Serum Concentrations of Ubiquitin C-Terminal Hydrolase-L1 and α II-Spectrin Breakdown Product 145 kDa Correlate with Outcome after Pediatric TBI

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Abstract

Predicting outcome after pediatric traumatic brain injury (TBI) is important for providing information to families and prescribing rehabilitation services. Previously published studies evaluating the ability of serum biomarkers to predict outcome after pediatric TBI have focused on three markers: neuron-specific enolase (NSE), S100B, and myelin-basic protein (MBP), all of which have important limitations. The study objectives were to measure serum concentrations of two novel serum biomarkers, ubiquitin C-terminal hydrolase (UCH-L1) and α II-spectrin breakdown product 145 kDa (SBDP145), in children with TBI and healthy controls and to assess the ability of these markers to predict outcome as assessed by a dichotomous Glasgow Outcome Scale (GOS) score. We also sought to compare the predictive ability of UCH-L1 and SBDP145 to that of the clinical gold standard, the Glasgow Coma Scale (GCS) score, and to that of the well-accepted biomarkers NSE, S100B, and MBP. Serum UCH-L1 and SBDP145 concentrations were significantly greater in subjects than in controls. The increase in UCH-L1 and SBDP145 was exclusively seen in subjects with moderate and severe TBI; there was no increase after mild TBI. Both markers had a significant negative partial correlation with the GCS after controlling for age. Both UCH-L1 and SBDP145 were correlated with GOS, and this correlation was stronger than the correlations with NSE, S100B, or MBP. These results suggest that these two markers may be useful in assessing outcome after moderate and severe pediatric TBI.

Key words: α II-spectrin breakdown product; outcome; pediatric; traumatic brain injury; ubiquitin C-terminal hydrolase

Introduction

THE USE OF SERUM BIOMARKERS to predict outcome after pediatric TBI has been the subject of multiple studies over the past decade (Beers et al., 2006,2007,2010; Fraser et al., 2010; Lo et al., 2009; Sandler et al., 2010). Previous studies have focused primarily on three brain-specific markers—neuron-specific enolase (NSE), S100B, and myelin-basic protein (MBP)—and have consistently shown an association between higher concentrations of serum biomarkers and poorer outcome after TBI (Berger 2006,2007; Guzel et al., 2008; Vos et al., 2010; Wiesmann et al., 2010; Yamazaki et al., 1995). Each of these markers has limitations. S100B is affected by extracranial injury, is only occasionally increased after mild TBI, and

has high normative concentrations in young children (Anderson et al., 2001; Bechtel et al., 2009; Berger et al., 2002; Portela et al., 2002; Unden et al., 2005). NSE is affected by hemolysis, a particularly important problem in infants and young children (Berger and Richichi, 2009), and MBP is rarely increased immediately after injury (Beers et al., 2007; Berger et al., 2006a), and therefore requires repeated measurements over time. There is a need for novel serum biomarkers that can assist in outcome prediction after pediatric TBI.

The neuron-specific cytoplasmic ubiquitin C-terminal hydrolase (UCH-L1) is a proteolytically stable and abundant protein which is found exclusively in neurons. Previous studies in adults have demonstrated that it is increased after TBI (Papa et al., 2010; Siman et al., 2008,2009). In addition, it

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may be a marker of neuronal loss after aneurysmal subarachnoid hemorrhage (Lewis et al., 2010), as well as a marker of abnormal blood-brain barrier function after severe TBI (Blyth et al., 2011). A recently published study by Douglas-Escobar and colleagues demonstrated increases in serum UCH-L1 concentrations in neonates after moderate to severe hypoxic ischemic encephalopathy (Douglas-Escobar et al., 2010). No studies have evaluated serum UCH-L1 concentrations after pediatric TBI.

