Chapter 2

Comparison of serum S-100 protein levels following stroke and head injury

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Abstract

**Objective:** Temporal changes in serum S-100 protein levels were compared between patients with ischemic stroke, transient ischemic attack (TIA) and head injury. In addition, S-100 levels were correlated with clinical severity and outcome.

**Methods:** Measurements were done with a LIA-mat® Sangtec® 100 using an automated immunoluminometric assay. Serum S-100 was measured in 21 stroke patients, 18 TIA patients and 10 head injury patients on days 1 (0-24 hours), 2, 3, 4, 5 or 6 and 8 or 9. In a control group of 28 healthy volunteers 1 measurement was done. For the stroke and TIA patients, National Institutes of Health Stroke Scale (NIHSS) scores were obtained on admission and on day 10. For the head injury patients, Glasgow Coma Scale (GCS) scores were obtained on admission and Glasgow Outcome Scale (GOS) scores were obtained after 6 months.

**Results:** Changes in serum S-100 levels over the first 3 days were significantly different between stroke and head injury patients (p=0.014) and between stroke and TIA patients (p=0.006). Peak concentrations of S-100 were most often observed on day 3 or 4 after stroke and on day 1 or 2 after head injury. In stroke patients, individual S-100 peak levels correlated well with the NIHSS score on admission (r=0.58 p=0.014) and the change in NIHSS score between day 10 and day 1 (r=0.65 p=0.005). In head injury patients, a good correlation between individual peak levels of S-100 and the GCS score on admission (r=-0.81 p=0.010) and the GOS score 6 months after the trauma was found (r=-0.87 p=0.004).

**Conclusions:** We conclude that there is a significant difference in temporal changes of S-100 levels between ischemic stroke and head injury patients. This suggests different pathophysiological mechanisms. The results of this study further confirm that peak levels of serum S-100 correlate with neurological deficit resulting from either stroke or head injury.
Introduction

S-100 is a calcium binding protein, which is mainly present in glial cells and Schwann cells, but also in other tissues such as melanocytes, adipocytes, chondrocytes and epidermal Langerhans cells. S-100 is also present in certain tumors such as schwannoma, glioma, melanoma and neuroblastoma. The presence of the S-100 beta isomer is restricted to glial cells and Schwann cells. S-100 has recently been described as a marker for disease severity in ischemic stroke patients and head injury patients. Increased concentrations have been found in cerebrospinal fluid and serum of both stroke and head injury patients. Several studies have shown a correlation between clinical and radiological data and S-100 levels. The main objective of this study was to compare the temporal profile of changes in S-100 levels between stroke, transient ischemic attack (TIA) and head injury patients. In addition, we correlated these changes with outcome.

Methods

Patients and Subjects
Serum was collected from 21 ischemic stroke patients (13 female; mean age ± standard deviation (S.D.); 66 ± 10 years), 18 TIA patients (8 female; mean age ± S.D.; 69 ± 15 years) and 10 head injury patients (4 female; mean age ± S.D.; 43 ± 21 years) admitted to the department of Neurology, University Hospital of Groningen. Stroke and TIA patients who suffered a previous stroke, head injury or CNS infection within the last 3 months were excluded. Head injury patients with epidural haematoma requiring surgery, previous head injury or CNS infection within the last 3 months were excluded. Patients with small epidural haematomas, subdural haematomas or subarachnoid hemorrhages as a result of the trauma were not excluded.

The control group consisted of 28 healthy blood donors (12 females, mean age ± S.D.; 44 ± 9 years) from the Blood Bank Noord Nederland. These samples were used to calculate a reference value for serum S-100 levels. Written informed consent was obtained from all patients and blood donors. This study was approved by the local Research Ethics Committee.

Measurement of disease severity and outcome
For the stroke patients, National Institutes of Health Stroke Scale (NIHSS) scores were obtained on admission and on day 10. Outcome was determined by subtracting the score on day 10 from the initial score. Patients were considered to have major neurological improvement when the difference between these scores was ≥ 4 points, no major change when the difference ranged from 3 to -3 and major neurological deterioration when the difference was ≤ -4 points. For the head injury patients, Glasgow Coma Scale (GCS) scores were
obtained on admission. The outcome was measured with the Glasgow Outcome Scale (GOS) score after 6 months (i.e. 1=deceased, 5=good recovery) 22, 23.

**Sample collection and measurement**

Samples were taken on days 1 (0-24 hours), 2, 3, 4, 5 or 6 and 8 or 9. In the control group 1 measurement per subject was done. Within 1 hour of collection, all samples were centrifuged and stored at -20°C until analysis. Measurements were done with a LIA-mat® Sangtec® 100 using an automated immunoluminometric assay. This measures S-100 beta only, and not other isomers of S-100.

**Statistical analysis**

To analyze the data, the direction coefficient of the regression line fitted through the data points of the first 3 days and the individual peak levels were used as summary measures 24. One-way analysis of variance (one-way ANOVA) was performed to compare the means of these summary measures. Correction for multiple comparisons was performed using the Dunnett T3 method for unequal variances 25. To investigate the relation between disease severity and both summary measures we used Spearman-rank correlation.

