Cell adhesion molecular markers in ischaemic stroke patients: correlation with clinical outcome and comparison with primary autoimmune disease

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Abstract

Introduction: Several studies have found increased concentration of proinflammatory, post-ischaemic markers during acute stroke, such as sICAM-1 and sVCAM-1, that may modulate the patients' prognosis. We have therefore evaluated the molecular profiles of sICAM-1 and sVCAM-1 in ischaemic stroke patients, to correlate them with neurological deficits and to compare them with the molecular levels obtained from patients with primary autoimmune disease.
Material and methods: Thirty-six first-ever ischaemic stroke patients (SG) as well as 32 healthy volunteers (CG) and 31 multiple sclerosis patients (MS), both as controls, were included in the prospective study. sICAM-1 and sVCAM-1 were determined at the 1st, 5th and 14th day after stroke in SG, comparing with a single sample level in CG and MS. Evaluation of the neurological deficit at the 1st and the 14th day after stroke was based on Rankin scale, functional independence measure (FIM), Orgogozo score and NIHS scales.
Results: In SG, sICAM-1 level was significantly lower at the 5th day vs. the initial serum level ($p < 0.05$). A non-significant linear increase of SG sVCAM-1 level was observed between the sampling days. All SG sICAM-1 levels were significantly higher vs. CG and lower vs. MS levels ($p < 0,05$). At the 14th day, SG sVCAM-1 level was significantly higher vs. CG levels ($p < 0.05$), but there were no significant differences between SG levels and MS levels. A significant correlation between sVCAM-1 levels at the 1st and 14th day and clinical outcome scales was assessed.
Conclusions: The observed sICAM-1 decrease at the 5th day after stroke may be associated with an anti-inflammatory effect in ischaemic penumbra. The sVCAM-1 profile indicates a chronic inflammatory state in stroke, and its determination seems to be a good molecular marker of the patients' neurological state at the beginning and two weeks after the ischaemic episode. The systemic inflammatory response by cell adhesion molecules that accompanies acute stroke is less intensive than that observed during the autoimmune response in primary autoimmune disease such as MS.

Key words: stroke, multiple sclerosis, sICAM-1, sVCAM-1, biomarker, cytokines, proinflammatory, soluble cell adhesion molecules.

Introduction

Both humoral and cellular secondary inflammation form an inseparable process, which accompanies acute ischaemic stroke [1-3] with evidence

that these responses may exacerbate tissue injury. Acute cerebral ischaemia followed by reperfusion therefore leads violently to the activation of proinflammatory cytokine production by the immunological cells which infiltrate the ischaemic core in the early and late phase of the process and which stimulate endothelial cells, neurons, astrocytes and microglia cells as well [4]. A secondary inflammatory or immunological response includes the increased expression of the cell adhesion molecules that play a very significant and critical role in both normal and in various pathophysiological disease states. The crucial role for this inflammation response after ischaemic stroke is in the leukocytes' adhesion to endothelial cells and their migration through the blood-brain barrier [4-6]. This inflammation response during cerebral ischaemia, through activation of endothelial cells, leads to excretion of soluble forms of cell adhesion molecules such as ICAM-1 (intercellular adhesion molecule-1) or VCAM-1 (vascular cell adhesion molecule-1) into the bloodstream [4-6]. These inflammatory cells can then move through the endothelium by diapedesis and release cytokines and enzymes that are important components in the progression of the ischaemic lesion. The profiles of soluble forms of these cell adhesion molecules in the serum, and their role during cerebral ischaemia, have not yet been defined in detail. It is also not understood whether there exists a correlation between the soluble forms of cell adhesion molecules and the neurological outcome in ischaemic stroke patients, but this may be interesting because we still need to find new prognostic factors and therapeutic targets in ischaemic stroke. The existing few data are therefore often conflicting [7-10], as some authors have provided evidence that members of the soluble cell adhesion molecules superfamily may be linked with the clinical outcome [7-9], like other inflammatory components in ischaemic stroke [11, 12]. But others have shown that soluble forms of cell adhesion molecules have no or very weak objective predictive value in the assessment of stroke patients [10]. It seems to be also very interesting to know the severity of this secondary immune response. The most valuable reference group on that point is the group of multiple sclerosis patients with their unquestionable immunological background.

