PAPER

Endothelial cell activation markers and delayed cerebral ischaemia in patients with subarachnoid haemorrhage

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Background: Endothelial cell activation may be connected with the pathogenesis of delayed cerebral ischaemia (DCI) after subarachnoid haemorrhage (SAH).
Aim: To assess the relationship between serial concentrations of circulating markers of endothelial cell activation (soluble intercellular adhesion molecule-1, soluble platelet selectin (sP-selectin), soluble endothelial selectin, ED1-fibronectin, Von Willebrand Factor (VWF) and VWF propeptide) and development of DCI.
Methods: 687 blood samples were collected from 106 consecutive patients admitted within 72 h after onset of SAH. Changes in levels were analysed in the last sample before and in the first sample after the onset of DCI (n = 30), and in subgroups with DCI occurring within 24 h after treatment of the aneurysm (n = 12) or unrelated to treatment of the aneurysm (n = 18). Patients without DCI (n = 56) served as controls.
Results: Concentrations of sP-selectin, but not of the other markers, were found to increase considerably after DCI unrelated to treatment of the aneurysm (increase 25 ng/ml, 95% CI 8 to 43), whereas they tended to decrease in the control patients without DCI (decrease 13 ng/ml, 95% CI −28 to 2.4). Surgery was found to profoundly influence the levels of the markers irrespective of the occurrence of DCI.
Conclusion: The rise in sP-selectin level during DCI is suggested to be the result of platelet activation, as levels of the other markers of endothelial cell activation were not increased after DCI unrelated to treatment. Whether a causal role of platelet activation is implicated in the development of DCI should be determined in further studies in which the relationship between concentrations of markers and treatment is taken into account.

Endothelial cell activation may have a role in the pathogenesis of delayed cerebral ischaemia (DCI) after subarachnoid haemorrhage (SAH).[1 2] Activated endothelial cells express several receptors on their cell membrane. The inflammatory cell adhesion molecules intercellular adhesion molecule-1 (ICAM-1), P (platelet)-selectin and E (endothelial)-selectin participate in rolling, firm adhesion and transmigration of leucocytes along the vessel wall. ICAM-1 is constitutively expressed in low quantities on leucocytes, fibroblasts, epithelial cells and endothelial cells. Its expression increases on stimulation by cytokines.[3] E-selectin is found only on activated endothelium. P-selectin is a membrane molecule of the α granules in platelets and of the endothelial Weibel–Palade bodies and is expressed on the cell membrane on activation of endothelial cells.[4] ED1-fibronectin (ED1-fn) is an adhesive glycoprotein that is synthesised in endothelial cells. The ED1 domain is included in fibronectin molecules in pathological conditions of the vessel wall.[5] Von Willebrand Factor (VWF) is a large adhesive glycoprotein participating in the adhesion of platelets. It is produced and released by vascular endothelial cells and, in much smaller amounts, by platelets. VWF is a marker of both acute and chronic endothelial cell activation. A recently developed assay is the measurement of VWF propeptide, which may serve as a marker of acute endothelial cell activation.[6]

The synthesis of VWF propeptide is linked with the synthesis of VWF, but its plasma half life is shorter than that of VWF.

We studied the relationship between serial concentrations of circulating markers of endothelial cell activation (soluble (s) ICAM-1, sP-selectin, sE-selectin, ED1-fn, VWF and VWF propeptide) and development of DCI in patients with SAH.

PATIENTS AND METHODS

Patients

From January 1999 to March 2001, we entered 106 consecutive patients with aneurysmal SAH into our study, who were admitted to our hospital within 72 h after onset of SAH. The diagnosis of aneurysmal SAH was based on the history, a CT scan showing blood in the basal cisterns and CT-angiography. Patients in whom death seemed imminent were not included. We obtained informed consent from all patients or their representatives. The institutional review board approved the study.

