The Journal Of The International Federation Of Clinical Chemistry And Laboratory Medicine

eJIFCC

THE REFERENCE LEVEL OF SERUM S-100B PROTEIN FOR POOR PROGNOSIS IN PATIENTS WITH INTRACRANIAL EXTRACEREBRAL HEMATOMA

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Abstract

BACKGROUND

S-100B protein, blood-brain barrier permeability marker, is one of a few biochemical indicators useful in the evaluation of traumatic brain injury. Our aim was to correlate serum concentration of S-100B with clinical condition and CT head scan findings as well as to estimate the level of the protein significant for clinical outcome prediction.

METHODS

The cohort of 41 subjects underwent clinical examination by the neurosurgeon, consciousness was evaluated with Glasgow Coma Scale (GCS). Diagnosis was established on the basis of CT head scans. Venous blood samples were collected before surgery. Serum concentration of S-100B protein was estimated using electrochemiluminesce immunoassays (ECLIA) on Cobas 6000 Analyzer (Roche Diagnostics). Clinical outcome was measured applying Glasgow Outcome Scale (GOS). Finally, data were analyzed with Statistica, v. 8.0 (StatSoft, Inc. 2007).

RESULTS

The average S-100B concentration was 0.95 ± 1.75 µg/L. Statistical analysis revealed significant correlation between S-100B and GCS, GOS and dimers–D concentration ($p<0.001$, Spearman correlation test). There were statistically significant differences in the S-100B concentration depending on the presence of brain oedema (1.29±2.02 vs. 0.06±0.03; $p<0.01$, Mann-Whitney test) or contusion foci (1.37±1.77 vs. 0.72±1.92; $p<0.01$) in CT scans. The S-100B concentration of 0.288 µg/L was determined as a cut-off point for unfavorable clinical outcome prediction (ROC, $p<0.001$).

CONCLUSIONS

Association between serum S-100B concentration and clinical, radiological or laboratory findings prove its usefulness as a diagnostic marker for assessment of brain trauma severity. The concentration of the protein >0.288 μg/L is associated with poor prognosis.

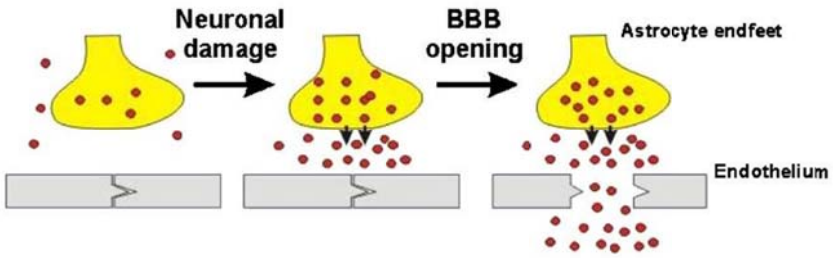
INTRODUCTION

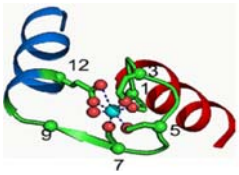
Craniocerebral trauma

Injuries are 4th most common cause of death all over the world [1]. The occurrence of head traumas in multiorgan injuries is over 80%, moreover ca. 40-50% head injuries are isolated, what makes it the most commonly injured organ of the human body. 50% of craniocerebral injuries are severe - mortality rate approaches 30-40% [2 ,3]. This is a consequence of traumatic brain injury (TBI) with systemic implications. Brain injuries may be divided into two groups: primary or secondary brain injury. First group includes contusion or laceration of the brain and diffuse axonal injury (DAI). Extracerebral hematoma is a consequence of vessel damage and can be primary or secondary cause of brain injury. Among mentioned pathologies hematomas give the biggest chance of successful surgical treatment, that can prevent the development of secondary brain injury like brain oedema, ischemia, hypoxia or infection. Extracerebral hematomas can be divided into epidural, acute subdural (≤3 days) or chronic subdural hematoma (>3 days) [3]. These pathological states can be treated pharmacologically or surgically, depending on the severity of the injury. As a result of appropriate posttraumatic diagnostic and therapeutic regimen some patients may survive. It is difficult to predict, at the time of initial presentation, the ultimate prognosis for a given patient [4], important also in the field of family counseling. Currently, the severity of craniocerebral injury is mainly determined based on neurological condition evaluation or neuroimaging results. Useful parameters that aid complete diagnosis are intracranial pressure measurements or biochemical analysis of cerebrospinal fluid (CSF) and blood.

