Clinical Utility of Serum Levels of Ubiquitin C-Terminal Hydrolase as a Biomarker for Severe Traumatic Brain Injury

**BACKGROUND:** Brain damage markers released in cerebrospinal fluid (CSF) and blood may provide valuable information about diagnosis and outcome prediction after traumatic brain injury (TBI).

**OBJECTIVE:** To examine the concentrations of ubiquitin C-terminal hydrolase-L1 (UCH-L1), a novel brain injury biomarker, in CSF and serum of severe TBI patients and their association with clinical characteristics and outcome.

**METHODS:** This case-control study enrolled 95 severe TBI subjects (Glasgow Coma Scale [GCS] score, 8). Using sensitive UCH-L1 sandwich ELISA, we studied the temporal profile of CSF and serum UCH-L1 levels over 7 days for severe TBI patients.

**RESULTS:** Comparison of serum and CSF levels of UCH-L1 in TBI patients and control subjects shows a robust and significant elevation of UCH-L1 in the acute phase and over the 7-day study period. Serum and CSF UCH-L1 receiver-operating characteristic curves further confirm strong specificity and selectivity for diagnosing severe TBI vs controls, with area under the curve values in serum and CSF statistically significant at all time points up to 24 hours (P < .001). The first 12-hour levels of both serum and CSF UCH-L1 in patients with GCS score of 3 to 5 were also significantly higher than those with GCS score of 6 to 8. Furthermore, UCH-L1 levels in CSF and serum appear to distinguish severe TBI survivors from nonsurvivors within the study, with nonsurvivors having significantly higher and more persistent levels of serum and CSF UCH-L1. Cumulative serum UCH-L1 levels > 5.22 ng/mL predicted death (odds ratio, 4.8).

**CONCLUSION:** Serum levels of UCH-L1 appear to have potential clinical utility in diagnosing TBI, including correlating to injury severity and survival outcome.

**KEY WORDS:** Biomarkers, Critical care, Diagnosis, Head injury, Outcome, Proteomics, UCH-L1

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**ABBREVIATIONS:** AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay; GCS, Glasgow Coma Scale; TBI, traumatic brain injury; UCH-L1, ubiquitin C-terminal hydrolase-L1
of TBI that allow the classification of patients most likely to
benefit from the treatment, and ultimately the determination of
the prognosis and risk of death of individual patients.

As a result, neurobiochemical markers have attracted increased
attention during the last decade. By analogy with biological
markers of acute myocardial infarction, several reviews have
highlighted the need for biomarkers that would provide early,
quantitative information about the extent of brain tissue
damage.6,7

Previously studied markers in large cohorts include neuron-
specific enolase,8-9 glial protein S100β,10-12 and myelin
basic protein.8,13 S100β, a calcium-binding protein mainly
expressed in astrocytes and released from dying cells, appears to
be a promising marker of TBI.14 However, S100β is also present
in oligodendrocytes, microglia, neurons, and extracerebral
tissue and is actively secreted independently of cell death.14
Neuron-specific enolase is present in the cytoplasm of neurons,
and a rapid appearance in serum after head injury has been
reported.15 Myelin basic protein, a proteolipid specific to the
myelin sheet of central myelin, has been shown to be released
after brain damage or demyelinating diseases.13 Although
a number of studies have suggested that these potential biomarkers correlate with the severity of injury, conflicting
results make it difficult to determine their utility for the routine
assessment of TBI patients.16,17

Glial fibrillary acidic protein and all-spectrin breakdown
products have also been reported as glial and axonal markers of
TBI, respectively.18-21 Recently, as a result of increased sophis-
tication of proteomics and other discovery techniques, investi-
gators have described ubiquitin C-terminal hydrolase-L1
(UCH-L1), neuron specific and concentrated in neuronal
soma,22 as a novel potential marker for brain injury.