Patients were under continuous observation, with monitoring of the Glasgow Coma Scale (GCS) Score, electrocardiogram, blood pressure, pulse rate, temperature, fluid balance and laboratory parameters at regular intervals. Compressive stockings were used to prevent deep venous thrombosis. Medical treatment consisted of oral nimodipine and maintenance of a neutral fluid balance. Outcome was assessed after 3 months with the modified Rankin Scale.[7]

Data collection

For each patient, an anonymised summary of the medical history was prepared, describing all episodes of clinical deterioration in detail. On the basis of this description, two observers independently assessed the occurrence of DCI. Clinical features of DCI were defined as a deterioration in

Abbreviations: DCI, delayed cerebral ischaemia; ED1-fn, ED1-fibronectin; GCS, Glasgow Coma Scale; ICAM, intercellular adhesion molecule; SAH, subarachnoid haemorrhage; sE-selectin, soluble endothelial selectin; sICAM, soluble intercellular adhesion molecule; sP-selectin, soluble platelet selectin; VWF, Von Willebrand Factor

consciousness (a decrease in the GCS Score by ⩾1 point) or the appearance of focal signs that lasted for at least 1 h and could not be explained by epileptic seizures, recurrent bleeding, hydrocephalus or metabolic disturbances. Causes for clinical deterioration other than DCI were excluded by using laboratory tests and CT scanning or MRI as soon as the deterioration was observed. The principal investigator reviewed all available initial and follow-up CT scans and MRIs for the presence of ischaemic lesions and, in case of doubt, a second observer also reviewed them. The amount of intracisternal and intraventricular blood was assessed on the initial CT scan by means of the Hijdra Score.[8] Radiological ischaemic changes were counted as new ischaemic lesions if they were not visible on the initial CT scan or MRI and persisted on follow-up scans. Cerebral angiography or transcranial Doppler ultrasound to show vasospasm were not routinely carried out. Observers were not aware of the laboratory results at the time of clinical and radiological assessment.

Table 1 Clinical characteristics of patients with or without DCI

	With DCI (n = 30)*	Without DCI (n = 56)
Mean age in years (SD)	57.3 (12.2)	53.7 (11.8)
Women	24 (80)	38 (70.0)
WFNS grade at admission		
I	12 (40)	27 (48)
II	11 (37)	12 (22)
III	1 (3)	4 (7)
IV	5 (17)	9 (16)
V	1 (3)	4 (7)
Amount of blood† > median	13 (45)	17 (31)
Rebleeding	5 (17)	4 (7)
DCI		
Clinical signs only	4 (13)	
Clinical and radiological signs	26 (87)	
Onset of DCI		
Spontaneous	9 (30)	
<24 h after operation	12 (40)	
<24 h after coiling	6 (20)	
<24 h after rebleeding	3 (10)	
Median day of onset of DCI (range)	4.0 (1–15)	
Treatment of aneurysm		
Clipping	18 (60)	46 (82)
Coiling	5 (17)	6 (11)
No treatment‡	7 (23)	4 (7)
Median day of operation (range)	3.0 (1–65)	9.3 (0–28)
Median day of coiling (range)	4.0 (2–17)	2.7 (2–3)
Rankin Score[8] after 3 months		
0	2 (7)	5 (9)
1	1 (3)	23 (41)
2	3 (10)	11 (20)
3	6 (20)	5 (9)
4	6 (20)	4 (7)
5	2 (7)	5 (9)
Death	10 (33)	3 (5)
Diabetes mellitus	0 (0)	2 (3.6)
Hypertension	11 (37)	13 (23)
Hypercholesterolaemia or treatment with statins	2 (6.7)	5 (8.9)
Infectious disease (during the entire hospital stay)	15 (50)	20 (36)

DCI, delayed cerebral ischaemia; WFNS, World Federation of Neurological Surgeons Scale.[10]
Values are n (%) unless otherwise stated.
*Patients without DCI but with new radiological ischaemic lesions, insufficient blood sampling or patients who died before day 3 are not included in this table.
†Amount of blood is graded according to Hijdra's method, with a maximum score of 30.[8] In the current series, the median was 24.
‡Because of poor clinical condition.

Blood sampling and laboratory investigations

We collected 687 blood samples from 106 patients. The first blood sample was taken within 72 h after onset of SAH. The additional samples were obtained early in the morning, three times a week on alternate days, irrespective of clinical deterioration.

