Background and Purpose—Symptomatic intracerebral hemorrhage (ICH) is a major complication of thrombolysis in patients with acute ischemic stroke. We analyzed whether baseline hemostatic markers could predict symptomatic ICH (SICH).

Methods—In a multicenter study of patients treated with intravenous tissue plasminogen activator (t-PA) within 3 hours of stroke onset, we analyzed the following variables: demographic data, vascular risk factors, blood glucose at admission, time from the onset of symptoms to t-PA infusion, blood pressure, neurological deficit measured by the National Institutes of Health Stroke Scale (NIHSS) score, early signs of ischemia on the baseline computed tomography (CT) scan, and protocol deviations. In blood samples, the following markers of coagulation/fibrinolysis were measured before treatment: fibrinogen, prothrombin fragments 1+2, Factor XIII, Factor VII, α2 antiplasmin, plasminogen activator inhibitor-1 (PAI-1), and thrombin-activatable fibrinolysis inhibitor. ICH was classified according to the European Cooperative Acute Stroke Study (ECASS) II criteria. SICH was defined as a parenchymal hematoma (PH1) or PH2 type, associated with an increase in ≥4 points on the NIHSS score appearing within 36 hours after infusion.

Results—we studied 114 patients. Mean age was 68.4 ± 12.7 years, and 61% were men. The median baseline NIHSS score was 14. Mean time to treatment was 153 ± 33 minutes. Eight patients had SICH (7%), and 18 patients (15.7%) had asymptomatic ICH. None of the baseline markers of coagulation/fibrinolysis were associated with SICH. In the multivariate analysis, only NIHSS on admission was an independent risk factor for SICH.

Conclusions—None of the hemostatic markers analyzed in our study predicted symptomatic cerebral hemorrhage in patients with ischemic stroke treated with t-PA. (Stroke. 2006;37:996-999.)

Key Words: hemorrhage ■ hemostatics ■ thrombolysis

Intravenous recombinant tissue plasminogen activator (t-PA) administered within 3 hours of symptom onset is an effective therapy for acute ischemic stroke,1,2 but the most serious risk of thrombolysis is intracerebral hemorrhage (ICH). A meta-analysis showed that recombinant t-PA (rt-PA) treatment was associated with an increase in symptomatic ICH (SICH; odds ratio, 3.1).2

Knowledge of risk factors for SICH may improve the patient selection and also the safety of this treatment. Many clinical and radiological variables3–6 and protocol deviations7 have been reported to predict ICH in patients treated with thrombolysis. In addition, some studies have found that higher levels of blood glucose,3 matrix metalloproteinase-9,8 thrombin-activatable fibrinolysis inhibitor (TAFI),9 and fibronectin10 and lower levels of PAI-19 are associated with the risk of cerebral hemorrhage.

Deficient or excessive levels of coagulation factors and fibrinolysis may give rise to differences in baseline procoagulant or profibrinolytic states. This baseline status may predispose to or protect against cerebral bleeding after rt-PA treatment. Only 1 study9 has analyzed the role of baseline hemostatic markers as a risk factor for SICH, and it found that lower levels of PAI-1 and higher levels of TAFI were associated with SICH. However, in this study, few markers were analyzed, and antigenic levels rather than functional levels were measured.

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In the present study, we analyzed whether baseline markers of coagulation and fibrinolysis could predict SICH in patients treated with intravenous t-PA based on the hypothesis that lower levels of fibrinolysis inhibitors are predictors of SICH.

Materials and Methods
We analyzed patients with ischemic stroke treated with intravenous t-PA within 3 hours of symptom onset in 3 tertiary hospitals that followed the same protocol (clinical criteria of National Institute of Neurological Disorders and Stroke1 and radiological criteria according to the European Cooperative Acute Stroke Study [ECASS] II11). One center prospectively included consecutive patients, whereas data from the remaining 2 centers were retrospectively collected. Informed consent was requested from all patients or their relatives.

