Evaluation of Serum S100B as a Surrogate Marker for Long-term Outcome and Infarct Volume in Acute Middle Cerebral Artery Infarction

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Background: An easily accessible and valid surrogate marker for interventional stroke trials is needed.

Objective: To investigate the usefulness of various S100B serum measures to predict long-term outcome and infarct volume in patients with acute stroke.

Design: Inception cohort study.

Setting: Tertiary care university hospital.

Patients: Thirty-nine patients (mean±SD age, 69.1±11.5 years) with acute nonlacunar middle cerebral artery infarction presenting less than 6 hours after symptom onset.

Main Outcome Measures: Functional outcome 6 months after stroke (modified Rankin scale score) and final infarct volume on day 7 by means of standardized volumetry of brain images. Serum S100B level was determined at hospital admission and 24, 48, 72, 96, 120, and 144 hours after symptom onset.

Results: Single S100B measures obtained 48 and 72 hours after stroke onset demonstrated the highest Spearman rank correlations with modified Rankin scale scores (p=0.68 and p=0.67, respectively; P<.001) and infarct volume (p=0.93 and p=0.94, respectively; P<.001). A 48-hour S100B value of 0.37 µg/L or less revealed a sensitivity of 0.87 and a specificity of 0.78 in predicting an independent functional outcome. In a multivariate model, S100B emerged as an outcome predictor that was independent of age, sex, stroke severity, etiology, lesion side, and risk factors.

Conclusions: Single S100B values obtained 48 and 72 hours after stroke onset provide the highest predictive values with respect to functional outcome and infarct volume in nonlacunar middle cerebral artery infarction. More complex measures of the S100B kinetic (ie, area under the curve or peak value) were not superior. Therefore, these single S100B measures appear to be useful surrogate end points in acute interventional stroke trials.

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IN ACUTE INTERVENTIONAL STROKE trials, functional outcomes as measured by various scales are used as the primary end points.1,2 This approach is limited by the low statistical power of these functional scales because of their ordinal scoring level. Furthermore, it has been shown that the choice of outcome measure may be crucial for the demonstration of therapeutic effects.3,4 Infarct volume measured during brain imaging may function as a secondary end point, but the optimal time point and the best imaging modality for infarct volume measurements remain undefined.4 Clinical long-term follow-up and repeated brain scans increase the associated labor and expenses. Therefore, there is a need for a surrogate marker in interventional stroke trials that is easily accessible, inexpensive, reliable, valid, and powerful with respect to clinically relevant outcome measures.

It has been shown previously that the astroglial protein S100B is elevated in peripheral blood in the first days after acute ischemic stroke.5-12 These authors described significant correlations between the cumulative S100B release estimated by the area under the curve (AUC), the final infarct volume, and the short-term outcome.7-12 Nevertheless, these promising findings have not established S100B as an end point in ongoing clinical trials, because the AUC, peak value, and other complex measures of the S100B kinetic require extensive blood sampling, which is not feasible in large-scale trials. Furthermore, the diagnostic accuracy of various single S100B values in predicting relevant outcome measures has not been evaluated prospectively, to our knowledge. Therefore, the objective of the present investigation was to test the predictive value of easily accessible S100B values...
for long-term outcome and infarct volume in a sample of patients experiencing stroke, for potential use in interventional stroke trials.

**STUDY POPULATION**

We prospectively included 39 consecutive patients admitted to our stroke unit within 6 hours of symptom onset with suggested acute ischemia in the middle cerebral artery (MCA) territory, which was verified by means of a perfusion or diffusion deficit as seen on magnetic resonance imaging performed directly after hospital admission. In case of magnetic resonance imaging contraindications (n = 5), patients were included based on computed tomographic findings. In these cases, MCA territorial infarction was verified by follow-up computed tomographic scan 24 hours after symptom onset. We excluded patients with lacunar infarctions as seen on brain imaging, bilateral lesions, coincidental intracranial hemorrhage, concomitant ischemia in the posterior circulation, or a history of stroke. After hospital admission, the neurological deficit was quantified using the National Institutes of Health Stroke Scale score. Baseline characteristics of the study population, as well as vascular risk factors, stroke etiology, and treatment, are shown in Table 1. All patients or next of kin gave informed consent for study participation and for determination of the S100B values. The study was approved by the ethics review committee of our university hospital.

