

Serum S-100B Protein as a Prognostic Factor in Patients with Severe Head Injury

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Objective : Despite the recent progress that has been made in intracerebral monitoring, it is still difficult to quantify the exact extent of primary brain damage after severe head injury. In this work, we investigate the role of S-100B protein as a serum marker of brain damage after severe head injury.

Methods : 21 patients with severe head injury (GCS score <9) were selected for this prospective study. A venous blood sample was taken as soon as possible after head injury and the serum concentration of S-100B protein was measured daily for five consecutive days. The serum level of S-100B protein was compared with the patients' outcome. The outcome was measured twice, at hospital discharge and after 6 months of follow-up using the Glasgow Outcome Scale(GOS).

Results : Those patients who died within two weeks (after head injury) had a significantly higher serum S-100B value than those who survived (median, 9.64ug/L versus 2.91ug/L). Seven (78%) of the nine patients who died had a maximum S-100B value of 2ug/L or higher, while three (25%) of the twelve surviving patients showed a maximum S-100B protein value of more than 2ug/L (P<0.05).

Conclusion : These results indicate that S-100B protein appears to be the most reliable index for estimating the extent of brain damage.

KEY WORDS : Head injuries · S-100B proteins · Prognosis · Mortality.

Introduction

The outcome of severe head injury is of great interest to the doctor, the patient and his or her family members. In spite of the significant progress that has been made in intracranial monitoring device, it is still difficult to quantify the exact extent of brain damage and its prognosis after severe head injury. Generally, the Computed Tomography(CT) findings and Glasgow Coma Scale(GCS) scores are used to establish the severity of the head injury¹⁹⁾. However, there are still some limitations associated with our ability to estimate the extent of brain damage and predict the outcome.

Some authors had reported that the initial GCS scores after head injury was able to predict the prognosis in only 44%, but that the predictive value of the prognosis was slightly higher i.e., 59%, if GCS and CT were considered together²²⁾. Therefore, it is necessary to establish the specific index for the severity of brain damage⁷⁻⁹⁾.

Vollmer tried to evaluate the outcome after severe head injury by investigating the level of adenylate kinase, aspartate aminotransferase, lactate dehydrogenase, and creatine kinase BB, but their findings were found to be unreliable due to the lack of specificity^{6,14,15)}. After Vollmer's work, various studies have been conducted to find a protocol or biochemical substance with high sensitivity and specificity for this index. In these studies, several kinds of biochemical materials, such as lactate dehydrogenase(LDH), creatine kinase-BB(CK-BB), neuron specific enolase(NSE), myelin basic protein(MBP), aspartate aminotransferase, and S-100B protein were found to be related to head injury¹⁻⁴⁾. Especially, S-100B protein was found in the serum only when the brain blood barrier was destroyed due to the brain damage. Therefore, the serum concentration of S100-B protein is able to indicate the extent of brain damage. Moreover, there is a close correlation between the concentration of serum S-100B protein and the prognosis⁵⁾.

In this work, the prognostic value of S-100B protein was

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investigated as a serum index for brain damage after severe head injury, and was compared with the GCS score and CT findings.

Materials and Methods

21 patients with a GCS score of less than 9 who were admitted to our hospital within 6 hours after severe head injury from September 2002 to May 2003 were included in this prospective study. These patients did not have medical history of other resuscitation, shock, acidosis, hypotension, hypoxia, neurological disease and spinal cord injury.

All patients underwent intubation. Additional mechanical ventilation was administered only when necessary. The patients underwent CT upon admission and the result was classified according to the Marshall Computed Tomographic Classification^{3,19)}. The GCS score and S-100B protein level of each patient were investigated. Additionally, the sex, age and history of surgical interventions of the patients were checked. The data so obtained were compared with the prognosis. The outcomes within 2 weeks and after 6 months were estimated using the Glasgow Outcome Scale(GOS) score.

