J Neurosurg 87:207–214, 1997

Hemostasis and fibrinolysis activation after subarachnoid hemorrhage

Seija Peltonen, M.D., Seppo Juvela, M.D., Ph.D., Markku Kaste, M.D., Ph.D., and Riitta Lassila, M.D., Ph.D.

Wihuri Research Institute and Departments of Neurosurgery and Neurology, Helsinki University Central Hospital, Helsinki, Finland

The authors assessed hemostasis and fibrinolysis serially: on admission and on the 1st and 7th days after surgery for subarachnoid hemorrhage (SAH), examining the complications of aneurysm rupture and its surgical repair. Of 32 patients, 25 with SAH were compared with seven control patients who underwent surgery for an unruptured intracranial aneurysm. On admission, patients with SAH had higher thrombin–antithrombin III complex (TAT) levels (13.3 $\pm$ 3.8 vs. 3.8 $\pm$ 0.6 ng/ml, $p = 0.01$), fibrin degradation product, D-dimer levels (1310 $\pm$ 220 vs. 556 $\pm$ 89 ng/ml, $p = 0.0001$), and leukocyte counts (14.6 $\pm$ 0.7 vs. 10.6 $\pm$ 1.8 $\times$ 10^9 cells/L, $p < 0.05$) than did control patients. Postoperative D-dimer values ($p = 0.007$) remained higher in the SAH group. Furthermore, admission D-dimer levels were higher in the patients in poor clinical condition than in those in good condition (2017 $\pm$ 377 vs. 934 $\pm$ 208 ng/ml, $p = 0.007$), and D-dimer levels were associated with the outcome at 3 months after admission. Additionally, thrombin generation and fibrinolytic markers measured on admission were related to clinical grade, amount of subarachnoid blood seen on computerized tomography (CT) scanning, and patient fatality. Patients with hypodense lesions verified on follow-up CT scanning or with persistent neurological deficits at 3 months had higher prothrombin fragments 1 and 2, TAT, D-dimer, and plasminogen activator inhibitor-1 values on the 1st day postoperatively than did patients without such lesions. In short, in patients with SAH, activation of coagulation and fibrinolysis was strongly associated with clinical state, patient fatality, and outcome at 3 months, and postoperatively this activation correlated with the development of brain infarction.

Key Words • subarachnoid hemorrhage • hemostasis • fibrinolysis • thrombin-antithrombin III complex • D-dimer • plasminogen activator inhibitor-1

Lack of relevant markers has made the clinical assessment of the evolution of cardiovascular diseases difficult.[14] Aneurysmal subarachnoid hemorrhage (SAH) with delayed ischemia and rebleeding causes high rates of morbidity and mortality.[6,11] Increased knowledge of the underlying pathogenetic mechanisms, as well as more advanced prognostic tools, would be highly valuable.

During acute thrombotic states such as pulmonary embolism and deep vein thrombosis, markers measuring thrombin generation (that is, thrombin–antithrombin III complex, [TAT]) and fibrinolytic degradation of cross-linked fibrin (that is, D-dimer) have recently been proven useful experimentally as well as clinically.[4,5,8] We have previously studied atherothrombosis by using indicators measuring coagulation and fibrinolysis activation in peripheral arterial occlusive disease, both in its subclinical stage and in acute or subacute thrombotic occlusion.[23,24] During thrombotic occlusion, TAT and plasminogen activator inhibitor-1 (PAI-1) were clearly associated with a poor outcome.[24] As in our study of peripheral arterial disease,[24] patients with acute ischemic stroke presented with a persistent hypercoagulable state.[28] Furthermore, the pathophysiology of bleeding disorders has been evaluated using these variables to assess hemostasis and fibrinolysis.[3]

Patients with aneurysm bleeding usually do not have a deficient coagulation system; instead, sudden local bleeding occurs at a site where the vascular wall has ruptured because of structural and proteolytic disturbances, such as is reported for vulnerable aortic aneurysms.[25] Therefore, in patients with aneurysm bleeding, in which various amounts of blood are in contact with extravascular matrix, such markers would measure thrombin generation and subsequent fibrinolysis. We performed serial assessments of coagulation and fibrinolysis and studied their association with outcome during the acute stage after subarachnoid bleeding that was accompanied by complications such as aneurysm rupture and its surgical repair. Patients undergoing an operation for an unruptured intracranial aneurysm were used as controls.

Clinical Material and Methods

Patient Population

This prospective study included 25 patients with SAH and seven patients with unruptured intracranial aneurysms

TABLE 1

*Clinical characteristics of 32 patients who underwent aneurysm surgery**

Characteristic	Patients W/ SAH (25 patients)	Controls (7 patients)
age (yrs)	46.9 ± 2.8	46.7 ± 3.3
sex (M/F)	16:9	4:3
BMI (kg/m^2)	26.4 ± 0.7	24.2 ± 1.6
hypertension	5	3
smoker	17	4
heavy drinker	3	2

* BMI denotes body mass index calculated as weight/(height)^2; heavy drinkers consumed alcohol on average at least 300 g/week; hypertension = patients receiving antihypertensive medication or with repeated blood pressure readings exceeding 160/95; smokers = current smokers.

(control group), all of whom were admitted to Helsinki University Central Hospital between December 1, 1991 and May 31, 1993. To collect and handle the samples within the time frame of the study, only one or two patients could be studied at a time. Consequently, patients with SAH and control patients were entered into the study according to this blood-collection restriction. The study design, which was approved by an institutional review board, was such that the clinician and the laboratory analysts were blinded to the data until both sets of analyses were brought together at the end of the study. After admission, the patients, their family members, and/or coworkers were interviewed about the patients' recent use of medications, smoking habits and alcohol intake, and previous diseases. Information on all patients was also collected from medical records from other hospitals and general practitioners to check diseases, medications, and blood pressure values. Clinical characteristics of the patients are presented in Table 1.

