Admission Fibrinolytic Profile Is Associated With Symptomatic Hemorrhagic Transformation in Stroke Patients Treated With Tissue Plasminogen Activator

Marc Ribo, MD; Joan Montaner, MD, PhD; Carlos A. Molina, MD, PhD; Juan F. Arenillas, MD, PhD; Esteban Santamarina, MD; Manuel Quintana; José Alvarez-Sabín, MD, PhD

Background and Purpose—Symptomatic intracranial hemorrhage (SICH) is the most feared complication after tissue plasminogen activator (tPA) stroke treatment. Endogenous fibrinolytic inhibitors play an essential role in the coagulation/fibrinolysis balance and may be involved in the bleeding process. We aim to determine the predictive value of pretreatment levels of fibrinolysis inhibitors (PAI-1, lipoprotein(a), TAFI, and homocysteine) on SICH.

Methods—Consecutive tPA-treated stroke patients with middle cerebral artery occlusion were studied. Baseline blood samples were obtained just before tPA administration and fibrinolysis inhibitors were determined. A second computed tomography (CT) scan was obtained at 24 hours or when a neurological worsening occurred to rule out SICH.

Results—Seventy-seven patients (40% women, age 75 years) were studied. Median admission National Institutes of Health Stroke Scale was 17 (range, 7 to 22) and mean time to treatment was 160 minutes. Six patients (7.9%) presented with a SICH. In analyses based on clinical and CT variables, no relation could be found with SICH. When laboratory data were analyzed, patients who experienced SICH showed lower baseline PAI-1 (21.7±3.5 ng/mL versus 31.8±12.1 ng/mL; P<0.01) and higher TAFI (216.7±78.4% versus 162.1±54.2%; P=0.03). Homocysteine and lipoprotein(a) were not related to SICH. The only factors associated with SICH were TAFI >180% (OR, 12.9; CI, 1.41 to 118.8; P=0.02) and PAI-1 <21.4 ng/mL (OR, 12.75; CI, 1.17 to 139.2; P=0.04). The combination of admission PAI-1 <21.4 ng/mL and TAFI >180% had a sensibility of 75% and a specificity of 97.6% (P<0.01) predicting SICH, with a positive predictive value of 75% and negative predictive value of 97.6%.

Conclusions—Baseline PAI-1 and TAFI levels predict SICH after stroke tPA therapy. In the future, these biomarkers could be used to improve thrombolysis safety. (Stroke. 2004;35:2123-2127.)

Key words: blood–brain barrier ■ complications ■ hemostasis ■ stroke, acute ■ thrombolysis

Intravenous recombinant tissue plasminogen activator (tPA) administered within 3 hours from symptom onset has been proven to be an effective therapy for acute ischemic stroke. However, its use in clinical practice is not generalized worldwide. The most feared complication of thrombolytic therapy is symptomatic intracranial hemorrhage (SICH), and it is probably the main reason that, at the present, only a small fraction of potentially eligible stroke patients are treated with tPA.

Identification of pretreatment predictors of SICH may help in selecting optimal candidates, limiting complications, and potentially allowing the extension of time window for tPA administration. Previous studies pointed out clinical (stroke severity, old age, high blood pressure), radiological (early CT changes), sonographic (proximal middle cerebral artery occlusion), and analytic factors (glucose, platelet count, MMP-9) as pretreatment predictors of SICH. However, the role of endogenous fibrinolysis inhibitors, determinants of the coagulation/fibrinolysis balance, remains unknown. After an acute ischemic event, the release of these inhibitors into the blood flow may lead to wide interindividual differences in the global fibrinolysis capacity, which are called to block or enhance tPA effects playing a key role in the development of SICH.

We aim to determine the impact of pretreatment levels of fibrinolysis inhibitors (plasminogen activator inhibitor [PAI]-1; lipoprotein a [Lp(a)]; thrombin-activated fibrinolysis inhibitor [TAFI]; and homocysteine on the development of SICH.

Patients and Methods
From February 2002 to October 2003, all patients with an acute (<3 hours from symptom onset) nonlacunar stroke admitted to the emergency department of a university hospital were prospectively studied. Seventy-six patients with a transient ischemic attack (cerebral blood flow transient occlusion who fulfilled...
established criteria for intravenous t-PA treatment (0.9 mg/kg) were included.

