Serum Neuron-Specific Enolase and S-100B Protein in Cardiac Arrest Patients Treated With Hypothermia

Marjaana Tiainen, MD; Risto O. Roine, MD, PhD; Ville Pettilä, MD, PhD; Olli Takkunen, MD, PhD

Background and Purpose—High serum levels of neuron-specific enolase (NSE) and S-100B protein are known to be associated with ischemic brain injury and poor outcome after cardiac arrest. Therapeutic hypothermia has been shown to improve neurological outcome after cardiac arrest. The aim of this study was to evaluate the effect of therapeutic hypothermia on levels of serum NSE and S-100B protein, their time course, and their prognostic value in predicting unfavorable outcome after out-of-hospital cardiac arrest.

Methods—Seventy patients resuscitated from ventricular fibrillation were randomly assigned to hypothermia of 33°C for 12 or 24 hours or to normothermia. Serum NSE and S-100B were sampled at 24, 36, and 48 hours after cardiac arrest. Neurological outcome was dichotomized into good or poor at 6 months after cardiac arrest.

Results—The levels of NSE (P=0.007 by analysis of variance for repeated measurements) but not S-100B were lower in hypothermia- than normothermia-treated patients. A decrease in NSE values between 24 and 48 hours was observed in 30 of 34 patients (88%) in the hypothermia group and in 16 of 32 patients (50%) in the normothermia group (P<0.001). The decrease in NSE values was associated with good outcome at 6 months after cardiac arrest (P=0.005), recovery of consciousness (P<0.001), and survival for at least 6 months after cardiac arrest (P=0.012).

Conclusions—Decreasing levels of serum NSE but not S-100B over time may indicate selective attenuation of delayed neuronal death by therapeutic hypothermia in victims of cardiac arrest. (Stroke. 2003;34:2881-2886.)

Key Words: heart arrest • hypothermia • nerve tissue protein S-100 • neuron-specific enolase

The outcome of successfully resuscitated patients is determined by the extent of hypoxic-ischemic cerebral injury. The duration and severity of the episode of global cerebral ischemia and secondary mechanisms of ischemia related to reperfusion contribute to the extent of brain damage. Recently, 2 independent randomized clinical trials have shown that lowering the body temperature to 33°C for 12 or 24 hours results in improved neurological outcome of comatose survivors of out-of-hospital cardiac arrest (CA).1,2 Induced hypothermia also increased the chances of survival.1

Neuron-specific enolase (NSE) is the neuronal form of intracytoplasmic glycolytic enzyme enolase. NSE has been shown to be located in neurons and neuroectodermal cells.3,4 It is a dimeric enzyme composed of 2 γ subunits, with a molecular weight of 78 kDa and biologic half-life of ∼24 hours. Neuronal damage and impairment of the blood-brain barrier integrity can be detected by the release of NSE into cerebrospinal fluid (CSF) and eventually into the blood. Increases in CSF and serum NSE levels have been reported after stroke, brain injury, and CA.5–7

The S-100B protein is an acidic Ca2+-binding protein with a molecular weight of ∼21 kDa and biologic half-life of 0.5 hours.8 This protein has 2 subtypes. The αβ form is found in astroglial cells, and the ββ form is found predominantly in astroglial cells and Schwann cells but has been demonstrated in some neoplasms and in melanocytes, adipocytes, and chondrocytes.9,10 Increased serum levels of protein S-100B have been reported after traumatic brain injury, stroke, CA, and cardiopulmonary surgery.11–14

Early prognosis of neurological outcome in patients resuscitated from CA is a major ethical, medical, and economic challenge. A false prediction of poor outcome can lead to early withdrawal of care and carries a risk of self-fulfilling prophecy. A falsely optimistic prediction may lead to unnecessary prolongation of intensive care therapy and might prevent admission of other patients who might benefit more. Several studies have found high serum NSE levels to be associated with poor outcome of CA patients.14–19 High serum S-100B levels on admission or at 12, 24, 48, or 72 hours after CA have been reported to correlate with an unfavorable neurological outcome.14,18,20–22

The effect of therapeutic hypothermia on the serum levels of these biochemical markers of hypoxic brain damage has not been studied. It is also not known whether the prognostic value of these markers is preserved in CA patients treated with hypothermia. The aim of this study was to evaluate the...
effect of therapeutic hypothermia on serum NSE and S-100B protein levels, their time course, and their prognostic value in predicting unfavorable outcome after out-of-hospital CA. Our hypothesis was that the neuroprotective effect of hypothermia would be accompanied by diminished release of these markers into the serum.