Biochemical parameters

Recently conducted studies showed that blood parameters can be easily available, precise and cost-effective markers of traumatic brain injury. There are numerous indices connected with the severity of TBI and prognosis. They belong to diverse biochemical families and their clinical significance or accurate diagnostic levels are still under investigation. Helmy et al. using hierarchical log linear analysis described the connection between the outcome and raised serum glucose (>7.1 mmol/L), low albumin (<30 g/dL), low hemoglobin (<13 g/dL ♂, <11.5 g/dL ♀) and elevated white cell count (>11.0 x 109/mL) [4]. Additionally, some authors claim, that the level of cortisone can reflect the severity of trauma [5 ,6]. Important group of blood markers in TBI includes coagulation/fibrinolysis parameters [7 ,8 ,9]. In the previous study, we have shown statistically significant correlation between INR and the outcome [10]. During further investigation we proved the correlation between the severity of the trauma and platelet count, fibrinogen and dimers-D concentration. Further group contains markers specific for brain tissue: S-100B protein, S-100A6 protein, neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), neurofilament heavy chain (NF-H) or creatine kinase (CK-bb) [6,11 ,12]. Most of these parameters are additionally used to assess blood-brain barrier dysfunction (Fig 1), that can be also estimated with markers like MMP -2, MMP-9, ICAM-1, IL-6 [13 ,14]. Among all above-mentioned parameters S-100B seems to be particularly sensitive and promising [15], therefore we decided to precisely analyze its diagnostic potential in patients with intracranial hematoma.



