Prognostic properties of the association between the S-100B protein levels and the mean cerebral blood flow velocity in patients diagnosed with severe traumatic brain injury

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Abstract. Craniocerebral injury (CBI) is tissue damage caused by a sudden mechanical force. CBI can result in neurological, neuropsychological and psychiatric dysfunctions. Currently, the severity of CBI is assessed using the Glasgow Coma Scale, brain perfusion pressure measurements, transcranial Doppler tests and biochemical markers. This study aimed to determine the applicability of the S-100B protein levels and the time-averaged mean maximum cerebral blood flow velocity (V_{mean}) as a means of predicting the treatment outcomes of CBI in the first 4 days of hospitalization. The results validated the standard reference ranges previously proposed for the concentration of S-100B (0.05-0.23 μg/l) and the mean of cerebral blood flow velocity (30.9 to 74.1 cm/sec). The following stratification scheme was used to predict the success of treatment: Patients with a Glasgow Outcome Scale (GOS) score ≥4 or GOS <4 were stratified into 'favorable' and 'unfavorable' groups, respectively. The favorable group showed relatively constant levels of the S-100B protein close to the normal range and exhibited an increase in V_{mean}, but this was still within the normal range. The unfavorable group exhibited a high level of S-100B protein and increased V_{mean} outside of the normal ranges. The changes in the levels of S-100B in the unfavorable and favorable groups were -0.03 and -0.006 mg/l/h, respectively. Furthermore, the rate of decrease in the V_{mean} value in the unfavorable and favorable groups were -0.26 and -0.18 cm/sec/h, respectively. This study showed that constant levels of S-100B protein, even slightly above the normal range, associated with an increase in V_{mean} was indicative of a positive therapeutic outcome. However, additional research is required to obtain the appropriate statistical strength required for clinical practice.

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Introduction

Craniocerebral injury (CBI) is a heterogeneous group of non-congenital tissue damages caused by a sudden mechanical force, which results in neurological, neuropsychological and psychiatric dysfunctions (1,2). Currently, CBI is one of the leading causes of death in addition to cardiovascular disease and cancer (3). A histogram of age-related CBI shows a bimodal distribution with the first and second maximums for subjects aged 15 and >75 years old, respectively (4). CBI is more common amongst men than among women, showing a ratio between 1.9:1-2.8:1 (4-8).

Current clinical diagnosis of CBI is based on a number of methods, including the Glasgow Coma Scale (GCS) (9), the measurement of cerebral perfusion pressure, the Transcranial Doppler Test (TCD) and analysis of the levels of biomarkers, allowing quantitative evaluation and treatment monitoring of brain tissue damage (10,11).

Amongst a variety of biomarkers of CBI, the serum levels of S-100B reflects the degree of posttraumatic brain damage (10,12-30). S-100B is secreted primarily by astrocytes in the cerebral cortex (31,32) and is present in large quantities in astroglial cells (33). It also plays an essential role in cell growth metabolism (34). S-100B secretion triggers autocrine and paracrine effects in glial cells, microgel cells and neurons (35). Furthermore, S-100B stimulates neuronal growth in nanomolar and picomolar concentrations (36), and induces apoptosis in micromolar concentrations (37).

TCD spectral analysis is used to determine the velocity of blood flow through the maximum systolic velocity, the end-diastolic velocity and the time-averaged mean maximum velocity (V_{mean}) in insolated blood vessels (38). The clinical practicality of V_{mean} values has been confirmed in the treatment of severe cases of traumatic brain injury (TBI) (39).

Given the importance of precise prognostic methods for the diagnosis and effectiveness of TBI treatment, advances in the

quality of the early diagnosis of CBI are essential. Therefore, the time-related changes in S-100B protein levels and the V_{mean}, as well as the associations between these parameters in patients diagnosed with severe CBI as defined by a GCS score ≤8 were investigated in the present study.

Materials and methods

Study subjects. All experiments and methods were performed following relevant guidelines and regulations (40). The Bioethics Committee of the Postgraduate Education Medical Center (Warsaw, Poland) approved the experimental protocols (approval no. 501-2-1-20-49/04). Informed consent was obtained from all subjects or their guardians, and a parent/legal guardian of subjects under 18 years of age.

The present study included patients with severe CBI (GCS score ≤8) admitted to the Department of Neurosurgery and Trauma of the Nervous System at the Medical Center of Postgraduate Education (Warsaw, Poland).

