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REVIEW ARTICLE

Reliability of S100B in predicting severity of central nervous system injury

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Abstract S100B is a protein biomarker that reflects CNS injury. It can be measured in the CSF or serum with readily available immunoassay kits. The excellent sensitivity of S100B has enabled it to confirm the existence of subtle brain injury in patients with mild head trauma, strokes, and after successful resuscitation from cardiopulmonary arrest. The extent of S100B elevation has been found to be useful in predicting clinical outcome after brain injury. Elevations of S100B above certain threshold levels might be able to reliably predict brain death or mortality. A normal S100B level reliably predicts the absence of significant CNS injury. The specificity of S100B levels as a reflection of CNS injury is compromised by the findings that extra-cranial injuries can lead to elevations in the absence of brain injury. This potential problem can most likely be avoided by measuring serial S100B levels along with other biomarkers and carefully noting peripheral injuries. Serum markers GFAP and NSE are both more specific for CNS injury and have little to no extra-cranial sources. Sustained elevations of S100B over 24 h along with elevations of GFAP and NSE can more reliably predict the extent of brain injury and clinical outcomes.In the future, S100B measurements might reliably predict secondary brain injury and enable physicians to initiate therapeutic interventions in a timelier manner. S100B levels have been shown to rise hours to days before changes in ICP, neurological examinations, and neuroimaging tests. S100B levels may also be used to monitor the efficacy of treatments.

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Introduction

It is important for physicians in neurological intensive care units (NICU) to be able to predict the presence and severity of central nervous system (CNS) injury. An accurate appreciation of the severity of CNS injury can help predict outcomes and rationally help to decide when the application of aggressive therapeutic interventions would be appropriate [1].

In patients with little or no chance for a meaningful recovery intensivists can appropriately initiate comfort measures and avoid subjecting the majority of patients to a severely debilitating survival. At the same time, they can allocate limited vital health care resources to patients with better chances for meaningful recovery. Patients with neurological injuries from a variety of causes are at risk of secondary injuries from pathophysiological mechanisms such as increased intracranial pressure, vasospasm, stroke, and seizures. The timely application of aggressive medical and surgical interventions in the NICU can frequently mitigate the clinical impact of secondary injury to the brain.

Standard methods to prognosticate the severity of initial brain injury and anticipate the onset of secondary injury have included the neurological examination, neuroimaging studies, intracranial pressure monitors, electrodiagnostic testing, and transcranial dopplers. These tests have limited reliability in critically ill patients who are frequently given sedatives, analgesics, and muscle relaxants, or are not

stable enough to leave the NICU for frequent neuroimaging studies. Therefore, over the past 50 years, intensivists have searched for biological markers that can reliably reflect the severity of injury to predict outcomes or are sensitive enough to detect the early onset of secondary injury.

The journey to find this biological ''Holy Grail'' led investigators in the 1970s and 80s to look in the cerebrospinal fluid [2–8]. Excitotoxic amino acids, various enzymes, oxidative stress markers, and inflammatory response molecules have been measured in the CSF of such patients. Correlations of the levels of these CSF markers in patients with various neurological illnesses have led to conflicting and unreliable results. These problems may be due in part to the variable presence of intraventricular blood or simply to the use of external ventricular drains (EVD) whose placement alone has been found to potentially influence the CSF levels of these markers. Another impediment to the usefulness of CSF markers in the NICU was the difficulty justifying the risks of placing an EVD in some patients who might not otherwise need one.

The difficulties associated with the use of CSF markers have led investigators to search for an ideal serum marker that might be highly specific for brain injury, is sensitive to minor injuries, appears rapidly in the serum and is easy to measure with lab tests whose results could be available in a very short time [9, 10]. Various glial and neuronal proteins such as S100B, GFAP, and neuron specific enolase (NSE), have been found to be elevated in the blood of patients after stroke, brain trauma, and hypoxic encephalopathy [11]. Early investigations indicate that S100B might be sensitive enough to identify the early stages of secondary CNS injury and accurate enough to measure the severity of CNS injury so that it could reliably predict clinical outcome.

The purpose of this paper is to review the current literature on S100B in order to determine the utility in measuring this biomarker during the management of patients with critical neurological illnesses.

Biochemistry of S100B proteins

S100 proteins are a family of dimeric cytosolic calcium binding proteins made up of an alpha and a beta isomer. S100 proteins are found in abundance in astroglial and Schwann cells and have been found in a few tumors such as schwannoma, gliomas, melanoma, and neuroblastoma [12–16]. The alpha and beta isomers have also been called S100A1 and S100B respectfully. There are also other rare A types numbering over 6. Most S100 proteins are found as dimmers, which are combinations of isomers in a molecule. Three types of dimers are usually found. A homodimer consisting of two S100A1 isomers has been labeled S100A1A1. Likewise, a homodimer of two S100B isomers has been labeled S100BB. One heterodimer combining the alpha and beta isomers has been found and is called S100A1B [17]. All three heterodimers have been found in glial cells, astrocytes, ependymal cells, oligodendrocytes, and Schwann cells in the central and peripheral nervous system [18–22]. The S100BB dimmer is 21 kDa in size and is predominantly represented in glial and Schwann cells [21–23]. Unless specifically noted, all references in this monograph to the S100BB homodimer should be considered equivalent to S100B.

S100 family members have been found in the following locations outside of the nervous system: melanocytes, adipocytes, chondrocytes, and epidermal Langerhans cells [24–27]. The Beta isomer of S100 has also been found in skeletal muscle, skin, and in both white and brown fat [22, 25].

Investigations have noted that various mechanisms can release S100B protein from glial cells into the extracellular space [28]. Astrocytic activation has been found to immediately follow primary brain injury [29]. S100B protein is involved in the astrocytes reaction to injury by regulating the Calcium influxes and stimulating astrocytic proliferation via interaction with transcription factors [30–33].

Serotonin can enhance the release of S100B via stimulation of astroglial 5-HT1A receptors [34]. Corticotropin-like intermediate-lobe peptide and adrenocorticotropic hormone (ACTH) have both been found to release S100B [35]. Activation of A(1) adenosine or mFlu3 metabotropic glutamate receptors has been shown to lead to the rapid release of S100B proteins [36].

S100B may be involved in the cellular response to ischemia. Extracellular Adenosine levels are found to be elevated shortly after stroke and traumatic brain injury due to rapid intracellular ATP depletion. S100 proteins were found to be released within one hour after the extracellular adenosine levels rose [37–39]. S100 family proteins appear to be released from proliferating astrocytes [40]. Experimental injury to the brain via trauma or stroke *has* been found to induce a reactive gliosis with a peak around 3–4 days after the injury [41, 42].

S100B appears to be released into the extra-cellular space near the injured tissue and can 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 [43]. S100B is removed from the serum by renal clearance with a serum half-life of 20–25 min [44]. Serum concentrations of S100B are not influenced by hemolysis because S100B was found to be absent in red blood cells [45].

S100B measurement techniques

Various methods have been developed to measure the S100B levels in serum including a radioimmune assay

(RIA), immunoradiometric assay (IRMA), fluoroimmune assay (FIA), enzyme-linked immunoabsorbent assay (ELISA), and optic immunization [46–49]. The ELISA technique has been advocated by some authors as being simple, inexpensive, convenient, highly sensitive, and can avoid isotope contamination [50, 51].