Treatment Regimen

Nimodipine treatment was initiated after diagnosis of a ruptured aneurysm and was continued for up to 21 days post-SAH. Patients undergoing surgery received betamethasone routinely (4 mg every 6 hours) starting the day before surgery and continuing in diminishing doses to the 6th postoperative day. Hypertensive or hypervolemic therapy was not used, and the patients did not receive antifibrinolytic agents. Six of the seven control patients received betamethasone, whereas nimodipine was not administered to this group. All patients with a ruptured aneurysm who were candidates for surgery (21 patients) and all seven patients with unruptured aneurysms were treated by clipping the lesion. The ruptured aneurysms were clipped within 4 days (mean 1.8 days) after SAH.

Clinical Monitoring and Outcome

The patients' clinical condition on admission, before surgery, and during the three blood samplings was graded according to the World Federation of Neurological Surgeons (WFNS) Grading Scale.[10] The presence of SAH was verified by means of a computerized tomography (CT) scan in all patients, and the thickness of the subarachnoid blood clot was noted. The CT scans were repeated after clinical deterioration, at discharge, and during follow-up review. In all except two patients, the origin of the aneurysm hemorrhage was confirmed. One patient with SAH confirmed by CT scanning died soon after the bleeding, but the location of the aneurysm rupture remained unknown because no angiogram was obtained and an autopsy was not performed. The second patient had a nonaneurysmal SAH.

Neurological examinations were performed daily after the patients were admitted. Delayed cerebral ischemia was defined as a gradual development of focal neurological signs and/or deterioration of consciousness and was not found to be caused by intracerebral hematoma, rebleeding, hydrocephalus, clipping of an arterial branch together with the aneurysm, infection, serum electrolyte disorder, or any other known reason. Causes of poor clinical condition and outcome were determined by repeated CT scans, a routine postoperative angiogram, laboratory investigations, or at autopsy.

Outcome was assessed at 3 months according to the Glasgow Outcome Scale (GOS)[17] score. Follow-up CT scans were also obtained at 3 months after the aneurysm rupture to identify permanent hypodense lesions consistent with infarction, lesions not seen on the admission CT scan and occurring in areas other than those of a possible previous intracerebral hematoma, or any lesion caused by clipping of the arterial branch containing the aneurysm. In those patients who died within 3 months post-SAH, the final CT scan was considered to be the follow-up scan if it was obtained later than 3 weeks after SAH to identify permanent hypodense lesions.[18]

Blood Samples

We collected three blood samples from 22 of the patients with SAH; two patients died before the second sampling, and one patient before the third sampling. The first samples were drawn on admission (between 7 a.m. and 10:30 p.m.) as soon as possible after the neurosurgeon made the diagnosis of SAH. Free-flowing blood was collected through a polytetrafluoroethylene cannula, and the first 3 to 6 ml of blood was discarded. Blood used for cell counts was collected in vials containing ethylenediamine tetraacetic acid, and blood for hemostatic and fibronolytic samples (nine volumes) was collected into polypropylene tubes containing special anticoagulating agents (one volume), as reported earlier.[23,24] Essentially, citrated plasma (0.1 M) was used to test fibrinogen, prothrombin fragments 1 and 2 (F1+2), and TAT levels, and sodium citrate was supplemented with aprotinin (0.2 trypsin-inhibiting U/ml) to test D-dimer levels. The hemostatic samples were centifuged for 10 minutes at 1800 G (22˚C) without delay, and the plasma was separated for freezing. Specifically, the guidelines of the Leiden Fibrinolysis Workshop were followed to collect and handle blood for determination of tissue plasminogen activator (TPA) and PAI-1 levels.[22]

The second and third blood samples were each collected at 7 a.m., while the patients were fasting. The second sample was obtained on the morning after surgery, and the third sample was collected in the 2nd week (range 6–14 days) after the onset of SAH. In patients with an unruptured aneurysm, the first sample was obtained on the