α II-Spectrin is primarily found in neurons and is abundant in axons and presynaptic terminals. It is processed into breakdown products (SBDP) of 145, 150, and 120 kDa. SBDP120 is produced by proteolytic cleavage with caspase-3, and is primarily associated with apoptosis. SBDP145 and SBDP150 are produced by cleavage with calpain and are primarily associated with necrosis. Several adult studies have demonstrated increased CSF concentrations of the SBDPs after severe TBI (Brophy et al., 2009; Mondello et al., 2010; Pineda et al., 2007). Cerebrospinal fluid (CSF) SBDP145 concentrations were also elevated in a rat model of controlled cortical impact (Pike et al., 2001; Ringger et al., 2004). No published studies have evaluated serum concentrations of the SBDPs in pediatric or adult TBI patients.

The objectives of the current study were therefore to (1) measure and compare serum concentrations of UCH-L1 and SBDP145 in children with mild, moderate, and severe TBI and in controls, (2) evaluate the ability of these markers to predict outcome as measured by the Glasgow Outcome Scale (GOS) score, and (3) compare the predictive ability of these markers to the predictive ability of the Glasgow Coma Scale (GCS) score, serum NSE, serum S100B, and serum MBP concentrations.

Methods

Subjects

The protocol was approved by the University of Pittsburgh Institutional Review Board. Children were eligible for enrollment as cases if they were less than 15 years of age and were admitted to Children's Hospital of Pittsburgh of University of Pittsburgh Medical Center (CHP) with a history of a closed head injury, had a head computed tomography (CT) scan as part of clinical care, and had blood collected for biomarker measurement within 24 h of presentation. Decisions about whether a head CT and/or hospital admission were warranted were left to the discretion of the treating physician. No head CTs were performed and no child was admitted to the hospital because he or she was enrolled in the study.

Children were eligible as controls if they presented to CHP and had blood collected either as part of well-child care (e.g., as part of a 1-year well-child examination which includes measurement of hemoglobin), or as part of an evaluation of an acute injury in which there was no concern for closed head injury. We considered there to be no concern for closed head injury if the treating physician did not order a head CT. In children with TBI due to child abuse, the diagnosis of abusive head trauma was made by the CHP Child Protection Team, which is consulted on all cases of possible physical abuse at the hospital; there was no disagreement within the Child Protection Team about the cause of injury in any of the cases in the current study. Defining whether an injury is inflicted based on the conclusion of a Child Protection Team is fre-

quently used in clinical studies of abusive head trauma (Hymel et al., 1997; Jenny et al., 1999; Keenan et al., 2003). Time of injury in cases of abusive head trauma was defined as the time at which a caretaker contacted EMS or brought the child for medical care. This is the way in which we have defined time of injury in prior biomarker studies (Berger et al., 2006b, 2009).

Measures

At time of presentation. All children received standard care for their injuries. A GCS score was assigned on arrival by the trauma team for each patient, as is standard practice in our institution. Demographics including age, gender, and etiology of injury (abuse or not abuse) were collected. We did not collect data about race. The presence or absence of loss of consciousness, clinical seizure, and vomiting was collected for each subject. Injury severity was classified as mild (GCS score 13–15), moderate (GCS score 9–12), or severe (GCS score $\leq$ 8). CT scans were evaluated by a pediatric neuroradiologist as part of routine clinical care. In subjects who had more than one CT scan performed, data were collected for the first CT. For the purposes of analysis, CT scans were dichotomized as "normal" or "abnormal." For those CT scans that were abnormal, data were collected about the presence or absence of skull fracture, subdural or subarachnoid hemorrhage, epidural hemorrhage, intracerebral hemorrhage, and cerebral edema.

