Early Neurobehavioral Outcome After Stroke Is Related to Release of Neurobiochemical Markers of Brain Damage

Michael T. Wunderlich, MD; Anne D. Ebert, PhD; Torsten Kratz, MD; Michael Goertler, MD; Stefan Jost, MD; Manfred Herrmann, MD, PhD

Background and Purpose—The study aimed to investigate the predictive value of neurobiochemical markers of brain damage (protein S-100B and neuron-specific enolase [NSE]) with respect to early neurobehavioral outcome after stroke.

Methods—We investigated 58 patients with completed stroke who were admitted to the stroke unit of the Department of Neurology at Magdeburg University. Serial venous blood samples were taken after admission and during the first 4 days, and protein S-100B and NSE were analyzed by the use of immunoluminometric assays. In all patients, lesion topography and vascular supply were analyzed and volume of infarcted brain areas was calculated. The neurological status was evaluated by a standardized neurological examination and the National Institutes of Health Stroke Scale (NIHSS) on admission, at days 1 and 4 on the stroke unit, at day 10, and at discharge from the hospital. Comprehensive neuropsychological examinations were performed in all patients with first-ever stroke event and supratentorial brain infarctions. Functional outcome was measured with the Barthel score at discharge from the hospital.

Results—NSE and protein S-100B concentrations were significantly correlated with both volume of infarcted brain areas and NIHSS scores. Patients with an adverse neurological outcome had a significantly higher and significantly longer release of both markers. Neuropsychological impairment was associated with higher protein S-100B release, but this did not reach statistical significance.

Conclusions—Serum concentrations and kinetics of protein S-100B and NSE have a high predictive value for early neurobehavioral outcome after acute stroke. Protein S-100B concentrations at days 2 to 4 after acute stroke may provide valuable information for both neurological status and functional impairment at discharge from the acute care hospital.

Key Words: health status ■ nerve tissue ■ neuron-specific enolase ■ neuropsychology ■ outcome ■ protein S-100 ■ stroke

In recent years, neurobiochemical markers of brain damage gained particular attention in the identification of stroke patients with an adverse neurological outcome. Neuron-specific enolase (NSE) and protein S-100B are the markers of brain damage that have been studied most often in clinical and experimental settings. Protein S-100B forms part of a large and diverse family of Ca$^{2+}$-binding proteins predominantly found in astrocytes and Schwann cells, and NSE, a dimeric isoenzyme of the glycolytic enzyme enolase, is found in the cytoplasm of neurons and cells with neuroendocrine differentiation. Both protein S-100B and NSE are considered specific neurobiochemical markers of brain damage after brain infarctions in humans or in animals and after brain damage caused by traumatic brain injury or by cardiac surgery. During the last few years, several studies have investigated release and kinetics of protein S-100B and/or NSE after acute stroke and their association with lesion volume, clinical status, and outcome. Because of small sample sizes and different approaches to the analysis of neurobiochemical markers (concentrations in cerebrospinal fluid versus peripheral blood), the results are heterogeneous. Furthermore, the majority of studies focus on severe neurological deficits after stroke.

The purpose of the present study was to investigate the relation between release patterns and serum concentrations of protein S-100B and NSE and early neurobehavioral outcome after stroke in a well-characterized group of patients. Our objectives were (1) to analyze the correlation of neurobiochemical markers and volume of infarcted brain areas and (2) to evaluate the association of NSE and protein S-100B with early neurological, neuropsychological, and functional outcome after stroke.

Subjects and Methods

Subjects
From a consecutive series of 81 patients with acute stroke admitted to the stroke unit of the Department of Neurology between February and September 1998, 58 patients were included in the study. Criteria...
TABLE 1. Demographic, Clinical, and Neuroradiological Data