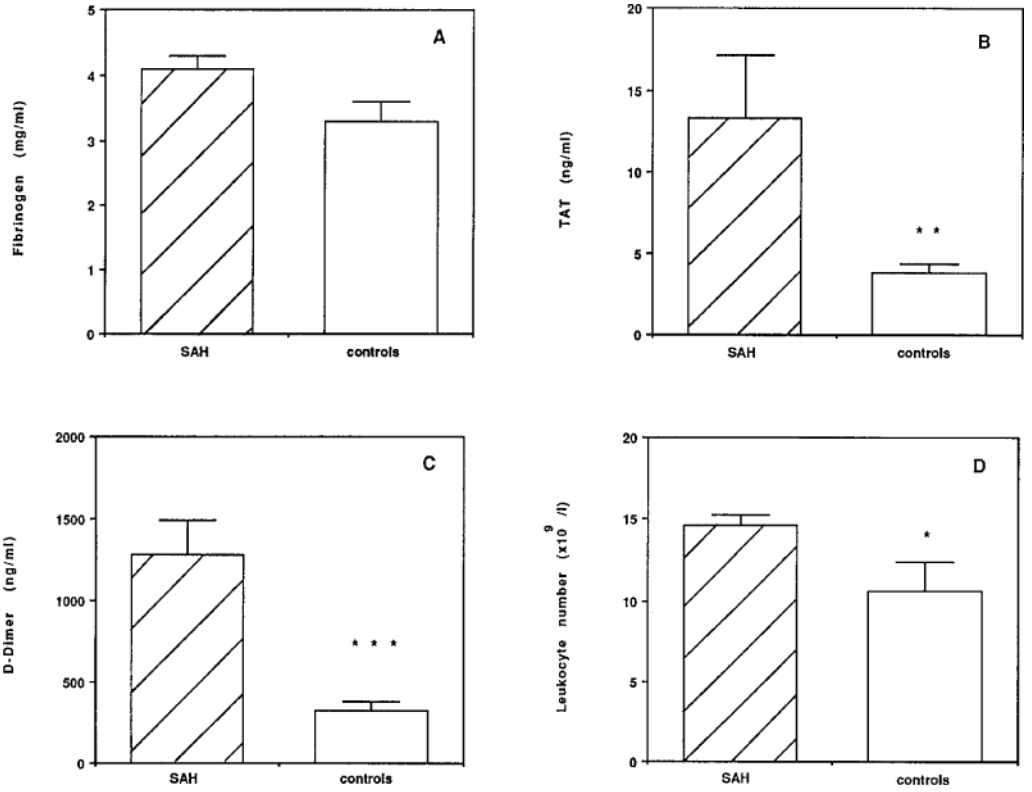

FIG. 1. Bar graphs showing levels of fibrinogen (A), TAT (B), D-dimer (C), and numbers of leukocytes (D) on hospital admission in the presence (SAH, 25 patients) and absence (control group, seven patients) of aneurysm bleeding. Values represent the mean ± the SEM. The probability values and statistical tests were as follows: $p = 0.08$, Mann–Whitney U-test (A); $p = 0.01$, Mann–Whitney U-test (B); $p = 0.0001$, Student's t-test (C); and $p < 0.05$, Student's t-test (D).

morning of surgery, the second on the morning after surgery, and the third within 5 to 8 days postsurgery.

Analysis of the Plasma Samples

The plasma processing occurred within 3 months after sample collection. Fibrinogen was determined by the functional method of Clauss (normal range 2–4 mg/ml). The levels of F1+2 (reference range 0.44–1.1 nmol/L [5th–95th percentile]), TAT (1–4.1 ng/ml [reference range 2.5th–97.5th percentile]), D-dimer (< 400 ng/ml), TPA antigen (age 25–34 years, 1–20 ng/ml [2.5th–97.5th percentile]; age 55–64 years, median 8.6 and 7.6 ng/ml, respectively), and PAI-1 antigen (18 ± 10 ng/ml, range 4–43 ng/ml) were analyzed with an enzyme-linked immunosorbent assay (Enzygnost F1+2 and Enzygnost TAT, Behringwerke AG, Marburg, Germany; Asserachrom D-Di, Diagnostica Stago, Asnieres-sur-Seine, France; TintElize TPA and TintElize PAI-1, Biopool, Umeå, Sweden). The D-dimer values obtained after serial dilutions and still exceeding 3000 ng/ml were counted as 3000 ng/ml.

Statistical Analysis

The data were analyzed using biomedical data package statistical programs (Version 1993; BMDP Statistical Software Inc., Los Angeles, CA). Categorical variables were compared by means of Fisher's exact two-tailed test or the Pearson chi-square test. Continuous variables, which were expressed as the mean ± standard error of the mean (SEM), were compared between groups by the Mann–Whitney U-test, Student's or Welch's t-test, or analysis of variance (ANOVA) with corrected multiple pairwise comparisons, as appropriate. In patients with no missing data, the effect of elapsed time after SAH and different grouping variables were compared by repeated-measures ANOVA and analysis of covariance. Associations of continuous variables were assessed using the Spearman rank correlation coefficient test.

Results

On admission, clinical status was markedly worse in the patients with SAH according to the WFNS classification (Grade 2.35 ± 1.35, mean ± SEM) than in the control group (all Grade 1) ($p = 0.02$). During collection of the first blood sample the grades in patients with SAH versus control patients were 1.96 ± 0.22 versus 1 ± 0 ($p = 0.02$). The TAT and D-dimer levels and numbers of leukocytes were significantly higher in the SAH than in the control group (Fig. 1). Moreover, fibrinogen levels tended to be higher among individuals in the SAH than in the control group. In contrast, F1+2, TPA, and PAI-1 did not differ between the two groups, with values of 1.7 ± 0.2 versus 1.2 ± 0.2 nmol/L, 8.5 ± 0.9 versus 9.7 ± 2.2 ng/ml, and

TABLE 2

Differences in hemostatic and fibrinolytic variables on admission between patients with SAH who survived and those who died

Variable	Alive (21 patients)	Dead (4 patients)	p Value
WFNS grade	1.6 ± 0.1	4.0 ± 0.4	0.001
fibrinogen (mg/ml)	3.9 ± 0.2	4.8 ± 0.3	<0.05
TAT (ng/ml)	11.6 ± 4.2	22.1 ± 9.6	0.02
D-dimer (ng/ml)	1029 ± 195	2602 ± 398	0.01
TPA (ng/ml)	7.1 ± 0.7	15.8 ± 2.5	0.01
PAI-1 (ng/ml)	9.8 ± 1.7	20.7 ± 4.4	0.02

5.9 ± 1.8 versus 6.4 ± 4.6 ng/ml in SAH and control groups, respectively. In the SAH group three patients died before a third blood sample could be obtained, and one died on Day 11. Among the four patients who died, levels of hemostatic and fibrinolytic variables on admission were significantly elevated when compared with values for those who survived (Table 2).

