We assayed plasma concentrations of fibrinogen, fibrinopeptide A, plasmin-\(\alpha_2\) plasmin inhibitor complex, D dimer, and antithrombin III activity in 40 patients with cerebral thrombosis and nine patients with cerebral embolism during the acute (<7 days), subacute (7–27 days), and chronic (>28 days) periods and compared these with 69 controls. In cerebral thrombosis, fibrinogen and fibrinopeptide A levels were elevated significantly in all stages \((p<0.001)\), whereas plasmin-\(\alpha_2\) plasmin inhibitor complex and D dimer levels were elevated significantly in the subacute and chronic periods. The antithrombin III activity was significantly decreased in the acute stage. The elevation of fibrinogen and plasmin-\(\alpha_2\) plasmin inhibitor complex levels in the acute stage was significantly greater in patients with an infarct size greater than 10 mm\(^2\) compared to patients with an infarct size less than 10 mm\(^2\). We observed similar changes in patients with cerebral embolism. These results suggest that enhanced coagulation exists at all stages and endogenous fibrinolysis is activated in the subacute and chronic periods in a large proportion of patients with cerebral thrombosis and embolism. (Stroke 1990;21:1663–1667)
fibrinolytic agents were not administered throughout the study period. None of the patients had received antiplatelet medication before or after the stroke.

We selected the controls from healthy individuals who participated in the annual mass examination in our districts and had not received any drug. Fourteen controls had hypertension. Fifteen patients with stroke had hypertension; four had diabetes mellitus. In both patient and control groups, we excluded subjects with a history of prior stroke, cardiac disease, occlusive peripheral vascular diseases, or hypercholesterolemia. Informed consent was obtained from all patients.

Blood was withdrawn by careful venipuncture into a prechilled tube containing 32 mg/ml sodium citrate for fibrinogen, and a tube containing an anticoagulant solution (500 IU/ml heparin, 10 KIU/ml aprotinin) for fibrinopeptide A, plasmin–α2 plasmin inhibitor complex, and D dimer. The tubes were centrifuged at 2,000g for 15 minutes, and plasma was stored at −20° C until assayed.

Fibrinogen concentration was measured by the thrombin time method. Antithrombin III activity was determined by the two-stage method using the chromogenic substrate H-D-Phe-Pip-Arg-pNA (S-2238)11 (Kabivitrum AB, Mölndal, Sweden). Concentrations of fibrinopeptide A were measured by competitive enzyme immunoassay,12 using Asserachrom FPA (Diagnostica Stago, Asnieres-sur-Seine, France). Plasmin–α2 plasmin inhibitor complex was assayed by an enzyme-linked immunosorbent assay one-step sandwich method (EIA-B; Teijin Ltd., Tokyo), using peroxidase-conjugated monoclonal anti-α2 plasmin inhibitor antibody.13 D dimer levels were measured by an enzyme-linked immunosorbent assay14 (Dimer test EIA, Agen Ltd., Brisbane, Australia).

A computed tomogram (CT scan) was performed on admission and 1 or 2 weeks thereafter. A clearer hypodense area usually was obtained at the second CT scan. Infarct size was calculated based on the maximum length and width of the hypodense area on the horizontal CT films in which the area appeared largest. The infarct size ranged from 0 to 4,028 mm², with a median of 49 mm². Four patients had infarcts larger than 1,000 mm².

Based on the results of assessment for normality of data, we used nonparametric procedures (Wilcoxon rank sum test) to compare data from various groups.

### Results

In patients with cerebral thrombosis (Table 1), levels of fibrinogen and fibrinopeptide A were significantly increased ($p<0.001$) at all periods compared with controls and showed no substantial changes between the periods, although fibrinogen levels were slightly lower in the acute period than in the subacute and chronic periods. Antithrombin III activity was significantly decreased in the acute stage and returned to control values in the subacute and chronic stages. Concentrations of plasmin–α2 plasmin inhibitor complex were not increased in the acute period but were significantly increased in the subacute and chronic periods ($p<0.001$). D dimer concentrations were elevated slightly in the acute period ($p<0.01$), significantly in the subacute period ($p<0.001$), and then decreased slightly in the chronic period.

In patients with embolic stroke (Table 1), fibrinogen levels were not increased in the acute period, but increased progressively in the subacute and chronic periods ($p<0.05$). Fibrinopeptide A levels were elevated in all periods ($p<0.001$). Antithrombin III activity was significantly decreased in the acute stage and returned to control values in the subacute and chronic stages. Plasmin–α2 plasmin inhibitor complex and D dimer levels were elevated slightly in the acute period and then increased in the subacute ($p<0.01$ for plasmin–α2 plasmin inhibitor complex and $p<0.05$ for D dimer) and chronic periods ($p<0.001$ for plasmin–α2 plasmin inhibitor complex and not significant for D dimer).