Venous blood samples for the determination of the S-100B level were obtained as soon as possible after admission, within 6 hours after trauma, and thereafter every 24 hours for a maximum of 5 days. The blood samples were immediately centrifuged, and the serum was stored at -20°C for analysis. The serum concentration of the S-100B protein was measured by means of an immunoradiometric assay kit (Sangtec medical, Dietzenbach, Germany). After dilution in phosphate buffer, the centrifuged serum was incubated for 1 hour in a plastic bead coated with monoclonal antibody to S-100 protein. During the incubation, S-100 was bound to the antibody-coated bead. Then, the bead was washed and the sample was incubated with 125I-labeled monoclonal antibody to S-100 protein for 2 hours. After washing it, the radioactivity bound to the bead was measured using a gamma counter. The maximum serum level of the measured serum concentrations was used for the analysis. Less than 0.13µg/L was considered as normal, 0.13~0.50µg/L as borderline and over 0.50µg/L as an abnormal increase. The abnormal increase was further classified into two conditions. 0.50~2.0µg/L was considered as a mild increase and over 2.0µg/L as a severe increase.

The statistical analysis was performed using SPSS 11.0, including Fisher's exact test and the Mann-Whitney test. Statistical significance was determined at the level of $p < 0.05$.

Results

The patients consisted of 16 males and 5 females. The average age of the patients was 34 (range from 4 to 70).

Table 1. Relationship between Glasgow Coma Scale(GCS) and mortality

Group	Survival	death	Predictive value
GCS 6,7,8	10	3	Negative Predictive Value=0.77
GCS 3,4,5	2	6	Positive Predictive Value=0.75

$P=0.029$, (Fisher Exact test), Specificity=0.83, Sensitivity=0.67, Accuracy=0.76 *There is a strong association between GCS and mortality

Table 2. Relationship between GCS and long-term outcome

Group	Favorable	Unfavorable	Predictive value
GCS 6,7,8	6	7	Negative Predictive Value=0.46
GCS 3,4,5	1	7	Positive Predictive Value=0.875

$P=0.133$, (Fisher Exact test), Specificity=0.86, Sensitivity=0.50, Accuracy=0.62 *That show the high specificity and relatively low sensitivity

However, age had no practical effect on the investigation, because there was no notable difference between the average ages of the survived and deceased patients. The average age of the survived and deceased patients were 32 and 35, respectively. The patients' outcome was classified into 4 conditions by means of the GOS score. A GOS score of 5 was considered as a mild disability, 4 as an moderate disability, 2-3 as an severe disability and 1 indicated that the patient was deceased.

Within 2 weeks after head injury, the number of deceased patients was 9 (43%). After 6 months, there were 5 patients with severe disability (24%), 6 with moderate disability (29%), and 1 with mild disability (4%). The average follow-up period for the survived patients was 12.5 months from 8 to 16 months). The causes of the patients' head injury consisted of 15 traffic accidents (72%), 3 fall-down accidents (14%), 2 assaults (10%) and 1 gunshot trauma (4%). The low GCS score group (3,4,5) consisted of 8 patients (38%) and the high GCS score group (6,7,8) consisted of 13 patients (62%). The outcome of the low GCS score group was 6 deaths (75%), 1 severe disability(12.5%) and 1 moderate disability (12.5%). In the high GCS score group, there were 3 deaths (23%), 4 severe disabilities (31%), 5 moderate disabilities (38%) and 1 mild disability (8%) (Table 1, 2) This result shows that the lower the GCS score was upon admission to the hospital was the higher the mortality was and this difference was statistically significant ($P=0.029$). Also, the lower the GCS score upon admission to the hospital, the lower the GOS score at 6 months after head injury. However, this difference was not statistically significant ($P=0.133$).

In the group of 15 patients who had an intracranial mass effect (type 4,5,6) on brain CT upon admission to the hospital, there were 7 deaths (47%), 4 severe disabilities (26.5%) and 4 moderate disabilities (26.5%). In the group of 6 patients who had no mass effect on brain CT upon admission to the hospital (type 1,2,3), there were 2 deaths (33%), 1 severe disabilities

Table 3. Relationship between Computed Tomography(CT) and mortality

Group	Survival	death	Predictive value
CT 1,2,3	4	2	Negative Predictive Value=0.67
CT 4,5,6	8	7	Positive Predictive Value=0.47

P=0.477, (Fisher Exact test), Specificity=0.33, Sensitivity=0.78, Accuracy=0.52 *There is only a slight relationship between CT and mortality