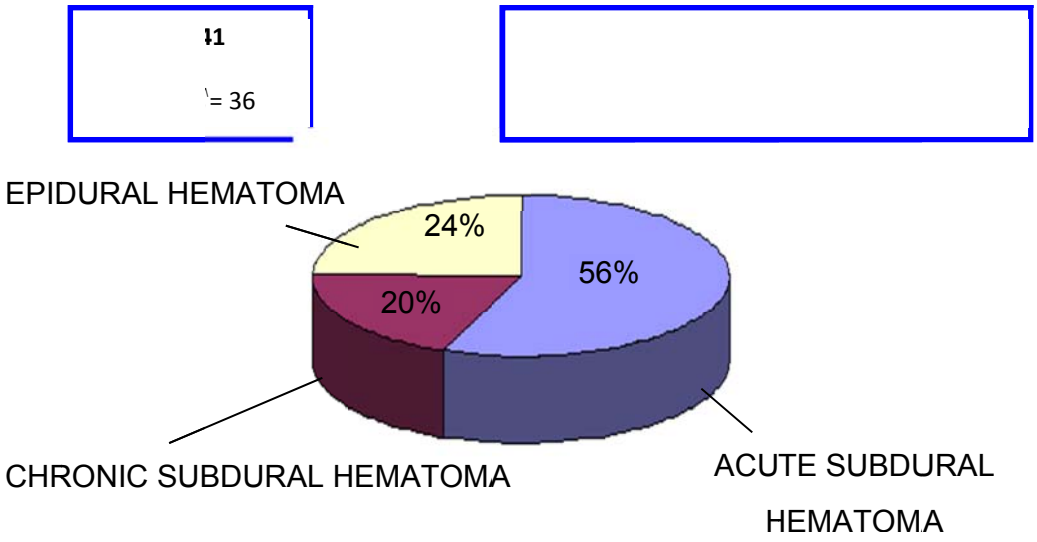


S-100B

S-100B was invented by Janigro D., Mayberg M. and Barnett G. and submitted to US Patent Office in 2001. It is a member of large group of Ca++ binding proteins, similar to calmodulin. There are at least 16 members of the family, which are located as a cluster on chromosome 1q21. On the contrary, S-100B gene is located separately at 21q22.3 [16]. In vivo it has a homodimeric structure and a molecular weight of 21 kDa. Every monomer is composed of two helix-loop-helix (EF-hand) motifs (Fig 2) connected by a central hinge region [17 ,18]. The C-terminal EF-hand contains the canonical Ca2+-binding loop, common to all EF-hand proteins (e.g., troponin C or calmodulin). The N-terminal EF-hand consists of 14 amino acids and is characteristic for S-100 proteins. Generally, the dimeric S-100 proteins bind four Ca2+ per dimer, but S-100B in addition to Ca2+ binds Zn2+ and even Cu2+. This suggests that S-100 protein–target interactions and cellular functions may be triggered by those ions [19]. S-100B has many proposed trargets, f.e. RAGE, IQGAP1, Tau protein and p53 [16,20 ,21 ,22]. The protein is expressed mainly in astrocytes and oligodendrocytes and plays a crucial role in neural growth and homeostasis. After traumatic or ischemic brain injury its concentration is elevated both in serum and in cerebrospinal fluid (CSF) [15].

PATIENTS AND METHODS

Our prospective clinical trial was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and approved by the Local Ethics Committee. Material included 41 patients operated on for a posttraumatic intracranial extracerebral hematoma in the Clinic of Neurosurgery and Neurotraumatology in Poznan, from Novemver 2008 to November 2009. Fig 3 presents basic characteristics of the cohort.



Subjects underwent clinical examination by the neurosurgeon followed by anaesthesiological consultation. Clinical evaluation regarded:

- Presence of neurological deficits
- Consciousness assessment with Glasgow Coma Scale (GCS) (Tab. 1)

Determination of the general risk of a surgery using American Society of Anaesthesiology (ASA) score (Tab. 2)

Table 1. Glasgow Coma Scale

	Patient's response to vocal or painful stimuli			GCS score (points)
	Eye	Verbal	Motor	
Min	1 (does not open the eyes)	1 (makes no sounds)	1 (no movements)	3 (brain death)
Max	4 (opens eyes spontaneously)	5 (converses normally)	6 (obeys commands)	15 (minor brain injury)

Table 2. ASA score

Healthy patient	1
Mild systemic disease	2
Severe systemic disease – that limits functions	3
Severe systemic disease – threat to life	4
Patient not expected to survive	5

Diagnosis was established on the basis of CT head scans. During neuroimaging: brain oedema, contusion foci, traumatic subarachnoid hemorrhage, and medial shift (MS) were additionally evaluated [23].

Patients with multi-organ injury, intracerebral or posterior cranial fossa hematomas, injuries to the facial skeleton, cerebral stroke during recent 6 months, neoplasms of central nervous system, epilepsy in anamnesis, Alzheimer's disease or metastatic neoplasms could not be included into the study group due to adverse clinical course of above mentioned pathologies or the possibility of non-specific rise of S-100B concentration in biological fluids.

Blood samples were collected during admission to the hospital, simultaneously to standard diagnostic procedure concerning blood cell count, electrolytes, coagulation parameters and blood gases assessment. Eppendorf tubes containing 100ul serum were stored at -20°C. Subsequently, concentration of S-100B protein was estimated using electrochemiluminescence immunoassays (ECLIA) on Cobas 6000 Analyzer (Roche Diagnostics).