**BLOOD SAMPLING AND S100B MEASUREMENTS**

Venous blood samples (2 mL) were drawn at hospital admission (baseline) and 24, 48, 72, 96, 120, and 144 hours after symptom onset. Blood samples were centrifuged (at 2703 g for 5 minutes), and serum was stored at −25°C. Two patients died during the study period of malignant MCA infarction, for whom final blood sampling occurred after 96 and 120 hours.

For measurement of the S100B serum concentrations, we used a commercially available monoclonal 2-site immunolumino-metric assay and a fully automatic LIA-mat system (Byk-Sangtec Diagnostica, Dietzenbach, Germany), which measures the β subunit of protein S100 as defined by 3 monoclonal antibodies (SMST 12, SMSK 23, and SMSK 28). The detection limit of this kit was 0.02 µg/L. Intra-assay and interassay variabilities were 2.8% to 6.4% and 2.2% to 10.7%, respectively.

**LONG-TERM OUTCOME**

Six months after stroke, functional outcome was assessed by a neurologist (O.C.S.) using the modified Rankin Scale (mRS). Patients who died within the first 6 months were scored as mRS 6. By means of this scale, functional outcome was dichotomized as independent (mRS score 0-2) or as dependent or dead (mRS score 3-5 or 6).

**INFARCT VOLUME**

Infarct volume was based on fluid-attenuated inversion recovery magnetic resonance imaging (n = 25) or computed tomographic scanning (n = 12) performed on day 7 after symptom onset. For infarct magnetic resonance imaging volumetry, we used commercially available software (MRvision; MRvision Inc, Winchester, Mass), and for computed tomographic scans we used public domain software (National Institutes of Health, Bethesda, Md). For the 2 patients who died between days 4 and 6, follow-up imaging was not obtained. Measurements of lesion volume were performed independently by 2 observers (O.C.S. and R.M.R.); one of them was blinded to all other data. The mean ± SD infarct volume measured by the blinded observer was 133.2 ± 137.0 mL, and that by the unblinded observer was 142.6 ± 171.0 mL. Interobserver agreement revealed a single-measure intraclass correlation coefficient of 0.98 (95% confidence interval, 0.96-0.99; Cronbach α = .99). Final analysis was based on the consensus achieved between both observers at joint reevaluation of their data previously obtained independently.

**STATISTICAL ANALYSIS**

All statistical analyses were performed using the SPSS 10.0 software package (SPSS Inc, Chicago, Ill). The S100B AUC and peak value (defined as the highest individual value followed by a subsequent decrease) were derived from each S100B kinetic. To assess the predictive value of S100B with respect to functional outcome, we first calculated bivariate correlations (Spearman ρ) between the S100B measures and the actual mRS values. Second, using receiver operating characteristic curve analysis, cut points for the corresponding S100B values were determined, and accuracy measures for predicting a dichotomized functional state 6 months after stroke (ie, independent vs dependent or dead) were derived from cross-tabulations. A χ² statistic was used to indicate significant findings. Third, multivariate stepwise logistic regression analysis was performed to estimate whether S100B (ie, 48-hour value and AUC) is independently predictive of functional outcome. To check multicollinearity between the independent variables, we performed bivariate Spearman ρ correlations. Despite significant correlation (P < .001) between the initial National Institutes of Health Stroke Scale scores and the corresponding S100B values (ρ = .58 and ρ = .60, respectively), no significant multicollinearity was found.