Sample stratification. The total number of patients included in the study was 60, consisting of 51 patients in the unfavorable group and 9 patients in the favorable group. The mean age ± standard deviation were 48.41±15.24 years (age range, 19-73 years) and 47.20±16.64 years (age range, 14-75 years) in the unfavorable and favorable groups, respectively.

The GCS score was calculated at admission using an internal encoded GCS calculator (41,42) written in Python (Python Software Foundation; python.org/).

According to the European Consortium of Brain Injury Guidelines, all patients were subjected to the standard diagnostic and therapeutic protocols (43). In patients for whom poor ventilation was suspected, a gasometric examination, using a CDI™ blood parameter monitoring system 500, was performed to optimize pCO_2 (range, 30-40 mmHg). Furthermore, hematocrit and hemoglobin levels were maintained at 30-40% and 12-14 g/dl, respectively.

On discharge from the Department of Neurosurgery, the patient's health was evaluated using the traditional Glasgow outcome scale (GOS) (44), which is comprised of the following five categories: 1, death; 2, persistent vegetative state; 3, severe disability; 4, moderate disability; and 5, low disability. For this study, patients with a GOS ≥4 and GOS <4 were classified as 'favorable' and 'unfavorable', respectively.

The inclusion criteria, based on an analysis of GOS as described above, resulted in a study group of 60 patients (48 men and 12 women). The clinical description of the study group after admission to the hospital according to the GCS and Marshall (MCTC) classification (45) is presented in Fig. 1.

The S-100B protein levels were measured in 5 ml venous blood samples collected from patients upon admission to the hospital. Subsequent blood samples were collected at 24-h intervals for 96 h. After clotting and centrifugation for 10 min at 2,000 x g at 4˚C, blood samples were stored for further use at -22˚C. The S-100B protein concentration was measured using a Anti-S-100 antibody kit (S1-61; cat. no. sc-53438; Santa Cruz Biotechnology, Inc.) according to the manufacturer's protocol (Liaison Sangtec 100; Sangtec Ltd.). The Sangtec 100 kit uses three different monoclonal antibodies (SMST12, SMSK 25 and SMSK 28) directed against the β-chains of the S-100B homodimer, and has a wide detection range (0.02-30 μg/l). Protein concentration was measured using a LIAISON analyzer (DiaSorin) calibrated with a freeze-dried Sangtec 100 Cal (Low/High) calibrator. The sensitivity threshold for this test was 0.02 μg/l.

V_{mean} was measured by subjecting patients to a transcranial Doppler examination using a Medasonic Transpect CDS Doppler (Medasonics, Inc.) in the power motion mode TCD (46,47). First, the arteries of the brain base, accessible through the temporal window, were examined. The middle cerebral arteries on the side of the dominant lesion or on the right side of the extent of the lesion were further analyzed. This examination was performed at 24-h intervals for 96 h after the patient was admitted to the Department of Neurosurgery.

Similar to that for the S-100B protein levels, the reference value for $V_{mean,}$ (<30.9 cm/s) was derived from the study in a group comprising 40 healthy volunteers (22 men and 18 women). The mean age of the reference was 43.4±9.17 years (range, 30-61 years).

Statistical analysis. A Shapiro-Wilk test (48) was used to assess the distribution of the parameters investigated. Parameters exhibited either skewed or normal distribution, and the subsequent analysis used was based on the distribution of the data. Data are presented as the mean ± standard deviation, and the minimum and maximum values. Differences between study groups (favorable vs. unfavorable) at a specific time were assessed by analyzing the bootstrapped difference in the means, in which a sample of 10,000 repeats with replacement was used (49). Differential statistics on continuous outcomes of S-100B protein concentration and V_{mean} were performed using a one-way aligned rank transform for nonparametric factorial ANOVA (50). The clinical treatment outcome factor encompassed two levels (favorable and unfavorable). Due to the shortcomings of current statistical methods in handling advanced nonparametric statistics, it was decided only to discuss one-way nonparametric factorial ANOVA results.

Given the repeated nature of the data and the mortality of the patients, the data was censored to balance the factorial ANOVA model. Post hoc analysis was performed using the estimated marginal means (emmeans) procedure. The velocity of time-dependent changes in a specific parameter is defined by the slope (tangent) of a line obtained from connected means at consecutive measurement times. $P<0.01$ was considered to indicate a statistically significant difference. All analyses were performed in R (51).