S100B is usually measured with one of 8 available assay kits available from 4 different manufacturers (Table 1). Serum is usually collected from the patient and allowed to clot and centrifuged. It can be stored at 70 degrees below zero Centigrade until analysis. Most of the assay kits are sensitive to all dimers of S100 that have a beta isomer, which therefore measures a combination of S100A1B and S100BB fractions. The CanAG Diagnostic company offers the only kits that measure only one dimmer, either S100A1B or S100BB.

Some assay techniques such as the 100-immunoradiometric (IRMA) assays from Sangtec Medical are less sensitive than other techniques with lower detection limits of 0.2 μg/l. These assays are not sensitive enough for the analysis of normal control subjects. However, the clinical use of these assays may not require greater degrees of sensitivity. Most investigations have shown that patients with documented brain injury tend to have elevations significantly over this 0.2 microgram per liter limit. Therefore, they may be clinically useful. Their clinical utility is also enhanced by their turn around time of less than 2 h.

The Syn X Pharma Inc./Nanogen company in the United States noted on their corporate website that their assay was for research purposes and not for use in clinical diagnostics.

There are few studies available that compare the accuracy of the various test kits. Chen, and associates, performed infra-batch and intra-batch analyses of an ELISA test kit available from Sangtec. They concluded that the test was accurate and reproducible after they found the coefficient of variation to be less than 8% [51]. Four different Assays were compared side by side in a melanoma study. Two manual (Sangtec 100 ELISA and CanAG S100 EIA) and two automated (LIASISON Sangtec 100 and Elecsys S100) assays were evaluated [52]. The study correlated the results to clinical measures and reference values. They concluded that all four methods proved reliable with some minor distinctions. The automated tests appear to have more rapid turn around times of less than 2 h while the manual tests took almost 24 h to obtain the results. They also noted that the manual methods were not as sensitive as the automated methods. The automated methods also demonstrated more accurate coefficients of variation. They also found that the linearity between the assays was acceptable except for the Santec 100 ELISA manual method, which was the least accurate method. One investigation found that the 100 LIA automated assay from Santec[r] proved to be specific for S100B monomer either in the S100A1B or S100BB dimmers [53]. It did not cross react with S100A1.

In a multi-center study of Melanoma patients, the clinical and analytical performance of the Elecsys[r] S100

Table 1 Eight assay kits from four different manufacturers

Assay Name	Protein(s) Detected	Antibodies	Manufacturer
CanAg S100A EIA	S100A1B only	Monoclonal	CanAG Diagnostic Sweden
CanAg S100BB EIA Manual test Sensitivity only to 0.2 μg/l	S100BB only	Monoclonal	CanAG Diagnostic Sweden
Elecsys[r] S100 Automated test Rapid turn around Sensitivity < 0.02 μg/l	S100A1B, S100BB	Monoclonal/ Monoclonal	Roche Diagnostic Germany
Nexus Dx tm S-100 ELISA	S100A1B, S100BB	Monoclonal/ Polyclonal	Syn X Pharma Inc. Nanogen, USA
Sangtec[r] 100 IRMA Most rapid turn around (< 2hr) Sensitivity only to 0.2 μg/l	S100A1B, S100BB	Monoclonal/ Monoclonal	Diasorin S.r.l. Sangtec, Italy
Sangtec[r] 100 ELISA Manual test Sensitive to 0.2 μg/l Longer turn around (24 h)	S100A1B, S100BB	Monoclonal/ Monoclonal	Diasorin S.r.l. Sangtec, Italy
LIA-mat[r] Sangtec[r] 100 Manual technique Longer turn around More Sensitive < 0.02 μg/l	S100A1B, S100BB	Monoclonal/ Monoclonal	Diasorin S.r.l. Sangtec, Italy
LIAISON[r] Sangtec[r] 100 Automated ''Sandwich'' technique Rapid turn around, < 2 h. Sensitivity < 0.02 μg/l	S100A1B, S100BB	Monoclonal/ Monoclonal	Diasorin S.r.l. Sangtec, Italy

S100B = homodimer S100BB, S100A1B = heterodimers consisting of S100A1 + S100B

IRMA = immunoradiometric assay, ELISA = Elecsys = electrochemiluminescence immunoassay

electrochemoluminescence immunoassay from Roche Diagnostics was evaluated [54]. Lot-to-Lot correlation showed a coefficient of 0.99. Sensitivity was 41%, the positive predictive value was 0.50 and the negative predictive value was 0.91. The Elecsys[r] assay was found to be very sensitive, valid, rapid, and reproducible. The investigators then went on to compare the Roche assay to a Sangtec[r] 100 immunoassay by Diasorin. They found that the two tests also correlated well.

A study measuring S100B levels in patients undergoing carotid endarterectomy or carotid artery stenting compared the Elecsys[r] S100 assay to the LIAISON-mat S100 System from DiaSorin [55]. This study found the results of the two assays to be in agreement except that the Elecsysr assay tended to produce slightly lower results than the LIA-mat[r] assay from Sangtec. An investigation of patients with minor head injury in Brazil used the Elecsys S100 assay and found it to be very sensitive but not very specific [56].

The LIAISON[r] Sangtec[r] 100 assay by DiaSorin measures S100B levels via three monoclonal antibodies (SMSK 12, SMSK 25, and SMSK 28) in a two-sided (''sandwich'') inmmunoluminometric assay using coated magnetic particles. DiaSorin claims that the serum detection limit of this assay is lower than 0.02ug/l. One investigation using the LIAISON assay evaluated healthy individuals and found that 95% of healthy individuals had serum levels below 0.15 μg/l [57]. The ''sandwich'' technique has become very popular since 2004 due to its improved sensitivity and it's rapid turn around of results in less than 2 h.

The Sangtec assay uses monoclonal antibodies raised against peptides derived from bovine S100B. A Swiss study evaluated the specificity of the bovine assay against human S100B proteins [58]. Human recombinant S100 peptides were substituted for the lyophilized bovine protein calibrators supplied by Sangtec. The researchers found that there was no interference or cross-reactivity by other S100 proteins tested. These included S100A6 and S100A4, which are commonly expressed in brain tissue. These results determined that the bovine assay was a specific and reliable method for measuring S100B levels in human serum.

Sampling sites of S110B protein

Most investigators have sampled S100B from arterial lines. However, some investigators have advocated the use of jugular venous lines to measure S100B in an attempt to avoid mixing in fractions of S100B that arise from extracranial sources [59, 60].

The measurement of biochemical markers in the cerebrospinal fluid has been shown to reflect the presence and extent of brain injury [61]. Some investigators have sampled S100B from CSF taken from external ventricular drains [62]. However, a few investigators have been concerned that the very placement of a ventricular catheter might alter the CSF S100B levels; thereby, confounding the specificity of the measurements in brain injury [63].

S100B levels in normal control populations

Any evaluation of serum S100B levels in patients with brain injury must assess the data in relation to the range of values seen in a normal control population. This is especially important when evaluating patients with relatively minor brain injuries.

S100B can be found in very low concentrations in normal controls with the use of sensitive assays. Nygaard found that 95% of patients with no previous history of neurological injury had S100B levels below 0.12 μg/l [64]. Sex and age in normal controls do not appear to have any significant influence on serum S100B levels [65]. Anderson reported a series of 495 healthy control patients who had a medium serum S100B level of 0.01 μg/l with 97.5% below 0.13 μg/l [66].