UCH-L1 is involved in either the addition or removal of
ubiquitin from proteins that are destined for metabolism (via the
ATP-dependent proteasome pathway),23 and its variants have
been associated with familial Parkinson disease.24 In primary
cultures, proteasome inhibitors have been shown to cause arrest
neurite outgrowth and “dying-back” degeneration.25,26 In focal
cerebral ischemia and spinal cord injury, increased protein
aggregates and decreased proteasome activity have been observed,
respectively.27,28 These data suggest that UCH-L1 plays an
important role in the removal of excessive, oxidized, or misfolded
proteins during both normal and neuropathological conditions
such as neurodegenerative disorders.29

Because of the important function of UCH-L1 and its high
brain specificity, we hypothesized a potential utility in the
assessment of TBI patients. This study examined whether UCH-L1 was significantly
upregulated in serum from severe TBI patients compared with
uninjured control subjects. Temporal profiles of UCH-L1 were
compared in serum and cerebrospinal fluid (CSF) from the same
severe TBI patient cohort. We also examined relationships
between elevations in serum levels of UCH-L1 and mortality
after TBI.

**METHODS**

**Design and Population**

This prospective case-control study enrolled patients who presented to
the University of Florida Trauma System (Shands Hospital in Gainesville
and Jacksonville, Florida) and the University of Pécs (Pécs, Hungary) over
a 12-month period. Inclusion criteria of TBI patients were as follows:

- a Glasgow Coma Scale (GCS) score of 8 and a ventricular intracranial
  pressure monitoring as part of their routine clinical care. Patients with
  TBI had minor concomitant injury of the thorax and/or abdomen and/or
  extremities. Patients with multiple trauma were excluded (Injury Severity
  Score > 15). Serum and CSF samples from severe TBI subjects were
  collected every 6 hours up to a maximum of 7 days after TBI. The CSF
  samples from severe TBI subjects were collected directly from
  ventriculostomy catheters, which were placed as a standard of care for
  severe TBI patients at these institutions. Because the time between injury
time and admission and the time to ventriculostomy placement vary,
sampling for CSF and blood UCH-L1 measurement was not always
possible at exactly the same number of hours after TBI for the first 24
hours. Therefore, we established 6-hour time intervals for sampling of
UCH-L1 and documented the time interval of measurement accord-
ingly. Samples were immediately centrifuged at 4000 rpm, frozen, and
stored at −70°C until the time of analysis. The CSF control samples
were obtained either from hydrocephalic patients who had ventriculo-
peritoneal shunts placed and had CSF samples taken intraoperatively or
from patients with an unruptured aneurysm who also had CSF samples
taken intraoperatively. Control patients were healthy subjects who had
a normal mental status at the time of enrollment and had no evidence of
acute brain injury or hemodynamic instability. This study was approved
by the Institutional Review boards of the University of Florida and Pécs
University. Informed consent was obtained from all patients and/or legal
authorized representatives from each site.

**Measurement of UCH-L1 Biomarker**

Samples were measured using a standard UCH-L1 sandwich enzyme-
linked immunosorbent assay (ELISA) protocol as described below. For
UCH-L1 sandwich ELISA, 96-well plates were coated with 100 μL per
well of capture antibody (500 ng per well of purified mouse monoclonal
anti-UCHL1, made in house) in 0.1 mol/L sodium bicarbonate, pH 9.2.
Plates were then incubated overnight at 4°C and emptied, and 300 μL
per well of blocking buffer (Startingblock T20-TBS) was added and
incubated for 30 minutes at ambient temperature with gentle shaking.
This was followed by either the addition of the antigen standard
(recombinant UCH-L1) for standard curve (0.05-50 ng per well) or
samples (5 μL CSF; 20 μL serum) in sample diluent (total volume,
100 μL/well). The plate was incubated for 2 hours at room temperature
and then washed with automatic plate washer (5 × 300 μL/well with
wash buffer, Tris-buffered saline with Tween). Detection rabbit poly-
clonal horseradish peroxide–conjugated anti–UCH-L1 (made in house,
50 μg/mL) in blocking buffer was then added to wells at 100 μL per
well and incubated for 1.5 hours at room temperature, followed by washing.
Finally, the wells were developed with 100 μL per well of chemilumi-
nescent substrate solution (SuperSignal ELISA, Femto Co: Pierce,
catalog No. 37075, Rockford, Illinois) with 1-minute incubation times.
The signal was read by a 96-well chemiluminescence microplate reader
(GloRunner DXL Luminometer, Turner BioSystems Inc, Sunnyvale,
California). To assess performance, we averaged results from > 30
independent experiments (each with duplicate samples) performed over

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a period of 6 months. The raw dose-response curves were used to calculate sample recovery (calculated calibrator concentration/input concentration) with Sigma Plot version 11.0 (4 parameter logistic function for curve fitting). The precision is defined as the coefficient variation of sample recovery.

