Clinical Significance of New Coagulation and Fibrinolytic Markers in Ischemic Stroke Patients

Noriko Ono, Tetsuji Koyama, Akira Suehiro, Ken-ichi Oku, Kunihiko Fujikake, and Eizo Kakishita

Background and Purpose: We investigated plasma levels of D-dimer products of crosslinked fibrin degradation products, thrombin-antithrombin III complex, and plasmin-α2-antiplasmin complex for detecting coagulation system activation in ischemic stroke patients to determine the possible effect of age on these marker levels.

Methods: We measured plasma levels of these three markers in 54 acute ischemic stroke patients within 5 days of stroke onset, in 44 chronic ischemic stroke patients over 3 months from onset, and in 50 age-matched healthy subjects. We divided the stroke patients into two subgroups, those with visible occlusion and those with nonvisible occlusion having obstruction of the major cerebral artery.

Results: The plasma levels of these three markers were significantly (p<0.01) higher in the stroke patients than in controls. Significant differences did not exist at any level between the patients and controls in the younger-aged subjects (<64 years of age), but did exist in the older-aged subjects (>75 years of age). An age-related increase of the marker levels was noted between stroke patients and controls. No significant difference in the three markers was found among any of the stroke patients.

Conclusions: Increased levels of these markers in stroke patients seem to be related mostly to age. (Stroke 1991;22:1369–1373)

Acute ischemic stroke is caused mainly by atherosclerotic or thromboembolic disorders in the cerebral circulation. Detecting and monitoring the coagulation system activation in acute ischemic stroke with conventional laboratory tests are difficult because the tests lack adequate sensitivity and specificity. However, some tests use specific intermediate breakdown products (fibrinopeptide Bβ 15-42) of fibrin polymer formation or D-dimer products (D-dimer) of crosslinked fibrin degradation products to detect coagulation and fibrinolytic system activation in thrombotic disorders as parameters of thrombin and plasmin generation.

Recently, the generation of thrombin and plasmin has been quantitatively measured by enzyme-linked immunosorbent assay (ELISA) using the thrombin-antithrombin III complex (TAT) and the plasmin-α2-antiplasmin complex (PAP) in venous thrombosis and disseminated intravascular coagulation.

In this study, we investigated the usefulness of these markers for detecting coagulation system activation in ischemic stroke patients. We then tried to determine which of the following had the greatest effect on these marker levels: risk factors, age, acute vascular endogenous response, and visibility of the occlusion on angiography.

Subjects and Methods

We divided 98 patients with ischemic cerebrovascular stroke into two groups based on neurological signs and findings from brain computed tomography (CT), magnetic resonance imaging (MRI), and cerebral angiography. The acute cerebral infarction group consisted of 54 patients (30 men and 24 women, mean±SD age 69.5±13.3 years) who had been admitted to Hanwa Memorial Hospital between September 1989 and May 1990. The second group consisted of 44 patients with old cerebral infarcts on brain CT scan who had been in stable condition for >3 months from stroke onset.

The first group of acute stroke patients were diagnosed by the presence of new infarct lesions on...
brain CT or MRI. These 54 patients were divided into two subgroups. The visible occlusion subgroup consisted of those having obstruction of the major cerebral artery and its branches accompanied by large infarcts at the territory of their circulation. This subgroup consisted of 29 patients (18 men and 11 women, 68.4±14.2 years), six with internal carotid artery occlusion, 18 with middle cerebral artery occlusion, three with basilar artery occlusion, one with superior cerebellar artery occlusion, and one with posterior cerebral artery occlusion. The second subgroup consisted of those patients with nonvisible occlusion who showed only small infarcts of the territory of the small perforators of the internal carotid system on brain CT and MRI. This subgroup consisted of 25 patients (12 men and 13 women, 70.9±12.4 years) with no occlusion of the major arteries on cerebral angiography. Nine patients from the visible occlusion subgroup and four from the nonvisible occlusion subgroup had atrial fibrillation. Other complications are listed in Table 1. Venous blood was drawn from the acute stroke patients for laboratory tests before angiography within 5 days of stroke onset (on the day of admission) to avoid the effects of anticoagulant and thrombolytic therapy.

The second group of 44 chronic stroke patients was also divided into two subgroups as for the acute stroke patients. One subgroup consisted of 19 patients (13 men and six women, 71.4±9.6 years) with large infarcts due to major artery occlusion and the other of 25 patients (13 men and 12 women, 68.2±11.8 years) with small infarcts without major artery occlusion. Their complications are listed in Table 1. Eighteen of these patients received antiplatelet agents, but no anticoagulant and thrombolytic therapy 2 months before blood sampling.

