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

Received April 6, 1994; revision accepted August 31, 1994.

From the Clinica Medica, Istituto di Medicina Interna e Malattie Dismetaboliche, Università di Napoli, and Unità di Trombosi e Aterosclerosi, I.R.C.C.S. “Casa Sollievo della Sofferenza,” S. Giovanni Rotondo, Italy.


Reprint requests to Giovanni Di Minno, MD, Clinica Medica, Istituto di Medicina Interna e Malattie Dismetaboliche, Via S. Pansini, 5, 80131, Napoli, Italy.

© 1994 American Heart Association, Inc.
Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Stroke - n (%)</th>
<th>Stroke + n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>109</td>
</tr>
<tr>
<td>Men</td>
<td>57 (52.3)</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>66.5</td>
</tr>
<tr>
<td>Age &gt;70 y</td>
<td>28 (26.4)</td>
</tr>
<tr>
<td>Previous ischemic episodes</td>
<td>–</td>
</tr>
<tr>
<td>Positive family history</td>
<td>27 (24.8)</td>
</tr>
<tr>
<td>Cigarette smokers</td>
<td>40 (36.7)</td>
</tr>
<tr>
<td>Alcohol consumers</td>
<td>36 (33.0)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>59 (54.1)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>36 (33.0)</td>
</tr>
<tr>
<td>LDL cholesterol &gt;135 mg/dL</td>
<td>27 (24.8)</td>
</tr>
<tr>
<td>TPA &gt;10 ng/mL</td>
<td>21 (19.4)</td>
</tr>
<tr>
<td>PAI-1 &gt;43 ng/mL</td>
<td>15 (13.8)</td>
</tr>
<tr>
<td>ACA IgG &gt;24 GPL</td>
<td>8 (7.3)</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; TPA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor type 1; ACA, antiphospholipid 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.

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 (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). The levels of TPA were higher in subjects with a history of stroke than in subjects without such history (10.30 ± 4.33 ng/mL in the former group versus 7.28 ± 3.98 in the latter; P < .0001) plasma levels of PAI-1 behaved similarly (39.50 ± 25.50 ng/mL in patients with a history of stroke versus 29.18 ± 13.68 in patients without a history of stroke).
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=0.45, P<.0001). On the other hand, according to previous data,29 TPA levels were significantly correlated with age (r=0.155, 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 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 behaved similarly (39.27±21.76 ng/mL in those with one ischemic event, 39.91±28.04 ng/mL in those with more than one event, and 29.18±13.68 ng/mL in subjects without a history of stroke, P<.0001). 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. TPA >10 ng/mL was detected in 21 of the subjects (47.7%) with a history of one cerebral ischemic event, in 32 (51.6%) of those with more than one cerebral ischemic event, and in 21 (19.4%) of those without a history of stroke (P<.0001). PAI-1 >43 ng/mL was detected in 12 (27.3%) of the patients with a history of one event, in 19 (30.6%) of those with more than one event, and in 15 (13.8%) 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. −2Log likelihood χ²: 247.64, P=.025; Model χ²: 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 antidiabetic drugs, a class of medication reported to enhance the fibrinolytic potential of the blood by slightly enhancing plasma levels of TPA antigen.32 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>32/47</td>
<td>.38</td>
</tr>
<tr>
<td>&lt;43 ng/mL</td>
<td>35.5%</td>
<td>68.1%</td>
<td>(.22-.64)</td>
</tr>
<tr>
<td>PAI-1</td>
<td>10/19</td>
<td>21/27</td>
<td>.59</td>
</tr>
<tr>
<td>&gt;43 ng/mL</td>
<td>52.6%</td>
<td>77.8%</td>
<td>(.30-.15)</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>
<tr>
<td></td>
<td>9/15</td>
<td>7/8</td>
<td>.33</td>
</tr>
<tr>
<td></td>
<td>60.0%</td>
<td>87.5%</td>
<td>(.05-.21)</td>
</tr>
</tbody>
</table>

PAI-1 indicates plasminogen activator inhibitor type 1; ACA, antcardioliopin antibodies; TPA, tissue-type plasminogen activator; and GPL, units for ACA (see text).
(Table 3) suggests that TPA plasma levels have a different impact on the identification of subjects with a history of cerebral ischemic events. A history of stroke was more common in individuals <70 years old with TPA plasma levels >10 ng/mL than in older persons with comparable concentrations of the fibrinolytic variable.

Among potential predictors of cerebral ischemia, conflicting data are present on ACA IgG. In some reports, almost one third of the ischemic events associated with this antibody positivity involve the cerebral district. Other reports dispute these figures and even question the relevance of ACA positivity with respect to ischemic events. In our setting, ACA IgG did not help discriminate between subjects with and without a history of stroke, nor did it interact with other variables in accounting for the previous ischemic event. However, such 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 with 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.

Acknowledgment

The authors wish to thank Drs Elena Tremoli and Rosanna Scala for helpful suggestions.

References


56. Hoffman CJ, Burns P, Lawson WE, Katz JP, Miller RH, Hultin MB. Plasma fibrinogen level is not elevated in young adults from
Abnormally high circulation levels of tissue plasminogen activator and plasminogen activator inhibitor-1 in patients with a history of ischemic stroke.
M Margaglione, G Di Minno, E Grandone, G Vecchione, E Celentano, G Cappucci, M Grilli, P Simone, S Panico and M Mancini

Arterioscler Thromb Vasc Biol. 1994;14:1741-1745
doi: 10.1161/01.ATV.14.11.1741

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/14/11/1741

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/