Activated Leukocyte Cell Adhesion Molecule and Prognosis in Acute Ischemic Stroke

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Background and Purpose—Biomarkers predicting mortality and functional outcome in stroke may be clinically helpful in identification of patients likely to benefit from intervention. Activated leukocyte cell adhesion molecule (ALCAM) is upregulated during neuroinflammation; we investigated whether ALCAM concentrations are associated with long-term mortality after ischemic stroke.

Methods—In 244 patients with acute ischemic stroke (age 69±13 years), samples of ALCAM were obtained serially from presentation to Day 5 and after 6 months. Patients with overt ischemic heart disease and atrial fibrillation were excluded. The patients were followed for 47 months with all-cause and cardiovascular mortality as end points.

Results—At follow-up, 72 patients (29%) had died, 43 due to cardiovascular causes. Patients with ALCAM in the fourth outcome in stroke would be clinically helpful.3

Conclusions—ALCAM levels measured at admission of acute ischemic stroke are associated with long-term mortality. (Stroke. 2011;42:00-00.)

Key Words: acute ischemic stroke ■ ALCAM ■ long-term prognosis

Acute ischemic stroke is a leading cause of death and serious, long-term disability in adults.1 Effective treatments are available, but such therapies are greatly dependent on early diagnosis and assessment of stroke severity.3,2 Thus, prompt identification of patients at increased risk for adverse outcomes from ischemic stroke might target individuals likely to benefit from intervention. In this context, rapidly measurable biomarkers predicting mortality and functional outcome in stroke would be clinically helpful.1

Although the pathogenesis of acute ischemic stroke is multifactorial, several prognostically meaningful aspects may be assessed with objective biomarkers. For example, inflammation is thought to play a critical role in several aspects of pathophysiology and disease exacerbation in acute ischemic stroke.4–6 Inflammation could promote plaque destabilization within a carotid atherosclerotic lesion, resulting in embolization and subsequent cerebral stroke, and inflammation may progress within freshly infarcted cerebral tissue, further promoting tissue damage.

Cell adhesion molecules are involved in attraction and recruitment of leukocytes into the site of inflammation and tissue damage, promoting transendothelial migration that involves a series of well-coordinated events between inflamed endothelium and activated leukocytes.5,7 Firm adhesion of leukocytes to the endothelial cells as well as leukocyte activation is mediated by receptors of the immunoglobulin gene superfamily. Given the involvement of cell adhesion molecules in atherogenesis and response to tissue damage as well as the high circulating levels of their soluble isoforms, soluble cell adhesion molecules have been shown to be associated with disease severity and outcome in various cardiovascular (CV) disorders, including stroke.8,9 Thus, high levels of soluble intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 have been detected in patients with ischemic stroke and in particular, intracellular adhesion molecule-1 levels on admission have been associated with adverse outcome in these patients.9–13

Also belonging to the immunoglobulin gene superfamily is activated leukocyte cell adhesion molecule (ALCAM), also...
denoted CD166. ALCAM is upregulated substantially during inflammation and replaces vascular cell adhesion molecule-1 during transmigration of leukocytes across central nervous system endothelium. ALCAM has been shown to be suitable as a prognostic marker for different types of cancer, but at present, there are no data on ALCAM levels as a biomarker in CV disorders. Based on its role in neuroinflammation, we prospectively studied patients with acute ischemic stroke to (1) describe the level of ALCAM during the early phase of ischemic stroke; and to (2) examine whether ALCAM concentrations are associated with long-term mortality after ischemic stroke.

Subjects and Methods
Between August 2003 and October 2004, 790 patients with acute ischemic stroke admitted to the Department of Neurology at Odense University Hospital, Odense, Denmark, were consecutively screened for inclusion in the study. Patients with overt ischemic heart disease (n = 177; ie, any prior myocardial infarction, stable or unstable angina pectoris, pathological Q-waves on the baseline electrocardiogram, previous coronary angioplasty or coronary bypass surgery), patients with current atrial fibrillation (n = 132), or patients with onset of stroke symptoms >7 days before admission (n = 75) were not included. In addition, 115 patients were excluded because of technical reasons or lack of compliance and 47 patients were unwilling to participate, rendering 244 patients eligible for inclusion in the study.

