High Serum Levels of Endothelin-1 Predict Severe Cerebral Edema in Patients With Acute Ischemic Stroke Treated With t-PA

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Background and Purpose—Severe cerebral edema is associated with poor outcome in patients with acute stroke. Experimental studies suggest that astrocytic endothelin-1 (ET-1) has deleterious effects on water homeostasis, cerebral edema, and blood brain barrier (BBB) integrity, which contribute to more severe ischemic brain injury. In this study we analyze the association between high serum levels of ET-1 and the development of severe cerebral edema in patients treated with t-PA.

Methods—One hundred thirty-four patients treated with t-PA according SITS-MOST (Safe Implementation of Thrombolysis in Stroke Monitoring Study) criteria were prospectively studied. Serum levels of ET-1, matrix metalloproteinase-9 (MMP-9), and cellular fibronectin (c-Fn) were determined by ELISA in serum samples obtained on admission, before t-PA bolus. Severe brain edema was diagnosed if extensive swelling caused any shifting of the structures of the midline was detected on the cranial CT performed at 24 to 36 hours. Stroke severity was evaluated before t-PA administration and at 24 hours by NIHSS. Functional outcome at 3 months was evaluated by the modified Rankin Scale (mRS).

Results—Nineteen patients (14.2%) developed severe brain edema. Median ET-1 (8.4 [6.7, 9.6] versus 1.9 [1.6, 3.2] fmol/mL, \(P<0.0001\)) and c-Fn (6.0 [4.1, 6.7] versus 3.2 [2.1, 4.6] mg/L, \(P<0.0001\)) serum levels were significantly higher in patients with severe cerebral edema. The best cut-off values for ET-1 and c-Fn serum levels for the prediction of severe brain edema were 5.5 fmol/mL (sensitivity 95% and specificity 94%) and 4.5 mg/L (sensitivity 73% and specificity 77%) respectively. ET-1 serum levels \(>5.5\) fmol/mL before t-PA treatment were independently associated with development of severe brain edema (OR, 139.7; CI95%, 19.3 to 1012.2; \(P<0.0001\)), after adjustment for baseline stroke severity, early CT signs of infarction, serum levels of cFn \(>4.5\) mg/L, and cardioembolic stroke subtype.

Conclusions—ET-1 serum levels \(>5.5\) fmol/mL are associated with severe brain edema in acute stroke patients treated with t-PA. These results suggest that ET-1 may be a new diagnostic marker for development of severe brain edema in patients with acute ischemic stroke treated with t-PA. (Stroke. 2008;39:2006-2010.)

Key Words: endothelin-1 ■ thrombolytic therapy ■ ischemic stroke ■ cerebral edema

Thrombolysis with tissue plasminogen activator (t-PA) is an effective therapy for acute ischemic stroke. Partial or complete early recanalization of the middle cerebral artery occurs in more than 50% of patients who receive intravenous t-PA treatment; however, early reperfusion does not avoid ischemic stunning, neurological worsening, or delayed ischemic injury in more than one-third of these patients.\(^{1-3}\) One of the reasons for deleterious outcomes after t-PA administration may be linked to the neurotoxicity of the drug on the neurovascular unit.\(^{4}\) In animal models, t-PA promotes the disruption of the blood-brain barrier (BBB) that results in edema and cerebral hemorrhage.\(^{5,6}\) Despite the advances in the overall management of acute stroke, the development of edema and swelling after cerebral ischemia remains the major cause of death in patients with large infarctions, and so a better understanding of the pathophysiology of brain edema is necessary for the improvement of its clinical management.\(^{7}\)

Patients who develop cerebral edema are currently not revealed by clinical, neuroimaging, or biochemical markers early enough, and with sufficient accuracy to indicate contraindications for thrombolytic treatment. High serum metalloproteinase-9 (MMP-9) and cellular fibronectin (c-Fn)
levels have been associated with malignant brain edema in clinical studies. Endothelin-1 (ET-1) may be a new biomarker of BBB disruption because ET-1 overexpression leads to further water accumulation and brain edema after middle cerebral artery occlusion in experimental models, and endothelin type A receptor antagonist shows a protective effect on brain edema and injury after transient middle cerebral artery occlusion in rats.

This study investigates the potential association between serum levels of ET-1, MMP-9, and c-Fn with the development of severe brain edema in patients with acute ischemic stroke treated with t-PA.