The time course of the changes in fibrinogen, TAT, and D-dimer values is presented in Fig. 2 for the SAH and control groups. The time from discovery of bleeding to collection of the first blood sample was 1.5 ± 0.2 days (range 0.2–3). Of the 25 patients with SAH, 21 underwent surgery. During the 1st postoperative day the WFNS grade for the SAH group was 2 ± 0.2 versus Grade 1 for all controls ($p = 0.01$), and at the time of the third blood sample the differences in patients' grades persisted, with values of 1.8 ± 0.2 among the SAH group versus 1 in the control group. The significant effects of surgery on the hemostatic parameters were apparent in both groups. During the 1st postoperative day only D-dimer levels remained notably elevated among the patients with SAH, whereas the differences in the other significant preoperative measures of surgery-associated activation of coagulation disappeared after the operation. In the patients with complete data from samples 1 and 2, the difference in D-dimer levels between the SAH and control groups was significant ($p = 0.007$), and so was the effect of time on D-dimer results ($p < 0.0001$). However, the relative increases in D-dimer levels were similar between the groups and from sample 1 to sample 2 (interaction, $p = 0.57$). In addition, TAT values increased from the first to the third blood sample ($p < 0.05$), and values nearly reached significance ($p = 0.08$) in the patients with SAH when compared with control values, but with no significant interaction between time and group ($p = 0.13$). Extensive thrombin generation (TAT 21.4 ± 5.9 ng/ml) continued in the SAH group during the week after the operation. In the control patients, thrombin generation was halved immediately postsurgery, but was still twice the basal level at 1 week ($p < 0.05$). However, TPA and PAI-1 values between the groups did not differ, and they did not change in response to the operation itself (data not shown).

We divided the patients with SAH into two groups according to their clinical state (17 patients with Grades 1–3 vs. eight with Grades 4–5). The D-dimer levels on admission among patients in poor clinical condition were significantly higher (2017 ± 377 ng/ml) than among those in good clinical condition (934 ± 208 ng/ml, $p < 0.007$). Additionally, the leukocyte number was higher on the

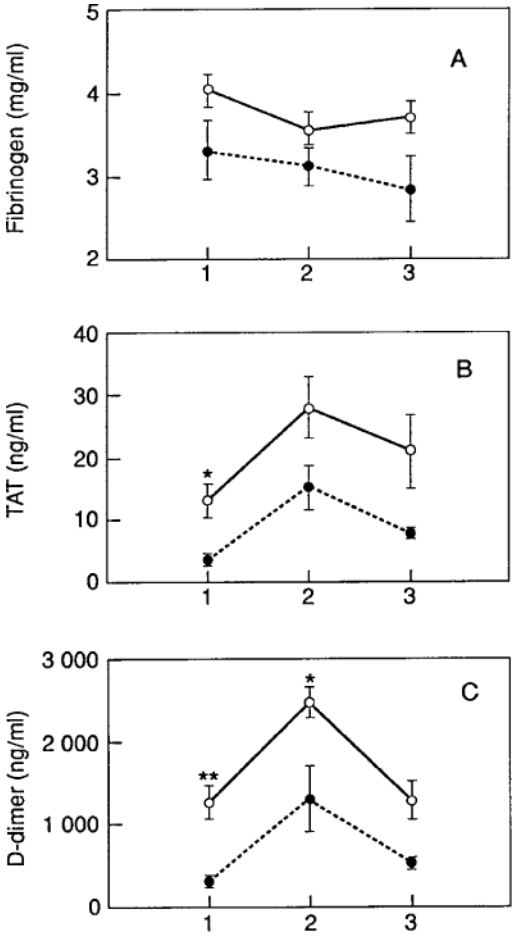

FIG. 2. Graphs demonstrating the time course of fibrinogen (A), TAT complex (B), and D-dimer (C) in patients with and without SAH who underwent surgical treatment. Values with their *error bars* represent the mean ± the SEM. The *solid line* denotes patients with SAH (21 patients) and the *broken line* denotes patients without aneurysm bleeding (seven individuals). Blood samples 1, 2, and 3 (x axis) were obtained on admission, on the 1st postoperative day, and on the 7th postoperative day. *$p < 0.01$ and **$p < 0.001$, Mann–Whitney U-test or Student's t-test, where pertinent.

1st postoperative day (18.6 ± 1.5 × 10^9 cells/L) among patients in poor clinical condition than it was (14.9 ± 1.1 × 10^9 cells/L) for the group in good condition ($p = 0.05$). Moreover, on admission to the hospital, the WFNS grade was significantly related to D-dimer, PAI-1, TPA, fibrinogen, and TAT levels (Table 3). That these correlations were less marked after surgery demonstrated the confounding effect of the operation on the hemostatic and fibrinolytic systems.

Furthermore, when we divided the patients with SAH into two groups according to severity of bleeding, nine either had no detectable clot or only a thin layer of subarachnoid clot (< 3 mm), whereas 16 had a thick clot ≥ 3 mm) or a hematoma. Those patients with more severe bleeding as seen on the CT scan exhibited higher TAT, TPA, and PAI-1 antigens on admission than did those with modest bleeding (Table 4), and the patients with severe

TABLE 3

Significant Spearman rank correlation coefficients between WFNS grade of patients with SAH and markers

Marker	Blood Sample No. (r_s)*		
	1	2	3
fibrinogen (mg/ml)	0.49	0.50	0.39
TAT (ng/ml)	0.47	—	—
D-dimer (ng/ml)	0.70	—	0.46
TPA (ng/ml)	0.60	0.62	—
PAI-1 (ng/ml)	0.60	—	—
F1+2 (ng/ml)	0.32	—	—
leukocytes (10^9 cells/L)	0.42	—	—

* Sample 1 was obtained on admission, sample 2 on the 1st postoperative day, and sample 3 on the 7th postoperative day (see *Blood Samples*). Abbreviations: r_s = correlation coefficient; — = not significant.