Some investigators have suggested that the ''normal range'' of S100B should be considered significantly higher, from 0.12 μg/l to 0.50 μg/l [67–69]. Vox reported the highest ranges of normal S100B levels, with 95% of subjects without obvious brain injury having values lower than 1.13 μg/l [70].

Savola noted that these studies that reported higher normal levels actually lacked normal controls [72]. He argued that 0.20 μg/l may be a more appropriate cut-off point to reliably differentiate mildly injured patients from normal controls, as long as there were no significant extracranial injuries. Romner reported that 99% of patients with mild head injuries and serum S100B levels below 0.20 μg/l had normal Ct-scans [73]. Townend reported that only 1% of patients with head injury and a S100B values below 0.32 μg/l developed severe disability [74].

Brain injuries and elevated S100B protein

The majority of the literature reported to date involves the elevation of S100B and other serum biomarkers in patients who have sustained traumatic brain injury. Elevations in serum S100B protein have been reported to reflect injury to the brain and increased permeability of the blood brain barrier [20]. CT scan findings of patients have been correlated with S100B serum levels [75]. S100B levels have been found to reflect various consequences of severe brain injury such as contusions, swelling, and diffuse axonal injury.

Non-traumatic forms of brain injury have been shown to be associated with elevated S100B. Patients with ischemic brain injury have been found to have elevated serum

S100B levels [76]. Hydrocephalus has been associated with elevated CSF and serum protein markers [77]. Aneurysmal subarachnoid hemorrhage has also been associated with elevated S100B levels [78]. CNS infections have been found to elevate S100B proteins in the serum and the levels can be used as a means of following the patient's response to therapy [79].

Sources of S100B outside of the central nervous system

The reliability of S100B as a measure of brain injury depends upon its specificity to brain tissue. Extra-cranial sources of S100B have been reported in muscle and adipose tissue [22, 25, 80, 81]. Basketball players, boxers, ice hockey players, and joggers have been shown to have elevated serum S100B levels [82, 83]. Serum S100B has been found to be elevated in 18 marathon runners without any evidence of brain injury [84]. In this study, researchers measured serum S100B, GFAP, and CK levels before the 25K race and 1, 3, and 20 h after completing the race. S100B levels before the race were less than 0.08 μg/l and rose to an average of 0.16 μg l. S100B and CK elevations were highly correlated for each individual ($r = 0.7$, $P = 0.001$). The elevations of these markers were found to be weakly proportional to the body weight (S100B: $r = 0.50$, $P < 0.05$) (CK: $r = 0.41$, $P = 0.08$). All of these elevations returned to the normal pre-race levels by 20 h after the race. They also noted that none of the runners had elevations in their serum GFAP. They concluded that S100B can be elevated from injury to skeletal muscle without brain injury.

It is difficult to interpret the significance of S100B elevations in patients with brain injury when variable amounts of skeletal muscle injury may randomly influence the elevations. This problem of S100B's lack of specificity to brain injury warrants crucial consideration in the evaluation of patients with traumatic brain injury (TBI) and multiple organ injuries.

One manner to differentiate between the S100B coming from the brain and the body is to look at serial S100B levels over a few days time. The release of S100B from injured skeletal muscle has been found to be short lived and back to normal levels within 20 h. Elevations of S100B after 24 h may more reliably reflect brain injury as long as continued muscle injury is not occurring such as in compartment syndromes. However, even in these patients, simultaneous measurements of serum CK can afford the ability to distinguish the cerebral component of S100B from the skeletal muscle component. This information places the reliability of early (<24 h) measurements of S100B in question when evaluating patients with TBI and simultaneous skeletal muscle injury. Delayed measurements of S100B after 24 h may still be reliable predictors of clinical outcome in these patients.

Elevations of S100B protein in the serum after heart surgery have been demonstrated to be due to multiple sources such as traumatized fat, muscle and bone marrow [66]. Blood taken from the wound during heart surgery contained traumatized fat, muscle and bone marrow. The S100B levels in this surgical blood were found to be significantly elevated up to 40 μg/l.

The elevations of serum S100B after multi-organ system trauma appears to be due to multiple extra-cranial sources in addition to the brain. In one study, 17 multi-system trauma patients without brain injury were found to have elevated serum S100B levels [85]. Patients with bone fractures were found to have the highest elevations of S100B ranging 2–10 μgm/l. Patients with thoracic contusions without fractures were found to have elevations of S100B between 0.5 μgm/l and 4 μgm/l. Anderson suggested that normal serum S100B levels more reliably predicted normal brain function than elevated levels predicted brain injury [66].

Savola, and associates, attempted to evaluate the relative contributions of different types of head and extracranial traumas on serum S100B levels by evaluating 379 consecutive trauma patients, which included 54 patients with cranial and extra-cranial injuries as well as 155 patients with pure extra-cranial injuries [72]. They measured S100B levels within 6 h of injury and correlated the levels to neurological examinations, CT scan findings, location, and severity of extra-cranial injuries and the injury severity score (ISS). They found that severe extra-cranial injuries without brain injury could significantly elevate S100B levels. However, they found that brain injury was associated with significantly higher levels of S100B. The median elevation of S100B from head trauma was 0.17 μg/l while the median elevation of S100B from pure extra-cranial trauma was significantly lower at 0.07 μg/l ($P < 0.001$). They confirmed previous findings that serum S100B levels correlated well with the severity of brain injury with median elevations in moderate to severe brain injury at 1.27 μg/l ($P < 0.001$). Severe purely extra-cranial injuries elevated the median S100B levels to 0.35 μg/l and minor extra-cranial injuries elevated the median S100B levels only to 0.07 μg/l. They identified that 61% of brain injured patients and only 26% of extra-cranially injured patients had levels above 0.13 μg/l (Pearson Chi Square test, $P < 0.001$). Only 4% of patients with pure extra-cranial lesions had levels greater than 0.50 μg/l; whereas, 67% of patients with moderate to severe head injury surpassed the 0.50% level. They also confirmed that normal S100B levels were a very reliable predictor of good neurological outcome.

Schmidt noted that serum NSE is usually derived from circulating red blood cells in normal patients and that hemolysis that occurs with systemic injury can lead to significant NSE elevations in patients without brain injury [86]. GFAP does not have significant extra-cranial sources and may be a more specific marker for brain injury [70, 87]. GFAP is a specific marker for well differentiated astrocytes in the CNS. Patients with hydrocephalus and stroke have been found to have elevations of GFAP [76, 77]. Normal GFAP levels in patients with elevated S100B levels may indicate that the S100B elevations were due to extra-cranial sources.

Cerebrovascular disease

Numerous investigations have noted that S100B was found to be elevated in patients with cerebral infarctions, and that the levels were proportional to the volume of infarction seen on scans and correlated to clinical outcomes [88–93].

S100B measurements may be sensitive enough to identify patients with subtle or subclinical ischemic deficits. Patients with TIAs and normal scans were found to have significantly lower elevations compared to patients with fixed neurological deficits and abnormalities on the scans [93]. Connolly reported that secondary rises in serum S100B after carotid endarterectomy might be a sensitive measure that reflects subclinical neurological deficits [94]. The S100B elevations found in the completed stroke patients were found to peak on day 3 after the onset of symptoms. The initial elevations were thought to be due to adenosine linked release in response to ischemia found in the peri-infarctional zone where cerebral perfusion continues and the blood brain barrier is found to be disrupted. The high levels of adenosine and S100B in the center of the infarction probably does not raise the serum levels due to the lack of perfusion in these regions. The peak on day 3 was thought to be due to the secretion of reactive astrocytes, which are found to be present around day 3 in the peri-infarctional zones [95–97].