Results

This study was carried out using two groups of patients stratified by the GOS score (52) at discharge; patients were classified into either an unfavorable (GOS score <4) and favorable (GOS score ≥4) group. No significant differences in age were found between the groups. Fig. 1 shows a general description of the severity of craniotrauma in patients assessed using the GOS and MCTC scores (53).

The reference range obtained from a healthy patients reference group consisting of 40 healthy volunteers [22 men and 18 women; 47.0±14.77 (age range, 21-80)] for the S-100B levels used in this study was 0.05-0.23 μg/l. A graphical and

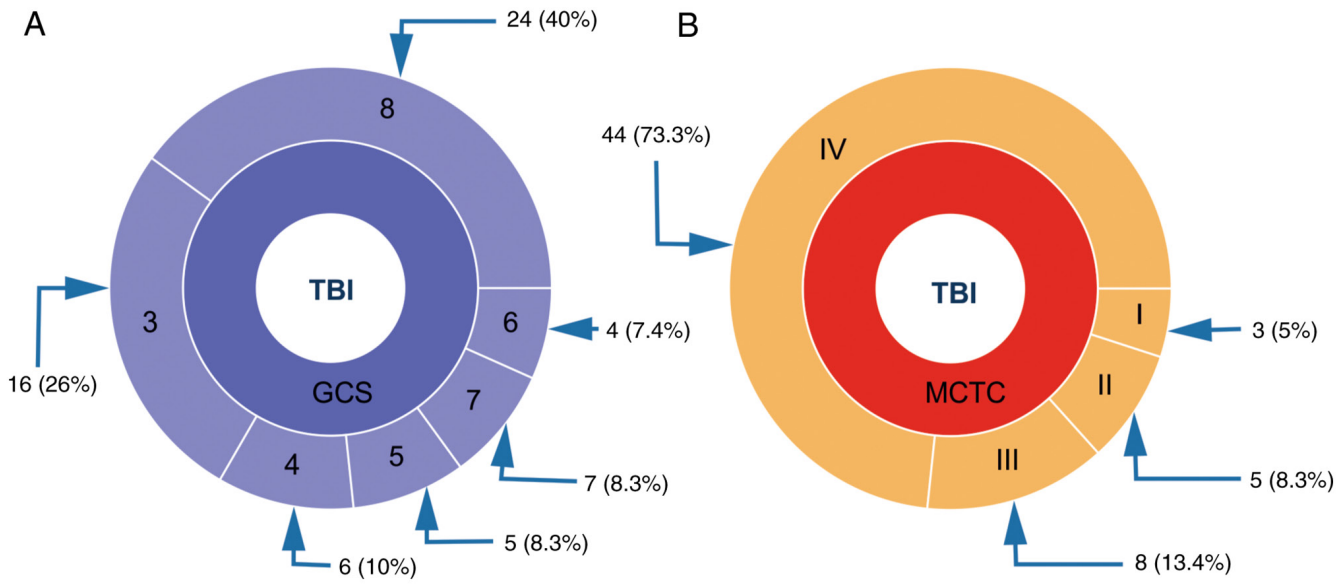

Figure 1. Clinical classification of patients admitted to the Department of Neurosurgery and Trauma of the Nervous System of the Medical Center of Postgraduate Education (Warsaw, Poland). (A) In group GCS, the numbers 3-8 correspond to GCS classification level and (B) MCTC distribution. Numerals correspond to the number of cases (percentage of cases). TBI, traumatic brain injury; GCS, Glasgow Coma Scale; MCTC, Marshall classification of traumatic brain injury.

numerical representation of the changes in S-100B levels is shown in Fig. 2 and Table I. The results showed that the patients in the unfavorable group had higher levels of S-100B than those in the favorable group at all measured time points. No statistically significant time-dependent differences, defined by the lack of an overlay between specific confidence intervals, in S-100B concentrations were found within the unfavorable group. However, a significant decrease in serum S-100B protein levels was found between measurements at 24 vs. 48 h, 24 vs. 72 h and 24 vs. 96 h in the favorable group. The difference in the S-100B decrease velocity between the two groups showed a relative decrease equal to 5.4, with velocities of $V_{S\text{-}100B\_U}$=-0.03 μg/l/h and $V_{S\text{-}100B\_F}$=-0.006 μg/l/h for the unfavorable and favorable groups, respectively.