TABLE 4

*Differences between markers in patients with SAH according to severity of bleeding on CT scans**

Sample & Marker	Severity of Bleeding (ng/ml)*		p Value
	None/ Thin Layer	Thick Layer/ Hematoma	
sample 1	9 patients	16 patients	
TAT	5.6 ± 0.9	17.2 ± 5.5	<0.05
TPA	5.6 ± 1.1	10.1 ± 1.2	<0.05
PAI-1	6.5 ± 1.8	14.4 ± 2.3	<0.05
sample 2	3 patients	16 patients	
TPA	5.5 ± 1.3	11.1 ± 1.2	<0.01
PAI-1	9.1 ± 3.5	16.1 ± 2.5	<0.05

* Thin layer denotes blood layer less than 3 mm and thick layer is greater than or equal to 3 mm seen in fissures or vertical cisterna.

bleeding were found to have elevated fibrinolytic enzymes on the 1st postoperative day.

Patients who displayed signs of brain infarction detectable as hypodense areas on the CT scans obtained 3 months after SAH had significantly increased F1+2, TAT, D-dimer, and PAI-1 antigen levels during the 1st postoperative day compared with those with no infarction, despite their similar grade during sampling (2.1 ± 0.2 and 2.2 ± 0.4, respectively) (Fig. 3). Consequently, patients with symptomatic brain ischemia and persistent neurological deficits had higher levels of TAT and PAI-1 antigen than did patients with no such deficits ($p < 0.05$; Fig. 4). Furthermore, D-dimer levels exceeded 3000 ng/ml in all three patients with neurological deficits. We next determined the specific cutoff values for the association between hemostatic variables and development of brain infarction. The sensitivity and specificity, respectively, of the cutoff values for the four variables were as follows: F1+2, 100% and 70% at > 1.5 nmol/L; TAT, 70% and 50% at > 23 ng/ml; D-dimer, 90% and 50% at > 2000

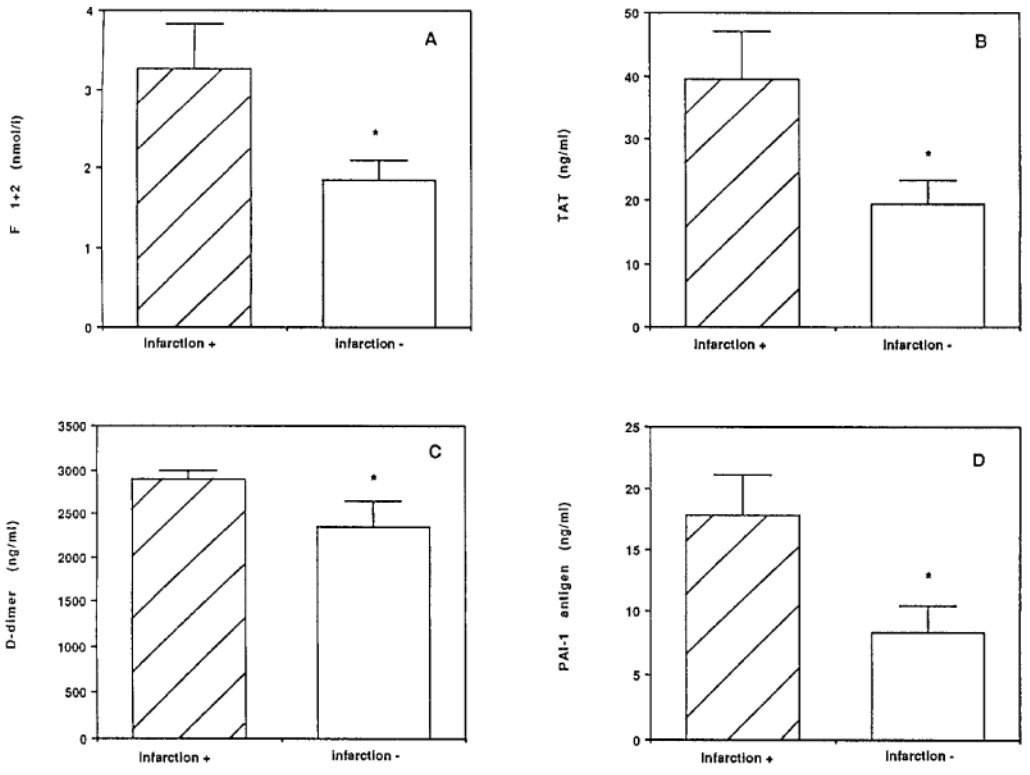

FIG. 3. Bar graphs showing the levels of F1+2 (A), TAT (B), D-dimer (C), and PAI-1 antigen (D) in the 10 patients with and the six without brain infarction, that is, a hypodense area on CT scans obtained 3 months after insult. Values (the mean ± the SEM) were obtained on the 1st postoperative day. *$p < 0.05$, Mann–Whitney U-test or Student's t-test, where pertinent.

