

Original Article

Increase of plasma S100B level in patients with moderate and severe traumatic brain injury

Shoucheng Fan, Hongjiang Wang, Junzhu Yin

Department of Neurosurgery, The People's Hospital of Xishuangbana Dai Nationality Autonomous Prefecture, Jinghong, Yunnan, China

Received August 13, 2016; Accepted September 28, 2016; Epub November 1, 2016; Published November 15, 2016

Abstract: Background: in this study, we investigated the S100B level in plasma of TBI patients and to explore the correlation between plasma S100B and neurological outcome in TBI patients. Methods: 82 patients with moderate and severe TBI and 60 age-matched controls were enrolled in this study. Samples of plasma were collected within 24 hours (as the initial value), at 72 and 120 hours post injury. Plasma S100B was measured with ELISA and neurological outcome was assessed by Glasgow Outcome Scale (GOS). We compared different levels of these indexes in two groups and further investigated the correlation between each other. Results: we found a significant elevation in the levels of the initial S100B in the plasma ($P < 0.05$), which lasted to 72 hours post injury. Furthermore, we found the plasma S100B in patients with diffuse axonal injury (DAI) was much higher than that in patients without DAI ($P < 0.05$). In addition, there was a positive relationship between the initial S100B and Glasgow Outcome Scale at 6 months post injury ($r = 0.714$, $P < 0.01$). Conclusions: we show that S100B increased in plasma of TBI patients, especially in patients with DAI and the initial plasma S100B is associated with the neurological outcomes.

Keywords: Traumatic brain injury (TBI), S100B, diffuse axonal injury

Introduction

The traumatic brain injury (TBI) is a major cause of death and disability in Western countries [1], and it has become a major public health problem in the United States, because 80,000 to 90,000 people are killed or disabled due to TBI every year [2]. With the development of national economy and transportation, the incidence rate of brain injury is also increasing in China year by year and with more and more lethal residues injured due to TBI [3].

The pathophysiological mechanism implicated in the cellular and molecular changes after TBI remains unclear, and the specific medical treatments for this are also very limited, and further, reliable biomarkers for early prediction of prognosis and functional recovery are very few [4, 5], especially in DAI patients. In recent years, many scholars have begun to explore brain neuron and glial cell-derived substances in fluid whether as an assessment of acute encephalopathy, including the severity of the TBI, effectiveness of treatment, prognosis and

recovery of brain function. This type of study, based on serum protein levels, focused on the neuroimaging, clinical scales and neurophysiological relevance; however, the study with cerebrospinal fluid (CSF) protein levels and their clinical relevance is very limited.

S100B is a low-molecular-weight, calcium-binding protein, which is glial-specific and expressed primarily by astrocytes. It has been shown that S100B is implicated in several neurological, neoplastic, and other types of diseases, including Alzheimer's disease, Down's syndrome, epilepsy, amyotrophic lateral sclerosis and type I diabetes. It has been suggested that the regulation of S100B has potential for the treatment of epilepsy [6-8].

Some studies have shown that there is almost no expression of S100B in normal brain tissues, which is consistent in mammals [9-11]. Found in animal experiments, S100B was significantly increased within the first three hours after the brain injury, reaching a peak at 24 hours, and lasted for nearly a week [12].

Increased plasma S100B in TBI patients

Compared to the wildtype mice, the mice with knock-out of S100B developed an increased astrocyte activity after brain insults [13].

Therefore, in this study, we focused on the plasma levels of S100B in patients with moderate and severe TBI. We also investigated the relationship between these indexes and neurological outcome.

Patients and methods

The People's Hospital of Xishuangbana Human Research Review Committee approved the study. Patients were selected if the time of injury could be ascertained to have occurred within a 24-hour window from insult to ventriculostomy, which was performed for diagnostic and therapeutic purposes. Patient's families gave informed consent for the use of CSF and blood samples of patients; and there were no additional risks associated with the study beyond standard patient care. Thirty TBI patients with a moderate or severe head injury, defined as a Glasgow Coma Scale (GCS) score of 12 or less on admission, and a ventricular catheter were recruited in the study from January of 2012 to October of 2012. There were 21 males and 9 females, with mean age of 48 ± 6 years.

