Acta Biomed 2012; 83: 5-20

Review

The value of the calcium binding protein S100 in the management of patients with traumatic brain injury

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Abstract. *Background:* From the first study in 1995 the role of calcium-binding protein S100B in Traumatic Brain Injury (TBI) has been variously investigated in many clinical works. The aim of this work is to analyze the recent published reports with a reference to serum and CSF levels and to identify a possible role of S100 in the management of TBI. *Methods:* A MEDLINE search with a various number of query related to "S100" and "TBI" was performed from 2000 to 2011. All identified articles and abstracts have been reviewed *Results:* Serum and CSF samples of the marker well correlate in most of the papers to the degree of intracranial injury as determined by CT scans. Furthermore patients with the higher levels of S100B show a worse prognosis. In the paediatric age a relationship with the outcomes in spite of difficulties to determine normal values is also observed. Some proposal about a clinical use of S100B to decrease the number of neuroradiological examinations are present. *Conclusions:* S100B shows some interesting potentialities, but we have not enough evidence to insert this marker of brain damage in the protocols for management of TBI. However its use in experts' hands in association with others clinical and radiological features may help to improve medical practice in the treatment of TBI. (www.actabiomedica.it)

Key words: S100, biomarkers, traumatic brain injury, outcome, paediatric, brain damage, mild TBI, CT

Introduction

While in certain medical fields a wide range of serum markers with a clear impact on patient's management is present (1), in the diseases of the central nervous system a wide choice of serum markers with adequate specificity and sensitivity are not available. Traumatic brain injury (TBI) have an epidemiological incidence of 235/100'000/year in Europe (2). TBI are usually classified according to the Glasgow coma scale (GCS) in severe (GCS 3-8), moderate (9-13) and minor (14-15) (3). In the case of patients with minor head injury without neurological deficits, the presence of an accepted serum biomarker could identify the presence of damaged brain tissue. This information would help the physician to decide if the patient should be clinically observed , if it is necessary to obtain CT or MRI scans and, if so, when such examinations should take place. Over the past decade the use of CT for minor head injury has become increasingly common, while its diagnostic yield has remained low. The increased use of CT adds substantially to health care costs (4, 5) and exposes a large number of adults and children each year to the potentially harmful effects of ionizing radiation . There is in fact a growing concern that early exposure to ionizing radiation may result in a substantial rise in lifetime risk of fatal cancer (6), inducing to start large multicenter studies to validate a specific protocol to identify very low-risk patients (7, 8).

Brenner and colleagues (9, 10) estimated that the lifetime cancer mortality risk attributable to the ioniz-

ing radiation to which a one-year-old child would be exposed through a single CT scan of the head was about 1 in 1500; they estimated the corresponding mortality risk for 10-year-old children as about 1 in 5000. In the United States, of approximately 600,000 abdominal and head CT examinations annually performed in children under the age of 15 years, a rough estimate is that 500 of these individuals might ultimately die from cancer attributable to the CT radiation (9, 10).

In addition, Hall and others (11) recently reported that low doses of ionizing radiation to the brain in infancy may influence cognitive abilities in adulthood. These risks may be small for a given individual, but when applied to a large population they may create a substantial public health risk. So we can understand how the absence of an ideal biomarkers, which makes possible the discrimination of high-low risk patients may influence the clinical practice in Emergency Medicine.

In severe TBI (GCS<9, comatose patients), the visible neurological deficits is usually related to the presence of brain damage. In these cases the serum levels of S100BB have been investigated to evaluate the relationship with outcomes .

An ideal serum marker should have: adequate sensitivity and specificity, high predictive values for brain injury, be released only after irreversible destruction of brain tissue, have a rapid appearance in serum and have temporal correlation with the onset of injury. The possibility of using serum S100B in patients with MHI was firstly reported in 1995 (12), then various glial and neuronal proteins such as S100B, Glial Fibrillar Acidic Protein (GFAP), and Neuron Specific Enolase (NSE), have been found to be elevated in the blood of patients after stroke, brain trauma, hypoxic encephalopathy and neurological disorders (2, 13, 14).

We focused this review on the role of S100 protein which is the most studied and investigated . We divide our analysis into five parts : an initial part to explain the nature of this peptide, a paragraph in which we consider the role of S100B in minor traumatic brain injury (MTBI, GCS 13-15), a section where we explore the value of S100B in severe TBI (GCS < 9). A separate approach is then carried out for pediatric population. Finally we have taken into consideration the possible cause of variability in the serum concentration which may act as confounding factor in the clinical practice.