Subjects and Methods

The protocol and consent procedures of this study were approved by the ethics committee of Helsinki University Central Hospital in accordance with institutional guidelines. A deferred consent was used for all patients. The patient’s family was informed about the trial, and they had the possibility to withdraw the patient anytime from the study. Each patient was informed about the trial both orally and in writing when possible.

Patients were randomized into the Hypothermia After Cardiac Arrest (HACA) trial between March 1997 and June 2000. All adult patients admitted to the emergency department of the Helsinki University Central Hospital after resuscitation from out-of-hospital CA were screened for the trial. The criteria for inclusion were 18 to 75 years of age, a witnessed CA, ventricular fibrillation or nonperforated CA and before randomization, evidence of hypotension (mean arterial pressure < 60 mm Hg) for > 30 minutes after ROSC and before randomization, evidence of hypoxia (arterial O2 saturation < 85%) for > 15 minutes after ROSC and before randomization, a terminal illness, preexisting coagulopathy, pregnancy, unavailability for follow-up, and enrollment in another study. Patients who met the inclusion criteria were randomly assigned to hypothermia or normothermia.

All CA data were collected implementing the Utstein style.23 CA was defined as the absence of both palpable pulse and spontaneous respiration. ROSC was defined as return of palpable pulse. In the Helsinki area, basic and advanced cardiac life support was provided by the 3-tiered Helsinki Emergency Medical Services. Outside Helsinki, basic life support was provided by the staff of regional fire brigade–based Emergency Medical Services and advanced cardiac life support by the emergency medical helicopter. All units are equipped with semiautomated defibrillators and are trained to intubate, insert intravenous catheters, and start medication.

After assessment of respiratory and hemodynamic function and initial neurological evaluation in the emergency department, patients were transferred to the Intensive Care Unit. All patients received standard intensive care management and monitoring, including mechanical ventilation, arterial catheters, central venous catheters, a Foley catheter with a temperature sensor, and a pulmonary artery catheter as necessary.

We used midazolam 0.125 mg · kg⁻¹ · h⁻¹ for sedation, fentanyl 0.002 mg · kg⁻¹ · h⁻¹ IV for analgesia, and pancuronium 0.05 mg · kg⁻¹ · h⁻¹ for complete muscle relaxation for a total of 32 hours. Target mean arterial pressure was 80 mm Hg, and crystalloid fluids, hydroxyethyl starch, or inotropic agents were used as necessary. No intravenous glucose was administered. Intravenous insulin was infused if the plasma glucose level exceeded 10 mmol/L and targeted at normoglycemia. Elevated position (30°) of upper trunk and head was maintained.

Patients randomized to normothermia were allowed to rewarmed passively to normoglycemia (core temperature < 38°C) and then were kept normothermic. Those randomized to hypothermia treatment were actively cooled externally to a core temperature 33 ± 1°C with a cooling device (Therakool, Kinetic Concepts Inc United Kingdom). The cooling device consists of a mattress and cover that delivers cold air over the entire body. Ice packs were applied on the patient’s axilla and groin to enhance cooling. Hypothermia (33 ± 1°C) was maintained for 24 hours from start of cooling, and patients were then allowed to rewarmed over 8 hours. Life support was maintained for at least 3 days in all patients and at least 7 days in patients responding to pain in any manner.

