Original Investigation

Time Course and Diagnostic Accuracy of Glial and Neuronal Blood Biomarkers GFAP and UCH-L1 in a Large Cohort of Trauma Patients With and Without Mild Traumatic Brain Injury

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IMPORTANCE Glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase L1 (UCH-L1) have been widely studied and show promise for clinical usefulness in suspected traumatic brain injury (TBI) and concussion. Understanding their diagnostic accuracy over time will help translate them into clinical practice.

OBJECTIVES To evaluate the temporal profiles of GFAP and UCH-L1 in a large cohort of trauma patients seen at the emergency department and to assess their diagnostic accuracy over time, both individually and in combination, for detecting mild to moderate TBI (MMTBI), traumatic intracranial lesions on head computed tomography (CT), and neurosurgical intervention.

DESIGN, SETTING, AND PARTICIPANTS This prospective cohort study enrolled adult trauma patients seen at a level I trauma center from March 1, 2010, to March 5, 2014. All patients underwent rigorous screening to determine whether they had experienced an MMTBI (blunt head trauma with loss of consciousness, amnesia, or disorientation and a Glasgow Coma Scale score of 9-15). Of 3025 trauma patients assessed, 1030 met eligibility criteria for enrollment, and 446 declined participation. Initial blood samples were obtained in 584 patients enrolled within 4 hours of injury. Repeated blood sampling was conducted at 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, and 180 hours after injury.

MAIN OUTCOMES AND MEASURES Diagnosis of MMTBI, presence of traumatic intracranial lesions on head CT scan, and neurosurgical intervention.

RESULTS A total of 1831 blood samples were drawn from 584 patients (mean [SD] age, 40 [16] years; 62.0% [362 of 584] male) over 7 days. Both GFAP and UCH-L1 were detectible within 1 hour of injury. GFAP peaked at 20 hours after injury and slowly declined over 72 hours. UCH-L1 rose rapidly and peaked at 8 hours after injury and declined rapidly over 48 hours. Over the course of 1 week, GFAP demonstrated a diagnostic range of areas under the curve for detecting MMTBI of 0.73 (95% CI, 0.69-0.77) to 0.94 (95% CI, 0.78-1.00), and UCH-L1 demonstrated a diagnostic range of 0.30 (95% CI, 0.02-0.50) to 0.67 (95% CI, 0.53-0.81). For detecting intracranial lesions on CT, the diagnostic ranges of areas under the curve were 0.80 (95% CI, 0.67-0.92) to 0.97 (95% CI, 0.93-1.00)for GFAP and 0.31 (95% CI, 0-0.63) to 0.77 (95% CI, 0.68-0.85) for UCH-L1. For distinguishing patients with and without a neurosurgical intervention, the range for GFAP was 0.91 (95% CI, 0.79-1.00) to 1.00 (95% CI, 1.00-1.00), and the range for UCH-L1 was 0.50 (95% CI, 0-1.00) to 0.92 (95% CI, 0.83-1.00).

CONCLUSIONS AND RELEVANCE GFAP performed consistently in detecting MMTBI, CT lesions, and neurosurgical intervention across 7 days. UCH-L1 performed best in the early postinjury period.

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A blood test for traumatic brain injury (TBI) is on the horizon, and private enterprises are commercializing TBI biomarkers for US Food and Drug Administration approval. Research in the field of TBI biomarkers and concussion has skyrocketed in the last decade.[1-3] Two promising brain injury biomarkers have emerged for mild TBI, namely, glial protein glial fibrillary acidic protein (GFAP) and neuronal protein ubiquitin C-terminal hydrolase L1 (UCH-L1). GFAP is an astroglial biomarker of injury and is found in the astroglial skeleton of both white and gray brain matter.[4] UCH-L1 is a neuronal brain injury biomarker found in high abundance in neurons and has previously been used as a histological biomarker.[5]