Evaluation of each study subject was performed by a senior neurologist blinded to the biomarkers; the presence of intracerebral or subarachnoid hemorrhage was ruled out by CT at the time of admission. Patient demographics along with past and present clinical history including medication were obtained by interview. Renal failure was defined as plasma creatinine >120 μM. Heart failure was considered present if the patients had a left ventricular systolic ejection fraction <50% and/or if a patient had previously been given the diagnosis of heart failure by a physician. If the patient was unable to take part in the interview, relatives participated. Medical history was retrieved by reviewing hospital records. Severity of the index stroke was assessed using the Scandinavian Stroke Scale. Stroke subtypes were classified according to the Oxfordshire Community Stroke Project. The study was approved by the local ethics committee and by the National Danish Registry Board.

Laboratory Analyses
Peripheral venous blood was collected immediately at admission, subsequently up to 5 days after admission, and once at 6 months. The specimens were collected in heparin-containing tubes, centrifuged for 10 minutes at 2000 g within 4 hours of collection, stored at −80°C, and thawed <3 times before analyses. Plasma ALCAM was quantified by an enzyme immunoassay in duplicate using commercially available matched antibodies (R&D Systems, Minneapolis, MN). The plasma samples were diluted 80 times before enzyme immunoassay measurements, minimizing any influence of different matrices. The inter- and intra-assay coefficient of variation was <10%. Cardiac troponin T (cTnT) was determined on an Elecsys 1010 (Roche Diagnostics Boehringer Mannheim, Mannheim, Germany) as previously described. The inter- and intra-assay coefficient of variation was <5%. As diagnostic cutoff value, we used 0.03 μg/L, reflecting the lowest cutoff value providing <10% imprecision. C-reactive protein (CRP) and creatinine (Jaffe method) were measured on Modular Analytics P (Roche Diagnostics, Basel, Switzerland). The inter- and intra-assay coefficients of variation were <5%.

Follow-Up
The primary outcome measure was all-cause mortality. Mortality data were obtained from the Danish Central Personal Registry, which registered all deaths in Denmark within 2 weeks. Cause-specific mortality was divided into CV (n = 43), cancer (n = 13), and other (n = 16; mainly chronic obstructive pulmonary disease [n = 4] and pneumonia [n = 1]). For statistical analysis, we focus on CV death and only as a group because a further division into more cause-specific groups will result in power issues and possibly obscure our findings. The median follow-up time was 4.4 years (interquartile range, 3.7 to 4.9 years) and follow-up was complete in 100% of subjects.

Statistics
To evaluate the difference of demographic and clinical characteristics, Fisher exact test, Wilcoxon rank sum test, and Student t test were applied where appropriate. Differences in the levels of ALCAM from Day 0 to 6 were evaluated by univariate repeated-measures analysis of variance a priori and Wilcoxon signed rank test a posteriori. Multivariable stepwise linear regression analyses were performed to identify determinants of ALCAM levels (log-transformed) including variables associated with increased ALCAM in Table 1 (all variables with P < 0.2 were included).

For mortality analyses, concentrations of ALCAM at the time of presentation and over the first several days after presentation were examined as a function of long-term follow-up. Receiver operating characteristic curves were established for ALCAM as a predictor of death at follow-up. Kaplan-Meier analysis with log-rank test was performed to compare mortality rate in quartiles of ALCAM. The Cox proportional hazards model was applied to assess the effect of ALCAM on survival at follow-up. Baseline variables were included in the adjusted model if they were imbalanced between patients that survived and those who died, as indicated by a univariate probability value <0.05. The following variables were entered into the multivariable model: age, presence of heart and/or renal failure, Scandinavian Stroke Scale score, CRP, and TnT levels >0.03 μg/L. In all...
Univariate Analyses
ALCAM and Outcomes After Ischemic Stroke