**Results**

**Stroke and TIA patients**

All stroke and TIA patients suffered from supratentorial focal cerebral ischemia. Of these patients 4 died on day 4 due to transtentorial herniation caused by edema and 2 died between day 11 and day 15 because of sepsis and respiratory insufficiency. Data on disease severity and outcome are shown in table 1.

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Stroke: n=21</th>
<th>TIA: n=18</th>
<th>Head Injury: n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease severity on admission</td>
<td>NIHSS range: 6-27 Mean±S.D.:14,1 ± 6,0</td>
<td>NIHSS range: 1-7 Mean±S.D.:3,3 ± 2,2</td>
<td>GCS range: 3-13 Mean±S.D.:10,3 ± 4,3</td>
</tr>
<tr>
<td>Outcome</td>
<td>improve: 6</td>
<td>improve: 18</td>
<td>GOS 1: 0</td>
</tr>
<tr>
<td></td>
<td>no change: 10</td>
<td></td>
<td>GOS 2: 0</td>
</tr>
<tr>
<td></td>
<td>deteriorate: 5</td>
<td></td>
<td>GOS 3: 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GOS 4: 2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>GOS 5: 4</td>
</tr>
</tbody>
</table>

Table 1. Data on disease severity and outcome

**Head injury patients**

CT-scans of the brain were classified according to Marshall et al. 26. Diffuse cerebral edema not compressing the cisterns and with little or no focal injury was present in 6 patients (diffuse injury II). A combination of both focal and diffuse injury was found in 4 patients
(diffuse injury III). These four patients had small epidural, subdural, subarachnoid or intraparenchymal hemorrhages. Data on disease severity and outcome are shown in table 1.

**Serum S-100**

Because of 2 outliers, the control group serum S-100 levels followed a non-normal distribution (Shapiro-Wilk test for normality in small samples, p=0.010). The mean value was 0.12 (median: 0.11 S.D.: 0.08 range: 0.01 to 0.34). Values exceeding 0.30 were considered abnormal (mean + 2 S.D.). In 16 TIA patients, 18 stroke patients and 8 head injury patients the data over the first 3 days were complete. These patients were used for analysis of the temporal profile. One-way ANOVA testing revealed a significant difference between the means of the direction coefficients (F=8.49 p=0.001). Post hoc testing showed significant differences between TIA and stroke patients (p=0.006), stroke and head injury patients (p=0.014) but not between TIA and head injury patients (p=0.917). Seventeen stroke patients, 16 TIA patients and 10 head injury patients were used in analysis of individual peak S-100 levels. Patients with missing values, but with clearly identifiable peak levels; i.e. those with one or more non-peak levels both before and after the missing value, were included in the analysis. The four patients who died on day 4 were therefore not included in the peak level analysis. One-way ANOVA testing showed significantly different mean peak levels (F=5.753 p=0.006). Post hoc testing indicated significant differences between TIA and stroke patients (p=0.020) and between TIA and head injury patients (marginal, p=0.055) but not between stroke and head injury patients (p=0.707).

In the TIA group S-100 values ranged from 0.01 to 0.73 ng/ml. Five out of 18 TIA patients had abnormal serum S-100 values on one or more time points. Out of 16 patients with identifiable peak levels, 7 patients had peak levels on day 1, 4 on day 2, 3 on day 3, 1 on day 4 and 1 on day 5. Most patients showed a curve resembling a straight horizontal line or a slightly decreasing line (figure 1).

![Figure 1: Serum S-100 (mean ± S.E.M.) in TIA, Stroke and Head Injury(HI) patients.](image)
In the stroke group S-100 levels ranged from 0.08 to 6.73 ng/ml. Nineteen out of 21 stroke patients had abnormal serum S-100 levels on one or more time points. Out of 17 patients with identifiable peak levels, 1 patient had a peak level on day 1, 2 on day 2, 8 on day 3, 3 on day 4 and 3 on days 5 or 6. Most patients showed a peaked curve with a maximum on day 3 or 4 (see figure 1 for summary). Individual S-100 peak levels correlated well with the NIHSS score on admission ($r=0.58$, $p=0.014$, not shown) and the change in NIHSS score between day 10 and admission when categorized in three outcome groups ($r=0.65$, $p=0.005$) (figure 2a). Individual direction coefficients also correlated significantly with outcome ($r=0.61$, $p=0.007$) (figure 2b).

Figure 2. (a) Correlation between outcome after 10 days and peak serum S-100 levels in stroke patients ($n=17$). (b) Correlation between outcome after 10 days and the direction coefficient of the regression line fitted through the data points of the first 3 days in stroke patients ($n=18$).
In the head injury group, S-100 values ranged from 0.1 to 3.48 ng/ml. Nine out of 10 patients had abnormal serum S-100 levels on one or more time points. All patients had identifiable peak levels. Eight patients had peak levels on day 1, one on day 2 and 1 on day 3 (n=10). Serum S-100 followed a gradual decline with a peak level on day 1 in most patients (see figure 1 for summary). A good correlation between the individual peak levels of S-100 and the GCS total score on admission (r=-0.81 p=0.010, not shown) and the GOS score 6 months after the trauma (r=-0.87 p=0.004) was found (figure 3a). Individual direction coefficients did not correlate significantly with outcome (r=-0.16 p=0.70)(figure 3b).