The aims of the present study were as follows:

1) to assess sICAM-1 and sVCAM-1 profiles during the first two weeks of cerebral ischaemia and to correlate these results with the neurological deficit (clinical outcome) scales and
2) to compare the severity of the inflammation process in ischaemic stroke patients with multiple sclerosis patients, based on sICAM-1 and sVCAM-1 levels in each subgroup.

Material and methods

Study design and patients

Ninety-nine patients (36 male and 63 female, mean age 55.1 years) were enrolled consecutively between January, 2004, and May, 2005 in the Neurological Department of the Medical University of Silesia, Katowice, Poland.

The patients were qualified for the study according to the following exclusion criteria:

i) no informed consent,
ii) presence of a malignancy,
iii) presence of an active inflammation process or infection,
iv) presence of autoimmune disorders,
v) preceding surgery prior to two weeks before the study,
vi) any state after myocardial infarction or stroke,
vii) current steroid therapy or other immunosuppressive treatment,
viii) carbohydrate balance disturbances (diabetes mellitus, glucose intolerance),
ix) statin therapy and
x) the presence of a carotid artery occlusion, as found by routine carotid artery ultrasound examination (Colour Power Doppler).

Patients with ischaemic stroke were also excluded from the study if the level of inflammation parameters (body temperature, WBC, CRP) during the hospitalization period suggested an infection.

The final analysis was performed in the three following patient subgroups:

a) study group (SG) – 36 patients (17 males and 19 females) with acute ischaemic stroke (confirmed by CT scans) with the age (mean ± SD) of 67.5 ± 10.9 years; neurological deficits were evaluated on the first and fourteenth hospitalization day using two different clinical scales: (1) *injury* (Orgogozo [13, 14] and National Institute of Health (NIH) score [15-18]); (2) *functional* (Functional Independence Measure (FIM) [19] and Rankin scale [20]); in this subgroup sICAM-1 and sVCAM-1 level determination was performed on the first, fifth and fourteenth day after stroke;
b) control group (CG) – 32 healthy volunteers (3 males and 29 females) with the age of (mean ± SD) 61 ± 8.1 years; in this subgroup sICAM-1 and sVCAM-1 level determination was performed from a single sample; in the control subgroup, atherosclerosis risk factor frequency was matched with the study subgroup; there was no significant difference in anthropometric (weight, BMI) and biochemical (glucose, lipid profile) parameters and blood pressure level as well;
c) multiple sclerosis (recently recognized – during the first relapse of the disease) group (MS) – 31 patients (16 males and 15 females) aged (mean ± SD) 36.8 ±10.8 years; measurements of sICAM-1 and sVCAM-1 levels were performed

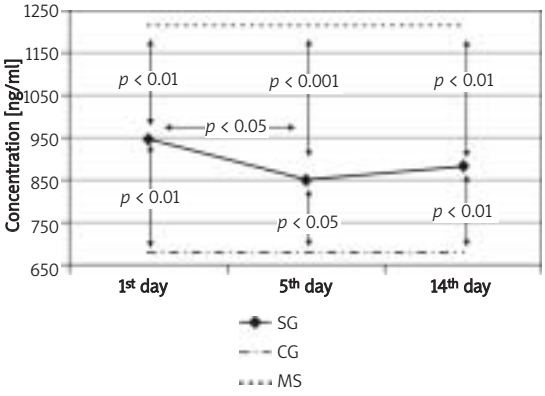

Figure 1. sICAM-1 profile in stroke patient group (SG) compared with sICAM-1 level in control group (CG) and in multiple sclerosis (MS) group respectively

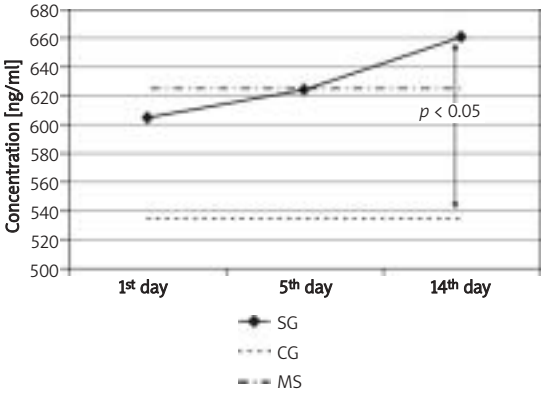

Figure 2. sVCAM-1 profile in stroke patient group (SG) compared with sVCAM-1 level in control group (CG) and in multiple sclerosis (MS) group respectively

in this subgroup before the introduction of immunosuppressive therapy.