Blood samples for NSE and S-100B were collected from the arterial catheter at 24, 36, 48, 72, and 96 hours after ROSC. Blood was allowed to clot for 20 to 30 minutes at room temperature and then centrifuged and frozen to <-18°C. Samples that showed visible hemolysis were not analyzed. NSE was quantified with a time-resolved immunofluorometric assay (DELFIA, Wallac). The detection limit is 1 μg/L; the upper reference limit is 12.5 μg/L. S-100B was quantified with an automated immunoluminometric assay (LIAISON, Sangtec Medical). This method detects the β subunit of S-100B. The detection limit is 0.02 μg/L, and the upper reference limit is 0.15 μg/L.

Assessment of Outcome

Standard neurological examination was performed daily during treatment at the Intensive Care Unit, on days 9 and 14, at discharge from hospital, and at 3 and 6 months after CA. The primary end point was a favorable neurological outcome 6 months after CA as assessed by the Pittsburgh Outcome Scale.24,25 This 5-category scale of cerebral performance categories (CPCs) is defined as follows: CPC 1, normal cerebral function; CPC 2, moderate cerebral disability;
CPC 3, conscious with severe disability; CPC 4, comatose or persistent vegetative state; and CPC 5, dead. For statistical analyses, neurological outcome was dichotomized into good (CPC 1 and 2) or poor (CPC 3, 4, and 5). Good outcome implied independent function. Neurological outcome was determined without knowledge of treatment assignment. We also recorded the following parameters: the best achieved CPC within 6 months after ROSC, change of CPC from prearrest level, recovery of consciousness (defined as ability to obey verbal command), and death.

Statistical Analysis

Categorical variables are given as counts and percentages. Data are given as median and interquartile range. The NSE and S-100B levels in the normothermia and hypothermia groups were compared by use of repeated-measures analysis of variance (ANOVA) after logarithmic transformation. Scheffé’s test was used as a posthoc test. Outcome data are binary, and χ² test or Fisher’s exact test was used to compare proportions between the hypothermia and normothermia groups. Continuous data were compared by use of the Mann-Whitney U test. Values of P<0.05 were considered statistically significant. The discriminative power of serum NSE and S-100B in predicting poor outcome was evaluated by receiver-operating characteristics (ROC) analysis. We used the StatsDirect (StatsDirect Ltd) statistical software to analyze data.

Results

Seventy unconscious (Glasgow Coma Scale <9) patients were enrolled in the study according to the inclusion criteria. Of those 70 patients, 36 were randomized to hypothermia treatment and 34 to normothermia treatment. Characteristics of enrolled patients are presented in Table 1. Statistically, the 2 treatment groups were comparable.

At 6 months, good neurological outcome was achieved in 69% (n=25) of hypothermia-treated patients (CPC 1, 22; CPC 2, 3) and in 47% (n=16) of normothermia-treated patients (CPC 1, 11; CPC 2, 5). Two patients (6%) had CPC 3, and 9 patients (25%) died after a median of 13 days (range, 1 to 116 days) in the hypothermia-treated group. In the normothermia-treated group, 4 patients (12%) had CPC 3, 1 patient (3%) had CPC 4, and 13 patients (38%) died after a median of 9 days (range, 1 to 147 days).

In both treatment groups, 1 patient died before 24 hours. Thus, serum NSE and S-100B could be measured from 35 hypothermic patients and 33 normothermic patients. For some patients, blood samples were not available at all time points. The difference in NSE values between 24 and 48 hours could be analyzed in 34 hypothermic patients and 32 normothermia-treated patients.
TABLE 2. Serum NSE and S-100B at 24, 36, and 48 Hours After ROSC

