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Relationship of Serum S100B and
Intracranial Injury in Children with Accidental Closed Head Trauma

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Medicine

by
Sarah Elisabeth Frasure
2008

Abstract

S100B, a calcium-dependent protein produced by astroglial cells in the central nervous system (CNS) and by chondrocytes, functions as both a neurotrophin and neurotoxin. It has a half-life in the systemic circulation of approximately six hours. We examined whether serum levels of S100B would be predictive of intracranial injury (ICI) in children, as detected by cranial computed tomography (CT), in children with closed head trauma (CHT). In addition, we evaluated the effect of long bone fractures on the level of S100B in children with both CHT and extracranial injuries such as long bone fractures. One hundred fifty-two children, who presented to the Pediatric Emergency Department of Yale -New Haven Children's Hospital, within six hours of accidental CHT, and required CT to exclude ICI, were prospectively enrolled. After informed consent from a caregiver, samples were obtained by venipuncture and analyzed for a quantitative serum level of S100B.

Of the 152 children enrolled in this study, 24 had an ICI. Mean S100B levels were significantly greater in children with ICI (0.212 $\mu\text{g/L}$ vs. 0.084 $\mu\text{g/L}$; $p<0.001$), in children with long bone fractures (0.220 $\mu\text{g/L}$ vs. 0.083 $\mu\text{g/L}$; $p<0.001$), and in children who were non-white (0.127 $\mu\text{g/L}$ vs. 0.081 $\mu\text{g/L}$; $p=0.03$). Sixty-two percent of children with ICI had venipuncture performed more than 120 minutes after head injury. After controlling for time of venipuncture, fractures, and race, mean S100B levels were still greater in children with ICI (0.409 $\mu\text{g/L}$ vs. 0.118

$\mu\text{g/L}$; $p < 0.001$). The discriminatory value of S100B to detect ICI, as determined by the area under the receiver operator characteristics (ROC) curve, was 0.67. Further study of S100B is necessary to determine whether this biochemical marker could serve as a useful adjunct in the evaluation of children with CHT.

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Introduction

Head trauma is a major cause of morbidity and mortality in children. Each year over 400,000 children under the age of 14 years present with closed head trauma (CHT) to Emergency Departments across the United States (1). Children less than 4 years of age are twice as likely to experience CHT as any other age group. In children under the age of one year, head injury is the leading cause of death (2).

Since no serum diagnostic marker exists to distinguish which children have intracranial injuries after CHT, the majority of symptomatic children who present with a head injury will undergo computed tomography (CT) to exclude intracranial injury (ICI). Despite the presence of such post-traumatic complaints as nausea, headache, vomiting, and loss of consciousness at the time of admission to the Emergency Department, most children with such symptoms and CHT will not have ICI (3). Thus, we wished to determine whether serum S100B levels could predict the presence of ICI by CT, in children who suffer CHT.

Children with CHT who undergo a CT scan sometimes require sedation to produce a more precise image of the brain. Yet both sedation and diagnostic imaging carry serious health risks to children. Adverse sedation events, defined as death and permanent neurological injury, are associated with drug overdose and drug interactions (4). Negative outcomes are particularly frequent in hospital settings when three or more drugs are employed to sedate a patient. Furthermore, the lack of specific pediatric labeling on medication makes children vulnerable to the possibility

of an adverse sedation event; both transcription mistakes and flawed dose dispensing occur in the United States with sufficient regularity to raise alarm (4). In addition to the risk of adverse outcome from sedation errors, children are also exposed to a significant amount of radiation when undergoing a CT scan. Although the recent development of the faster helical CT scan has reduced the need for sedation in pediatric patients, a much higher radiation dose is required (5). Children are significantly more radiosensitive than adults, as their larger numbers of dividing cells are most susceptible to radiation induced neoplastic transformation (6).