Prognostic implications

Since several studies have identified that serum S100B levels reliably correlated with clinical outcomes; this information may be helpful in guiding families and clinicians in choosing the most appropriate treatment. If the levels reach a threshold that reliably predicts poor outcome despite aggressive therapy then these treatments could be avoided and comfort measures considered.

The clinical utility of this test will require more reliable information describing what the threshold for poor outcome really is. This will require a standardization of the different methodologies that measure S100B similar to the international normalized ratios (INR) developed to measure a standardized coumadin effect in patients.

Therapeutic implications

Some investigators have advanced a theory that the temporal pattern of S100B elevations might be able to predict the extent of reperfusion that might occur after the administration of intravenous or intra-arterial thrombolytic drugs [93]. Early reperfusion was predicted to release the significant amounts of S100B that was located in the ischemic core of the infarction thereby elevating the serum levels. However, one investigation noted that two patients who presented with middle cerebral artery occlusion were successfully treated with reperfusion within 6 h. These early reperfusion patients demonstrated significantly lower S100B levels [98].

Hypoxic brain injury and cardiac arrest

Elevations of S100B have been reported after cardiac arrest, hypothermic circulatory arrest, and during cardiopulmonary bypass surgery [99–101]. The majority of patients who suffer a cardiac arrest will remain unconscious for some period of time with 20% resulting in a vegetative state [102].

Investigations have correlated the S100B elevations to clinical outcomes of patients after successful cardiopulmonary resuscitation. Martens evaluated 63 patients following cardiac arrest and correlated serum and CSF S100B and NSE measurements at 24 h for the serum and at 48 h for the CSF [103]. Death or vegetative state developed in 55% of the patients. The remaining 45% regained consciousness. The study found the most specific predictor of poor outcome was a serum S100 value > 0.7 μg/l with a specificity of 96% and positive predictive value (PPV) of 95%. A subsequent study of 58 patients who suffered out of hospital cardiac arrests found less striking results using a serum S100B value of 0.7 μg/l at admission [104]. This study was able to predict that consciousness would be regained with a specificity of 85% and PPV of 84%.

Using biochemical markers such as S100B to predict clinical outcome after cardiac arrest may be confounded by extracranial release of S100B from soft tissue and skeletal injuries after chest compression and defibrillation as well as renal dysfunction due to hypoperfusion. However, Rosen found that the extracranial component of the S100B elevations was short lived and prolonged evaluation of S100B of serial levels may more accurately reflect CNS

injury [99]. An elevated serum S100B level (>0.2 μg/l) on day 1 of a cardiac arrest predicted death within 2 weeks in 71% of their patients. However, if S100B was still elevated (>0.2 μg/l) on the second day there was a positive predictive value for mortality improved to 100%. This indicates that sustained elevations of S100 may be more predictive of ongoing CNS damage and subsequent poor outcomes.

Hachimi–Idrissi reported that inducing mild hypothermia to a core body temperature of 33 degrees Celsius significantly reduced serum S100B levels during the first 24 h following a cardiac arrest [105]. The normothermic cohort group was found to have a slight but insignificant decrease in S100B levels. The patients treated with mild hypothermia were found to have better survival and clinical outcomes. Therefore, serum S100B levels may be able to reflect the benefits of medical interventions in patients with cardiopulmonary arrest.

Future investigations may be able to use serum S100B levels and clinical outcome scales to measure the neuroprotective effects of new interventions. Optimal clinical outcomes might require adjustment or changes in the ongoing medical intervention if subsequent serum S100B levels do not respond to treatment.

Cerebral trauma

Minor brain injury

The sensitivity of serum S100B elevations to reflect minor brain injury is best determined by evaluating investigations of patients with minor brain injury either to minor trauma or in response to minor ischemic events due to decreased cerebral perfusion pressure or emboli occurring during cardiopulmonary bypass surgery or hypotensive events. The use of serum S100B elevations to predict early secondary brain injury is another measure of the test's sensitivity.

Minor head injury with Glasgow Coma scores (GCS) between 13 and 15 have been noted to be associated with significantly elevated serum S100B levels in 20–38% of patients [67, 68, 106]. Patients with elevated serum S100B levels were found to have poor neuropsychological outcomes and post-concussion symptoms [107–110]. Waterloo reported that elevated serum S100B levels 12 h after minor head injury predicted deficits on measures of reaction time, attention, and speed of information processing in patients evaluated 12 months after the injury [109].

The S100B levels have been found to be correlated to the extent of brain contusions found on CT-scans [110–112]. Some patients with elevated S100B levels were found to have normal CT-scans after head trauma [106]. In these patients, diffuse axonal injury was presumed to be the cause of their brain injury and elevated S100B levels. MRI scans have been found to be abnormal in some though not all of these patients. Ingebrigtsen reported a series of 50 patients with minor head injuries where serum S100B levels were correlated to neurobehavioral outcome and MRI scan imaging [106]. All patients had normal CT scans and a GCS between 13 and 15 at the time of injury. Serum S100B levels were measured in the ER and hourly for 12 h. MRI scans were performed within 48 h. Blinded neuropsychological evaluations were performed within 48 h and 3 months after the injury. The researchers found that 14 (28%) of their patients had detectable levels of S100B in the serum. Five (10%) of their patients were found to have contusions on the MRI scans that were not detectable by CT scanning. They concluded that S100B elevations were a valid measure of the presence and severity of minor traumatic brain injury because four out of these five patients with contusions found on MRI scan were found to have elevations of S100B in their serum and there was a trend for neuropsychological abnormalities of attention, memory, and information processing in patients with elevated S100B. The one patient with contusions found on MRI scan who did not have an elevated S100B level underwent the first measurement over 6 h after the injury and might have been positive if the measurements were performed closer to the time of the injury. They noted that 36% of patients with initially elevated S100B levels returned to normal within 6 h. Nine patients with elevated S100B levels did not have an abnormality seen on MRI scanning. They noted that these patients with normal MRI scans had significantly lower elevations in their S100B levels compared to the patients with contusions seen on MRI scanning. Their follow-up neuropsycholgical data was available for only 72% of patients. However, there were no significant differences in the percentage of S100B positive patients evaluated at three months or in the group lost to follow-up. All of the subtests demonstrated greater neuropsychological deficits for patients with elevated S100B serum levels. However, these trends were found to be statistically significant in only one out of nineteen subtests. The large standard deviation and small number of patients in this study may have been responsible for the lack of statistical significance.

Some investigations have identified individuals with normal serum S100B levels despite objective signs of significant brain injury on CT scanning and neurological examination [106–112]. These false negative examples raise the question as to what exact pathophysiological mechanisms are required for elevations of S100B to occur. Carefully controlled animal investigations might be able to identify the relative elevations of S100B levels after injuries that do not lead to Ct scan abnormalities such as

diffuse axonal injury, agonist induced neuroexcitation injury, or ischemic brain injury.