Clinical and Transcranial Doppler Protocol
A detailed history of vascular risk factors was obtained from each patient. To identify potential mechanism of cerebral infarction, a set of diagnostic tests was performed; when indicated, patients also underwent special coagulation tests, transthoracic/transesophageal echocardiography, and Holter monitoring. With this information and the neuroimaging data, previously defined etiologic subgroups were determined. Clinical examination was performed every hour during the first 6 hours and at 12, 24, and 48 hours after stroke onset. Stroke severity and neurological improvement or worsening were assessed by using the National Institutes of Health Stroke Scale (NIHSS). A standard transcranial Doppler examination was performed in the emergency room on admission before tPA administration using 1-channel 2-MHz equipment (transcranial Doppler 100 mol/L; Spencer Technologies). A standard set of diagnostic criteria was applied to assess arterial occlusion. Proximal MCA occlusion was defined as the absence of flow or the presence of minimal flow signal throughout the MCA at an insolation depth between 45 and 65 mm, accompanied by flow diversion in the ipsilateral anterior carotid artery (ACA) and posterior carotid artery (PCA), according to the thrombolysis in cerebral ischemia (TIBI) grading system.

CT and Intracranial Hemorrhage
On admission, all patients underwent a CT scan that was repeated at 24 to 48 hours to evaluate the presence of hemorrhagic transformation. Whenever a neurological worsening (NIHSS decrease ≥4 points) occurred, an additional CT scan was immediately performed to rule-out SICH. CT scans were reviewed by a neuroradiologist with extensive experience in acute stroke who was blinded to clinical details and laboratory data. Hydropenia on the pretreatment CT was retrospectively classified as absent, involving less than one third of the MCA territory, or involving one third or more of the MCA territory. Presence and type of hemorrhagic transformation was defined according to previously published criteria. Hemorrhagic transformations were categorized as SICH when a neurological deterioration accompanied the presence of blood at any site in the brain on the CT scan, or as asymptomatic (AICH) when no neurological worsening was observed.

Blood Sampling and Laboratory Assays
Peripheral blood samples were drawn from each patient at study entry, just before tPA administration. EDTA and citrate tubes were used to collect the blood. Plasma was immediately separated by centrifugation at 3000 rpm for 15 minutes and stored at −80°C until analysis. Plasma homocysteine levels were determined with the Fluorescence Polarization Immunoassay (Axis Biomedical). PAI-1 (citrate tubes; TintElize, Biopool), Lp(a) (EDTA tubes; Macra, Trinity Biotech), and TAFI (EDTA tubes; Zymutest, Hyphen BioMed) levels were determined with commercially available enzyme-linked immunosorbent assays (ELISA). For TAFI levels, results are expressed as a percentage of a control pooled plasma of healthy subjects provided with the kit. Analyses were performed in duplicate according to the manufacturer’s instructions. The mean intra-assay coefficients of variation were <10% for all molecules.

Statistical Analyses
Descriptive and frequency statistical analysis were obtained and comparisons were made using the SPSS 10.0 statistical package. Statistical significance for intergroup differences was assessed by the Pearson χ2 or the Fisher exact test for categorical variables, and the Student t test and ANOVA for continuous variables. Pearson correlation coefficient was used to determine correlations between the fibrinolysis inhibitors and other continuous variables. When indicated, Mann–Whitney U and Spearman tests were used. To calculate the sensitivity and specificity of biomarkers to predict SICH, a receiver–operator characteristic (ROC) curve was config-

Fibrinolysis Inhibitors Levels According to Symptomatic Intracranial Hemorrhage

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>No (n=71)</th>
<th>Yes (n=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1, ng/mL</td>
<td>32.9</td>
<td>32.5</td>
<td>21.7</td>
<td>0.001</td>
</tr>
<tr>
<td>TAFI, %</td>
<td>167.2</td>
<td>162.4</td>
<td>216.7</td>
<td>0.027</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>9.14</td>
<td>7.9</td>
<td>23</td>
<td>0.162</td>
</tr>
<tr>
<td>Hcy, μmol/L</td>
<td>12</td>
<td>12</td>
<td>11.2</td>
<td>0.661</td>
</tr>
</tbody>
</table>

Values are mean, median, or percent, as appropriate.

