Elevated Serum S100B Levels Indicate a Higher Risk of Hemorrhagic Transformation After Thrombolytic Therapy in Acute Stroke

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Background and Purpose—Intracerebral hemorrhage constitutes an often fatal sequela of thrombolytic therapy in patients with ischemic stroke. Early blood–brain barrier disruption may play an important role, and the astroglial protein S100B is known to indicate blood–brain barrier dysfunction. We investigated whether elevated pretreatment serum S100B levels predict hemorrhagic transformation (HT) in thrombolized patients with stroke.

Methods—We retrospectively included 275 patients with ischemic stroke (mean age of 69±13 years; 46% female) who had received thrombolytic therapy within 6 hours of symptom onset. S100B levels were determined from pretreatment blood samples. Follow-up brain scans were obtained 24 hours after admission, and HT was classified as either hemorrhagic infarction (1, 2) or parenchymal hemorrhage (1, 2).

Results—HT occurred in 80 patients (29%; 45 hemorrhagic infarction, 35 parenchymal hemorrhage). Median S100B values were significantly higher in patients with HT (0.14 versus 0.11 µg/L; P=0.017). An S100B value in the highest quintile corresponded to an OR for any HT of 2.87 (95% CI: 1.55 to 5.32; P=0.001) in univariate analysis and of 2.80 (1.40 to 5.62; P=0.004) after adjustment for age, sex, symptom severity, timespan from symptom onset to hospital admission, vascular risk factors, and storage time of serum probes. A pretreatment S100B value above 0.23 µg/L had only a moderate sensitivity (0.46) and specificity (0.82) for predicting severe parenchymal bleeding (parenchymal hemorrhage 2).

Conclusions—Elevated S100B serum levels before thrombolytic therapy constitute an independent risk factor for HT in patients with acute stroke. Unfortunately, the diagnostic accuracy of S100B is too low for it to function in this context as a reliable biomarker in clinical practice. (Stroke. 2007;38:2491-2495.)

Key Words: biomarker ▪ blood–brain barrier ▪ intracerebral hemorrhage ▪ S100B ▪ thrombolysis

Intravenous recombinant tissue plasminogen activator is the only proven effective therapy for patients with acute ischemic stroke within the first few hours of symptom onset.1,2 However, the benefits of early vessel recanalization are partly diminished by a significant rate of symptomatic intracerebral hemorrhage.3

Different potential risk factors for cerebral hemorrhagic transformation (HT) after thrombolytic therapy have been identified (eg, age, severity of neurological symptoms, high blood pressure, history of diabetes mellitus, heart disease, lower platelet count, pretreatment with antiplatelet agents, and early CT abnormalities).3–7 Pathophysiological, animal studies on focal cerebral ischemia have shown that endothelial damage and immediate blood–brain barrier (BBB) dysfunction are important stages in the development of HT after thrombolytic therapy.8–11 Recently, MRI-based studies have confirmed the rapid occurrence of BBB opening in ischemic stroke in humans too and its association with postthrombolytic hemorrhagic complications.12–14

The calcium-binding protein S100B was recently reported to be a serum marker of BBB dysfunction.15–18 In humans, S100B concentrations were found to be up to 40 times higher in cerebrospinal fluid than in serum and were shown to increase rapidly in the case of hyperosmotic-induced BBB damage reflecting the leakage of S100B from cerebrospinal fluid into serum.16,17 In this context, we hypothesized that elevated pretreatment serum S100B levels might be independently associated with HT after thrombolytic therapy in the hyperacute phase of ischemic stroke. If this proved to be the case, it would further substantiate the importance of BBB

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disruption in the pathogenesis of HT. In addition, we evaluated the diagnostic accuracy of serum S100B in predicting clinically relevant parenchymal hematoma in patients with stroke receiving thrombolysis.

Methods

Study Protocol

We retrospectively identified a total of 275 patients (mean age 69±13 years; 46% female) who had received thrombolytic therapy for the treatment of acute cerebral ischemia within the first 6 hours of symptom onset from prospective, independent studies in the field of stroke biomarkers that had been performed in 3 tertiary care hospitals: the Department of Neurology, Vall d’Hebron Hospital, Barcelona, Spain (121 patients between March 2003 and July 2005); the Department of Neurology, University of Magdeburg, Germany (65 patients between July 2002 and June 2005); and the Department of Neurology, University of Frankfurt am Main, Germany (89 patients between October 2002 and December 2004). In the 3 study centers, all patients or their next of kin had given informed consent for participation in open biomarker research studies, including the determination of serum S100B. The corresponding study protocols had been approved by the local ethics review committees.