**Acute Injury and Outcome Measures**

We compared levels of UCH-L1 in control and TBI patients at each of the specified time points. Acute injury magnitude was functionally assessed by the GCS on admission. The 24-hour postinjury GCS score was used as an end point because the GCS of many patients was confounded by other factors, including the presence of alcohol, sedative drugs, and neuromuscular blockade agents in the first several hours after injury. As a result, the GCS is more limited in the first hours after injury and improves with repeated evaluations. Postacute outcome was assessed by the Glasgow Outcome Scale score at 3 months after injury. Assessment Glasgow Outcome Scale score was obtained by direct patient contact or via telephone interview with the patient and/or a family member. For statistical analyses, the outcome considered was mortality.

**Data Analysis**

For statistical analysis, biomarker levels were treated as continuous data, measured in nanograms per milliliter and expressed as mean ± SEM. Data normality was assessed with the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to test differences in biomarker concentration between 2 groups. A receiver-operating characteristic curve was constructed to explore the diagnostic ability of the biomarker to distinguish between uninjured controls and TBI patients at different time points after injury. Univariate logistic regression analysis was used to evaluate the prognostic ability of CSF and serum levels of UCH-L1 separately to predict the probability of death (Glasgow Outcome Scale score, 1) 3 months after injury. The C statistic indicates an overall measure of classification accuracy (representing the overall proportion of individuals correctly classified), with the value of 1.0 representing perfect accuracy. For analysis of the data, UCH-L1 concentrations were divided in thirtiles, with the lowest thirtile serving as the reference group. Statistical significance was set at P = .05. All analyses were performed with the statistical software package Sigma Plot version 11.0 (Systat Software, Inc).

**RESULTS**

**UCH-L1 Assay Performance and Clinical Study Design**

This study enrolled 95 severe TBI subjects. Patient characteristics in injury severity are shown in Table 1. There were 167 normal control subjects who provided blood samples (Table 1); CSF samples were also taken from 24 control patients who had CSF taken intraoperatively as a part of routine clinical care for mainly hydrocephalus patients (Table 1).

Highly sensitive UCH-L1 sandwich ELISA has been constructed and optimized for both CSF and serum detection for the purpose of this study. The antigen calibrator is recombinant His-tag human UCH-L1 produced in Escherichia coli and affinity purified with a Ni⁺ column. These preparations of UCH-L1 are routinely > 95% pure by sodium dodecyl sulfate-gel electrophoresis analysis (Figure 1A). The sandwich ELISA uses 2 antibodies specifically raised against human UCH-L1. The capture antibody is a mouse monoclonal antibody (IgM class) raised against recombinant His-tag UCH-L1 mentioned above. The detection antibody is a rabbit polyclonal antibody (IgG class) raised against a 50-amino acid epitope derived from a region of human UCH-L1. Both antibodies were tested against recombinant His-tag UCH-L1 protein by immunoblotting and human brain and other tissue lysate and shown to be specifically detecting a single band of UCH-L1 target protein with high intensity. Recombinant UCH-L1 has slightly higher molecular weight than native ICH-L1 protein (24 kDa) as a result of the presence of N-terminal His-tag and leader sequence (Figure 1A). Results in Figure 1A also show that UCH-L1 is highly

<table>
<thead>
<tr>
<th>TABLE 1. Summary of Demographic and Clinical Data for Severe Traumatic Brain Injury Cases and Controls Included in This Study*</th>
</tr>
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<tbody>
<tr>
<td><strong>Severe TBI, n = 95</strong></td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
</tr>
<tr>
<td>Minimum-maximum, y</td>
</tr>
<tr>
<td>Female/male, n (%)</td>
</tr>
<tr>
<td>Ethnicity, %</td>
</tr>
<tr>
<td>Not Hispanic or Latino</td>
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<tr>
<td>Hispanic or Latino</td>
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<tr>
<td>Injury GCS (range)</td>
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<tr>
<td>ED GCS (range)</td>
</tr>
<tr>
<td>Mechanism of injury, %</td>
</tr>
<tr>
<td>Motor vehicle</td>
</tr>
<tr>
<td>Motor cycle</td>
</tr>
<tr>
<td>Fall</td>
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<tr>
<td>Assault</td>
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<tr>
<td>Other</td>
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*CSF, cerebrospinal fluid; ED, emergency department; GCS, Glasgow Coma Scale; NA, not applicable; TBI, traumatic brain injury.