Biochemistry

S100 proteins are a family of dimeric cytosolic calcium binding proteins made up of an alpha and a beta isomer. They are found in abundance in astroglial and Schwann cells and have been found in a few tumors such as schwannoma, gliomas, melanoma, and neuroblastoma (15-19). The alpha and beta isomers have also been called S100A1 and S100B respectfully. Other rare A types numbering over 6 also exists. The S100 genes include at least 13 members, which are located as a cluster on chromosome 1q21. The name of this protein group derives from its solubility in ammonium sulfate, which is 100% at a neutral pH (20).

Most S100 proteins are found as dimers. Three types of dimers are usually found: S100A1A1, S100BB, S100A1B. All three heterodimers have been found in glial cells, astrocytes, ependymal cells, oligodendrocytes, and Schwann cells in the central and peripheral nervous system but they have been found also in adipocytes, melanocytes, chondrocytes and epidermal Langerhans cells (20-23). It has an important role in regulating phosphorylation mediated by the protein kinase C on growth-associated protein 43, which is involved in axonal growth and synaptogenesis during development, synaptic remodeling, and long-term potentiation. S-100B interacts with and stabilizes microtubule-associated proteins such as tau and microtubule-associated protein 2 (MAP-2) (24). For this reason higher S100B concentrations in serum and CSF have been found in the earlier stages of Alzheimer disease, when plaque formation is more active (25).

The protein's action is in relationship with its concentration: at nanomolar levels S-100B stimulates neurite outgrowth and enhances survival of neurons, but at micromolar levels it stimulates the expression of inflammatory cytokines and induces apoptosis (26).

Some investigations have clarified that various mechanisms may release S100B protein from glial cells into the extracellular space. It happens during as-

trocytic reaction that immediately follow primary brain injury. S100B protein is involved in this by regulating the Calcium influxes and stimulating astrocytes proliferation via interaction with transcription factors (27-29).

Moreover S100 family proteins appear to be released from proliferating astrocytes (30). Experimental injury to the brain via trauma or stroke induce a reactive gliosis with a peak around 3-4 days after the injury (31, 33).

S100B appears to be released into the extra-cellular space near the injured tissue and may enter into the serum from the brain through a disrupted blood brain barrier or into the CSF and then into the blood via the arachnoid villi (33). S100B is removed from the serum by renal clearance with a serum half-life of 20-25 min (34). Other values for the halftime have been reported in relationship with the severity of injury, with a value of three hours in severely head injured patients (35). Serum concentrations of this marker are not influenced by hemolysis because S100B was found to be absent in red blood cells (36).

The reported median plasma concentration of S-100B in blood of 200 healthy blood donors between 18 and 65 years of age is 0.05 μg/L. There is no gender difference, but plasma concentrations of S-100B slightly decrease with age, without a statistically significant correlation (37).

Minor head injury

Although CT scans have been widely available for almost three decades, no consensus on the indications for their use in MTBI without clinical symptoms is present . Two different approaches have been reported to assure the safe discharge of patients following minor head trauma. One of inpatient monitoring for 3-5 days and cranial computerized tomography (CCT) only in the case of deteriorating neurological status (38), the second of routine CCT and subsequent discharge in the absence of lesions (4, 39, 40). Both approaches have disadvantages. One is associated with exposure to ionizing radiation and sedation, which is required during the examination in infants and young children. The other is the high cost and the additional resources required for inpatient observation. Recent analysis have shown that only 4-7% of MTBI patients presenting to EDs in the U.S. have incurred any type of brain injury visible on CT scan, and only 0.5% have a potentially life-threatening epidural haematoma (8).

This is a particular interesting issue because in this group of patients we may prove the sensibility and the predictive negative value of S100B.

In a recent review and meta-analysis Unden and Romner (41) reported a sensitivity of 97% of S100B in identifying the presence of brain damage and a specificity of 40%, with a corresponding negative predictive value (NPV) of 99. These values seems to demonstrate a reliability of the S100 as a biomarker of brain damage.