Biomarker measurement. Blood was collected as soon as possible after arrival to the hospital. Blood samples were spun at 5000 rpm for 10 min. Serum was then removed and stored at -70°C until analysis. Serum UCH-L1 and SBDP145 were measured using a previously described in-house sandwich ELISA (colorimetric format for UCH-L1, chemoluminescent format for SBDP145) at Banyan Biomarkers Inc. (Liu et al., 2010). The lower limit of detection for the UCH-L1 and SBDP145 assays were 0.025 ng/mL and 0.25 ng/mL, respectively. The investigators at Banyan were blinded to the clinical characteristics of the subjects. NSE, S100B, and MBP concentrations had been measured in the serum samples as part of previously published studies by our group (Berger et al., 2005, 2006b).

Outcome assessment. Outcome was assessed at hospital discharge and at 3–4 months and/or 6–8 months after hospital discharge using the GOS score (1 = good outcome, 5 = death). Outcome assessment was performed either by the clinical team at hospital discharge and/or by a trained research technician at a scheduled clinic visit and was collected as part of separate studies evaluating the relationship (Beers et al., 2007; Berger et al., 2007). When subjects had outcome evaluated at more than one time point, the GOS obtained at the later evaluation was used for analysis.

Statistical analysis

SPSS 19.0 was used for all analyses. Descriptive statistics were used to describe the study population. Nonparametric Kruskal-Wallis and Mann-Whitney tests were used to evaluate differences among groups for UCH-L1 and SBDP145. Pearson's correlations were used to assess the relationship between biomarker concentrations and GCS and GOS scores. Chi-square testing was used to evaluate the relationship between clinical variables and CT results and GOS. For the

purposes of analysis, GOS was classified as a dichotomous variable (good outcome=GOS 1–2, poor outcome=GOS 3–5). For all analyses, $p < 0.05$ was considered significant.

Results

Demographics and clinical data

A total of 49 subjects were enrolled from 4/1/2000 until 6/30/2004: 39 cases and 10 controls. Of the 39 cases, 16 had severe TBI, 12 had moderate TBI, and 11 had mild TBI. The etiology of injury was child abuse in 10 cases. The mean (SD) age of all subjects was 4.1 (3.8) years with a range of 1 week to 12.4 years. Sixty percent of the subjects were male. There was no difference in age or gender between cases and controls. Among the 39 cases, 28 had loss of consciousness, clinical seizure, and/or vomiting, 9 had none of these symptoms and data were unavailable for 2 subjects. Among the 39 cases, 11 had a normal head CT, 6 had an isolated skull fracture, and the remaining 22 had some type of intracranial hemorrhage with or without a skull fracture.

Outcome was collected at a mean (SD) of 3.7 (3.1) months after enrollment with a range of 0–8 months. Sixty-five percent of subjects had a good outcome (GOS 1), 2.5% had moderate disability (GOS 2), 23.5% had severe disability (GOS 3), and 7.5% died (GOS 5). No subjects were in a vegetative state (GOS 4). Serum was collected a median of 3.9 h after injury with a range of 0.5–43.7 h.

UCH-L1 and SBDP145 after TBI

Overall, Kruskal-Wallis tests revealed significant differences between cases and controls for both UCH-L1 ($\chi^2_3 = 14.64, p = 0.002$) and SBDP145 ($\chi^2_3 = 8.89, p = 0.031$; Fig. 1a and b). There were significant differences in UCH-L1 concentrations between controls and cases with severe TBI ($p = 0.001$) and moderate TBI ($p = 0.003$), but not mild TBI ($p = 0.132$). There were no differences in SBDP145 concentrations between controls and cases with severe TBI ($p = 0.077$), moderate TBI ($p = 0.107$), or mild TBI ($p = 0.967$), though the lack of difference in the moderate and severe TBI groups was likely due to small sample size given the overall significance. Time after injury did not have a significant relationship with either UCH-L1 ($r = -0.016, p = 0.921$) or SBDP145 ($r = 0.037, p = 0.821$). There was no difference in the UCH-L1 or SBDP145 concentrations based on etiology of injury (child abuse or not).

