Serum S-100 and Neuron-Specific Enolase for Prediction of Regaining Consciousness After Global Cerebral Ischemia

Patrick Martens, MD; Andreas Raabe, MD; Per Johnsson, MD, PhD

Background and Purpose—The aim of our study was to assess the use of S-100 protein (S-100) and neuron-specific enolase (NSE) in serum and cerebrospinal fluid (CSF) for the prediction of patients’ regaining consciousness after acute global cerebral ischemia.

Methods—Sixty-four unconscious patients were followed until the return of consciousness or until death/vegetative state. Serum and CSF samples for measurement of S-100 and NSE using an immunoradiometric assay technique were obtained 24 hours (serum) and 48 hours (CSF) after the acute event and correlated with patient outcome.

Results—Values for serum S-100 protein, serum NSE, CSF S-100, and CSF NSE were significantly different in the 2 outcome groups. A serum S-100 value of >0.7 µg/L was found to be a predictor of not regaining consciousness, with a high positive predictive value (95%) and high specificity (96%).

Conclusions—S-100 protein used as serum marker 24 hours after acute global cerebral ischemia gives reliable and independent information on the outcome of the patient that is comparable or superior to that obtained with CSF markers. Therefore, S-100 may be a serum marker of brain cell damage useful for clinical assessment of these patients. (Stroke. 1998;29:2363-2366.)

Key Words: biological markers cerebral ischemia, global heart arrest nerve tissue protein S-100 neuron-specific enolase prognosis

Almost 80% of the patients with restored circulation after resuscitation remain unconscious for variable lengths of time. Approximately 20% of patients in a coma after hypoxic-ischemic injury will enter a vegetative state as the hypoxic ischemia results in bihemispheric damage, with relative preservation of the brain stem functions. It is estimated that the cost for the care of severely brain-damaged survivors runs in the billions of dollars each year. On the other hand, recent advances in pharmacological neuroprotective treatment emphasize the importance of an improved technique for objective assessment of this patient category. Clinicians therefore continue to search for accurate methods, such as biochemical markers, to enable early prediction of the outcome of these patients.

S-100 protein is a dimeric acidic calcium-binding protein found intracellularly and extracellularly in the brain. It has a molecular weight of approximately 21 kDa. The two isomeric subunits, αβ and ββ, are present in high concentrations in glial (astrocytes) and Schwann cells. The protein is eliminated or metabolized by the kidney, and the biological T1/2 of approximately 24 hours.

Neuron-specific enolase (NSE) is the neuronal form of the intracytoplasmic glycolytic enzyme enolase, which was first found in extracts of brain tissue (neuronal cell bodies and axons) and later in neuroendocrine cells (APUD cells) and neuroendocrine tumors, including small-cell lung cancer. It is a dimeric γγ enzyme with a molecular weight of 78 kDa and a biochemical T1/2 of approximately 24 hours. These markers have different but complementary biokinetic properties.

The brain is exceptionally vulnerable to global ischemia of any duration. Cardiac arrest may therefore produce cerebral damage that can be detected by the release of the above-mentioned cellular enzymes into cerebrospinal fluid (CSF) and eventually to the blood in a proportion corresponding to the extent of cellular brain injury.

The aim of this prospective study was to investigate the relationship between the severity of global cerebral ischemia and the levels of S-100 and NSE found in serum 24 hours and in CSF 48 hours after the acute event. Our objectives were (1) to investigate whether these markers correlate with outcome and (2) to compare the diagnostic and prognostic power between serum and CSF measurements.

Subjects and Methods
Between September 1995 and December 1997, 64 patients were admitted to the intensive care unit of the St Jan Hospital in Brugge, Belgium, following acute global cerebral ischemia (cardiac arrest). All patients were consecutively included in the study only if they remained...
unconscious (Glasgow Coma Scale score of <9) and artificially ventilated for >24 hours. All patients received advanced life support according to American Heart Association guidelines and were neurologically examined daily. For the purpose of this study, outcome was dichotomized into 2 groups: group 1 consisted of patients who died or remained in vegetative state, and group 2 consisted of patients who regained consciousness (ie, obeyed simple verbal commands). Regaining consciousness was considered an end point for follow-up, regardless of outcome at discharge or at 6 months. Therefore, patients who eventually died of multiorgan failure but who had clearly regained consciousness after cardiac arrest were classified in group 2. Conversely, group 1 was composed of patients whose outcome was death/vegetative state due to cerebral failure.