More than 600,000 abdominal and head CT examinations are performed annually in the United States in children under the age of 15 years. Approximately 500 of these patients will eventually develop cancer attributable to the CT radiation (7). Not only is the brain highly radiosensitive, but the risk of brain cancer as a result of radiation exposure increases with decreasing age. Although the development of reduced-exposure pediatric CT scanners has been recommended, no such machines currently exist. Thus, the majority of symptomatic children who present to the Emergency Department with CHT undergo CT, as there is no other diagnostic method to evaluate the presence of ICI. Less than 10% of such scans in children will demonstrate ICI (8).

In order to limit the use of CT imaging, investigators have established clinical criteria that may help to predict which patients who arrive at the Emergency Department with head injury are at risk for ICI. Oman et al. studied the sensitivity of

conditions such as loss of consciousness, skull fracture, neurological deficit, persistent vomiting, and abnormal behavior, to determine which patients with head trauma would likely have ICI (9). Thus, 1666 children with head trauma who underwent CT imaging in twenty-one participating Emergency Departments were examined. Of this sample, 8% (138) of patients had ICI as detected by CT. The independent use of clinical criteria, such as loss of consciousness and abnormal behavior, correctly identified ICI in 136 out of 138 patients. Therefore, the use of clinical criteria successfully recognized the majority of patients with CHT who were at risk for ICI.

Dunning et al. evaluated a similar clinical decision rule for the management of children with CHT in order to limit the number of patients who undergo diagnostic imaging. This prospective NEXUS II (National Emergency X-Radiography Utilization Study) cohort study enrolled 22,772 patients less than 16 years old who presented to ten emergency departments across England with CHT (10). CT scans were ordered for children with any of the following conditions: witnessed loss of consciousness, history of amnesia, three or more episodes of vomiting, seizure, GCS < 14, signs of basilar skull fracture, a focal neurological deficit, or if the mechanism included a motor vehicle crash, high-speed injury, or fall greater than 3 meters in height. Intracranial injuries of four patients were missed using this algorithm, demonstrating a sensitivity of 98%, and specificity of 87% (10). Validation of this study is required, however, before emergency physicians can

confidently apply this algorithm as they assess which children should undergo a CT scan for CHT.

One of the biggest limitations of such clinical decision rules is the type of wording and inter-observer reliability of clinical predictors such as headache. A patient who describes his/her headache as 'severe' following ICI is perhaps more likely to get a CT scan than a patient who fails to further delineate the nature of his/her headache. Sun et al. examined the difference in clinicians' interpretations of clinical predictors used to determine whether to obtain a CT scan for a pediatric patient with ICI (11). Although some definitions, such as 'abnormal mental status' and 'skull fracture' were similar between the two studies, the definitions for clinically relevant 'vomiting' and 'headache' were different. Whereas the UC Davis study defined 'vomiting' as 'history of vomiting,' the NEXUS II study defined 'vomiting' as 'inclusive of recurrent, projectile or forceful emesis after trauma, or vomiting associated with altered sensorium.' In a similar fashion, the UC Davis definition of 'headache' was much broader than the definition provided by NEXUS II. Sun et al determined that the use of the specific NEXUS II decision instrument would have mislabeled a significant number of children with clinically relevant intracranial injury as not being at risk for such (11). Thus, clinical decision rules require carefully worded definitions and high inter-observer reliability if they are to be utilized appropriately and are one of the limitations of the use of such an instrument.

Because of the complex issues that clinicians must consider when evaluating children for ICI after CHT, we examined the ability of a serum biomarker, S100B, to correctly predict which children had ICI after CHT. The group of S100 calcium binding proteins in the human body includes approximately 16 units, each having a unique pattern of cellular expression. The family of proteins was named S100 as a result of their solubility in a solution of 100 % saturated ammonium sulfate at neutral pH (12). Furthermore, the S100 genes are situated together on chromosome 1q21. Individuals with Down syndrome, epilepsy, and Alzheimer's disease express higher baseline levels of S100B than healthy individuals.