Current evidence indicates that both serum GFAP and UCH-L1 are detectable in serum in less than 1 hour after a mild TBI and are able to distinguish between patients with mild TBI and other trauma patients without acute brain injury after injury.[6-10] GFAP and UCH-L1 levels are significantly elevated in patients with TBI with intracranial lesions on computed tomography (CT) and, in patients with mild TBI, can distinguish between those with a normal and an abnormal CT scan of the brain.[6-8,11,12] The sensitivity of GFAP and UCH-L1 for detecting intracranial lesions on CT ranges between 94% to 100% in both children and adults.[6-10] Notably, GFAP and UCH-L1 are elevated in patients with mild TBI requiring neurosurgical intervention and can predict with high sensitivity which patients with mild TBI will require neurosurgery.[6,7] Moreover, GFAP performs considerably well in detecting intracranial lesions in polytrauma trauma patients with mild TBI who have substantial extracranial injuries and fractures[8,9] and detects axonal injury on magnetic resonance imaging 3 months after TBI.[11] The results of these studies suggest that both GFAP and UCH-L1 are specific for brain injury. Preliminary studies have shown benefit in combining these 2 biomarkers for predicting CT lesions.[12]

Trauma patients are seen at different times after injury, and there is an inadequate understanding of the temporal profiles of GFAP and UCH-L1 in human serum, particularly after a mild TBI. Most important, there is insufficient knowledge as to when these biomarkers should be used for the clinical evaluation of the trauma patient with suspected mild TBI. This study evaluated the temporal profiles of serum glial and neuronal serum biomarkers GFAP and UCH-L1 in a large cohort of trauma patients seen at an emergency department (ED) with and without mild to moderate TBI (MMTBI) and assessed their diagnostic accuracy for detecting traumatic intracranial lesions on head CT and neurosurgical intervention over time. Because of the heterogeneous nature of TBI and the problems with injury classification, we included patients with TBI with a Glasgow Coma Scale (GCS) score of 9 to 15 (mild to moderate). However, the focus of our study centered on mild TBI; 97.8% (318 of 325) of our patients with TBI had a GCS score of 13-15.

Key Points

Question What is the time course and diagnostic accuracy of serum glial and neuronal biomarkers glial fibrillary acidic protein and ubiquitin C-terminal hydrolase L1 for detecting mild to moderate traumatic brain injury (MMTBI), traumatic intracranial lesions on head computed tomography (CT), and neurosurgical intervention?

Findings In this cohort study, a total of 1831 blood samples were drawn in 584 trauma patients over 7 days. UCH-L1 peaked earlier than GFAP, but GFAP was detectable for a longer period of time and was associated with the severity of injury.

Meaning GFAP appeared to consistently detect MMTBI, CT lesions, and neurosurgical intervention across 7 days, whereas UCH-L1 performed best in the early postinjury period.

Methods

Study Population

This prospective cohort study enrolled a convenience sample of adult trauma patients seen at the ED of a level I trauma center (Orlando Regional Medical Center) within 4 hours of injury from March 1, 2010, to March 5, 2014. This study was approved by the Orlando Regional Medical Center Institutional Review Board, and written informed consent was obtained from each patient or his or her legal authorized representative before enrollment.

Eligibility for MMTBI was determined by the treating physician (including L.P., S.S., P.G., K.D.W., and other nonauthors) based on a history of blunt head trauma followed by either loss of consciousness, amnesia, or disorientation in patients seen at the ED within 4 hours of injury with a GCS score of 9 to 15. Eligibility was also prospectively verified by the research team (including L.P., C.F.B., C.N.T., N.J.A., M.A.L., C.A.H., D.I.M.G., and other nonauthors) before enrollment. Head CT scans were performed at the discretion of the treating physician. Patients were excluded if they (1) were younger than 18 years; (2) had no history of trauma as their primary event (eg, syncope or seizure); (3) had known dementia, chronic psychosis, or active central nervous system pathology; (4) were pregnant; (5) were incarcerated; or (6) had a systolic blood pressure less than 100 mm Hg.

The non-TBI general trauma group included patients with a GCS score of 15 seen at the ED with a traumatic mechanism of injury but without TBI. They experienced similar mechanisms of injury as the MMTBI group, but all had a normal mental status since injury (as verified by the research team) and had no evidence of acute brain injury or hemodynamic instability. These patients were carefully screened to ensure that they had no loss of consciousness, no amnesia, and no alteration in sensorium at any time after injury. The purpose of enrolling both patients with TBI and general trauma patients was to simulate the real-world setting in which TBI biomarkers would be used.

