Hematogenous Factors and Prediction of Delayed Ischemic Deficit After Subarachnoid Hemorrhage

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Background and Purpose: Delayed ischemic deficits contribute to the high morbidity and mortality rates associated with subarachnoid hemorrhage. We evaluated the potential usefulness of measuring coagulation and hemorheological variables and cardiolipin antibodies for prediction of delayed ischemic deficit after subarachnoid hemorrhage.

Methods: Consecutive patients with subarachnoid hemorrhage were studied. Coagulation and hemorheological variables and cardiolipin antibodies were measured on admission, within 7 days of subarachnoid hemorrhage. A subset of patients was studied on admission and at two subsequent occasions.

Results: Sixty-nine patients were studied. Sixty-one of these were without clinical manifestations of vasospasm at admission, and 16 developed delayed ischemic deficit during their hospitalization. None of the laboratory variables measured were significantly different between patients with or without later development of delayed ischemic deficit. Elevation of the fibrin fragment D-dimer was found in the group of eight patients admitted with ischemic symptoms and in 49% (34 of 69) of all patients, but this was not associated with delayed ischemic deficit. Sixteen patients were studied on three occasions; this group showed a significant decrease in hematocrit, an increased white blood cell count, and no change in fibrinogen concentration. Fibrin D-dimer levels rose significantly after surgery (from 5.01±0.69 to 5.53±0.58 In-ng/ml, p<0.025) and after onset of delayed ischemic deficit (from 4.71±0.64 to 5.84±0.34 In-ng/ml, p<0.01).

Conclusions: Hemostatic measurements, hemorheological variables, and cardiolipin immunoreactivity did not predict delayed ischemic deficit in this population. (Stroke 1992;23:1404-1409)

KEY WORDS • blood coagulation disorders • cerebral infarction • fibrinogen • subarachnoid hemorrhage
static mechanisms.\textsuperscript{18-21} An extensive hemorrheological evaluation was also performed: whole blood viscosity, plasma viscosity, red blood cell aggregation, and red blood cell deformability were measured. We report herein that these hematogenous factors do not predict delayed ischemic deficit after SAH.

**Subjects and Methods**

In this prospective study, conducted in accordance with guidelines from the University of Southern California Research Committee, we evaluated consecutive patients with SAH admitted to the Neurosurgical Intensive Care Unit at Los Angeles County–University of Southern California Medical Center. Diagnosis of acute SAH was made based on clinical presentation, with confirmation by hemorrhagic or xanthochromic cerebrospinal fluid, and/or subarachnoid blood on brain computed tomographic (CT) scan or magnetic resonance imaging (MRI). We excluded patients with traumatic SAH (n=5), renal failure (n=1), and pregnancy (n=1); we also excluded two patients with an initial diagnosis of SAH who subsequently had angiographic evidence of arteriovenous malformations. No patient in this study had recent (less than 1 month) myocardial infarction, deep vein thrombosis, hepatic failure, significant trauma, or evidence of dehydration or sepsis.

All patients were evaluated at admission and received complete physical and neurological examinations. The Hunt and Hess grading system\textsuperscript{22} was used to determine initial severity of SAH, the Glasgow Coma Scale\textsuperscript{23} and Toronto Stroke Scale\textsuperscript{24} were used daily to quantify changes in neurological status, and the Glasgow Outcome Scale\textsuperscript{25} was used to determine disability at discharge. Brain CT scan was performed on admission for every patient and repeated as needed. CT scan was used to define the presence of hydrocephalus; cerebral angiography was performed for all patients, with the exception of some with Hunt and Hess grades 4 and 5. Patients routinely received 3–5 I intravenous fluids daily. Beginning with the eighteenth patient entered into the study, the calcium channel antagonist nimodipine was used from time of admission for every patient at a dose of 60 mg every 4 hours. Dexamethasone was also given to most patients from time of admission (Hunt and Hess grades 3–5) or during the perioperative period (grades 1 and 2).