The S100 proteins consist of dimers, each of which has a subunit composition of $\alpha\alpha$, $\beta\beta$, or $\alpha\beta$. S100AA and S100BB (also known as S100B) predominate, and are located in different cells throughout the human body. Whereas S100A is found in myocardial cells, acinar cells, renal tubules, and muscle fibers, S100B is concentrated primarily in the central nervous system, and to a lesser degree in chondrocytes and adipocytes (11). In the brain, S100B links the variation of intracellular calcium levels to alterations in cell function. S100B regulates neuron extension, calcium fluxes, and inhibition of PKC-mediated phosphorylation, astrocytosis and axonal proliferation. S100B seems to be most abundant in glial cells, although its presence in other neuronal cells has also been described. At low concentrations S100B promotes neuronal development, survival, and repair (13). High extracellular concentrations of S100B, on the other hand, seem to initiate

neuronal cell death via both nitric oxide release from astrocytes, and induced apoptosis. Thus, S100B likely functions both as a neurotrophin and a neurotoxin after closed head injury, depending on its concentration and the time elapsed since the injury occurred (14).

As a result of its relatively large molecular weight of 20 kDa, S100B is normally unable to pass through the brain-blood barrier into the systemic circulation (12). Therefore, its concentration is negligible in the bloodstream of healthy individuals. When the brain-blood barrier is disrupted after ICI, however, serum S100B levels rise significantly. Serum S100B has a half-life of approximately six hours, and is readily excreted in urine (15). Thus, it is imperative to measure serum levels of S100B shortly after CHT for it to be useful as a marker for ICI. An inexpensive enzyme-linked immunoassay (ELISA) exists (Can Ag Diagnostics AB, Gothenburg, Sweden) that can accurately determine the concentration of serum S100B; the cost is approximately \$9 per patient. In contrast, each CT scan costs approximately \$350 and carries the risk of radiation exposure, often combined with the risk of sedation. If serum S100B levels accurately correlate with the presence of ICI after CHT, we could determine which children would most likely require a CT scan after CHT, prevent exposure to radiation and sedation, and reduce medical costs.

Over the past ten years, researchers have examined the extent to which serum biomarkers, such as S100B, GFAP, IL-6, and NSE, could determine the presence of

ICI after CHT in adults. Results of these studies suggested that S100B might be more useful than other biomarkers. Specifically, S100B levels have been demonstrated to be elevated in adults with traumatic brain injury, cerebral infarction, spontaneous subarachnoid hemorrhage, intracranial tumors, meningitis, hydrocephalus, and spinal cord compression (11,16). Thus, to evaluate S100B as a marker for ICI after CHT, children with a history of brain tumors, hydrocephalus, spinal cord injuries, or Down syndrome would be excluded in the patient population to be studied.

In order to determine the significance of an elevated serum S100B level in patients with ICI, however, one must be able to compare it with serum S100B levels in patients without brain abnormalities. Nygaard et al. attempted to define the baseline level of serum S100B in healthy adults. Blood was obtained from 110 patients between the ages of 20 and 89 years undergoing surgery with spinal anesthesia (17). Exclusion criteria included neurological disease or malignancy. They employed a commercially available kit (Sangtec Medical, Bromma, Sweden) for the analysis of S100B in serum, and identified the lowest detectible value as 0.2 $\mu\text{g/L}$. In this study S100B was not detected in any patient. Therefore, Nygaard et al. concluded that S100B is normally less than 0.2 $\mu\text{g/L}$ in the serum of healthy adults (17). With the establishment of an adult baseline S100B level, it is reasonable to study the relationship between S100B and ICI. Raabe et al. investigated S100B as a serum marker of brain damage after severe traumatic brain injury in adults (18).

Venous blood samples were taken at admission and every 12 hours thereafter for 10 days from 84 patients with severe traumatic brain injury as detected by CT scan. S100B values above 0.5 $\mu\text{g/L}$ were considered elevated. A strong correlation between abnormal CT scan results and initial S100B levels above 0.5 $\mu\text{g/L}$ was noted. Furthermore, 39% of patients succumbed to their injuries within 6 months. Patients who died had a significantly higher median serum S100B values (2.7 $\mu\text{g/L}$) compared with surviving patients (0.54 $\mu\text{g/L}$) (17). In most patients S100B levels remained elevated over the course of the first few days after brain injury, irrespective of outcome. Patients with a poor neurological outcome, however, had persistently elevated levels of S100B, which may reflect continuing secondary brain injury after the initial insult. Additional studies by Vos et al. demonstrated that serum levels of S100B at the time of admission help to predict the extent of ICI in patients with severe CHT (19).