Concentrations of hemostatic markers in the acute period of cerebral thrombosis were compared between patients with infarct sizes smaller than 10 mm² and larger than 10 mm² (Table 2). We found a more conspicuous elevation of fibrinogen ($p<0.05$), plasmin–α2 plasmin inhibitor complex ($p<0.01$), and D dimer levels (not significant) in patients with larger

### Table 1. Hemostatic Markers in Cerebral Thrombosis and Embolism

<table>
<thead>
<tr>
<th></th>
<th>Fibrinogen (mg/dl)</th>
<th>FPA (ng/ml)</th>
<th>AT-III (%)</th>
<th>PIC (ng/ml)</th>
<th>D dimer (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=69)</td>
<td>261±42</td>
<td>2.0±1.0</td>
<td>90±10</td>
<td>0.8±0.3</td>
<td>100±15</td>
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<tr>
<td>Thrombosis</td>
<td></td>
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</tr>
<tr>
<td>Acute (n=40)</td>
<td>304±63*</td>
<td>14.1±12.5*</td>
<td>82±14†</td>
<td>1.2±1.3</td>
<td>259±281‡</td>
</tr>
<tr>
<td>Subacute (n=27)</td>
<td>344±100*</td>
<td>11.5±12.7*</td>
<td>89±18</td>
<td>1.5±1.0*</td>
<td>444±474*</td>
</tr>
<tr>
<td>Chronic (n=20)</td>
<td>343±71*</td>
<td>15.3±12.7*</td>
<td>92±19</td>
<td>1.4±0.7*</td>
<td>333±244</td>
</tr>
<tr>
<td>Embolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute (n=9)</td>
<td>286±89</td>
<td>6.0±2.4*</td>
<td>75±20†</td>
<td>1.5±1.5</td>
<td>221±71</td>
</tr>
<tr>
<td>Subacute (n=5)</td>
<td>317±35†</td>
<td>20.3±12.6*</td>
<td>86±10</td>
<td>1.4±0.5‡</td>
<td>271±105‡</td>
</tr>
<tr>
<td>Chronic (n=7)</td>
<td>390±169†</td>
<td>10.7±5.7*</td>
<td>87±27</td>
<td>1.6±0.9*</td>
<td>329±183</td>
</tr>
</tbody>
</table>

Values are mean±SD. FPA, fibrinopeptide A; AT-III, antithrombin III; PIC, plasmin–α2 plasmin inhibitor complex.

*‡‡‡p<0.001, 0.05, 0.01 compared with control.
infarcts (Table 2). A similar tendency persisted in the chronic stage (Table 2).

The fibrinogen concentration exceeded the upper 95% confidence level of controls in 6% (one of 16) of patients with an infarct size smaller than 10 mm², and in 42% (10 of 24) of patients with an infarct size larger than 10 mm². The concentration of plasmin–α₂ plasmin inhibitor complex over the upper 95% confidence level of controls was obtained in 0% (zero of 16) of patients with the smaller infarct size and in 33% (eight of 24) of patients with the larger infarct size.

There was no correlation between chronic elevation of hemostatic markers and continued symptoms of cerebral ischemia.

**Discussion**

As previously reported,¹⁵ our study showed that fibrinogen concentrations in the acute period of cerebral thrombosis were significantly increased compared with controls. Although the fibrinogen levels of our patients before the stroke are unknown, the sustained elevation of fibrinogen levels in the subacute and chronic periods may indicate an increased baseline fibrinogen level in patients with stroke. It is already known that an elevated serum fibrinogen concentration is a risk factor for stroke.¹⁵

Fibrinogen is converted to fibrin monomer with liberation of fibrinopeptide A. This process is catalyzed by thrombin and inhibited by antithrombin III, which forms thrombin–antithrombin III complex. The increase of fibrinopeptide A and the decrease of antithrombin III are therefore very sensitive and specific markers of thrombin generation in vivo and of activation of the coagulation system.¹⁶ Low concentrations of fibrinopeptide A in normal subjects reflect a baseline level of thrombin action accounting for a small proportion (3%) of fibrin catabolism.¹⁷ Elevated levels of fibrinopeptide A have been found in patients with thrombotic and inflammatory disorders,¹⁸–²³ including the acute phase of ischemic stroke.¹⁰,²⁴,²⁵ We found a sustained elevation of fibrinopeptide A levels from acute through chronic phases of both thrombotic and embolic stroke. The reduction of antithrombin III activity in the acute stage and its recovery to the control values in the later stages is consistent with previous findings²⁶ and probably reflects the consumption of active antithrombin III in the acute stage.