Blood was collected from the medial cubital vein by an evacuated tube system in 3.1% citrate (1:10) within 72 h of onset of the haemorrhage and was centrifuged immediately at 2000×g for 15 min at 4℃. The supernatant was separated and centrifuged a second time. Plasma samples were stored at −70℃. We assessed sICAM-1, sP-selectin and sE-selectin with commercially available ELISAs (Kordia, Leiden, The Netherlands). VWF and VWF propeptide were measured at the Department of Blood Coagulation, Sanquin, Amsterdam, The Netherlands, as described previously.[6]

We measured circulating ED1-fn with an ELISA (developed at the University Medical Centre Utrecht, Utrecht, The Netherlands) in which the primary antibody was IgM mAb 3E2 (Sigma, St Louis, Missouri, USA) against ED1-fn; ligand capture was detected with peroxidase-conjugated rabbit anti-human fibronectin (DAKO, Glostrup, Denmark).[9]

Data analysis

To investigate the relationship between changes in concentrations of the markers and occurrence of DCI, we compared concentrations in paired blood samples taken within 72 h before and after onset of DCI. The patients were divided into three subgroups: (1) onset of DCI unrelated to treatment of the aneurysm; (2) onset of DCI within 24 h after clipping of the aneurysm; and (3) onset of DCI within 24 h after coiling. If DCI was diagnosed within 24 h shortly after rebleeding, patients were included in the first subgroup (DCI unrelated to treatment). Patients without clinical features of DCI but with new radiological ischaemic lesions were excluded from the analysis. The remaining patients without DCI served as controls. Within the control group, we also compared concentrations before and after surgery. Concentrations are reported as mean (SD). We used the paired t test for the detection of differences between serial concentrations. Finally, we compared the changes in concentrations in patients with and in those without DCI, by using the independent samples t test. As levels of some of the markers may be influenced by the occurrence of an infectious disease, we also analysed the changes in these paired samples after exclusion of patients with an infection between the two samples. The presence of an infection had to be proved by microbiological cultures or chest radiographs compatible with clinical signs and symptoms.

RESULTS

Patients

Of the 106 patients entered into the study (fig 1), 12 were excluded because of radiological ischaemic lesions but no DCI, 7 because of a missing blood sampling either before or after onset of DCI, and 1 because occurrence of DCI could not be determined. Table 1 shows the clinical characteristics of the patients that could be included in the analysis. Of the 30 patients with DCI, 26 had clinical evidence of ischaemia with compatible lesions on CT scan or MRI, whereas four had only clinical deficits. Clinical symptoms consisted of a decrease of 3–8 (median 5.5) in the GCS Score (n = 8), a decrease in both the GCS Score and focal signs (n = 8) or only of focal signs (n = 14). Of 14 instances of DCI before day 4, 13 occurred within 24 h after treatment of the aneurysm. The median time interval between the onset of DCI and the last blood sampling before DCI was 1 (range1–3) day, and that between the onset of DCI and the first blood sampling after onset of DCI was 2 (range 1–3) days.

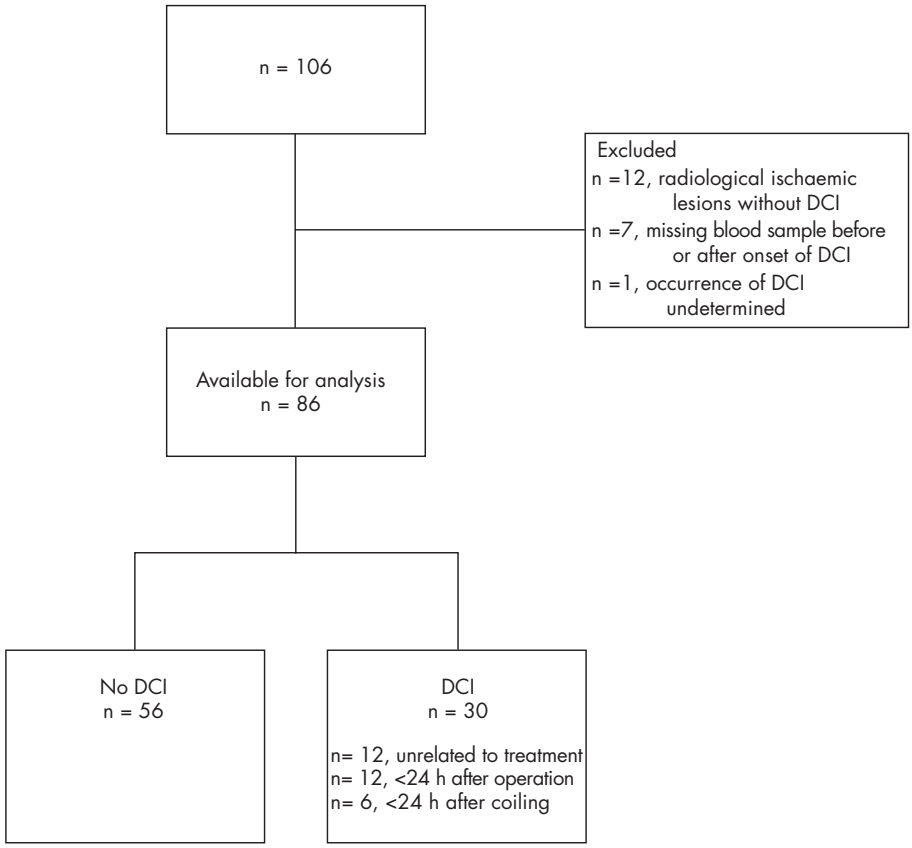

Figure 1 Flow chart of selection of subgroups and reasons for exclusion. DCI, delayed cerebral ischaemia.