The following data were recorded for each patient: (1) demographics (age and sex), (2) vascular risk factors (high blood pressure, diabetes mellitus, ischemic heart disease, atrial fibrillation, smoking habit, hypercholesterolemia, and previous cerebral ischemia), (3) Dose of t-PA, (4) blood glucose at admission, (5) time from symptom onset to t-PA infusion, (6) blood pressure at admission, (7) severity of the neurological deficit measured by the National Institutes of Health Stroke Scale (NIHSS) score, (8) rate of protocol deviations, (9) previous treatment with any antithrombotic agent, (10) early signs of ischemia on the baseline CT scan (hypodensity, sulcal effacement, loss of basal ganglia differentiation, loss of the insular ribbon, and loss of the gray-white matter distinction), and (11) etiology of the cerebral infarction, according to the Trial of Org 10172 in Acute Stroke Treatment criteria.12 The functional outcome at 3 months was measured by the modified Rankin scale (mRS) score. A favorable outcome was defined as an mRS score of 0 to 1 and a poor outcome as an mRS score >1.

Hemorrhagic Events
We classified the ICH type as either parenchymal hematomas (PH1, PH2) or hemorrhagic infarcts (HI1, HI2) according to the ECASS II criteria.14 SICH was defined as a PH2 associated with neurological worsening (an increase in ≥4 points on the NIHSS score within 36 hours after infusion) and without an alternative explanation for the neurological worsening. Asymptomatic ICH was considered in all PH or HI within 36 hours after infusion without neurological worsening.

Markers of Coagulation and Fibrinolysis
For several years, the 3 hospitals systematically collected blood samples in patients treated with t-PA, obtaining informed consent before sampling. Blood samples obtained by venipuncture were collected in one tenth of 0.129 mol/L sodium citrate before treatment in all patients. Platelet-poor plasma was obtained by centrifugation at 3000 rpm for 20 minutes at room temperature. Plasma was frozen in aliquots at −40°C until analyzed. A normal plasma pool was prepared by mixing plasma from 100 healthy blood donors.

In one center, we analyzed markers of coagulation in all blood samples, using the same methodology for all. We analyzed the following markers: fibrinogen (by the von Clauss method with thrombin from BioMerieux), prothrombin fragments 1+2 (ELISA method from Dade Behring), Factor XIII (Pefakit FXIII incorporation assay from Pentapharm), and Factor VII (ELISA Diagnostica Stago). We measured the following fibrinolysis inhibitors: α2-antiplasmin (chromogenic methods from Chromogenix), PAI-1 (using a Chromolize PAI-1 activity test from Biopool), and TAFI (antigen was analyzed using an ELISA method from Hyphen BioMed and functional TAFI [by Actichrome TAFI kit from American Diagnostica]) using the normal plasma pool as a standard. The results were expressed as a percentage of normal plasma.

Statistical Analysis
We compared the level of markers in the SICH group with the nonsymptomatic ICH group using univariate analysis. Contingency tables and the χ² test were used for categorical variables and the Student t test for quantitative variables. Median NIHSS scores were compared with the Mann–Whitney U test. A multivariate logistic regression analysis was performed with the variables that reached a P<0.1 in the univariate analyses. The results were considered statistically significant when P<0.05.

Results
A total of 114 patients were included in the study. Mean age was 68.4±12.7 years, and 61% were men. The median NIHSS score on admission was 14 (range 4 to 26).

Etiologic stroke subtypes were classified as cardioembolic (47.4%), large-artery atherotrombotic (19.3%), small-vessel (2.6%), unusual (1.8% arterial dissection), or undetermined (28.9%). Thrombolysis was initiated on average 153±33 minutes after symptom onset. In 12 patients (10.5%), a protocol deviation occurred. Eleven patients were treated beyond the 3-hour limit, and 1 patient scored >25 points on the NIHSS. Table 1 summarizes the univariate analyses of demographic, clinical, radiological, and etiological stroke subtype data. The variables significantly associated with SICH were older age (P=0.001), higher NIHSS score on admission (P=0.05), and longer time to treatment (P=0.03).