Patients with TBI were excluded if they were thought to have infection, peripheral bleeding disorder, tumor and other concomitant traumatic injuries, such as thoracic and abdominal injuries. Patients with TBI were further grouped into the moderate (GCS >8) or severe (GCS <9) arm. 15 age and gender-matched control subjects (10 males and 5 females with mean age of 47 ± 7 years) were patients with acute headache, in whom head CT were performed to exclude intracranial disorders. Control group had paired plasma collected at the time of head CT. Plasma samples were collected in tubes with sodium heparin. After collection, the samples of plasma were centrifuged at 3000 rpm for 10 minutes at 4 Celsius Degree, and the supernatant was collected, aliquoted, and stored at -80 Celsius Degree.

ELISA for measurement of plasma S100B

Human S100B Quantikine® ELISA kit (Shanghai, China) was used to measure plasma S100B level. Microplates were pre-coated with specific primary antibodies for S100. The specific secondary antibodies were conjugated to horseradish peroxidase. The solution was stopped with sulfuric acid. All patients with TBI had

paired samples at all time points and there were no missing data.

Neurological outcome assessment

We used the GOS to evaluate the long-term neurological outcome for TBI patients at six months post injury. From 4-5, it is considered as good outcome; while, less than 4 (0-3), it is considered as poor outcome.

Statistical analyses

Statistical analyses were performed using the GraphPad Prism software package (version 6.0; GraphPad Software Inc., San Diego, CA). Data are expressed as mean \pm standard error of the mean (SEM). Two paired groups were compared with t test and multiple groups were compared with one-way ANOVA. Furthermore, the relationship between the parameters was tested by Pearson analysis. The ROC was used to assess the acute density of S100B for the assessment. Significance was set as a *P* value of less than 0.05.

Results

S100B level in TBI patients and the control group

Plasma S100B (71.34 ± 3.93) ng/mL levels in TBI patients within 24 hours was significantly different from that in controls ($P<0.05$) (**Table 1**). In addition, the plasma S100B level in patients with severe TBI was significantly higher than that in patients with moderate TBI (82.68 ± 7.22 vs. 42.42 ± 4.94) (**Table 2**). Furthermore, the blood level of S100B was further increased in patients with DAI compared to those without DAI (75.86 ± 9.50 vs. 62.00 ± 4.86).

S100B changes over time

The plasma S100B level reached the peak within 24 hours post injury, which lasted at least 72 hours, and there was no statistical difference between the 24-hour point (71.34 ± 13.93) and 72-hour point (49.63 ± 8.93), but both of them were different from the 120-hour point (26.15 ± 4.89).

ROC curve for S100B and neurological outcome

All TBI patients underwent GOS assessment at six months after onset by an experienced neu-

Increased plasma S100B in TBI patients

Table 1. Plasma S100B levels in TBI patients and the control group

Group	n	S100B
TBI group	60	71.34±3.93
Control group	20	3.51±1.53
P		P<0.05

Table 2. Plasma S100B levels in moderate and severe TBI patients

Group	n	S100B
Severe TBI	33	82.68±7.22
Moderate TBI	27	42.42±4.94
P		P<0.05

Table 3. A positive correlation between S100B and GOS

Time point	Correlation	P
24 hours	0.475	0.008
72 hours	0.420	0.021
120 hours	0.655	0.001

rosurgeon that was blinded to the laboratory data. The average score in severe TBI group was 2.38±0.64, while that in moderate group was 3.91±0.89.

Furthermore, the ROC curve was based on the initial plasma S100B and GCS to correlate them with the long-term neurological outcome assessment. In **Table 3**, ROC analysis indicated that both S100B and GCS status are the prediction of patients' neurological outcome. The comparison of ROC curves, however, indicated that the S100B provided a significantly better prediction of neurological outcome in TBI patients (AUC: 0.819 vs. 0.353). On the ROC curve for S100B, the corresponding specificity at 75.11 ng/mL was 100%, and the sensitivity was 64.7% (**Figure 1**).

Discussion

In this study, we found increased levels of S100B in the plasma of patients with moderate and severe TBI. Moreover, the plasma S100B level lasted 72 hours post brain injury, and recovered to normal level within 120 hours post injury. Furthermore, the initial plasma S100B was significantly higher in patients with DAI compared to those without DAI, which indi-

cated increased S100B might be related to the axonal injury specifically.