Patients were evaluated daily for the possible presence of the following: 1) rebleeding, diagnosed by presence of neurological deterioration with evidence of new bleeding on CT scan, MRI, or lumbar puncture; 2) electrolyte disturbance; and 3) delayed ischemic deficit, defined as neurological deterioration (focal neurological signs and/or decreased level of consciousness) in the absence of rebleeding, electrolyte disturbances, or hydrocephalus and confirmed by vasospasm on angiography or infarction on CT scan or MRI.

Initial blood samples for hemostasis and hemorrheological assays were collected from all patients on admission, before initiation of nimodipine and/or dexamethasone treatment and before any major surgical procedure, and within 7 days after SAH. In addition, for 16 of the final 22 patients entered into this study, selected hematologic variables were measured on three occasions during 1–4 days, 5–8 days, and 9–12 days after SAH.

Blood for hemorrheological studies was collected into 5-ml tubes containing 1.5 mg/ml ethylenediaminetetraacetic acid (EDTA), and all hemorrheological analyses were performed within 4 hours after venipuncture. Hematocrit, leukocyte count, and platelet count were measured using an automated hematology analyzer (Minos STX, ABX, Horsham, Pa.). Whole blood viscometry (0.5 and 94.5 sec\textsuperscript{-1}) was performed at 25°C using a small-volume Couette viscometer (model LS-30, Contraves AG, Zurich, Switzerland). Plasma viscosity was measured at 25°C using a rolling ball viscometer (Haake Mess-Technik GmbH, Karlsruhe, FRG). Red blood cell aggregation was measured by the zeta sedimentation ratio technique\textsuperscript{26,27} using a Zetafuge (Coulter Electronics, Hialeah, Fla.). With this method, a 3-minute period of low-gravity centrifugation of blood in a 2-mm i.d. tube causes the erythrocyte-plasma interface to move toward the tube bottom. This interface determines the "hematocrit" of the suspension and is called the zetacrit. The zeta sedimentation ratio is calculated as the ratio of the true hematocrit to the zetacrit times 100 and increases with increasing red blood cell aggregation.\textsuperscript{26,27} Erythrocyte deformability was measured using a red blood cell filtration system, the Cell Transit Time Analyzer.\textsuperscript{28} This instrument measures the individual transit times of 1,000 erythrocytes through cylindrical micropores 5 \( \mu \)m in diameter by 15 \( \mu \)m long by means of a computer-based conductometric system, then averages them to provide a mean value as well as various percentiles of the distribution. Red blood cell deformability and red blood cell velocity, the latter being inversely proportional to transit time, are highly correlated.\textsuperscript{28}

Fibrinogen was measured based on the turbidimetric rate of the formation of fibrin polymer (Du Pont ACA, Wilmington, Del.). Blood for coagulation assays was collected into tubes containing buffered sodium citrate (9 parts blood to 1 part sodium citrate); after centrifugation at 2,000g for 5 minutes, plasma was divided into multiple aliquots and frozen at \(-80^\circ\)C until assayed. Fibrin \( \alpha \)-dimer and PAI-1 antigen were measured in duplicate by enzyme immunoassay (American Diagnostica, Greenwich, Conn.). Free protein S antigen was quantitated by electroimmunodiffusion (American Diagnostica, Greenwich, Conn.) and expressed as percent of control plasma. Plasma thrombomodulin levels were determined by enzyme immunoassay using monoclonal antibodies, as previously described.\textsuperscript{17} Anticardiolipin antibodies were measured by an enzyme-linked immunosorbent assay method, and results were expressed in phospholipid units. The test was standardized using four anticardiolipin antibodies–positive serum samples with known immunoglobulin (Ig) G and IgM phospholipid units; five normal serum samples, as well as five anticardiolipin antibodies–positive serum samples, were run as secondary standards in each assay. In our laboratory, samples with more than 15 IgG phospholipid units or more than 6 IgM phospholipid units are considered positive.\textsuperscript{20} Note that because plasma was used for anticardiolipin antibodies assays in this investigation, possible serum–plasma differences were evaluated. Anticardiolipin antibodies assays were thus performed on both serum and plasma from 34 individuals; assays on plasma versus serum for IgG and IgM isotype varied
Sixty-nine consecutive patients admitted with a diagnosis of SAH were entered into the study (Table 1). Eight patients had ischemic deficits at the time of initial examination and blood sampling; 16 of the remaining patients later developed delayed ischemic deficit. The time from onset of SAH to blood sampling in patients with ischemic symptoms at admission and those who developed delayed ischemic deficit was 2.8±1.2 days and 3.2±1.9 days, respectively. Eighteen patients (26%) had hydrocephalus, four patients (6%) sustained rebleeding, and 12 patients (17%) died. Thirty-nine patients (57%) had a good or moderate recovery, with later development of delayed ischemic deficit. Thrombomodulin levels in SAH patients were roughly comparable with those of normal controls in a previous investigation. 16 Eleven patients had antiphospholipid antibodies; one had increases of both IgG (47 phospholipid units) and IgM (28.5 phospholipid units) levels, and the remainder had only elevated IgM anticardiolipin antibodies. Of the 11 patients with increased PAI-1 levels, one had symptoms of vasospasm on admission, five did not; this trend was not significant.