Nevertheless in the same review are listed 6 patients who showed low S100B blood levels and a positive CT: 2 traumatic subarachnoid hemorrhages, 2 skull fractures , one subdural hematoma and one small brain contusion. Other five cases are reported in two more articles (42, 43), including also patients with GCS 15 and CT scan positive for left temporal epidural haematoma, temporal subarachnoid hemorrhage and subdural haematoma which required evacuation.

In this view we have to underline the poor relationship that exists between S100Bb and epidural hematoma (a common lesion in mild head injured patients) witnessed by a study (44) where 3/5 epidural hematoma (in one case with a concomitant cerebral herniation) showed a normal serum S100B level (<0.2 microg/l). This evidence is easily explainable by the fact that patients harbouring epidural hematomas have no direct damage to glial and neuronal cells and consequently the level of a marker of damage to these cells-line could remain normal. In a clinical setting of mild head injured patients it is difficult to accept as a clinical/radiological guide a markers which, even in a very few case, may fail to identify an intracranial haematoma.

In last years, some different studies (42, 45-55) (summarized in table 1) have investigated the sensitivity and specificity of early (first six hours post-trauma) serum sample of S100B. Particularly some authors (51) underlined that adding the measurement of S-100B concentration to the clinical decision rules for

Table 1. Value of serum S100B concentrations as predictor factor of CT positivity in adult population who sustained a minor head trauma

	Patients (% of CT+)	Cut-off value	Time of sampling	Sensitivity	Specificity
Ingebrigtsen, 2000, *Brain Inj* (45)	182 (5%)	0,20 ug/l	At admission	90%	65%
Biberthaler 2001, *World J Surg* (46)	52 (28,8%)	0,1 ng/ml	At admission	100%	40,5%
Mussack 2002, *Shock* (47)	139 (13,6%)	0,21 ng/ml	At admission	100%	50%
Biberthaler, 2002, *Eur J Med Res* (48)	104	0,12 ng/ml	Within 2 hours	100%	46%
Biberthaler 2004, *Unfallchirurg* (49)	75 (18,6%)	-		Significant correlation	Significant correlation
De Boussard, 2004, *Brain Inj* (50)	66	0,1 ug/l	At admission	61%	77%
Biberthaler 2006, *Shock* (51)	1309 (7,1%)	0,1 ug/l	At admission	99%	30%
Bazarian, 2006, *Restor Neurol Neurosci* (52)	86	0,1 ug/l	Within 4 hours post- injury	75%	65%
Poli de Figuereido, 2006, *Clinics (Sao Paulo)* (53)	50	0,1 ug/l	Within 3 hours post-injury	100%	20%
Muller 2007, *J Trauma* (54)	226 (9,3%)	0,1 ug/l	1 hour after injury	95%	31%
Bouvier, 2009, *Ann Biol Clin* (55)	105	0,1 ug/l	Within 3 hours post-injury	100%	33%
Morochovic, 2009, *Eur J Neurol* (42)	102	0,1 ug/l	Within 6 hours post-injury	83,3%	28%

CT+: presence of a brain injury at imaging

a CCT scan in patients with MHI could allow a 30% reduction in scans. It is unreasonable to expect an actual 100% sensitivity or NPV from a diagnostic test and in some clinical situation, a very high sensitivity and even higher NPV is acceptable only if combined with other diagnostic variables.

A different approach was carried out from other authors who tried to evaluate the role of an admission S100B sample to predict neurological deterioration and outcome in minor head trauma. For Bloomfield et al (15) the sensitivity of S100B is best demonstrated in patients with mild brain injury where it accurately correlates with clinical outcomes. A normal S100B level reliably predicts good neurological outcomes. Mild elevations of S100B correlate with post-concussion syndromes. Moderate elevations correlate with neuropsychological evidence of significant disability. The role as prognostic factor in MTBI have been investigated in some studies (42, 55-66) (summarize in table 2) and resulted controversial. Five studies (45, 56-59) showed a significant role as prognostic factor and seven studies (60-66) demonstrated the absence of a significant correlation between early serum S100B concentrations and long-term outcome. The follow-up evaluations are related to the onset of post-concussional syndrome measured with Rivermead Post-Concussion Symptoms Questionnaire (58, 60, 63), with a relevant clinical change (45, 56, 57, 59, 62, 64-66) or with neuropsychological tests (60, 61).