Activated fibrinolysis in patients with stroke has been indicated by an increase of fragment E,²⁷ an increase of fibrin monomer,¹–⁵ or its soluble complex,²⁸ and a decreased half-life of fibrinogen.²⁹ The degradation of cross-linked fibrin is catalyzed by plasmin. This process is regulated by plasminogen, a precursor of plasmin, and α₂ plasmin inhibitor, which reacts with plasmin to form plasmin–α₂ plasmin inhibitor complex and is degraded in the reticuloendothelial system. Prior results on plasminogen levels in patients with stroke have been controversial; researchers have found levels to be increased,¹–⁵ unchanged,³⁰ and decreased.⁹ Direct measurement of plasmin in plasma is difficult because it is rapidly bound to α₂ plasmin inhibitor to become an enzymatically inactive complex.³¹ Plasmin–α₂ plasmin inhibitor complex is therefore a direct indicator of in vivo plasmin generation. In the present study, concentrations of plasmin–α₂ plasmin inhibitor complex were significantly increased in the subacute and chronic periods, but not in the acute period.

D dimer is the principal breakdown fragment of fibrin, consisting of fragment D moieties of two adjacent fibrin monomers covalently bound by the cross-links between their γ chain remnants.³²–³⁴ In accelerated fibrinolysis, plasmin–α₂ plasmin inhibitor complex and D dimer are increased, and plasminogen and α₂ plasmin inhibitor levels are reduced.³⁵ Significant increase of D dimer levels indicating enhanced fibrin breakdown has been reported in deep vein thrombosis³⁶,³⁷ and unstable angina or acute myocardial infarction.³⁸ We found that D dimer levels increased slightly in patients with stroke in the acute period, increased further in the subacute period, and then decreased in the chronic period.

**Table 2.** Comparison of Hemostatic Markers by Infarct Size

<table>
<thead>
<tr>
<th>Infarct size</th>
<th>Fibrinogen (mg/dl)</th>
<th>FPA (ng/ml)</th>
<th>AT-III (%)</th>
<th>PIC (ng/ml)</th>
<th>D dimer (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute stage</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;10 mm² (n=16)</td>
<td>271±45</td>
<td>14.4±12.1</td>
<td>83±10</td>
<td>0.6±0.1</td>
<td>155±83</td>
</tr>
<tr>
<td>≥10 mm² (n=24)</td>
<td>321±69*</td>
<td>15.6±13.2</td>
<td>86±13</td>
<td>1.6±1.7†</td>
<td>298±352</td>
</tr>
<tr>
<td>Chronic stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 mm² (n=10)</td>
<td>309±63</td>
<td>21.1±13.8</td>
<td>99±17</td>
<td>1.1±0.3</td>
<td>151±57</td>
</tr>
<tr>
<td>≥10 mm² (n=10)</td>
<td>378±60*</td>
<td>9.4±7.8</td>
<td>84±14</td>
<td>1.7±0.8</td>
<td>463±243*</td>
</tr>
</tbody>
</table>

Values are mean±SD. FPA, fibrinopeptide A; AT-III, antithrombin III; PIC, plasmin–α₂ plasmin inhibitor complex.

*p<0.05.
fp<0.01.
Our findings on plasmin–α2 plasmin inhibitor complex and D dimer suggest that fibrinolytic reactions occur later than activation of coagulation. This is supported by the findings of Feinberg et al, who showed that the ratio of fibrinopeptide A to fibrinopeptide B-B1-42, an index of thrombin activity relative to plasmin activity, was elevated in the acute phase and decreased thereafter.

Our results show that the infarct size is significantly related to concentrations of fibrinogen and plasmin–α2 plasmin inhibitor complex in the acute stage of cerebral thrombosis and to concentrations of fibrinogen and D dimer in the chronic stage. We do not know whether the greater elevation of these variables is the cause or result of larger infarcts. However, it does not seem to be simply a reflection of increased tissue injury because only four patients had infarcts larger than 1,000 mm³, which may have induced systemic coagulation–fibrinolysis abnormalities, and because the elevation of fibrinogen and plasmin–α2 plasmin inhibitor levels persisted in the chronic period. It therefore may be possible, at least in some patients, that higher fibrinogen levels may have contributed to generating larger infarcts, which in turn induced greater fibrinolysis.

We found no substantial difference between cerebral thrombosis and embolism in the overall changes of fibrinopeptide A, plasmin–α2 plasmin inhibitor complex, and D dimer concentrations after stroke.

In conclusion, in a substantial proportion of patients with cerebral thrombosis and embolism, enhanced coagulation exists at all stages, and endogenous fibrinolysis is activated in the subacute and chronic periods. The individual variation (standard deviation) of hemostatic markers was much greater in stroke patients than in controls (Table 1), indicating that concentrations of hemostatic markers were increased remarkably in some patients, while unchanged in others. Further studies are needed to determine whether the activated coagulation and fibrinolysis in the chronic period may be indicative of the risk of stroke recurrence.

References

**KEY WORDS** • antithrombin III • cerebral embolism and thrombosis • fibrinogen
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