Sixteen of the final 22 patients entered into this study had fibrin D-dimer levels above the normal range for our laboratory (i.e., >230 ng/ml): six of eight patients with ischemic deficit on admission, six of 16 who developed delayed ischemic deficit, and 22 of 45 without delayed ischemic deficit. Increased fibrin D-dimer was found in patients with ischemic deficit on admission compared with those who did not have delayed ischemic deficit after admission (Table 2). Sixteen percent (11 of 69) of the patients had PAI-1 levels above the normal range (i.e., >48 ng/ml); these 11 patients with increased PAI-1 consisted of two of eight patients with ischemic deficit on admission, four of 16 who developed delayed ischemic deficit, and five of 45 without delayed ischemic deficit. Thrombomodulin levels in SAH patients were roughly comparable with those of normal controls in a previous investigation. 16 Eleven patients had antiphospholipid antibodies; one had increases of both IgG (47 phospholipid units) and IgM (28.5 phospholipid units) levels, and the remainder had only elevated IgM anticardiolipin antibodies. The 11 patients with increased PAI-1 levels, one had symptoms of vasospasm on admission, five developed delayed ischemic deficit, and five did not; this trend was not significant.

A comparison between baseline laboratory data for the 16 subjects with delayed ischemic deficit and the 45 subjects who did not have this complication showed that none of the measured variables were significantly different between the two groups (Table 2). These results are not altered if we analyze as a single group those patients with ischemic symptoms at admission and those with later development of delayed ischemic deficit. Sample size was adequate to detect a 50% difference between groups for fibrinogen, fibrin D-dimer, thrombomodulin, protein S, low- and high-shear whole blood viscosity, plasma viscosity, zeta sedimentation ratio, and transit time with a power of 80% (α=0.05). Cerebral angiography, performed in 61 patients, was normal in four patients. These four patients had an uneventful course, and their hemostatic and hemorheologic profiles did not differ significantly from those of aneurysm patients without delayed ischemic deficit (data not shown).

Forty-nine percent (34 of 69) of patients in this study had fibrin D-dimer levels above the normal range for our laboratory (i.e., >230 ng/ml): six of eight patients with ischemic deficit on admission, six of 16 who developed delayed ischemic deficit, and 22 of 45 without delayed ischemic deficit. Increased fibrin D-dimer was found in patients with ischemic deficit on admission compared with those who did not have delayed ischemic deficit after admission (Table 2). Sixteen percent (11 of 69) of the patients had PAI-1 levels above the normal range (i.e., >48 ng/ml); these 11 patients with increased PAI-1 consisted of two of eight patients with ischemic deficit on admission, four of 16 who developed delayed ischemic deficit, and five of 45 without delayed ischemic deficit. Thrombomodulin levels in SAH patients were roughly comparable with those of normal controls in a previous investigation. 16 Eleven patients had antiphospholipid antibodies; one had increases of both IgG (47 phospholipid units) and IgM (28.5 phospholipid units) levels, and the remainder had only elevated IgM anticardiolipin antibodies. The 11 patients with increased PAI-1 levels, one had symptoms of vasospasm on admission, five developed delayed ischemic deficit, and five did not; this trend was not significant.
TABLE 2. Laboratory Data for Study Population at Baseline