Written consent for the study participation was obtained from all of the patients. The study was approved by the Local Ethics Committee of the Medical University of Silesia (NN-013-153/02).

Chemical analysis

Venous blood samples were drawn after a 12-h overnight fast. The sICAM-1 and sVCAM-1 serum level measurements were performed in serum using enzyme-linked immunosorbent assay using commercially available standard kits (ELISA – Bender MedSystems®), with the method sensitivity: 3.3 ng/ml for sICAM-1 and 0.9 ng/ml for sVCAM-1 respectively.

Statistical analysis

All results were expressed as means ± SD. The statistical significance of changes was analysed using Student paired and unpaired *t*-test. In correlation analyses Spearman's rank value was used. In all statistical analyses a *p* value smaller than 0.05 was considered significant. Obtained results were analyzed based on Statistica 5.1 PL software (StatSoft®).

Results

sICAM-1

The intercellular adhesion molecule sICAM-1 serum levels in the patient subgroup with acute cerebral ischaemia were as follows (mean ± SD): the 1st day of stroke – 947.2 ±280.4 ng/ml, the 5th day after stroke – 851 ±307.9 ng/ml, the 14th day after stroke – 882.5 ±276 ng/ml. sICAM-1 level at the fifth day after stroke was significantly lower compared to the initial level ($p < 0.05$). The sICAM-1 levels were significantly higher in the SG compared to the CG; for that subgroup sICAM-1 level was 676.9 ±273 ng/ml. Statistical significance values for each sICAM-1 level in the study subgroup (SG) were as follows (*Student paired t-test*): 1st day vs. CG – $p < 0.001$, 5th day vs. CG – $p < 0.05$, 14th day vs. CG – $p < 0.01$. The sICAM-1 serum level in the multiple sclerosis patient group (MS) was 1213.1 ±353.7 ng/ml and was significantly higher compared with all values obtained in the SG. Observed changes had *p* values as follows (*Student unpaired t-test*): 1st day vs. MS – $p < 0.01$), 5th day vs. MS – $p < 0.001$, 14th day vs. MS – $p < 0.01$ (Figure 1).

sVCAM-1

The patients' sVCAM-1 serum level profile – 1 from SG with cerebral ischaemia was as follows (mean ± SD): the first day after stroke – 604.8 ±247.2 ng/ml, the fifth day after stroke – 624.4 ±280.1 ng/ml, the fourteenth day after stroke – 660.8 ±246.1 ng/ml. A linear increase of the sVCAM-1 serum level was observed but with no statistical significance. The obtained serum values of sVCAM-1 in the control group (534.1 ± 201.4 ng/ml) were significantly lower than observed in the SG only at the fourteenth day after cerebral ischaemia with *p* value < 0.05 (*Student unpaired t-test*). There were no significant differences between the SG and the MS with respect to sVCAM-1 serum levels. The sVCAM-1 serum level in the multiple sclerosis group was – 625 ±308.7 ng/ml (Figure 2).

Neurological deficit

The sVCAM-1 serum level obtained at the first and fourteenth day after stroke correlated significantly with the neurological deficit estimated using both injury and functional stroke scales (Table I). Depending on the scale score scheme, the Spearman rank value was positive or negative, but always indicates a better clinical state for patients with a higher sVCAM-1 level (Figures 3, 4). There was no significant correlation between the initial level

Table I. Correlations between sVCAM-1 levels (1st and 14th day of stroke) and clinical state of patients, assessed by injury [Orgogozo and National Institute of Health (NIH)] and functional [Rankin and Functional Independence Measure (FIM)] scales at the 1st and 14th day respectively (r = Spearman rank value)