Eight patients (7%) presented SICH. We also detected asymptomatic hemorrhagic complications in 18 additional patients (15.7%) with the following subtype distribution: 8 HI1, 8 HI2, and 2 PH1. We did not detect any asymptomatic PH2.

| TABLE 1. Main Characteristics of Patients With and Without SICH |
|---------------------------------|-----------------|-----------------|---|
|                                 | SICH (n=8)      | Non-SICH (n=106)| P  |
| Sex distribution (% men)        | 37.5            | 63.2            | 0.25 |
| Age (y; mean, SD)               | 77.8±5.9        | 67.7±12.9       | 0.001|
| Dose rt-PA (mg; mean, SD)       | 58.1±9.3        | 66.2±11.1       | 0.15 |
| Time to treatment (min; mean, SD)| 171.3±15.9    | 152.1±33.3      | 0.03 |
| NIHSS score on admission (median; range) | 17 (15–21) | 13 (4–26) | 0.05 |
| Previous treatment with any antithrombotic (%) | 37.5 | 30.1 | 0.69 |
| Protocol deviation (%)          | 12.5            | 10.3            | 0.9  |
| Blood glucose at admission (mg/dL; mean, SD) | 172.9±85.6 | 135.78±65.7 | 0.16 |
| Systolic blood pressure (mm Hg; mean, SD) | 164.1±15.2 | 154.4±27.7 | 0.36 |
| Diastolic blood pressure (mm Hg; mean, SD) | 81.5±13.9 | 83.9±13.4 | 0.29 |
| Early signs of ischemia (%)     | 62.5            | 45.2            | 0.46 |
| Etiological subtypes (%)        | 48              |                 |     |
| Atherothrombotic                | 0              | 20.8            |     |
| Cardioembolic                   | 75             | 45.3            |     |
| Small-vessel                    | 0              | 2.8             |     |
| Unusual                         | 0              | 1.9             |     |
| Undetermined                    | 25             | 29.2            |     |
TABLE 2. Results of Coagulation and Fibrinolysis Markers in Patients With and Without SICH

<table>
<thead>
<tr>
<th></th>
<th>SICH (n=8)</th>
<th>Non-SICH (n=106)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3.9±1.1</td>
<td>3.7±1.1</td>
<td>0.7</td>
</tr>
<tr>
<td>α2-antiplasmin (%)</td>
<td>87.8±6.6</td>
<td>92.2±14.9</td>
<td>0.13</td>
</tr>
<tr>
<td>Factor XII (%)</td>
<td>114.5±35.8</td>
<td>132.3±41.4</td>
<td>0.21</td>
</tr>
<tr>
<td>Antigenic TAFI (%)</td>
<td>89.3±14.7</td>
<td>86.0±15.5</td>
<td>0.56</td>
</tr>
<tr>
<td>Functional TAFI (%)</td>
<td>84.6±25.5</td>
<td>97.2±25.0</td>
<td>0.17</td>
</tr>
<tr>
<td>PAI-I (IU)</td>
<td>5.6±5.5</td>
<td>9.6±13.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Factor 1+2 (µ mol/ml)</td>
<td>1.2±0.54</td>
<td>1.3±1.2</td>
<td>0.76</td>
</tr>
<tr>
<td>Factor VII (%)</td>
<td>76.6±17.6</td>
<td>81±20.2</td>
<td>0.52</td>
</tr>
</tbody>
</table>

The results of the hemostatic markers at baseline are shown in Table 2. None of the markers analyzed were associated with SICH, although patients with SICH had lower levels of antifibrinolytic factors: α2-antiplasmin (P=0.13), functional TAFI (P=0.17), and PAI-I (P=0.11).

In the analysis of the variability between hospitals, we did not detect significant differences between the SICH rate (P=0.1) or vascular risk factors. However, we detected significant differences in some of the baseline hemostatic levels: antigenic TAFI (P=0.01), functional TAFI (P=0.001), and Factor XIII (P=0.01).

In the multivariate analysis (performed with age, NIHSS, and time to treatment), only NIHSS on admission was risk factor for SICH (odds ratio, 1.3; 95% CI 1.0 to 1.6; P=0.036).