Table 4. Relationship between CT finding and long-term outcome

Group	Favorable	Unfavorable	Predictive value
CT 1,2,3	3	3	Negative Predictive Value=0.50
CT 4,5,6	4	11	Positive Predictive Value=0.73

P=0.299, (Fisher Exact test), Specificity=0.43, Sensitivity=0.79, Accuracy=0.67

Table 5. The association between mortality and the serum S-100B protein level

Group	Survival	death	Predictive value
S-100B < 2	9	2	Negative predictive Value=0.82
S-100B > 2	3	7	Positive Predictive Value=0.7

P=0.024, (Fisher Exact test), Specificity=0.75, Sensitivity=0.78, Accuracy=0.76 *There is a highly significant association between the serum peak S-100B protein level and mortality

Table 6. The association between the serum peak S-100B protein level and 6 month long-term outcome

Group	Favorable	Unfavorable	Predictive value
S-100B < 2	5	6	Negative Predictive Value=0.45
S-100B > 2	2	8	Positive Predictive Value=0.8

P=0.221, (Fisher Exact test), Specificity=0.71, Sensitivity=0.57, Accuracy=0.62

(17%), 2 moderate disability (33%) and 1 mild disability (17%) (Table 3, 4). From these results, it was revealed that if the patient had a mass lesion on brain CT upon admission to the hospital, the mortality was increased and the prognosis of the patient was poor. However, this result was not statistically significant (P=0.477, 0.299). It was observed that the serum concentration of S-100B protein was over 0.5 μ g/L in all patients with severe head injury. The average S-100B value of the patients who died within 2 weeks after head injury was 9.64 μ g/L and that of those patients who survived was 2.91 μ g/L. In the 11 cases in which a mild increase (0.5~2.0 μ g/L) in the S-100B protein level was observed, there were 2 deaths (18%). In the 10 cases in which an severe increase was observed (>2.0 μ g/L), there were 7 deaths (70%). This result shows that the higher the serum concentration of S-100B protein was upon admission to the hospital, the higher the mortality was, and this difference was statistically significant (P=0.024). Among those patients with a mild increase in the S-100B protein level, 6 months after the severe head injury, there were 4 pa-

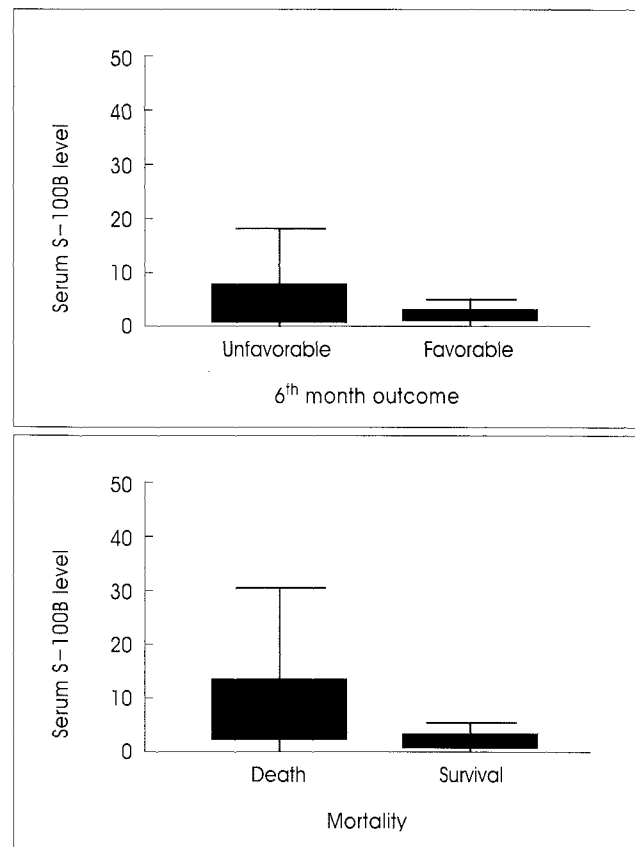


Fig. 1. Boxplot showing median, interquartile range and outliers for the venous concentrations of the peak S-100B protein levels in both the survival & deceased groups after severe head injury. *There was a significant difference between those patients who died and those who survived (Mann-Whitney test).

tients with severe disability (36.5%), 4 with moderate disability (36.5%) and 1 with mild disability (9%). In the group of 10 cases who had an severe increase in the S-100B protein level (>2.0 μ g/L), there were 1 patient with severe disability (10%) and 2 patients with moderate disability (20%) (Table 5, 6), (Fig. 1). These results imply that the S-100B protein level reflected the extent of the severe head injury and helped estimate the prognosis. However, this result was not statistically significant (P=0.221).