**Results**

**Baseline Characteristics**
Baseline clinical characteristics of the study subjects are shown in Table 1. At presentation, the median ALCAM concentration in the group as a whole was 39.9 ng/mL (interquartile range, 34.2 to 46.8 ng/mL). In a multivariable stepwise linear regression model, the following were found to be independent predictors of presenting ALCAM concentration in acute patients with ischemic stroke: age ($t=4.03; P<0.001$), gender ($t=-2.74, P=0.007$), and a history of heart and/or renal failure ($t=2.24, P=0.026$).

When examining the temporal pattern of ALCAM from symptom onset, we found no time-dependent difference during the first 5 days (Figure 1). However, a significant increase in ALCAM levels was observed after 6 months as compared with baseline ($P<0.001$; Figure 1). The reason for this pattern is not clear, but because cerebral atheroscleroses are likely to be common features in most of this homogenous group of patients with ischemic stroke, it could potentially reflect accelerated atherogenesis after the vascular events.

**ALCAM and Outcomes After Ischemic Stroke**

**Univariate Analyses**
At follow-up (median, 4.4 years), 72 patients (29%) had died. ALCAM concentrations at admission were significantly higher in decedents than in survivors and notably, the same pattern was also seen for the other time points during follow-up (Table 2).

Receiver operating characteristic analysis indicated that the level of ALCAM at admission had reasonable accuracy for the prediction of mortality at follow-up (Table 2); ALCAM levels from different days after admission had largely similar areas under the curve values (Table 2), underscoring the stability of ALCAM levels during the early phase after acute ischemic stroke. To this point, the mean ($\pm$ SD) intra-individual variability in ALCAM levels during the first 5 days was $10.2\%\pm 5.4\%$ (range, 1.1 to 48.9). Because the area under the curve for ALCAM was the same through the first several days after presentation, we examined levels at admission for identifying the cut point for ALCAM as a predictor of death. In quartile analysis, an apparent threshold effect was observed in patients at or above the highest quartile for ALCAM ($\geq46.8$ ng/mL). This was confirmed in Kaplan-Meier analyses using these quartiles (Figure 2A). An ALCAM concentration $\geq 46.8$ ng/mL had 45% sensitivity, 84% specificity, positive predictive value of 54%, and negative predictive value of 78% for death at 1 year.

When looking at clinical variables in relation to high and low ALCAM levels (ie, fourth quartile versus the lower 3), a few associations were noted (Table 3). Patients with increased circulating ALCAM concentrations were older and had decreased hemoglobin levels. They also tended to be women and had a history of heart and/or renal failure. In contrast, no significant differences in ALCAM levels were observed for other variables, including stroke subtypes, previous ischemic stroke, hypertension, diabetes mellitus, blood pressure, or levels of cTnT, creatinine, and CRP.

**Multivariate Analyses**
The unadjusted hazard ratio for fourth quartile ALCAM and other variables associated with increased mortality are given in Table 4. In multivariate analysis, baseline variables (Table 1) were included if they were imbalanced between patients who survived and those who died (ie, $P<0.05$ in univariate analyses). After adjustment for these variables (ie, Scandinavian Stroke Scale score, TnT, CRP, age, and prior heart and/or renal failure), ALCAM levels in the fourth quartile remained an independent predictor of long-term mortality.
ALCAM in Relation to the Cause of Death

During follow-up, 43 patients died of CV causes, 13 patients died of cancer, and 16 patients died of other causes. The highest ALCAM levels were observed in cancer (median, 49.4; interquartile range, 38.9 to 52.7; P = 0.037 versus survivors) followed by CV (44.8 [38.4 to 51.8; P = 0.001 versus survivors) and other causes (42.6 [34.0 to 51.2]; P = 0.3 versus survivors). ALCAM was a significant predictor of CV death, also after adjustment in multivariate analysis (Table 4). Cancer did not remain significant in multivariate analysis due to low numbers. Figure 2B shows the Kaplan-Meier analyses for CV death. As can be seen, the early divergence in the fourth quartile was mostly due CV death, and this association with “short-term” mortality supports that ALCAM may be specific to stroke-related mortality and not coincidentally associated.