<table>
<thead>
<tr>
<th>Stroke subtypes, No. (%)</th>
<th>Supratentorial Infarctions</th>
<th>Infratentorial Infarctions</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (%)</td>
<td>43 (74)</td>
<td>15 (26)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>67 (± 14.4)</td>
<td>62 (± 11.1)</td>
</tr>
<tr>
<td>Sex, male/female, No. (%)</td>
<td>24 (56)/19 (44)</td>
<td>12 (80)/3 (20)</td>
</tr>
<tr>
<td>Time to admission, mean (SD), h</td>
<td>6 (± 5.4)</td>
<td>12 (± 7.0)</td>
</tr>
<tr>
<td>NIHSS score on admission, mean (SD)</td>
<td>9.9 (± 8.8)</td>
<td>3.3 (± 2.4)</td>
</tr>
<tr>
<td>NIHSS score day 1, mean (SD)</td>
<td>9.9 (± 9.0)</td>
<td>2.1 (± 1.9)</td>
</tr>
<tr>
<td>NIHSS score day 4, mean (SD)</td>
<td>6.6 (± 6.9)</td>
<td>1.7 (± 1.8)</td>
</tr>
<tr>
<td>NIHSS score day 10–12, mean (SD)</td>
<td>3.9 (± 6.6)</td>
<td>1.0 (± 1.2)</td>
</tr>
<tr>
<td>Barthel score on discharge, mean (SD)</td>
<td>68.5 (± 37.5)</td>
<td>92 (± 11.6)</td>
</tr>
<tr>
<td>Lesion location, left/right, No. (%)</td>
<td>23 (53)/20 (47)</td>
<td>12 (80)/3 (20)</td>
</tr>
<tr>
<td>LACI indicates lacunar infarcts; TACI, total anterior circulation infarcts; PACI, partial anterior circulation infarcts; POCI, posterior circulation infarcts.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LACI</td>
<td>9 (15.5)</td>
<td></td>
</tr>
<tr>
<td>TACI</td>
<td>12 (20.7)</td>
<td></td>
</tr>
<tr>
<td>PACI</td>
<td>15 (25.9)</td>
<td></td>
</tr>
<tr>
<td>POCI</td>
<td>15 (25.9)</td>
<td></td>
</tr>
<tr>
<td>Not classifiable</td>
<td>7 (12.1)</td>
<td></td>
</tr>
<tr>
<td>Lesion volume, mean (SD), mL (n = 58)</td>
<td>36.6 (± 90.2)</td>
<td>7.6 (± 15.3)</td>
</tr>
<tr>
<td>Etiology;† No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardioembolism</td>
<td>21 (36.2)</td>
<td></td>
</tr>
<tr>
<td>Small-artery occlusion</td>
<td>10 (17.2)</td>
<td></td>
</tr>
<tr>
<td>Large-artery atherosclerosis</td>
<td>10 (17.2)</td>
<td></td>
</tr>
<tr>
<td>Undetermined</td>
<td>17 (29.3)</td>
<td></td>
</tr>
</tbody>
</table>

for exclusion were hemorrhagic stroke or ischemic brain infarctions in the course of cerebral hemorrhage and concomitant major cardiac, renal, hepatic, and tumor diseases potentially interfering with standardized assessment of neurological or neuropsychological status. Table 1 shows demographic, clinical, and neuroradiological data as well as information on subtypes of cerebral infarctions and etiology.23 Forty-three patients suffered from supratentorial infarctions, and 15 patients exhibited cerebellar or brain stem infarctions. Thirty patients had a first single and unilateral supratentorial stroke. The mean time between the onset of stroke and admission to the stroke unit was 6.9 hours (± 13.7).

Methods

Neuropsychological Examinations
Serial venous blood samples were collected at admission (t1: mean time after stroke onset, 10 ± 9.6 hours; n = 52), and at days 1 (t2: 22 ± 9.4 hours; n = 56), 2 (t2: 46 ± 9.6 hours; n = 55), 3 (t3: 70 ± 9.9 hours; n = 49), and 4 (t4: 94 ± 10.2 hours; n = 47) after admission to the stroke unit. Blood was allowed to clot, and after centrifugation within 30 minutes (1000g, 10 minutes), serum was stored at −80°C for later analysis. Serum protein S-100B and NSE were analyzed by the use of immunoluminometric assays and a fully automated LIA-mat system (Byk-Sangtec Diagnostica). Sangtec 100 measures the β-subunit of protein S-100 as defined by 3 monoclonal antibodies. The detection limit of the kit is 0.02 µg/L, and the range of protein S-100B serum concentrations of healthy subjects is reported to be <0.12 µg/L. NSE measurement is based on monoclonal antibodies that bind to the γ-subunit of the enzyme, and the minimal measurable concentration is <1.0 µg/L.

