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 electrochemiluminescence 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 targets, 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 November 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)

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-Whitney test p<0.05). Statistical analysis (Mann-Whitney 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, concomitant 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 B-subunit of these dimeric protein, and they will therefore detect any S-100 protein that contains at least one B-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, adipocytes, 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 heterogeneity 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 associated 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.
References

1. World Health Organization : Regional burden of disease estimates for 2004. The world health report 2004 - changing history.
2. Słowinski K.: Multiorgan injuries. In: Fibak J.: Surgery - review. PZWL, Warsaw:1998, 411
3. Trojanowski T.: CNS trauma. In: Noszczyk W.: Surgery, Vol.1, PZWL, Warsaw:2006: 269-275
4. Helmy A., Timofeev I., Palmer C.R., Gore A., Menon D.K., Hutchinson P.J.: Hierarchical log linear analysis of admission blood parameters and clinical outcome following traumatic brain injury. Acta Neurochir. 2010, 152:953-957
5. Kwon SK, Kovesdi E, Gyorgy AB, Wingo D, Kamnaksh A, Walker J, Long JB, Agoston DV.: Stress and traumatic brain injury: a behavioral, proteomics, and histological study. Front Neurol. 2011;2:12.
6. Graham MR, Myers T, Evans P, Davies B, Cooper SM, Bhattacharya K, Grace FM, Baker JS.: Direct hits to the head during amateur boxing is associated with a rise in serum biomarkers for brain injury. Int J Immunopathol Pharmacol. 2011;24:119-25.
7. Bayir A., Kalkan E., Kocak S., Ak A., Cander B., Bodur S.: Fibrinolytic markers and neurologic outcome in traumatic brain injury. Neurol. India 2006, 54: 363 – 365
8. MacLeod J. B., Lynn M., McKenney M. G.: Early coagulopathy predicts mortality in trauma. J. Trauma 2003, 54: 66-71
9. Takahashi H., Urano T., Takada Y., Nagai N., Takada A.: Fibrinolytic parameters as an admission prognostic marker of head injury in patients who talk and deteriorate. J. Neurosurg 1997, 86: 768-772
10. Guzniczak P., Zaborowski M., Kaluzny A., Anczykowski G.: Coagulation parameters as predictive factors in patients with posttraumatic intracranial extracerebral hematomas treated surgically. Neuroskop 2009;11: 29-33
11. Böhmer AE, Oses JP, Schmidt AP, Perón CS, Krebs CL, Oppitz PP, Davila TT, Souza DO, Portela LV, Stefani MA.: NSE, S-100B and GFAP levels as outcome predictors in severe traumatic brain injury patients. Neurosurgery. 2011 (in press)
12. Gyorgy A, Ling G, Wingo D, Walker J, Tong L, Parks S, Januszkiewicz A, Baumann R, Agoston DV.: Time-dependent changes in serum biomarker levels after blast traumatic brain injury. J Neurotrauma. 2011;28:1121-6
13. Michalski D, Pelz J, Weise C, Kaczka J, Boltze J, Groesche J, Kampmad P, Schneider D, Hobohm C, Hartig W.: Early outcome and blood-brain barrier integrity after co-administered thrombolysis and hyperbaric oxygenation in experimental stroke. Exp Stroke Med. 2011;16:3:5
14. Rodriguez-González R, Sobrino T, Rodríguez-Yáñez M, Millán M, Brea D, Miranda E, Moldes O, Pérez J, Lomas DA, Leira R, Dávalos A, Castillo J.: Association between neuroserpin and molecular markers of brain damage in patients with acute ischemic stroke. J Transl Med. 2011;11:58.
15. March N., Cavaglia M., Fazio V., Bhudia S., Hallene K., Janigro D.: Peripheral markers of blood-brain barrier damage. Clinica Chimica Acta 2004;342: 1-12
16. Heizmann CW: S-100B Protein in Clinical Diagnostics: Assay Specificity. Clinical Chemistry 2004;50: 249-251
17. Heizmann CW, Fritz G, Schäfer B. W. S-100 proteins: structure, functions and pathology. Front Biosci 2002;36:d1356-d1368.
18. Fritz G, Heizmann CW.: 3D-structures of the Ca2+ and Zn2+-binding S-100 proteins. In: Bode W, Messerschmidt A, Cygler M, eds. Handbook of metalloproteins, Vol. 3. Chichester, NY.
19. Heizmann CW, Cox JA: New perspectives on S100 proteins: a multifunctional Ca2+, Zn2+ and Cu2+-binding protein family. Biometals 1998;11:383-397.
20. Mbele Gaël O., Deloume J.C., Gentil Benoit J., Delphin C., Ferro M., Garin J., Takahashi M., Baudier J.: The zinc- and calcium-binding S-100B interacts and co-localizes with IQGAP1 during dynamic rearrangement of cell membranes. J. Biol. Chem. 2009; 277: 49998-50007