Discussion

Among a cohort of patients with acute ischemic stroke, we found that plasma levels of ALCAM were consistently higher across the first several days after stroke in those patients who subsequently experienced an adverse outcome. Furthermore, ALCAM values remained elevated after 6 months in dece-
dents and displayed only minor intraindividual variability indicating suitability as a stable biomarker for adverse events in these patients. Importantly, elevated ALCAM levels remained an independent predictor of all-cause and CV mortality also after adjustment in multivariate analysis for established risk factors such as age, the presence of heart and renal failure, cTnT and CRP levels, and severity of the index stroke. To the best of our knowledge, this is the first report assessing the prognostic value of circulating ALCAM levels in cerebrovascular disease, implicating a potential pathogenic role in the development of atherosclerosis and its major clinical consequences such as stroke.

The dynamic processes that take part during the development of an acute ischemic stroke involve plaque destabilization, embolization of thrombus materials, and tissue damage within the brain. These processes involve inflammation as a common mediator, and molecules that could reflect these inflammatory pathways have been used as biomarkers to predict the evolution of ischemic stroke. Thus, several studies have shown an association between leukocyte count, fibrinogen, or CRP and a higher risk following stroke. Circulating adhesion molecules reflect the inflammatory interaction between endothelial cells and leukocytes. Although there are several data on the association between these molecules and adverse outcome among patients with coronary artery disease, acute coronary syndromes, and heart failure, there are limited data of their ability to predict long-term mortality in patients with ischemic stroke. Campbell et al showed that soluble vascular cell adhesion
molecule-1 was associated with recurrent stroke in patients with previous stroke or transient ischemic attack. In a small study, Blum et al showed that patients with acute ischemic stroke who improved clinically during the first days of hospitalization demonstrated a decrease in circulating levels of E-selectin, intracellular adhesion molecule-1, and vascular cell adhesion molecule-1. However, data regarding ALCAM that was not correlated with CRP or cTnT, may suggest that ALCAM reflects other pathogenic processes than those mirrored by more established biomarkers. Certainly, ALCAM has been implicated in stabilization of the immunologic synapse, T cell activation, and monocyte transendothelial migration, and has previously been found to give prognostic information in malignancies. We report the value of ALCAM levels for prognostication after acute ischemic stroke. Plasma ALCAM displayed very low intra-individual variation during the acute phase and circulates at high levels making it a potentially reliable biomarker for clinical use. However, although our data may suggest that ALCAM is relatively specific and will identify individuals who truly are at risk, there will still be a high mortality rate among patients with low ALCAM levels, limiting its use.

Although the present study cannot inform whether ALCAM plays a mechanistic role in complications after stroke, it is tempting to hypothesize that elevated ALCAM values are pathogenic in this setting. The lack of association of ALCAM with stroke severity could suggest that increased ALCAM levels may predispose individuals to an adverse outcome rather than merely reflect the ischemic insult. Indeed, acute or chronic inflammatory diseases preceding stroke may contribute to outcome. Furthermore, the recruitment of leukocytes and platelets in the cerebral microvasculature is widely regarded as a pivotal step in the inflammatory response associated with cerebral ischemia. Increased ALCAM levels could therefore contribute directly to atherosclerosis and its cerebrovascular complications by altering blood–brain barrier function and accelerating the recruitment of leukocytes into the cerebral microvasculature. In addition, experimental studies implicate a role for CD4 T and CD8 T cells in cerebral microvascular dysfunction. Because ALCAM, through its interaction with CD6 on T cells, is required for optimal T cell activation (promoting an inflammatory T cell phenotype), it might also contribute to inflammation and tissue injury associated with brain ischemia through T cell-mediated mechanisms. Highly relevant to the setting of ischemic stroke, a recent study demonstrated that ALCAM expression on blood–brain barrier cells was upregulated in active multiple sclerosis and that ALCAM blockade restricted the transmigration of CD4 T cells and monocytes across blood–brain barrier endothelium, identifying ALCAM as a potential target for the therapeutic dampening of neuroinflammation. The current study has some limitations. The number of events was relatively low, limiting the use of subanalyses. Another stroke classification may have changed the ability of ALCAM to identify stroke subtypes. The predictors of mortality entered in multivariate analysis in the present study were identified by univariate analysis. To avoid overfitting of the model, other common risk factors for poor outcome in patients with stroke, for example, smoking, were not included. On the other hand, the longitudinal follow-up and assessment of ALCAM levels in serial plasma samples represent strengths of the current study. The exclusion criteria represent both strength and a weakness. By excluding patients with atrial fibrillation, overt ischemic heart disease, or patients with onset of stroke symptoms >7 days before admission, all states characterized by an increased inflammatory