*Undetermined in 8 controls.
enriched in human brain tissue and almost exclusively expressed in brain, with a very minor presence in testis and large intestine, making it an excellent brain injury marker candidate. The configured and optimized UCH-L1 sandwich ELISA has a linear dynamic range of at least 4 orders of magnitude and a detection limit of 0.01 ng/mL in CSF, and it is linear for at least 3 orders of magnitude with a detection limit of at least 0.3 ng/mL for serum (Figure 1). Assay in serum gave lower chemiluminescence signals than CSF, given the same analyte (UCH-L1) concentrations, because it is known that serum has strong matrix signal suppression effects. The precision (percent coefficient of variation) over the linear range is 10% and 15% over the detection range for CSF and serum analysis, respectively.

**UCH-L1 as a Serum Marker in Diagnosing Severe TBI**

The TBI patients had significantly elevated serum and CSF levels of UCH-L1 after injury compared with uninjured control subjects at all time intervals after injury (Figure 2). As expected, CSF values of UCH-L1 were substantially higher and more sustained than levels of UCH-L1 in serum (Figure 2). There was a significant overall correlation between median concentrations of UCH-L1 in CSF and serum ($r_s = 0.59$, $P < .001$). Both CSF and serum UCH-L1 levels are highly detectable over the 7-day time course. Both the temporal CSF and serum profiles show that the earliest time points have the highest signals, followed by a rapid decay. However, some delayed increases were also observed, suggesting possible secondary insult or injury (Figure 2).

The UCH-L1 levels were highly readily detectable at the earliest time point (ie, within 6 hours of injury) measured in serum (at $5.32 \pm 1.10$ ng/mL) and within the first 24 hours (at $2.09 \pm 0.43$ ng/mL). Both are significantly above normal control serum levels (Table 2).

The CSF UCH-L1 levels were again highly detectible within 6 hours of injury (at $171.1 \pm 27.94$ ng/mL) and within the first 24 hours (at $110.60 \pm 14.53$ ng/mL). Both are significantly above normal control serum levels ($7.69 \pm 2.78$ ng/mL; Table 2). Overall, the mean CSF level of UCH-L1 in TBI patients was $66.21 \pm 9.72$ ng/mL compared with $7.6 \pm 2.78$ ng/mL in CSF controls (Table 2).
Figure 3 presents receiver-operating characteristic analyses of serum and CSF levels of UCH-L1 in patients with severe TBI vs controls at 6, 12, 18, and 24 hours after injury. Area under the curve (AUC) values are also plotted over these time periods. The AUC values in serum and CSF were statistically significant at all time points \((P < .001)\). Consistent with the marked decline in serum levels of UCH-L1 within the first 24 hours, AUC values also showed decreases in serum (Figure 3C and 3D). In contrast, AUC values in CSF remained relatively constant, consistent with the more sustained elevation in that compartment (Figure 3A and 3B).

**Serum and CSF UCH-L1 Level Correlation to Severity of TBI**

The UCH-L1 levels in CSF and serum were also compared against TBI severity by analyzing dichotomized GCS patient groups (GCS score of 3-5 vs 6-8). The GCS scores on admission were used for this analysis. Patients with GCS scores of 3 to 5 (generally reflecting more severe injury) indeed had higher CSF and serum UCH-L1 levels within 12 hours after injury than patients with GCS scores of 5 to 8 \((P = .07\) and \(P = .02\), respectively, Mann-Whitney test; Table 2).