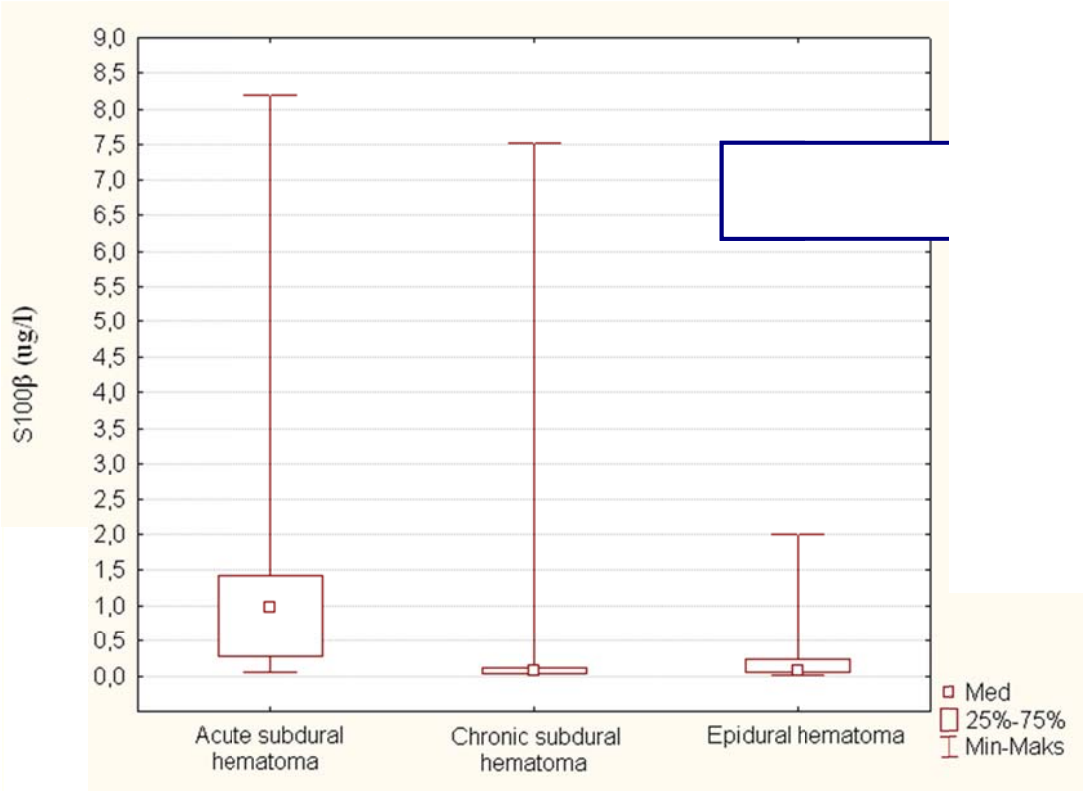
Clinical outcome was measured applying Glasgow Outcome Scale (GOS) (Tab. 3) at the discharge from the hospital [24, 25, 26].

Glasgow Outcome Scale	GOS score (points)
Good Recovery (normal activity even though minor deficits)	5
Moderate Disability (patient is independent as far as daily life is concerned, disabilities are present)	4
Severe Disability (patient depends upon others for daily support)	3
Persistent Vegetative State (patient chronically unconscious)	2
Death	1

Finally, database was created and data were statistically analyzed. Kruskal-Wallis, Spearman, Mann-Whitney tests were performed with Statistica, v.8.0 (StatSoft, Inc. 2007). Receiver Operator Characteristic (ROC) was plotted using Analyse-it® (Analyse-it Software, Ltd.) for Microsoft® Excel™.

