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Detection of soluble intercellular adhesion molecule–1 and vascular cell adhesion molecule–1 in both cerebrospinal fluid and serum of patients after aneurysmal subarachnoid hemorrhage

Mehmet Yasar Kaynar, M.D., Taner Tanriverdi, M.D., Ali Metin Kafadar, M.D., Tibet Kacira, M.D., Hafize Uzun, Ph.D., Seval Aydin, Ph.D., Koray Gumustas, Ph.D., Ahmet Dirican, Ph.D., and Cengiz Kuday, M.D.

Departments of Neurosurgery, Biochemistry, and Biostatistics, Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey

Object. The aim of this study was to explore whether levels of intercellular adhesion molecule–1 (ICAM-1) and vascular cell adhesion molecule–1 (VCAM-1) are elevated in the cerebrospinal fluid (CSF) and serum of patients after aneurysmal subarachnoid hemorrhage (SAH).

Methods. This prospective clinical study focused on 21 patients who had recently suffered an SAH due to aneurysmal rupture and 15 control patients with hydrocephalus who had no other central nervous system disease. Cerebrospinal fluid and serum samples obtained within the first 3 days and on the 5th and 7th days of SAH were assayed for ICAM-1 and VCAM-1 by using quantitative enzyme-linked immunosorbent assays.

Levels of soluble forms of ICAM-1 ($p = 0.00001$) and VCAM-1 ($p = 0.009$) in the patients' CSF and those of ICAM-1 ($p = 0.00001$) and VCAM-1 ($p = 0.00001$) in their serum were found to be elevated after SAH compared with levels in the CSF and serum of control patients with hydrocephalus. In addition, when the authors compared the increased levels of adhesion molecules in the CSF and serum of patients after SAH, the only statistically insignificant difference that they found was between the levels of VCAM-1 in serum obtained on Days 5 and 7 after SAH ($p = 0.27$).

Conclusions. Adhesion molecules are a group of macromolecules that may participate in the inflammatory process, a common pathway leading to vasospasm after SAH. Leukocyte adherence to the vascular endothelium, which is induced by adhesion molecules, has been believed to be the initial signal of the development of vasospasm. The authors have demonstrated the synchronized elevation of two adhesion molecules in both CSF and serum following aneurysmal SAH. Blocking of ICAM-1 as well as VCAM-1 by monoclonal antibodies post-SAH may provide a beneficial effect on vasospasm.

Key Words • adhesion molecule • aneurysm • intercellular adhesion molecule–1 • subarachnoid hemorrhage • vascular cell adhesion molecule–1

Despite the contemporary management of ruptured cerebral aneurysms, cerebral vasospasm is still the leading cause of high morbidity and mortality rates following SAH. An association between the release of blood breakdown products in the subarachnoid space and vasospasm has been established; however, the precise pathopysiological mechanisms for this and what elements have roles in the development of vasospasm remain to be elucidated. The cumulative body of evidence in recent years has indicated that by regulating inflammation, adhesion molecules play a crucial role in the development of vasospasm after SAH due to an aneurysm rupture.[2,9,10,15,16,19,22] The upregulation of adhesion molecules has been shown in various inflammatory cerebral diseases, including bacterial meningitis, encephalitis, and multiple sclerosis.[2,9,22] The expression of adhesion molecules on the endothelium and vessel wall in atherosclerotic lesions and in ischemia–reperfusion injury has also been demonstrated, supporting the notion that these molecules play an important role in the immune-mediated inflammatory cascade of events.[3,6,8]

During the last decade, experimental studies have implicated adhesion molecules in the development of vasospasm after SAH. Handa and associates[10] demonstrated significant expression of ICAM-1 on the endothelial layer of the BA following SAH induced by an intracisternal injection of arterial blood in rats. Sills, et al.,[19] exposed an association between endothelial ICAM-1 upregulation and FA vasospasm after exposure to blood in a rat FA model. Inhibition of experimental vasospasm with antiadhesion molecules has also been demonstrated. Oshiro and colleagues[17] showed inhibition of experimental vasospasm by using anti–ICAM-1 mAb in an FA model in rats. In a rabbit model of SAH, Bavbek and coworkers[1] demonstrated that monoclonal antibodies against ICAM-1 and its ligand, CD18, decrease BA vasospasm. Inhibition of vasospasm was accomplished by Clatterbuck, et al.,[5] who used mAbs directed against LFA-

Abbreviations used in this paper: ACoA = anterior communicating artery; BA = basilar artery; CNS = central nervous system; DIND = delayed ischemic neurological deficit; FA = femoral artery; ICA = internal carotid artery; ICAM-1 = intercellular adhesion molecule–1; LFA-1 = lymphocyte function–associated antigen–1; mAb = monoclonal antibody; MCA = middle cerebral artery; PCoA = posterior communicating artery; SAH = subarachnoid hemorrhage; TCD = transcranial Doppler; VCAM-1 = vascular cell adhesion molecule–1.

