Astroglial Protein S-100 Is an Early and Sensitive Marker of Hypoxic Brain Damage and Outcome After Cardiac Arrest in Humans

Bernd W. Böttiger, MD; Stefan Möbes, MD; Rolf Glätzer, MD; Harald Bauer, MD; André Gries, MD; Peter Bärtsch, MD; Johann Motsch, MD; Eike Martin, MD

Background—The results of early conventional tests do not correlate with cerebral outcome after cardiac arrest. We investigated the serum levels of astroglial protein S-100 as an early marker of brain damage and outcome after cardiac arrest.

Methods and Results—In 66 patients undergoing cardiopulmonary resuscitation after nontraumatic cardiac arrest, blood samples for the evaluation of S-100 were drawn immediately after and 15, 30, 45, and 60 minutes; 2, 8, 24, 48, and 72 hours; and 7 days after initiation of cardiopulmonary resuscitation. Moreover, the serum levels of neuron-specific enolase were determined between 2 hours and 7 days. If patients survived for >48 hours, brain damage was assessed by a combination of neurological, cranial CT, and electrophysiological examinations. Overall, 343 blood samples were taken for the determination of S-100. Maximum S-100 levels within 2 hours after cardiac arrest were significantly higher in patients with documented brain damage (survivors and nonsurvivors, 3.70±0.77 mg/L) than in patients without brain damage (0.90±0.29 mg/L). Significant differences between these 2 groups were observed from 30 minutes until 7 days after cardiac arrest. In addition, the positive predictive value of the S-100 test at 24 hours for fatal outcome within 14 days was 87%, and the negative predictive value was 100% (P<0.001). With regard to neuron-specific enolase, significant differences between patients with documented brain damage and those with no brain damage were found at 24, 48, and 72 hours and 7 days.

Conclusions—Astroglial protein S-100 is an early and sensitive marker of hypoxic brain damage and short-term outcome after cardiac arrest in humans. (Circulation. 2001;103:2694-2698.)

Key Words: heart arrest ■ cardiopulmonary resuscitation ■ ischemia ■ brain ■ outcome

Brain damage is one of the major causes of morbidity and mortality after cardiac arrest and cardiopulmonary resuscitation (CPR) in hospitalized patients. Early assessment of brain damage and prediction of cerebral outcome after cardiac arrest, therefore, may affect postarrest treatment strategies. Various attempts, including neurological evaluation, cranial CT, electroencephalogram, somatosensory evoked potentials, and measurement of cerebral oxygen consumption, have been made to assess brain damage in comatose patients soon after cardiac arrest. Early neurological and electrophysiological evaluations, however, do not predict cerebral outcome. A serum marker that reflects the severity of brain damage as accurately as biochemical markers do in myocardial injury, however, would improve early evaluation and quantification of post–cardiac arrest brain damage. Biochemical markers of hypoxic brain damage such as neuron-specific enolase (NSE), however, have also shown promising results only at later stages (ie, >24 hours after cardiac arrest). Recently, S-100 has been found to be a promising marker for central nervous system injury.

Protein S-100 is part of a large family of calcium-binding proteins, and evidence exists that S-100 regulates calcium-dependent cellular signaling in neuronal differentiation, outgrowth, and apoptosis through different concentrations. Elevated serum levels of S-100 have been documented in patients suffering from different types of brain damage, including stroke, minor and severe head injury, and brain damage associated with extracorporeal circulation and later stages of global cerebral ischemia. No data are available, however, concerning serum levels of S-100 during the early phase after global cerebral ischemia from cardiocirculatory arrest in humans. In an attempt to find an early marker of hypoxic brain damage and outcome, we investigated the serum levels of astroglial protein S-100 during CPR and after restoration of spontaneous circulation (ROSC).

Methods

Patients

After approval from the local Ethics Committee and informed consent from patients without brain damage or the relatives of...
patients with brain damage had been obtained, patients who had undergone CPR after nontraumatic cardiac arrest before admission to the hospital and who were covered by the local physician-staffed advanced cardiac life support system were studied. Venous blood samples were taken during CPR and after ROSC by an independent physician who was not responsible for the patient’s care. According to the recommendations of the Utstein Consensus Conference, patient survival was assessed with regard to ROSC and hospital admission (all patients were admitted to the same hospital). In addition, short-term outcome (14 days’ survival) was assessed, and patients were divided into 4 groups (as shown in Figure 1): (1) no brain damage (patients discharged from the hospital fully oriented and without any communication defects; ie, cerebral performance category 3), (2) documented brain damage (ie, according to neurological, cranial CT, and electrophysiological evaluations systematically performed between 48 hours and 96 hours after cardiac arrest; to focus on all patients, the data of surviving and nonsurviving patients were combined here; see also Table 1), (3) no ROSC, and (4) patients who died soon after ROSC before assessment of brain damage.