Cerebral flow impairment was analyzed using V_{mean} levels as a function of hospitalization time. The respective data are presented in Table II and Fig. 3. Analysis showed that the patients in the unfavorable group had a significantly lower V_{mean} value than those in the favorable group. Statistically significant differences in V_{mean} were observed between 24 and 96 h and between 48 and 96 h in the unfavorable group. The relative difference in the V_{mean} increase between the unfavorable and favorable groups was 1.44 (V_{mean_U}=0.26 cm/sec/h, V_{mean_F}=0.18 cm/sec/h, respectively).

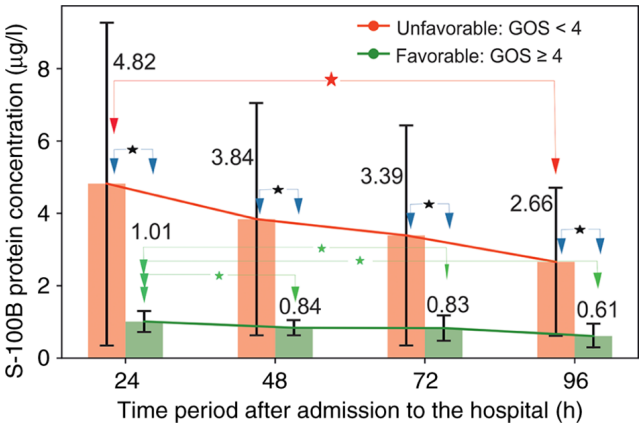

Figure 2. Changes in S-100B protein concentration stratified by GOS level evaluated on discharge from the Department of Neurosurgery. The error bars represent the standard error of S-100B concentration at each specific time point. The blue arrows show differences in the means between the unfavorable and favorable groups. The red arrow shows the statistically significant differences in S-100B levels within the unfavorable group at different time points. The green arrow shows the statistically significant differences within the favorable group at different time points. *P<0.01.

Discussion

Due to the poor prognosis of the treatment outcomes of TBI patients (54-63), rendered by the insufficient discriminatory capacity of the current prognostic indicators, the present study assessed the clinical applicability of S-100B protein levels and the V_{mean} as potential prognostic factors.

Briefly, this study introduced and validated a novel concept for predicting a treatment outcome in patients in a favorable and unfavorable group using an amalgam of physical and biochemical parameters collected during the initial hospitalization stage, encompassing the first 4 days after hospital admission.

Over the past 20 years, studies have focused on patient treatment outcomes following a TBI (64-66). Mercier *et al* (56) summarized 41 reports on the applicability of correlations between TBI and the S-100B levels in patients with severe TBI for long-term prognosis. However, the report described unfavorable treatment results for increased levels of S-100B protein. The present study improved on predicting treatment outcomes based on a combination of physical and biochemical parameters collected during the initial stage of hospitalization.

Table I. Changes in S-100B levels stratified by the Glasgow Outcome Scale score on discharge from the Department of Neurosurgery.

A, Unfavorable group

Time, h	Mean, mg/l	Standard deviation	Min, mg/l	Max, mg/l	Number of subjects
24	4.82	4.45	0.76	19.8	51
48	3.84	4.21	0.47	16.8	48
72	3.39	4.04	0.38	17.83	40
96	2.66	3.05	0.136	16.7	37
Mean	3.68	3.94	0.44	17.78	-

B, Favorable group

Time, h	Mean, mg/l	Standard deviation	Min, mg/l	Max, mg/l	Number of subjects
24	1.01	0.29	0.71	1.6	9
48	0.84	0.21	0.62	1.3	9
72	0.83	0.35	0.51	1.5	9
96	0.61	0.24	0.39	1.1	9
Mean	0.82	0.27	0.56	1.38	-

Table II. Changes in time-averaged mean maximum cerebral blood flow velocity stratified by Glasgow Outcome Scale score on discharge from the Department of Neurosurgery.

A, Unfavorable group

Time, h	Mean, mg/l	Standard deviation	Min, mg/l	Max, mg/l	Number of subjects
24	32.06	11.31	5	67	51
48	39.73	16.53	6	75	48
72	38.78	20.77	5	120	40
96	45.43	25.1	6	145	37
Mean	39.00	18.43	5.50	101.75	-

B, Favorable group

Time, h	Mean, mg/l	Standard deviation	Min, mg/l	Max, mg/l	Number of subjects
24	41.78	7.17	32	56	9
48	51.56	15.53	36	87	9
72	52	11.43	39	75	9
96	60.38	14.99	44	91	9
Mean	51.43	12.28	37.75	77.25	-

The therapeutic outcomes of treatment were validated against the GOS, which was evaluated on the 4th day of hospitalization. Patients with a GOS score <4 or GOS score ≥4 were stratified into unfavorable and favorable groups, respectively. The mean age of the study sample was 48.73±16.3 years, which is slightly higher than previously reported (67,68). However, no significant differences were determined were found between the age distribution of the present study and those of Jain *et al* (67) and Jennett (68) using a Student's t-test. The mortality rate of the patients in the current study was 26.6%, which is higher than previously reported (69). The reasons for these differences are unknown.