Study Procedures

All initial patient assessments were made by board-certified emergency medicine physicians (including L.P., S.S., P.G., K.D.W., and other nonauthors) trained in a formal 1-hour session on evaluating patient eligibility for the study. After initial screening, a meticulous secondary assessment was conducted by the research team in the ED to ensure that each patient met inclusion criteria and to verify any exclusion. All prehospital and ED records were reviewed; patients, families, and witnesses (if available) were carefully questioned; and

the final determination was made by the emergency physician together with the research team. Patient classification was performed prospectively. Blood samples were obtained from each patient with MMTBI and each trauma patient within 4 hours of the reported time of injury. Repeated blood sampling was conducted for as long as the patient remained in the hospital at 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, and 180 hours after injury. Once patients were discharged, blood sampling was discontinued. For each blood draw, a single vial of approximately 5 mL of blood was collected and placed in clot tubes with a serum separator and allowed to clot at room temperature. The blood was centrifuged within 30 minutes, and the serum was placed in barcoded aliquot containers and stored in a freezer at −70°C until it was transported to a central laboratory. There, the samples were analyzed in batches using sandwich enzyme-linked immunosorbent assays to GFAP and UCH-L1. Laboratory personnel processing the samples were masked to the clinical data. After assessment and treatment in the ED, patients were either discharged home or admitted to the hospital based on the severity of their injuries, and patient management was not altered by the study.

Trauma patients underwent standard CT scan of the head based on the clinical judgment of the treating physician. The pattern of CT scan ordering at this level I trauma center is such that most patients with blunt head injury with subsequent symptoms have a head CT scan performed as part of usual care. Physicians also ordered CT scans of the head in the general trauma control participants based on mechanism of injury or clinical circumstances. Computed tomography examinations were interpreted by board-certified radiologists, who recorded location, extent, and type of brain injury. Radiologists were masked to the study protocol but had the usual clinical information.

Outcome Measures

The performance of GFAP and UCH-L1 in detecting brain injury was evaluated over a 7-day period. The main outcome measures included the performance of the biomarkers over time for (1) detecting the presence of MMTBI and distinguishing trauma patients with MMTBI from those without MMTBI, (2) identifying traumatic intracranial lesions on CT scan, and (3) having a neurosurgical intervention.

Intracranial lesions on CT included any acute traumatic intracranial lesions visualized on CT scan, including intracranial hemorrhage (epidural, subdural, or subarachnoid hemorrhage) or contusion, cerebral edema, diffuse axonal injury, midline shift of intracranial contents or signs of brain herniation, and pneumocephalus. Neurosurgical intervention was defined as either death within 7 days secondary to brain injury or the need for any of the following procedures within 7 days: craniotomy, elevation of skull fracture, intracranial pressure monitoring, or intubation for brain injury.[13]

Statistical Analysis

Descriptive statistics with means and proportions were used to describe the data. For statistical analysis, biomarker levels were treated as continuous data, measured in nanograms per milliliter, and expressed as medians with interquartile ranges (IQRs). Data were assessed for equality of variance and distribution. Logarithmic transformations were conducted on nonnormally distributed data. Group comparisons were performed using independent-sample *t* test with variance consideration and the χ^2 test. Receiver operating characteristic curves were created to explore the ability of the biomarkers to identify the presence of a TBI, detect intracranial lesions on CT scan, and predict those having a neurosurgical intervention. Estimates of the areas under the curves (AUCs) were obtained, with an AUC of 0.5 indicating no discrimination and an AUC of 1.0 indicating a perfect diagnostic test. Classification performance was assessed by the sensitivity, specificity, and positive and negative predictive values with 95% CIs. All analyses were performed using a statistical software package (SPSS, version 22.0; IBM Corporation).