Neuroradiological Examinations
All neuroradiological examinations were based on cranial CT scans. All scans were performed in standardized slices without contrast enhancement soon after admission (mean, 6.9 ± 7.6 hours after infarction) and were repeated within the first week (mean, 76.6 ± 60.0 hours after infarction). We analyzed the cranial CT data of all subjects using the public domain NIH Image program (developed at the US National Institutes of Health and available online at the Internet at http://rsb.info.nih.gov/nih-image/). Lesions were evaluated with respect to lesion topography (on the basis of Damasio and Damasio24 and Matsui and Hirano25) and territories of vascular supply (according to Damasio and Damasio,24 including the territories of the deep perforators of the carotid system of Ghika et al26). Neuroradiological evaluations were performed independently by 2 members of our group (M.T.W. and T.K.); 1 of them (T.K.) was blind to all other data. The mean difference of the calculation of lesion volume between evaluators was 1.5 mL (SD ± 9.4 mL), and 95% of all differences of measurements were within 1 SD. Inter-rater correlation was calculated r = 0.96 (P < 0.001).

Neurological and Neuropsychological Assessments
All subjects underwent a standardized neurological examination on admission, at days 1 and 4 on the stroke unit, at days 10 to 12 on the ward, and at discharge from the hospital. The neurological deficit was quantified by the use of the National Institutes of Health Stroke Scale (NIHSS).27 We performed comprehensive neuropsychological examinations in all patients with a first-ever supratentorial stroke event and without any clouding of consciousness or severe disorders of attention (n = 23). The neuropsychological examination started with a bedside screening (speech and language, calculation, visuo-perceptual and visuoconstructional performance, apraxia, memory, executive functions, and attentional performance) on day 3 after admission. In case of neuropsychological impairments, a comprehensive and standardized neuropsychological evaluation was performed 1 week after admission. In all patients, the functional outcome at discharge was rated with the Barthel score.

Statistical data evaluation was performed with nonparametric tests for independent and related samples (Mann-Whitney U, Wilcoxon, and Friedman tests). The threshold for significance was set at P ≤ 0.05. In cases of multiple comparisons, we applied an α-adjustment (Bonferroni correction) to compensate for type I errors, and only P values < 0.01 were considered significant.

Results

Neuroradiological and Neurobiochemical Data
On 55 cranial CT scans, ischemic infarction could be identified corresponding to the acute stroke symptoms; 3 scans showed no clearly demarcated brain infarctions. The lesions in supratentorial stroke mostly involved temporal and parietal brain areas and were mainly supplied by medial and posterior branches of the middle cerebral artery and the lenticulostriate arteries. Seventeen patients showed lesions in the territory of the posterior circulation system. Mean absolute lesion volume was calculated to be 36.6 mL (± 90.2) in supratentorial and 7.6 mL (± 15.3) in infratentorial infarctions.

Figure 1 shows the release patterns of protein S-100B and NSE. Maximal protein S-100B concentrations were found on
day 2 on the stroke unit (between 55 and 66 hours after stroke onset). NSE concentrations reached maximal values on the day of admission (between 7 and 18 hours after stroke onset). After a decrease from admission to the end of day 1 on the stroke unit (Wilcoxon signed rank test: $z = -1.903$, $P = 0.057$), we found a continuous secondary increase. Volume of lesion and serum concentrations of both neurobiochemical markers were highly and significantly correlated (protein S-100B: $r^2 = 0.09$, $P = 0.002$; $t_1$: $r^2 = 0.29$, $P < 0.001$; $t_2$: $r^2 = 0.76$, $P < 0.001$; $t_3$: $r^2 = 0.81$, $P < 0.001$; $t_4$: $r^2 = 0.78$, $P < 0.001$; NSE: $t_1$: $r^2 = 0.15$, $P = 0.005$; $t_2$: $r^2 = 0.10$, $P = 0.021$; $t_3$: $r^2 = 0.69$, $P < 0.001$; $t_4$: $r^2 = 0.57$, $P < 0.001$; $t_5$: $r^2 = 0.63$, $P < 0.001$). Both absolute concentrations and temporal patterns of protein S-100B and NSE did not differ significantly with respect to location or vascular supply of the infarcted brain area. Cardioembolism as cause of the brain infarction resulted in significantly higher protein S-100B release compared with small-artery occlusion or large-artery atherosclerosis.