**Methods**

**Study Population and Patients Characteristics**

We studied 134 acute ischemic stroke patients from 4 university hospitals treated with t-PA within 3 hours from symptom onset following the SITS-MOST criteria. Patients were continuously monitored during the first 24 hours in acute stroke units and were prospectively evaluated by investigators at each center using cerebral CT and neurological and functional scales according to the SITS-MOST registry during a follow-up period of 90 days. The protocol was approved by the Ethics Committees of the participating centers, and informed consent was signed by patients or their relatives. For the purpose of this investigation, additional exclusion criteria were prior disability (modified Rankin Scale [mRS] >1) and known infectious, inflammatory, or cancer diseases at the time of treatment.

**Clinical Variables**

Stroke severity was quantified before t-PA administration and at 24 hours by using the National Institute of Health Stroke Scale (NIHSS). Neurological deterioration was diagnosed when the NIHSS worsened ≥4 points between the 2 examinations. Poor outcome was defined as mRS ≥2 at 90 days.

**Neuroimaging Variables**

CT scans were carried out on admission and at 24 to 36 hours after thrombolytic therapy. Early CT signs of infarction were evaluated on admission, and hypodensity volume, hemorrhagic transformation (HT), and brain edema were assessed at 24 to 36 hours. HT was classified according to the ECASS-2 criteria. HT was considered as being symptomatic when it was associated with early neurological deterioration. Brain edema was classified as grade I if effacement of the cortical sulci, grade II if ventricular asymmetry, and grade III if shifting of the structures of the midline were observed. Malignant edema was diagnosed if midline shift and compression of the basal cisterns were associated with a decrease in the level of consciousness to somnolence or stupor compared with the baseline clinical status on admission. Patients with brain edema classified as grade III and those who developed malignant MCA were grouped as severe cerebral edema. Hypodensity volume was calculated from CT images using the formula 0.5axbxc, where a and b are the largest perpendicular diameters and c is the slice thickness. CT scans were evaluated by investigators blinded to the laboratory determinations and clinical outcome.

**Laboratory Determinations**

Serum samples were taken immediately after admission (within 3 hours of stroke onset and before t-PA treatment), and stored at −80°C. Serum levels of ET-1, c-Fn, and MMP-9 were determined with commercially available quantitative sandwich enzyme-linked immunoabsorbent assay kits obtained from Biomedica Medizin-produkte GMBH; Biohit Plc; and Biotrack, Amersham Pharmacia, respectively. Biomarker concentrations were measured in a central laboratory by investigators blinded to the clinical outcome and neuroimaging findings. Clinical investigators were unaware of the laboratory results until the end of the study, once the database was closed.

**Statistical Analysis**

Results are expressed as percentages for categorical variables and as mean (SD) or median (quartiles) for the continuous variables depending on whether they were normally distributed or not. Proportions were compared using the chi-square test, and the Student t test or the Mann–Whitney test were used to compare continuous variables between groups, as appropriate.

**Table 1. Baseline Clinical Characteristics, Vascular Risk Factors, Stroke Subtype, Biochemical Parameters, and Neuroimaging Findings in Patients With or Without Severe Brain Edema**