TABLE 1

*Clinical characteristics of patients with SAH**

SAH Case No.	Age (yrs), Sex	Hunt & Hess Grade	Fisher Grade	Symptomatic Vasospasm	Aneurysm Location	GOS Score at 6 Mos
1	65, F	III	2	yes	PCoA	death
2	67, F	II	3	no	ACoA	good recovery
3	58, M	III	3	yes	ICA	severe disability
4	71, F	V	3	yes	MCA	death
5	67, F	II	3	yes	PCoA	death
6	32, F	III	4	no	MCA	mild disability
7	27, M	II	2	no	ACoA	good recovery
8	52, F	II	2	no	MCA	good recovery
9	43, F	II	2	no	PCoA	good recovery
10	32, M	III	3	no	ACoA	death
11	32, M	II	3	no	ACoA	good recovery
12	17, F	II	2	no	ICA	good recovery
13	44, F	II	3	no	ICA	mild disability
14	38, M	III	2	yes	ACoA	persistent vegetative state
15	42, M	II	2	no	PCoA	good recovery
16	52, M	II	2	no	ACoA	good recovery
17	54, F	II	2	yes	ICA, MCA	mild disability
18	56, F	III	3	yes	OphA	severe disability
19	36, M	II	3	no	ACoA, MCA	good recovery
20	59, F	II	2	no	MCA	good recovery
21	31, F	II	2	no	ACoA	good recovery

* OphA = ophthalmic artery.

1, a surface molecule of leukocytes that interacts with ICAM-1, in an FA model in rats. In a recent study, the prevention of cerebral vasospasm by a humanized anti–CD11/CD18 mAb administered after experimental SAH in nonhuman primates was shown.[4]

Although encouraging findings from these experimental studies have been obtained, the putative role of adhesion molecules in human vasospasm following aneurysmal SAH is not yet adequately known. Polin, et al.,[18] demonstrated elevation of soluble E-selectin, ICAM-1, VCAM-1, and L-selectin in the CSF of patients after aneurysmal SAH. Nissen and colleagues[16] demonstrated that P- and L-selectin are increased in the serum of patients after aneurysmal SAH and proposed that these selectin superfamilies, rather than ICAM-1 and VCAM-1, may be involved in the pathophysiology of DIND after SAH. Mack, et al.,[14] found a correlation between soluble ICAM-1 levels in serum and functional outcomes in patients following aneurysmal SAH. Mocco and coworkers[15] have also shown the same correlation in patients with vasospasm, evidenced by daily monitoring of TCD velocities. In addition, a strong correlation between clinical outcome and the concentrations of soluble adhesion molecules in the CSF of patients with acute intracerebral hemorrhage has also been demonstrated. The results of these experimental and clinical studies strongly indicate that adhesion molecules may be involved in the pathogenesis of vasospasm after aneurysmal rupture.

In this prospective clinical study, our aim was to examine soluble ICAM-1 and VCAM-1 levels in the CSF and serum of patients at various time points after aneurysmal SAH.

Material and Methods

Patient Population

Ethical approval for this study was obtained from the Human Investigations Committee at Istanbul University, and all patients or the next of kin, if the patient was unconscious, provided informed consent. We studied patients who were referred to our neurosurgical unit between January and June 2003 with the diagnosis of SAH established by computerized tomography scanning. We excluded patients who had any kind of infection at the time of CSF and serum collection, in which adhesion molecules might play a part. The sole inclusion criterion was admission of the patients to our unit within the first 3 days of SAH.

Demographics of Patients and the Control Group

The study population consisted of 21 patients with aneurysmal SAH and 15 patients with hydrocephalus without any other known CNS diseases. Among the patients with SAH, seven had ACoA aneurysms, four had PCoA aneurysms, four had MCA bifurcation aneurysms, three had ICA aneurysms, one had an ophthalmic artery aneurysm, one had both ICA and MCA aneurysms, and one had both ACoA and MCA aneurysms.

The average age of the patients with SAH was 46.4 years (range 17–71 years). The Glasgow Outcome Scale,[13] which was applied to these patients at hospital discharge, showed good recovery in 11 patients, mild disability in three, and severe disability in two. Four patients died before discharge and one demonstrated a persistent vegetative state.

The average age of the control group was 50.2 years (range 15–81 years). Nine patients had normotensive hydrocephalus and six had hydrocephalus due to aqueduct stenosis. Summaries of demographic information for the patients with SAH and the control group are provided in Tables 1 and 2, respectively.