### Neurological, Neuropsychological, and Functional Outcome

NIHSS scores showed a continuous and significant improvement between admission and discharge (Friedman test: $\chi^2 = 63.1$, $df = 5$, $P < 0.001$). Detailed neuropsychological assessments 1 week after stroke (mean, 7.6 ± 2.1 days) could be performed in 23 patients with first-ever supratentorial infarctions. Patients with and without neuropsychological assessments did not differ significantly with respect to stroke scale scores, volume of lesion, or serum concentrations of neurobiochemical markers. Fifteen patients (65%) exhibited severe impairments most marked in attentional performance, executive functions, and memory performance (for details, see Table 2).

Patients with cerebellar or brain stem lesions showed a better functional outcome at discharge from the hospital (Barthel score, 92 [±11.6] versus 68.5 [±37.5] in patients with supratentorial infarctions; $P = 0.08$, Mann-Whitney $U$ test).

### Table 2. Neuropsychological Deficits in Patients With First Unilateral Supratentorial Infarction

<table>
<thead>
<tr>
<th>Domain of deficits</th>
<th>All</th>
<th>LH Lesions</th>
<th>RH Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphasia</td>
<td>15</td>
<td>6 (40)</td>
<td>5 (56)</td>
</tr>
<tr>
<td>Apraxia</td>
<td>3 (20)</td>
<td>2 (22)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Visuo-perceptive impairment</td>
<td>5 (33)</td>
<td>1 (11)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>Visuo-constructional impairment</td>
<td>3 (20)</td>
<td>2 (22)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Memory impairment</td>
<td>10 (67)</td>
<td>6 (67)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>Deficits in executive functions</td>
<td>12 (80)</td>
<td>7 (78)</td>
<td>5 (83)</td>
</tr>
<tr>
<td>Attention deficits</td>
<td>10 (67)</td>
<td>6 (67)</td>
<td>4 (67)</td>
</tr>
</tbody>
</table>

LH indicates left hemisphere; RH, right hemisphere.
Interactions Between Release of Neurobiochemical Markers and Neurological, Neuropsychological, and Functional Outcome

We found high and significant correlations between both neurobiochemical markers and NIHSS scores. Protein S-100B serum concentration began to be significantly associated with neurological status 2 days after the stroke event, and the highest values were found between NIHSS scores at discharge and protein S-100B values at day 4 on the stroke unit ($r^2=0.67$, $P<0.001$). Figure 2 shows determination coefficients between both protein S-100B and NSE release and NIHSS scores at discharge. The graph demonstrates that patients with an adverse neurological outcome had a significantly higher and longer release of both markers. The association between release of neurobiochemical markers and neurological status followed the same pattern in patients with supratentorial and infratentorial lesions.

Functional outcome (assessed by Barthel score) at discharge from the hospital was significantly correlated with serum concentrations of both neurobiochemical markers 2 to 4 days after admission. Again, the highest correlations were found between late protein S-100B and NSE values and Barthel scores (protein S-100B at day 4: $r^2=0.31$, $P<0.001$; NSE at day 4: $r^2=0.32$, $P<0.001$).

Patients with neuropsychological impairment showed numerically higher protein S-100B concentrations (Figure 3). However, no significant differences between groups could be calculated. NSE values were comparable in both groups.

Multivariate analysis based on a stepwise linear regression model with lesion volume, protein S-100B, and NSE concentrations as independent variables and NIHSS score at discharge as dependent variable showed protein S-100B release as the only variable with a significant predictive value. Protein S-100B serum concentration at day 4 after admission ($R^2=0.67$, $\beta=0.82$, $P<0.001$) and protein S-100B at day 1 ($R^2$ change $=0.09$, $\beta=-0.39$, $P=0.001$) explained 75% of the neurological outcome at discharge.