**Serum and CSF UCH-L1 Level Correlation to Severe TBI Outcome**

We then examined serum and CSF UCH-L1 level correlation to severe TBI outcome in terms of patient survival within the 7-day study period. Within the first 6 hours after injury, the CSF levels of UCHL1 for nonsurvivors were significantly higher than those of survivors (CSF, 292.1 ± 47.17 vs 67.16 ± 22.32 ng/mL; \(P = .01\), Mann-Whitney \(U\) test) and over the duration of the study (CSF, 97.51 ± 10.93 vs 34.33 ± 3.2 ng/mL; \(P < .001\)), respectively (Figure 4). Importantly, serum levels of UCHL1 for survivors were also significantly higher than those of non-survivors within the first 6 hours (serum, 8.42 ± 2.58 vs 1.00 ± 0.66 ng/mL, respectively; \(P = .01\)) and over the duration of the study.
FIGURE 3. Ubiquitin C-terminal hydrolase-L1 (UCH-L1) receiver-operating characteristic (ROC) curves comparing 24-hour postinjury traumatic brain injury cerebrospinal fluid (CSF; A) and serum (C) UCH-L1 levels vs CSF controls or serum normal controls, respectively. Area under the curve (AUC) over time was also plotted (B and D).
been associated with neurodegenerative diseases including Parkinson, Huntington, and Alzheimer diseases.\textsuperscript{32} Neuroproteomic work in our laboratory identified UCH-L1 as a protein with 2-fold increases in abundance in the injured cortex 48 hours after controlled cortical impact in a rat model of TBI.\textsuperscript{29} We have also characterized UCH-L1 as a novel biomarker for brain ischemia and TBI in rats.\textsuperscript{33} There are limited studies assessing UCH-L1 in human cardiac arrest in TBI.\textsuperscript{34} We previously examined UCH-L1 levels in CSF as a biomarker of severe TBI.\textsuperscript{35} Siman et al\textsuperscript{36} also reported an elevation of UCH-L1 signals, among a panel of other putative markers, in CSF of severe TBI patients. This study is the first to systematically assess UCH-L1 in human serum after TBI and to compare levels with those found in CSF of the same patients (Figures 2 and 3 and Table 2). We confirmed that UCH-L1 protein is present in human serum and that its levels are significantly elevated after severe TBI using ELISA analysis. Additionally, UCH-L1 was detectable in blood very early after injury, related to injury magnitude and an early predictor of mortality. In this study, UCH-L1 levels in serum distinguished between severe TBI patients and uninjured control subjects. Levels of UCH-L1 were significantly elevated in serum from injured subjects within the first 6 to 24 hours after injury and averaged over the 7-day duration of study.

Figure 2 also illustrates that the temporal profile of UCH-L1 was characterized by secondary increases after the initial injury. Future studies are needed to determine whether these increases were associated with secondary insults such as increased intracranial pressure or hypotension in the same patients. Similar secondary increases were also observed in patients who died (see below). Because our study was not designed to obtain samples sooner than 6 hours after injury, we were unable to describe the time course of UCH-L1 release in CSF or serum closer to the time of injury. In practice, it is difficult to obtain CSF samples sooner than 6 hours after injury. Ongoing studies by our group taking blood samples from patients at the site of injury could provide insights into UCH-L1 levels and serum within the first 1 to 2 hours after insult.

Although preliminary, receiver-operating characteristic curves demonstrated the potential utility of measures of UCH-L1 in serum and CSF for differentiating between TBI patients and control subjects (Figure 3). Because we have yet to determine the final cutoffs of UCH-L1 values for normal and TBI subjects, sensitivity and specificity analyses and predictive power calculations were not conducted in this group of patients. Additionally, as shown by this work and others,\textsuperscript{37,38} the temporal profile of changes in biomarker levels is an important factor in determining diagnostic utility. Serum levels were most reliably associated with TBI when measured within the first 6 hours after injury and dropped off markedly thereafter. In contrast, sustained levels of UCH-L1 in CSF resulted in relatively constant AUC values. Receiver-operating characteristic values for UCH-L1 in CSF were similar to those previously reported.\textsuperscript{35} Importantly, elevations of UCH-L1 levels in CSF and serum in the first 12 hours after TBI were significantly increased in survivors compared to non-survivors (over study duration and 6 hours after injury). *P = .05, Mann-Whitney U test.

**FIGURE 4.** Comparison of ubiquitin C-terminal hydrolase-L1 (UCH-L1) levels in cerebrospinal fluid (CSF; A) and serum (B) in survivors and non-survivors (over study duration and 6 hours after injury). *P = .05, Mann-Whitney U test.
associated with initial severity of injury as measured by the GCS score on admission (Table 2).

