic event, in 32 (51.6%) of those with more than one cerebral ischemic event, and in 15 (27.3%) of those with a history of one event, in 19 (30.6%) of those with more than one event, and in 15 (23.0%) of those without a history of stroke (\( P < .020 \)).

### Table 2. Factors Associated With Previous Cerebral Ischemic Events

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>Wald Statistic</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>.040</td>
<td>7.586</td>
<td>.0059</td>
</tr>
<tr>
<td>Positive family history</td>
<td>-.347</td>
<td>4.454</td>
<td>.0348</td>
</tr>
<tr>
<td>PAI-1, ng/mL</td>
<td>.022</td>
<td>4.967</td>
<td>.0258</td>
</tr>
<tr>
<td>TPA, ng/mL</td>
<td>.145</td>
<td>11.208</td>
<td>.0008</td>
</tr>
</tbody>
</table>

PAI-1 indicates plasminogen activator inhibitor type 1; TPA, tissue-type plasminogen activator.

Data are from the whole patient sample (n = 215), logistic regression analysis. \( \chi^2 = 247.64, P = .025 \); Model \( \chi^2 = 44.75, P = .0001 \).

Plasma levels of these variables correlated minimally with the time interval elapsed from the last stroke.

### Discussion

Stroke is a major thrombotic complication of atherosclerosis. However, with two recent exceptions,\(^{30,31}\) the involvement of the fibrinolytic system in this ischemic event is poorly understood. In the present report, we document that TPA and PAI-1 levels are abnormally high in subjects with a history of ischemic stroke just as in those with severe angina pectoris or coronary artery disease. This is true when the patient population is analyzed as a whole as well as when it is evaluated according to the number of ischemic episodes. The effect appears little related to the use of drugs. As few as 2 subjects in the group with a history of events among those without a history were using oral antidiabetics; a class of medication reported to enhance the fibrinolytic potential of the blood by slightly enhancing plasma levels of TPA antigen.\(^{22}\) Moreover, the findings did not change when the subjects who were using such drugs were excluded from the analysis (data not shown). Among subjects attending our metabolic ward, TPA was the strongest discriminator between subjects with and those without a history of cerebral ischemic events. The well-known age-dependent increase in TPA appeared to affect the strength of the association minimally. Moreover, interaction analysis

### Table 3. Interaction Between PAI-1, Age, Family History, ACA IgG, and TPA Levels in Patients With a History of Stroke

<table>
<thead>
<tr>
<th>Variable</th>
<th>TPA-&lt;10 ng/mL</th>
<th>TPA+&gt;10 ng/mL</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1</td>
<td>43/121</td>
<td>12/47</td>
<td>.38</td>
</tr>
<tr>
<td>PAI-1 &lt;43 ng/mL</td>
<td>35.5%</td>
<td>68.1%</td>
<td>.22-.64</td>
</tr>
<tr>
<td>PAI-1 &gt;43 ng/mL</td>
<td>10/19</td>
<td>21/27</td>
<td>.59</td>
</tr>
<tr>
<td>Age &lt;70 y</td>
<td>31/100</td>
<td>32/43</td>
<td>.27</td>
</tr>
<tr>
<td>Age &gt;70 y</td>
<td>21/39</td>
<td>20/30</td>
<td>.73</td>
</tr>
<tr>
<td>Family history -</td>
<td>31/96</td>
<td>31/47</td>
<td>.40</td>
</tr>
<tr>
<td>Family history +</td>
<td>21/43</td>
<td>20/25</td>
<td>.38</td>
</tr>
<tr>
<td>ACA IgG &lt;24 GPL</td>
<td>44/125</td>
<td>46/66</td>
<td>.39</td>
</tr>
<tr>
<td>ACA IgG &gt;24 GPL</td>
<td>35.2%</td>
<td>69.7%</td>
<td>.25-.60</td>
</tr>
</tbody>
</table>

PAI-1 indicates plasminogen activator inhibitor type 1; ACA, anticardiolipin antibodies; TPA, tissue-type plasminogen activator; and GPL, units for ACA (see text).
There is a negative correlation between PAI-1 values of the control subjects in this study were agreement with these figures. On the other hand, the lack of significance may merely be the reflection of too small a sample size. The lack of association between familial risk and previous ischemic events when the population was split according to the number of ischemic episodes makes this speculation conceivable.