Outcome at 3 months was favorable in 47 patients (41.2%) and poor for 67 (58.8%). SICH was associated with a significantly worse outcome; the percentage of patients with an mRS score of 0 to 1 was 0% in patients with SICH and 44% in patients without SICH (P=0.02). Mortality was also higher in patients with SICH (75% versus 11%; P=0.0001).

Discussion

In this multicenter study of 114 patients treated with intravenous t-PA, none of the baseline markers of coagulation/fibrinolysis were significantly associated with SICH. In agreement with other studies, the severity of the neurological deficit, as measured by the NIHSS score, was a risk factor for symptomatic cerebral bleeding.

The mechanisms underlying hemorrhage after thrombolytic therapy are still unclear. In previous studies, markers of endothelial lesion or extracellular matrix disruption, such as metalloproteinases or cellular fibronectin, have been related with ICH. Although the vessel wall integrity is important to restrain hemorrhage, hemostatic markers may also play an important role in the risk of bleeding. In agreement with this reasoning, in previous randomized trials of anticoagulation in acute stroke patients, the incidence of SICH was more frequent in the anticoagulated group that in the placebo arm, and the higher the dose of heparin, the higher this percentage.

On the other hand, the same dose of t-PA may have different effects in different patients depending on their baseline procoagulant or profibrinolytic state. Decreased or increased levels of some of these factors may influence the delicate balance between coagulation and fibrinolysis.

In our study, we hypothesized that a decrease in the physiological levels of fibrinolytic inhibitors or in factors that participate in clot stability (Factor XIII) could be predictors of SICH.

In agreement with our hypothesis, mean levels of fibrinolysis inhibitors were lower in patients with SICH (although nonsignificantly), possibly indicating a relative baseline over-activation of fibrinolysis in these patients. We cannot rule out the possibility that a study with a larger sample of patients could provide significant differences.

Although only 8 patients had SICH in our study, we studied a large sample of 114 patients treated with t-PA, and none of hemostatic marker were a risk factor for SICH, suggesting that physiological variations in these baseline markers may play a small role as predictors of SICH. In addition, to consider using these markers in t-PA treatment decision-making, they should be highly sensitive and specific to predict SICH as well as easily and quickly measurable, but this is not currently the case.

In a single-center study of 77 patients treated with t-PA, in which 6 patients developed SICH, lower baseline plasma PAI-I and higher antigenic TAFI levels were associated with SICH. The paradoxically high antigenic TAFI levels were explained by the authors by a low TAFI affinity to fibrin that leads to higher levels of circulating TAFI and a high fibrinolytic state or by the presence of TAFI polymorphisms that might interfere with the ELISA results. A fact to consider is that in this study, antigenic levels rather than functional levels were measured. The antigenic TAFI determination measures the quantity of TAFI present in plasma, irrespective of whether this protein is active or not. The functional assay measures its activity (ie, the capacity to be transformed into the enzyme): TAFI activated and the activity that the enzyme formed presents.

Of 114 patients evaluated in our study, 26 patients had also been included in the previous study. However, in our analysis, the concentration of antigenic TAFI levels was similar in both groups. Furthermore, the concentrations of functional TAFI and PAI-I were not significantly lower in patients with SICH. Possible explanations for these discrepancies are the different methodology used between hospitals and the small sample of patients with SICH in both studies.

Our study has some limitations. First, the number of patients with symptomatic hemorrhage was small, and it is possible that we did not have significant differences for type II error; and with a larger sample, the difference could be statistically significant. Second, we did not analyze the platelet count and other biological markers reported as risk factors for ICH as fibronectin or matrix metalloproteinase. Third, storage aspects, such as the time of freezing and the time from blood collection until the samples were frozen, may influence the results; this would explain the variability in hemostatic levels detected among hospitals in our study.

In conclusion, this study demonstrated that neurological severity on admission is a powerful predictor of SICH, and that none of the baseline markers of coagulation and fibrino-
lysis analyzed predicted SICH. Further studies with these and other hemostatic markers of coagulation and fibrinolysis with larger samples may help to evaluate the influence of these markers as risk factors for SICH.

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Pretreatment Hemostatic Markers of Symptomatic Intracerebral Hemorrhage in Patients Treated With Tissue Plasminogen Activator

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