Moreover Ruan et al (67) evaluated the economic impact of S-100B as a pre-head CT screening test on Emergency Department management of adult pa-

Table 2. Value of serum S100B concentrations as prognostic factor in adult population who sustained a minor head trauma

	N	Time of sample	Cut-off value	Outcome measures	Time of outcome	Specificity	Sensitivity
Mussack, 2000, *Acta Neurochir Suppl* (56)	80	0, 6, 24 hours on admission	Comparison with control group	Later clinical course	-	Significant correlation	Significant correlation
Ingebrigtsen, 2000, *Brain Inj* (45)	182	At admission	Detectable/ Undetectable	At least 1 symptoms of PCS	3 months	Significant correlation	Significant correlation
De Krujik, 2002, *Acta Neurol Scand* (57)	104	Within 6 h post trauma	Mean comparated to control group	Development of vomit	-	Significant correlation	Significant correlation
Savola, 2003, *Eur J Neurol* (58)	172	Within 6 h post trauma	0,50 ug/l	modified RPCSQ_	1 month	93	27
Stranjalis, 2004, *J Neurotrauma* (59)	100	Within 3 h post trauma	0.15 ug/L	short-term to return to work or to undertake certain activities	1 week	Significant correlation	Significant correlation
De Boussard, 2005, *J Rehab Med* (60)	97	At admission	0.15 ug/l	Neuropsychological test	3 month	No correlation	No correlation
	97	At admission	0.15 ug/l	RPCSQ_	3 month	No correlation	No correlation
Stapert, 2005, *Eur Neurol* (61)	-	Within 6 h post trauma	0.22 ug/l	Neuropsychological tests	median of 13 days (7-21)	No correlation	No correlation
Sojka, 2006, *Brain Inj* (62)	88	On admission and 7 h later	Logistic regression	IES questionnaire for post-traumatic stress-related symptoms	15 +/- 4 months	Significant correlation	Significant correlation
Bazarian, 2006, *Brain Inj* (63)	35	Within 6 h post trauma	Linear correlation	RPCSQ_	3 month	No correlation	No correlation
Lima, 2008, *Injury (64)*	50	At admission	-	quality of life, anxiety and depression, and PCS	18 months	No correlation	No correlation
Bouzat, 2009, *Ann Fr Anesth Reanim* (65)	67	Within 12 h post trauma	Comparison with control group	decrease from initial GCS, or any treatment for neurological deterioration	7 days	No correlation	No correlation
Kleinert, 2010, *Zentralbl Chir (66)*	73	Within 3 h post trauma	-	Development of PCS	-	No correlation	No correlation

N: number of patients
IES: Impact of event scale
RPCSQ: Rivermead Post-Concussion Symptoms Questionnaire
PCS : Post concussion Syndrome

tients with mild traumatic brain injury. According to the result of this study , two situations exist in which the use of S100B sample has a favorable economic impact: when CT scan rates for patients with isolated MTBI relatively high (> 78% of cases); and when the time for imaging is longer than that for obtaining S-100B test results.

Despite its high sensitivity and excellent negative predictive value, S-100B assay has low specificity and low positive predictive value, limiting however its ability to reduce hospital costs and avoid CT use in mild injured patients .

Severe head injury

The application of biomarkers in this group of patients is linked with the value of serum or CSF level of S100B as a predictor of outcomes. Even if most papers, summarized in table 3, indicate S100B as a reliable prognostic factor, the low specificity of this marker is confirmed by some authors who have identified more adequate predictors of brain deterioration such as NSE or GFAP (68-70).

In the treatment of severe head injury, the first goal for the use of a biochemical marker should be to discriminate between subjects who will die irrespective of treatment and those who have a chance of survival. In the clinical setting, no marker at the moment allows the decision of whether or not to treat.

The highest S-100B serum levels are usually detected in the first blood sample after a severe head injury. In patients that did not survive, the serum level of S-100B remained elevated for days, but in patients that survived the level of S-100B decreased within the first 36 h after moderate TBI (71).