Specimen Handling

Seventy-eight (total 156) samples were assayed for ICAM-1 and 78 for VCAM-1. For each patient, serial blood and CSF samples were collected at the same time within 3 days and on the 5th and 7th days post-SAH. The blood and CSF samples were collected by venipuncture and lumbar puncture, respectively. In the control group, the blood samples were collected by venipuncture and the CSF samples were obtained during ventriculoperitoneal shunt placement surgery. Samples from the control group were obtained only once. As soon as possible, each 10-ml CSF and blood specimen was centrifuged at 10,000 rpm for 15 minutes and the supernatant was stored at −70°C until assayed.

TABLE 2

*Clinical characteristics of control patients**

Control Case No.	Age (yrs), Sex	Diagnosis
1	34, F	HAS
2	15, M	HAS
3	74, M	NPH
4	14, F	HAS
5	45, F	NPH
6	44, M	NPH
7	50, F	NPH
8	81, M	NPH
9	65, M	NPH
10	78, F	NPH
11	55, F	NPH
12	78, M	NPH
13	43, F	HAS
14	42, M	HAS
15	35, F	HAS

* HAS = hydrocephalus due to aqueduct stenosis; NPH = normotensive hydrocephalus.

TABLE 4

*Adhesion molecule levels in the CSF and serum of control patients**

Control Case No.	CSF ICAM-1	Serum ICAM-1	CSF VCAM-1	Serum VCAM-1
1	2.62	195	6.4	395
2	0.0	216	13.8	286
3	3.3	172	4.9	348
4	0.7	168	26.2	372
5	3.5	185	31.3	303
6	3.8	156	9.2	296
7	0.0	194	22.3	388
8	4.3	205	34.8	429
9	12.6	178	66.3	376
10	1.7	186	5.4	365
11	2.0	193	21.6	349
12	0.0	165	43.9	407
13	1.1	219	30.8	398
14	0.7	148	44.6	362
15	0.9	157	6.3	351

* Values represent nanograms per milliliter.

Soluble ICAM-1 and VCAM-1 levels were quantitatively measured in CSF and serum in nanograms per milliliters by using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). A dilution of 1:10 was used for the ICAM-1 and VCAM-1 assays and the absorption measurements were obtained at 450 nm (with a correction of 630 nm to eliminate optical imperfections in the plate) by using a microtiter plate reader (Automated Microplate Reader [model EC311sx]; Bio-Tek Instruments, Winooski, VT). All samples and standards were run in duplicate.

Statistical Analysis

Data were analyzed with the aid of the SPSS statistical program (SPSS, Chicago, IL). Statistical analyses were performed using the nonparametric Mann–Whitney U-test. A probability value less than 0.05 was considered statistically significant.

Results

Sixty-three CSF and 63 serum samples from patients with SAH and 15 CSF and 15 serum samples from patients in the control group were obtained for this prospective clinical study. The samples were tested for the presence of ICAM-1 and VCAM-1. The results in the SAH and control groups are provided in Tables 3 and 4, respectively, and a summary of the statistical data is provided in Table 5.

TABLE 3

*Adhesion molecule levels in the CSF and serum of patients with aneurysmal SAH within the first 3 days and on Days 5 and 7 posthemorrhage**

SAH Case No.	CSF ICAM-1			Serum ICAM-1			CSF VCAM-1			Serum VCAM-1		
	Day ≤ 3	Day 5	Day 7	Day ≤ 3	Day 5	Day 7	Day ≤ 3	Day 5	Day 7	Day ≤ 3	Day 5	Day 7
1	18.6	21.0	25.0	397	429	466	26.3	30.3	31.6	561	572	574
2	12.3	15.0	24.0	428	435	439	38.7	40.4	45.8	598	603	622
3	31.0	42.0	63.0	456	475	497	49.5	52.6	51.3	677	692	714
4	46.0	58.0	88.0	465	499	546	8.6	12.7	23.6	693	699	736
5	92.0	118.0	146.0	469	516	598	107.3	112.8	110.4	682	703	742
6	96.0	122.0	149.0	475	527	619	98.5	105.6	119.7	688	692	714
7	20.0	29.0	33.0	382	392	447	38.6	43.9	56.8	572	592	408
8	25.0	28.0	31.0	375	412	439	50.8	59.7	62.6	561	570	583
9	2.6	5.0	19.0	368	405	457	35.3	43.6	61.8	526	529	542
10	6.3	8.0	11.0	403	443	479	6.9	12.8	13.9	538	552	578
11	7.9	10.0	18.0	418	437	461	74.2	81.6	98.7	546	576	581
12	3.2	5.6	8.7	385	396	405	56.9	61.8	65.6	519	525	533
13	37.0	43.0	59.0	446	461	476	44.7	52.7	63.8	582	599	648
14	45.0	49.0	74.0	429	439	497	39.3	41.6	43.8	571	587	619
15	29.0	37.0	52.0	405	466	519	4.2	5.9	19.8	542	576	583
16	3.4	10.0	19.0	369	372	398	67.8	75.6	77.9	519	536	542
17	19.0	21.0	36.0	407	419	458	72.6	79.8	86.7	538	547	551
18	56.0	62.0	79.0	445	516	593	79.3	81.6	85.9	717	746	754
19	22.0	29.0	31.0	395	447	486	81.6	82.3	82.7	674	699	735
20	7.6	12.0	14.0	409	432	443	21.7	29.6	35.8	643	652	678
21	9.5	14.0	17.0	377	381	390	16.8	19.3	28.2	630	649	662

* Values represent nanograms per milliliter.

