The relationship between S100B protein serum levels, injury severity and Glasgow Outcome Scale values in children with CNS injuries

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Abstract

OBJECTIVES: The S100B protein subgroup is a thermolabile acidic calcium-binding protein, which was first described in association with the central nervous system. Destruction of nerve tissue results in S100B protein release from astrocytes and elevation of its levels in cerebrospinal fluid. If the blood-brain barrier is also damaged, S100B can pass into the systemic circulation and elevated blood levels of S100B can be detected. High S100B serum levels in patients with head injuries are predictive of possible development of secondary brain injury and may be related to the extent of permanent injury to the CNS .

MATERIAL AND METHODS: The authors present results obtained from a group of 39 children aged 0 (newborns) to 17 years with an isolated craniocerebral injury.

RESULTS: In our group of 39 children (aged 0–17 years) we observed excellent GOS group (GOS – Glasgow Outcome Scale 4 or 5) in 33 patients at the time of transfer from our intensive care unit to the neurological department. There were no deaths and only 6 children were in the poor GOS group (GOS 2 or 3). A second GOS evaluation was performed 6 months later: at this time 36 children were in the excellent GOS group and only 3 children remained in the poor GOS group.

CONCLUSIONS: Due to high variability in S100B protein serum levels in children (dependent on age and gender) no correlation between initial S100B levels and GOS was observed for our group of patients. Our results indicate that the rate of decrease of S100B protein levels back to normal values is more meaningful than its absolute value.

ORIGINAL ARTICLE

INTRODUCTION

Craniocerebral injury is the leading cause of morbidity and mortality in children (Brichtová & Kozák 2008). This consideration creates a need for a specific biomarker which facilitates targeted treatment and provides a method of predicting outcomes. The S100B protein was first described in the central nervous system. High levels of S100B (S-100 B, beta-beta dimer) are found in central nervous tissue glial cells (primarily astrocytes) and in peripheral cells such as Schwann cells and adipocytes. However, only in glial cells do we find that the protein homodimer consists of alpha subunits. In 95% of healthy adults, S100B serum levels are lower than 0.1 μg/l (median 0.04 μg/l). In adulthood, there are no age and gender differences in S100B levels; however, in children, S100B levels vary with age (Portela *et al.* 2002), which reflects its neurotrophic role. Destruction of nerve tissue and neurodegenerative disabilities results in elevated levels S100B protein in the CSF (cerebrospinal fluid) in response to its release from astrocytes. If the blood-brain barrier is also damaged, S100B can pass into the systemic circulation and elevated S100B levels can be detected in the blood (Townend *et al.* 2002; Thornhill *et al.* 2000). Elevated S100B serum concentrations have been associated with brain trauma, cerebral ischemia of any etiology, brain infection, and hypoxic damage to the CNS. In uncomplicated recoveries, S100B levels return to normal values within a week. High initial S100B serum levels shortly after injury, reflects cell damage and increased permeability of the blood-brain barrier (BBB). It is, therefore, an indicator of high-risk patients who are likely to develop secondary injuries to the CNS (central nervous system) (Townend *et al.* 2002). While absolute levels suggest high-risk patients, protein level fluctuations seem to be an indicator of severity of permanent damage to the CNS and permanent neuronal deficits. It is important to note that the extent of permanent damage does not correlate with the severity of injury (Thornhill *et al.* 2000).