The reference range for the normal concentration of S-100B established in the present study was 0.05-0.23 μg/l. The lower limit of the reference range was similar to that shown in previous studies. However, the upper limit was twice as high (70). In addition, the upper reference limit obtained in the present study was twice the value reported for patients

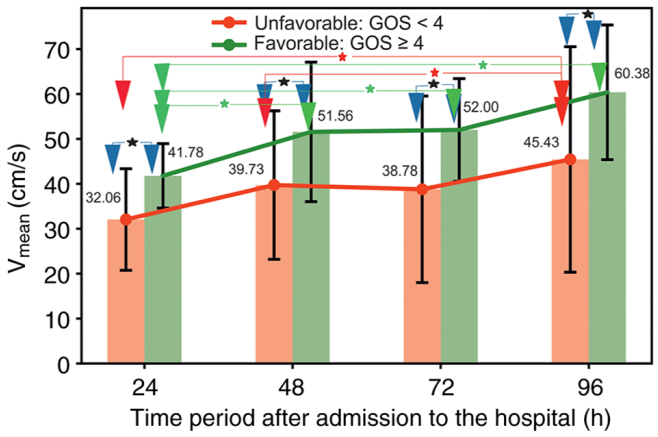

Figure 3. Changes in V_{mean} stratified by the GOS score evaluated on discharge from the Department of Neurosurgery. Errors bars represent the standard error of S-100B concentration at each specific time point. The blue arrows show the differences in means between the unfavorable and favorable groups. The red arrow shows the statistically significant differences in S-100B levels in the unfavorable group. The green arrow shows statistically significant differences in the favorable group. *P<0.01. GOS, Glasgow Outcome Scale; V_{mean}, time-averaged mean maximum cerebral blood flow velocity.

with an isolated head injury for whom CT scans for mild TBI were negative (71,72). Furthermore, an apparent discrepancy was found between the pathological levels of S-100B reported in this study (>0.235 μg/l) and those of a previous study (>0.5 μg/l) (73).

The present study is amongst only a few to report the association between time-dependent changes in S-100B levels and the outcome of TBI treatment (74-76). A striking discrepancy was observed between the data in the present study and those reported by Gyorgy *et al* (74), where it was previously shown that there was no correlation between the severity of TBI and serum S-100B levels. Additionally, the study by Gyorgy *et al* (74) also showed an increase in the S-100B levels between 7-72 h after injury, whereas the current study showed a pronounced decrease in the S-100B levels. However, a comparison of the results of this study with those reported by Shakeri *et al* (16), revealed a lack of statistical differences between S-100B protein levels stratified into favorable and unfavorable groups in both studies. The observed difference between this and the latter study may be due to different analytic techniques, such as the use of monoclonal antibodies in the present study and ELISA in the ins the study by Gyorgy *et al* (74). Furthermore, the present study employed advanced techniques for the analysis of nonparametric data, such as one-way aligned rank transformation for nonparametric factorial ANOVA and bootstrap analysis. Such an approach allowed for identification of the subtle differences between S-100B levels and the mean cerebral blood flow velocity between the favorable and unfavorable outcome groups.

The results of the present study agree with a report by Raabe and Seifert (73), which indicated that serum levels of S-100B were significantly higher in patients with unfavorable outcomes than in those with favorable outcomes. In patients with favorable outcomes, high initial levels of S-100B returned to normal levels within 96 h. Furthermore, both studies revealed a noticeable difference in serum S-100B levels between the unfavorable and favorable groups, with an apparent decrease in S-100B levels within 3-4 days after hospitalization. The present study also revealed a decrease in S-100B concentration velocity of 0.03 μg/l/h in the unfavorable group. This result shows that patients in the unfavorable group require 153 h (6.3 days) to reach the normal reference range.