TABLE 5

*The mean CSF and serum concentrations of adhesion molecules in patients in the SAH and control groups**

Parameter	CSF ICAM-1†‡	Serum ICAM-1†‡	CSF VCAM-1†§	Serum VCAM-1†‖
control group (15 patients)	2.48	182.46	24.5	361.66
SAH group (21 patients)				
Day ≤3 post-SAH	28.067	414.429	48.552	598.905
Day 5 post-SAH	35.171	442.762	53.886	614.095
Day 7 post-SAH	47.462	481.571	60.305	625.857

* Values represent nanograms per milliliter.
† Differences between levels in the control and SAH groups were statistically significant at $p < 0.00001$.
‡ Differences among levels measured at the three time points post-SAH were also statistically significant at $p < 0.00001$.
§ Differences between levels in the control and SAH groups were statistically significant at $p = 0.009$.
‖ Comparison between level measured within the first 3 days and those measured on Day 5 and on Day 7 showed statistically significant differences ($p = 0.00001$ and $p = 0.01$, respectively).

Graphic representations of the ICAM-1 and VCAM-1 levels in CSF and serum in patients in the SAH and control groups are provided in Figs. 1 and 2.

Levels of ICAM-1 in CSF

The CSF levels of ICAM-1 markedly differed between in the SAH and control groups. In the control group, CSF samples from three patients had no detectable quantity of ICAM-1 and the mean concentration of this adhesion molecule was 2.48 ± 3.15 ng/ml. In contrast, CSF samples from all patients who had experienced SAH had quantifiable ICAM-1 during the first 3 days and on the 5th and 7th days post-SAH. The mean values in this group were 28.06 ± 5.8 ng/ml within the first 3 days, 35.17 ± 7.1 ng/ml on Day 5, and 47.46 ± 8.8 ng/ml on Day 7. This difference in concentrations between groups was statistically significant ($p = 0.00001$). In addition the mean elevated levels of ICAM-1 measured post-SAH at the three time points were statistically significant when compared with each other ($p = 0.00001$).

Levels of ICAM-1 in Serum

The levels of ICAM-1 in the serum of patients in the SAH and control groups were also markedly different. The mean concentration of ICAM-1 in the control patients was 182.46 ± 21.65 ng/ml compared with 414.43 ± 7.46, 442.76 ± 9.73, and 481.57 ± 13.82 ng/ml in the SAH group within the first 3 days and on the 5th and 7th days after the hemorrhage, respectively. The differences in concentration between the two groups of patients was statistically significant ($p = 0.00001$). In addition, the mean elevated levels of serum soluble ICAM-1 measured post-SAH at the three time points were statistically significant when compared with each other ($p = 0.00001$).

Levels of VCAM-1 in CSF

The levels of VCAM-1 averaged 24.5 ± 17.9 ng/ml in the control group compared with 48.55 ± 6.48, 53.89 ± 6.58, and 60.30 ± 6.52 ng/ml in the SAH group within the first 3 days and on the 5th and 7th days posthemorrhage, respectively. This difference in levels between the patient groups was statistically significant ($p = 0.009$). In addition the differences among the mean elevated levels of VCAM-1 measured at the three time points post-SAH were also statistically significant ($p = 0.00001$).

Levels of VCAM-1 in Serum

The levels of VCAM-1 in the serum of patients with SAH were significantly elevated when compared with levels in the control group. In the control group, the mean levels of VCAM-1 were 361.66 ± 41.38 ng/ml. In contrast, in patients with SAH these levels were 598.9 ± 14.51, 615.0 ± 14.82, and 625.86 ± 20.12 ng/ml within the first 3 days and on the 5th and 7th days post-SAH, respectively. Comparisons between the levels measured on those 3 days and the level in the control group provided statistically significant differences ($p = 0.00001$). When we compared the elevated level of VCAM-1 measured within the first 3 days with levels measured on Day 5 and Day 7, there was a statistically significant difference ($p = 0.00001$ and $p = 0.01$, respectively). When we compared the elevated level measured on Day 5 with that measured on Day 7 post-SAH, however, no statistically significant difference was demonstrated ($p = 0.27$).