As the median day of onset of DCI was day 4, we analysed paired samples from the 56 control patients with SAH and without DCI taken in the same period of 2–7 days after onset of SAH. The control group was divided into three subgroups in the same way as those with DCI: (1) patients who did not undergo any treatment within this time frame; (2) patients whose aneurysms were operated during this period; and (3) patients whose aneurysms were coiled. We also analysed the changes in these paired samples after exclusion of patients with an infection between the two samples.

Laboratory results

Concentrations of sP-selectin markedly increased in the subgroup of patients with DCI unrelated to treatment of the aneurysm (25 ng/ml, 95% CI 8.0 to 43), whereas they decreased in the controls (−13 ng/ml, 95% CI −28 to 2.4; table 2). The difference was statistically significant (−38 ng/ml, 95% CI −66 to −11). The changes in sP-selectin concentrations in the three patients with DCI within 24 h after rebleeding (25 ng/ml, 95% CI −4.4 to 53) showed the same trend as in the remaining nine patients (26 ng/ml, 95% CI 1.4 to 51).

In the entire group of patients with DCI (n = 30), we found a statistically significant rise in VWF concentrations (21 nmol/l, 95% CI 9.6 to 32; table 2). This increase, however, was found mainly in patients in whom DCI occurred within 24 h after treatment of the aneurysm, but not in those with DCI unrelated to the treatment of the aneurysm. Moreover, VWF levels also increased in the patients without DCI, especially after surgery, but also if the aneurysm had not been treated within the sampling period.

We found a statistically significant decrease in concentrations of sICAM-1 (−117 ng/ml, 95% CI −193 to −42) and sE-selectin (−16 ng/ml, 95% CI −32 to −0.6) in the patients in whom DCI occurred within 24 h after operation. In the patients with DCI unrelated to treatment, we found no appreciable changes in sICAM-1 or sE-selectin concentrations.

ED1-fn and VWF propeptide concentrations did not show any marked changes in the patients with DCI. In the control patients, however, ED1-fn concentrations increased significantly (2.9 μg/l, 95% CI 1.4 to 4.5) in the patients who underwent surgery for their aneurysm.

After the exclusion of patients with an infection between the two samples (with DCI, n = 4; without DCI, n = 11), or of those with only clinical DCI without radiological ischaemic abnormalities (n = 4), the main findings of the study remained significant ($p<0.05$) despite the smaller numbers.

DISCUSSION

The main finding of our study was an increase in sP-selectin levels after DCI, which was not related to treatment of the aneurysm in patients with an aneurysmal SAH. In patients without DCI, sP-selectin levels tended to decrease during the same period after onset of SAH. We found no increase in sP-selectin levels in the patients with onset of DCI occurring within 24 h after surgery, but a rise in sP-selectin levels may not have been evident in these patients, as sP-selectin levels in the controls decreased during operation. We found only one previous report[11] on sP-selectin levels in patients with SAH, which described higher mean levels of sP-selectin in patients with DCI. The analysis included serial samples taken before as well as after the onset of DCI and showed a trend towards higher levels of sP-selectin after onset. The timing and influence of surgery, however, was not accounted for. Studies in patients with ischaemic stroke also reported increased levels of sP-selectin.[12 13] The question is whether this increase in sP-selectin originates from endothelial cells,

Table 2 Comparison of changes in serial concentrations of markers of endothelial cell activation after SAH in patients with or without DCI, and in subgroups of these patients according to treatment of the aneurysm