Romner reported a series of 278 patients with a full spectrum of head injuries and noted that nondetectable serum S100B protein levels (<0.20 μg/l = lower limit of sensitivity of their testing method) predicted a normal CT scan with negative predictive value of 99% [73]. This finding was supported by other investigators who consider that S100B levels below 0.20 μg/l reliably predicted good clinical outcomes.

Neurosurgeons and emergency room physicians have been searching for a reliable sign that would identify which patients with minor head injuries would develop life threatening deteriorations. This knowledge would allow us to appropriately triage high risk patients to intensive care units for close observation and lower risk patients to regular floor beds or possibly even early discharge. The use of S100B serum elevations as a measurement of the severity of brain injury has been demonstrated in the investigations reviewed. However, none of these investigations have even attempted to correlate S100B elevations to the risk of neurological deterioration in patients after minor head injuries. Until these studies are performed, we will not know how reliable S100B measurements will be in the triage of patients with minor head injuries.

Severe brain injury

Clinicians have searched for reliable methods to predict the clinical outcome of patients who sustain severe brain injury [113–115]. The advances in intracranial monitoring and neuroimaging have improved the reliability of our predictions [116].

Attempts to predict clinical outcome or mortality using the admission GCS or CT-scan findings have not been as reliable as we would like. Petzold found that the initial GCS of 3–8 predicted fatal outcome with a sensitivity of only 62% [117]. This is partially due to the use of intubation and intravenous sedation, anesthetics and neuromuscular blockade in the early resuscitation efforts [118].

The Traumatic Coma Data Bank (TCDB) was established in the early 1980s to enable the analysis of various high-level statistical methods to predict outcome in a well documented computerized database of over 1000 patients who were treated for severe head injuries with a standardized protocol. The database collected a consistent and large amount of clinical and radiographic data from the emergency room and ICUs on these patients. The database also collected outcome data taken 6 months after the injury. CT-scan results were classified in a manner that took into consideration the status of the basilar cisterns and shift of midline. This database found the following statistically significant prognostic factors: age, best motor response, papillary response, papillary size, eye opening, verbal response, oculocephalic reflex, midline shift, presence of extra or intracerebral lesions, ICP, pulse, and presence of multiple injuries. Race, sex, blood pressure, blood alcohol, and time from injury to admission were not found to be statistically significant in predicting outcome. The use of computerized statistical models taking into consideration these group predictors theoretically could yield the highest predictive accuracy. Choi and Barnes described the comparison of four basic statistical prediction models on this TCDB [119]. They compared the discriminant function method, the logistic regression method, the nearest neighbor method and the prediction tree method. The analysis of these comparisons revealed that the upper limit of accuracy in predicting outcome of patients based purely on ER and ICU data was only 80%. This was a significant advance. However, the 20% inaccuracy rate using this aggressive predictive method made it fall short of the goals to develop the ability to predict clinical outcomes enough to reliably influence treatment strategies.

Several investigations have reported that S100B serum level elevations reliably reflect severe brain injury and that the extent of S100B elevation can reliably predict functional outcomes and even potentially predict patient mortality [59, 70, 71, 107, 111, 112, 120–125]. These investigations typically evaluated patients who sustained a severe traumatic brain injury with Glasgow Coma Scores (GCS) following resuscitation of 8 or less. All patients were treated aggressively with surgical decompression of mass lesions and medical management designed to maintain normal ICP and cerebral perfusion pressure (CPP). Serum S100B levels were evaluated at the time of admission and serial levels taken usually on a daily basis during the course of their treatment in the ICU. Elevations at the time of admission were correlated with clinical outcomes using the CT-scan findings, GCS, Injury Severity Score (ISS), neurological examinations, or Glasgow Outcome Score (GOS) at various intervals from discharge up to one year after the injury. Some investigators utilized blinded observers.

These investigators uniformly found that serum S100B levels were directly proportional to the severity of injury and inversely proportional to the Glasgow outcome score. Vos found that S100B, NSE, and GFAP serum levels correlated significantly with the ISS and CT findings [70]. However, these biomarker levels did not correlate significantly with age, sex, or GCS.

They found that Patients with poor outcomes (GOS 1–3) had significantly higher serum S100B levels than patients with better outcomes (GOS 4–5) with a P value of <0.01. Mean S100B levels ranged from 1.1 μg/l to 4.9 μg/l in patients with poor outcomes. Patients with better outcomes demonstrated significantly lower S100B levels ranging

between 0.3 μg/l and 1.6 μg/l. Some of these studies calculated the cut-off value or threshold of S100B elevation that could reliably predict poor outcomes with figures ranging between 2.0 μg/l and 2.5 μg/l [51, 59, 107, 111, 112, 124]. Some investigators also found that GFAP and NSE were elevated in a similar manner as S100B protein.

Serum S100B levels were found to be elevated more in patients who developed hypotension, hypoxia, or absent pupillary light reflexes. Serum levels of S100B did not correlate well with the hospital admission GCS. However, Raabe reported that serum S100B levels in 44 patients with severe head injury correlated very well with CT scan findings of injury measured by the CT-TCDB [112]. Herrmann reported a similar ability for serum S100B levels to predict CT scan findings in 66 patients with severe head injuries [108].

Influence of multiple organ injury on S100B levels

Many patients with severe traumatic brain injuries also sustain significant injuries to multiple organ systems. Anderson, and associates, found S100B levels to be elevated in patients with multiple organ systems even without traumatic brain injury [66]. They found that the median S100B elevation of these patients was 0.01 μg/l. Pelinka attempted to determine whether the measurement of S100B serum levels was a reliable marker for TBI in a series of patients with and without multiple organ trauma [126]. They studied 23 patients with TBI alone, 23 patients with TBI and multiple organ trauma, and 9 patients with multiple organ trauma without TBI. They found that all patients with multiple organ trauma demonstrated elevated S100B levels whether or not TBI was present. Therefore, S100B levels are sensitive to injury outside of the brain. They found that the initial S100B levels were highest in non-survivors with TBI and multiple organ injury followed by survivors with TBI and multiple organ injury. Therefore, they commented that S100B serum levels taken during the first 24 h do not reliably predict clinical outcome in this patient population. They advocated the daily measurement of serum S100B markers as a reliable means of predicting clinical outcome. They further stated that secondary S100B elevations could be used as a reliable marker for the development of secondary brain injury enabling intensivists to initiate clinical interventions at an earlier timeframe.

Savola, et al, measured serum S100B levels in 379 consecutive trauma patients and evaluated the influence of extra-CNS injuries on S100B levels [72]. Significant head trauma was found in 224 patients 54 of who also had simultaneous extra-cranial injuries. They also evaluated 155 patients with pure extra-cranial injuries without evidence of brain injuries on scans or neurological evaluations. Therefore, they were able to compare the S100B elevations in groups of patients with pure closed head injuries, pure extra-cranial injuries, or combinations of the two.

They evaluated the relative contribution of various extra-cranial injuries by subdividing the patients into groups with no injury, small injuries, and large injuries. Patients without extra-cranial injuries had a median S100B level of 0.02. Patients with soft tissue contusions, wounds, sprains, luxations, and small fractures were placed into the small injury group and had a median S100B level of 0.07. Significant elevations did not occur until patients sustained large fractures and abdominal injuries. This group with large extra-cranial injuries had a median S100B level of 0.35.