	Orgogozo	NIH	Rankin	FIM
sVCAM-1 (1st day)	$r = 0.390$ $p < 0.05$	$r = -0.302$ $p = 0.08$ (NS)	$r = -0.361$ $p < 0.05$	$r = 0.347$ $p < 0.05$
sVCAM-1 (14th day)	$r = 0.388$ $p < 0.05$	$r = -0.373$ $p < 0.05$	$r = -0.374$ $p < 0.05$	$r = 0.327$ $p = 0.06$ (NS)

of sVCAM-1 and clinical outcomes on the 14th day of stroke ($r = 0.306$ vs. Orgogozo, $r = -0.318$ vs. NIH, $r = 0.200$ vs. FIM, $r = -0.223$ vs. Rankin scale respectively). The sICAM-1 serum levels obtained on the 1st and 14th day during the observation period did not correlate significantly with the neurological deficit estimated by injury and functional stroke scales.

Discussion

In different autopsy studies based on the immunocytochemical technique, the expression of ICAM-1 and VCAM-1 on the surface of damaged cells was assessed during the first week after acute brain ischaemia. ICAM-1 was observed on the epithelial cells and VCAM-1 on both epithelial cells and astrocytes [21-23]. The animal model based studies also proved the important role of cell adhesion molecules in the ischaemia/reperfusion process [24-26].

The assessment of adhesion molecule levels in patients with cerebrovascular diseases needs special caution because of the association with risk factors for atherosclerosis development [27, 28]. The presence of atherosclerotic changes is