To quantify the relationship between S100B level and infarct volume, we calculated bivariate Spearman ρ correlations. Furthermore, to estimate final infarct volume from S100B values, we performed univariate linear regression analysis after log

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**Table 1. Characteristics of the 39 Patients With Acute Middle Cerebral Artery Infarction***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Age, y</td>
<td>69.1 ± 11.5</td>
</tr>
<tr>
<td>Female sex</td>
<td>14 (35.9)</td>
</tr>
<tr>
<td>Left middle cerebral artery territory</td>
<td>21 (53.8)</td>
</tr>
<tr>
<td>Time to stroke unit admission, h</td>
<td>1.7 ± 1.1</td>
</tr>
<tr>
<td>Time to first brain scan, h</td>
<td>2.5 ± 1.4</td>
</tr>
<tr>
<td>National Institutes of Health Stroke Scale score at admission</td>
<td>14.8 ± 6.3</td>
</tr>
<tr>
<td>Vascular risk factors</td>
<td>Arterial hypertension 28 (71.8)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8 (20.5)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>20 (51.3)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>11 (28.2)</td>
</tr>
<tr>
<td>Stroke etiology</td>
<td>Cardioembolic 24 (61.5)</td>
</tr>
<tr>
<td>Large-vessel disease</td>
<td>11 (28.2)</td>
</tr>
<tr>
<td>Other or unknown</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>Treatment</td>
<td>Thrombolysis 20 (51.3)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>22 (56.4)</td>
</tr>
</tbody>
</table>

*Data are given as mean±SD or as number (percentage).
The S100B AUC ranged from 7.0 to 931.9 µg/L·h in 3 patients, and 120 hours after stroke in 3 patients. 72 hours after stroke in 9 patients, 96 hours after stroke in 8 patients, 48 hours after stroke in 10 patients, 24 hours after stroke in 3 patients, and 144 hours after stroke in 1 patient. The S100B serum concentrations at hospital admission (baseline) ranged from 0.04 to 0.36 µg/L (mean±SD, 0.12±0.07 µg/L). In 33 (85%) of 39 patients, an S100B peak value according to our definition (ie, with subsequent decrease) could be detected, with peak concentrations ranging from 0.08 to 12.20 µg/L (mean±SD, 2.49±3.41 µg/L). Peak values occurred 24 hours after stroke in 8 patients, 48 hours after stroke in 10 patients, 72 hours after stroke in 9 patients, 96 hours after stroke in 3 patients, and 120 hours after stroke in 3 patients. The S100B AUC ranged from 7.0 to 931.9 µg/L·h (mean±SD, 196.5±267.5 µg/L·h).

Transformation for normalizing the S100B and infarct volume distribution. Because several consecutive statistical tests were performed, α adjustment according to the modified Bonferroni procedure was applied at each step of analysis.

RESULTS

S100B KINETIC

The S100B serum concentrations at hospital admission (baseline) ranged from 0.04 to 0.36 µg/L (mean±SD, 0.12±0.07 µg/L). In 33 (85%) of 39 patients, an S100B peak value according to our definition (ie, with subsequent decrease) could be detected, with peak concentrations ranging from 0.08 to 12.20 µg/L (mean±SD, 2.49±3.41 µg/L). Peak values occurred 24 hours after stroke in 8 patients, 48 hours after stroke in 10 patients, 72 hours after stroke in 9 patients, 96 hours after stroke in 3 patients, and 120 hours after stroke in 3 patients. The S100B AUC ranged from 7.0 to 931.9 µg/L·h (mean±SD, 196.5±267.5 µg/L·h).

LONG-TERM OUTCOME

Functional outcome 6 months after stroke was obtained in 38 patients; 1 patient was not available for follow-up. Nine patients had already died (mRS score, 6). Fourteen patients were moderately or severely disabled (mRS score, 3-5), and the remaining 15 patients were slightly disabled or showed no symptoms (mRS score, 0-2).