Relationship of UCH-L1, SBDP, NSE, S100B, and MBP, with GCS scores

Preliminary analysis revealed a significant positive correlation between age and GCS score ($r = .530, p = 0.001$). The decision was therefore made to control for age when evaluating the relationship between GCS and UCH-L1 and SBDP145. Both UCH-L1 and SBDP145 had a significant negative partial correlation with GCS score (Table 1). The correlation of UCH-L1 and SBDP145 with GCS was stronger than the correlation with NSE, S100B, or MBP.

Relationship of UCH-L1, SBDP, NSE, S100B, and MBP with clinical symptoms and head CT results

There was no relationship between the presence of clinical symptoms and abnormalities on head CT or

FIG. 1. Box-and-whisker plots demonstrating serum UCH-L1 (a) and SBDP145 (b) concentrations in controls and cases with mild, moderate, and severe TBI. The black horizontal line in each box represents the median, with the boxes representing the interquartile range (UCH-L1, ubiquitin C-terminal hydrolase; SBDP145, spectrin breakdown product 145 kDa; TBI, traumatic brain injury).

between the presence of clinical symptoms and biomarker concentrations.

There also was no relationship between the presence of an abnormal CT or a skull fracture and biomarker concentrations. There was, however, a moderate age-adjusted correlation between the presence of subdural hemorrhage and

TABLE 1. PEARSON'S CORRELATION BETWEEN UCH-L1, SBDP145, NSE, S100B, MBP, AND GCS SCORE

	UCH-L1	SBDP145	NSE	S100B	MBP
GCS score	-0.388*	-0.431*	0.077	-0.191	0.025

* $p < 0.05$.

UCH-L1, ubiquitin C-terminal hydrolase; SBDP, spectrin breakdown product; NSE, neuron-specific enolase; MBP, myelin-basic protein; GCS, Glasgow Coma Scale.

increased concentrations of SBDP145 ($r = -0.536$, $p = 0.001$), and UCH-L1 ($r = -0.612$, $p < 0.000$).

Relationship of UCH-L1, SBDP, GCS, NSE, S100B, and MBP with binary GOS score

Preliminary analysis revealed a significant positive correlation between age and GOS score ($r = .331$, $p = 0.040$). All correlations were therefore adjusted for age. Higher concentrations of both UCH-L1 and SBDP were associated with worse outcome (Table 2). There was no correlation between NSE, S100B, or MBP concentrations and outcome.

Correlations between UCH-L1, SBDP, NSE, S100B, and MBP

A significant positive correlation existed between UCH-L1 and SBDP145 ($r = .485$, $p = 0.000$). UCH-L1 and NSE also had a significant positive correlation ($r = .400$, $p = 0.006$). All other correlations among biomarker scores were non-significant.

Discussion

This is the first study to report increased serum concentrations of both UCH-L1 and SBDP145 in children with TBI. The correlation of both markers with GCS and GOS scores, even in this relatively small sample, suggests that these markers may have potential both in the quantitative assessment of injury severity and in the prediction of outcome. The fact that both UCH-L1 and SBDP145 were more highly correlated with GCS and the dichotomous GOS than any of three well-accepted markers of pediatric TBI (NSE, S100B, and MBP) further supports their potential clinical use. The correlation between UCH-L1 and SBDP145 and GCS may have particular clinical importance in young children, given the known limitations of GCS scores in this population (Durham et al., 2000). As part of this preliminary study, we only measured UCH-L1 and SBDP145 concentrations at a single, early time point. It is possible that their concentrations at later time points may be more predictive of outcome, as has been shown with other biomarkers such as NSE (Beers et al., 2007; Berger et al., 2007).

The correlation between UCH-L1, SBDP145, and NSE concentrations is not surprising, given that increases in all three represent neuronal injury/death. Unlike NSE, however, UCH-L1 and SBDP145 are not found in red blood cells and are unaffected by hemolysis, one of the significant limitations of NSE. This suggests an important potential clinical advantage.