METHODS

Patients with isolated trauma to the CNS, hospitalized in our ICU (intensive care unit) from 2004–2008 were included in our study. The age of affected children ranged from 0–17 years, with an average age of 8.94 years. The male:female ratio was 31:8. The average value of the GCS on admission was 7.538 and the average GOS was 4.17. Coagulable blood was drawn to determine S100B protein serum levels. The blood draw protocol was that the first sample was obtained immediately after admission to the bed ward (hour zero) and then at hour 6, 12, 24, 36, 48, and 72. Measurements were performed using the ECLIA immunoassay method and a Roche Elecsys 2010 apparatus. We also performed a GCS evaluation on admission or got the result from the emergency department, and we performed a GOS at the time of transfer to the pediatric neurology department. An additional GOS evaluation was performed 6 months after the injury. The average initial S100B protein level was 1.878 μg/l, with a maximum of 13.15 μg/l and a minimum of 0.061 μg/l. The average S100B protein level at discharge was 0.222 μg/l, with a maximum of 2.01 μg/l and a minimum of 0.012 μg/l. Inclusion criteria were: parental consent and an isolated traumatic brain injury. Exclusion criteria – no parental consent, patients with polytrauma, patients with a history of epileptic seizures during the 10 days prior to injury, children with Down syndrome, retarded development or any neurological disease. Patients with polytrauma were excluded because of the possibility of increased S100B serum levels due to S100B released from adipocytes, as well as issues described below. Down syndrome patients normally show elevated S100B levels, which was why they were excluded. Children with burns, fractures of long bones or chest wall contusions were also excluded from the group. Several studies have been published showing that bone marrow is a potential extracerebral source of S100B protein (Routsi *et al.* 2006) and S100B protein levels have also been shown to increase in response to burn injury (Lindberg *et al.* 2008). CT finding and neurosurgical interventions, if needed, were recorded for each patient. GOS was evaluated at the time of transfer to the department of pediatric neurology, which was generally 10–15 days after injury. The S100B protein cut-off value obtained when using the Elecsys Roche Diagnostics analytic system in adults is 0.105 μg/l. In children, S100B protein levels are higher and vary with age (Portela *et al.* 2002), additionally, girls have higher levels than boys. The median and range of S100B protein levels for 0–1 year of age is 0.95 μg/l (median) and 0.44–2.55 μg/l, which is significantly higher than the median and range for children aged 2–7 years (0.73 μg/l, 0.44–1.06 μg/l, respectively). The highest individual concentrations of S100B protein were detected in children during their first year of life and between 9 and 10 years of age (Gazzolo *et al.* 2003). From 16 years of age, S100B values approach those of adults. Physiological fluctuation of levels with age is due to one of the functions of the S100B protein, i.e. controlling maturation of the mammalian brain.

RESULTS

By means of statistical analysis, we attempted to test whether there was a relation between the S100B protein levels and GCS and GOS. We also looked for a potential relationship between the rate of S100B protein decrease, at a specific times, and GCS, and GOS. Simple statistics (Table 1) show the average levels of S100B protein at a given hour for the whole group. They also show by what amount the S100B level decreased between two measured blood samples. To determine the relationship, the Pearson's correlation coefficient (Table 2) was used, which is a measure of linear relationships between

a paired variables (it is affected by outlying values). It showed a relationship between S100B levels, at a specific hour, and GCS and GOS; it also showed how statistically significant the decreases were in S100B protein levels, between two blood samples, all in relation to GCS and GOS.

This measurement shows that the relationship of S100B protein level taken at hour zero and GCS on admission was statistically significant (at the 5% level of significance). That is, the severity of initial impairment correlates with higher S100B levels. The measurement also indicates that there is no statistically significant correlation between the initial S100B protein level and GOS, i.e. the patient's condition at discharge. The correlation between S100B protein level at hour 72 and GOS was found to be significant. Also the correlation of protein level decrease from hour 0 to hour 6, in relation to GCS, as well as the protein level decrease from hour 0 to hour 72, in relation to GCS, was significance (p=0.05). A statistically significant correlation of protein level decrease from hour 24 to hour 72, in relation

Tab. 1. Basic statistics.

Variable	N	Mean	Std Dev	Median	Min	Max
_0_hod	39	1.878	3.268	0.507	0.061	13.150
_6_hod	39	0.689	1.517	0.170	0.022	7.210
_12_hod	39	0.406	0.840	0.130	0.034	4.410
_24_hod	39	0.320	0.681	0.091	0.026	3.057
_48_hod	39	0.242	0.553	0.082	0.017	2.590
_72_hod	39	0.223	0.442	0.068	0.012	2.010
cortdif6_0	39	–1.189	2.750	–0.310	–12.384	0.146
cortdif12_0	39	–1.472	2.957	–0.347	–12.567	0.893
cortdif24_0	39	–1.558	3.028	–0.395	–12.872	0.933
cortdif48_0	39	–1.637	3.148	–0.429	–12.981	2.133
cortdif72_0	39	–1.655	3.146	–0.436	–12.991	1.223
cortdif12_6	39	–0.283	0.833	–0.032	–4.190	0.747
cortdif24_6	39	–0.369	0.930	–0.054	–4.153	0.787
cortdif48_6	39	–0.447	1.243	–0.081	–5.118	1.987
cortdif72_6	39	–0.466	1.243	–0.085	–5.200	1.077
cortdif24_12	39	–0.369	0.930	–0.054	–4.153	0.787
cortdif48_12	39	–0.447	1.243	–0.081	–5.118	1.987
cortdif72_12	39	–0.466	1.243	–0.085	–5.200	1.077
cortdif48_24	39	–0.078	0.392	–0.012	–1.868	1.200
cortdif72_24	39	–0.097	0.391	–0.022	–1.879	0.717
cortdif72_48	39	–0.019	0.209	–0.010	–0.910	0.711
gcs	39	7.538	4.103	7.000	3.000	14.000
gos	39	4.179	0.854	4.000	2.000	5.000
gdifabs	39	3.359	3.580	3.000	–2.000	9.000
gdifrel	39	1.740	0.735	1.600	0.600	2.800