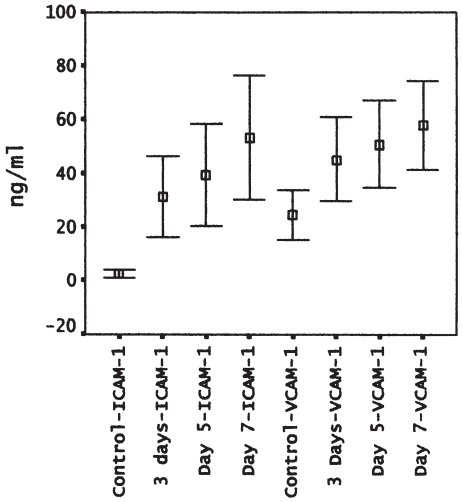


FIG. 1. Graph showing a comparison of levels of ICAM-1 and VCAM-1 in the CSF of patients with SAH and control patients. *Squares* represents the means ± standard errors of the means and *bars* denote the range of values. The differences in the levels of these adhesion molecules between the two groups of patients were statistically significant (for ICAM-1 $p < 0.00001$; for VCAM-1 $p < 0.009$).

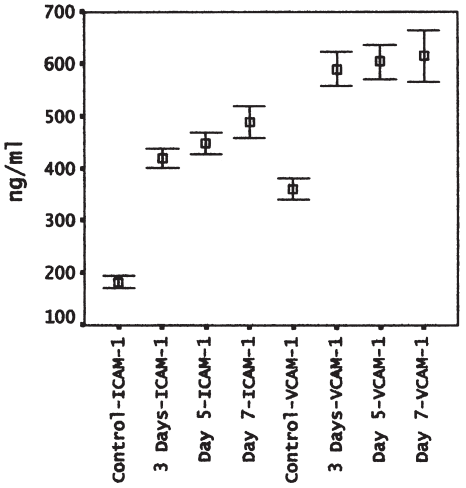


FIG. 2. Graph showing a comparison of levels of ICAM-1 and VCAM-1 in the serum of patients with SAH and control patients. *Squares* represent the means ± standard errors of the means and *bars* denote the range of values. The differences in the levels of these adhesion molecules between the two groups of patients were statistically significant (for both ICAM-1 and VCAM-1 $p < 0.00001$).

Other Factors

In this study, the SAH group was composed of eight male and 13 female patients with a mean age of 46.4 years, and the control group included seven male and eight female patients with a mean age of 50.2 years. There was no statistically significant difference between groups with regard to age or sex ($p = 0.5$ for age and $p = 0.6$ for sex). Among these patients, age and sex; Fisher grade;[7] Hunt and Hess grade[12] at presentation; history of hypertension, coronary artery disease, diabetes mellitus, or smoking; leukocytosis on admission; symptomatic vasospasm; and the GOS score had no effect on elevated levels of ICAM-1 and VCAM-1. Table 6 summarizes data for a comparison of levels of adhesion molecules in the control group and in patients in the SAH group with poor outcomes.

Discussion

Two members of the immunoglobulin superfamily, ICAM-1 and VCAM-1, are known to be adhesion molecules thought to participate in the pathogenesis of vasospasm after SAH. Detailed descriptions on the location and function of each of these molecules have appeared in the literature. It is beyond the scope of this paper to provide similar detailed descriptions, but suffice to state that ICAM-1 can be induced by interleukin-1, interferon-γ, and tumor necrosis–α, and is mainly expressed by the vascular endothelium, macrophages, and lymphocytes. By binding to its ligand, CD18, which is present on circulating leukocytes, ICAM-1 causes firm adhesion of leukocytes to the vascular endothelium and mediates diapedesis, which is known to be one of the most crucial steps in the inflammatory process. On the other hand, VCAM-1 is expressed on the vascular endothelium in response to cytokine production, and binds to its ligand, late antigen–4, which is expressed on lymphocytes and monocytes. The main function of this adhesion molecule is to support adhesion.[11,21]