Table 2 provides results of the bivariate correlation (Spearman ρ) between various S100B measures and the mRS scores, revealing highly significant coefficients ranging from 0.53 to 0.68. This indicates that higher S100B values were associated with higher mRS scores. Single S100B values obtained 48 and 72 hours after stroke onset were correlated with the corresponding mRS scores as high as the AUC and the peak value. Derived using receiver operating characteristic curve analysis, we calculated cut points that provided optimal sensitivities and specificities. Based on these cut points, Table 2 provides accuracy measures predicting functional state 6 months after stroke. The 48- and 72-hour S100B values provided an 82% overall accuracy, and the AUC and peak value also did not provide superior accuracy. In a multivariate stepwise logistic regression model that included age, sex, lesion side, National Institutes of Health Stroke Scale score, vascular risk factors, and stroke etiology, S100B emerged as an independent outcome predictor (Table 3).

INFARCT VOLUME

Final infarct volume varied between 2.8 and 555.5 mL (mean±SD, 150.5±172.3 mL). Using univariate linear regression analysis (Table 4), all S100B values were significantly associated with final infarct volume, whereas single S100B values obtained 48 and 72 hours after stroke onset, as well as the AUC and peak value, provided the highest correlation coefficients. Scatterplots and the corresponding regression curves are displayed in the Figure for the 48-hour S100B value, AUC, and peak value.
In the present study, single measures of the S100B serum concentration obtained 48 and 72 hours after stroke onset were highly correlated with long-term outcome and infarct volume. For each of these single S100B values, the diagnostic accuracy of predicting an independent state of the patient was as high as for the more complex measures of the S100B kinetic (ie, the AUC or peak value) and reached an overall accuracy of 82%. Furthermore, based on a single S100B value, infarct volume could be calculated using a linear model. Therefore, we conclude that single S100B values obtained 48 and 72 hours after stroke onset may be ideal to use as easily accessible and valid surrogate markers for future interventional trials of nonlacunar MCA infarctions.

There are good arguments favoring S100B as a measure of effectiveness in different types of acute interventional stroke trials. For thrombolytic studies, it has been shown that lesion volume may constitute a meaningful surrogate marker of successful treatment. A post hoc analysis of the imaging data of the National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Stroke Trial showed significantly smaller lesion volumes in the recombinant tissue plasminogen activator–treated group. Furthermore, it has been shown that S100B level, in particular a 48-hour S100B value of less than 0.4 µg/L, is an indicator of MCA recanalization within 6 hours of stroke onset, based on a high sensitivity and specificity (overall accuracy, 95%). Therefore, early and sufficient recanalization of an occluded intracranial artery is associated with smaller infarcts and better outcome. Thereby, the effectiveness of a recanalizing intervention can be closely gauged by serum S100B level, and a single value appears to be as predictive as more complex measures of the S100B kinetic.

The failure of previous investigations of various neuroprotective agents was not necessarily because of the ineffectiveness of the agents under study but, rather, because of shortcomings with respect to study design and patient selection criteria. For example, the optimal time point and duration for the application in humans of one neuroprotective drug that was evaluated are unknown. It is likely but not proved that patients with potentially salvageable but hypoperfused brain tissue (“ischemic penumbra”) may obtain benefits from neuroprotective treatment. In this context, S100B level may function as a useful marker of tissue damage, helping to identify optimal conditions for the application of a neuroprotective drug. In a recent study investigating the effect of recombinant human erythropoietin (rhEPO) in acute MCA infarction within 8 hours after stroke onset, S100B level was used as a secondary end point. The authors reported a significantly better outcome 30 days after stroke as measured by the Barthel Index and a trend toward an improved outcome as measured by the mRS score in the rhEPO-treated group. Supporting our hypothesis, the S100B serum values were significantly lower in the rhEPO-treated group compared with the placebo-treated group (mean S100B level, 0.76 vs 0.43 µg/L on day 7 after stroke). Unfortunately, in this small trial (21 rhEPO-treated and 19 placebo-treated patients), the S100B values obtained 3 days after stroke onset did not differentiate between the 2 treatment groups, suggesting no positive effect of rhEPO at that time point. This is not in conflict with our results, because we compared patient groups with an independent vs a dependent or dead status 6 months after stroke (Table 2). In their rhEPO-treated group, Ehrenreich et al reported only 3 additional patients who were independent 30 days after stroke according to the mRS score, which is not significant. Furthermore, more than 10% of all patients in their trial experienced a lacunar stroke, in which S100B levels do not increase significantly. On the other hand, it is conceivable that neuroprotective strategies may have positive effects on the S100B kinetic at a later stage compared with recanalizing interventions because of inhibition of secondary tissue damage. Nevertheless, results of the rhEPO trial suggest that S100B may be a useful surrogate marker in neuroprotective proof-of-principle studies.