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ischemic symptoms on admission</th>
<th>Delayed ischemic deficit</th>
<th>No delayed ischemic deficit</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>8</td>
<td>16</td>
<td>45</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.3±14.8</td>
<td>49.4±16.5</td>
<td>46.5±14.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>35.2±6.9</td>
<td>37.7±4.8</td>
<td>39.0±6.2</td>
</tr>
<tr>
<td>White blood cells (10³/ml)</td>
<td>14.9±7.1</td>
<td>12.3±4.7</td>
<td>12.9±4.9</td>
</tr>
<tr>
<td>Platelets (10³/ml)</td>
<td>218±59</td>
<td>265±56</td>
<td>262±69</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>382±167</td>
<td>341±90</td>
<td>339±104</td>
</tr>
<tr>
<td>Low-shear blood viscosity (cP)</td>
<td>33.2±17.1</td>
<td>34.1±14.0</td>
<td>37.8±13.5</td>
</tr>
<tr>
<td>High-shear blood viscosity (cP)</td>
<td>5.6±1.7</td>
<td>5.7±0.9</td>
<td>6.1±1.1</td>
</tr>
<tr>
<td>Plasma viscosity (cP)</td>
<td>1.76±0.16</td>
<td>1.66±0.1</td>
<td>1.69±0.15</td>
</tr>
<tr>
<td>Zeta sedimentation ratio</td>
<td>55.4±13.2</td>
<td>54.5±8.8</td>
<td>55.5±8.4</td>
</tr>
<tr>
<td>Red blood cell transit time (msec)</td>
<td>3.11±0.32</td>
<td>2.98±0.32</td>
<td>2.94±0.45</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>36.2±16.6</td>
<td>37.3±33.1</td>
<td>29.4±17.3</td>
</tr>
<tr>
<td>D-dimer (log-transformed ng/ml)</td>
<td>6.11±0.77*</td>
<td>5.32±1.09</td>
<td>5.25±0.90</td>
</tr>
<tr>
<td>Thrombomodulin (ng/ml)</td>
<td>37.3±30.3</td>
<td>29.0±14.2</td>
<td>30.6±9.7</td>
</tr>
<tr>
<td>aCL IgG isotype (%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>aCL IgM isotype (%)</td>
<td>1 (13%)</td>
<td>5 (31%)</td>
<td>5 (11%)</td>
</tr>
<tr>
<td>Free protein S (% control)</td>
<td>69.2±23.3</td>
<td>73.5±23.9</td>
<td>71.5±21.5</td>
</tr>
</tbody>
</table>

Values are mean±SD. Blood and plasma viscosity, zeta sedimentation ratio, and red blood cell transit time were measured for the first 47 patients (six with ischemic symptoms at sampling time, 12 with delayed ischemic deficit, and 29 without delayed ischemic deficit). cP, centipoise; PAI-1, plasminogen activator inhibitor 1; D-dimer, fibrin D-dimer; aCL, anticardiolipin antibodies; Ig, immunoglobulin.

*p<0.05 compared with patients without delayed ischemic deficit.

**Table 3. Data for 16 Patients Sampled Three Times**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Days 1–4</th>
<th>Days 5–8</th>
<th>Days 9–12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scale scores</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Glasgow Coma Scale</td>
<td>13±3</td>
<td>14±2</td>
<td>14±2</td>
</tr>
<tr>
<td>Toronto Stroke Scale</td>
<td>13±21</td>
<td>13±18</td>
<td>20±30</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>36.3±4.3</td>
<td>30.5±5.5*</td>
<td>29.1±5.4*</td>
</tr>
<tr>
<td>White blood cells (10³/ml)</td>
<td>13.3±4.1</td>
<td>15.9±5.1</td>
<td>17.2±5.7†</td>
</tr>
<tr>
<td>Platelet count (10³/ml)</td>
<td>261±59</td>
<td>233±80</td>
<td>279±91</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>304±68</td>
<td>307±118</td>
<td>313±113</td>
</tr>
<tr>
<td>Fibrin D-dimer (ln-ng/ml)</td>
<td>5.19±0.63</td>
<td>5.34±0.84</td>
<td>5.46±0.54</td>
</tr>
<tr>
<td>aCL IgG isotype</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>aCL IgM isotype</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Data are mean±SD. All three periods refer to time after subarachnoid hemorrhage. aCL, anticardiolipin antibodies; lg, immunoglobulin.