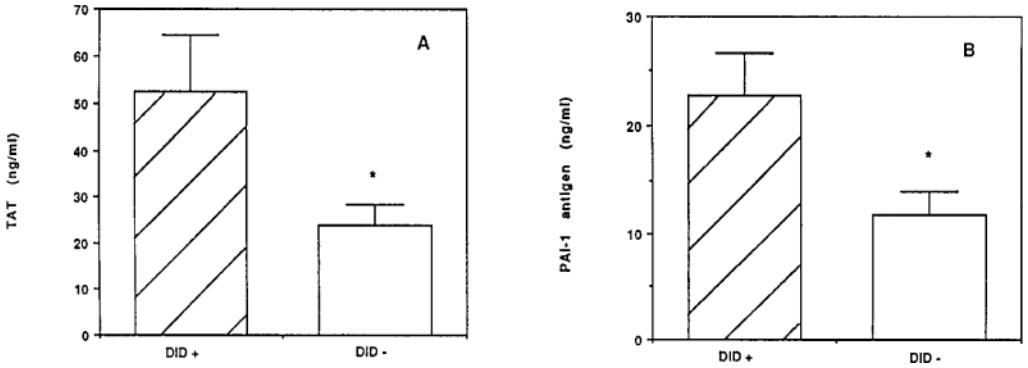

FIG. 4. Bar graphs showing the TAT and PAI-1 antigen levels in three patients with (+) and 16 without (−) persistent neurological deficits (delayed ischemic deterioration, DID). Values (the mean ± the SEM) were obtained on the 1st postoperative day. *$p < 0.05$, Mann–Whitney U-test or Student's t-test, where pertinent.

ng/ml; and PAI-1, 80% and 50% at > 8 ng/ml, respectively.

Clinical status, both at admission and during the 1st and 7th postoperative days, was associated with later disability assessed using the GOS. The patients who reached an independent state at 3 months, compared with those in a dependent state or those who died, had a lower clinical WFNS grade on admission (1.44 ± 0.12 vs. 3.29 ± 0.42, $p = 0.0004$), on the 1st postoperative day (1.73 ± 0.59 vs. 3 ± 0.82, $p = 0.01$), and on the 7th postoperative day (1.39 ± 0.61 vs. 3.75 ± 0.96, $p = 0.001$). In the patients either dead or with significant disability at 3 months post-SAH, values for fibrinogen and D-dimer on admission to the hospital and 1 week post-SAH were significantly elevated (Table 5). The same was true with TPA and PAI-1 antigens during admission and 1 day postoperatively. Finally, Table 6 illustrates the internal consistency of the measurements of the hemostatic and fibrinolytic system. Of note are the significant correlations between leukocyte number and TAT, as well as between the former and D-dimer.

Discussion

In this study we have shown that in patients with SAH, markers assessing activation of coagulation and fibrinolysis were strongly related both to clinical state and to long-term outcome at 3 months. Similar results have previously been reported by Fujii, et al.,[12] and by Itoyama, et al.[15] The study by Fujii, et al., was designed to be retrospective and did not include a control group. Almost 10-fold higher levels of TAT were reported in that study in comparison with ours, a discrepancy likely caused by a delay in contact of blood and anticoagulant during blood collection because of the use of a multiple-syringe system. However, D-dimer levels were closer to those found in our study, probably because of the less vulnerable marker that mainly measured plasmin-derived degradation of cross-linked fibrin.[2] In contrast with these two previous studies, we also chose a control group and took serial samples from the patients to control prospectively for the confounding effects of time and surgery on the results. We showed that during the 1st week after patients underwent surgery for SAH, activation of coagulation and fibrinolysis exceeded that in the control patients who were surgically treated for an unruptured aneurysm. Moreover, a novel finding was that the degree of thrombin generation on the 1st postoperative day was associated with the development of brain infarction during recovery.

The finding that the clinical state, thickness of the subarachnoid blood clot on the CT scan, and the outcome of the patients were all associated with activation of hemostasis and fibrinolysis indicates that the extent of bleeding and the resolution of the expanding clot play an intimate role in the progression and regression of SAH. We controlled for the effects of surgical intervention on hemostasis and fibrinolysis by testing a group of patients who had unruptured aneurysms. In the control patients the effect of an intracranial operation on the coagulation system was clearly illustrated by the absence of any activation associated with SAH. This was the case although their surgical trauma was somewhat less than that of the SAH patients, but this likely reflects the prevalence of tissue factor in the brain and the vascular target of surgery. A similar finding was reported in another study by Fujii, et al.[13] It can be concluded that thrombin generation and its sequelae leading to plasmin activation and degradation of cross-linked fibrin were significantly enhanced in the patients with SAH in comparison with the control patients. Also, after the operation the pattern of the decrease in measured variables was similar, but the level at which they remained in the SAH group indicates a long-lasting activation state of blood coagulation and fibrinolysis that endured for approximately 2 weeks after SAH.