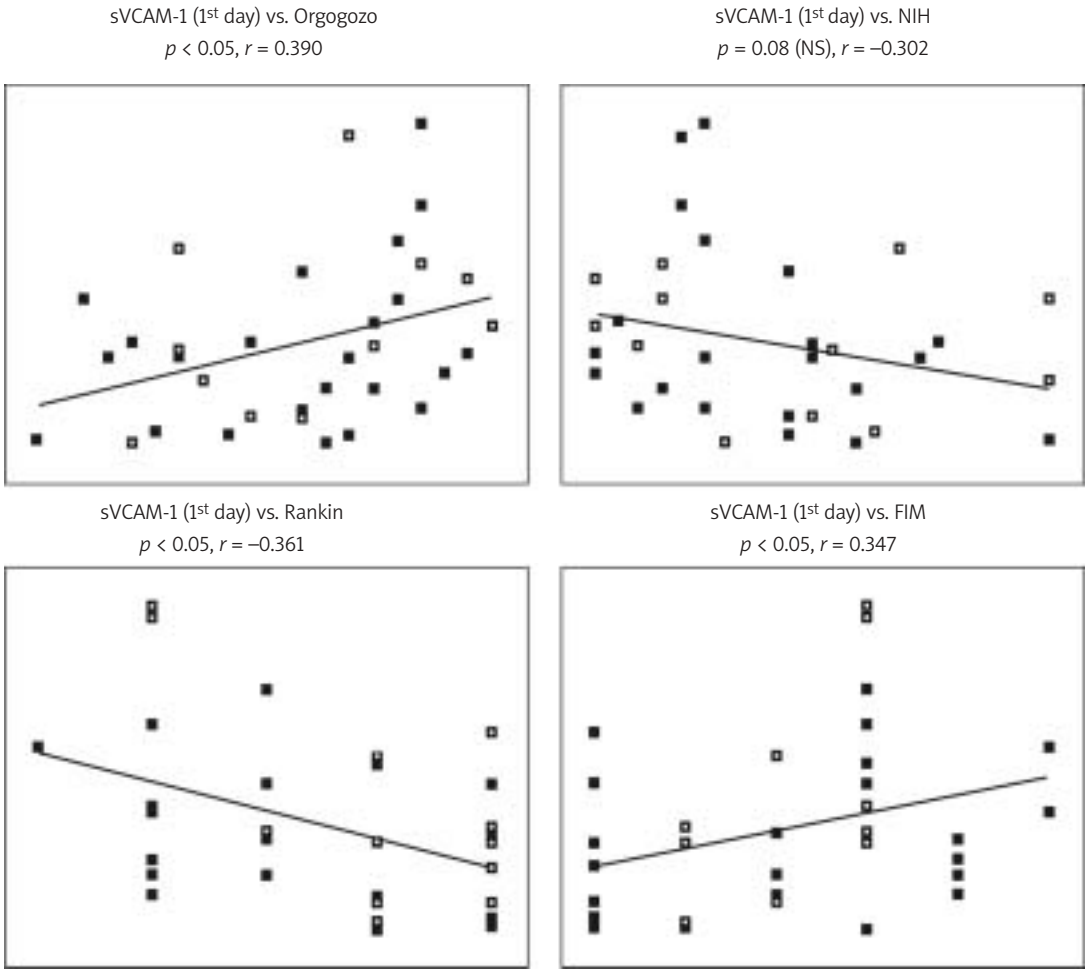

Figure 3. Correlation between sVCAM-1 level (1st day) and clinical state of patients assessed on the 1st day of stroke, based on the injury [Orgogozo and National Institute of Health (NIH)] and functional [Rankin and Functional Independence Measure (FIM)] scales

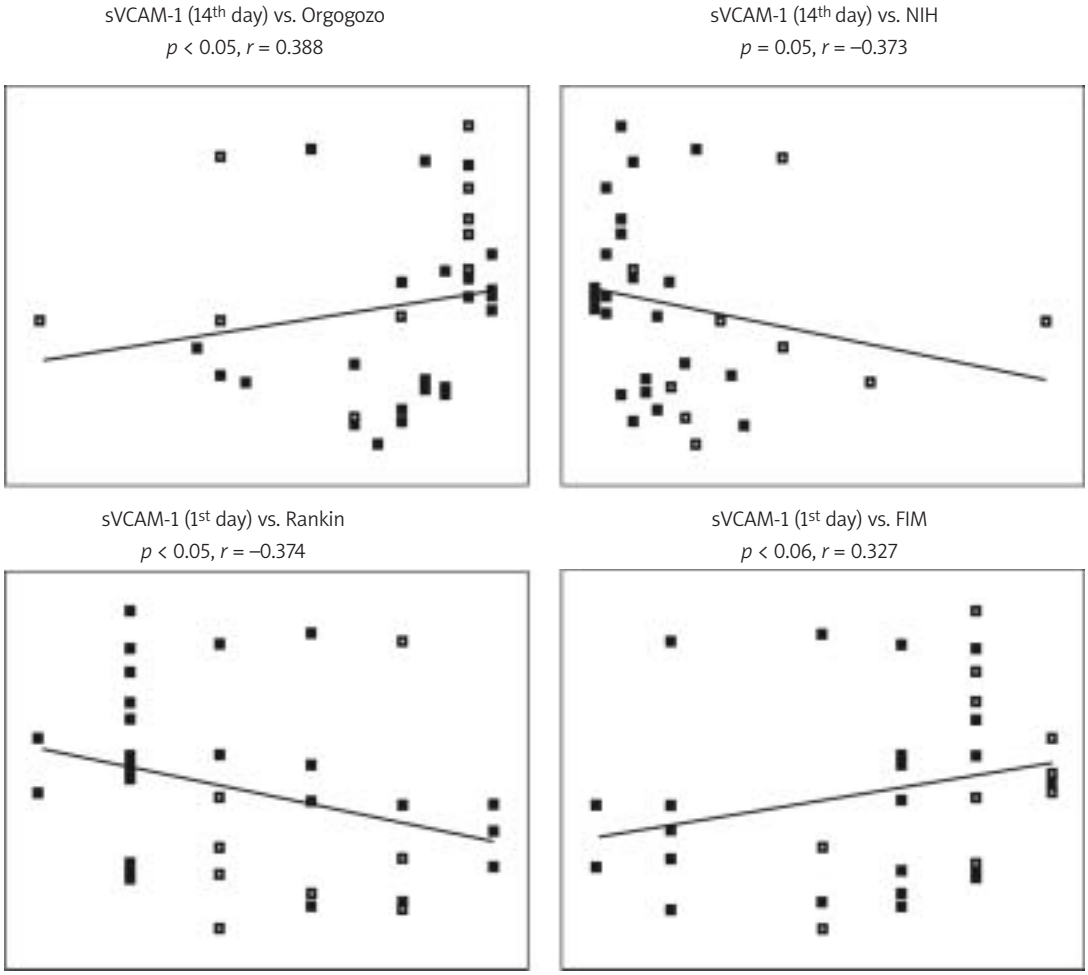

Figure 4. Correlation between sVCAM-1 level (14th day) and clinical state of patients assessed on the 14th day of stroke, based on the injury [Orgogozo and National Institute of Health (NIH)] and functional [Rankin and Functional Independence Measure (FIM)] scales

