Markers of a Hypercoagulable State Following Acute Ischemic Stroke

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Background and Purpose: The nature of hematologic disorders in different stroke subtypes remains uncertain. The purpose of this study was to clarify the differences in the coagulation and fibrinolytic activities among ischemic stroke subtypes.

Methods: We performed sequential measurements of hematologic parameters in 21 patients with acute cardioembolic stroke, 10 patients with atherothrombotic stroke, 23 patients with lacunar stroke, and 20 age-matched controls.

Results: A marked elevation of plasma concentrations of the thrombin-antithrombin III complex and crosslinked D-dimer was observed only in cardioembolic stroke within 48 hours of onset ($p<0.01$), persisting for one month with a gradual decline. In atherothrombotic stroke, however, the level of crosslinked D-dimer was not elevated at the onset, but increased seven days after onset ($p<0.01$). No significant changes in these marker levels were observed in lacunar stroke.

Conclusions: Our findings suggest that the nature of altered coagulation and fibrinolysis are different in various subtypes of ischemic stroke, and that an assessment of these hematologic parameters may be useful for the early classification of these subtypes. (Stroke 1992;23:194-198)

Brain infarction can be distinguished into several subtypes with different pathogenetic mechanisms. Recognition of varying hematologic involvement in each stroke subtype may lead to more accurate diagnoses and therapeutic intervention tailored to these differences. Although recent investigations have focused on the coagulation or fibrinolytic conditions following acute ischemic stroke,1-8 the nature and characteristics of the hematologic disturbances in different strokes subtype have not been fully explored. The results previously obtained have been conflicting and failed to distinguish cardioembolic stroke from thrombotic stroke. Since hypercoagulability may be induced by several complicated factors, such as elevated function of platelets and leukocytes, perturbation of the endothelium, hypercoagulability of plasma, or increased blood viscosity, the data obtained should be analyzed in relationship to stroke subtype. In our previous studies,9,10 analyses of the extrinsic coagulation factors and the molecular markers of hemostasis suggested the presence of different characteristics of coagulation disorders among subtypes of acute ischemic stroke. In the present investigation, we propose to further clarify the differences in the coagulation and fibrinolytic systems among ischemic stroke subtypes.

Subjects and Methods

Subjects were selected from 73 consecutive ischemic stroke patients admitted to the Stroke Care Unit of the National Cardiovascular Center within 48 hours after onset from January 1988 to March 1989. The subjects consisted of 21 cardioembolic, 10 atherothrombotic, and 23 lacunar stroke patients. Nineteen patients diagnosed as unclassified stroke were not included in this study. The age, sex, and risk factors, including cardiac disorders, in each stroke subset are summarized in Table 1. Diagnosis of cardioembolic stroke was made by criteria previously reported.10,11 We applied continuous electrocardiography or two-dimensional echocardiography in all patients to detect a potential cardiac source of emboli.11 Brain computed tomography (CT) and cerebral angiography were performed to confirm the presence of findings characteristic of embolic stroke.11,12 The abrupt onset of maximal neurological deficit or evidence of systemic embolism also was used to support a diagnosis of cardioembolic stroke. Atherosclerotic occlusion of a major cerebral artery was confirmed by cerebral angiography in every patient with atherothrombotic stroke. Lacunar stroke
was diagnosed by the presence of an appropriate neurological syndrome and CT finding. Additionally, the absence of vascular pathology such as an ulcerative plaque in the aortic arch or carotid arterial wall was confirmed by cerebral angiography or duplex ultrasonography.

Ongoing venous thrombosis or pulmonary embolism was not clinically detected in any patient during the study. Patients with liver dysfunction, renal dysfunction, infection, or malignancy and those on anticoagulant or antiplatelet agents were excluded.

Twenty healthy men with a mean age of 61.6 years were used as age-matched controls. No control had a history of cardiovascular, cerebrovascular, peripheral vascular disease, or other major medical illnesses.

Blood samples were taken from all patients within 48 hours after onset but before the commencement of the therapy (day 1 of hospitalization; acute stage), and on the seventh day (day 7; subacute stage) and the 30th day after onset (day 30; chronic stage). Venous blood was drawn from the antecubital vein of the nonparalyzed arm using the two-syringe technique. After discarding the first 3 ml, blood was collected into a siliconized glass tube that contained one-tenth volume of 3.8% trisodium citrate. Plasma was separated by centrifugation at 2,300g for 15 minutes at room temperature and stored at −70°C until tested. Blood samples were obtained similarly in the controls.

The measurements for antithrombin III biological activity were performed by the amidolytic method. Antithrombin III antigen was measured by an immunoturbidometric method using NOR-Partigen® (Behringwerke A.G., Marburg, FRG). Plasma concentrations of thrombin-antithrombin III complex and crosslinked dimer were measured using commercially available enzyme-linked immunosorbent assay kits (Behringwerke A.G., Marburg, FRG, and Agen Ltd., Brisbane, Australia).