Results
We included 77 patients (39.5% women) in the study, with a mean age of 70±12 years (range, 26 to 91 years) and with an acute MCA occlusion. Baseline characteristics of the patients are shown in the Table. Median NIHSS score on admission was 17 (range, 7 to 22), and mean time to treatment was 160 minutes. According to Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria, stroke causes were: cardioembolic, 39.5% (n=30); atherothrombotic, 25% (n=19); undetermined, 22.1% (n=17); dissection, 5.3% (n=4); and unknown origin, 9.1% (n=7). On baseline CT scan, 30 patients (47%) presented early ischemic changes; none of them involved one third or more of the MCA territory. On the follow-up CT scan, 17 patients (22.4%) presented a hemorrhagic transformation (1 HI-1, 5 HI-2, 1 PH-1, 6 PH-2); from those 17 patients, 6 (7.9%) experienced SICH (all PH2).

Fibrinolysis Inhibitors
No correlation could be found between all 4 studied fibrinolysis inhibitors, indicating that each molecule varied independently from the others, and no differences for biomarkers levels were found among TOAST etiologic subgroups (data not shown).

PAI-1
PAI-1 plasma levels (mean 33.1±12.2 ng/mL) were within our laboratory standard rates (32.9±10.07 ng/mL). No differences in baseline PAI-1 levels were found regarding age, gender, vascular risk factors, time to treatment, or initial stroke severity. Patients who presented SICH after t-PA infusion showed lower baseline PAI-1 levels than the rest of the patients (21.7±3.5 ng/dL versus 31.8±12.1 ng/dL; P<0.01); moreover, graded PAI-1 levels were observed according to the degree of bleeding (Figure 1).

TAFI
Plasma TAFI levels were significantly higher than our laboratory reference range for healthy controls (167.2±57.8% versus 109.4±29.4%; P<0.001). No differences in baseline TAFI levels were found regarding age, gender, or previous vascular risk factors. However, TAFI appeared to be negatively correlated with the time from symptoms onset (r=−0.292; P=0.019). Patients who presented SICH after t-PA infusion had higher baseline TAFI levels than those
without SICH (216.71 ± 78.4% versus 162.1 ± 54.2% \(P=0.027\)), and graded TAFI levels were observed according to the degree of bleeding (Figure 2).

**Lp(a)**

The determined Lp(a) plasma levels were not significantly higher than those in a reference healthy group (median, 9.14 mg/dL versus 5.8 mg/dL; \(P=0.285\)). Lp(a) was correlated with age (\(r=0.4; \ P=0.001\)) and fibrinogen levels on admission (\(r=0.263; \ P=0.048\)). No relationship between Lp(a) and other analytical parameters could be found. SICH was not related to pretreatment Lp(a) levels (\(P=0.162\)).

**Homocysteine**

Baseline homocysteine (median, 12; range, 10.4 to 14.8 \(\mu\)mol/L) was slightly more than the laboratory normal range (5.6 to 12.2 \(\mu\)mol/L) and appeared to be correlated with patient age (\(r=0.53; \ P=0.001\)) and negatively correlated with the time from symptom onset (\(r=-0.32; \ P=0.037\)). No differences could be found according to hemorrhagic transformation after tPA treatment or clinical outcome.

**Predictors of SICH**

Main baseline demographic and routine laboratory data of patients with and without SICH were studied; none of them was associated with SICH. The presence of early ischemic changes on admission CT scan was not related to later SICH. Among admission variables, only PAI-1 and TAFI baseline levels were significantly different between both groups (Table). A ROC curve determined the cut-points that better predict further development of SICH for TAFI > 180% (OR, 12.9; CI, 1.41 to 118.8; \(P=0.02\)) and for PAI-1 < 21.4 ng/mL (OR, 12.75; CI, 1.17 to 139.2; \(P=0.04\)). After adjusting for potential confounders that were shown to predict SICH (age, baseline NIHSS, diabetes), the logistic regression model determined that PAI-1 < 21.4 ng/mL remained the only independent predictor of SICH. The combination of admission PAI-1 < 21.4 ng/mL and TAFI > 180% had a sensitivity of 75% and a specificity of 97.6% (\(P<0.01\)) predicting SICH, with a positive predictive value of 75% and negative predictive value of 97.6%.