Mortality within 3 months after severe TBI was significantly associated with increased levels of UCH-L1 in serum and CSF within 6 hours after injury and averaged over the study duration (Figure 4).

These data are comparable to a previous report that patients who did not survive to 6 weeks had significantly higher values of UCH-L1 in CSF 24 hours after injury compared with survivors. A cumulative serum UCH-L1 level > 5.22 ng/mL was a predictor of death, whereas a cumulative CSF UCH-L1 level did not predict death (Table 3). This is potentially related to the mechanism of release of UCH-L1 from the brain to the circulation. The mechanism by which UCHL-1, a neuron-specific protein, is transported from the brain compartment into the circulation is unknown. We hypothesize that UCHL-1 effluxes into the extracellular fluid on neural cell disruption, leaks by blood-brain barrier breakdown, and equilibrates with the blood. Therefore, serum UCH-L1 levels could more accurately reflect cerebral brain injury and blood-brain barrier dysfunction/disruption, explaining the difference in the predictive value between CSF and serum. Interestingly, Majetschak et al measured CSF levels of ubiquitin over 7 days in 6 patients with TBI and found that ubiquitin levels progressively recovered in survivors but continued to increase until death in nonsurvivors. Although this study assessed the parent compound ubiquitin and not UCH-L1, the data illustrate how elevations in this protein family can be associated with poor outcome. Recently, Brophy

**TABLE 3.** Crude Odds Ratio of Cumulative Cerebrospinal Fluid and Serum Levels of Ubiquitin C-Terminal Hydrolase-L1 (by Thirtiles) for the Prediction of Death (Glasgow Outcome Scale, 1) at 3 Months After Severe Traumatic Brain Injury by Univariate Logistic Regression

<table>
<thead>
<tr>
<th>Variables</th>
<th>Glasgow Outcome Scale Score, 1</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>C Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCH-L1 in CSF, ng/mL</td>
<td>≤ 130.76</td>
<td>1</td>
<td>Referent</td>
<td>0.65</td>
</tr>
<tr>
<td>130.77-505.95</td>
<td>0.55 (0.12-2.55)</td>
<td>0.44</td>
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<td></td>
</tr>
<tr>
<td>&gt; 505.95</td>
<td>2.25 (0.52-9.70)</td>
<td>0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCH-L1 in serum, ng/mL</td>
<td>≤ 0.69</td>
<td>1</td>
<td>Referent</td>
<td>0.67</td>
</tr>
<tr>
<td>0.7-3.96</td>
<td>2 (0.52-7.69)</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 3.96</td>
<td>4.8 (1.15-20.09)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*CI, confidence interval; CSF, cerebrospinal fluid; OR, odds ratio; UCH-L1, ubiquitin C-terminal hydrolase-L1.*
et al. evaluated exposure and biokinetic parameters of UCH-L1 in CSF and serum. They found a statistically significant increase in the median amount and peak concentration of UCH-L1 in serum and a shorter time to achieve the peak concentration in nonsurvivors compared with survivors. As with acute diagnosis of injury and injury magnitude, there may be critical periods after injury during which levels of markers provide more reliable predictions of long-term outcome. The half-life of UCH-L1 is similar in both CSF and serum, ranging from 7 to 9 hours. Ongoing kinetic studies of UCH-L1 in CSF and serum will further define the optimal frequency and timing of sampling after TBI in humans. Figure 4 illustrates important differential features of the temporal profile of UCH-L1 in CSF and serum of patients who survived vs patients who died. Nonsurvivors had more sustained levels of biomarkers in both compartments. These more sustained levels were associated with secondary transient increases. It is important for future studies to determine the relationship between these temporal characteristics of biomarkers changes and the occurrence of secondary insults after TBI. Such studies are ongoing by our group. In addition, ongoing studies will address other clinical variables such as sex and age that are known to influence clinical outcome in the biochemical response to TBI.