Biomarker Analysis

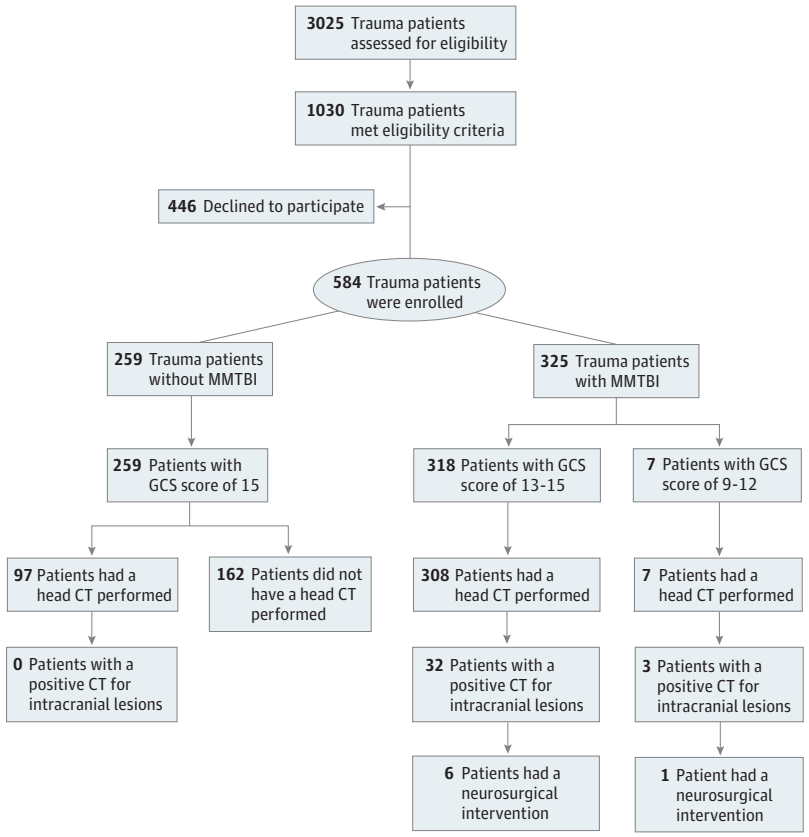
Serum GFAP and UCH-L1 levels were measured in duplicate for each sample using a validated enzyme-linked immunosorbent assay platform (Banyan Biomarkers Inc). For the GFAP assay, the lower limit of quantification (LLOQ) is 0.030 ng/mL, and the upper limit of quantification (ULOQ) is 50.000 ng/mL. The lower limit of detection (LLOD) is 0.008 ng/mL. For the UCH-L1 assay, the LLOQ is 0.100 ng/mL, and the ULOQ is 9.000 ng/mL. The LLOD is 0.045 ng/mL. Any samples yielding a signal over the quantification or calibrator range were diluted and reassayed.

Results

Over the study period, 3025 trauma patients were screened, and 1030 patients met eligibility criteria for enrollment. There were 446 trauma patients who were eligible and declined to participate and 584 trauma patients who were enrolled. Demographic characteristics between enrolled patients and those not enrolled were similar. Enrolled patients had a mean (SD) age of 40 (16) years (age range, 18-83 years), and nonenrolled patients had a mean (SD) age of 41 (17) years (age range, 18-88 years) ($P = .39$). The proportion of men was 62.0% (362 of 584) for enrolled patients vs 58.7% (262 of 446) for nonenrolled patients ($P = .48$). Race/ethnicity was also not significantly different ($P = .07$).

Of 584 enrolled participants, 325 (55.7%) had trauma with MMTBI, and 259 (44.3%) had trauma without MMTBI. Among patients with TBI, 318 of 325 (97.8%) had mild TBI and a GCS score of 13 to 15, and only 7 of 325 (2.2%) had moderate TBI and a GCS score of 9 to 12. All trauma patients without MMTBI had a GCS score of 15. **Figure 1** shows the distribution of enrolled patients. Computed tomography scans of the head were performed in 315 of 325 (96.9%) patients with MMTBI and in 97 of 259 (37.5%) trauma patients without MMTBI. Intracranial lesions were found in 35 of 325 (10.8%) patients with MMTBI and in none of the trauma patients without MMTBI. The distribution of clinical characteristics of all enrolled patients is summarized in **Table 1**. Neurosurgical interventions were performed in 7 of 325 (2.2%) patients with MMTBI and

Figure 1. Flow Diagram of Screened and Enrolled Patients



CT indicates computed tomography; GCS, Glasgow Coma Scale; and MMTBI, mild to moderate traumatic brain injury.

in none of the trauma controls. Five patients were intubated for worsening mental status (one received hyperosmolar therapy), and 2 patients had craniotomies for repair or elevation of open skull fractures.