**Discussion**

The present study demonstrates that admission PAI-1 and TAFI levels determined before tPA treatment in acute stroke assist in risk prognostication for further SICH. In some patients, these biomarkers may influence the coagulation/fibrinolysis balance toward a state favorable for the bleeding process. Pretreatment PAI-1 levels have shown to influence the tPA-mediated recanalization rates, but this is the first study involving fibrinolysis inhibitors in bleeding complications after thrombolysis in stroke.

Previous studies have demonstrated that hemorrhagic transformation after tPA treatment is associated with reperfusion. Hemorrhagic infarctions (HI-1, HI-2) are associated with early recanalization (short ischemia time) and little endothelial or blood–brain barrier damage (low fibronectin or MMP-9 levels), with consequent clinical improvement and good outcome. However, large parenchymal hematoma (PH-2) are associated with delayed recanalization and higher levels of endothelial damagers (glucose, MMP-9) leading to...
to reperfusion on a damaged vascular bed, extended bleeding, and neurological deterioration. Low platelet counts and antiplatelet therapy have been also associated with SICH after tPA treatment, suggesting that the coagulation/fibrinolytic balance is implicated in the bleeding process. In vitro studies support the concept that fibrinolytic enzymes interact with the brain microvascular endothelium and thus affect the integrity of the blood–brain barrier through active cell contraction. A profibrinolytic state could favor blood extravasation and help small hemorrhagic infarctions to become large parenchymal hematomas.

Low PAI-1 levels have been related to bleeding disturbances. In this study, we measured PAI-1 in the very early phase of ischemic stroke (mean time, 160 minutes) just before tPA administration. We observed that PAI-1 <21.4 ng/mL was associated with SICH, supporting the hypothesis that very low PAI-1 levels may confer an enhanced fibrinolytic state, predisposing to intracranial bleed.

TAFI removes carboxy-terminal lysyl and arginyl residues from partially degraded fibrin, preventing the plasminogen-mediated fibrin degradation. Plasma TAFI levels had been shown to be elevated in the first hours after ischemic stroke; however, limited and incomplete information is available about this marker and its effects, particularly in tPA-treated patients. In our patients, elevated TAFI levels (>180%) were associated with later SICH. This apparent paradox may be explained by the TAFI consumption theory: in those cases in which there is a high affinity between fibrin and TAFI leading to elevated TAFI activity, there may be a consumption of circulating TAFI. Thus, elevated circulating TAFI levels would indicate low activity and therefore high fibrinolytic state. In fact, a previous study demonstrated that elevated TAFI levels were associated with good response to tPA treatment and early MCA recanalization or a lower risk of coronary events, presuming an optimal fibrinolytic state for clot dissolution.

The other studied fibrinolysis inhibitors, homocysteine and Lp(a), did not show any relation with SICH and probably do not play a significant role in the bleeding process.

The combination of admission PAI-1 <21.4 ng/mL and TAFI >180% had a sensibility of 75% and a specificity of 97.6% predicting SICH, with a positive predictive value of 75% and negative predictive value of 97.6%. Obtaining information about the fibrinolysis/coagulation status before tPA administration could assist physicians in risk stratification of SICH and adopting decisions like endovascular mechanical retrieval of the clot without using tPA. At present, methods able to determine plasma levels of these biomarkers are not short enough to be used in the emergency room. Our results should stimulate investigations toward generation of ultrarapid and reliable methods able to give physicians this important information before treatment. It will be also very interesting to correlate these findings with techniques able to instantly measure the global fibrinolytic capacity and to bring them to the daily practice.

This pilot study was conducted with a reduced number of patients with SICH, limiting the statistical power of our results; therefore, conclusions need further confirmation in a larger cohort of patients in a multicenter study. The causal relation between PAI-1 and TAFI levels with SICH cannot be demonstrated strictly with this study. However, because there is a succession in time between biomarker determination and development of SICH, we considered both biomarkers as predictors of SICH.

Conclusions
Baseline PAI-1 and TAFI levels are associated with SICH development after stroke tPA therapy. In the future, these biomarkers could be used to improve thrombolysis safety in stroke patients. Risk quantification of SICH, particularly with a good negative predictive value, may help physicians feel more confident with tPA treatment and therefore could help generalize fibrinolytic therapy in the clinical practice.

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References


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