associated with chronic endothelium activation which can provoke the over-expression of cell adhesion molecules [29, 30]. That is why some authors used a few control groups to compare with stroke patients to distinguish the influence of chronic production of soluble cell adhesion molecules on their final level during stroke. In the study conducted by Shyu *et al.* [31] comparing the results obtained in the acute ischaemic stroke patient group with age and atherosclerosis risk factors matched with a control group and with young healthy volunteers, significantly higher sICAM-1 level in stroke patients vs. both control groups was found. There was also no correlation between the sICAM-1 level and the percentage of extracerebral artery occlusion in the ischaemic stroke patient subgroup, even if the occlusion was larger than 50%. Similar (inclusion) criteria were used by Blann *et al.* [21], who demonstrated significantly higher sVCAM-1 levels in ischaemic stroke patients vs. healthy controls and patients with carotid artery atherosclerosis at the beginning and at 3 months after an acute ischaemic stroke. However, prognostic follow-up made by Ehrensperger *et al.* [32] showed that there is no association between sICAM-1 and carotid artery stenosis. Neither baseline nor subsequent sICAM-1 levels at the seven post-stroke day were predictive of risk for future ischaemic events. Fassbender *et al.* [33] in their study compared sICAM-1 and sVCAM-1 levels in an ischaemic stroke patient group with a control group matched in age and in atherosclerosis risk factors, and found that the sVCAM-1 level was significantly higher in the ischaemic stroke patient group throughout the observation period: the sICAM-1 level was higher in the 8th and 10th h after initiation of ischaemic stroke and lower in the 4th h than in the control group, demonstrating that changes of soluble cell adhesion molecules are the acute, immunological response after an acute ischaemic event. An interesting observation was made by

the group of Bitsch *et al.* [10], which showed that there are no significant differences between sICAM-1 and sVCAM-1 levels comparing patients with irreversible ischaemic stroke and patients with TIA (transient ischaemic attack). Their findings may indicate that the crucial role for the soluble cell adhesion molecule releasing process is at the very early phase of a cerebral ischaemic event. A recent study showed significantly higher sICAM-1 and sVCAM-1 serum levels in patients with acute ischaemic stroke compared with healthy controls and additionally demonstrated that the serum concentration of these molecules is independent even of age, sex and atherosclerosis risk factors [34].

The results obtained in our study confirm that the immune system plays an important role in the ischaemic cascade that is initiated after an acute ischaemic event. In patients with acute ischaemic stroke, without an underlying carotid artery occlusion, a significant increase of the sICAM-1 level on the 1st, 5th and 14th day of stroke compared with an age and atherosclerosis risk factors matched control group was found. The soluble VCAM-1 level at the 14th day after ischaemic stroke was also significantly higher than in the control group. For assessment of the severity of immune process activation, sICAM-1 and sVCAM-1 levels obtained in the study group were also compared with multiple sclerosis new-onset patients' samples before immunosuppressive therapy. A study analysing sICAM-1 and sVCAM-1 level in patients with intracerebral bleeding compared with MS patients showed three times higher level of sICAM-1 and sVCAM-1 in the cerebrospinal fluid in a subgroup of patients who died after ischaemic stroke compared to MS patients, and no significant difference in patients who survived after ischaemic stroke vs. MS patients [35]. The significantly higher levels obtained in the present study in MS patients vs. acute ischaemic stroke patients and a control group indicates that the primary immune response in multiple sclerosis has higher intensity than secondary inflammation during acute ischaemic stroke. However, sVCAM-1 level does not differ significantly in the MS group vs. both the stroke and the control group. The explanation may be the significantly younger age in the MS patient subgroup and the higher chronic activation of the endothelium in older patients with acute ischaemic stroke and in the control group, which obviously had more atherosclerosis risk factors.