Ingebrigtsen et al. sought to validate S100B as a biomarker of brain injury after mild CHT. Fifty patients with minor head injuries, normal CT scans, and Glasgow Coma Scale (GCS) of 13-15 were enrolled in this prospective study (20). Patients underwent MRI scans in order to reveal any subtle intracranial injuries not observed by CT. Blood was drawn at admission, and every hour for 12 hours. Elevated S100B levels were detected in 28% of patients. Levels of serum S100B were highest immediately after trauma and subsequently declined. In addition, MRI

scans discovered intracranial injuries not previously detected by CT scan in 5 patients, 4 of whom had high S100B levels (20).

Pediatric studies have examined the relationship between S100B and ICI in children with CHT. As with adult studies, it is important to determine the baseline serum S100B levels in the bloodstream of healthy infants and children. An inverse relationship between age and serum S100B levels was noted by Amer-Whalin et al (21). Infants have significantly higher normal serum S100B level than those of either children or adults. Maschmann et al. reported the serum S100B levels in 66 healthy neonates as 2.4-3.5 $\mu\text{g/L}$, which is significantly greater than the mean S100B level in adults of 0.2 $\mu\text{g/L}$ (22). Blood was collected in 12-24 hour intervals over the first seven days of life. Maschmann et al. concluded that amplified protein turnover in neuronal cells, combined with increased permeability of the brain-blood barrier in infants, leads to the subsequent escape of S100B from the central nervous system.

Spinella et al. examined the difference between serum S100B in 136 healthy children, and 27 children with traumatic brain injury (23). The mean serum S100B level in the control group of children was 0.3 $\mu\text{g/L}$, and was characterized by an expected moderate inverse relationship with age. Blood was drawn within a mean of 7 hours after CHT in 27 pediatric patients. A serum S100B level of 2 $\mu\text{g/L}$ demonstrated 86% sensitivity and 95% specificity to predict poor outcome after 6 months (23). Although a strong association between high S100B levels and poor

outcome was noted, a study with a larger number of patients is required to determine whether S100B is indeed an independent predictor of outcome after ICI in children.

Similarly, Berger et al. measured the serum concentrations of S100B in children after mild, moderate, and severe traumatic brain injury (24). Forty-five children between the ages of 0 and 13 years with closed head injury were enrolled prospectively. Blood was drawn upon arrival to the Emergency Department, and every 12 hours for 5 days thereafter. However, the relationship of the time of injury and the time of first blood draw was not mentioned in this study. No correlation between the presence of vomiting, loss of consciousness, or post-traumatic seizure and serum S100B levels was demonstrated. After 12 hours, an abnormally high S100B level was noted only in patients with severe head injury. The short half-life of S100B would explain why there was no sustained increase of S100B in patients with mild or moderate head injury. Although 49% of the patients had abnormal S100B values, 70% of the CT scans were normal. Thus, a significant number of subjects with high S100B values had normal CT scans (24). They did not demonstrate any relationship between the level of S100B and extracranial injuries, such as long bone fractures.

In order to explore the failure of CT to visualize subtle ICI, Akhtar et al. examined the relationship between high S100B levels and MRI scans in children with traumatic brain injury and negative CT scans (25). The authors wished to

determine how often both MRI and S100B were abnormal in pediatric patients with negative CT scans. Seventeen children between the ages of 5 and 18 years were enrolled in the study. Blood was drawn 6 hours and 12 hours after admission to the Emergency Department. Akhtar et al. determined that 41% of patients with negative CT scans had identifiable lesions on MRI. There was no difference in S100B concentrations between children with positive and negative MRI, although concentrations were abnormally elevated in both cases (25). Furthermore, S100B concentrations were higher at both time intervals in children with head and other bodily injuries compared with patients who had sustained only head injury. This result indicates that the principal extracranial sources of S100B, chondrocytes and adipocytes, when traumatized, likely release S100B into the bloodstream, and must be carefully considered in future studies.