The involvement of the immune–inflammatory response has been implicated in the pathogenesis of vasospasm after SAH in both animal and human studies. Handa and associates[10] showed the induction of ICAM-1 expression on the endothelium, media, and adventitia of the BA after SAH, which was induced by an intracisternal injection of blood in rats. In their study, a correlation between the degree and timing of arterial spasm and the leukocyte inflammatory response was also demonstrated. In an FA model of vasospasm in rats, Sills, et al.,[19] demonstrated early induction of ICAM-1 on the vessel endothelium. At that study the influx of inflammatory cells was correlated with the time and location of increased ICAM-1 expression. Furthermore, in recent years, the upregulation of adhesion molecules has also been shown in ischemia–reperfusion injury models and a variety of cerebral inflammatory diseases in humans.[2,8,9,22] In animal studies these findings indicate that expression of adhesion molecules may play a pivotal role in inflammation in the CNS and may cause or initiate vasospasm after SAH. The results of these animal studies have led to the development of antiadhesion strategies, which seemed to provide satisfactory results. Oshiro and colleagues[17] demonstrated that anti–ICAM-1 mAbs, which were administered systemically starting 3 hours after exposure of blood to the FA, resulted in a significant inhibition of vasospasm in the rat FA model. This inhibition was also correlated with a reduction in the number of infiltrating macrophages and granulocytes in the periadventitial space of the blood-exposed vessel. In a rabbit model of SAH, Bavbek, et al.,[1] reported attenuation of vasospasm following an intracisternal administration of mAbs directed against ICAM-1 and its ligand, CD18. Using the same model, Clatterbuck, et al.,[5] demonstrated the ability to block vasospasm of the FA by using mAbs to LFA-1, the molecule on the surface of leukocytes that interacts with ICAM-1. In a more recent study, Clatterbuck, et al.,[4] extended their previous work involving the systemic administration of an mAb for a blockade of leukocyte migration to a nonhuman primate model of chronic, posthemorrhagic cerebral vasospasm. Those researchers demonstrated that administration of humanized anti–LFA-1 and macrophage antigen-1, which are expressed on the surface of a leukocyte, prevents experimental cerebral vasospasm in nonhuman primates, despite the unaltered presence of hemoglobin in the subarachnoid space. The downregulation of ICAM-1 by a periadventitial release of ibuprofen, a nonselective cyclooxygenase inhibitor, has also been proven.[20] These experimental studies, in which antiadhesion molecule strategies were used, clearly support the hypothesis that inflammation plays a role in cerebral vasospasm after SAH.

Despite the growing body of evidence from animal studies that demonstrate the importance of leukocyte–endothelial adhesion molecule interaction in the molecular chain of events leading to posthemorrhagic vasospasm, a limited number of human studies have provided evaluations of ICAM-1 and VCAM-1, both of which have been shown to have a crucial role in the development of vasospasm after aneurysmal SAH. It may be possible that animal models of SAH produce vasospasm that differs from that observed in humans in both time course and anatomy. Therefore, it is clear that clinical studies designed to evaluate adhesion molecules in patients with aneurysmal SAH may provide direct evidence. For this purpose, some investigators have begun to perform clinical studies to explore whether lev-

TABLE 6

*Comparison of mean CSF and serum concentrations of adhesion molecules in control patients and patients in the SAH group with good and bad outcomes**

Parameter	SAH Group		Control Group (15 patients)	Significance
	Good Outcome (14 patients)	Bad Outcome (7 patients)		
CSF ICAM-1			2.48 ± 3.1	S
Day ≤3	21.03 ± 24.0	42.12 ± 27.8		
Day 5	27.18 ± 29.7	51.14 ± 35.3		NS
Day 7	36.47 ± 35.3	69.42 ± 44.2		
CSF VCAM-1			24.52 ± 17.9	S
Day ≤3	50.17 ± 26.7	45.31 ± 37		
Day 5	55.84 ± 27.6	49.2 ± 36.9		NS
Day 7	64.7 ± 27.4	51.0 ± 34.3		
serum ICAM-1			182.46 ± 21.6	S
Day ≤3	402.7 ± 31.3	437.7 ± 29		
Day 5	427.2 ± 40.5	473.8 ± 37.3		NS
Day 7	459.7 ± 57.3	525.1 ± 54		
serum VCAM-1			361.6 ± 41.3	S
Day ≤3	582.2 ± 57.1	634.1 ± 74.2		
Day 5	596 ± 57.4	650.1 ± 77.2		NS
Day 7	598.7 ± 85.6	673.8 ± 80.3		

* Values are expressed as mean nanograms per milliliter ± standard deviation. Abbreviations: NS = not statistically significant; S = statistically significant.

els of soluble adhesion molecules, particularly ICAM-1 and VCAM-1, are elevated in CSF or blood after SAH and to evaluate the role of regulating leukocyte adhesion to, and migration across, the vascular endothelium. The first clinical prospective study was performed by Polin, et al.,[18] who showed elevated levels of soluble ICAM-1, VCAM-1, E-selectin, and L-selectin in the CSF of 17 patients after SAH when compared with 16 control patients. The increase in adhesion molecules was more pronounced for ICAM-1 and E-selectin than for VCAM-1 and L-selectin. The authors concluded that the expression of adhesion molecules, particularly ICAM-1 and E-selectin, may be a critical step in the inflammatory process that occurs after SAH in humans. In a recent prospective study, mean serum concentrations of ICAM-1; VCAM-1; platelet–endothelial adhesion molecule; and E-, P-, and L-selectin were compared between patients with and without DIND after aneurysmal SAH. Of all the adhesion molecules tested in this study, P- and L-selectin were thought to be involved in the pathophysiology of DIND. Although the results for ICAM-1 and VCAM-1 were not statistically significant, they did demonstrate a strong statistical trend in patients with DIND.[16] Mack, et al.,[14] showed a significant correlation between serum ICAM-1 levels and outcomes in patients with SAH. According to these authors, serum soluble ICAM-1 levels appear to be strong predictors of poor outcome in patients with Hunt and Hess Grades I and II. In a more recent clinical study, Mocco and coworkers[15] examined soluble ICAM-1 levels at various time points after aneurysmal SAH, and analyzed them with respect to changes in vessel flow dynamics and morphological characteristics by monitoring TCD velocities and performing cerebral angiography. In patients in whom vasospasm was documented by both TCD studies and angiography a highly significant mean rate of increase in soluble ICAM-1 levels was found during the perivasospasm period.