The S100B is one of the various members of the S-100 protein family. This 92 amino-acid protein is encoded by the S100B gene located on chromosome 21q22.3 [14]. Although S100B is mainly produced by astroglial and Schwann cells, additional extracerebral sources have been also identified [15]. As protein S100B expression is considerably increased in several types of cancers, mainly of neuroectodermal origin, this biomarker is now used for follow-up and therapeutic monitoring of a variety of malignancies including melanoma, malignant peripheral nerve sheath tumors, schwannomas, paraganglioma stromal cells, histiocytoma, and clear cell sarcomas [15]. Importantly, the European Society for Medical Oncology (ESMO) has recently concluded that protein S100B is the most accurate blood test in the follow-up of melanoma patients [16].

In recent years, the clinical usefulness of protein S100B has extended far beyond the boundaries of cancer diagnostics. A meta-analysis of 22 studies concluded that the sensitivity of this biomarker is as high as 98.7% for a positive CT scan in patients with mild TBI, with a value of specificity approximately 51% [17]. Further decreasing the cut-off value to 0.20 µg/L would allow identifying brain injury with 99.6% sensitivity and 47.0% specificity. Importantly, it was recently demonstrated that the high negative predictive values of protein S100B at ED admission would safely allow withholding CT scans in up to 50% of patients with mild TBI, so decreasing the total healthcare cost for patient management by approximately 30% [18]. It is also noteworthy that the measurement of S100B has consistently overwhelmed that of other potential biomarkers of brain injury, especially neuron-specific enolase (NSE) [19, 20].

Indeed, the future of TBI diagnostics is still unwritten, and other innovative biomarkers such as the neurofilament medium polypeptide (NFM) protein may soon be ready for prime time [21]. In the meanwhile, it seems reasonable to conclude that the measurement of protein S100B represents a valuable perspective for evaluating both children and adults presenting to the ED with mild TBI, provided that an optimal diagnostic cut-offs for ruling out brain lesions can be settled.

Increased plasma S100B in TBI patients

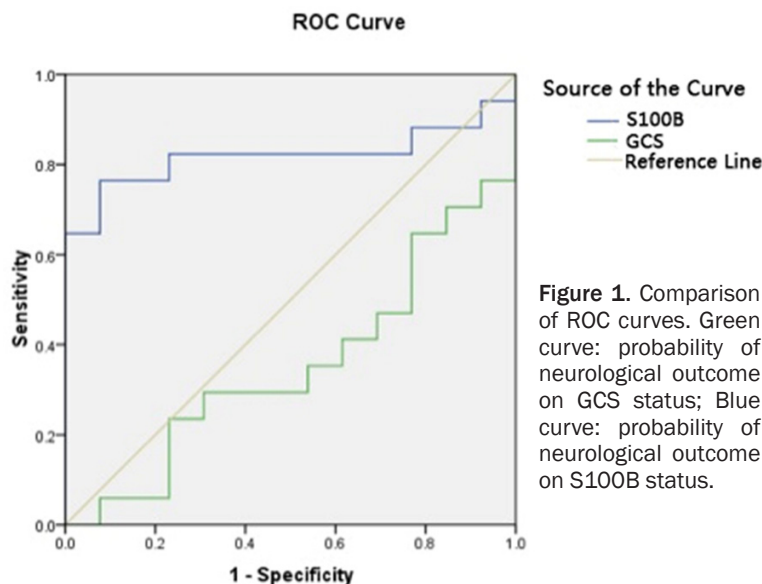


Figure 1. Comparison of ROC curves. Green curve: probability of neurological outcome on GCS status; Blue curve: probability of neurological outcome on S100B status.

Up to now, although some studies have found the increased S100B after TBI, nevertheless, few of them correlate the altered MMPs with the neurological outcome, especially the association with axonal injury. Here, we used the ROC curve to find the initial plasma S100B could predict the neurological outcome with higher sensitivity and specificity, and this further strengthened the interest of targeting S100B to treat patients with TBI.