We sought to determine whether S100B could serve as a diagnostic marker of ICI in children. As a result we designed a prospective study in a large cohort of children to establish whether serum levels of S100B in children with CHT could accurately predict which children would likely have ICI, as detected by CT scan. We hypothesized that mean serum S100B levels would be higher in children with an abnormal CT scan when compared to patients with unremarkable CT scans. Our study would improve on previous studies in that we would evaluate a larger cohort of patients with CHT, examine the relationship of S100B and the presence of extracranial injuries, such as long bone fractures, as well as the relationship of S100B to the time after injury when venipuncture was performed to obtain serum

for analysis. We would also evaluate how clinical symptoms after injury, such as loss of consciousness (LOC), headache and vomiting, and how clinical signs, such as the presence of altered mental status, as determined by a patient's GCS, affect serum levels of S100B. A serologic diagnostic marker, such as S100B, would be a very useful clinical tool for Emergency Department physicians to assess which children with CHT should undergo diagnostic imaging and possibly sedation, and which children would not benefit from such a procedure.

Material and Methods

At the Pediatric Emergency Department of Yale-New Haven Children's Hospital, 350 children per year less than 18 years old require CT for evaluation of CHI. Of this sample, 15% have ICI. With a two-sided 0.05 significance level, group sizes of 15(+ICI) and 135 (-ICI) will provide 80% power to observe a three-fold increase in the level of S100B in the (+) ICI group compared to the (-) ICI group.

All children who presented to the Emergency Department within six hours of closed head injury, and required a CT scan, were eligible for enrollment in the study. Exclusion criteria were history of seizure within seven days prior to sustaining head injury, penetrating head injury, encephalopathy, pre-existing developmental delay, Down syndrome, cerebral palsy, renal insufficiency, or intravascular hemolysis.

Written informed consent was obtained from the parent or guardian. We utilized a standardized data entry sheet to enroll each patient, recording the medical record number, mechanism and date of injury, initial Glasgow Coma Scale, presence of post-traumatic complaints, presence of intracranial injury or skull fracture on CT scan, existence of extra-cranial injuries (bone fractures, abdominal injuries), and the need for inpatient admission. The following post-traumatic complaints were noted at the time of admission: headache, nausea, vomiting, loss of consciousness, dizziness, and altered mental status. Injury severity was classified as mild (GCS 13-15),

moderate (GCS 9-12) or severe (GCS \leq 8). Every child who was enrolled in the study underwent a CT scan to exclude intracranial injury. An attending radiologist as well as an attending pediatric neurosurgeon provided the final interpretation of each CT scan.

A patient's medical record was reviewed if he/she was admitted to the hospital from the Pediatric Emergency Department for the treatment of traumatic brain injury. The following data were noted: admission to the pediatric intensive care unit, need for surgical intervention, need for medical intervention, and number of days of hospitalization.

In order to measure S100B levels, venous blood samples were obtained from each enrolled patient in the Emergency Department within six hours of closed head trauma. Blood was allowed to clot for 30 minutes, and was subsequently centrifuged (800-1000 RPM for 10 minutes) within six hours. Serum samples were frozen at -20 °C until further analysis, at which point S100B concentrations were measured using an immunoluminometric assay. Sangtec 100 (Can Ag Diagnostics AB, Gothenburg, Sweden) is an enzyme-linked immunoassay designed to detect the level of S100B in serum. Standard and control reagents, as well as wash buffer, are reconstituted with de-ionized water. Fifty μ L each of standard, control reagent, and patient sample were pipetted into test wells. One hundred fifty μ L of tracer was added to the wells and incubated for 2 hours. After washing the wells with buffer, 100 μ L of TMB substrate was added to each well. The samples were incubated on a

plate shaker at 800 RPM for 15 minutes at room temperature. The reaction was stopped by adding 100 μ L of TMB stop solution. The absorbance of each well was read over a 15-minute period at 450 nm using a microplate reader.