Although MBP is a marker of axonal injury, the lack of correlation with MBP concentrations with those of the other markers was not unexpected; the MBP value used in the current study was the initial concentration, but MBP concentrations peak 48–72 h after TBI, and the peak concentration is likely a more accurate representation of axonal injury than the initial concentration (Beers et al., 2007; Berger et al., 2007).

The lack of an increase in either marker in children with mild TBI may represent a lack of neuronal or axonal injury in these subjects, a lack of sensitivity of the assays to minor injury or a combination of both. While all subjects in the study were clinically diagnosed with a mild TBI and had a GCS score of 15, it is well-recognized that the GCS is insensitive as a method of assessing injury severity in mild TBI; there is wide spectrum of injury severity within the group of children diagnosed with mild TBI. The subjects with mild TBI in this study were likely a similarly heterogeneous group, and within this group was a subset of patients who had only an isolated closed head injury without neuronal or axonal injury. In this subset of subjects we would not expect to have increased biomarker concentrations, and therefore the lack of an increase in biomarkers in these cases is not due to a lack of sensitivity of the marker, but is due to a true lack of cellular injury. It is also possible that there was no increase in either marker because of a lack of sensitivity of the ELISA itself; the ELISAs for both UCH-L1 and SBDP145 were developed relatively recently, and as these assays continue to be refined, the lowest detection limit will likely improve. Future studies of UCH-L1 and SBDP145 after mild TBI will be needed in order to better assess the range of injury severity within the population of subjects with mild TBI. It would also be helpful to assess more sensitive outcome measures including post-concussive symptoms, although this is difficult in young children.

The study was limited primarily by the small number of subjects. In particular, the lack of significance of the increase in SBDP145 in subjects with moderate and severe TBI despite the overall significance in subjects with TBI versus controls is almost certainly due to small sample size.

In addition, the lack of correlation of NSE and S100B concentrations with outcome may also be due to small sample size. A larger study of 152 children with TBI published by our group several years ago demonstrated a relationship between early concentrations of both these markers and outcome (Berger et al., 2007). In a smaller pediatric study evaluating serial biomarker concentrations, the time to peak NSE and S100B, but not initial NSE or S100B, was correlated with outcome (Beers et al., 2007).

In conclusion, this is the first study to measure the concentration of UCH-L1 and SBDP145 after pediatric TBI. Despite our small sample size, the results demonstrate an increase in both markers in moderate and severe TBI, as well as a robust relationship with both GCS and GOS scores. The relationship between both UCH-L1 and SBDP145 and these well-established measures of injury severity and outcome was stronger than the relationship between NSE, S100B, or MBP and these same clinical parameters. Although the predictive ability of UCH-L1 and SBDP145 was not as strong as that of GCS ($r = -0.415$, -0.383 , and -0.476 , respectively), these preliminary data suggest a possible role for UCH-L1 and SBDP145 in the assessment of injury severity and/or prediction of outcome after pediatric TBI.

TABLE 2. PEARSON'S CORRELATION BETWEEN UCH-L1, SBDP145, GCS, NSE, S100B, MBP, AND GOS SCORE

	SBDP145	UCH-L1	NSE	S100B	MBP	GCS
Binary GOS score	0.383*	0.415*	-0.105	0.183	-0.213	0.476**

* $p < 0.05$.

** $p < 0.01$.

UCH-L1, ubiquitin C-terminal hydrolase; SBDP, spectrin breakdown product; NSE, neuron-specific enolase; MBP, myelin-basic protein; GCS, Glasgow Coma Scale; GOS, Glasgow Outcome Scale.

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Author Disclosure Statement

Kevin K.W. Wang and Ronald L. Hayes own stock, receive royalties from, and are officers of Banyan Biomarkers Inc., and as such may benefit financially as a result of the outcomes of this research or work reported in this publication. No competing financial interests exist for the other authors.

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