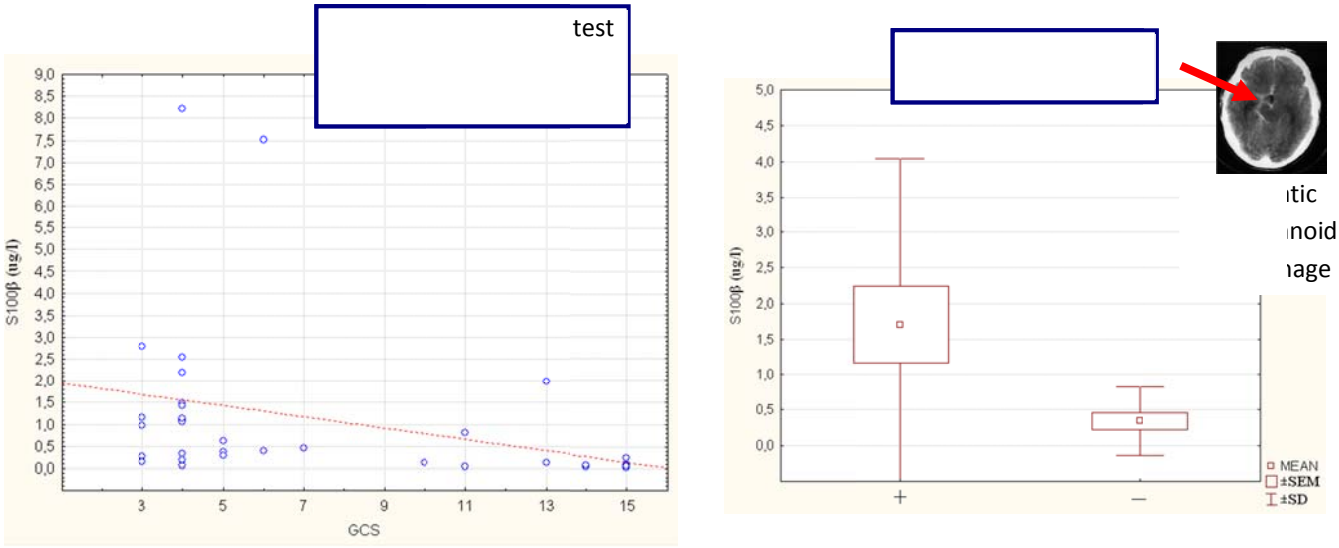
RESULTS

The average S-100B concentration was 0.95 ± 1.75 µg/L (range: 0.022 – 8.210 µg/L). The median of GCS score was 10 points (range: 3-15 points). Statistical analysis revealed significant differences in the concentration of S-100B depending on clinical diagnosis: acute subdural vs. chronic subdural vs. epidural hematoma (1.2±1.72 vs. 1.0±2.63 vs. 0.31±0.6 µg/L; Kruskal-Wallis test, $p<0.05$) (Fig 4).



S-100B indicates severity of TBI

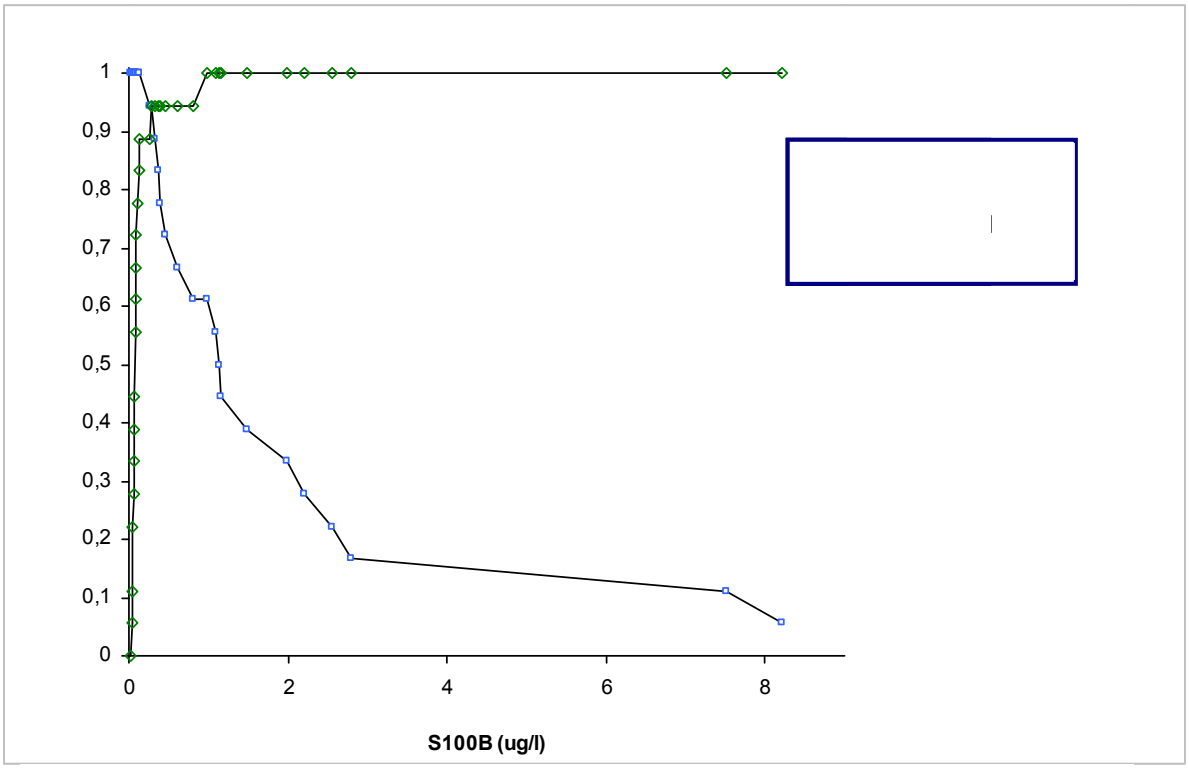
Mann-Whitney test revealed statistically significant difference in the concentration of S-100B protein between conscious and unconscious patients on admission to the hospital (0.22±0.46 vs. 1.58±2.18 µg/L; p<0.001). Statistical analysis revealed significant correlation between S-100B and both GCS (r=-0.69) (Fig 5) and ASA (r=0,67) scores (p<0,001). Moreover, statistical differences were found regarding the presence of significant neurological deficits (1.18±1.97 vs 0.33±0.63 µg/L, Mann-Whithey test p<0.05). Statistical analysis (Mann-Whithey test) of CT head scanning results revealed significant differences in the concentration of S-100B depending on the presence of: brain oedema (1.29±2.02 vs. 0.06±0.03 µg/L; p<0.01), contusion foci (1.37±1.77 vs. 0.72±1.92 µg/L; p<0.01), traumatic subarachnoid hemorrhage (1.7±2.34 vs. 0.34±0.48 µg/L; p<0.001) (Fig 6) and midline-shift (MS) greater than 15 mm (1.48±2.1 vs. 0.88±1.83 µg/L; 0.05<p<0.1 -tendency to obtain statistically significant difference). Furthermore, significant correlation between S-100B and dimers-D concentration was proved (r=0.64; p<0.001). There were no statistical dependence regarding age, concominant diseases or alcohol abuse.