Serum S100B levels were calculated for ten control subjects without closed head injury, 128 children with closed head injury and normal cranial CT scans, and 24 children with closed head injury and abnormal CT scans. We calculated an independent sample t-test to compare the mean level of serum S100B between children with ICI as detected by CT, and children without ICI. In addition, analysis of covariance was utilized to adjust for group differences, such as age, race, and gender. Descriptive statistics were used to determine the assumptions required for these tests, and necessary transformations or non-parametric tests were performed to meet testing assumptions. In addition, we evaluated the best combination of sensitivity and specificity for a particular level of S100B, in order to differentiate between those subjects with a positive from those with a negative scan by using the area under the ROC curve to determine the discriminatory value of S100B to detect ICI.

Results

Between April 2005 and October 2006, 466 children with closed head trauma (CHT) were evaluated at the Pediatric Emergency Department of Yale-New Haven Children's Hospital. One hundred fifty-two children (33%) were prospectively enrolled: 24 with ICI as detected by CT scan, and 128 children without ICI. There were no significant differences between the two groups with respect to age, gender, or race (*Table 1*). Injury mechanisms included falls (42%), pedestrians struck by vehicle (23%), motor vehicle crashes (13%), and sports collisions (9%). Twenty-five children had long bone fractures. None of the children with ICI required operative intervention, and all were discharged to home after hospitalization.

Time of venipuncture after injury was significantly later in children with intracranial injury (62% with ICI vs. 34% without ICI; $p=0.03$). Mean S100B levels were significantly greater in children with intracranial injury ($0.213 \mu\text{g/L}$ vs. $0.0844 \mu\text{g/L}$; $p=0.0001$), in children with fractures ($0.220 \mu\text{g/L}$ vs. $0.0832 \mu\text{g/L}$; $p<0.001$), and in children who were non-white ($0.127 \mu\text{g/L}$ vs. $0.0805 \mu\text{g/L}$; $p=0.03$) (*Table 1*). After controlling for time of venipuncture, fractures, and race, mean S100B levels were still greater in children with intracranial injury ($0.409 \mu\text{g/L}$ vs. $0.118 \mu\text{g/L}$; $p=0.0001$) (*Table 2*). The discriminatory value of S100B to detect closed head injuries (AUC) was 0.67. When $0.050 \mu\text{g/L}$ of S100B was chosen as the cut-off value, sensitivity was 75%, specificity 56%, positive predictive value 20% and

negative predictive value was 90%. After patients with long bone fractures were excluded, however, the discriminatory value increased to 0.69. The sensitivity became 73%, specificity 52%, positive predictive value 23%, and the negative predictive value was 89%.

There were no significant differences between the two groups with respect to loss of consciousness, vomiting, or amnesia following CHT. The group of children who suffered an intracranial injury as diagnosed by CT scan, however, was significantly more likely to have a palpable scalp hematoma and a GCS < 12 in the Emergency Department (*Table 3*). Children without ICI were significantly more likely to complain of headache following the CHT.

Patient Demographics

	Age (Years)	% Male	% Caucasian	% Fractures	Blood Draw > 120 Minutes
(+) ICI	6.9	58%	63%	24%	62%
(-) ICI	9.8	71%	54%	23%	34%
P value	0.01	Not significant	Not significant	Not significant	0.03

Table 1. There were no significant differences between the two groups with respect to age, gender, or race. Time of venipuncture after injury was significantly later in children with intracranial injury (p=0.03).

Mean S100B Levels ($\mu\text{g/L}$)

	ICI as detected by CT scan	Caucasian	Long bone fractures	ICI after controlling for all other factors
Yes	0.213 <i>(0.109-0.416)</i>	0.081 <i>(0.068-0.105)</i>	0.220 <i>(0.11-0.426)</i>	0.409 <i>(0.24-0.697)</i>
No	0.084 <i>(0.068-0.105)</i>	0.127 <i>(0.092-0.175)</i>	0.083 <i>(0.07-0.103)</i>	0.118 <i>(0.089-0.156)</i>
P value	0.001	0.03	0.008	0.001

Table 2. Mean S100B levels were significantly greater in children with intracranial injury ($p=0.0001$), in children with fractures ($p=0.0008$), and in children who were non-white ($p=0.03$).