Peripheral blood samples of S-100 and NSE were obtained from patients 24 hours after confirmed global cerebral ischemia. In 34 patients still unconscious 48 hours after the acute event, a lumbar puncture (LP) was performed for analysis of CSF S-100 and CSF NSE. In each outcome group there is 1 pair of missing data serum.

Analysis of S-100 and NSE
S-100 was analyzed using a monoclonal 2-site immunoradiometric assay (Sandtest 100; AB Sangtec Medical). The method is defined by the 3 monoclonal antibodies SMST 12, SMT 25, and SMSK 28. The monoclonal antibodies detect the S-100 isoforms αβ and ββ, which are specific for astroglial cells. The serum samples were diluted with phosphate buffer and incubated with a plastic bead coated with monoclonal S-100 antibodies. During incubation S-100 is bound to the antibody-coated bead. After 1 hour of incubation the beads were washed to remove any unbound material and incubated with 125I-labeled anti–S-100 antibody. After a 2-hour incubation and subsequent washing, the amount of radioactive label bound to immobilized S-100 was measured by a gamma counter. Although the detection limit of the kit (at the time of investigation) according to the company was 0.2 μg/L, values between 0 and 0.2 μg/L could be detected in our laboratory.

NSE was measured by a standardized monoclonal 2-site, single-incubation immunoradiometric (Sandwich) assay (Profilitigen NSE IRMA, AB Sangtec Medical). This method has a sensitivity or minimum measurable NSE value of <0.5 μg/L, γ-enzolase and a reference range of 0–12.5 μg/L in the serum of healthy individuals (95th percentile).

The peak concentration that can be measured without a dilution of the sample is 200 μg/L.

Statistical Analysis
For statistical analysis, values of S-100 given as <0.2 μg/L, which is the lower sensitivity limit of this test, were set to 0.19 μg/L. Because of the nonparametric distribution, all values were presented as median with their interquartile range. Group comparisons were performed with the Mann-Whitney U test. Spearman rank correlation coefficients were calculated for the enzyme levels in serum and CSF. Positive predictive value, negative predicting value, and specificity predicting poor outcome were obtained with use of predefined cutoff levels. Odds ratio indicating the relative risk were calculated with their 95% confidence intervals from χ2 analysis. A value of P<.05 was considered significant. For statistical analysis, the SPSS software package (SPSS Inc) was used.

Results
One patient was excluded from analysis, because he died of cardiac failure before his level of consciousness could be assessed. Twenty-eight of the remaining 63 patients (45%) regained consciousness after initial survival. Three of the 28 woke up with severe disability and eventually died. All 35 patients who could never obey simple commands died; therefore, 38 patients (60%) died after global cerebral ischemia. No patient in our study remained in a permanent vegetative state. Among the 28 who regained consciousness, only 7 patients underwent an LP.

Serum S-100, serum NSE, S-100 CSF and NSE CSF were significantly higher in patients who never regained consciousness compared with those who regained consciousness (Table 1). Differences between both groups were highest for serum S-100 protein (0.78 μg/L versus 0.19 μg/L; P<.00029), followed by NSE-CSF (180 μg/L versus 15.9 μg/L; P<.00046), serum NSE (21.2 μg/L versus 15.2 μg/L; P<.001) and S-100 CSF (22.4 μg/L versus 2.6 μg/L; P<.0024) (Figure 1).

S-100 and NSE correlated well with each other in the CSF (r=0.74, P<.001). Both CSF concentrations also correlated with their serum concentrations (r=0.66, P<.001 for NSE; r=0.42, P<0.05 for S-100). On the other hand S-100 and NSE did not correlate at all with each other in serum (r=0.15, P=0.25).