*p<0.01, †p<0.05 compared with days 1–4.

Discussion

Prior stroke investigations have emphasized the prominence of abnormalities of hemostasis, hemorheology, and cardiolipin immunoreactivity; most of these studies, however, have focused on demonstrating abnormalities after ischemic stroke. Subarachnoid hemorrhage patients represent a unique population in that they are at high risk for brain infarction within a relatively brief period of time, i.e., approximately 2 weeks after hemorrhage. In this prospective study we found that neither hemostatic findings, hemorheological variables, nor cardiolipin immunoreactivity predicted the occurrence of cerebral infarction in patients with SAH. In a subgroup of patients studied repeatedly, we did note a decrease in hematocrit over the course of the 2-week period of evaluation, most likely reflecting the effect of aggressive fluid therapy. In addition, leukocyte count increased during this period.

To the best of our knowledge, this is the first attempt to measure plasma levels of several hemostatic proteins (i.e., fibrin D-dimer, PAI-1, protein S, and thrombomodulin) as well as cardiolipin antibodies in a substantial group of SAH patients. Patients who subsequently developed delayed ischemic deficit did not have significant elevations of fibrin D-dimer, PAI-1, or thrombomodulin, nor did they have decreased protein S. Note that the proportion of patients with elevated baseline fibrin D-dimer (i.e., 49%) was high, suggesting coagulation activation in a substantial number of these SAH patients; this abnormality did not, however, predict...
delayed ischemic deficit. Rather, fibrin dimer was elevated in patients admitted with ischemic symptoms and tended to increase after delayed ischemic deficit; fibrin dimer also increased after surgery. Elevated fibrinogen after SAH occurred despite a significant diminution in hematocrit. Second, aggressive fluid therapy resulting in hemodilution might have partially counteracted this increase apparently mediated by interleukins 1 and 6. In the present investigation (Table 3), we found virtually no increase (less than 1%) in fibrinogen concentration at days 5–8 compared with days 1–4 and only a 3% increase for days 9–12 compared with days 1–4; these changes in fibrinogen concentration were markedly less than the 36% increase found in a previous study of SAH patients. Despite the lack of change in fibrinogen concentration, leukocyte count increased significantly. There are, we believe, several potential explanations for these findings. First, systemic administration of steroids may have contributed to leukocytosis; changes in leukocyte count after injury appear to be caused by a mechanism distinct from that of increased fibrinogen concentration. Second, aggressive fluid therapy resulting in hemodilution might have partially masked the rise in fibrinogen concentration; note that hematocrit did decrease during the evaluation period (Table 3). However, in a previous study, increased fibrinogen after SAH occurred despite a significant diminution in hematocrit. Finally, each of the 16 patients in this group received oral nimodipine (60 mg every 4 hours) after admission. The evolution of fibrinogen levels in this SAH group was similar to our findings in acute ischemic stroke patients receiving oral nimodipine; the latter patients had no increase in fibrinogen during the 3-week period after stroke. The evolution of fibrinogen levels in stroke patients may warrant further investigation.

In conclusion, hematogenous factors, including hemostatic and hemorheological variables and cardiopulmonary function, did not predict delayed ischemic deficit in this SAH population. The coagulation system was found to be activated in a substantial number of SAH patients, but this activation was independent of the development of delayed ischemic deficit. Our results suggest that in patients in the presence of current therapeutic measures given to SAH patients, systemic hemostatic and rheological factors do not play a major role in the pathogenesis of delayed ischemic deficit.

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References
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