21. Baudier J, Cole R.D: Interactions between the microtubule-associated tau proteins and S100b regulate tau phosphorylation by the Ca2+/calmodulin-dependent protein kinase II. J. Biol. Chem. 2006;263: 5876–83.
22. Lin J, Yang Q, Yan Z, Markowitz J, Wilder P.T, Carrier F, Weber D. J: Inhibiting S100B restores p53 levels in primary malignant melanoma cancer cells. J. Biol. Chem. 2010;279: :34071-7.
23. Guzniczak P, Kaluzny A, Zaborowski M, Jankowski R.: Analysis of preoperative CT head scanning in patients with posttraumatic intracranial haematomas. Neuroskop 9 2007;9: :106-112.
24. Akavipat P, Sookplung P, Kasewsinghpa P, Maunsaiyap P.: Prediction of discharge outcome with the full outline of unresponsiveness (FOUR) score in neurosurgical patients. Acta Med. Okayama 2011;65: :205-210.
25. Perel P, Edwards P, Shakur H, Roberts I.: Use of the Oxford Handicap Scale at hospital discharge to predict Glasgow Outcome Scale at 6 months in patients with traumatic brain injury. BMC Med Res Methodol. 2008;8:72.
26. Fuller G.W, Yeoman P.: A simple hospital discharge score predicts Glasgow Outcome Scale at 12 months in patients with traumatic brain injury. Crit Care. 2010; 14: :303.
27. Wiesmann M, Steinmeier E, Magerkurth O, Linn J, Gottmann D, Missler U. Outcome prediction in traumatic brain injury: comparison of neurological status, CT findings, and blood levels of S-100B and GFAP. Acta Neurul Scand. 2010;121:179-85
28. Ueno T, Irguro Y, Yamamoto H, Sakata R, Kakhina Y, Nakamura K.: Serial measurement of serum S-100B protein as a marker of cerebral damage after cardiac surgery. Ann Thorac Surg. 2003;75:1892-7
29. Samola O, Pyhtinen J, Leino TK, Siitonen S, Niemela O, Hillborn M.: Effects of head and extracranial injuries on serum protein S100B levels in trauma patients. J. Trauma 2004;56:1229-34
30. Rainer T, Lesko M, Sacho R, Lecky F, Childs C.: Predicting outcome after severe traumatic brain injury using the serum S100B biomarker: Results using a single (24h) time-point. Resuscitation 2009;80: :341-345
31. Gartner W, Ilhan A, Neziri D, Base W, Weissel M, Wöhrer A, Heinzl H, Waldhör T, Wagner L, Preusser M.: Elevated blood markers 1 year before manifestation of malignant glioma. Neuro Oncol. 2010;12:1004-8
32. Steiner J, Bogerts B, Schroeter ML, Bernstein HG.: S100B protein in neurodegenerative disorders. Clin Chem Lab Med. 2011;49:409-24
33. Liu J, Wang H, Zhang L, Xu Y, Deng W, Zhu H, Qin C.: S100B transgenic mice develop features of Parkinson's disease. Arch Med Res. 2011;42:1-7
34. Lu C, Li J, Sun W, Feng L, Li L, Liu A, Li J, Mao W, Wei H, Gao L, Zhang X, Huang Z, Meng X, Wang Y. Elevated plasma S100B concentration is associated with mesial temporal lobe epilepsy in Han Chinese: a case-control study. Neurosci Lett. 2010;484:139-42.
35. Beer C, Blacker D, Bynevelt M, Hankey GJ, Pudddey IB.: Systemic markers of inflammation are independently associated with S100B concentration: results of an observational study in subjects with acute ischaemic stroke. J Neuroinflammation. 2010;7:71
36. Marginean I, Stanca D, Vacaras V, Soritaou O, Margiean M, Muresanu D.: Plasmatic markers in hemorrhagic stroke. J Med Life. 2011;4:148-50
37. Kavalci C, Genschallac H, Durukan P, Cevik Y.: Value of biomarker-based diagnostic test in clinical diagnosis of hemorrhagic-ischemic stroke. Bratisl Lek Listy. 2011;112:398-401
38. Lin J, Yang Q, Wilder PT, Carrier F, Weber DJ.: The calcium-binding protein S100B down-regulates p53 and apoptosis in malignant melanoma. J Biol Chem. 2010;285:27487-98
39. Loppin M, Quillien V, Adamski H, Ollivier I, Garlantézec R, Chevrier-Breton J.: Protein S100 beta and Melanoma Inhibitory Activity (MIA): a prospective study of their clinical value for the early detection of metastasis in malignant melanoma. Ann Dermatol Venereol. 2007;134:535-40.
40. Sawauchi S, Taya K, Murakami S, Ishi T, Ohtsuka T, Kato N, Kaku S, Tanaka T, Morooka S, Yuhki K, Urashima M, Abe T.: Serum S-100B protein and neuron-specific enolase after traumatic brain injury. No Shinkei Geka. 2005;33:1073-80.
41. Undén J, Bellner J, Astrand R, Romner B. Serum S100B levels in patients with epidural haematomas. Br J Neurosurg. 2005;19:43-5.
42. Davidsson J, Risling M.: A new model to produce sagittal plane rotational induced diffuse axonal injuries. Front Neurol. 2011;2:41
43. Vajtr D, Průsa R, Houst’ava L, Sámal F, Kukacka J, Pachl J.: Biochemical and immunohistochemical markers of brain injury. Soud Lek. 2006;51:36-41.