The immune response during stroke represents a kinetic process [4-6]. For that reason, it is very important to obtain information about the profiles of cell adhesion molecules during ischaemic stroke. Blann *et al.* [21] found a significant increase of the sVCAM-1 serum level after the 12th day of acute ischaemic stroke, which remained high during the three-month observation period. Fassbender *et al.* [33] revealed a permanent sVCAM-1 increase until the 5th day after ischaemic stroke but without any significant difference. The presented results are similar to Bitsch *et al.* [10], who obtained a significantly higher sICAM-1 level on the first day vs. the 5th day after ischaemic stroke and a repeated significant increase on the 14th day. In the present work, the sICAM-1 serum level significantly decreased on the 5th day vs. the initial level. The obtained increase on the 14th day was not statistically significant as compared with the 5th day. However, our obtained data concerning sICAM-1 conflict with a previous study (based on a similar schedule of sampling) which showed that sICAM levels were similar at each sampling period and did not differ from those of controls [36]. During the two weeks of observation, we observed a permanent increase of sVCAM-1 serum level, which was however not significant. The sICAM-1 serum level increased on the 1st day of stroke, suggesting high endothelial cell activation and preparation for infiltration of the vessel wall by neutrophils. The sICAM-1 serum level decrease at the 5th day vs. the initial level may be associated with the higher binding of soluble forms of cell adhesion molecules with the receptors. The second explanation may be the hypothesis of an endogenous molecular neuroprotection mechanism called "precon-ditioning" which can induce ischaemic tolerance [37, 38]. The most probable explanation of that phenomenon is the suppression of genes for proinflammatory molecules such as cytokines, cell adhesion molecules and transcription factors [39]. Repeated sICAM-1 level increase may suggest secondary endothelium activation in the late phase of ischaemic stroke that can be associated with ischaemic penumbra remodelling. In the second phase of inflammation during stroke a few days later, monocyte infiltration plays a crucial role [40, 41]. The later increase of sVCAM-1 level in stroke patients suggests the important role of this molecule in the monocyte infiltration process.

Inflammation and immune system activation may lead to deterioration of the neurological state of patients. A study performed by Blum *et al.* [9] showed that stroke patients who improved clinically within the 1st h days of hospitalization demonstrated remarkable inhibition of ICAM-1, VCAM-1 and E-selectin soluble form levels at the 4th day of stroke vs. admission levels. The results of our study suggest that sVCAM-1 can be a good molecular marker of neurological deficit in stroke patients: sVCAM-1 levels on the 1st and 14th day after ischaemic stroke positively correlated with

neurological deficit assessed by stroke scales on the 1st and 14th day respectively. Similar results were obtained by Bitsch *et al*. [10]. However, Chinese authors demonstrated that an initial increased level of sICAM-1 is associated with neurological deterioration during the first week after ischaemic stroke as compared with stroke patients without deterioration [8]. The prognostic value of sVCAM-1 and sICAM-1 on in-hospital mortality in patients with ischaemic stroke was examined recently by Rallidis *et al.* [7]. They found that high sICAM-1 level, determined within the first 12 h of stroke, was associated with early death in middle-aged patients.

Cerebrovascular diseases are one of the main causes of handicap of adults and the third cause of death. So we still need good prognostic markers to select those patients that need initiation of additional therapeutic methods especially in the acute phase of stroke. The results presented here suggest that the soluble forms of ICAM-1 and VCAM-1 seem to play an important role in stroke pathology and may have prognostic value in stroke patients. Cell adhesion molecules that are presented on the epithelial cells' surface may also be a therapeutic target in the (near) future.

In conclusion, the significant decrease of sICAM-1 level on the 5th day of stroke may be associated with the limitation of acute inflammation in the ischaemic core, as part of the preconditioning-induced neuroprotective mechanism. However, sVCAM-1 indicates a chronic inflammatory state in ischaemic stroke. The sVCAM-1 profile determination seems to be a good molecular marker of the clinical state in acute ischaemic stroke patients. The activation of cell adhesion molecules in the inflammation response that accompanies ischaemic stroke is less intensive than the primary autoimmune process in multiple sclerosis patients before immunosuppressive therapy.

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