Indeed, it was intriguing that in the blood samples obtained on the 1st postoperative day, thrombin generation measured as TAT and F1+2, as well as D-dimer and PAI-1 antigen, were increased in those patients who developed brain infarction, which was detected as a hypodense area on the CT scans at 3 months. The reason for this new observation remains unknown, but because the patients' clinical condition did not explain the observations, a thrombotic mechanism may be involved, which, in certain patients, could also reflect endothelial dysfunction in small vessels after the operation. It is well known that such hypodense lesions consistent with cerebral infarction may be caused by delayed cerebral ischemia or symp-

TABLE 5

*Marker levels and outcome in 25 patients with SAH divided into two groups according to GOS score**

Sample & Marker	Group I	Group II	p Value
sample 1	18 samples	7 samples	
fibrinogen (mg/ml)	3.8 ± 0.2	4.8 ± 0.3	0.01
D-dimer (ng/ml)	896 ± 191	2270 ± 362	<0.005
TPA (ng/ml)	7.0 ± 0.8	12.3 ± 2.2	<0.05
PAI-1 (ng/ml)	9.4 ± 1.9	17.3 ± 3.3	<0.05
sample 2	18 samples	4 samples	
TPA (ng/ml)	7.2 ± 1.0	14.8 ± 1.2	0.001
PAI-1 (ng/ml)	11.2 ± 1.9	22.2 ± 5.9	0.03
sample 3	18 samples	4 samples	
fibrinogen (mg/ml)	3.5 ± 0.2	5.0 ± 0.5	0.002
D-dimer (ng/ml)	1102 ± 222	2245 ± 497	<0.05

* Group I = GOS scores of I and II; Group II = GOS scores of III to V.

TABLE 6

Significant Spearman rank correlation coefficients between marker levels on admission in 25 patients with SAH

Marker	Sample 1 (r_s)
fibrinogen vs TAT	0.47
fibrinogen vs TPA	0.42
TAT vs D-dimer	0.50
TAT vs TPA	0.49
TAT vs F1+2	0.45
TAT vs leukocyte count	0.44
D-dimer vs TPA	0.39
D-dimer vs F1+2	0.42
D-dimer vs leukocyte count	0.31
TPA vs PAI-1	0.68

tomatic arterial vasospasm, but these can also be caused by surgery (for example, by spatula pressure or injury to small perforating arteries during surgical manipulation).[18] It is therefore possible that these patients, who are subjected to a greater increase in activation of coagulation, may also develop hypodense lesions more easily. Because hemostatic variables did not correlate with delayed ischemia in the blood samples obtained on admission and at 1 week after hemorrhage and surgery, it is unlikely that activation of coagulation and fibrinolysis are associated with large-vessel cerebral vasospasm. Furthermore, as reported by Ameriso, et al.,[1] preoperative D-dimer is not associated with delayed ischemic deficit, although that study did not monitor D-dimer levels immediately after surgery.

Of specific interest was the behavior of F1+2 in this study. In contrast to the enzyme-inhibitor complex TAT, F1+2 were not elevated in the first sample after the bleeding event. We have previously obtained similarly discrepant TAT and F1+2 results during massive arterial thrombosis in the lower extremities and in experimental thrombosis in which fibrin was allowed to be generated.[24,26] Diquélou, et al.,[9] reported the same inconsistency in an experimental model of thrombosis using nonanticoagulated blood. Because prothrombin fragments have been reported to bind to phospholipids,[16] we suggest that F1+2 are retained in thrombus or clotting blood. Therefore, the postoperative elevation of F1+2 that is associated with a later brain infarction seems clearly significant. In addition to increased F1+2, a surprising finding was that circulating levels of the enzymes of the fibrinolytic system, TPA and PAI-1, did not rise in the context of bleeding in SAH. We have no explanation for this or for the fact that TPA and PAI-1 remained constant after clipping of the aneurysm. In a study assessing the effects of intracranial surgery in connection with clipping of the unruptured aneurysm, Fujii, et al.,[13] noted a rapid increase in TPA, PAI-1, and plasmin-α_2–antiplasmin complexes, which, however, returned to baseline levels at approximately 12 hours after the surgery. In an operation with more extensive tissue damage, such as total hip replacement, functional PAI-1 in particular has been reported to increase, which explains postoperative thrombotic complications.[19] The connection with inflammation following the bleeding event seems obvious. This was evidenced by early leukocytosis, but also by the correlation between both TAT and D-dimer and leukocyte count.[21] Thrombin activates platelets and endothelial cells to express P-selectin, which is an adhesive signal for leukocytes, and, for instance in monocytes, induces tissue-factor synthesis.[7] On the other hand, neutrophils secrete elastase, and monocytes/macrophages secrete cathepsin D, both of which participate in fibrinolysis,[27] which is not dependent on plasminogen activators.

Conclusions

Our study illustrates relationships between coagulation and fibrinolysis activation and the early as well as the long-term outcome of patients suffering SAH. A novel finding was that thrombin and plasmin generation, as well as the amount of circulating PAI-1 at 24 hours postsurgery, are clearly associated with the development of infarction detectable 3 months postoperatively. Thus, coagulation activation that is caused soon after surgery by endothelial dysfunction and/or thrombotic mechanisms, seems to explain the delayed cerebral ischemia. Based on the practical implications of the current study, we believe that it is worthwhile to monitor the activation of coagulation in connection with the operation and early recovery period after SAH. We have approximated reliable cutoff values for the variables used, but these have to be set separately for each laboratory. In the future it would be valuable to conduct randomized trials to test the hypothesis that early-phase low-molecular-weight heparin, which has proven to be beneficial after ischemic stroke,[20] can prevent or reduce the risk of post-SAH infarction, which is strongly associated with disability and death.

Acknowledgments

The authors thank Dr. Vesa Manninen for his constant encouragement. The excellent technical assistance of Tuula Järvenpää for performing the hemostatic and fibrinolytic assays is gratefully acknowledged.