	With DCI						Without DCI					
	n	Before	After	Change	95% CI	p Value	n	First*	Second	Change	95% CI	p Value
VWF (nmol/l)												
All†	30	109 (37)	130 (45)	21	9.6 to 32	<0.01	56	88 (31)	113 (51)	25	13 to 36	<0.01
No intervention‡	12	118 (26)	125 (33)	7	−6.9 to 22	0.28	34	91 (36)	109 (57)	18	2.8 to 33	0.02
Clipping	12	107 (46)	130 (51)	23	3.4 to 43	0.03	17	81 (23)	118 (36)	37	25 to 49	<0.01
Coiling	6	97 (37)	141 (59)	44	9.1 to 79	0.02	5	91 (11)	119 (63)	28	−61 to 116	0.43
sP-selectin (ng/ml)												
All	30	99 (54)	107 (64)	8	−2.2 to 18	0.12	56	115 (77)	103 (66)	−12	−24 to −1.2	0.03
No intervention	12	94 (68)	119 (83)	25	8.0 to 43	<0.01	34	131 (86)	118 (74)	−13	−28 to 2.4	0.10
Clipping	12	95 (49)	88 (48)	−7	−18 to 4.9	0.23	17	94 (56)	74 (33)	−20	−36 to −2.6	0.03
Coiling	6	116 (34)	119 (46)	3	−27 to 31	0.84	5	82 (30)	94 (71)	12	−70 to 93	0.71
sICAM-1 (ng/ml)												
All	30	389 (141)	349 (125)	−40	−81 to 0.9	0.06	56	366 (135)	390 (156)	24	−20 to 67	0.28
No intervention	12	334 (152)	360 (147)	26	−16 to 68	0.21	34	348 (129)	404 (160)	56	−0.1 to 114	0.05
Clipping	12	442 (133)	325 (119)	−117	−193 to −42	<0.01	17	397 (141)	363 (143)	−34	−100 to 30	0.27
Coiling	6	393 (104)	375 (97)	−118	−86 to 50	0.53	5	389 (163)	385 (190)	−4.0	−290 to 282	0.97
sE-selectin (ng/ml)												
All	30	43 (24)	36 (23)	−7	−16 to 1.7	0.11	56	45 (23)	41 (31)	−4	−11 to 2.0	0.17
No intervention	12	35 (18)	37 (27)	2	−12 to 18	0.69	34	53 (23)	50 (36)	−3	−12 to 6.5	0.52
Clipping	12	44 (27)	28 (11)	−16	−32 to −0.6	0.04	17	35 (18)	27 (9.3)	−8	−17 to 2.2	0.12
Coiling	6	58 (25)	48 (27)	−10	−30 to 11	0.28	5	32 (14)	28 (14)	−4	−34 to 26	0.72
ED1-fn (μg/l)												
All	30	4.7 (6.0)	6.0 (5.0)	1.3	−0.1 to 2.6	0.07	56	3.6 (3.4)	5.9 (5.1)	2.3	1.1 to 3.5	<0.01
No intervention	12	3.8 (3.3)	4.2 (3.6)	0.4	−1.3 to 2.1	0.61	34	3.5 (3.3)	5.1 (4.6)	1.6	−0.1 to 3.3	0.06
Clipping	12	5.9 (8.5)	8.4 (6.4)	2.5	−0.3 to 5.2	0.08	17	3.5 (3.3)	6.4 (5.2)	2.9	1.4 to 4.5	0.01
Coiling	6	4.1 (4.2)	4.7 (2.6)	0.6	−3.1 to 4.2	0.70	5	4.2 (4.4)	9.3 (7.7)	5.1	−0.7 to 11	0.07
VWF propeptide (nmol/l)												
All	30	8.2 (2.1)	9.0 (2.6)	0.8	0.0 to 1.7	0.05	56	7.8 (2.6)	8.7 (3.6)	0.9	−0.1 to 1.9	0.07
No intervention	12	8.3 (1.7)	8.9 (2.0)	0.6	−0.8 to 1.9	0.39	34	7.9 (2.9)	9.0 (3.9)	1.1	−0.2 to 2.3	0.09
Clipping	12	8.6 (2.5)	9.7 (3.4)	1.1	−0.6 to 2.9	0.17	17	7.6 (2.4)	8.0 (2.6)	0.4	−1.5 to 2.2	0.67
Coiling	6	7.2 (1.8)	8.0 (1.6)	0.8	−0.5 to 2.0	0.17	5	7.8 (1.2)	9.3 (5.1)	1.5	−4.4 to 7.4	0.53

DCI, delayed cerebral ischaemia; ED1-fn, ED1 fibronectin; SAH, subarachnoid haemorrhage; sICAM, soluble intercellular adhesion molecule; sE-selectin, soluble endothelial selectin; sP-selectin, soluble platelet selectin; VWF, Von Willebrand Factor.