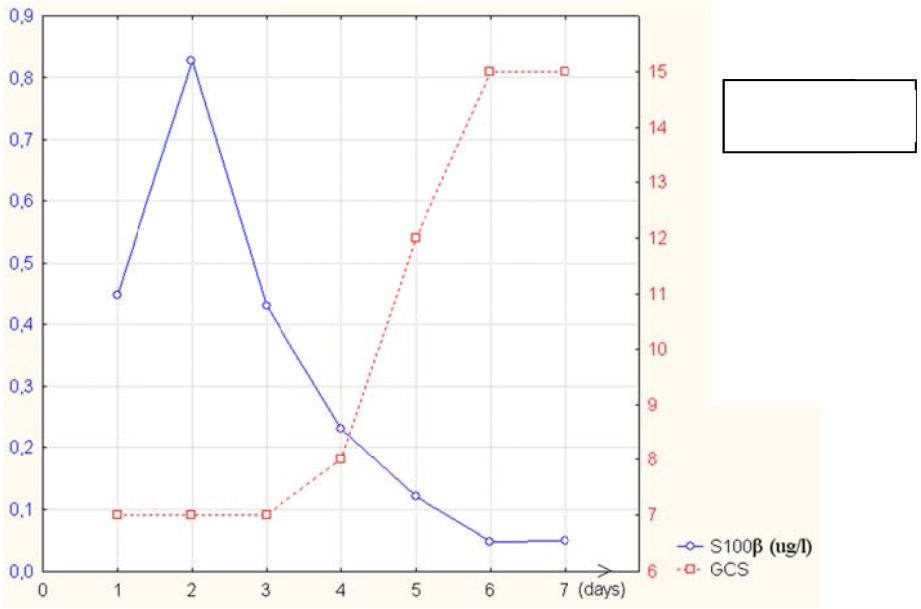
S-100B as clinical outcome indicator

Statistical analysis revealed significant correlation between the S-100B concentration on admission and GOS score (r=-0.81; p<0.001). Additionally, Mann-Whitney test (p<0.01) revealed statistically significant difference in the concentration of S-100B protein between deceased (1.96±2.27 µg/L) vs. survivors (0.57±1.5 µg/L).

Poor clinical outcome was defined as GOS score <4. This group consisted of patients: permanently unable to unassisted existence, in persistent vegetative state or deceased, despite surgical treatment. Using ROC analysis (AUC=0.98, CI:0.93-1.00; p<0.001) we established the value of S-100B concentration = 0.288 µg/L, that with high sensitivity (94.4%) and specificity (94.4%) enables preoperative qualification of the patient to the group of high or low risk of poor clinical outcome (Fig 7). Area under a curve (AUC) for prediction of death equals 0.87 (CI:0.76-0.99, p<0.001), with 90.9% sensitivity and 60.8% specificity for our estimated cut-off point.