A correlation between S-100B and primary end points (GCS, CT, direct brain damage) in patients with severe head injury has been reported in several studies in severe TBI . These studies show that S-100B levels correlate well with outcome measured as Glasgow Outcome Scale (GOS) (72-83), Extended Glasgow Outcome Scale (GOSE) (84), death or brain death (68, 70, 82, 85, 86), presence of emergency neurosurgery procedure (82, 87), intracranial pressure (ICP) (71, 72, 79, 87-89), cerebral perfusion pressure (CPP) (71, 72), mean arterial pressure (71), neuropsychological outcome (90). Even if all studies investigate the concentrations of serum S100B, no differences in the prediction of long-term outcome after severe TBI made by serum concentrations of S100B, S100A1B and S100BB are present (87).

For death or unfavourable outcome Vos et al (84) showed that S100B is a stronger predictor than age, ISS, pupillary reactions, and GCS score, while for Wiessman et al (91) serum levels on admission and after 24 h were of higher predictive value than CCT findings or clinical examination.

Early serum S100B are clearly related to the extent of primary injury and direct brain damage (clinical and radiological evidence) as confirmed in some recent studies (43, 72, 86, 87, 92, 93). Specifically for the radiological evidence it is reported (86) that initial serum S100B protein is correlated with neuroimaging (Marshall classification, 94) severity. In particular, the group of patients with Marshall grade NEML (VI) presented with the highest initial S-100B protein levels in serum, following by those with Marshall grade III or IV. This is in accordance with the fact that severe TBI subjects with Marshall grade I or II have the mildest type of severe TBI from the neuroradiological point of view. In addition, subjects with grade EML (V) presented with lower S-100B levels, since they had already undergone craniotomy (the craniotomy had preceded the initial serum S-100B measurements) and the increased intracranial pressure had already been relieved. This is in accordance with the finding that surgery reduces S-100B levels.

Many studies (70, 85, 86, 89, 91, 95) have demonstrated different level of serum S100B in the three group of minor (lower), moderate and severe (higher) traumatic brain injury, relating the post-traumatic GCS and serum marker level. Specifically Korfias et al (86) reported that patients with lower GCS scores had 1.5- to 3 time higher S-100B levels. However we have to consider that the level of consciousness may be unreliable when patients are intubated at the trauma scene with the use of anesthetics, sedatives, and neuromuscular blockade (91).

An important aspect is the possibility to elaborate a model to predict outcome in TBI considering clinical or radiological manifestations associated to serum

Table 3. Validation of post-TBI S100B values to predict outcome in severe TBI

	N	Cut-off-value	Time of sampling	Type and Timing of outcome	Sensitivity	Specificity	Blood or CSF
Raabe, 2000, *Neurosurg Rev* (72)	25	0,2 ug/l	Adm and daily for 10 days	GOS at 6 months	SSC	SSC	Blood
Rothoerl, 2001, *Acta Neurochir Suppl* (73)	44	2 ug/l	Within 1 to 6 h PT	GOS at 11 months	75%	82%	Blood
Pleines, 2001, *J Neurotrauma* (74)	13	Means and linear regression	First 2 weeks PT	contusion sizes on the CT scans	SSC	SSC	CSF
	13	Means and linear regression	First 2 weeks PT CT scans	contusion sizes on	Correlation but not statistically significant	Correlation but not statistically significant	Blood
	13	Means and linear regression	First 2 weeks PT	GOS	SSC	SSC	CSF and Blood
Woertgen, 2002, *Brain Inj* (75)	51	0.5 ug/l	Adm	GOS and quality of life	SSC	SSC	Blood
Dimopulou, 2003 *Neurology* (85)	47	1 ug/l	adm and every 24 hours for 6 days	Brain death in first 6 days post-injury	SSC	SSC	Blood
Hu, 2004, *Zh Wei Zh Ji Ji YX* (76)	66	Comparison between different groups of outcome	Adm and every 24 hours for a maximum of 3-7 days	GOS at 6 months	SSC	SSC	Blood
Vos, 2004, *Neurology* (77)	85	1,12 ug/l	Adm	GOS and death at 6 months	100% for death and 88% for GOS 1-3	41% for death and 43% for GOS 1-3	Blood
Li, 2004, *Chin J Traumatol* (78)	40	-	Within 12 h PT	GOS	SSC	SSC	Blood
Hayakata, 2004, *Shock* (79)	23	Peak CSF sample	Adm and at 6, 12,24, 48, 72, 96 hours	Increased ICP, mass volume, GOS at 6 months	SSC	SSC	Blood and CSF
Ucar, 2004, *J Trauma* (80)	48	1.3 +/- 2.5 ug/l: unfavorable	Within 12 h PT and at 24, 48, 72 h PT	GOS at 6 and nine months	No statistically significant	No statistically significant	Blood
	48	62.2 +/- 21.8 ug/l: unfavorable	Within 12 h PT and at 24, 48, 72 h PT	GOS at 6 and nine months	SSC	SSC	CSF
Da Rocha, 2006, *Clin Chem Lab Med* (81)	23	0.79 ug/l	Adm, 24 h and 7 days PT	GOS at ICU discharge, death	-	73% at admission for fatal outcome	Blood