Post-traumatic Brain Injury Complaints

	LOC	Hematoma	Headache	Vomiting	Amnesia	GCS < 12
(+) ICI	50%	91%	24%	29%	17%	29%
(-) ICI	55%	51%	48%	17%	27%	2%
P value	NS	0.001	0.05	NS	NS	0.001

Table 3. There were no significant differences between the two groups with respect to loss of consciousness, vomiting, or amnesia following closed head trauma. The group of children who suffered an intracranial injury as diagnosed by CT scan, however, was significantly more likely to have a hematoma, and a GCS < 12 in the Emergency Department.

Discussion

The majority of children who present to the Emergency Department with closed head trauma (CHT) will not have intracranial injury (ICI) (23). We wished to determine whether serum S100B levels could be utilized to predict the presence of ICI after CHT by CT scan. A serologic assay could help to decide which children CHT would most likely benefit from diagnostic imaging to exclude ICI. While we found that mean serum levels of S100B are higher in children with ICI than those without ICI, the discriminatory value of S100B to detect ICI was only 0.67. Thus, S100B may not be an accurate screening tool to detect ICI in children after CHT.

There are both strengths and weaknesses of this study. The strengths include large sample size (152 children), and a diverse spectrum of injury mechanisms (falls, motor vehicle accidents, sports). Also, there were no significant differences between the two groups (with and without ICI) with respect to age, gender, or race. Weaknesses include the short half-life of S100B, a characteristic that, in retrospect, does not make it a useful screening tool for ICI in children who present for medical evaluation more than six hours after CHT. A number of our subjects with ICI arrived at Yale-New Haven Children's Hospital after having been referred from smaller hospitals, thus increasing the time between injury and venipuncture, and may have had lower levels of S100B, thus reducing the ability of S100B to detect ICI in this group of children.

In this study, children with both ICI and long bone fractures had significantly higher S100B levels than those without. Our results in children differ from data compiled by Berger, who did not find that serum levels of S100B were affected by the presence of fractures. Our results suggest that extra-cerebral sources of S100B, such as that from long bone fractures, affect the specificity of S100B to detect ICI.

Two studies in the adult population have evaluated the relevance of extra-cerebral sources of S100B in the setting of ICI. Uden et al. examined S100B levels after uncomplicated bone fractures in adult patients without neurological disease. Blood was drawn from 55 adults who presented to the Emergency Department with orthopedic fractures no older than 24 hours (26). Patients with head injury were excluded from this study. S100B values of greater than 0.150 $\mu\text{g/L}$ were considered elevated. Twenty-nine percent of subjects had S100B values above that cut-off value. The authors determined that patients with hip, tibia, and radial fractures were more likely to have high S100B values than those with smaller fractures to hands or feet (26). Had Uden et al. chosen a lower cut-off value, such as 0.050 $\mu\text{g/L}$ (utilized in our study), they would have had many more subjects with elevated S100B values in the setting of orthopedic fractures. Although S100B could function as a marker of brain injury, the existence of extra-cerebral sources of S100B makes it difficult to interpret an elevated S100B value in a patient with both ICI and bone fractures.

Savola et al. studied the S100B values of 224 adults with head trauma (mild, moderate, or severe), and 155 adults with an assortment of extra-cranial injuries, including soft tissue contusions, sprains, and small and large fractures (27). Patients with head trauma had significantly higher median S100B values (0.170 $\mu\text{g/L}$) than patients with extra-cranial injuries (0.070 $\mu\text{g/L}$). Furthermore, serum S100B values correlated with the severity of brain injury; individuals with severe brain trauma had higher values of S100B than patients with mild or moderate ICI. Savola et al. also noted that extra-cranial injury independently increased S100B levels (27). Soft tissue contusions, sprains, luxations, and small fractures led to mildly elevated S100B levels in only 3% of patients. Large fractures and abdominal injuries that were obtained in the absence of brain injury, however, significantly increased S100B values, suggesting that large extra-cranial injuries may decrease the positive predictive value of S100B as a diagnostic marker of brain trauma in patients with multiple injuries. At present, other than this current study, there has been no other study to evaluate the effect of extra-cranial injury on the serum levels of S100B in children with CHT.