Tab. 2. Pearson Correlation Coefficients (N=39).
Prob > |r| under H0: Rho=0

Variable	gcs	gos	gdifabs	gdifrel
_0_hod	–0.377	–0.303	–0.360	–0.354
0#hod	0.018	0.061	0.024	0.027
_6_hod	–0.188	–0.317	–0.140	–0.086
6#hod	0.251	0.049	0.395	0.601
_12_hod	–0.272	–0.507	–0.190	–0.118
12#hod	0.094	0.001	0.245	0.473
_24_hod	–0.231	–0.477	–0.151	–0.077
24#hod	0.157	0.002	0.359	0.639
_48_hod	–0.234	–0.472	–0.155	–0.089
48#hod	0.152	0.002	0.345	0.592
_72_hod	–0.172	–0.432	–0.094	–0.027
72#hod	0.296	0.006	0.570	0.869
cortdif6_0	0.345	0.186	0.351	0.373
CortDif6_0	0.032	0.258	0.029	0.019
cortdif12_0	0.340	0.191	0.344	0.358
CortDif12_0	0.034	0.244	0.032	0.025
cortdif24_0	0.355	0.220	0.355	0.365
CortDif24_0	0.026	0.178	0.027	0.022
cortdif48_0	0.351	0.232	0.346	0.352
CortDif48_0	0.029	0.156	0.031	0.028
cortdif72_0	0.368	0.254	0.361	0.364
CortDif72_0	0.021	0.118	0.024	0.023
cortdif12_6	0.069	0.066	0.063	0.038
CortDif12_6	0.677	0.691	0.702	0.818
cortdif24_6	0.138	0.168	0.118	0.084
CortDif24_6	0.402	0.307	0.474	0.610
cortdif48_6	0.126	0.176	0.102	0.066
CortDif48_6	0.446	0.283	0.537	0.690
cortdif72_6	0.169	0.233	0.138	0.096
CortDif72_6	0.305	0.153	0.404	0.562
cortdif24_12	0.138	0.168	0.118	0.084
CortDif24_12	0.402	0.307	0.474	0.610
cortdif48_12	0.126	0.176	0.102	0.066
CortDif48_12	0.446	0.283	0.537	0.690
cortdif72_12	0.169	0.233	0.138	0.096
CortDif72_12	0.305	0.153	0.404	0.562
cortdif48_24	0.071	0.162	0.043	0.010
CortDif48_24	0.667	0.326	0.796	0.954
cortdif72_24	0.208	0.342	0.157	0.104
CortDif72_24	0.204	0.033	0.341	0.528
cortdif72_48	0.255	0.337	0.212	0.177
CortDif72_48	0.117	0.036	0.195	0.282

to GCS and GOS, was also interesting. This means that the absolute value of the S100B protein level relative to the GOS of the patient was less meaningful compared to the rate of decrease of S100B levels, especially starting from hour 24. The decrease between hour 0 and all the other time-points also shows a statistically significant relation to the GCS.

CONCLUSION

The relationship between S100B protein levels and the severity of injury and GCS has already been thoroughly documented (Berger *et al.* 2007). The objective of this study was to answer the question whether monitoring S100B in the pediatric population would have any clinical value. Due to great variability (age and gender) of S100B protein serum levels in children, we were unable to find a correlation between initial protein levels and GOS in our study group. Our results indicate that the speed with which S100B protein level decrease toward normal values is more meaningful than absolute S100B values. Children in whom protein levels returned to normal within 6 hours after injury all achieved a GOS score of 5. Children in whom protein levels decreased to normal after hour 72 achieved GOS scores of 2 or 3.

