Hemostatic Markers in Acute Transient Ischemic Attacks

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Background and Purpose: Hemostatic abnormalities have been shown previously in stroke patients. The purpose of this study was to assess the activity of selected parameters of the coagulation system in acute reversible cerebral ischemia.

Methods: We measured fibrinopeptide A, thrombin-antithrombin III, and D-dimer in 36 patients in both the acute (<7 days) and postacute stage (1 and 3 months) after a transient ischemic attack (TIA). The results were compared with those of 20 asymptomatic patients with a history of remote TIA and 65 age- and sex-matched controls.

Results: Mean fibrinopeptide A and thrombin-antithrombin III values were elevated in the acute stage after a TIA (P < .02) compared with levels at 1 month. In contrast, D-dimer was significantly increased at all three time points after the event when compared with remote TIA (P < .05) or control subjects (P < .001). No association could be found between marker levels and clinical outcome or the degree of cervical atherosclerosis as assessed by duplex ultrasonography.

Conclusions: These findings suggest that after acute reversible cerebral ischemia, there is early transient activation of thrombogenesis and ongoing fibrinolysis. (Stroke. 1994;25:282-286.)

Key Words: cerebral ischemia, transient • dimers • fibrinopeptides • hemostatics • thrombin
with a myocardial infarction within the preceding 6 months, unstable angina, or an anginal attack within the previous 2 weeks were also excluded because these conditions have been shown to be associated with abnormal hemostatic marker levels.9,10

The control group comprised age- and sex-matched volunteers who were either hospital employees, spouses of patients followed in the neurology clinic, or patients followed in the clinic without a prior history of cerebrovascular events or symptoms suggestive of cerebral or retinal ischemia. These control individuals were recruited during the study period concurrently to our TIA patients. For comparison, markers were also measured in a group of patients with a history of remote TIA who had been asymptomatic for at least the past year.

Patients in the TIA group had blood drawn for measurement of FPA, D-dimer, and TAT levels at least once within the first 7 days and repeated at 1 month and 3 months after their qualifying event. Patients in both the control and remote TIA groups had a single blood sample drawn for measurement. In addition, all patients had a complete blood count and prothrombin time, partial thromboplastin time, electrolyte, liver, renal, and lipid profiles. Most patients also had an electrocardiogram and, if there was any clinical or electrographic suspicion of cardioembolism, an echocardiogram was done to further rule out a cardiac source. TIA patients also had a CT scan of the brain, reviewed by a neuroradiologist, to rule out a possible new cerebral infarct in the territory of the qualifying event as well as a duplex ultrasound of the cervical arteries to assess the degree of extracranial atherosclerosis.

All patients had blood drawn by the same study nurse (F.B.) to minimize variability in technique. Special care was taken to draw blood atraumatically. For D-dimer and TAT, blood was collected in tubes containing 0.1 vol Na citrate and immediately centrifuged at 3000 rpm for 10 minutes; plasma was then collected and frozen at –70°C until assayed. Assays were done by enzyme-linked immunosorbent assay (ELISA) technique following the specifications supplied with the commercial kits (Asserachrom D-Dimer, Diagnostica Stago, Asnières-sur-Seine, France, and Enzygnost TAT micro, Behring, Marburg, Germany, for D-dimer and TAT, respectively). For FPA, blood was drawn without a tourniquet (to minimize local thrombin activation) and collected in tubes containing an anticoagulant solution supplied with the kit (Asserachrom FPA, Diagnostica Stago, Asnières-sur-Seine, France); it was then processed and assayed in a standardized fashion.

Patients with TIA were treated medically or surgically as judged appropriate by the consultant neurologist. For patients who underwent anticoagulant therapy, angiography, or surgery, only blood samples drawn before the intervention were included in the data analysis. Patients in the TIA group were seen at regular intervals in the neurology clinic. The occurrence of further vascular events defined as either a single or multiple TIAIs, stroke, myocardial infarction, and cardiac or cerebrovascular death were recorded during the follow-up period. For patients with stroke or myocardial infarction during the study period, only blood samples collected before these further vascular events were included in the data analysis.