<table>
<thead>
<tr>
<th>Sampling Time, h</th>
<th>Hypothermia, μg/L</th>
<th>Normothermia, μg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSE, 24</td>
<td>10.2 (8.3–15.5)</td>
<td>9.3 (6.2–19.2)</td>
</tr>
<tr>
<td>NSE, 36</td>
<td>9.8 (7.0–17.2)</td>
<td>9.1 (5.4–18.8)</td>
</tr>
<tr>
<td>NSE, 48</td>
<td>7.9 (5.9–13.9)</td>
<td>8.6 (5.2–20.2)</td>
</tr>
<tr>
<td>S-100B, 24</td>
<td>0.13 (0.10–0.18)</td>
<td>0.13 (0.09–0.22)</td>
</tr>
<tr>
<td>S-100B, 36</td>
<td>0.12 (0.10–0.17)</td>
<td>0.13 (0.09–0.26)</td>
</tr>
<tr>
<td>S-100B, 48</td>
<td>0.12 (0.09–0.17)</td>
<td>0.12 (0.10–0.24)</td>
</tr>
</tbody>
</table>

Values are medians (interquartile range).

The time course of serum NSE and S-100B levels is presented in Figures 1 and Figure 2. NSE levels were lower in the hypothermia group compared with the normothermia group (P=0.007 by ANOVA for repeated measurements). Median serum NSE and S-100B levels at 24, 36, and 48 hours after ROSC in the hypothermia and normothermia groups are presented in Table 2. A decrease (defined as any decrease) in NSE values between 24 and 48 hours was observed in 30 of 34 patients (88%) in the hypothermia group and 16 of 32 patients (50%) in the normothermia group (P<0.001). The decrease in NSE values was associated with good outcome at 6 months after ROSC (P=0.005). It was also associated with regaining consciousness (P<0.001), no change to prearrest CPC (P<0.001), and survival for at least 6 months after ROSC (P=0.012). Only 1 patient (in the hypothermia group) died within 6 months after achieving good CPC. A decrease in S-100B values was observed between 24 and 48 hours in 17 of 34 patients (50%) in the hypothermia group and in 15 of 33 patients (45%) in the normothermia group. Decreased S-100B values had no relation to outcome.

The prognostic value of serum NSE and S-100B in predicting unfavorable outcome was evaluated by ROC analysis in both groups. Cutoff values resulting in a specificity of at least 95% in predicting poor outcome are presented in Table 3. ROC curves for serum NSE and S-100B at 48 hours after ROSC for both treatment groups are presented in Figure 3.

TABLE 3. AUC Values and Cutoff Values With at Least 95% Specificity Predicting Poor Outcome for Serum NSE and S-100B Protein at 24, 36, and 48 Hours After ROSC

<table>
<thead>
<tr>
<th>Sampling Time, h</th>
<th>Hypothermia, μg/L</th>
<th>Specificity, %</th>
<th>Sensitivity, %</th>
<th>AUC</th>
<th>Normothermia, μg/L</th>
<th>Specificity, %</th>
<th>Sensitivity, %</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSE</td>
<td>At 24 h</td>
<td>31.2</td>
<td>96</td>
<td>22</td>
<td>0.72</td>
<td>13.3</td>
<td>100</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>At 36 h</td>
<td>26.0</td>
<td>96</td>
<td>30</td>
<td>0.85</td>
<td>12.6</td>
<td>100</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>At 48 h</td>
<td>25.0</td>
<td>96</td>
<td>25</td>
<td>0.80</td>
<td>8.8</td>
<td>100</td>
<td>76</td>
</tr>
<tr>
<td>S-100B</td>
<td>At 24 h</td>
<td>0.21</td>
<td>100</td>
<td>30</td>
<td>0.65</td>
<td>0.19</td>
<td>100</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>At 36 h</td>
<td>0.21</td>
<td>96</td>
<td>20</td>
<td>0.69</td>
<td>0.49</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>At 48 h</td>
<td>0.23</td>
<td>96</td>
<td>22</td>
<td>0.63</td>
<td>0.12</td>
<td>100</td>
<td>88</td>
</tr>
</tbody>
</table>

AUC indicates area under ROC curve.

Discussion

Our study provides biochemical corroboration of the clinical outcome results of 2 recent randomized trials on therapeutic hypothermia in CA.11–12 Hypothermia resulted in rapidly decreasing levels of serum NSE, reflecting amelioration of secondary ischemic neuronal injury, and serum levels of NSE were significantly different in hypothermic and normothermic patients.