Discussion

Peak levels of protein S-100B serum concentrations were found at the second day after stroke, whereas a first NSE peak was recorded after admission, followed by a second increase from days 2 to 4. The release patterns of protein S-100B confirm the results of previous clinical studies that had examined sequential S-100B levels after stroke.6,9,18,28 Experimental data gained from middle cerebral artery occlusion in a rat model showed a significant increase of NSE starting 2 hours after focal ischemia.4 The different temporal patterns of NSE and protein S-100B release may be attributed to different pathophysiological mechanisms after focal ischemia. NSE serum levels correspond to the ischemia-induced cytoplasmic loss of NSE in neurons and are detectable before irreversible neuronal damage takes place.11 The first NSE peak within 7 to 18 hours after stroke onset may reflect the initial damage of neuronal tissue, whereas a second increase may be attributed to secondary mechanisms of neuronal damage due to edema and increase of intracranial pressure. In accordance with this assumption, we found a continuous or secondary increase of NSE serum concentrations in 5 of 7 patients with a deterioration of clinical status. The delayed peak level of serum protein S-100B concentration may reflect later responses in the pathophysiological cascade and microglial reaction to ischemia. Both necrotic cell damage in the penumbral zone of focal infarctions and a breakdown of membrane integrity due to cytotoxic and vasogenic edema may provide leakage of protein S-100B from cytosol to the extracellular space.5,21 We found numerically high and significant correlations between both NSE and protein S-100B serum concentrations and the volume of infarcted brain areas. This result corroborates the data of previous clinical or
Biochemical Markers Predict Neurobehavioral Outcome After Stroke

experimental studies on focal ischemia and indicates that both neurobiochemical markers mirror the extent of substantial brain damage.

Our data show a significant association between NSE and protein S-100B concentrations and both neurological and functional status at discharge from the hospital. This result could be interpreted as an epiphenomenon of the association between volume of lesion and degree of neurological impairment. However, if the correlation between neurobiochemical parameters and neurological outcome was controlled for the volume of lesion, the partial correlation coefficients dropped but remained significant (protein S-100B at day 4: \( r^2 = 0.24, P = 0.001 \)). The majority of previous studies failed to demonstrate a significant correlation between NSE release and outcome after stroke.\(^{18 - 20}\) Büttner and coworkers\(^{21}\) reported a significant correlation between protein S-100B and neurological status at admission. They failed, however, to calculate significant correlations between protein S-100B values and the functional outcome 4 weeks after stroke onset. Significant correlations between initial serum and/or cerebrospinal fluid concentrations of protein S-100B and clinical and/or functional outcome were reported by Fassbender et al.\(^{21}\) Mislant et al.\(^{18}\) and Abraha et al.\(^{5}\) The comparison of different studies that use protein S-100B as a potential predictor of stroke outcome is hampered by 2 major obstacles. First, the authors used different techniques as well as commercially available or self-developed kits, the sensitivity and specificity of which are not comparable. Second, a variety of different scores and scales were applied for the assessment of neurological status or functional outcome after stroke. Some of the measurements (eg, Glasgow Coma Scale or Glasgow Outcome Scale) are hardly appropriate to provide detailed information on neurological or functional outcome after stroke. In the present study we used a newly developed immunoluminometric assay with a lower detection threshold. Furthermore, we applied more detailed assessments of the neurological, neuropsychological, and functional outcome. Both measurements of serum markers and neurobehavioral assessments provide a wider range of data, which may be responsible for the numerically higher and more significant correlations we found between neurobiochemical and neurobehavioral data.

As far as we know, the present study was the first to investigate the release of neurobiochemical markers of brain damage and neuropsychological disorders after stroke. Patients with neuropsychological deficits exhibited numerically higher protein S-100B serum concentrations than patients without neuropsychological impairment. The difference did not reach statistical significance, but we must consider that patients who were not able to perform a standardized neuropsychological assessment were excluded from data analysis. These patients had significantly higher protein S-100B values than patients without neuropsychological deficits and probably will display deficits when neuropsychological assessment can be performed.

Protein S-100B and NSE were both associated with neurological and functional outcome at the time of discharge from the hospital. However, a multivariate comparison of release patterns of both markers based on a linear regression analysis demonstrated that only protein S-100B provides significant information on neurological outcome. The same was true with respect to the comparison of neuropsychologically impaired versus unimpaired patients. Protein S-100B therefore seems to have a higher predictive value than NSE.

The present studies on neurobiochemical markers in stroke patients concentrate on the relation between release patterns and neurobehavioral disorders. Whether the kinetics of protein S-100B release after brain damage may allow insight into brain repair mechanism or plasticity requires further research. To investigate these questions and to analyze the potential value of neurobiochemical markers with respect to subtle long-term neuropsychological deficits, studies with a neuropsychological follow-up examination are needed.

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**References**


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