Statistical Methods

Descriptive statistics for categorical variables are reported as percentages. For continuous variables, means and standard errors are given. Comparisons between the study groups for marker levels were carried out using Wilcoxon rank sum tests. To compare the clinical variables between the TIA, remote TIA, and control groups, χ² tests were performed. Within the TIA group, pairwise Wilcoxon signed rank sum tests were used to assess differences in marker levels over the three time points. Values of P<.05 were not reported as significant.

Results

Thirty-six patients presenting within 7 days after a TIA were entered. Demographic and clinical data from the TIA, remote TIA (n=20), and control (n=65) groups are shown in Table 1. All three groups were similar in respect to age but there were significantly more men in the remote TIA group as compared with both the TIA and control groups (P<.0001 and P<.05, respectively) and ischemic heart disease, which was more frequent in TIA patients than in control subjects (P<.0001). The index event was in the carotid territory (28 hemispheric and 4 retinal) in all but 4 of the TIA patients. Fourteen of these patients reported no history of previous cerebrovascular events whereas 21 of them had already experienced prior TIAIs and 5 had a remote history of ischemic stroke. Twenty-eight of the 36 TIA patients had their first blood sample drawn within 2 days after the onset of symptoms. Nine patients provided a single sample whereas all others provided samples on at least two separate occasions during the first week. CT scans were done at 4 or more days after the index event in 32 of the TIA patients to minimize the risk of missing late-appearing hypodense lesions. Only 3 patients, for logistic reasons, underwent CT scanning within 48 hours of their event and 1 patient with a retinal TIA did not have a CT scan. No infarct
Table 2. Hemostatic Marker Levels

<table>
<thead>
<tr>
<th></th>
<th>FPA, ng/mL</th>
<th>TAT, ng/mL</th>
<th>D-Dimer, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIA group (&lt;7 days)</td>
<td>6.75±1.54*</td>
<td>7.8±2.4†</td>
<td>949±262§</td>
</tr>
<tr>
<td>n=36</td>
<td></td>
<td>n=36</td>
<td>n=36</td>
</tr>
<tr>
<td>TIA group (1 month)</td>
<td>3.29±0.72</td>
<td>6.2±2.5</td>
<td>1196±385§</td>
</tr>
<tr>
<td>n=26</td>
<td></td>
<td>n=27</td>
<td>n=27</td>
</tr>
<tr>
<td>TIA group (3 months)</td>
<td>2.75±0.96</td>
<td>5.3±1.9</td>
<td>751±128§</td>
</tr>
<tr>
<td>n=22</td>
<td></td>
<td>n=22</td>
<td>n=22</td>
</tr>
<tr>
<td>Remote TIA group</td>
<td>4.38±1.78</td>
<td>5.0±1.9</td>
<td>478±62</td>
</tr>
<tr>
<td>n=20</td>
<td></td>
<td>n=20</td>
<td>n=20</td>
</tr>
<tr>
<td>Control group</td>
<td>3.63±0.5</td>
<td>3.7±0.3</td>
<td>434±39</td>
</tr>
<tr>
<td>n=63</td>
<td></td>
<td>n=65</td>
<td>n=65</td>
</tr>
</tbody>
</table>

FPA indicates fibrinopeptide A; TAT, thrombin-antithrombin III; and TIA, transient ischemic attack. Values represent mean±SEM.

*P<.02 compared with TIA group at both 1 month and 3 months.
†P<.05 compared with control group.
‡P<.02 compared with TIA group at 1 month.
§P<.001 compared with control group.
||P<.05 compared with remote TIA group.

was seen on CT in 29 patients while old infarcts in a different vascular territory from the current TIA were present in 6 patients. At the time of the initial blood collection, all 36 TIA patients were taking antiplatelet drugs (29 on aspirin, 4 on aspirin and dipyridamole, and 3 on ticlopidine) and all 20 remote TIA patients were taking aspirin. None of the control patients were taking antiplatelet or nonsteroidal anti-inflammatory drugs.

During follow-up, 2 TIA patients received Coumadin and 1 underwent carotid angiography followed by endarterectomy and were thus excluded from further analysis.