(continued)

Table 3 ***(continued)*****.** Validation of post-TBI S100B values to predict outcome in severe TBI

	N	Cut-off-value	Time of sampling	Type and Timing of outcome	Sensitivity	Specificity	Blood or CSF
Watt, 2006, *Brain Injury* (90)	23	Comparison with control group	Within 12 h PT and daily for 7 days	neuropsychological test within 2 weeks from PTA	SSC, maximum for PTA duration (WPTA Scale)	SSC, maximum for PTA duration (WPTA Scale)	Blood
Korfias, 2007, *Intens Care Med* (86)	112	0.5 ug/l	Adm and every 24 h for a maximum of 7 days	1 month survival, 1st week worsening	SSC	SSC	Blood
Nylen, 2008, *Acta Neurochir* (87)	59	0.55 ug/l (S100B), and 0.17 mg/L (S100BB)	on days 1, 2, 3, 4, 6, 8 and once between days 11 and 14	One year follow-up, ICP, need of surgery	-	100% for GOS 1-3	Blood
Kirchhoff, 2008, *Eur Journ Med Res* (97)	21	Comparison with control group	after positioning the ventricular catheter and 12, 24, 48 and 72 h PT	ICP >15 mmHg	SSC	SSC	Blood and CSF
Olivecrona, 2009, *JNeurNeurosPsych* (70)	48	-	Within 6-37 h PT and every 12 h for 5 days	Death at 3 and 12 months	33,3%	95%	Blood
Rainey, 2009, *Resuscitation* (82)	100	0.53 ugL/1.	Within 22-26 h PT	GOS at 3 months or death	82% for GOS less of 4; 83% for death	60% for GOS less of 4; 49% for death	Blood
	100	0.53 ugL/1.	Within 22-26 h PT	Emergency Neurosurgery	No statistically significant	No statistically significant	Blood
Murillo-Cabezas, 2010, *Brain Inj* (83)	87	day 2: >2.37 ug/l; day 3: >1.41 ug/l	Adm and 24, 48 and 72 hours PT	GOS at 12 months	SSC	SSC	Blood
Vos, 2010, *Neurology* (84)	79	1,13 ug/l	Adm	GOSE at 6 months	25%	100%	Blood
Stein, 2011 *Neurocrit Care* (88)	23	Linear regression	Twice daily for 7 days	ICP>20 and CPP <60 mmHg in first 7 days	SSC	SSC	CSF
Bohmer 2011, *Neurosurgery* (68)	20	14.34 ng/mL	Within 2-4 h post hospitalization and daily for 7 days	Brain death in first 7 days	60% for survival-non survival	100% for survival-non survival	CSF

N: number of patients
Adm: at admission
WPTA: Westmead Post-traumatic Scale
GOS: Glasgow Outcome Scale
GOSE: Extended Glasgow Outcome Scale
PT: Post Trauma
SSC: statistically significant correlation
ICU: intensive care unit

S100B concentrations (84, 96) Specifically the highest predictive accuracy for unfavorable outcome is obtained by combining S100B with the presence of a mass lesion.

The evaluation on different biological fluid sample have also been carried out (68, 74, 80, 88, 93, 97) with the results that urine concentration are not predictive of CT results. The concentration found in CSF showed in one study better correlation than serum concentrations with the brain contusion size (74). The CSF concentration have been shown to predict ICP, CPP, brain death and outcomes (68, 79, 88, 98). Anyway, if we move to CSF biomarker concentrations, it seems that sensitivity and specificity are higher for NSE compared with S100B (68).

In the same directions other authors matched S100B serum concentrations with concentrations of other two more important biomarkers of brain damage (GFAP and NSE) to test CT positive predictive power (69) or prognostic value (70, 77, 84) on follow-up without univocal results.