They also subdivided the patients without extra-cranial injuries into graded brain injury groups. They found that the S100B levels correlated significantly with the severity of brain injury ($P < 0.001$). The first group had no head injury, was neurologically and radiographically normal and their median S100B levels was 0.02. The next group who sustained a closed head injury without loss of consciousness and any neurological symptoms had a median S100B level of 0.10. The third group sustained a mild brain injury with a loss of consciousness for less than 24 h, were complaining of neurological symptoms and had a median S100B level of 0.15. The Last group with moderate to severe brain injury had a loss of consciousness greater than 24 h, demonstrated focal deficits on neurological examination, had abnormalities on CT-scanning and had a median S100B level of 0.94.

They correlated the brain injury and extra-cranial injury groups as seen in Table II. They found that the largest S100B elevations occurred in patients with severe head injuries and severe extra-cranial injuries with a median S100B level of 4.01 μg/l. Head trauma patients alone had a significantly higher median S100B level of 0.17 μg/l than patients with pure extra-cranial injuries who had a median level of 0.07 μg/l ($P < 0.001$). The levels correlated to the severity of head injury and extracranial injuries. Patients with severe head injuries had significantly higher S100B levels (median 1.27 μg/l) than patients with mild head injury (median 0.16 μg/l).

Large extra-cranial injuries were found to significantly elevate serum S100B levels. However, small extra-cranial injuries had very little influence on S100B levels compared to brain injury. They also found that a normal S100B level after a closed head injury would very reliably rule out significant brain injury.

Savola, and associates, also evaluated the relative reliability of S100B cut-off values of 0.13, 0.20 and 0.50 μg/l in predicting the presence and extent of brain injury. They found that the cut-off level of 0.13 μg/l did

Table 2 S100B elevations correlated with severity of head injury and extra-cranial injury.

	No CHI or , Normal Neuro	CHI with Normal Neuro	CHI with Mild Brain Injury	CHI with Moderate to Severe Brain Injury	All
No Extra-cranial Injury	0.02	0.10	0.15	0.94	0.15
	0.0–0.1	0.06–0.17	0.07–0.28	0.22–1.66	0.06–0.28
	n = 8	*n* = 27	*n* = 131	*n* = 12	*n* = 178
Small Extra-cranial Injury	0.07	0.15	0.24	0.53	0.08
	0.03–0.12	0.09–0.27	0.13–0.48	0.34–5.75	0.04–0.16
	n = 148	*n* = 7	*n* = 30	*n* = 6	*n* = 191
Large Extra-cranial Injury	0.35	0.05	0.93	4.01	0.82
	0.20–0.64		0.80–13.50	2.08–6.42	0.32–3.73
	n = 7	*n* = 1	*n* = 4	*n* = 6	*n* = 18
All	0.07	0.10	0.16	1.27	*N* = 384
	0.03–0.13	0.06–0.18	0.07–0.32	0.32–3.33	
	n = 163	*n* = 35	*n* = 165	*n* = 24	

CHI = closed head injury

not allow them to reliably determine the relative contributions of brain injury from extra-cranial injuries. Serum levels of S100B greater than 0.13 μg/l was seen in 61% of the head injury patients and in over 26% of the patients with extra-cranial injuries. They felt that 0.50 was a better cut-off level because only 4% of purely extra-cranial injured patients and 67% of the patients with moderate to severe brain injuries exceeded this level. However, 2 out of the 7 patients with large extra-cranial injuries had levels over 0.50 μg/l.

The weakness of Savola's investigation include the lack of serial serum S100B levels, the lack of careful radiographic correlations, and the lumping of all patients with abnormal scans or focal neurological deficits into one group. Thus, the investigators were not able to determine the relative contributions of various surgical procedures on the S100B levels. They were also not able to correlate the S100B level to clinical outcomes.

Penetrating brain injuries

Most of the studies reported above evaluated patients with closed head injuries. Patients with penetrating brain injuries may have a different propensity to elevate S100B serum levels. Pelinka, and associates, noted that the highest initial S100B level they found was in a surviving patient with a gunshot wound to the brain without any peripheral organ injury [126]. They noted that all the other patients sustained closed head injuries and that patients with penetrating injuries would most likely have higher S100B levels and require a separate study.

Prediction of death

The need to develop criteria to clinically predict brain death in patients is universally agreed upon. Various confounding factors present in some patients, have been found to reduce the reliability of brain death determination [127, 128]. These factors include shock, hypothermia, metabolic disturbances, facial trauma, pre-existing papillary abnormalities, and CNS depressant medications. It is also very difficult to clinically determine brain death in very young children whose cranial nerve responses have not yet fully developed.

The measurement of the Glascow Coma Scale (GCS) has been shown to be a poor predictor of brain death. In one study of patients with severe head injury (GCS 3–8) the GCS predicted mortality with a sensitivity of only 62% [117]. Monitoring ICP in these patients has been thought to improve the reliability of predicting mortality. However, the sensitivity of high ICP to predict mortality has been reported to be only between 69% and 80% [129].

The discovery of markers that would reliably predict patient mortality would be very helpful for families and physicians who would consider further aggressive therapy as unrealistic in the face of certain death. Investigators have evaluated the reliability of various biomarkers, including S100B, in the prediction of brain death.

Regner, and associates, measured S100B levels in patients with severe brain injuries and found that patients who met brain death criteria had significantly higher S100B levels compared to surviving patients [130]. However, many patients with TBI have associated multiple traumatic injuries to their bodies that might significantly

elevate S100B levels. Pelinka, and associates, attempted to measure the relative contributions of TBI and multiple trauma to S100B elevations [126]. They compared S100B serum levels in 23 patients with pure TBI, 9 patients with pure multiple trauma without TBI, and 23 patients with a combination of TBI and multiple trauma. Initial levels of S100B were found to be markedly elevated in both surviving and non-surviving patients. They found that 48 h after the injury the S100B levels of the survivors and non-survivors became significantly different. All of the 10 pure TBI patients who became brain dead had elevated serum S100B levels beyond 48 h or demonstrated a triphasic response with a secondary rise during the time that they suspected secondary brain injury to occur [126]. All 13 survivors except for one demonstrated S100B levels that dropped and remained low 48 h or earlier after the trauma. The one patient who survived despite an elevated S100B level after 48 h was due to a transient elevation in the wake of an operation. In the 23 patients with TBI and multiple trauma, S100B levels were also found to be uniformly increased during the initial 48 h. Survivors and non-survivors once more demonstrated distance differences in their S100B levels after 48 h. However, these differences were not found to be as significant as they noted in the patients with pure TBI. The 15 surviving patients demonstrated delayed decreases in the S100B levels after 48 h. Non-surviving patients followed a different course. The 8 non-survivors who died from multiple organ failure demonstrated a triphasic course with an initial elevation followed by a decrease and then a thermal elevation shortly before death. The 2 patients with TBI and multiple trauma who became brain dead did not demonstrate a terminal rise in S100B levels. The 9 patients with pure multiple trauma without TBI also demonstrated a uniform initial increase in S100B levels. All 8 of the survivors again demonstrated the biphasic course of delayed decreases of S100B levels. The one patient who did not survive after multiple trauma without TBI passed away within 8 h after the injury. Therefore, delayed S100B levels were not measured. They also compared the predictive value of S100B serum levels to GCS and the ISS and a combination of the GCS and ISS (TRISS). They found that the TRISS score underestimated mortality from brain death when the trauma was limited to the brain. In TBI patients without injury to multiple organ systems the actual brain death mortality was three times higher than predicted by the TRISS score. They also found that S100B levels did not correlate well with CT scan findings, localization or extent of injury found on CT, or the GCS. Pelinka concluded that S100B levels were reliable predictors of both mortality and clinical outcome in surviving patients after multiple trauma with or without TBI. However, he pointed out that serial S100B measurements were necessary and that the levels taken over 24 h after the injury were far more reliable than levels taken only during the first day of injury.