Figure 3. (a) Correlation between peak S-100 levels and outcome in head injury patients (n=10). (b) Correlation between outcome after 6 months and the direction coefficient of the regression line fitted through the data points of the first 3 days in head injury patients (n=8).

Discussion

This study was primarily conducted to compare the temporal profile of serum S-100 levels between stroke patients, TIA patients and head injury patients. Furthermore, we correlated S-
100 levels with clinical severity and outcome. Almost all stroke patients showed an increase in serum S-100 over the first 3 days with peak levels on day 3 or 4. In contrast, most head injury patients showed peak levels on day 1 or 2 with a gradual decrease over the first 3 days. TIA patients showed little variation in S-100 levels over time, but sometimes a slight decrease over the first few days was observed. We demonstrated that these changes in S-100 levels were significantly different between stroke and head injury patients. How can this difference be explained?

S-100 beta is present in glial cells throughout the brain but not in neurons. It is secreted into the extracellular space. In vitro studies have shown that it can be released from astroglial cells in a number of ways: activation of A(1) adenosine or mGlu3 metabotropic glutamate receptors, by stimulation of astroglial 5-HT1A receptors and by adrenocorticotropic hormone (ACTH) and corticotrophin-like intermediate-lobe peptide. S-100 beta is also secreted from proliferating astrocytes. Secretion of S-100 by extracellular adenosine occurs very rapidly, within 1 hour. Extracellular adenosine levels are elevated early after head injury and stroke because of rapid intracellular ATP depletion. S-100 release by 5-HT1A receptor stimulation and ACTH-like substances is probably somewhat slower. Reactive astrocytes appear early after experimental trauma and stroke and peak intensities of immunohistochemical staining are usually observed after approximately 3 or 4 days in both conditions. In humans, the peri-infarct region is intensely stained with antibodies against MRP-8 and -14 (which belong to the S-100 protein family) within the first 3 days. Labeled cells were found selectively in the peri-infarctional area. This is consistent with studies in animals. Increased adenosine and glutamate levels in the extracellular space can cause immediate secretion of S-100. S-100 reaches the bloodstream easily because of impaired blood-brain barrier function. Late release is probably due to release from reactive astrocytes. Thus, all four mechanisms of excretion may be present during stroke and during head injury. In stroke however, high levels of adenosine occur in a region that is not perfused with blood, i.e. the core of the infarct. S-100 accumulated in this region cannot be released into the bloodstream. Spontaneous reperfusion could result in an early rise of serum S-100 but usually this does not occur in the first few days. It is thus likely that the core of the infarct does not contribute to any observed rise in S-100 serum levels. The pattern of reactive astrogliosis observed in animal studies and in human studies resembles the serum S-100 temporal profile in stroke patients, and offers a likely explanation. In head injury patients the acute rise in serum S-100 is due to massive adenosine and glutamate induced S-100 release in heavily damaged but perfused brain areas. S-100 diffuses into the bloodstream and this accounts for raised serum levels shortly after the trauma. Direct mechanical injury to astrocytes could also contribute to this. Increased values on day 3 and later reflect reactive astrogliosis as is the case for stroke patients, although the intensity might be slightly less when compared to stroke. If there is early reperfusion, the core of the infarct releases the S-100 produced by adenosine and glutamate, leading to early peak levels comparable with head injury. This is a possible explanation for early peak levels in some stroke and TIA patients. If our theory is correct,
then S-100 could be used as a serum marker for successful thrombolysis after intravenous thrombolytic drugs. Late reperfusion will probably not contribute to any rise in serum S-100 because of hemostasis and distal clot formation in microvessels in combination with proteolysis of S-100.

Although the number of patients in this study is rather small, it confirms that peak serum S-100 levels are correlated with neurological outcome in stroke patients and head injury patients. The rate of change of S-100 levels during the first 3 days correlates with outcome in stroke patients, but not in head injury patients. However, head injury patients with bad outcome seem to have larger changes (either positive or negative) compared to those with better outcome (see figure 3b). In this study, patients with the highest levels did not always have the worst outcome (see figure 2a) and low peak levels were not uniformly correlated with a good outcome. Patients with peak levels lower than 1.0 ng/ml did all have a moderate to good outcome, for both head injury and stroke patients. Larger studies are needed to further determine the usefulness of S-100 and to verify the hypothesis described above.

In conclusion, we postulate that raised serum S-100 levels reflect peri-infarctional reactive gliosis in stroke. In head injury, adenosine induced S-100 release, serotonin and ACTH induced release, direct mechanical trauma to astrocytes, and reactive gliosis may all contribute to S-100 release. In our opinion, there is a role for S-100 as a co-predictor of outcome in stroke patients and head injury patients.

References

1. Isobe T, Takahashi K, Okuyama T. S-100a0 (alpha alpha) protein is present in neurons of the central and peripheral nervous system. *J Neurochem* 1984;43:1494-6.