The highest positive predictive values for predicting poor outcome were 0.95% for serum S-100 (cutoff value, 0.7 μg/L) and 0.96% for NSE CSF (cutoff value, 50 μg/L). Highest sensitivity values were 93% for S-100 CSF and 89% for NSE CSF (Table 2). Specificity was highest with serum S-100 (96%) and serum NSE (89%). Relative risk of death (given with 95% confidence intervals in parentheses) was increased 31-fold (4 to 250) for an increase in serum S-100 >0.7 μg/L, 8.6-fold (1.5 to 16) for an increase in S-100-CSF >6 μg/L (2 to 180), and 41.7-fold (3.6 to 500) for an increase in NSE-CSF >50 μg/L.

Patients with poor outcome after global cerebral ischemia were significantly younger than those who regained consciousness (72 years versus 58 years, P<.01).

Discussion
Early clinically unfavorable neurological findings after cardiac arrest do not exclude survival with an acceptable quality

<table>
<thead>
<tr>
<th>TABLE 1. Values for Serum S-100 and NSE and CSF S-100 and NSE in Patients Who Regained Consciousness and Those Who Did Not</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regained Consciousness</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Serum S-100 (μg/L)</td>
</tr>
<tr>
<td>0.9 (0.19–0.29)</td>
</tr>
<tr>
<td>0–5.34</td>
</tr>
<tr>
<td>[n=27]</td>
</tr>
<tr>
<td>Serum NSE (μg/L)</td>
</tr>
<tr>
<td>6.5–35.6</td>
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<tr>
<td>[n=27]</td>
</tr>
<tr>
<td>CSF S-100 (μg/L)</td>
</tr>
<tr>
<td>0.21–20</td>
</tr>
<tr>
<td>[n=7]</td>
</tr>
<tr>
<td>CSF NSE (μg/L)</td>
</tr>
<tr>
<td>1.9–50</td>
</tr>
<tr>
<td>[n=7]</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>1–84</td>
</tr>
<tr>
<td>[n=28]</td>
</tr>
</tbody>
</table>

Values are median (in micrometers per liter), with interquartile ranges in parentheses. n indicates number of measurements. Median values are significantly different for all parameters (see text).
of life. Moreover, it is not possible to perform a decisive clinical evaluation of patients under mechanical ventilation with sedation and muscle relaxants. In this study awakening is considered an end point because it separates those who are totally disabled from those with less-than-total disability. In contrast to stroke, trauma, and intracerebral hemorrhage, hypoxia-ischemia after cardiac arrest is global, with impairment primarily of areas in the brain and with a high demand for oxygen and energy.

New technical approaches to separate unfavorable from favorable prognosis in patients after global cerebral ischemia have been suggested. The invasive or cumbersome nature of some of the techniques that study, for instance, cerebral blood flow and metabolism, have precluded their routine application in humans. Temporary loss of EEG, auditory, and somatosensory evoked responses can be encountered in comatose patients who survive cardiac arrest, making repeated reevaluations necessary. Monitoring of cerebral oxygen extraction needs further investigation before it can be used in routine practice.

Brain cell damage and leakage of brain cytosolic enzymes after global cerebral ischemia result in increased levels of these enzymes in serum and CSF. In particular, the peak creatine phosphokinase (CPK) activity in the CSF has been used for the prediction of the severity of the insult and risk of permanent brain damage. The appearance of CPK (brain type) in blood after cardiac arrest indicates global ischemia but is not a reliable indicator for outcome, in part because of a rapid and individually variable inactivation in the body.

Roine et al and more recently Fogel et al have suggested that NSE is useful for assessment of brain damage measured both in serum and CSF. Roine found a sensitivity of 40% and a specificity of 98% for a serum NSE cutoff value of 17 μg/L. Based on serial sampling, Fogel proposes different cutoff values: 175 μg/L for CSF-NSE and 33 μg/L for serum NSE, with a sensitivity of 60% for the latter. However, the routine use of these assays with retrospectively determined cutoff values requires further validation.