The present study harbors some shortcomings. Because of the small sample size, the defined S100B cut points and the corresponding accuracy measures may be uncertain and should be reconfirmed in a larger sample.

Table 4. Bivariate Correlation and Univariate Linear Regression Analysis Between the S100B Measures and Final Infarct Volume

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spearman ρ</th>
<th>P Value‡</th>
<th>Regression Coefficient (95% Confidence Interval)*</th>
<th>Constant (95% Confidence Interval)</th>
<th>Correlation Coefficient</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>S100B value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.47</td>
<td>.003</td>
<td>1.5 (0.5 to 2.5)</td>
<td>3.2 (2.2 to 4.2)</td>
<td>0.47</td>
<td>.004</td>
</tr>
<tr>
<td>24h</td>
<td>0.85</td>
<td>&lt;.001</td>
<td>1.0 (0.8 to 1.3)</td>
<td>2.3 (2.1 to 2.5)</td>
<td>0.84</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>48h</td>
<td>0.95</td>
<td>&lt;.001</td>
<td>1.0 (0.9 to 1.1)</td>
<td>2.0 (1.9 to 2.1)</td>
<td>0.95</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>72h</td>
<td>0.94</td>
<td>&lt;.001</td>
<td>1.0 (0.8 to 1.1)</td>
<td>2.0 (1.9 to 2.1)</td>
<td>0.93</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>96h</td>
<td>0.91</td>
<td>&lt;.001</td>
<td>0.9 (0.8 to 1.0)</td>
<td>2.0 (1.9 to 2.1)</td>
<td>0.93</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>120h</td>
<td>0.91</td>
<td>&lt;.001</td>
<td>0.9 (0.8 to 1.0)</td>
<td>2.1 (2.0 to 2.2)</td>
<td>0.93</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>144h</td>
<td>0.91</td>
<td>&lt;.001</td>
<td>1.0 (0.8 to 1.1)</td>
<td>2.2 (2.1 to 2.4)</td>
<td>0.92</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Area under the curve</td>
<td>0.96</td>
<td>&lt;.001</td>
<td>1.0 (0.9 to 1.1)</td>
<td>–0.1 (–0.3 to 0.1)</td>
<td>0.95</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>µg/L · h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak value</td>
<td>0.94</td>
<td>&lt;.001</td>
<td>1.0 (0.9 to 1.1)</td>
<td>1.9 (1.8 to 1.9)</td>
<td>0.95</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*For linear regression, values were log transformed.
†Slope of regression line.
‡P<.006 indicates significant findings.
help to establish S100B as an easily accessible, inexpensive, and valid surrogate marker in future stroke trials.

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REFERENCES


Figure. Scatterplots of different S100B measures in relation to final infarct volume. Individual values and regression lines with the 95% confidence intervals are displayed.

Furthermore, as suggested in the Figure, there may be a potentially nonlinear relationship between the various S100B measures and the final infarct volume. All statistical analyses used in the present study assume linearity of the relationship and are displayed.