<table>
<thead>
<tr>
<th>Severe Brain Edema</th>
<th>No n=115</th>
<th>Yes n=19</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age, y</td>
<td>67.1 (12.3)</td>
<td>69.7 (10.9)</td>
<td>0.410</td>
</tr>
<tr>
<td>Women</td>
<td>42 (36.5%)</td>
<td>4 (21.1%)</td>
<td>0.145</td>
</tr>
<tr>
<td>Mean (SD) time from stroke onset to tPA treatment, min</td>
<td>144.1 (33.9)</td>
<td>149.7 (30.9)</td>
<td>0.451</td>
</tr>
<tr>
<td>Vascular risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of hypertension</td>
<td>60 (52.2%)</td>
<td>6 (31.6%)</td>
<td>0.215</td>
</tr>
<tr>
<td>History of diabetes</td>
<td>20 (17.4%)</td>
<td>5 (26.3%)</td>
<td>0.262</td>
</tr>
<tr>
<td>Clinical characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (quartiles) NIHSS on admission</td>
<td>13 (8, 19)</td>
<td>18 (15, 20)</td>
<td>0.032</td>
</tr>
<tr>
<td>Mean (SD) tPA doses, mg</td>
<td>68.8 (11.3)</td>
<td>72.9 (13.0)</td>
<td>0.469</td>
</tr>
<tr>
<td>Stroke subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atherothrombotic</td>
<td>22 (19.1%)</td>
<td>4 (21.1%)</td>
<td>0.102</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>45 (39.1%)</td>
<td>13 (68.4%)</td>
<td></td>
</tr>
<tr>
<td>Lacunar</td>
<td>9 (7.8%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Undetermined</td>
<td>39 (33.9%)</td>
<td>2 (10.5%)</td>
<td></td>
</tr>
<tr>
<td>Cardioembolic vs no cardioembolic stroke</td>
<td>45 (39.1%)</td>
<td>13 (68.4%)</td>
<td>0.016</td>
</tr>
<tr>
<td>Neuroimaging findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early CT signs of infarction</td>
<td>31 (27.0%)</td>
<td>14 (73.7%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Biochemistry and vital signs, mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean systolic blood pressure, mm Hg</td>
<td>150.9 (22.1)</td>
<td>155.7 (25.2)</td>
<td>0.718</td>
</tr>
<tr>
<td>Mean diastolic blood pressure, mm Hg</td>
<td>81.8 (12.8)</td>
<td>79.7 (13.7)</td>
<td>0.924</td>
</tr>
<tr>
<td>Maximum Body temperature first 24 hours, °C</td>
<td>37.1 (0.5)</td>
<td>37.3 (0.5)</td>
<td>0.251</td>
</tr>
<tr>
<td>Glucose levels, mg/dL</td>
<td>139.2 (53.2)</td>
<td>117.6 (27.7)</td>
<td>0.085</td>
</tr>
<tr>
<td>Leucocytes, ×10³/mL</td>
<td>8.7 (2.5)</td>
<td>9.2 (1.8)</td>
<td>0.201</td>
</tr>
</tbody>
</table>
Receiver operating characteristic (ROC) curves were configured to establish cut-off points for biomarkers levels that optimally predicted the development of severe cerebral edema. Accordingly, the impact of serum ET-1, c-Fn, and MMP-9 levels on outcome and brain edema formation was assessed by logistic regression analysis after adjusting for the main baseline related variables in the univariate analyses (enter approach and probability of entry $P < 0.05$).

Interactions between ET and other prognostic factors of brain edema were studied.

### Results

A total of 19 patients (14.2%) developed severe brain edema during the first 24 hours after stroke onset. Table 1 shows the main characteristics of patients by brain edema groups. The severity of neurological deficits on admission was significantly higher and cardioembolic stroke subtype and early CT signs of infarction were significantly more frequent in patients who developed severe brain edema as compared to those without. Patients with severe brain edema showed greater hypodensity volume (189.4 [164.4] versus 37.2 [54.1] cc; $P < 0.0001$), higher frequency of symptomatic hemorrhagic transformation (32% versus 12%; $P < 0.0001$), and poorer functional outcome at 3 months (89% versus 49%; $P = 0.001$).

Serum levels of ET-1, c-Fn, and MMP-9 were higher in the group of patients who developed severe brain edema, although the difference was only significant for ET-1 and cFn levels (Table 2). The higher serum ET-1 concentrations were the greater the intensity of brain edema (Figure).

In the explanatory analysis, the best cutoff value of ET-1 obtained from the receiver operating characteristic curve was $5.5 \text{ fmol/mL}$, which predicted the development of severe brain edema with a sensitivity of 95% and specificity of 93% (area under the curve 0.994; $P = 0.005$), whereas the best cutoff value of serum c-Fn levels was 4.5 mg/L which predicted the development of severe brain edema after t-PA administration with a sensitivity of 73% and specificity of 77%.

Serum ET-1 levels $>5.5 \text{ fmol/mL}$ before t-PA treatment were associated with severe brain edema (OR, 187.0; CI95%, 33.5 to 1041.7; $P < 0.0001$); this association remained after adjustment for baseline stroke severity, early CT signs of infarction, serum levels of cFn $>4.5 \text{ mg/L}$, and cardioembolic stroke subtype (OR, 139.7; CI95%, 19.3 to 1012.2; $P < 0.0001$; Table 3). No interactions were found.

### Discussion

Our study shows that serum ET-1 levels $>5.5 \text{ fmol/mL}$ are associated with the development of severe cerebral edema in patients with acute ischemic stroke treated with t-PA with a sensitivity of 95% and specificity of 94%. This result suggests that ET-1 is a powerful biological marker of risk of developing brain edema. Therefore, it may be used as a future therapeutic target in patients with ischemic stroke treated with t-PA.