Compared to Raabe and Seifert (73), the present study showed that the increase in S-100B protein levels observed in the favorable group did not return to normal, and was on average equal to 0.61 mg/l on day 4, remaining at levels around three times higher than the standard reference value (0.23 μg/l) and 12 times the value reported by Raabe and Seifert (73). Furthermore, the velocity of the decrease in serum S-100B concentration in the favorable group was 0.006 μg/l/h, indicating that a favorable patient would need 130 h of hospitalization before reaching the S-100B standard reference range. This observation may indicate that the difference in 30 h between the unfavorable and favorable groups in reaching the standard S-100B reference range is crucial for patient recovery. The lack of changes can lead to irreversible neuronal dysfunction with a consequent increase in extracellular calcium levels and the activation of toxic nitric oxide (77,78). Thus, the extended time required to recover S-100B levels may be a primary cause of increased mortality. Comparison of this study with a meta-analysis of 39 studies on a total of 1,862 patients (56) confirmed the results presented in the present study. That is, S-100B serum levels in the unfavorable group in the range of 2.16-14.0 μg/l.

The results of the present study also corroborate with those of previous studies (79-82), which showed that the initial concentration of S-100B is of paramount importance for the prediction of the outcome of TBI. In addition, the present study also substantiated the previous findings relating to the clinical significance of S-100B levels up to 3 days after hospital admission (83-89).

Cerebral flow dynamics determined by TCD examination has been one of the most popular neurosurgical diagnostic tools since the 1980s (90,91). This technique allows for analysis of abnormalities in cerebral circulation in patients with craniocerebral trauma (91-95). The impairment of cerebral flow reflected in V_{mean} is of paramount importance for determining the treatment outcomes of patients with severe craniocerebral trauma (96,97). In the present study, an abnormal V_{mean} level was defined by values <30.9 cm/s, which is in agreement with previous reports (39,98).

The present study showed that in the successful group, the majority of the patients exhibited a V_{mean} >30.9 cm/s (the threshold value defining the healthy subjects) during the first 24 h of hospitalization. However, in the unfavorable group, the number of patients with V_{mean} >30.9 cm/s was notably lower. This observation indicates the direct applicability of V_{mean} for predicting treatment outcomes amongst patients with severe CBI.

The velocity of the increase in V_{mean} in the unfavorable group was V_{mean_U}=0.26 cm/sec/h. This value was significantly lower than those previously reported (99,100) showing an adverse outcome for the following cases: An increase in V_{mean} equivalent to 2.08 cm/sec/h during the first 24 h (99) and V_{mean} equivalent to 2.7 cm/sec/h after 3 days of hospitalization (100). This observation indicates that the velocity of changes in V_{mean} may have a prognostic value in the clinical setting. However,

due to the discrepancy between the previous (99,100) and this report, further studies are required.

Analysis of the relationship between the parameters that define the unfavorable treatment outcomes led to the following observations. A significant time-dependent decrease in S-100B levels (a negative velocity equivalent to 0.03 μg/l/h) was associated with a statistically significant increase in V_{mean} levels (a positive velocity of 0.26 cm/sec/h). A favorable outcome was defined by the lack of changes in S-100B levels and a time-dependent increase in V_{mean} with a velocity of 0.18 cm/sec/h.

In conclusion, the present study is the first to report on the associations between S-100B protein levels and V_{mean} to predict patient treatment outcomes in those who have suffered a TBI or CBI, to the best of our knowledge. It was established that within the first 4 days of hospitalization, a constant level of S-100B protein even slightly above the normal range, associated with an increase in V_{mean}, was a predictor of successful treatment outcomes. Moreover, following the conclusion of the study by Thelin *et al* (22), which suggested that S100B could be used as a versatile screening, monitoring and prediction tool in the management of TBI patients, the present study revealed that serum concentration of S100B itself was of limited use in predicting TBI outcomes. However, additional studies are required to validate this observation and to obtain the appropriate statistical power. Moreover, the limited clinical applicability of currently studied CBI markers indicates the need for the continuous search for other markers, which exhibit improved specificity and sensitivity in a clinical environment.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SD collected and analyzed the data. SD, MZ, AZ and RT collected the data and wrote the manuscript. RT analyzed the data. All authors have read and approved the final manuscript. SD, RT and MZ have seen and confirmed the authenticity of the raw data.

Ethics approval and consent to participate

The Bioethics Committee of the Postgraduate Education Medical Center in Warsaw (Warsaw, Poland) approved the experimental protocols (approval no. 501-2-1-20-49/04). Informed consent was obtained from all subjects or their guardians and a parent/legal guardian of subjects under 18 years of age.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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