The sensitivities of the GCS score, CT findings, and S-100B protein level were 67%, 78% and 78% and the specificities were 83%, 33%, 75%, respectively. The corresponding prediction accuracies of the mortality were 76%, 52% and 76%, respectively (Table 7). If both the GCS score and S-100B protein level were considered together, the prediction accuracy of the mortality was decreased by 57%.

Discussion

It is important for medical personnel to be able to estimate the prognosis after severe head injury. By predicting the pr-

Table 7. Predictive values of score system associated with mortality after severe head injury

	Accuracy (%)	Specificity (%)	Sensitivity (%)	Positive Predictive Value(%)	Negative Predictive Value(%)
GCS	76	83	67	75	77
CT	52	33	78	47	67
S-100B	76	75	78	70	82

*This table shows that the accuracy of Glasgow Coma Scale(GCS) is the same as that of S-100B protein, that the specificity of S-100B protein is lower than that of GCS, and that the sensitivity of S-100B protein is higher than that of GCS

agnosis, it is possible to establish an appropriate treatment strategy quickly and to choose a precise treatment method. Despite the recent progress made in clinical and diagnostic equipment, it is still difficult to predict the prognosis immediately after head injury. It is known that a neurological examination within 24 hours after head injury; the GCS score, the existence of pupil reflex and an abnormal brain stem reflex, etc., allow for a prediction to be made with an accuracy of only 44%⁹⁾. Due to the popularization of brain CT, it is now possible to diagnose the head injury easily and the treatment more rapidly. However, in spite of the combined use of a neurological examination and brain CT, the prognosis can only be predicted with an accuracy of 59%⁷⁾.

Therefore, a number of studies have been conducted on the potential use of biochemical substances to predict the prognosis, instead of relying on GCS and CT. Since the level of a given biochemical substance quantifies the extent of primary head injury more precisely, a more accurate prediction can be obtained. S-100 protein has many subtypes. Among them, type B is called S-100B protein. S-100B protein was originally discovered by Moore¹⁰⁾. It exists mainly in glial and Schwann cells. S-100B protein is found in the cerebrospinal fluid(CSF) only when the brain tissue is destroyed^{11,23)}. Due to its relatively large molecular weight (M=22,000), it is found in serum only when the blood-brain barrier(BBB) is destroyed. Furthermore, it exists for a relatively short time, because its half-life is only 2 hours²¹⁾.

In extra-brain tissue, S-100B protein is found in adipose cells, chondrocytes, etc. However, it is present in such a small amount that it does not have a critical effect on the serum concentration of S-100B protein. Since S-100B protein is not influenced by hemolysis after blood sampling and can be maintained in a stable state without centrifugation or refrigeration, it has been used in research into brain tumors, multiple sclerosis, subarachnoid hemorrhage, acute encephalitis, brain injury, etc¹³⁾. By taking advantage of these properties, Persson et al. investigated the S-100B protein concentration in the CSF of subarachnoid hemorrhage patients and proved that it was sufficiently correlated with prognosis to be used as a prognostic factor for many brain diseases¹²⁾. After Persson's work, the number of

studies on the correlation between S-100B protein and prognosis in brain disease and injury has significantly increased¹⁷⁾. Rothoerl et al. compared the serum concentration of S-100B protein between groups of patients having a GCS score of 9-12 and those of patients less than 9. He observed that the concentration of S-100B protein in patients with severe head injury was remarkably high. Based on this observation, he proved that the level of S-100B protein can quantify the extent of brain damage. Moreover, he showed that in the group with a low GCS score of less than 9, when the concentration of S-100B proteins was low, the outcome was favorable, whereas otherwise the outcome was poor. Finally, he proved that the S-100B protein level can be used as an independent prognostic factor for severe head injury¹⁸⁾.