Positive findings in these animal and human studies led us to investigate whether both CSF and serum concentrations of adhesion molecules are increased and can be correlated with Hunt and Hess neurological grade, degree of SAH (Fisher grade), symptomatic vasospasm, and outcome. Moreover, this is the first prospective clinical study in which an attempt has been made to investigate soluble ICAM-1 and VCAM-1 in both CSF and serum in patients after aneurysmal SAH. Cerebrospinal fluid and serum concentrations of ICAM-1 are significantly higher in patients with SAH compared with control patients. The concentrations continued to increase at all time points at which ICAM-1 was measured. The same is true for VCAM-1. The increased levels of ICAM-1 and VCAM-1 in both CSF and serum may indicate that the process leading to a post–SAH cascade occurs in local (brain) and systemic compartments. Because of the vascular endothelial damage and blood–brain barrier breakdown that occur after SAH, these adhesion molecules can be shed into the CSF. Thus, the migration of inflammatory cells into the CNS, in addition to local destruction of the vascular endothelium and leukocytes in the subarachnoid space, may be the origin of increased CSF levels of ICAM-1 and VCAM-1. Similarly, shedding of these two molecules into the systemic circulation may partly explain the origin of increased serum levels of such molecules. Whether the increase in levels of ICAM-1 and VCAM-1 is simply a byproduct produced by vasospasm or represents a signal initiating inflammation and leading to vasospasm remains to be elucidated. Currently, the only evidence in hand to indicate the latter is the ability of antibodies to ICAM-1 and its ligand, CD18, to lessen experimental vasospasm in the rabbit,[1] FA,[4] and primate[5] models. The relevance of such experimental studies to human vasospasm has not yet been demonstrated. Additional experimental studies must be performed to find whether these molecules are involved in the pathogenesis of vasospasm or merely appear as byproducts before any clinical application of anti–ICAM-1 and anti–VCAM-1 treatment can be made. Although the adhesion molecules tested in our study did not differ statistically when Fisher grade, symptomatic vaso-

spasm, and outcome at 6 months were reviewed, patients with particularly high CSF levels of ICAM-1 (SAH Cases 4, 5, 6, 14, and 18) and high CSF levels of VCAM-1 (SAH Cases 5, 6, and 18) had Fisher Grade 3, unfavorable outcome, and/or symptomatic vasospasm. Data on adhesion molecules obtained in only these five patients can hardly provide proof of such a relationship, but the results may provide a strong impetus for additional clinical investigations of such a possibility. A positive correlation seems to be present between the elevated levels of adhesion molecules, particularly ICAM-1, and poor outcome; however, the reason for this poor outcome has not yet been explained clearly. Investigators have hypothesized that this relationship may be ascribed to the role ICAM-1 plays in the pathophysiology of human vasospasm.[14–16,18] We insist that the statistically insignificant relationship between outcome and the levels of adhesion molecules evaluated in our study may be due to the small number of patients with SAH. Another interesting finding seen in our study is that there was no correlation between the measured levels of adhesion molecules and initial leukocytosis. Such conflicts also may be attributed to the small number of the patients studied here.

Conclusions

Elevated levels of ICAM-1 and VCAM-1 in both CSF and serum may indicate that the process leading to poor outcome occurs in the brain and systemic circulation. Based on our results, we therefore suggest that application of anti–ICAM-1 and anti–VCAM-1 treatments delivered systemically or intrathecally may be helpful. Additional studies are required to elucidate whether adhesion molecules are the consequence of vasospasm or vice versa. As the pathophysiological sequence of events leading to vasospasm after SAH becomes better understood, it is possible that VCAM-1 and ICAM-1 will provide novel therapeutic targets for antivasospasm management in the future. In addition, it seems that it may be essential to obtain control values of these molecules from healthy volunteers associated with larger patient cohorts for a better understanding of the role of such molecules.