Inevitably, there were some limitations in our study. First, we held a small number of patient samples with a small statistical power. Even though, a statistical correlation was identified, which indicate the strong predicting role of S100B in TBI session. Second, we only focused on the S100B, actually, there were other calcium-associated proteins might be involved in the neuronal injury. Last, despite this promising evidence, a major drawback remains. The cut-off value used for ruling out a positive CT scan in patients with mild TBI varied widely across different studies, with thresholds comprised between 0.10 and 0.60 $\mu\text{g/L}$. This is mostly attributable to different study populations and the analytical techniques used for measuring protein S100B. As for this issue, Bouvier et al. measured the serum concentration of protein S100B by a commercial chemiluminescent immunoassay in a population of 409 healthy children aged 0-16 years [22], reporting that the serum value decreased in parallel with the age, i.e. from 0.97 $\mu\text{g/L}$ in chil-

dren aged 0-3 months, to 0.58 $\mu\text{g/L}$ in those aged 4-9 months, 0.31 $\mu\text{g/L}$ in those aged 10-24 months, and 0.20 $\mu\text{g/L}$, in children aged 2-16 years, a value that was found to be virtually identical to that of the adult population. Indeed, this is an essential information, wherein different protein S100B cut-offs should be used for assessing the risk of brain lesions in children according to their age. Another important finding that emerged from the study of Bouvier et al. is that the two commercially available chemiluminescent immunoassays display a satisfactory correlation

($r=0.92$), but the overall bias remains too high to permit using identical diagnostic thresholds (i.e. values were found to be 27% higher with the DiaSorin than with the Roche immunoassay). This is not surprising, as similar information has also been published by the STIC-S100 study group [23]. Notably, Bouvier et al. also observed that the reference range of protein S100B in serum was significantly higher in healthy black-skinned than in healthy white-skinned volunteers, thus highlighting further the need to personalize the diagnostic cut-offs of this biomarker. Future study is required to increase the patients sample and detailed stratifying based on patients' characteristics.

In conclusion, our results show that plasma S100B is increased in TBI patients, especially in those with traumatic axonal injury, and the initial S100B is associated with the neurological outcomes. Therefore, we propose that S100B may be a predicting factor for the neurological outcome and monitoring index in TBI sessions.

Disclosure of conflict of interest

None.

Address correspondence to: Hongjiang Wang, Department of Neurosurgery, The People's Hospital of Xishuangbana Dai Nationality Autonomous Prefecture, Jinghong 666100, Yunnan, China. E-mail: sn19n074@126.com