The vascular pathophysiology in the acute phase of stroke involves metabolic and hemodynamic changes, resulting in

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**Table 2. Baseline Serum Levels of Molecular Markers in Patients With or Without Severe Brain Edema**

<table>
<thead>
<tr>
<th></th>
<th>Severe Brain Edema</th>
<th>No n=115</th>
<th>Yes n=19</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1 levels, fmol/mL</td>
<td>1.9 (1.6, 3.2)</td>
<td>8.4 (6.7, 9.6)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>cFn levels, mg/L</td>
<td>3.2 (2.1, 4.6)</td>
<td>6.0 (4.1, 6.7)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>MMP-9 levels, ng/mL</td>
<td>108.6 (68.9, 170.5)</td>
<td>131.0 (70.3, 199.9)</td>
<td>0.288</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Crude (Model I) and Adjusted (Model II) OR of Severe Brain Edema for ET-1 Serum Levels $>5.5 \text{ fmol/mL}$**

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>CI 95%</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET-1 $&gt;5.5 \text{ fmol/mL}$</td>
<td>187.0</td>
<td>33.5 to 1041.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS basal</td>
<td>0.9</td>
<td>0.8 to 1.2</td>
<td>0.953</td>
</tr>
<tr>
<td>Early CT signs of infarction</td>
<td>3.7</td>
<td>0.5 to 26.5</td>
<td>0.193</td>
</tr>
<tr>
<td>Cardioembolic vs no cardioembolic stroke</td>
<td>5.3</td>
<td>0.8 to 36.9</td>
<td>0.090</td>
</tr>
<tr>
<td>ET-1 $&gt;5.5 \text{ fmol/mL}$</td>
<td>139.7</td>
<td>19.3 to 1012.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>cFn $&gt;4.5 \text{ mg/L}$</td>
<td>2.3</td>
<td>0.4 to 14.6</td>
<td>0.374</td>
</tr>
</tbody>
</table>

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**Figure.** Serum ET-1 levels by brain edema subtype. Boxplots show median values (horizontal line inside the box), quartiles (box boundaries), and the largest and smallest observed values (lines drawn from the end of the box) of ET-1.
the blood brain barrier breakdown. The basal cerebrovascular tone favors partial vasoconstriction and plays an important role in the regulation of cerebrovascular blood flow in response to changes in perfusion pressure as well as alterations in metabolic and endothelial factors. In this acute phase, ET-1 regulates the endothelial function mediating a receptor-dependent vasoconstriction or vasodilatation, and participates in the increase of permeability of the BBB. It has been suggested a relationship between ET-1 and the development of severe cerebral edema in experimental models of transient and permanent MCA occlusion. Furthermore, high plasma ET-1 levels have been found in patients who suffer ischemic stroke. Taken together, these observations are in agreement with our results which show an independent association between the development of severe cerebral edema and high serum levels of ET-1.

The cause of the increase of serum ET-1 levels in stroke patients is yet unclear. This phenomenon may represent a nonspecific overexpression of ET-1 by the systemic vascular endothelium in response to stress associated with the acute cerebral infarction. Alternatively, high serum ET-1 levels may reflect the generation of ET-1 in the injured endothelial cells of the ischemic cerebral microvessels. Hypoxia is known to stimulate ET-1 synthesis, and elevated thrombin concentrations within the ischemic region may also contribute to the induction of ET-1 release.

The limited sample size of the present study and consequently the low number of patients who develop severe brain edema, as well as the possibility that edema interferes with the estimation of the infarct volume, weaken the strength with which we may draw our conclusions. Besides, ELISA kits can only be either accepted or are valid as screening analytic tests, because this technique is slow and expensive and is therefore not applicable in daily clinical practice. However, the robustness of the results gives support to the need to develop a faster analytic method for determining the best cut off points and for measuring serum ET-1 at emergency departments and to test their accuracy in a large multicenter study.

In clinical practice, thrombolytic treatment should be used carefully in patients with a high risk of hemorrhagic transformation and brain edema. Nowadays, this selection mainly rely on early CT findings such as the ASPECTS score or the 1/3 MCA hypodensity rule, although new neuroimaging and biochemical predictors have been described. High serum levels of c-Fn, a component of the basal lamina which plays an important role in maintaining microvascular integrity, have been associated with malignant brain edema and hemorrhagic transformation. The present study shows that ET-1 is a new biomarker with an intense relationship with the development of severe cerebral edema in patients treated with thrombolysis, but causality needs to be tested in future studies.

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Disclosures

None.

References


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