Table 2 summarizes FPA, d-dimer, and TAT levels obtained in the TIA, remote TIA, and control groups. FPA levels were found to be significantly elevated in the acute (<7 days) but not the postacute phase (1 month and 3 months) after a TIA as compared with values in the control group (P<.05) (Fig 1). No significant differences were demonstrated between the TIA and remote TIA groups, but within the TIA group FPA was significantly elevated in the acute phase as compared with levels at 1 month and 3 months after the event (P<.02 each). These data suggest that FPA is elevated acutely after a TIA and subsequently decreases to baseline levels by 1 month after the event. Levels for TAT, another marker of thrombin activation, were also elevated in the acute phase after TIA as shown by higher levels at <7 days than at 1 month (P<.02). No other significant differences could be shown for TAT levels between the TIA group and either the remote TIA or control groups.

In contrast, d-dimer levels were found to be elevated in TIA patients in both the acute and postacute phases (<7 days, 1 month, and 3 months) after the index event as compared with values in the control group (P<.001). It was also elevated at 1 and 3 months after the event compared with the remote TIA group (P<.05) (Fig 2). No difference could be shown between levels at the three time points within the TIA group. None of the three markers were significantly different between the remote TIA and control groups.

During the follow-up period (mean, 13 months; range, 4 to 31 months), 18 TIA patients remained asymptomatic, 12 had recurrent TIAs, 3 had an ischemic stroke, 1 patient had a TIA followed by a stroke, and 2 died from cardiac-related causes. We did not find any significant differences for any of the three markers when comparing the 18 patients who remained symptom free with the 18 patients who had vascular events during the follow-up period. In addition, no association could be found between the results of cervical ultrasound (stenosis ≥50% compared with <50%) and marker levels in TIA patients.

Discussion

The primary goal of this study was to determine if abnormalities in levels of hemostatic markers were...
ischemic stroke. In this regard, patients with TIAs than those recently reported in patients with acute control groups. FPA levels have also been shown to be
elevation may play a role in acute reversible cerebral
ischemia. This is further supported by the absence of
visible ischemic lesions on CT scan. For these reasons, TIAs may represent a better model for studying the potential role of hemostatic abnormalities in certain subgroups of patients with cerebral ischemia. Vicari et al. reported elevated FPA levels in patients with TIAs but their study group was made up of only 12 patients and levels were not measured acutely (mean time of sampling, 4.5 weeks). In the few other series studying exclusively patients with TIA, newer, more sensitive ELISA-based assays were not yet available. Marra et al. found an elevation of factor VIII in their TIA patients, but testing was carried out at least 8 weeks after the event and FPA, TAT, and D-dimer were not measured. Fisher and Francis reported elevated D-dimer levels in their stroke but not in their TIA patients, and no elevation in FPA levels was found in either group. This is at variance with our findings. Possible explanations for these differences include younger average age, use of anticoagulants in some patients, small sample size, and different timing of blood sampling in the Fisher and Francis study. Other groups have also included patients with TIA in their series, however, no specific information or separate analysis was provided for this subgroup. In our study, we have found that abnormalities of sensitive markers of blood coagulation exist in some patients with acute reversible cerebral ischemia.

The most striking abnormalities were found for FPA, an oligopeptide released from the a chain of fibrinogen during its proteolytic cleavage by thrombin. In this regard, FPA is a marker of thrombosis since it reflects thrombin activity during the first step leading to production of cross-linked fibrin from fibrinogen. We found FPA levels to be significantly elevated in the acute phase after a TIA as compared with an age- and sex-matched control group. Indeed, the most prominent difference between the two groups was the actual cerebrovascular event itself (since the groups had relatively similar clinical profiles), suggesting that thrombin activation may play a role in acute reversible cerebral ischemia. This is further supported by the absence of any significant elevation in FPA at 1 month and 3 months after the vascular event when values decreased to levels comparable to both the remote TIA and control groups. FPA levels have also been shown to be elevated in other disease states where acute thrombosis is thought to play a role, such as completed stroke, unstable angina, and acute myocardial infarction. The levels we obtained in acute TIA are somewhat lower than those recently reported in patients with acute ischemic stroke. In this regard, patients with TIAs may be comparable to those with reversible coronary
ischemia, such as unstable angina, where FPA levels were also found to be elevated in the acute period. Despite these reports of thrombin activation in acute thrombotic conditions such as cerebral and coronary ischemia and its temporal association with the TIAs in our study, the precise role of thrombin activation in these clinical events is still open to question. Indeed, we are not aware of any definitive experimental or clinical evidence showing a primary role for thrombin generation in the pathogenesis of these conditions.