Blood Samples and Biochemical Markers

For the evaluation of serum levels of S-100 (S-100 IRMA, AB Sangtec Medical; sensitivity, 0.2 μg/L), blood samples were drawn immediately after and 15, 30, 45, and 60 minutes; 2, 8, 24, 48, and 72 hours; and 7 days after CPR was initiated. Samples for the determination of S-100 were put into specific tubes (Citrate Vacuum tubes, Boehringer Mannheim). After admission to the intensive care unit, additional blood samples were drawn into EDTA tubes (Vacutainer tubes, Boehringer Mannheim) for the determination of NSE (Prolifigen R, AB Sangtec Medical; normal range <12.5 μg/L) at 2, 8, 24, 48, and 72 hours and 7 days after CPR was initiated. In an attempt to simplify blood sampling during CPR and in the prehospital setting, NSE was not determined before 2 hours after cardiac arrest.

In all patients, blood samples during CPR and immediately after ROSC were drawn from the external jugular vein via a separate 12-gauge venous catheter inserted opposite to the infusion site. In each case, the first 10 mL of blood was discarded. After admission to the intensive care unit, blood samples were taken through a central venous catheter after the first 10 mL of blood was discarded. Immediately after sampling, the blood in the tubes was mixed, carefully avoiding the formation of foam, and placed on a mixture of water and ice to ensure a constant temperature of ±4°C, which was controlled by a thermometer. The tubes were centrifuged within 1 hour of collection at 1500 g for 15 minutes (4°C). The plasma was then separated and stored as aliquots in plastic tubes at −70°C until it was assayed. All assays were performed in a blinded manner by an independent member of the laboratory staff.

Statistical Analysis

ANOVA followed by the Scheffé test, Wilcoxon test, and Fisher’s exact test were used for statistical analysis. Data are mean±SEM; a probability value of P<0.05 was considered statistically significant. Because the duration of out-of-hospital cardiac arrest can only be estimated, these data were excluded from statistical analysis.

Results

Astrogial Protein S-100

Overall, 66 patients (24 female, 42 male; mean age, 66 years [range, 25 to 91 years]; Figure 1) were studied. The causes of cardiac arrest were primary cardiogenic, including acute myocardial infarction and pulmonary embolism (n=60); intoxication (n=3); status asthmaticus (n=2); and subarachnoid hemorrhage (n=1). According to the recommendations of the Utstein Consensus Conference, the outcome in these

<table>
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<tr>
<th>No.</th>
<th>Sex/Age, y</th>
<th>Duration of Arrest, min</th>
<th>Initial Rhythm</th>
<th>Duration of CPR, min</th>
<th>Diagnosis</th>
<th>Definite Diagnosis of Persistent Brain Damage</th>
<th>Time of Death, d</th>
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<td>20</td>
<td>AMI</td>
<td>Neuro (CPC4), CCT, EEG</td>
<td>8</td>
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AMI indicates acute myocardial infarction; AVB III°, third-degree atrioventricular blockade; CCT, cranial CT; CPC4, cerebral performance category 4 (ie, coma or vegetative state); EEG, electroencephalogram; neuro, neurological evaluation(s); PE, acute pulmonary embolism; SAH, subarachnoid hemorrhage; VF, ventricular fibrillation; and ?, estimated.
patients is given in Figure 1. Overall, 343 blood samples were taken. It was not possible to obtain blood from each patient at every time point.

Significant differences in the levels of S-100 between patients surviving without brain damage (CPC1; n = 9) and those with documented brain damage (n = 12; ie, 4 surviving and 8 nonsurviving patients; see Table 1) were observed during the entire study period. Even very early during CPR, significant differences were observed between the 2 groups (Figure 2). Moreover, maximum S-100 levels within 2 hours after cardiac arrest were significantly higher in patients with documented brain damage (3.70 ± 0.77 μg/L; P < 0.05) and in patients without ROSC (3.44 ± 0.58 μg/L; P < 0.05) than in patients with no brain damage (0.90 ± 0.29 μg/L), whereas patients who died before assessment of brain damage showed intermediate levels (2.36 ± 0.41 μg/L; Figure 3). The odds ratio of having brain damage was 15 (95% CI, 2.02 to 111.22) if S-100 serum levels were elevated within 2 hours after cardiac arrest. At 48 hours after cardiac arrest, an S-100 serum level of >1.10 μg/L revealed a specificity of 100% for the diagnosis of brain damage. The positive predictive value of the S-100 test at 2 hours for fatal outcome within 14 days was 79%, and the negative predictive value was 100% (P < 0.01). At 24 hours, the corresponding figures were 87% and 100% (P < 0.001), and at 48 hours, they were 75% and 100% (P < 0.01), respectively (Table 2).