To assess the dynamics of changes in the S100B concentration during the clinical course after head trauma, we finally decided to evaluate S-100B in 24h intervals during one week period. Evaluation was performed in randomly chosen case. Results shown in Fig 8 indicate that S-100B protein has a potential as a clinical course monitoring parameter. Further investigation is needed.



DISCUSSION

As previously mentioned, S-100B can be measured both in blood and CSF. Lumbar puncture performed to achieve biological material has numerous limitations and contraindications like the rise of intracranial pressure, what is a common pathology in the course of TBI. Thus, we have chosen peripheral venous blood sampling as a non-invasive procedure, what is in accordance with clinical practice. S-100B concentration doesn't decrease rapidly after trauma, what was proven earlier [27]. The fact, that in our study S-100B concentration was elevated in chronic hematomas as well as results from our case study confirm complex dynamics of S-100B concentration, what should be further investigated.

S-100 proteins have a wide distribution throughout the body. They can be found in Schwann cells, melanocytes, glial cells, chondrocytes, adipocytes, myoepithelial cells, macrophages, Langerhans cells, dendritic cells or keratinocytes. However, individual members of the S-100 protein family show a tissue- and cell-type-specific expression pattern, f.ex. S-100A1 (heart, kidney, and striated muscles), S-100A7 (skin), S-100B (brain tissue). Heizmann proved that diagnostic assays for S-100B don't reveal cross-reactivity with the other homologous members of this protein family, therefore are specific and reliable for measurement of S-100B in human body fluids [16]. Nevertheless, most immunoassays used in S-100B concentration evaluation contain monoclonal antibodies directed against the ß-subunit of these dimeric protein, and they will therefore detect any S-100 protein that contains at least one ß-subunit. Two such dimers are known: S-100BB (homodimeric structure as was described previously, specific for glial cells and Schwann cells) and S-100 A1-B (found in glial cells, melanocytes, adipocyetes, chondrocytes and epidermal Langerhans cells). The two dimers are known to be analyzed together, what certainly influence specificity of the assay [28]. However, it has been shown, that extracranial sources of S-100B (fat, muscle) do not appear to lead to a significant rise in S-100B levels [29 , 30]. Moreover, regarding the fact of different posttraumatic clinical course, one of the exclusion criteria in our study was multiorgan injury. Therefore, we additionally decreased the risk of nonspecific rise of S-100B due to soft tissues injuries. Thus, we have no reason to consider the elevated concentration of S-100B protein as of extracranial origin.

S-100B protein is undetectable in the blood of healthy subjects. Its concentration measured in body fluids may be composed of residues released from damaged cells as well as secreted under pathological conditions. Apart from traumatic brain injuries, S-100B plays a putative role in several neurological diseases, where rise of blood S-100B level is connected with structural or functional blood-brain barrier impairment. Gartner et al. showed that concentration of S-100B protein is elevated not only in already diagnosed CNS neoplasm, but may be detected long before clinical manifestation of malignant glioma [31]. Steiner et al. demonstrated the role and characteristics of S-100B in neurodegenerative disorders, i.e. Alzheimer's disease and amyotrophic lateral sclerosis [32]. Liu et al. proved that S-100B plays an important role in pathogenesis of Parkinson's disease [33]. Moreover, it's been reported that the protein is associated with mesial temporal lobe epilepsy [34]. Numerous studies indicates the importance of S-100B in cerebral stroke [35, 36]. Some studies indicate even a role of S-100B in differentiation between ischemic and hemorrhagic stroke [37]. Generally, due to lack of disease specificity, reliance on S-100B concentrations for differential diagnostic purposes in cases of suspected neurologic disorders is not recommended. Furthermore, it is important to remember, that elevated level of S-100B protein of extracranial origin can be diagnosed in patients with malignant melanoma [38, 39].