The absolute level of S100B elevation during the first 24 h after injury might be a reliable predictor of mortality or clinical outcome despite the warnings of Pelinka. Vos reported on a series of 85 patients with severe head injury where the initial S100B levels accurately predicted mortality. Vos, and his investigators, found that patients who died had a statistically significant higher initial serum S100B level than patients who survived with a P value of <0.001 [70]. They also noted that all patients with elevations of S100B over 1.13 μg/l liter died. Cut-off values for other biomarkers were also reliably able to predict death or poor outcome: NSE (21.7 μg/l), GFAP (1.5 μg/l). These cut-off values predicted death with a sensitivity of 0.85 GFAP, 0.85 for NSE and 1.0 for S100B. The negative predictive value of GFAP was 0.88, NSE was 0.86 and S100B was 1.0. Therefore, when any given patient's levels were below these cut-off values, they had a 86–100% chance of surviving. However, the specificity for these cut-off values was only 0.52 for GFAP, 0.48 for NSE and 0.41 for S100B. The positive predictive value for these cut-off values was 0.46 for GFAP, 0.45 NSE and 0.46 for S100B. This level of sensitivity and specificity might not be adequate to make clinical decisions. Therefore, the authors performed a logistic regression analysis and found that the reliability of mortality prediction increased when they used a combination of NSE and GFAP biomarker levels and the GCS. The ability of these serum markers in predicting either death or poor clinical outcome (crude odds ratio with a 95% confidence interval) was found to be more reliable than neuroimaging findings, GCS, or ISS.

One important feature of Vos's investigation was that it only correlated biomarkers measured within the first day of injury [70]. Other investigations looked at the temporal pattern of biochemical markers measured over the ensuing days after the injury. Another short-coming of Vos's investigation is that 89% of the patients studied also had multiple traumatic injuries. He also did not differentiate the cause of death between brain death and multiple organ failure. It would have been interesting if Vos analyzed subsets of TBI patients with or without multiple trauma and between brain death versus multiple organ failure.

Dimopoulou reported an investigation of 47 patients with severe brain injury (post-resuscitation GCS 3–8) where 17 developed the criteria for brain death [120]. All patients underwent serial S100B measurements at the time of admission and every 24 h for 6 days. The median S100B level of patients progressing to brain death was 2.32 μg/l. Survivors had a statistically significant lower median S100B level at 1.04 μg/l ($P = 0.0028$).

Petzold evaluated a series of 21 patients with severe head injuries (GCS 3–8) and correlated daily S100B levels

to ICP measurements and outcomes [117]. The mean serum S100B levels were significantly higher ($P = 0.048$) in patients who died (110 pg/ml) compared to patients who survived (37 pg/ml). They reported that initial serum S100B levels above a cut-off level of 60 pg/ml predicted mortality with a sensitivity of 100% within the first 24 h and a specificity of 83% on admission and 75% on day 1.

One study identified that brain death in three patients was preceded 72 h earlier by a secondary increase of the S100B levels [131]. This secondary rise in S100B levels might be able to improve the reliability in predicting eventual mortality. This leads us to evaluate the temporal patterns of S100B elevations after head injury.

Time frame data

Ingebrigtsen reported that S100B elevations in patients with minimal head injury were found to be highest right after the injury with rapid declines seen within the following 6 h [106]. These rapid declines in serum S100B with time after injury have been reported in other investigations [111, 120, 121, 132, 133]. This rapid decline was reported in a cardiac surgery series [134]. Hermann reported significant declines in the daily mean S100B serum concentrations [30, 50, 70] in patients with GCS > 13 [108]. They reported that patients with GCS <12 did not demonstrate the same declines (1400, 1000,1200,1000) in daily serum S100B levels. Therefore, it is important to understand the timing of blood sampling in relationship to the time of injury in each study in order to properly evaluate the findings.

Studies correlating S100B levels with severe head trauma have documented sustained and sometimes increasing elevations that correlate to severity of head trauma and outcome measures. These studies usually measure S100B levels at the time of admission and on a daily basis up to one week. These sustained elevations might reflect a significant original injury or ongoing secondary injury to the brain. Secondary injury to the brain might be occurring when initially elevated levels demonstrate a secondary rise on subsequent days [131].

S100b and secondary brain injury

Secondary brain injury evolves as a consequence of the initial brain trauma leading to a cascade of physiological mechanisms such as ischemia, brain swelling, increased ICP, inflammation, axonal degeneration, and programmed cell death. The clinical consequences of secondary brain injury can be significant. Changes in the ICP, neurological examination and neuroimaging have enabled us to initiate medical and surgical interventions to treat patients who have developed secondary brain injury. Waiting until secondary brain injury has led to these changes might be too late. Ideally, we could initiate interventions during the early phases of secondary brain injury before permanent damage has developed. Some investigations have identified that serum S100B measurements might afford us the opportunity to identify patients in the early stages of secondary brain injury. Intervention earlier in the process might be more effective in mitigating the impact of secondary injury to the brain and improve clinical outcomes.

The pathophysiological mechanisms of major secondary brain damage start to occur on a cellular level long before there are alterations measurable by current neuromonitoring techniques such as ICP, neurological examination or neuroimaging findings. There appears to be an initial phase of cytotoxic injury followed later by a vasogenic phase that eventually leads to increased intracranial pressure. The elevated ICP then leads to decreases in the CPP and subsequent further ischemic injury [135].

A reliable marker that reflected the extent of cytotoxic injury could potentially enable us to initiate therapy before the physiological cascade has developed to the point of increased ICP or changes seen on the patient's scan or neurological examination. It is frequently difficult for clinicians to know when such interventions are helping to reduce secondary injury in a timely manner. Clinical measures such as neurological examinations, scans, intracranial pressure monitors and electrodiagnostic tests are frequently difficult to interpret due to the need for sedating medications and other factors. Therefore, most investigations need to wait for long term clinical outcomes to be measured before they can reliably determine the worth of any interventions.

Many investigators have postulated that serum or CSF measurements of S100B and other brain proteins might be reliable candidates for the prediction of secondary brain injury. However, most of the assays required too much time to be useful as an ongoing measure of secondary injury. Recently, assays have been developed that may be able to reduce the turn around time to less than two hours making the technique potentially useful in guiding ongoing clinical interventions.

Jackson found that the half-life of serum S100B is only a few hours and that initial elevations after minor brain injury returns back to normal levels within a few hours [133]. Any sustained elevations in serum S100 levels probably indicate ongoing secondary injury. Any secondary increases of S100B levels most certainly reflect mechanisms that are responsible for secondary brain injury.

Some investigators have noted that secondary increases in S100B levels preceded increased ICP and neuroimaging findings in patients who subsequently developed secondary brain injury, which culminated in brain death [131].

Petzold reported that the initial serum S100B levels were able to significantly predict mortality 3–4 days before ICP readings predicted mortality [117].