We could say that certainly the potentialities of this protein are strong and interesting, with the opportunity to positively influence medical practice but the literature is strongly inhomogeneous. The absence of a common cut-off value and of a common timing of samples (which is particularly important for molecule with short half-life as S100B) makes it difficult to compare different studies.

Children

The avoidance of unnecessary CT is even more important in asymptomatic children with TBI. Consequently the importance of biomarkers of brain damage is oriented in this population more to the abovementioned application than to the outcome prediction.

Diagnostic value

In the last years a great attention has been given to validate protocols to detect the low-risk children who sustained a MTBI (7, 8) in order to avoid unnecessary exposure of children to ionizing radiation. The life-time cancer mortality of approximately 1/1500 in a 1-year-old child has to be considered (9). The estimated corresponding mortality risk for 10-year-old child is about 1 in 5000 (9, 10).

In two recent studies on MTBI children (98, 99) the calculated serum S100B sensibility to predict a CT positive for intracranial injury is 100% within 6 hours from trauma. Furthermore in one study (99) it is shown how it may be more reliable than clinical signs including GCS, neurologic examination (which often is difficult in pediatric patients) and the medical history of the trauma such as unconsciousness and amnesia.

The topic, anyway, is still controversial: 3 different studies of the last years investigate the role of the biomarkers comparing two groups of pediatric patients: MRI positive versus MRI negative patients (100); CT positive versus CT negative patients (101); symptomatic versus asymptomatic patients (102). In all cases the authors assessed the absence of reliability of S100B in distinguishing the two clusters and particularly in predict the imaging identifications of intracranial injury.

The application of serum S100B protein as a clinical biomarker of brain damage in pediatric cases of mild TBI meets three principal obstacles: variability in relation with age, extracranial sources of S100B and timing of samples.

An analysis was carried out in some studies (103-105) to investigate if the mechanism of TBI (inflicted vs non inflicted) could influence serum S100B value. The mean concentration, peak concentration, and the time to peak were not associated with the dynamics of injury, even if it could influence the long-term outcome (worst in inflicted TBI).

Patient age is one of the most important factor in serum biomarkers' concentration. Gazzolo et al (106) evaluated the normal levels of S100B in serum in 1004 children to create a reference curve and found that concentrations of the protein in children 0-15 years of age presented a pattern consisting of a decrease from 0 to 7 years followed by an increase from 7 to 13 years and, finally, by a second decrease in S100B from 14 to 15 years of age. The highest individual concentrations were observed in the first year of life and between 9 and 10 years of age. The authors used these data to provide a reference curve indicating S100B concentrations in peripheral blood at different ages in the two

Table 4. Value of S100B as predictor factor in pediatric population

	Patients	Age	GCS	Target of predictivity	Timing	Cut off level	Sensitivity CSF	Specificity
Akhtar, 2003, *Ped Crit Care Med* (100)	17	5-18 years	3-15	MRI +	Within 6 hours and 12 hours post trauma	0.20 ug/L	No statistically significance	No statistically significance
Berger, 2005, *J Neuros Ped* (103)	*1*00	0-13 years	3-15	CT+	At admission and 12 to 24 hours post trauma	0,017 ng/ml	77%	72%
Bechtel, 2009, *Pediatrics* (101)	152	0-18 years	3-15	CT+	Within 6 hours	50 ng/L	75%	56%
Castellani, 2009, *Acta Pediatrica* (98)	109	0-18 years	13-15	CT+	Within 6 hours	0.16 ug/L	100%	42%
Geyer, 2009, *J Neuros Ped* (102)	148	6 months-15 years	13-15	Presence of clinical signs	Within 6 hours	Means value	No statistically significance	No statistically significance
Hallen, 2010, *J Trauma* (99)	111	1-18 years	3-15	CT+	At admission and 6 hours later	0.195 ug/L	100%	88%

CT+ or MRI+: presence of a brain injury at imaging

genders. A different study (107) on the contrary established an upper normal reference level of 0.16 microg/L in children from 3 to 18 years.

It is therefore clear that serum concentrations of S100B are related to patients' age .

The sampling time of S100B in relationship with the time of head injury is also an important factor that may influence its serum concentrations. Delayed serum measurements of S100B following a head injury may lead to erroneous conclusions regarding the severity and extent of neuronal damage, because of the relatively short half-life of the S100B protein (26, 33).