TAT levels were also found to be elevated in the acute (<7 days) phase after a TIA as compared with levels at 1 month after the event. It is thought that antithrombin III (AT-III) acts by neutralizing activated thrombin resulting in the formation of TAT complexes. This creates a neoantigen that can be measured by ELISA. Recently, several groups have reported either increases in TAT or decreases in AT-III in patients with completed stroke. It remains unclear whether this so-called hypercoagulable state antedates deep vein thrombosis, and disseminated intravascular coagulation. D-Dimer is a byproduct of the digestion of cross-linked fibrin by plasmin. It is thought to be a marker of fibrinolysis as reflected by plasmin activity. Elevated D-dimer levels throughout the sampling period might suggest a prolonged dynamic state of increased thrombus formation and breakdown. Alternatively, it might signal an elevation of non-thrombus-associated "soluble fibrin" serving as an independent substrate for proteolysis by plasmin and perhaps reflecting a hypercoagulable state as suggested by Marnder. The design of our study does not allow us to confirm whether this so-called hypercoagulable state antedates or is a consequence of the TIA.

Differences in the distribution of ischemic heart disease and hypertension between the TIA and control groups raise the possibility that these variables may play a confounding role. Four arguments can be made against this possibility. First, to our knowledge, there is no convincing evidence from the available literature to indicate that hypertension or stable ischemic heart disease significantly affects the marker levels that we measured. Second, the transient nature of the elevation for both FPA and TAT, in conjunction with the TIA (only elevated at <7 days), argues against a major role for these risk factors that did not change over time in TIA patients. This was not the case for D-dimer, which remained elevated throughout the study period in the TIA group, raising the possibility that differences in group characteristics were responsible. Nevertheless, patients with remote TIAs, who had a risk-factor profile almost identical to our acute TIA group, had d-dimer levels that were not elevated compared with the control group, indicating that levels eventually decrease despite
this prolonged initial elevation. Third, the absence of elevation of any of these markers in the remote TIA group, despite also having a significantly higher prevalence of hypertension than the control group, does not support a confounding role for this risk factor. Finally, a statistical analysis to assess the association between hypertension or ischemic heart disease with FPA, TAT, and d-dimer levels showed no significant differences for any of these marker levels in our control group.

The potential of these hemostatic markers as prognostic indicators could not be shown in this study, suggesting a lack of association between marker levels (presumably a reflection of the activity of the coagulation system) and the likelihood of future vascular events. Nevertheless, with such small patient numbers in each subgroup and relatively short follow-up period, it may be premature to conclude that no association exists.

At the time of entry, the great majority of TIA patients were taking aspirin, which has potent antiplatelet activity, but little is known about its effect on the levels of hemostatic markers considered here. Hampton et al.19 looked at 49 patients with remote TIAs who had been assigned either aspirin or placebo and found lower FPA levels in the aspirin group. Szczeklik et al.20 have shown that FPA levels measured in blood emerging from skin incisions were significantly lower 2 hours after a single 500-mg dose of aspirin. These data suggest that aspirin may have, if anything, attenuated the rise in FPA found in our TIA group. As for TAT and d-dimer levels, Franke et al.21 in a recent study of acute stroke patients, could show no difference between those who were or were not receiving aspirin.

Finally, although we have shown that significant abnormalities in hemostatic marker levels are present transiently in patients with TIAs, confirmation by other groups in larger numbers of patients will be necessary to further define the role of thrombin activation and fibrinolysis in the pathogenesis of reversible cerebral ischemia as well as the clinical utility of these laboratory tests in both diagnosis and prognosis.

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References

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