Neuron-Specific Enolase
In 33 patients (9 female, 24 male; mean age, 67 years [range, 34 to 85 years]), 137 blood samples were taken. Fourteen patients died before assessment of brain damage (maximum NSE levels, 28.6 ± 11.5 μg/L). Nine patients survived without neurological deficit (CPC1), and 10 patients were shown to have suffered severe brain damage. When the serum levels of NSE in patients without neurological deficit (CPC1) and in those with documented brain damage were compared, significant differences were observed at 24, 48, and 72 hours and 7 days (Figure 4).

Discussion
The present data demonstrate that there is a marked difference in S-100 levels after cardiac arrest between patients who had suffered brain damage and those who had not. By determining levels of astroglial protein S-100 in blood samples, differences between the 2 groups can be detected very soon after the initiation of CPR procedures. Interestingly, patients without ROSC also showed significantly elevated levels of S-100, suggesting that the duration of cardiac arrest and cerebral ischemia in these patients might have been longer than in those without brain damage. Patients who died soon after ROSC without neurological evaluation showed intermediate values, which was expected, because this group most likely represents a mixture of patients with and without brain damage. In addition to the association of S-100 levels with brain damage, S-100 levels between 2 hours and 48 hours predict short-term outcome after cardiac arrest.

| Table 2. Comparison of S-100 Levels and Short-Term Outcome (14 Days’ Survival) in Patients After Out-of-Hospital Cardiac Arrest |
|-----------------|-----------|-----------|-------|-----|
|                  | Deceased | Alive     | Total | P   |
| **S-100 Serum Levels and Time Point** |           |           |       |     |
| 2 h              |           |           |       |     |
| <0.2 μg/L        | 0         | 5         | 5     |     |
| ≥0.2 μg/L        | 22        | 6         | 28    |     |
| Total            | 22        | 11        | 33    | <0.01 |
| 24 h             |           |           |       |     |
| <0.2 μg/L        | 0         | 8         | 8     |     |
| ≥0.2 μg/L        | 13        | 2         | 15    |     |
| Total            | 13        | 10        | 23    | <0.001 |
| 48 h             |           |           |       |     |
| <0.2 μg/L        | 0         | 7         | 7     |     |
| ≥0.2 μg/L        | 9         | 3         | 12    |     |
| Total            | 9         | 10        | 19    | <0.01 |

Figure 2. S-100 serum levels at different time points after initiation of CPR in patients without brain damage (n = 9) and in those with documented brain damage (n = 12; numbers in figure reflect number of blood samples analyzed per group and time point); *P < 0.05 vs no brain damage.

Figure 3. Maximum S-100 serum levels (S-100max) within 2 hours after initiation of CPR in patients without brain damage (n = 9), in patients with documented brain damage (n = 12), in patients without ROSC (n = 27), and in those who died before neurological assessment within 48 hours after cardiac arrest (n = 18); *P < 0.05 vs no brain damage.