References

1. Ameriso SF, Wong VLY, Ishii H, et al: Hematogenous factors and prediction of delayed ischemic deficit after subarachnoid hemorrhage. **Stroke 23:**1404–1409, 1992
2. Amiral J, Grosley M, Mimilla F, et al: Monoclonal antibodies to

different neo-epitopes on fibrinogen and fibrin degradation products. **Blood Coagul Fibrinolysis 1:**447–452, 1990
3. Bauer KA: New markers for *in vivo* coagulation. **Curr Opin Hematol 1:**341–346, 1994
4. Boneu B, Bes G, Pelzer H, et al: D-dimers, thrombin antithrombin III complexes and prothrombin fragments 1 + 2: diagnostic value in clinically suspected deep vein thrombosis. **Thromb Haemost 65:**28–32, 1991
5. Bounameaux H, Cirafici P, de Moerloose P, et al: Measurement of D-dimer in plasma as diagnostic aid in suspected pulmonary embolism. **Lancet 337:**196–200, 1990
6. Broderick JP, Brott T, Tomsick T, et al: Intracerebral hemorrhage more than twice as common as subarachnoid hemorrhage. **J Neurosurg 78:**188–191, 1993
7. Celi A, Pellegrini G, Lorenzet R, et al: P-selectin induces the expression of tissue factor on monocytes. **Proc Natl Acad Sci USA 91:**8767–8771, 1994
8. Declerck PJ, Mombaerts P, Holvoet P, et al: Fibrinolytic response and fibrin fragment D-dimer levels in patients with deep vein thrombosis. **Thromb Haemost 58:**1024–1029, 1987
9. Diquélou A, Lemozy S, Dupouy D, et al: Effect of blood flow on thrombin generation is dependent on the nature of the thrombogenic surface. **Blood 84:**2206–2213, 1994
10. Drake CG: Report of World Federation of Neurological Surgeons Committee on a universal subarachnoid hemorrhage grading scale. **J Neurosurg 68:**985–986, 1988 (Letter)
11. Fogelholm R, Hernesniemi J, Vapalahti M: Impact of early surgery on outcome after aneurysmal subarachnoid hemorrhage. A population-based study. **Stroke 24:**1649–1654, 1993
12. Fujii Y, Takeuchi S, Sasaki O, et al: Hemostasis in spontaneous subarachnoid hemorrhage. **Neurosurgery 37:**226–234, 1995
13. Fujii Y, Tanaka R, Takeuchi S, et al: Serial changes in hemostasis after intracranial surgery. **Neurosurgery 35:**26–33, 1994
14. Gerschlick AH: Are there markers of the blood-vessel wall interaction and of thrombus formation that can be used clinically? **Circulation 81 (Suppl 1):**I28–I34, 1990
15. Itoyama Y, Fujioka S, Takaki S, et al: Significance of elevated thrombin-antithrombin III complex and plasmin-α_2-plasmin inhibitor complex in the acute stage of nontraumatic subarachnoid hemorrhage. **Neurosurgery 35:**1055–1060, 1994
16. Jackson CM: Mechanisms of prothrombin activation, in Colman RW, Hirsh J, Marder VJ, et al (eds): **Hemostasis and Thrombosis. Basic Principles and Clinical Practice.** Philadelphia: JB Lippincott, 1987, pp 135–147
17. Jennett B, Bond M: Assessment of outcome after severe brain damage. A practical scale. **Lancet 1:**480–484, 1975
18. Juvela S: Aspirin and delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. **J Neurosurg 82:**945–952, 1995
19. Kassis J, Hirsh J, Podor TJ: Evidence that postoperative fibrinolytic shutdown is mediated by plasma factors that stimulate endothelial cell type I plasminogen activator inhibitor biosynthesis. **Blood 80:**1758–1764, 1992
20. Kay R, Wong KS, Yu YL, et al: Low-molecular-weight heparin for the treatment of acute ischemic stroke. **N Engl J Med 333:** 1588–1593, 1995
21. Kishimoto T: The biology of interleukin-6. **Blood 74:**1–10, 1978
22. Kluft C, Verheijen JH: Leiden Fibrinolysis Working Party: blood collection and handling procedures for assessment of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1). **Fibrinolysis 4 (Suppl 2):**155–161, 1990
23. Lassila R, Peltonen S, Lepäntalo M, et al: Severity of peripheral atherosclerosis is associated with fibrinogen and degradation of cross-linked fibrin. **Arterioscler Thromb 13:**1738–1742, 1993
24. Peltonen S, Lassila R, Rossi P, et al: Blood coagulation and fibrinolysis activation during sudden arterial occlusion of lower extremities—an association with ischemia and patient outcome. **Thromb Haemost 74:**1442–1446, 1995
25. Schneiderman J, Bordin GM, Engelberg I, et al: Expression of fibrinolytic genes in atherosclerotic abdominal aortic aneurysm wall. A possible mechanism for aneurysm expansion. **J Clin Invest 96:**639–645, 1995
26. Siljander P, Carpen O, Lassila R: Platelet-derived microparticles associate with fibrin during thrombosis. **Blood 87:** 4651–4663, 1996
27. Simon DI, Ezratty AM, Loscalzo J: The fibrin(ogen)olytic properties of cathepsin D. **Biochemistry 33:**6555–6563, 1994
28. Takano K, Yamaguchi T, Uchida K: Markers of hypercoagulable state following acute ischemic stroke. **Stroke 23:**194–198, 1992

Manuscript received August 20, 1996.
Accepted in final form January 16, 1997.
This study was supported by grants from the Paavo Nurmi Foundation, the Aarne Koskelo Foundation, and the Finnish Heart Research Foundation.

Address reprint requests to: Riitta Lassila, M.D., Ph.D., Wihuri Research Institute, Kalliolinnantie 4, FIN-00140 Helsinki, Finland.