*First and second samples are sequential samples taken between day 2 and day 7 after onset of SAH in patients without DCI.

†Including three patients with rebleeding.

‡Clipping or coiling of the aneurysm.

platelets or both. In our study, we did not find any evidence of endothelial cell activation after DCI unrelated to treatment in the analyses of the other markers. Therefore, we suggest that the increased levels of sP-selectin are the result of increased release from platelets. Two other findings support a role for platelets in the development of DCI after SAH. Firstly, activation and aggregation of platelets has been described in patients with SAH and DCI;[14] secondly, antiplatelet treatment may reduce the risk of DCI.[15]

Our findings with respect to sICAM-1 and sE-selectin in patients with DCI are in agreement with the only other longitudinal study of patients with SAH and DCI, which found a trend towards lower mean levels of sICAM-1 and sE-selectin in patients with DCI.[11] A pairwise comparison of values before and after onset of DCI was, however, not reported. Increased levels of sICAM-1 in plasma have been reported in patients with SAH after the onset of vasospasm, as documented with transcranial Doppler ultrasound or angiography.[16] Clinical or radiological signs of ischaemia were not mentioned in this report, and the method of determining the day of onset of vasospasm was not clearly stated.

We found four reports on the relationship between high levels of VWF and DCI[17–19] or intracranial vasospasm.[20] In our previous study,[17] the observed increase in VWF levels after DCI may well have been attributable to surgery or endovascular procedure. In another study,[18] the timing of blood sampling with respect to occurrence of DCI was not stated. In a recent publication[19] on early levels in the same patients as in this study, we reported that increased initial levels of VWF were associated with poor outcome and with occurrence of DCI. These findings do not necessarily contradict the results of the present study, as the rise in VWF levels in a subset of the patients may be an acute-phase phenomenon that reflects the severity of the SAH and subsequently contributes to delayed complications such as cerebral ischaemia. The fourth study described increased VWF levels preceding vasospasm associated with clinical symptoms in most patients.[20] Increased VWF levels after surgery are a well-known phenomenon.[21] The contradictions with our results may partly be explained by methodological differences and by confounding because of the influence of surgery. In a previous study,[17] we reported increased ED1-fn levels in patients with SAH. A marked rise in ED1-fn levels was found in seven patients with DCI, but five of these patients developed DCI during or shortly after endovascular or operative treatment. This is consistent with the findings of increased levels after surgery in this study. Probably, in patients with SAH the increase in ED1-fn is associated with surgery and is not related to involvement in the pathogenesis of DCI.

From our results, it is evident that surgery profoundly influences the levels of markers of endothelial cell activation and that subdivision of the patients according to treatment of the aneurysm is essential. Whereas levels of ED1-fn and VWF were increased after surgery, those of sICAM-1 and sE-selectin decreased during surgery. Although unexpected, this finding was convincing, as it was consistently observed after surgery in at least two of the three inflammatory adhesion molecules, in both the patients with DCI and in the controls. Decreased levels of sICAM-1 and sE-selectin may be a consequence of decreased synthesis or expression, or of increased utilisation. This decrease after surgery, however, may also be a consequence of haemodilution because of

intravenous administration of fluids during operation. Too few patients in our study underwent endovascular treatment for their aneurysms to ascertain an influence of this treatment on endothelial cell activation markers.

Our outcome event of interest was the occurrence of DCI, not of radiological vasospasm. As 30% of patients with vasospasm do not develop DCI, and 30% of patients with DCI have no vasospasm,[22] DCI is a more relevant outcome measure than vasospasm from a clinical point of view. Therefore, we did not routinely carry out transcranial Doppler investigations or angiography.

The results of our study do not support the hypothesis that endothelial cell activation contributes to the development of DCI. A role for endothelial cell activation, however, cannot be excluded. Locally increased levels in the cerebral circulation may not be detectable in the systemic circulation. Also, the number of patients in the subgroups, especially in the subgroup with DCI unrelated to treatment, may have been too small to detect a marked increase in levels.

In conclusion, we hypothesise that sP-selectin released from activated platelets may participate in the pathogenesis of DCI after SAH. Our findings support the rationale of further studies on the efficacy of antiplatelet treatment or, more specifically, treatment with anti-P-selectin monoclonal antibodies in patients with SAH. A causal role for P-selectin in the development of DCI after SAH, however, has yet to be established.

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Competing interests: None.

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