Although these data are encouraging, we recognize that there are limitations to this study. The present study was performed in a limited cohort of patients with severe TBI, a heterogeneous injury modality. This was a preliminary pilot study focused on GCS as the most widely used method used to evaluate the initial magnitude of injury. Future studies should assess the biomarker value against other measures of injury severity such as duration of coma and duration of posttraumatic amnesia. In addition, in the present study, only limited data are available regarding neuroimaging. The Marshall score was assessed by a trained neuroradiologist at only one of the sites. To avoid interobserver variability in the interpretation of neuroimaging and to add a potential confounding condition, we are designing a study that will include initial CT scans obtained on admission that will be interpreted by a qualified neuroradiologist at a central location. Because UCH-L1 is a specific marker of neurons in the central nervous system, it is likely that serum UCH-L1 is derived entirely from the brain. Ongoing studies of patients with peripheral polytrauma are directly assessing the effect of extracranial injuries on UCH-L1 values. These studies will provide further evidence of the brain specificity of UCH-L1.

Nevertheless, CSF and serum levels present within the first 6 hours after injury provide a framework for future clinical studies examining the utility of UCH-L1 as a potential biomarker of mild, moderate, and severe TBI in larger sample sizes.

CONCLUSION

The present study significantly extends the findings reported in previous research in animal models and human CSF after severe head injury. Taken together, the data provide strong support for the hypothesis that UCH-L1 levels can reliably diagnose the occurrence and magnitude of injury and predict outcome.

Disclosure

This study was supported in part by the National Institutes of Health (R01 NS049175-01, R01 NS052831-01, and R01 NS051431-01), supported in part by Department of Defense awards DAMD17-03-1-0772 and DAMD17-03-1-0066, National Institutes of Health awards, and Navy grant N00014-06-1-1029 (University of Florida). The opinions or assertions contained herein are the private views of the authors and are not to be construed as official, or as reflecting true views of the Department of the Army or Department of Defense. Drs Mondello, Budi, Robichaud, Gabrielli, Brophy, and Papa are consultants for Banyan Biomarkers, Inc. L. Akindy is an employee of Banyan Biomarkers, Inc. Drs Wang and Hayes own stock, receive royalties from, and are officers of Banyan Biomarkers Inc and thus may benefit financially as a result of the outcomes of this research or work reported in this publication. Dr Tortella reports no disclosures.

REFERENCES


**COMMENTS**

This is an important contribution representing an ambitious study to discover new biomarkers of traumatic brain injury (TBI). Ubiquitin C-terminal hydrolase-L1 (UCH-L1) was identified from unbiased proteomic studies to be one of the major neuronal proteins found to be elevated in cerebrospinal fluid (CSF) and serum after TBI. This study is a significant contribution to the development of UCH-L1 as a biomarker in that it includes a large number of patients (n = 95) with severe TBI, 24 controls subjects who contributed ventricular CSF, and 167 control subjects who contributed serum. The major conclusion of the study is that UCH-L1 is increased within 6 hours of injury, and although levels fall over the next several hours, they remain significantly elevated in serum for at least 36 hours and in CSF for up to 100 hours. There was also a relationship between UCH-L1 levels and injury severity and an association between UCH-L1 elevations and mortality.

The study has several important weaknesses. The main one is that we are not provided information on injury severity beyond the Glasgow Coma Scale, which is a unidimensional and very imperfect measure of injury severity. Duration of coma and duration of posttraumatic amnesia are generally recognized to be better measures of injury severity than initial Glasgow Coma Scale. We are also provided with little information about the neuroimaging results on these patients. If biomarkers are to fulfill their promise of allowing finely grained phenotyping of injury severity and injury type, future studies will have to take full advantage of the best available clinical information.

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Clinical advances in neurotrauma (indeed, throughout the spectrum of neurodegenerative disease) are hampered by the absence of valid biomarkers of disease severity, treatment, and recovery. The investigators behind this report have been at the forefront of the search for a biomarker in traumatic brain injury (TBI) over the last decade. The present study provides important data on the potential value of ubiquitin C-terminal hydrolase-L1 (UCH-L1) as a TBI biomarker. Significant clarification remains, including distinguishing changes in serum concentrations of UCH-L1 after TBI from other forms of non-central nervous system trauma and a more careful assessment of the relationship between UCH-L1 levels and functional impairments from TBI beyond the crude measure of the 3-month Glasgow Outcome Scale score. Nevertheless, the strength of this initial data likely augurs the inclusion of UCH-L1 in a forthcoming panel of biomarkers that measure injury and prognosis in TBI.

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