The control group consisted of 50 healthy volunteers averaging 66.0±11.0 years (32 men and 18 women).

Blood samples obtained from the patients and control groups were mixed with one-tenth volume 3.8% sodium citrate anticoagulant, then centrifuged at 1,000g at 10°C for 15 minutes. The supernatant fractions were stored at −80°C. Blood samples for the D-dimer were collected into tubes containing 2,000 units heparin and 4,000 KIU aprotinin.

The plasma levels of D-dimer, TAT, and PAP were measured by ELISA using a Dimertest EIA kit (AGEN Biomedical Corp., Brisbane, Australia) for D-dimer, Enzaiest EIA kit (Behring Werke Corp., Marburg, FRG) for TAT, and α2-PI complex EIA kit (Teijin Corp., Tokyo, Japan) for PAP. Prothrombin time, activated partial thromboplastin time, and plasma level of fibrinogen were measured by conventional methods, and the serum levels of fibrin degradation products were measured by latex agglutination assays.

Blood test results are given as mean±SEM, and statistical analyses were performed by analysis of variance for unpaired values between groups.

### Results

The plasma levels of D-dimer, TAT, and PAP in 50 healthy persons were 109.0±9.5 ng/ml, 2.94±0.42 ng/ml, and 0.63±0.05 μg/ml, respectively. The plasma levels of these markers in all stroke patients (n=98) were significantly (p<0.01) higher than those in the control group (Table 2). The D-dimer level in the older-aged (≥75 years) control group was significantly (p<0.05) higher than in the middle-aged (65–74 years) control group. However, TAT and PAP levels for the control group were not significantly different in each age group. Among all stroke patients, the D-dimer level in the older-aged group was significantly (p<0.01) higher than that in the younger-aged (≤64 years) group, but not significantly different from that in the middle-aged group. The TAT and PAP levels in the older-aged group were significantly (p<0.01 and p<0.01, respectively)

### Table 1. Clinical Findings in Acute and Chronic Stroke Patients and Healthy Controls

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>Previous stroke</th>
<th>Neurological deficits</th>
<th>Sampling time from onset</th>
<th>Infarct lesions on CT or MRI</th>
<th>Major arterial occlusion</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute stroke</td>
<td>54</td>
<td>69.5±13.3</td>
<td>-</td>
<td>+</td>
<td>≤5 days</td>
<td>+</td>
<td>-</td>
<td>13, 19, 35, 12</td>
</tr>
<tr>
<td>Visible occlusion</td>
<td>29</td>
<td>68.4±14.2</td>
<td>-</td>
<td>+</td>
<td>≤5 days</td>
<td>+</td>
<td>-</td>
<td>9, 16, 5</td>
</tr>
<tr>
<td>Nonvisible occlusion</td>
<td>25</td>
<td>70.9±12.4</td>
<td>-</td>
<td>+</td>
<td>≤5 days</td>
<td>-</td>
<td>4, 10, 19, 7</td>
<td></td>
</tr>
<tr>
<td>Chronic stroke</td>
<td>44</td>
<td>69.6±10.9</td>
<td>+</td>
<td>+</td>
<td>&gt;3 months</td>
<td>+</td>
<td>-</td>
<td>7, 19, 21</td>
</tr>
<tr>
<td>Visible occlusion</td>
<td>19</td>
<td>71.4±9.6</td>
<td>+</td>
<td>+</td>
<td>&gt;3 months</td>
<td>+</td>
<td>5, 9, 6, 10</td>
<td></td>
</tr>
<tr>
<td>Nonvisible occlusion</td>
<td>25</td>
<td>68.2±11.8</td>
<td>+</td>
<td>+</td>
<td>&gt;3 months</td>
<td>-</td>
<td>2, 10, 13, 11</td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td>50</td>
<td>66.0±11.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>...</td>
</tr>
</tbody>
</table>

CT, computed tomography; MRI, magnetic resonance imaging; AF, atrial fibrillation; DM, diabetes mellitus; HT, hypertension; HL, hyperlipidemia; -, no; +, yes.