Outcome

Few studies which investigate the role of early S100B in head injured children are available. Some papers assessed a sensitivity from 80% to 90% for early serum sample to predict an unfavorable outcome in pediatric patients (105, 108, 109), One study shows also a correlation between urinary levels and outcome with a sensitivity of 83% (110). In all these studies samples are collected in the first 12 hours post-injury.

Clinical assessment with the GCS scale well correlate with brain trauma outcome (111-114), but the timing of GCS and its components may influence its predictive value. The highest predictive value is reported (96, 115) for motor component of GCS recorded at the time of Pediatric Intensive Care Unit (PICU) discharge. In a recent study Lo et al (96) found in a pediatric population that pairing the GCS with a single biomarker level greatly enhanced outcome prediction, increasing the sensitivity and specificity from 75% to 96% and 100% respectively. The best association is found for GCS score at PICU discharge associated with S100B serum level (> 0,04 ng/ml) in first day post injury.

Other source of variability

Extracranial sources

We know that S100B is also present out of nervous system, in adipocytes, melanocytes, chondrocytes and epidermal Langerhans cells. Extracranial sources

are therefore a relevant confounding factor in using these markers to predict the presence of an intracranial injury and long-term outcomes (19-23).

Some studies indicates that serum levels in multiple organ trauma are higher than the levels in an isolated head injury, since both brain injuries and extracranial injuries related to trauma independently increase serum S100B (50, 116-121). Studies in which this difference is not found are also available (81, 82). In the absence of head trauma, large extracranial injuries (large fractures, abdominal injuries, thoracic contusions without fractures, burns) increase S100B levels considerably, whereas small extracranial injuries (i.e., soft tissue contusions, wounds, sprains, luxations, or small fractures) only rarely are related to elevated values. In a study of 55 traumatic patients (120) the maximum increase was observed for fracture of homerus, femur, radius, tibia and hip. The S100B released could originate from the bone tissue itself or from the adipose tissue around and inside the bones. (116, 118, 120, 121).

Moreover some investigations were performed in sports athletes finding a correlation between increase of serum S100B and post-activity (122, 123).

A proposed methods to distinguish the cerebral component of S100B from the skeletal muscle component is the simultaneous measurements of serum CK to correct the S100B value. In a study of Bazarian et al (124) corrected S-100B improved the prediction of initial positive head CT and of three-month headache when compared to uncorrected S-100B.

It is also important to underline that a raised concentration of the markers may be due to different pathological conditions such as septic shock, haemorragic shock, or organ dysfunction of various degrees, dilated cardiomyopathy, cardiac arrest, cardiac surgery and others (125-128). The role of alcohol level to influence serum concentrations of the marker was hypothesized from different authors (129-131) as well as the chronic assumption of beta-adrenergic receptor agonists and phosphodiesterase inhibitors which may increase the S100B levels (56).

Race of individuals

Abdesselam et al (132) analyzed blood samples from 136 healthy individuals, divided into three groups according to race: Black (B), Asian (A), and Caucasian (C). Mean values for serum S-100B were significantly higher in groups B and A as compared with group C. Serum S-100B concentrations did not significantly differ between groups B and A.

These differences in serum S-100B according to skin color may be related to increased expression of this protein by healthy melanocytes in blacks and, to a lesser extent, in Asians, compared with Caucasians. Melanocytes from black individuals have been shown to have a higher metabolic activity than those from Caucasians, including increased melanin synthesis (133).

Conclusions

In traumatic brain injury S100B protein may have different interesting applications. The importance in minor traumatic brain injury is related to the opportunity of select low-risk patients to avoid an unnecessary radiation exposure. Despite some positive reports, two problems are present: a high false positive rate and a variable sensitivity, with some cases with GCS 15 and serum levels under cut-off but with intracranial lesions on CT scan. As prognostic factor, a correlation has been found between S100B levels and severity of injury, Glasgow Outcome Scale and frequency of Post-Concussional Syndrome. However, to predict outcome of TBI patients serum S100B can be used only in combination with clinical factors. In the pediatric population the predictive value of biomarkers to identify an intracranial pathology appears lower than in adult populations. This issue could be caused by a more important role of confounding factor which might independently influence serum concentrations such as age, race, and multiple trauma .

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Accepted: 15th May 2012
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