Table 4. The Effects of ALCAM on All-Cause and CV Mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>All-Cause Mortality (n=72)</th>
<th>CV Mortality (n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wald</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALCAM (Q4 vs Q1–Q3) (admission)</td>
<td>18.7</td>
<td>3.01 (1.86–5.18)</td>
</tr>
<tr>
<td>SSS score</td>
<td>15.0</td>
<td>0.97 (0.96–0.99)</td>
</tr>
<tr>
<td>Troponin T &gt;0.03 μg/L</td>
<td>28.1</td>
<td>5.15 (2.81–9.45)</td>
</tr>
<tr>
<td>Log C-reactive protein</td>
<td>16.7</td>
<td>2.91 (1.74–4.86)</td>
</tr>
<tr>
<td>Age/10</td>
<td>27.4</td>
<td>1.77 (1.43–2.19)</td>
</tr>
<tr>
<td>Prior heart and/or renal failure</td>
<td>19.9</td>
<td>3.31 (1.96–5.61)</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALCAM (Q4 vs Q1–Q3)</td>
<td>5.0</td>
<td>2.01 (1.09–3.69)</td>
</tr>
<tr>
<td>SSS score</td>
<td>8.7</td>
<td>0.97 (0.95–0.99)</td>
</tr>
<tr>
<td>Troponin T &gt;0.03 μg/L</td>
<td>8.2</td>
<td>3.09 (1.43–6.71)</td>
</tr>
<tr>
<td>Log C-reactive protein</td>
<td>6.1</td>
<td>2.42 (1.20–4.89)</td>
</tr>
<tr>
<td>Age/10</td>
<td>6.5</td>
<td>1.42 (1.09–1.85)</td>
</tr>
<tr>
<td>Prior heart and/or renal failure</td>
<td>1.6</td>
<td>1.56 (0.79–3.09)</td>
</tr>
</tbody>
</table>

ALCAM indicates activated leukocyte cell adhesion molecule; CV, cardiovascular; HR, hazard ratio; Q, quartile; SSS, Scandinavian Stroke Scale; CI, confidence interval.
component, it seems likely that the ALCAM levels do not merely reflect inflammatory responses in concurrent inflammatory disorders. However, the exclusion of many patients also represents a weakness because the relevance of our findings to the general and heterogeneous stroke population remains unknown.

In the present study, we show that plasma levels of ALCAM during acute ischemic stroke were associated with all-cause and CV mortality during long-term follow-up. ALCAM has some unique properties as compared with other adhesion molecules such as the role in T cell activation and involvement in neuroinflammation that could contribute to its ability to predict adverse outcome in stroke and may also implicate its role as a mediator in these processes. Our findings suggest that ALCAM should be added to the list of inflammatory biomarkers that should be further investigated in CV disorders, including ischemic stroke. Although several biomarkers are inferior to CRP and cTnT in patients with ischemic stroke and other CV disorders, studies on other markers may be of importance to characterize the network of mediators that are involved in the development of these complex disorders.

Disclosures
None.

References


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