In our study, we found cutoff values considerably lower than those reported by Fogel et al. The sensitivities of our cutoff values are intermediate to those mentioned by Roine et al and Fogel et al. One rationale for analyzing not only NSE but also S-100 is the different distribution of these markers within the gray and white matter. S-100 is present mainly in glial cells (astrocytes) and NSE mainly in neurons. Despite this difference in cellular origin and biological T1/2 between S-100 and NSE, a high correlation between the 2 markers could be found in CSF (r = 0.74, P < 0.001), a finding which is in accordance with the findings of Roine et al, as well as for S-100 (r = 0.42, P < 0.05). This might suggest a close interaction between microglial and neuronal cell damage and a similar pattern of diffusion of both markers between CSF and the capillaries.

Our results suggested that serum S-100 in particular was a valid and reliable predictor at 24 hours after the event of global cerebral ischemia using the proposed cutoff value of 0.7 μg/L.

Although the sampling times (24 hours after cardiac arrest for serum and 48 hours for CSF) were arbitrarily chosen, the delay in CSF sampling by LP as opposed to intraventricular sampling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cutoff Value</th>
<th>PPV, %</th>
<th>NPV, %</th>
<th>Sens, %</th>
<th>Spec, %</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>S-100 0.7 μg/L</td>
<td>95</td>
<td>63</td>
<td>55</td>
<td>96</td>
<td>31</td>
<td>4–250</td>
</tr>
<tr>
<td></td>
<td>NSE 20 μg/L</td>
<td>86</td>
<td>65</td>
<td>51</td>
<td>89</td>
<td>8.6</td>
<td>1.5–16</td>
</tr>
<tr>
<td>CSF</td>
<td>S-100 6 μg/L</td>
<td>93</td>
<td>60</td>
<td>93</td>
<td>60</td>
<td>18.8</td>
<td>2–180</td>
</tr>
<tr>
<td></td>
<td>NSE 50 μg/L</td>
<td>96</td>
<td>63</td>
<td>89</td>
<td>83</td>
<td>41.7</td>
<td>3.6–500</td>
</tr>
</tbody>
</table>

PPV indicates positive predictive value (predicting poor outcome); NPV, negative predictive value (predicting regaining consciousness); Sens, sensitivity; Spec, specificity; OR, odds ratio; and CI, confidence interval.

For clinical use it is important that serum markers demonstrate high specificity and high PPV for predicting poor outcome (serum S-100). CSF parameters are characterized by a high sensitivity and high PPV.
can be justified by taking into account the slow equilibration of concentration gradients within the CSF. Only 20% of the CSF circulates downward into the subarachnoid space of the spinal cord. In addition, periods of global ischemia may result in brain swelling, with impairment of the passage of CSF through the fourth ventricle. In earlier reports from this center it was shown that enzyme levels (CPK, GOT, LDH) from cisternal CSF correlated well with those from spinal CSF but reached their peak 12 hours earlier. Furthermore, early LP may be dangerous and contraindicated because of medically induced thrombolysis, eg, for treatment of acute myocardial ischemia or due to elevated intracranial pressure.

Moreover, intracranial hypotension can follow LP. However, protracted cerebral hypoperfusion in the presence of elevated oxygen consumption during the postresuscitation syndrome might justify a delayed CSF examination. Nevertheless, a routinely available, reliable, and early serum marker with low detection limits for irreversible brain damage would be an ideal substitute for CSF sampling. Particularly high serum values would preclude the need for an LP. One major practical drawback is the current limited availability and speed of the assays. This would, however, be alleviated by the development of bedside tests.

In contrast to the data of Fogel et al, we found in our study a significant difference between the median age of patients who died and those who regained consciousness. The unfavorable outcome among younger patients could be explained by the inclusion of children with poor neurological outcome despite return of spontaneous circulation. It also might show that restarting the heart in patients with irreversible brain damage is more frequently achieved in younger patients.

Finally, it is important to realize that by performing CSF sampling after 48 hours only in patients whose level of consciousness remains questionable, we end up with very few patients in the favorable outcome group who underwent an LP. Therefore, specificities and sensitivities of the serum cutoff values are not entirely comparable with those of the CSF cutoff values.

**Conclusion**

Serum S-100 may provide independent biochemical information about the extent of brain damage after acute global cerebral ischemia. The diagnostic and prognostic value of serum S-100 in our study was comparable or superior to the results of CSF NSE analysis of previous reports.

**Acknowledgment**

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**References**

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