In our study we focused on patients with posttraumatic intracranial hematomas as a relatively homologous subgroup of TBI. Although we know considerable number of studies evaluating S-100B in TBIs, the are only a few assessing its level in defined pathological states, that together create a group of TBI. Sawauchi et al. demonstrated elevated S-100B level in patients with acute subdural hematoma with unfavorable outcome [40]. Undén et al. evaluated the concentration of S-100B in epidural hematomas, but the study was based only on five clinical cases [41]. Studies on animal models suggest that S-100B is of minor importance in isolated diffuse axonal injury [42]. Other reports demonstrate the correlation between S-100B and glial cells damage in multifocal cerebral contusion [43].

Finally, dynamics of S-100B increase in biological fluids can be connected with secondary insults following TBI [44, 45].

We decided to evaluate serum S-100B concentration in accordance to the presence of neurological disorders, consciousness state (GCS), computed tomography results and clinical outcome (measured with GOS). These clinical parameters are well known in the literature and widely-accepted elements of standard diagnostic and therapeutic procedures. Additionally, we assessed the association with ASA score and coagulation parameters, what was a topic of our previous work [10]. Clinical parameters described by other authors regarded different clinical scales and biochemical markers. Apart from GCS, Injury Severity Score (ISS) was proven to correlate with clinical outcome, while APACHE II score or length of stay didn't reveal such association [4]. Clinical scales describing outcomes included Full Outline of Unresponsiveness (FOUR), Discharge Disability Score (DDS) or Oxford Handicap Scale (OHS), that are similar and strictly correlate with GOS [24,25,26]. Attempts to use Marshall Computed Tomographic Classification (MCTC) in initial cranial computed tomography assessment demonstrated, that it is preferable to use combinations of individual CT predictors rather than MCTC [46]. Ruan et al calculated, that using S-100B as a screening tool in mild TBIs instead of CT based only upon presenting symptoms, will lower costs, if blood test results require less time than imaging and head CT scan rates for patients [47]. Several associations between S-100B and other biochemical parameters are known. Sawauchi et al. proved statistically significant correlation between the initial S-100B and neuron-specific enolase (NSE) in patients with subdural hematoma [40]. Furthermore, simultaneous rise in blood level after TBI was reported for S-100B together with glial fibrillary acidic protein (GFAP), neurofilament heavy chain (NF-H), myelin basic protein (MBP) or creatine kinase (CK-bb) [27,5,12,6]. Concentration of these biomarkers correlated with clinical outcome after the treatment. Similar studies were performed on inflammatory system parameters, like membrane attack complex of complement C5b9 or cortisol [44]. Further investigation is necessary to establish which constellation of serum markers is most suitable for monitoring patients with TBI.

The awareness of being at increased risk of unfavorable clinical outcome is for the patient and his family at least of the same importance as counseling regarding the risk of death. Hence, we decided to use GOS<4 points as a point of reference. In our study, we received cut-off point of S-100B for unfavorable outcome prediction equal to 0.288 µg/L. According to ROC analysis, both sensitivity and specificity of the test are at a very high level. For prediction of death the same cut-off point provides lower sensitivity and specificity. Similar dependence was obtained by Rainey et al: the authors calculated cut-off point for unfavorable outcome prediction (GOS<4) at a level of 0.53 µg/L, with 82% sensitivity and 60% specificity (for death prediction the same level was characterized by sensitivity 83% and specificity 49%) [30]. Furthermore, Gonzalez-Mao et al proposed S-100B cut-off point for death prognosis of 1.5 µg/L, with sensitivity 73 % and specificity 75.5 % [48]. The comparison reveals important differences in literature regarding S-100B concentration considered as a cut-off point. Possible explanation of that fact could be a significant heterogenity of study groups (mild/severe traumatic brain injuries). In our study, we focused on relatively homogenic clinical group of patients composed of posttraumatic intracranial hematomas, thus we believe, that our results are internally valid and representative especially for this subgroup of TBI.

CONCLUSIONS

Associations between serum concentration of S-100B and clinical findings prove its usefulness as a diagnostic marker for assessment of brain trauma severity. S-100B >0.288 µg/l is asspciated with unfavorable clinical outcome and can be applied as a valuable screening tool specifying patient's prognosis on admission to the hospital.

CONFLICT OF INTERESTS

None declarable.

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