Figure 4. Serum levels of NSE at different time points after initiation of CPR in patients without brain damage (n = 9) and in those with documented brain damage (n = 10); *P < 0.05 vs no brain damage.
Within the past few years, various attempts have been made to assess brain damage in comatose patients soon after cardiac arrest.\textsuperscript{3-10} Most of them, however, exhibit major limitations. A recent meta-analysis of the clinical and electrophysiological predictors of poor outcome in anoxic-ischemic coma based on data from >4500 patients suggested that the absence of N20 components of somatosensory evoked potentials in comatose patients with anoxic brain injury and the absence of pain or pupillary responses at 72 hours after cardiac arrest confirm that continuation of life support is futile.\textsuperscript{11} The results of early neurological and electrophysiological evaluations, however, do not predict cerebral outcome in these patients.\textsuperscript{11} Therefore, recent attention focuses on biochemical markers of hypoxic brain damage.\textsuperscript{13-16,22} To be an ideal marker for brain injury, the marker should be proved to be released from neurons or glial cells in experimental settings, for example cell cultures. Such proofs exist for NSE\textsuperscript{22} but not for S-100. NSE, however, also exists in platelets and in erythrocytes, and there is a constant turnover of NSE in blood, making changes specifically associated with brain damage in serum levels difficult to evaluate.\textsuperscript{14,16} Moreover, it is well known that platelets are markedly activated and hemolysis may occur during early reperfusion after cardiac arrest.\textsuperscript{33} Therefore, even though at least a proportion of NSE is released from neurons, the determination of NSE, particularly during early reperfusion after cardiac arrest, may not be brain specific. This is in line with the fact that NSE, as in the present study, has shown promising results at later stages after cardiac arrest.\textsuperscript{13-16} In contrast, release from blood cells in particular circumstances is not the case with S-100. The presence of S-100 in serum indicates cellular brain injury and damage to the blood-brain barrier.\textsuperscript{25-29} S-100 is a protein with calcium-binding capacity. There are 19 S-100 proteins, of which S-100A1 and S-100B (formerly known as S-100\textalpha{} and S-100\textbeta{}, respectively) may represent a homodimer or heterodimer.\textsuperscript{25,28,29} At least 4 of the possible subtypes are known to be represented in human tissue: S-100A1 (striated muscles, heart, and kidneys), S-100A1B (astroglial cells), S-100B (astroglial and Schwann cells), and S-100BB (astroglial cells).\textsuperscript{25,28,29} The test system used in the present study detects the \( \beta \)-subunit of S-100 and is therefore highly specific for the assessment of astroglial injury. Astroglial cells are the most common cells in the brain. They form a 3D network that constitutes a supporting framework for neurons.\textsuperscript{28,29} Astroglial cells are known to be as sensitive as neurons to hypoxic stress. Therefore, a marker for astroglial cell damage may indirectly reflect neuronal damage.\textsuperscript{17-19} Because of the estimated biological half-life of \( \approx 2 \) hours,\textsuperscript{23} constantly elevated levels of S-100 in patients with documented brain damage, as observed in the present study, may reflect a continuous release from damaged tissue. At 48 hours after cardiac arrest, all patients with S-100 levels of \( \geq 1.1 \) \( \mu \)g/L \textit{were subsequently diagnosed in the present study as having brain damage. Interestingly, the positive and negative predictive values of the S-100 test at 2 hours to 48 hours for fatal outcome within 14 days were very high. This suggests that, in addition to its association with brain damage, S-100 represents an early marker of short-term outcome after cardiac arrest, which is in full accordance with recent data presented by Rosen and coworkers.\textsuperscript{22} In survivors without brain damage, the mean values of S-100 have also been found to be transiently increased during and in the early phase after CPR. Such a transient increase in S-100 serum levels can also be observed in cardiac surgical patients without cerebral symptoms during and early after extracorporeal circulation.\textsuperscript{19-21} At least 2 explanations for that finding exist: First, small amounts of S-100 may be released from tissue outside the brain (S-100 seems to be present, at least in a limited amount, in adipose and other tissue as well,\textsuperscript{25,28,29} and it cannot be ruled out that some early effects in other tissues can be a confounding factor). Second, temporary brain edema with blood-brain barrier dysfunction and/or minor brain damage induced by cardiac arrest and CPR may occur in patients without persistent neurological dysfunction.\textsuperscript{22} It has been demonstrated that after short periods of global cerebral ischemia due to cardiocirculatory arrest, minor patterns of brain damage can be observed. Predilection areas of minor brain damage are the selectively vulnerable areas of the brain, including the hippocampal CA1 sector, the striatum, and the thalamic reticular nucleus.\textsuperscript{34,35} In the present study, the absence of brain damage in survivors was assessed according to clinical criteria evaluated in previous studies (CPC1).\textsuperscript{1,2,31} Therefore, it cannot be excluded that minor neurological sequelae like impaired memory, concentration problems, and other psycho-organic syndromes may have occurred in these patients. The present data suggest that the determination of S-100 serum levels makes it possible to assess overall cerebral outcome and survival after cardiac arrest earlier than with any other method.\textsuperscript{11} From an ethical point of view, however, the significance of one single marker should not be overestimated. Low serum levels of S-100 after cardiac arrest should, however, prompt maximum therapeutic efforts even in patients in whom good neurological outcome is thought to be unlikely from a clinical point of view. Moreover, clinically relevant neurological impairment may not increase in a linear fashion with the amount of cell damage in the brain. Overall, the present data suggest that both biochemical markers of brain damage, NSE and S-100, could be of value in our diagnostic arsenal in difficult cases and enhance the ensemble of different diagnostic approaches.

In conclusion, astroglial protein S-100 is an early and sensitive marker of severe brain damage and short-term outcome after cardiac arrest in humans. Therefore, the determination of S-100 serum levels may help to assess comatose patients early after cardiac arrest.

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article is dedicated to Prof Douglas Chamberlain on the occasion of his 70th birthday.

References


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