The decrease in serum NSE values after CA was associated with recovery of consciousness, good neurological outcome, and survival for at least 6 months. Increasing levels of serum NSE were associated with poor outcome. A decreasing S-100B level was not associated with outcome, nor did the time course of serum S-100B differ between the 2 treatment groups. The decrease in the levels of NSE but not S-100B may reflect attenuation of selective neuronal death by hypothermia. Neurons are more sensitive to hypoxic injury than astrocytes. Although they studied a different mechanism of neuronal injury, Berger et al26 have reported a selective increase in NSE but not S-100B in CSF in children with inflicted traumatic brain injury.

ROC analysis of serum NSE and S-100B at different time points revealed that cutoff values predicting unfavorable outcome with a specificity of at least 95% were higher in the hypothermia group. This may reflect the ability of hypothermia to provide cerebral protection despite initial neuronal damage. However, the sensitivity of these tests was remarkably poor in the hypothermia group. Our cutoff values for serum NSE in hypothermic patients assessed by ROC analysis are higher than14 or similar to16–17,19 those in previous studies on normothermic CA patients. In our normothermic patients, the cutoff values were only slightly elevated or were within the reference range. Several previous reports have suggested higher cutoff values for serum S-100B, but with the methods used in these studies, the detection limits were much higher (0.1 to 0.2 μg/L).14,20–21 One study using a method similar to ours resulted in almost the same cutoff value.18

This study has some limitations. Because the level of NSE in CSF was not measured, evidence of the effect of therapeutic hypothermia remains elusive. However, a high correlation between serum and CSF NSE values has been reported.7,14 Repeated lumbar punctures may be contraindicated because...
of induced thrombolysis or elevated intracranial pressure. It cannot be entirely excluded that the decrease in serum NSE levels seen in patients assigned to hypothermia could be related to a reduction of cerebral blood flow by hypothermia. The hypothermia lasted 24 hours, and with this assumption, one would expect to see a rise in the levels of NSE at 48 hours. The confounding factors for both of these markers are well known. NSE can be found in red blood cells and platelets. To avoid falsely high NSE values, samples with visible hemolysis were not analyzed. Anderson et al27 have demonstrated that there are extracerebral sources of S-100B contamination in cardiac operations with cardiotomy suction. It has not been proved that all the S-100B detected in the peripheral blood of CA patients could have originated from the brain even at 48 hours after CA. Finally, although power analysis suggested that our sample size was adequate, the relatively small sample size always causes the analyses to be disposed to possible type II error.

Hypothermia has multiple mechanisms of action in mitigating cerebral ischemic injury. It has been shown to reduce metabolic rate and oxygen consumption; to diminish excitotoxic action; to suppress the production of superoxide anions, nitric oxide, and different cytokines; and to maintain the integrity of the blood-brain barrier.28 Induced hypothermia requires muscle relaxation, sedation, and mechanical ventilation, which may complicate the clinical assessment. Therefore, more reliable tests predicting unfavorable neurological outcome in these patients are needed. Our results suggest that the time course of serum NSE between 24 and 48 hours after CA may help in clinical decision making. However, the use of therapeutic hypothermia seems to reduce the prognostic value of both serum NSE and S-100B in outcome prediction.

Acknowledgments
This study was supported by the Finnish Neurology Foundation, Laerdal Foundation, Maire Taponen Foundation, Helsinki University Central Hospital, and the European Union (Biomed2, for the HACA study). Kinetic Concepts Inc, United Kingdom (Wareham, UK) provided the cooling device used in the study. We would like to thank Henrik Alfthan, PhD, for his assistance in analysis of serum S-100B samples.

References
Serum Neuron-Specific Enolase and S-100B Protein in Cardiac Arrest Patients Treated With Hypothermia
Marjaana Tiainen, Risto O. Roine, Ville Pettilä and Olli Takkunen

*Stroke*. 2003;34:2881-2886; originally published online November 20, 2003;
doi: 10.1161/01.STR.0000103320.90706.35

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/34/12/2881

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org/subscriptions/