### Table 2. Comparison of Plasma Levels of D-dimer, TAT, and PAP Between Healthy Controls and All Stroke Patients

<table>
<thead>
<tr>
<th>Marker</th>
<th>All stroke patients (n=98)</th>
<th>Healthy controls (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer (ng/ml)</td>
<td>237.1±18.5*</td>
<td>109.0±9.5</td>
</tr>
<tr>
<td>TAT (ng/ml)</td>
<td>6.98±0.72*</td>
<td>2.94±0.42</td>
</tr>
<tr>
<td>PAP (μg/ml)</td>
<td>1.00±0.05*</td>
<td>0.63±0.05</td>
</tr>
</tbody>
</table>

Values are mean±SEM. D-dimer, D-dimer products; TAT, thrombin–antithrombin III complex; PAP, plasmin–α2-antiplasmin complex. *p<0.01 different from control by analysis of variance.
higher than those in the younger-aged group, but not significantly different from those in the middle-aged group for all stroke patients (Table 3). Considerable together, the D-dimer, TAT, and PAP levels of all stroke patients were significantly higher than those of the healthy controls in both the middle-aged and older-aged groups (p<0.01, p<0.05, and p<0.01, respectively, for both age groups) (Table 3). The difference of each marker level among the patients and control groups increased with age.

Although the D-dimer level of the visible occlusion subgroup was slightly higher than that of the nonvisible occlusion subgroup in acute stroke, there were no differences in TAT and PAP of both subgroups (Table 4). There was also no difference between the two age-matched groups (data not shown). In patients with old infarctions, there was no significant difference in these markers between the visible occlusion and nonvisible occlusion subgroups for chronic stroke patients (data not shown).

Although the D-dimer, TAT, and PAP levels were not significantly different between the age-matched acute and chronic stroke groups, the levels of the three markers increased with age in the acute stroke group, and the D-dimer and PAP levels increased with age in the chronic stroke group (Table 5).

The data for stroke patients and healthy persons did not differ significantly for prothrombin time, activated partial thromboplastin time, fibrinogen level, or level of fibrin degradation products (data not shown).

### Discussion

The average of each marker level of 50 healthy persons age-matched with stroke patients in this study was higher than the normal level previously reported for young, healthy persons. These marker levels increased slightly with age even in healthy persons in our study. In the stroke patients, all three markers were significantly higher than those of age-matched healthy controls, and those of the older-aged group were significantly higher than those of the younger-aged group. This age-related increase in the plasma level of the three markers agrees with the findings reported by Bauer et al. Furthermore, we noted that the difference of each marker level between patients and healthy persons increased with age.

Many stroke patients have some risk factors, such as diabetes mellitus, hyperlipidemia, hyperuricemia, obesity, hypertension, heavy smoking, advanced age, and atrial fibrillation. The plasma D-dimer level is known to increase in patients with atrial fibrillation, and TAT and PAP levels are reported to increase in patients with diabetes mellitus. As stroke patients simultaneously have many risk factors, it is very difficult to establish whether changes in the markers are due to the risk factors or the thromboembolism itself.

To avoid the effects of anticoagulant and thrombolytic therapy, as well as recanalization, we obtained only one sample before angiography to measure the marker levels in acute stroke patients. Therefore, the changes of marker levels in our data for these patients should show little effect of angiography, therapy, or recanalization and should reflect the influence of risk factors, onset mechanism, acute vascular endogenous response, and infarct size in thromboembolism.

There was no significant difference in the three marker levels between the visible and the nonvisible occlusion subgroups in acute stroke patients. The number of risk factors was not very different between the two subgroups (Table 1). Except for age, these risk factors in themselves do not seem to cause differences in the marker levels between the subgroups, although they might be closely correlated to the onset mechanism in acute stroke. Major artery occlusion is caused mainly by emboli from sources in the left atrium, with fibrillation and localized arteriosclerotic lesions. On the other hand, a lacunar infarction is caused mainly by thrombosis produced by

### Table 3. Comparison of Plasma Levels of D-dimer, TAT, and PAP With Aging for Healthy Controls and All Stroke Patients

<table>
<thead>
<tr>
<th>Marker</th>
<th>≤64 years (n=28)</th>
<th>65-74 years (n=29)</th>
<th>≥75 years (n=41)</th>
<th>≤64 years (n=17)</th>
<th>65-74 years (n=23)</th>
<th>≥75 years (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer (μg/ml)</td>
<td>144.5±19.9</td>
<td>226.3±37.1*</td>
<td>306.0±29.2*</td>
<td>114.2±21.5</td>
<td>91.1±9.8</td>
<td>141.5±19.3*</td>
</tr>
<tr>
<td>TAT (ng/ml)</td>
<td>4.24±0.78</td>
<td>6.24±0.95†</td>
<td>9.31±1.34‡</td>
<td>2.65±0.46</td>
<td>2.92±0.60</td>
<td>3.41±0.62</td>
</tr>
<tr>
<td>PAP (μg/ml)</td>
<td>0.70±0.07</td>
<td>1.13±0.21§</td>
<td>1.17±0.08‡</td>
<td>0.52±0.06</td>
<td>0.66±0.07</td>
<td>0.71±0.16</td>
</tr>
</tbody>
</table>

Values are mean±SEM. D-dimer, D-dimer products; TAT, thrombin-antithrombin III complex; PAP, plasmin-α2-antiplasmin complex.