44. Bellander BM, Olafsson IH, Ghatan PH, Bro Skejo HP, Hansson LO, Wanecek M, Svensson MA.: Secondary insults following traumatic brain injury enhance complement activation in the human brain and release of the tissue damage marker S100B. Acta Neurochir (Wien). 2011;153:90-100.

45. Kleindienst A, Meissner S, Eyupoglu IY, Parsch H, Schmidt C, Buchfelder M.: Dynamics of S100B release into serum and cerebrospinal fluid following acute brain injury. Acta Neurochir Suppl. 2010;106:247-50.

46. Maas AI, Hukkelhoven CW, Marshall LF, Steyerberg EW: Prediction of outcome in traumatic brain injury with computed tomographic characteristics: a comparison between the computed tomographic classification and combinations of computed tomographic predictors. Neurosurgery. 2005;57:1173.

47. Ruan S, Noyes K, Bazarian JJ.: The economic impact of S-100B as a pre-head CT screening test on emergency department management of adult patients with mild traumatic brain injury. J Neurotrauma. 2009;26:1655-64

48. Gonzalez-Mao C., Reparaz-Andrade A; Alvarez-Garca E.; Posada Pacrez P.; Del Campo-Pacez V; Andrade-Oliviac M.: Biomarker S100B and Glasgow Coma Scale (GCS): Prognostic indicators in traumatic brain injury (TBI). Clin Chem Lab Med 2011;49:Suppl

49. Zhou Y, Frey TK, Yang JJ: Viral calcioomics: Interplays between Ca2+ and virus. Cell Calcium, 2009;46:1-17