References

1. Bavbek M, Polin R, Kwan AL, et al: Monoclonal antibodies against ICAM-1 and CD18 attenuate cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits. **Stroke 29:** 1930–1936, 1998
2. Cannella B, Raine CS: The adhesion molecule and cytokine profile of multiple sclerosis lesions. **Ann Neurol 37:**424–435, 1995
3. Clark WM, Lauten JD, Lessov N, et al: Time course of ICAM-1 expression and leukocyte subset infiltration in rat forebrain ischemia. **Mol Chem Neuropathol 26:**213–230, 1995
4. Clatterbuck RE, Gailloud P, Ogata L, et al: Prevention of cerebral vasospasm by a humanized anti-CD11/CD18 monoclonal antibody administered after experimental subarachnoid hemorrhage in nonhuman primates. **J Neurosurg 99:**376–382, 2003
5. Clatterbuck RE, Oshiro EM, Hoffman PA, et al: Inhibition of vasospasm with lymphocyte function-associated antigen–1 monoclonal antibody in a femoral artery model in rats. **J Neurosurg 97:** 676–682, 2002
6. Endres M, Laufs U, Merz H, et al: Focal expression of intercellular adhesion molecule-1 in the human carotid bifurcation. **Stroke 28:**77–82, 1997
7. Fisher CM, Kistler JP, Davis JM: Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. **Neurosurgery 6:**1–9, 1980
8. Frijns CJM, Kappelle LJ, van Gijn J, et al: Soluble adhesion molecules reflect endothelial cell activation in ischemic stroke and in carotid atherosclerosis. **Stroke 28:**2214–2218, 1997
9. Granert C, Raud J, Xie X, et al: Inhibition of leukocyte rolling with polysaccharide fucoidin prevents pleocytosis in experimental meningitis in the rabbit. **J Clin Invest 93:**929–936, 1994
10. Handa Y, Kubota T, Kaneko M, et al: Expression of intercellular adhesion molecule 1 (ICAM-1) on the cerebral artery following subarachnoid hemorrhage in rats. **Acta Neurochir 132:**92–97, 1995
11. Haraldsen G, Kvale D, Lien B, et al: Cytokine-regulated expression of E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in human microvascular endothelial cells. **J Immunol 156:**2558–2565, 1996
12. Hunt WE, Hess RM: Surgical risk as related to time of intervention in the repair of intracranial aneurysms. **J Neurosurg 28:** 14–20, 1968
13. Jennett B, Bond M: Assessment of outcome after severe brain damage. A practical scale. **Lancet 1:**480–484, 1975
14. Mack WJ, Mocco J, Hoh DJ, et al: Outcome prediction with serum intercellular adhesion molecule–1 levels after aneurysmal subarachnoid hemorrhage. **J Neurosurg 96:**71–75, 2002
15. Mocco J, Mack WJ, Kim GH, et al: Rise in serum soluble intercellular adhesion molecule–1 levels with vasospasm following aneurysmal subarachnoid hemorrhage. **J Neurosurg 97:**537–541, 2002
16. Nissen JJ, Mantle D, Gregson B, et al: Serum concentration of adhesion molecules in patients with delayed ischaemic neurological deficit after aneurysmal subarachnoid hemorrhage: the immunoglobulin and selectin superfamilies. **J Neurol Neurosurg Psychiatry 71:**329–333, 2001
17. Oshiro EM, Hoffman PA, Dietsch GN, et al: Inhibition of experimental vasospasm with anti-intercellular adhesion molecule-1 monoclonal antibody in rats. **Stroke 28:**2031–2038, 1997
18. Polin RS, Bavbek M, Shaffrey ME, et al: Detection of soluble E-selectin, ICAM-1, VCAM-1, and L-selectin in the cerebrospinal fluid of patients after subarachnoid hemorrhage. **J Neurosurg 89:**559–567, 1998
19. Sills AK Jr, Clatterbuck RE, Thompson RC, et al: Endothelial cell expression of intercellular adhesion molecule 1 in experimental posthemorrhagic vasospasm. **Neurosurgery 41:**453–461, 1997
20. Thai QA, Oshiro EM, Tamargo RJ: Inhibition of experimental vasospasm in rats with the periadventitial administration of ibuprofen using controlled-release polymers. **Stroke 30:**140–147, 1999
21. Wellicome SM, Kapahi P, Mason JC, et al: Detection of a circulating form of vascular cell adhesion molecule-1: raised levels in rheumatoid arthritis and systemic lupus erythematosus. **Clin Exp Immunol 92:**412–418, 1993
22. Wilcox CE, Ward AM, Evans A, et al: Endothelial cell expression of the intercellular adhesion molecule-1 (ICAM-1) in the central nervous system of guinea pigs during acute and chronic relapsing experimental allergic encephalomyelitis. **J Neuroimmunol 30:** 43–51, 1990

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Address reprint requests to: Taner Tanriverdi, M.D., P.K: 4, Cerrahpasa, 34301, Istanbul, Turkey. email: tanerato2000@yahoo.com.