* p<0.01, † p<0.05 different from healthy controls by ANOVA.

§ p<0.01, ‡ p<0.05 different from younger-aged stroke group by ANOVA.

† p<0.05 different from middle-aged healthy controls by ANOVA.

### Table 4. Comparison of Plasma Levels of D-dimer, TAT, and PAP Between Visible and Nonvisible Occlusion Groups in Acute Stroke Patients

<table>
<thead>
<tr>
<th>Marker</th>
<th>Visible occlusion (n=29)</th>
<th>Nonvisible occlusion (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer (μg/ml)</td>
<td>302.0±46.3</td>
<td>227.0±27.0</td>
</tr>
<tr>
<td>TAT (ng/ml)</td>
<td>8.08±1.45</td>
<td>7.53±1.47</td>
</tr>
<tr>
<td>PAP (μg/ml)</td>
<td>1.05±0.11</td>
<td>0.96±0.08</td>
</tr>
</tbody>
</table>

Values are mean±SEM. D-dimer, D-dimer products; TAT, thrombin-antithrombin III complex; PAP, plasmin-α2-antiplasmin complex.
general arteriosclerosis. The fact that there was little difference in the marker levels between the visible and nonvisible occlusion subgroups suggests that different onset mechanisms in the formation of thromboemboli would not cause differences in the marker levels in acute stroke. Acute thromboembolism directly damages the vascular endothelium, which activates the coagulation and fibrinolytic system, and is quickly followed by the release of some vasoactive substances such as adrenaline, noradrenaline, vasopressin, and adenosine diphosphate, which intensify vasoconstriction and platelet aggregability. Therefore, the vascular endogenous response itself may activate the coagulation and fibrinolytic system and affect the marker levels. However, there were few differences in the marker levels due to acute vascular endogenous response related to the presence or absence of visible occlusion. Infarct size in brain CT scan was apparently larger in the visible occlusion subgroup than in the nonvisible occlusion subgroup, although exact measurements were not possible due to edema and hemorrhage in the infarct area, use of thrombotic therapy, and differing periods from stroke onset.

Feinberg et al. reported that the D-dimer level gradually increased and peaked at 2 weeks after the onset of acute stroke. Blood–brain barrier breakdown, observed by contrast-enhancement CT21,22 and radioisotope scanning,24–26 often reaches its maximum at 2–4 weeks after stroke onset. Infarct-damaged brain tissue itself might activate blood coagulation after leakage of brain tissue components into the vascular system. To avoid the effect by angiography, drug therapy, and recanalization, we obtained only one sample per patient before angiography and within 5 days of stroke onset. Therefore, the marker levels in our samples probably were affected very little by infarction. Examination of the changes of marker levels in the visible and nonvisible occlusion subgroups showed no differences related to different onset mechanisms in acute stroke patients.

The lack of difference among the three marker levels in acute and chronic stroke patients suggests that these levels might be changed very little by the acute vascular endogenous response. The differences in D-dimer and TAT levels between acute and chronic stroke patients increased in the older-aged group, suggesting that age-related changes might mask the acute vascular endogenous response.

In conclusion, the levels of D-dimer, TAT, and PAP in acute stroke patients were strongly affected by age rather than by an acute vascular endogenous response to stroke or visible occlusion on cerebral angiography.

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References


TABLE 5. Comparison of Plasma Levels of D-dimer, TAT, and PAP Between Acute Stroke Patients and Chronic Stroke Patients

<table>
<thead>
<tr>
<th>Marker</th>
<th>Acute stroke patients (n=54)</th>
<th>Chronic stroke patients (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤64 years (n=17)</td>
<td>≥75 years (n=25)</td>
</tr>
<tr>
<td></td>
<td>65–74 years (n=12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤64 years (n=11)</td>
<td>≥75 years (n=16)</td>
</tr>
<tr>
<td>D-dimer (ng/ml)</td>
<td>145.8±26.5</td>
<td>255.2±68.6</td>
</tr>
<tr>
<td>TAT (ng/ml)</td>
<td>4.45±1.01</td>
<td>6.52±1.59</td>
</tr>
<tr>
<td>PAP (μg/ml)</td>
<td>0.74±0.10</td>
<td>1.07±0.21</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

Different from chronic younger-aged stroke patients by ANOVA.

*p<0.01, tp<0.05 different from acute younger-aged stroke patients by analysis of variance (ANOVA).

KEY WORDS: blood coagulation • cerebral ischemia • fibrinolysis
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