Increased plasma S100B in TBI patients

References

- [1] Bruns J, Hauser WA. The Epidemiology of Traumatic Brain Injury: A Review. *Epilepsia* 2003; 44: 2-10.
- [2] Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil* 2005; 21: 375-378.
- [3] Jiang JY; Chinese Head Trauma Study Collaborators. Head trauma in China. *Injury* 2013; 44: 1453-7.
- [4] Begaz T, Kyriacou DN, Segal J, Bazarian JJ. Serum biochemical markers for post-concussion syndrome in patients with mild traumatic brain injury. *J Neurotrauma* 2006; 23: 1201-1210.
- [5] Kochanek P, Berger R, Bayir H, Wagner AK, Jenkins LW, Clark RS. Biomarkers of primary and evolving damage in traumatic and ischemic brain injury: diagnosis, prognosis, probing mechanisms, and therapeutic decision making. *Curr Opin Crit Care* 2008; 14: 135-141.
- [6] Azeh I, Mäder M, Smirnov A, Beuche W, Nau R, Weber F. Experimental pneumococcal meningitis in rabbits: the increase of matrix metalloproteinase-9 in cerebrospinal fluid correlates with leucocyte invasion. *Neurosci Lett* 1998; 256: 127-130.
- [7] Khrestchatisky M, Jourquin J, Ogier C, Charton G, Bernard A, Tremblay E, Rivera S. [Matrix metalloproteinases and their inhibitors, modulators of neuro-immune interactions and of pathophysiological processes in the nervous system]. *J Soc Biol* 2002; 197: 133-144.
- [8] Kanoh Y, Ohara T, Kanoh M, Akahoshi T. Serum matrix metalloproteinase-2 levels indicate blood-CSF barrier damage in patients with infectious meningitis. *Inflammation* 2008; 31: 99-104.
- [9] Rosenberg GA, Navratil M, Barone F, Feuerstein G. Proteolytic cascade enzymes increase in focal cerebral ischemia in rat. *J Cereb Blood Flow Metab* 1996; 16: 360-366.
- [10] Gasche Y, Fujimura M, Morita-Fujimura Y, Copin JC, Kawase M, Massengale J, Chan PH. Early appearance of activated matrix metalloproteinase-9 after focal cerebral ischemia in mice: a possible role in blood-brain barrier dysfunction. *J Cereb Blood Flow Metab* 1999; 19: 1020-1028.
- [11] Heo JH, Lucero J, Abumiya T, Koziol JA, Copeland BR, del Zoppo GJ. Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. *J Cereb Blood Flow Metab* 1999; 19: 624-633.
- [12] Lin Y, Pan Y, Wang M, Huang X, Yin Y, Wang Y, Jia F, Xiong W, Zhang N, Jiang JY. Blood-brain barrier permeability is positively correlated with cerebral microvascular perfusion in the early fluid percussion-injured brain of the rat. *Lab Invest* 2012; 92: 1623-1634.
- [13] Wang X, Jung J, Asahi M, Chwang W, Russo L, Moskowitz MA, Dixon CE, Fini ME, Lo EH. Effects of matrix metalloproteinase-9 gene knock-out on morphological and motor outcomes after traumatic brain injury. *J Neurosci* 2000; 20: 7037-7042.
- [14] Krohn M, Dressler J, Bauer M, Schober K, Franke H and Ondruschka B. Immunohistochemical investigation of S100 and NSE in cases of traumatic brain injury and its application for survival time determination. *J Neurotrauma* 2015; 32: 430-440.
- [15] Lippi G, Aloe R, Numeroso F, Cervellin G. The significance of protein S-100B testing in cardiac arrest patients. *Clin Biochem* 2011; 44: 567-75.
- [16] Dummer R, Hauschild A, Guggenheim M, Jost L, Pentheroudakis G; ESMO Guidelines Working Group. Melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010; 21 Suppl 5: v194-7.
- [17] Heidari K, Vafaei A, Rastekenari AM, Taghizadeh M, Shad EG, Eley R, Sinnott M, Asadollahi S. S100B protein as a screening tool for computed tomography findings after mild traumatic brain injury: systematic review and meta-analysis. *Brain Inj* 2015; 1-12. [Epub ahead of print].
- [18] Cervellin G, Benatti M, Carbuicchio A, Mattei L, Cerasti D, Aloe R, Lippi G. Serum levels of protein S100B predict intracranial lesions in mild head injury. *Clin Biochem* 2012; 45: 408-11.
- [19] Lippi G, Cervellin G. Diagnostic approach to the mild head trauma of the adult in Emergency Medicine: between biomarkers and imaging. *Recenti Prog Med* 2013; 104: 120-32.
- [20] Childs C, Martínez-Morillo E, Wai AP, Zu MM, Diamandis A, Diamandis EP. Exploring the relationship between serum biomarkers, acute intracerebral changes and outcome after severe traumatic brain injury (TBI). *Clin Chem Lab Med* 2013; 51: e195-7.
- [21] Martínez-Morillo E, Childs C, García BP, Álvarez Menéndez FV, Romaschin AD, Cervellin G, Lippi G, Diamandis EP. Neurofilament medium polypeptide (NFM) protein concentration is increased in CSF and serum samples from patients with brain injury. *Clin Chem Lab Med* 2015; 53: 1575-84.

Increased plasma S100B in TBI patients

- [22] Bouvier D, Duret T, Rouzaire P, Jabaudon M, Rouzaire M, Nourrisson C, Bourgne C, Pereira B, Evrard B, Sapin V. Preanalytical, analytical, gestational and pediatric aspects of the S100B immuno-assays. *Clin Chem Lab Med* 2016; 54: 833-42.
- [23] Laribi S, Kansao J, Borderie D, Collet C, Deschamps P, Ababsa R, Mouniam L, Got L, Leon A, Thoannes H, Santin A, Kouyoumdjian JC, Dahyot-Fizelier C, Millet C, Golmard JL, Beaudoux JL; Stic-S100 Study Groupa. S100B blood level measurement to exclude cerebral lesions after minor head injury: the multicenter STIC-S100 French study. *Clin Chem Lab Med* 2014; 52: 527-36.