It is of interest to relate our findings to some previous reports on fibrinolytic abnormalities in patients prone to arterial thrombosis. The majority of the investigators report PAI-1 levels around 20 ng/mL in normal subjects. The values of our apparently healthy volunteers are in agreement with these figures. On the other hand, the PAI-1 values of the control subjects in this study were 1.5 times higher. In their study on young survivors of myocardial infarction, Hamsten et al have shown that raised levels of PAI-1 were related to serum triglyceride levels. Our patients and without a history of stroke exhibit normal plasma levels of cholesterol and triglycerides and are entirely comparable with respect to the levels of these plasma lipids. In addition to lipids, other risk factors are known to be associated with raised PAI-1 levels. As pointed out in the description of the subjects, those without a history of stroke had been attending the metabolic ward because of the presence of one or more risk factors.

A strong, long-term relation between impaired fibrinolytic activity and incidence of ischemic heart disease has been suggested by several reports. TPA activates the conversion of plasminogen to plasmin, thus promoting fibrinolysis. However, despite this, the association of high levels of TPA and ischemic events is increasingly recognized. In three reports, increased levels of TPA were associated with coronary artery disease. In a recent 7-year follow-up study, TPA antigen has been shown to be a risk factor for long-term mortality in patients with angina pectoris and coronary artery stenosis. After completion of the present report, a large-scale prospective study has shown a predictive power of TPA antigen on stroke. To clarify the apparently paradoxical association of ischemic events with the plasma levels of a factor that promotes fibrinolysis, one should consider that TPA covalently binds to a series of inhibitors of fibrinolysis including PAI-1, α2-antiplasmin, and α2-macroglobulin. The enzymatically active fraction of TPA (TPA activity) is the portion of this enzyme that is not bound to PAI-1 or to any other inhibitor. Concentrations of TPA antigen above normal ranges have been reported in subjects with high plasma PAI-1 levels. There is a negative correlation between TPA antigen and TPA activity in plasma samples. Circulating levels of TPA inversely correlate with the ex vivo sensitivity of clots to lysis (M. Colucci, et al, unpublished data, 1994). Actually, an increase in TPA antigen is thought to reflect an inhibitory effect of PAI-1 on TPA activity. Thus, the combined data are consistent with the concept that the levels of TPA rise with the increase in PAI-1 inhibition, so that high levels of either factor reflect reduced fibrinolysis.

The mechanisms leading to high levels of TPA in those patients are still a matter of investigation. TPA and PAI are released from perturbed endothelial cells. Especially when combined, risk factors may trigger a vascular injury that in turn leads to inflammatory and proliferative events. TPA has been suggested as a marker of preclinical atherosclerosis in apparently healthy individuals. On the other hand, raised plasma levels of PAI-1 in the bloodstream have been associated with certain genetic variations at the PAI-1 locus. The latter possibility is now under intensive investigation in this laboratory. The extent to which raised levels of fibrinolytic indices reflect a vascular injury and/or the effect of a molecular variation is unclear and cannot be ruled out by the present data. However, despite these uncertainties, the abnormally high levels of fibrin associated with hypofibrinolytic states may greatly amplify an inflammatory and proliferative response.

These data show that in a group of subjects attending a metabolic ward, TPA plasma levels—whether alone or in combination with PAI-1 or certain vascular risk factors—identify subjects with a history of cerebral episodes. They also indicate that other factors (PAI-1, positive family history, and age) significantly affect this clinical condition. As of now, it is not clear whether, in those subjects, an index combining measurements of a risk factor and fibrinolytic variables would be a better marker of arterial risk than the fibrinolytic parameters or the risk factor evaluated independently. We believe that information from studies focusing on this issue will help reconcile recent evidence and old dogmas in atherosclerosis and identify new strategies in vascular medicine.

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