Patient inclusion criteria were: (1) initiation of thrombolysis within 6 hours of symptom onset; and (2) absence of intracerebral hemorrhage on initial CT or MRIs. Exclusion criteria were: (1) age >90 years; (2) previous intracerebral hemorrhage; (3) cerebral infarction within the past 3 months; (4) other central nervous system diseases; (5) serum creatinine level >2 mg/dL; and (6) any malignant diseases.

At all 3 hospitals, the severity of clinical symptoms on hospital admission was quantified according to the National Institutes of Health Stroke Scale. On hospital discharge, disability was determined using the modified Rankin Scale. Stroke etiology was classified according to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria. Baseline characteristics of the study population are shown in the Table.

### Baseline Characteristics of the Study Population (Mann-Whitney U or χ²)

<table>
<thead>
<tr>
<th></th>
<th>No HT</th>
<th>HT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>68.4±13.1</td>
<td>68.9±11.6</td>
<td>0.814</td>
</tr>
<tr>
<td><strong>Percent female</strong></td>
<td>44.1</td>
<td>48.8</td>
<td>0.482</td>
</tr>
<tr>
<td><strong>Median National Institutes of Health Stroke Scale (25th to 75th percentile)</strong></td>
<td>14 (10–19)</td>
<td>17 (13–21)</td>
<td>0.003*</td>
</tr>
<tr>
<td><strong>Median time to admission, h (range)</strong></td>
<td>2.0 (1.5–3.0)</td>
<td>2.1 (1.5–3.0)</td>
<td>0.320</td>
</tr>
<tr>
<td><strong>Median modified Rankin Scale discharge (range)</strong></td>
<td>4 (1–5)</td>
<td>4 (2–5)</td>
<td>0.004*</td>
</tr>
<tr>
<td><strong>Stroke etiology, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large vessel</td>
<td>25.5</td>
<td>20.0</td>
<td>0.331</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>52.1</td>
<td>58.8</td>
<td>0.315</td>
</tr>
<tr>
<td>Small vessel</td>
<td>1.0</td>
<td>0.0</td>
<td>0.360</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>21.4</td>
<td>21.3</td>
<td>0.985</td>
</tr>
<tr>
<td><strong>Thrombolytic therapy, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>89.7</td>
<td>82.5</td>
<td>0.097</td>
</tr>
<tr>
<td>Intraarterial</td>
<td>10.3</td>
<td>17.5</td>
<td>0.097</td>
</tr>
<tr>
<td><strong>Risk factors, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>54.7</td>
<td>62.5</td>
<td>0.236</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>18.7</td>
<td>26.3</td>
<td>0.160</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>34.9</td>
<td>23.8</td>
<td>0.072</td>
</tr>
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</table>

*Significant findings (P<0.05).

Blood Sampling and S100B Measurements

Venous blood samples were collected on hospital admission, always before thrombolytic therapy. The blood probes were centrifuged immediately to separate the serum, which was then frozen at −25°C. Thereafter, long-term storage was done at −80°C in all 3 study centers. Determination of S100B was performed in Frankfurt for the samples from Barcelona and Frankfurt and in Magdeburg for the samples from Magdeburg. The transport of the samples from Barcelona to Frankfurt was held on dry ice using a professional transportation company. All measurements of the serum S100B concentration were performed using the same type of a commercially available monoclonal 2-site immunoluminometric assay on a fully automated LIA-mat system (DIASORIN), which measures the beta subunit of the S100 protein as defined by 3 monoclonal antibodies (SMST 12, SMSK 25, and SMSK 28). The detection limit of this test is 0.02 μg/L. Intra- and interassay variability vary from 2.8% to 6.4% and from 2.2% to 10.7%, respectively. From 25 patients with stroke, serum S100B was determined both in Magdeburg and in Frankfurt revealing an intraassay correlation coefficient of 0.933 (95% CI: 0.853 to 0.970, P<0.001). In healthy individuals aged 30 to 70 years, a median S100B serum concentration of 0.06 μg/L (interquartile range: 0.03 to 0.10 μg/L) was reported. It has been described that S100B serum concentration can raise during long-term storage. Therefore, we included storage time as a cofactor in the statistical analyses (see subsequently). However, there was no significant correlation between storage time and S100B values in our data set (Spearman rho=0.042, P=0.486).