Statistical analyses were performed with an analysis of variance and subsequent Scheffe’s method to compare the values obtained from the various groups. Differences with a value of p<0.05 were considered significant.

**Results**

Hematologic data at the time of admission (day 1) in each subset and the controls are shown in Table 2. The level of antithrombin III activity was significantly lower in cardioembolic stroke patients than in the controls (p<0.05), but antithrombin III antigen levels were not significantly different from the controls in any of the stroke subsets. The levels of plasma thrombin–antithrombin III complex and crosslinked d-dimer were significantly higher in cardioembolic stroke patients than those in the controls at the acute stage (p<0.01). The levels of these two molecular markers in atherothrombotic and lacunar stroke patients did not differ from those in the controls.

The level of antithrombin III activity in cardioembolic stroke gradually increased over time and no differences from the controls were observed on day 7 and day 30. The level of antithrombin III antigen in this subset did not significantly change during the study (Figure 1). Antithrombin III activity and antithrombin III antigen levels in atherothrombotic stroke and lacunar stroke did not differ significantly from those in the controls throughout the study (data not shown). Serial changes in the levels of the thrombin–antithrombin III complex and crosslinked d-dimer in each stroke subtype are shown in Figure 2. In cardioembolic stroke, the plasma concentration of thrombin–antithrombin III complex was elevated on day 1 (p<0.01), then declined as time elapsed, and the difference from the controls became not significant on day 30 (Figure 2A). Elevation of the plasma concentration of crosslinked d-dimer was also observed in cardioembolic stroke. This level declined gradually, but remained significantly higher than in the controls throughout the study (p<0.01) (Figure 2A). In atherothrombotic stroke, however, thrombin–antithrombin III complex and crosslinked d-dimer levels on day 1 were not significantly greater than those in the controls. The level of crosslinked d-di-
TABLE 2. Hematologic Studies in Each Stroke Subtype Within 48 Hours After Onset (Day 1) and Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cardioembolic stroke</th>
<th>Atherothrombotic stroke</th>
<th>Lacunar stroke</th>
<th>Age-matched control</th>
</tr>
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<tbody>
<tr>
<td>Antithrombin III activity (%)</td>
<td>88.8±3.6*</td>
<td>95.2±2.9</td>
<td>97.1±3.2</td>
<td>102.1±2.0</td>
</tr>
<tr>
<td>Antithrombin III antigen (%)</td>
<td>93.0±3.3</td>
<td>94.0±3.7</td>
<td>94.4±4.2</td>
<td>102.6±2.7</td>
</tr>
<tr>
<td>Thrombin-antithrombin III complex (ng/ml)</td>
<td>10.2±1.9†</td>
<td>4.3±0.7</td>
<td>3.0±0.2</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>Crosslinked d-dimer (ng/ml)</td>
<td>607.0±167.6†</td>
<td>171.3±29.4</td>
<td>115.3±15.5</td>
<td>82.1±9.1</td>
</tr>
</tbody>
</table>

*p<0.05, †p<0.01, respectively, different from age-matched controls by Scheffe's method. Each value represents mean±SEM.

mer elevated significantly on day 7 (p<0.01), and declined on day 30 (Figure 2B). Both the thrombin-antithrombin III complex and crosslinked d-dimer in lacunar stroke patients did not show any significant changes during the study (Figure 2C).

The sensitivity and specificity of the thrombin-antithrombin III complex and crosslinked d-dimer for the differentiation of cardioembolic stroke from the other two stroke subtypes is presented in Table 3. When the cut-off values of 5 and 300 ng/ml for their parameters were set, both of these markers when measured within 48 hours (day 1) after stroke onset demonstrated useful sensitivities and specificities to identify cardioembolic stroke.

**Discussion**

We have previously reported significant decrease in the activities of antithrombin III and protein C, and a marked increase in the thrombin-antithrombin III complex and crosslinked d-dimer in acute cardioembolic stroke. In the present study, acute coagulation and fibrinolytic activation were again confirmed only in cardioembolic stroke patients, but not in the other two stroke subtypes, as represented by the increased thrombin-antithrombin III complex and crosslinked d-dimer levels measured. The gradual decline of these two markers within seven days after stroke onset was also found only cardioembolic stroke. In contrast, the level of crosslinked d-dimer rose moderately for 7 days in atherothrombotic stroke, but did not significantly change in the lacunar stroke group. The difference in the time course as well as the levels of these two hemostatic markers during the acute phase suggests that the nature of the altered coagulation and fibrinolytic state appears to be different depending on stroke pathophysiology. The molecular markers of hemostasis measured in our study suggest that they might be useful for acutely identifying cardioembolic patients from other ischemic stroke subtypes.