Some investigators have identified patients with secondary brain injury whose S100B serum levels demonstrated a secondary elevation over 24 h prior to the clinical deterioration [126, 131]. These findings open the door to the use of serial serum S100B measurements in order to identify patients who are developing the early phase of secondary brain injury and to initiate clinical interventions in a timelier manner. However, this would require rapid turn around of lab results in order for this test to be useful. Early laboratory techniques required too much time to allow S100B to be a useful therapeutic test. However, recent techniques have reduced the turn around time down to 2 h making the test a more practical clinical tool. Interventions can be initiated at the first sign of S100B elevations. The efficacy of interventions can be monitored by following the response of the S100B levels and correlated to clinical outcome studies. Thus, S100B monitoring may offer a potential window of opportunity for earlier clinical intervention that may successfully mitigate the clinical impact of secondary brain injury and improve clinical outcomes.

Utility of other biochemical markers

S100B has been advocated by many investigators as the most reliable single biomarker [107, 112, 120, 121]. NSE is a cytoplasmic glycolytic enzyme of neurons [136–139]. NSE and S100B serum levels in trauma patients have been found to correlate well to each other and to clinical outcomes and neuroimaging findings of contusion volume and presence of subarachnoid hemorrhage [70, 112, 121]. Some investigators prefer NSE due to its reflection of damage to neurons. Other authors report that S100B and NSE complement each other and note that elevated S100B with normal NSE levels correlates well with extra-cranial release of S100B [140].

Glial fibrillary acidic protein (GFAP) is a monomeric intermediate filament protein of astrocytes. GFAP measurements appear to have the advantage that no known extra-cranial source exits at this time. GFAP concentrations in the CSF have been found to be elevated in normal pressure hydrocephalus, stroke, and in dementia [141–143]. Increased GFAP immunoreactivity was found adjacent to cortical contusions in patients who died after head injury [144]. GFAP can be easily measured from the blood [145, 146]. GFAP serum levels in stroke patients were found to correlate to both infarction volume seen on imaging studies and to clinical outcome [76]. GFAP is more likely an indicator of cell destruction [76]. S100B appears to be more of an indicator of glial activation and less likely to indicate cell destruction. Therefore, GFAP and S100B may be complementary by providing different information regarding the severity of brain injury and the ongoing secondary cellular events. Two separate investigations reported that GFAP serum levels after severe brain injury could reliably predict clinical outcome and mortality [70, 112]. Pelinka, and associates, simultaneously compared GFAP and S100B serum levels in 92 patients with severe TBI and correlated these levels to clinical outcome using the GOS, and various clinical factors such as GCS, ICP, cerebral perfusion pressure (CCP), and CT scan findings using the Marshall classification [147]. They found that both GFAP and S100B levels remained elevated for days in patients who did not survive. Patients who survived had initially elevated levels that decreased within 36 h after the TBI. They found that both GFAP and S100B serum levels were accurate for the prediction of mortality after TBI with area under the curve values between 0.72 and 0.84 (0.5 is random chance and 1.0 is 100% sensitivity and specificity). They concluded that GFAP and S100B levels were more accurate in predicting mortality if sampled more than 36 h after the TBI. However, they did not study patients with pure TBI and of the 33 nonsurvivers, 9 of them suffered multiple organ failure or hemorrhagic shock. They did point out that GFAP levels did not increase in patients with hemorrhagic shock without TBI. Therefore, the combination of GFAP and S100B measurements along with accurate documentation of extra-cranial injuries is important for clinicians to consider when attempting to estimate the extent of cerebral injury and predict CNS outcomes.

H-FABP and B-FABP are similar protein markers that have been reported to be more sensitive than S100B in measuring the degree of brain injury in patients who experience minimal brain injury or electroconvulsive shock therapy [148]. Studies evaluating the clinical usefulness of H-FABP and B-FABP measurements are ongoing.

Conclusions

Brain injury clearly directly correlates with elevations of serum S100B levels. The sensitivity of S110B is best demonstrated in patients with mild brain injury where it correlates accurately 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. Significantly elevated S100B levels have been found to predict mortality. The exact thresholds for S100B elevations that predict brain injury and especially brain death are still being worked out. Various confounding factors have been identified that compromise the specificity of S100B. However,

strategies to overcome these confounding issues are being developed in the hope that S100B measurements will become a useful clinical tool in the neuro ICU.

Specificity issues due to extracranial sources of S100B

The specificity of S100B for brain injury is compromised by its tendency to become elevated by injuries outside of the CNS. This problem can be very significant in patients with simultaneous brain and extra-cranial injuries, patients who undergo various surgical procedures during their hospital stay, or patients who survive cardiopulmonary resuscitation efforts. All of these have been found to significantly elevate S100B levels. However, the following strategies appear to overcome the issue of S100B specificity:

(1) List and quantitate all peripheral injuries.
(2) Monitor all surgical procedures.
(3) Perform serial daily S100B measurements.
(4) Simultaneously measuring S100B, GFAP, and NSE.

Patients with minor peripheral injuries, such as soft tissue wounds, small fractures, and sprains tend to have minor elevations of S100B with rapid normalization within 24 h. Patients with more severe peripheral injuries such as large fractures and abdominal injuries have been shown to have significantly elevated S100B levels. However, these elevations also have a short half life and tend to normalize within 24–48 h. Mild brain injury has been associated with mild S100B levels that normalize within 24 h. However, moderate to severe brain injury has been associated with significant elevations of S100B that last beyond 48 h. Therefore, sustained elevations of S100B beyond 48 h may more accurately reflect the amount of brain injury with little contribution from peripheral injuries. Therefore serial S100B measurements have been demonstrated to be more reliable in its prediction of the presence and extent of brain injury even in patients with peripheral injuries. GFAP and NSE biomarkers are more specific reflections of brain injury that are not elevated in patients with peripheral injuries. Therefore, sustained elevations of S100B with simultaneous elevations of GFAP and NSE more reliably predicts the presence of significant brain injury.

Prediction of brain death

Many investigations have reported that serum S100B levels reliably predict clinical outcome in brain injured patients. Some reports indicate that S100B levels above a certain threshold could reliably predict mortality from brain death. This information can be crucial for physicians and families who are trying to decide upon appropriate therapeutic interventions and when to withhold treatment. However, more work will be needed to confirm the reliability of these thresholds before they can be clinically useful.

Prediction of secondary brain injury

Delayed increases in the S100B levels have been identified hours to days before ICP elevations, neuroimaging changes, or clinical deterioration. Therefore, delayed increases in S100B levels might in the future become a reliable predictor of impending secondary brain injury. Aggressive intervention may be initiated in a more timely manner in the hopes of avoiding or mitigating the clinical impact of secondary brain injury. A reliable marker that reflected the extent of cytotoxic injury could potentially enable us to initiate therapy before the physiological cascade has developed to the point of increased ICP, changes seen on the patient's scan, or permanent neurological deterioration. Future investigations will be able to correlate serial S100B levels to ongoing ICP, neurological examinations, and long term outcome studies. In the near future, clinicians will be able to monitor the response of S100B levels to their interventions. Adjustments to the patients' treatment regimens will be possible to optimally reduce S100B levels and stop the physiological mechanisms before permanent neurological deterioration could develop. The future use of serial S100B monitoring promises to significantly improve neurological outcomes in the treatment of patients with brain injuries.

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