Hemorrhagic Transformation

All patients underwent a follow-up brain scan (92% CT, 8% MRI), which was routinely performed approximately 24 hours after symptom onset. Presence and type of HT were defined according to previously published criteria: hemorrhagic infarction was defined as a petechial infarction without space-occupying effect and parenchymal hemorrhage (PH) was defined as hemorrhage with mass effect. Both types of intracerebral hemorrhage were subclassified (hemorrhagic infarction-1: small petechiae along the margins of the infarct; hemorrhagic infarction-2: confluent petechiae within the infarcted area; PH-1: hematoma involved <30% of the infarcted area with only slight space-occupying effect; PH-2: hematoma involved >30% of the infarcted area with substantial mass effect). A T2* sequence was used in the follow-up MRIs to determine the incidence of hemorrhage.

The classification of brain scans was performed independently by a neurologist and a radiologist from each center who were blind to all other data. The final analysis was based on the consensus reached by both observers on joint reevaluation of their original data.

Statistical Analysis

Statistical analyses were performed using the SPSS 10.0 software package (SPSS Inc). First, we investigated the probability of HT in quintiles of increasing serum S100B. For this purpose, we calculated the OR for any HT in each quintile with the lowest quintile serving as the reference. Second, to estimate the association between elevated S100B concentrations and the risk of HT, univariate and multivariate logistic regressions were performed. Finally, the diagnostic accuracy of elevated S100B values in predicting clinically relevant parenchymal bleeding (PH-2) was calculated. For descriptive analyses, we provided median values and percentiles; differences were tested using the Mann-Whitney U test or χ² statistics.

Results

Descriptive Analysis

HT was present in 80 (29%) of the 275 patients included. Of these, 28 had hemorrhagic infarction-1, 17 had hemorrhagic infarction-2, 22 had PH-1, and 13 had PH-2. As shown in the
Table, there were considerable differences in the initial severity of clinical symptoms (National Institutes of Health Stroke Scale score) and short-term outcome (modified Rankin Scale on hospital discharge) between the groups. Serum S100B values were significantly higher in patients with HT compared with patients without HT (median 0.14 μg/L [25th to 75th percentile 0.07 to 0.27 μg/L] versus 0.11 μg/L [0.07 to 0.16 μg/L]; P=0.017). As shown in the Figure, there was no linear relationship between S100B and the risk of HT. Only in the highest quintile (S100B >0.21 μg/L), the OR for any HT was significantly higher than in the lowest quintile (2.85 [95% CI: 1.28 to 6.38]; P=0.011).

**Logistic Regression**

Using univariate logistic regression analysis, a serum S100B value in the highest quintile (S100B >0.21 μg/L) as compared with the remaining quintiles corresponded to an OR of 2.87 (1.55 to 5.32; P=0.001) for any HT. After adjustment for several additional factors (age, sex, National Institutes of Health Stroke Scale score, time interval between symptom onset and hospital admission, history of arterial hypertension, history of diabetes mellitus, history of hypercholesterolemia, and storage time of serum probes), an elevated S100B (ie, in the highest quintile) emerged as an independent predictor of HT after thrombolytic therapy with an OR of 2.80 (1.40 to 5.62; P=0.004). Furthermore, National Institutes of Health Stroke Scale score (P=0.013) and diabetes mellitus (P=0.039) were independently associated with HT in this model. The independent association between S100B and HT remained still significant after the exclusion of those patients (n=32) who received thrombolytic therapy beyond the 3-hour time window (OR: 3.16; 1.48 to 6.75; P=0.003).

**Diagnostic Accuracy**

To evaluate the clinical usefulness of elevated pretreatment S100B in identifying patients who will experience severe parenchymal bleeding (PH-2) after thrombolysis, we calculated the diagnostic accuracy of elevated S100B using receiver operating characteristic analysis. A cutoff point of 0.23 μg/L provided a sensitivity of 0.46 and a specificity of 0.82 for the prediction of PH-2 (positive predictive value 0.12, negative predictive value 0.97, area under the curve 0.661, P=0.01).

**Discussion**

This study first identified pretreatment serum levels of the astroglial protein S100B to be an independent risk factor for cerebral bleedings after thrombolytic therapy in patients with acute ischemic stroke.