Since Rothoerl's achievement, a lot of research have been conducted into the correlation between the S-100B protein level and prognosis in severe head injury. According to Raabe's experiment, those patients with a GCS score of less than 9 and S-100B protein concentration of over 2 μ g/L showed a high mortality. When the serum concentration of S-100B protein was over 3.8 μ g/L, the mortality was 100%. Therefore, he concluded that the concentration of S-100B protein was correlated with both mortality and prognosis¹³⁻¹⁶⁾. In this work, 6 of 9 patients with S-100B protein concentration of more than 4.0 μ g/L died. This shows that there is coherence among the findings of Rothoerl, Raabe and this work. According to the findings of our work, mean value of serum S-100B protein concentration was 4.55 μ g/L in the group with a low GCS score of less than 9. The average S-100B value of the patients who died within 2 weeks after head injury was 9.64 μ g/L and that of those patients who survived was 2.91 μ g/L. 2 of 11 patients with S-100B protein concentration of less than 2.0 μ g/L died. But 7 of 10 patients with S-100B protein concentration of more than 2.0 μ g/L died. Our result indicate a strong association of serum S-100B value and mortality after severe head injury. Herrmann et al. showed that an initial S-100B value above 0.14 ng/ml has a high predictive value for short-term and long term neuropsychological deficits in traumatic brain injury²⁶⁾. Woertgen et al. investigated the correlation between the GCS score, the CT findings, the S-100B protein level and prognosis in severe head injury patients whose GCS scores were less than 9. They reported that the prediction rates of the GCS score, CT findings and S-100B protein level were 66%, 59% and 83%, respectively^{7,22-25)}. Therefore, the S-100B protein level was found to be a more powerful independent prognostic factor than CT or GCS.

The same tendency was observed in this work, wherein the accuracies of the GCS score, CT findings and S-100B protein level were found to be 76%, 52% and 76%, respectively. The sensitivity and specificity of the S-100B protein level for the

prediction of the prognosis were found to be 78% and 75%, respectively. In conclusion, the concentration of S-100B protein is an important biochemical marker for the prognosticator in patients with severe head injury. Recently, studies have also been conducted to find the correlation between the S-100B protein level in the CSF and prognosis. According to Ucar's work, the concentration of S-100B protein in the CSF is a more accurate predictive factor than that in the serum²⁰. Therefore, it is necessary to conduct an additional studies on the correlation between S-100B protein level in the CSF and prognosis.

Conclusion

From the investigation of the correlation between the indexes, CT, GCS and S-100B protein, and the prognosis of patients with severe head injury, it was shown that the S-100B protein level has a sensitivity of 78%, a specificity of 75% and an accuracy of 76% in the prediction of the prognosis. Therefore, we conclude that S-100B protein is the most reliable index for estimating the extent of brain damage. Also, in those patients with severe brain injury, an increase in the S-100B protein level in the serum is closely correlated with mortality within 2 weeks of head injury.

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Commentary

The authors performed excellent prospective study of serum S-100B protein in 21 patients with severe brain injury from September 2002 to May 2003. Similar study was obtained by Raabe in 1999. However, In that article, the first serum sample was obtained between 2 hours and 40 hours after head trauma. S100-B protein can be measured in arterial or venous system, is not affected by hemolysis, and remains stable for hours without need of immediate centrifugation and freezing of the sample. However, as mentioned in this article, the biological half-life is only 2 hours. So it is very reasonable that first serum sample was not obtained later than 6 hours after trauma in this manuscript comparing with Raabe's article. S100-B protein is highly specific for the nervous tissue. It is metabolized in kidney and excreted in the urine. Therefore, evaluating for renal function is important to interpret the meaning of S100-B serum level, which is not considered in

this paper.

Including this prospective study, there are lots of clinical and experimental evidences that serum S-100B protein may have potential as a serum marker of brain damage. However, further studies with larger data are required to duplicate these results, give further evidence of specificity and investigate the correlation with secondary insults. We need to pay more attention to clarifying the mechanism of S100-B protein release after brain damage.

elation with secondary insults. We need to pay more attention to clarifying the mechanism of S100-B protein release after brain damage.

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