Spinella et al (2003) monitored S100B levels in 27 children with TBI (traumatic brain injury). 6 of these children died. Lo *et al.* (2009) divided a group of 28 children with TBI into those with a good GOS scores (4 or 5) and those with a poor GOS scores (1, 2 or 3). Deaths are not clearly mentioned, however, the poor GOS group consisted of 4 patients. Our sample included 39 children aged 0–17 years. Excellent GOS (4 or 5) had been achieved in 33 patients by the time of transfer to the neurological department of our ICU. There were no deaths and only 6 children were in the poor GOS group (2 or 3). At the second GOS evaluation, which took place after 6 months, there were 36 patients in the excellent GOS group and only 3 children in the poor GOS group. Our results and comparison with other works show that elevated S100B levels are not an indication for a CT scan on admission. The exact clinical condition of the patient and the mechanism and circumstances of the injury represent the best indication for a CT examination on admission. However, the elevation of S100B levels has certain significance in a fully monitored patient during hospitalization. In this case, it may be an indication for a follow-up CT scan. What is the therapeutic significance of elevated S100B levels? Persistent elevated S100B levels may be important for decision-making regarding invasive monitoring of the patient or may facilitate the decision about an eventual tracheostomy in ventilated patients. These facts indicate that an experienced pediatric intensive care physician and a timely neurosurgical intervention performed with the maximum possible gentleness with regard to the child's developing brain play the crucial role in the recovery of child with a TBI.

DISCUSSION

There have been various studies investigating S100B levels in adults and children. Because of the various methods employed, the results are difficult to compare. S100B levels can be determined using chemiluminescence immunoassay, electro-chemiluminescence, immunoradiometric assay, ELISA, and immunofluorometric assay. The studies examined the levels of S100B in the serum, CSF, urine, but also using MR spectroscopy (Berger 2006; Berger & Kochanek 2006; Kleindienst *et al.* 2005; Shore *et al.* 2007).

S100B values measured in patient samples can vary depending on the determination method used. Therefore, laboratory findings must always include information regarding which test was used for S100B determination. The S100B values determined from a patient's sample using different tests cannot be directly compared with each other and doing so could cause an incorrect medical interpretation. If the test procedure employed to determine S100B levels, and which serves for treatment monitoring, is changed, it is necessary to compare the results of both test options before the change is made. Another problem of affecting the usefulness of this and other CNS biomarkers in children is that, in contrast to adults, there is no established normal level. In healthy adults between 18–65 years of age, the median S100B serum concentration is 0.052 μg, ranging from 0.023 to 0.097 μg, with no age or gender differences. The recommended cutoff for S100B level elevation in adults ranges from 0.2 to 0.5 μg (Berger 2006).The higher threshold would be less sensitive but more specific. Determination of normal levels in children is difficult. Spinela *et al.* (2003) determined, in a group of 136 healthy children, that the median serum level of S100B was 0.3 μg. This value correlated with age. Portella *et al.* (2002) arrived at approximately the same median. Berger *et al.* (2007) determined in their patient cohort a median of 0.016 ng/ml (ELISA method). On a set of 394 children admitted for elective surgery (extracerebral surgery), Castellani *et al.* (2008) found an upper normal limit of 0.16 μg/l (electro-chemiluminescence method, Roche). In this study, age ≤ 3 years was missing because of a small number of patients. The largest study was probably the one conducted by Gassolo *et al.* (2003) on a set of 1004 healthy children aged 1 month to 15 years. Their study showed age and gender differences and normal levels of S100B were significantly higher in girls than in boys. The highest values were found in children in the first year of life, another increase was observed between the ages of 7 and 13 years and from 13 years on the values decreased.

The authors assume that the high level observed during the first year of life was due to CNS development and maturation together with different permeability characteristics relative to the blood-brain barrier. The increase between 7 and 13 years of age can attributed to

the neurotrophic role of the protein in the acceleration of development and growth in this age group.

The median for the first year of life, according to this study, was 0.81–0.9 μg/l (M-F); for a group of 6 year olds, the median was 0.6–0.86 μg/l. The method used was Sangtec Medical immunoluminometric assay. A final caveat regarding the use of S100B levels in the evaluation of CNS injury severity is its extracerebral production in patients with neurological disease, premature babies, post-hypoxic conditions, encephalopathy, Down syndrome, multiple trauma, fractures of long bones, chest contusion and many other diseases and conditions (Nigaard *et al.* 1997, Maschmann *et al.* 2000, Nagdyman *et al.* 2001).

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