Of our sample of 152 patients with CHT, 24 children (16%) had ICI by CT. One may argue that MRI would have revealed a larger number of traumatic lesions than did CT alone. Thus, we may have mistakenly categorized children with brain injury undetectable by CT scan, but potentially visible on MRI, in the group of

children without ICI. If such patients with ICI detected only by MRI had elevated S100B levels, such an error would falsely elevate the mean S100B value of the group without ICI as detected by CT alone, thus narrowing the statistical difference between the two groups. Akhtar et al. examined 17 children between 5-18 years of age who presented to the Emergency Department with traumatic brain injury and had a negative CT scan (25). Patients subsequently underwent a standard non-contrast MRI with added FLAIR and GRE sequences. S100B values for each patient were also recorded. Forty-one percent of patients with a negative CT scan had positive findings on MRI, emphasizing the superior sensitivity of MRI in recognizing subtle ICI. All brain lesions were visible on FLAIR sequence, and 77% of lesions were visible on axial T-2 images. Although S100B levels were elevated, there was no significant difference in S100B concentrations when children with positive MRI findings were compared to those with unremarkable MRI scans (25). The use of MRI in the Emergency Department setting is impractical, given the time needed to complete the study and the requirement for sedation to render the young patients motionless. It is also important to note that none of the patients in our study who had ICI as detected by CT alone, and those in Ahktar's study with ICI as detected by MRI alone, required operative intervention for ICI. While the development and use of "quick-brain" MRI in the Emergency Department would make the detection of ICI in children with CHT easier, its use is not likely in the Emergency Department setting in the near future.

While S100B may not be a reliable screening tool for ICI in children after CHT, it may serve as a prognostic tool for outcome after ICI. Currently a patient's Glasgow Coma Scale (GCS) is utilized to help predict his or her ability to recover neurological function after traumatic brain injury. Feickert et al. examined the relationship between ICI and GCS in pediatric patients (28). In this retrospective study, 150 children with traumatic brain injury and initial $GCS \leq 8$, who were treated in the pediatric intensive care unit, were enrolled. All patients underwent CT scans; 43.3% had a head fracture and 57.9% were diagnosed with an ICI. Low GCS was highly predictive of poor neurological outcome. The likelihood of survival for patients with $GCS < 5$ was 0.18 compared with 0.94 for patients with $GCS \leq 8$ to 5 ($p < 0.0001$) (28). Similarly, in our study, the group of children who suffered an ICI as diagnosed by CT scan was significantly more likely to have a $GCS < 12$ in the Emergency Department. If S100B levels correlate with GCS, one may argue that patients with ICI who also have high S100B levels will likely have a worse neurological prognosis than patients with lower S100B values. Herrmann et al. analyzed S100B levels in the first three days after traumatic brain injury in 69 patients between the ages of 16 and 65 years (29). GCS were obtained in the Emergency Department and ten days after traumatic brain injury. Comprehensive neuropsychological testing was performed in 39 patients two weeks after hospital admission and in 29 patients approximately 6 months after traumatic brain injury. Initial S100B levels greater than $0.140 \mu\text{g/L}$ had the highest predictive power of

short and long-term neuropsychological disorders. Thus, S100B could serve in conjunction with GCS as a predictive marker of long-term neurological outcome after traumatic brain injury.

Conclusion

After controlling for time of venipuncture, fractures, and race, S100B levels were higher in children with ICI than those without such injury. However, the discriminatory value used to detect S100B was only 0.67. This study demonstrates that S100B may not be a practical screening device of ICI in children who present to the Emergency Department with CHT. The short half-life of the protein, combined with its higher average value in non-Caucasian children than in age-matched Caucasian counterparts, and its higher numeric value in children with bone fractures, do not make S100B a sensitive or specific biologic marker of ICI in children. Additional study of S100B is necessary in order to determine whether it can serve as a useful adjunct to evaluate children for intracranial injury CHT. Future evaluation of S100B in children with CHT and ICI may demonstrate its usefulness in predicting neurological recovery after ICI.

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