What is the pathophysiological background of our findings? A number of experimental studies have revealed that BBB opening can occur in the early stages of cerebral ischemia and that degradation of basal lamina components and subsequent microvascular damage are key determinants of HT after thrombolysis. Furthermore, recently published studies describe the detection of early BBB opening in human stroke by means of MRI and speculate about a higher risk of thrombolysis-induced HT for patients with early BBB changes. Our research into a serum marker that would indicate this early disruption was largely stimulated by the work of Kanner et al who demonstrated a sudden increase in serum S100B concentration in the case of hyperosmotic-induced BBB disruption. However, our study did not monitor BBB disruption directly, because this is of course difficult to do in the acute phase of human stroke. Nevertheless, which arguments support the conclusion that serum S100B indeed reflects early BBB alterations in acute cerebral ischemia? Several previous reports show conclusively that BBB opening can occur in the early stages of cerebral ischemia and that degradation of basal lamina components and subsequent microvascular damage are key determinants of HT after thrombolysis. Against this background, it is not surprising that we found serum S100B values in the normal range in the majority of our patients, although they experienced acute stroke. Following the penumbra concept, final infarct size is not definitively determined in the early stages of ischemic stroke, because successful thrombolytic therapy can result in small infarctions despite extensive misery perfusion initially. Thus, the argument that our findings simply reflect a surrogate for final infarct volume and tissue damage is not justified. It seems to be more plausible that
S100B reflects BBB disruption at this stage of ischemic stroke, allowing this protein to be identified in the bloodstream at an elevated concentration. However, because we identified high levels of S100B in any HT subtype, this protein provides only qualitative information about BBB opening. It is not specific enough to show BBB leakage extension and thus provide quantitative information reflecting the degree of HT. In either case, our results further substantiate the importance of early BBB opening, which occurs in some patients and predisposes them to hemorrhagic complications under thrombolytic treatment.

Two other prospective studies focused on BBB markers for predicting the development of HT after thrombolytic therapy. Montaner and coworkers found that in 41 patients with acute stroke, matrix metalloproteinase-9 was the most powerful predictor of PH occurrence in a multiple logistic regression model (OR: 9.62 [1.31 to 70.26]; P = 0.025). Furthermore, Castellanos and coworkers found high plasma cellular fibronectin levels to be significantly associated with subsequent HT in 87 patients with stroke treated with tissue plasminogen activator (multivariate OR: 2.1 [1.3 to 3.4]; P = 0.002). Nevertheless, there are some substantial limitations to all previous studies, including the sample sizes are too small to include a sufficient number of patients with hemorrhagic complications, especially if the focus were on severe parenchymal bleedings (ie, PH-2). Multivariate analysis is further restricted by not including all covariates for which an association has previously been found. Consequently, all biomarker studies in this field to date, including this one, should be regarded as pilot studies that did not definitively elucidate the clinical relevance of any single marker or a panel of different markers.

To date, various strategies have been used to optimize and augment thrombolytic treatment in patients with acute stroke: (1) identification of patients with a sustained “tissue at risk” beyond the 3-hour limit, as evidenced by a persistent diffusion/perfusion mismatch on the MRI; (2) the use of thrombolytic agents that are probably associated with a lower risk of HT; and (3) the combination of recombinant tissue plasminogen activator with ultrasound insolation to enhance recanalization rates. On the other hand, the effectiveness of acute stroke treatment can also be improved by holding off giving patients thrombolyis if they are at much greater risk of experiencing cerebral hemorrhagic complications. Unfortunately, there is as yet no reliable risk indicator enabling these high-risk patients to be sufficiently identified. This may be a promising clinical application for serum biomarkers, but the diagnostic accuracy of those investigated to date (ie, matrix metalloproteinase-9, fibronectin, S100B) does not appear to be good enough for them to function as a reliable test in acute stroke management. This is further complicated by the observation that the occurrence of HT under thrombolytic therapy is influenced by many different factors (eg, antiplatelet pretreatment, stroke severity, diabetes mellitus, preexisting cerebral small vessel disease), which may affect different pathophysiological mechanisms (eg, endothelial dysfunction, BBB disruption, hemostasis). It is therefore unlikely that a single risk indicator will be capable of predicting HT complications with a high degree of accuracy.

In conclusion, this study showed that S100B can provide independent information about the individual risk of a patient with acute stroke experiencing cerebral hemorrhagic complications under thrombolytic therapy. Unfortunately, its predictive value alone is not good enough for it to function as a diagnostic test. Because serum biomarkers in general may provide valid information in this context, large-scale prospective studies should be undertaken to determine their actual potential.

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Disclosures

None.

References


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