Recurrent embolization frequently occurs during the first few weeks after the onset of cardiogenic brain embolism, especially within 2 weeks, but the pathophysiology is uncertain. We previously reported that patients at high risk for recurrence have greater degrees of coagulation and fibrinolytic disorders than low-risk patients at the time of initial embolization. The persistence of hypercoagulable state during the

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Serial changes in antithrombin III activity (○) and antithrombin III antigen (●) in cardioembolic stroke measured on day 1 of hospitalization (within 48 hours), day 7, and day 30 of onset. Values are mean±SEM. *p<0.05, different from the controls by Scheffe's method.

![Figure 2](http://stroke.ahajournals.org/)

**Figure 2.** Thrombin-antithrombin III complex (TAT; open column) and crosslinked d-dimer (XDP; dashed column) in patients with cardioembolic stroke (panel A), atherothrombotic stroke (panel B), and lacunar stroke (panel C) examined on day 1 of hospitalization (within 48 hours), day 7, and day 30 of onset, and in age-matched controls. Values are mean±SEM. **p<0.01, *p<0.05, respectively, different from the controls by Scheffe's method.
first few weeks after onset in the present study may contribute to the early recurrent embolization.

The mean value of the thrombin–antithrombin III complex in atherothrombotic and lacunar stroke did not differ from the controls. Thus, thrombin generation in atherothrombotic stroke was not excessive at onset. If thrombin generation occurs at the site of an atherosclerotic plaque or within the penetrating artery, it may be difficult to detect the presence of local thrombi in situ by measuring coagulation parameters in the peripheral venous blood. It may be argued that a difference in the size of thrombi has caused such a difference in the level of coagulation parameters. However, this cannot fully explain the results because serial changes of hematologic markers in each subtype were different. In our previous study, a majority of patients with atherothrombotic or lacunar stroke had evidence of plasma hypercoagulability, which was partly related to an increase in factor VII activity. We suggested that the plasma in these patients had a hypercoagulability profile as one of the risk factors for the prethrombotic state. In the present study, the serial change in the plasma concentration of crosslinked d-dimer showed that fibrinolytic response was present immediately after onset in cardiogenic stroke but somewhat later in atherothrombotic stroke. Impaired fibrinolytic activity has been reported in various systemic atherothrombotic disorders. It is possible that thrombotic process develops on atherosclerotic plaques as a consequence of local activation of coagulation, which easily may be brought about by some unknown stimuli under the prethrombotic state. Such phenomena may also be accelerated by endogenous fibrinolytic insufficiency or plasma hypercoagulability during acute stage of atherothrombotic stroke.

The importance of antithrombin III as a major physiological inhibitor of the coagulation mechanism is documented by the high incidence of thromboembolic events in patients with congenital antithrombin III deficiency. Within 48 hours after cardioembolic stroke, antithrombin III antigen was not significantly different from controls, despite a significant decrease in antithrombin III activity and an increase in thrombin–antithrombin III complex. This suggests that the coagulopathy in acute cardioembolic stroke is not the result of a decrease in the amount of antithrombin III, as observed in antithrombin III deficiency, but because of a consumption coagulopathy caused mainly by brisk thrombin generation within the heart.

### Table 3. Sensitivity and Specificity of Molecular Markers Measured Within 48 Hours After Onset (Day 1) for Identifying Cardioembolic Stroke From Atherothrombotic and Lacunar Stroke

<table>
<thead>
<tr>
<th>Markers</th>
<th>Cut-off value (ng/ml)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin-antithrombin III complex</td>
<td>5</td>
<td>15/17 (88.2)</td>
<td>31/37 (83.8)</td>
</tr>
<tr>
<td>Crosslinked d-dimer</td>
<td>300</td>
<td>12/15 (80.0)</td>
<td>30/39 (76.9)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages.

The immunologically measured value of antithrombin III may be close to the sum of free antithrombin III and antithrombin III–protease complexes in this study, both activity and antigen of antithrombin III in atherothrombotic stroke and lacunar stroke did not differ from the controls at any stage, suggesting that antithrombin III activity is not related to a prethrombotic state in most such stroke patients. Patients with acute ischemic stroke with low antithrombin III activity probably are cardioembolic.

Hematologic parameters in patients with lacunar stroke were not much different from those in the controls throughout the study and were consistent with the previous studies. The pathophysiology of lacunar infarction may differ from atherothrombotic stroke in that ischemia is caused by obstruction of small penetrating arteries in the presence of lipohyalinosis or microatheroma. We should search other pathogenetic mechanisms of stroke when significant hematologic disorders are detected in a patient with so-called lacunar syndrome.

In conclusion, coagulation and fibrinolytic parameters differ in relationship to stroke subtype. It may be difficult to distinguish subtypes of stroke on clinical features alone, and our study suggests that thrombin–antithrombin III complex or crosslinked d-dimer can be used acutely as markers for the differentiation of cardioembolic stroke from the other two stroke subtypes. Screening of the prethrombotic state using such sensitive hemostatic molecular markers may be useful for the assessment and identification of stroke subtypes.

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### References


**KEY WORDS** • antithrombin III • blood coagulation disorders • cerebral ischemia
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