There were a total of 1831 blood samples drawn in 584 patients (1243 with MMTBI and 588 trauma controls). All patients had serum samples drawn within 4 hours of injury, with a mean (SD) time from injury to serum sample collection of 3.0 (0.8) hours. The mean (SD) times from injury to initial sampling were 3.0 (0.9) hours for trauma patients with MMTBI and 3.1 (0.8) hours for trauma patients without MMTBI. Five hundred eighty-four patients had samples drawn between injury and 4 hours, and the numbers of patients having samples obtained at 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, and 180 hours after injury were 429, 136, 107, 96, 88, 81, 57, 50, 41, 38, 28, 25, 13, 13, 10, 12, 11, 8, and 4, respectively.

Among trauma control patients, 333 of 588 (56.6%) samples were below the LLOD and 130 (22.1%) were below the LLOQ for GFAP. In patients with TBI, 269 of 1243 (21.6%) samples were below the LLOD and 175 (14.1%) were below the LLOQ for GFAP. Among trauma control patients, 93 of 588 (15.8%) samples were below the LLOD and 87 (14.8%) were below the LLOQ for UCH-L1. In patients with TBI, 146 of 1243 (11.7%) samples were below the LLOD and 143 (11.5%) were below the LLOQ for UCH-L1.

The time course of GFAP and UCH-L1 in all trauma patients is shown in **Figure 2**A. The serum concentration of GFAP was detectible within 1 hour of injury and reached a peak at 20 hours after injury. Concentrations steadily decreased over 72 hours. GFAP levels were still detectable at 168 hours (7 days) after injury but remained at lower levels between 72 and 180 hours after injury. In contrast, UCH-L1 rose more rapidly after injury and reached a peak at 8 hours. Concentrations of UCH-L1 decreased steadily over 48 hours and had small peaks and troughs over 7 days.

In the patients with MMTBI, glial injury biomarker GFAP levels were significantly higher (median, 0.112 ng/mL; IQR, 0.030-0.462 ng/mL; range, 0.008-8.078 ng/mL) compared with the trauma controls (median, 0.008 ng/mL; IQR, 0.008-0.030 ng/mL; range, 0.008-0.773 ng/mL) ($P < .001$). The levels of neuronal injury biomarker UCH-L1 were also significantly higher (median, 0.258 ng/mL; IQR, 0.109-0.627 ng/mL; range, 0.045-9.000 ng/mL) compared with the trauma controls (median, 0.171 ng/mL; IQR, 0.100-0.417 ng/mL; range, 0.045-4.241 ng/mL) ($P < .001$). The temporal profiles of serum GFAP and UCH-L1 in trauma patients with and without MMTBI are shown in Figure 2B. Concentrations of GFAP were significantly higher ($P < .05$) in patients with TBI than in patients without TBI at every time point after injury over 7 days except at 144, 156, and 168 hours after injury. Concentrations of UCH-L1 were significantly higher in patients with TBI at enrollment and 4, 8, 12, and 16 hours after injury. The ability of GFAP and UCH-L1 to distinguish trauma patients with and without MMTBI was assessed over 7 days by calculating the AUC

Table 1. Characteristics of Enrolled Patients[a]

Characteristic	Trauma Patients		Total (N = 584)
	With MMTBI (n = 325)	Without MMTBI (n = 259)	
Age, mean (SD) [range], y	39 (16) [18-78]	41 (16) [18-83]	40 (16) [18-83]
Male sex, No. (%)	212 (65.2)	150 (57.9)	362 (62.0)
Race/ethnicity, No. (%)			
Asian	6 (1.8)	2 (0.8)	8 (1.4)
Black	60 (18.5)	73 (28.2)	133 (22.8)
Hispanic	60 (18.5)	59 (22.8)	119 (20.4)
Native American	1 (0.3)	3 (1.2)	4 (0.7)
Middle Eastern	1 (0.3)	0	1 (0.2)
White	190 (58.5)	120 (46.3)	310 (53.1)
Other	7 (2.2)	2 (0.8)	9 (1.5)
GCS score in ED, No. (%)			
9-12	7 (2.2)	0	7 (1.2)
13	3 (0.9)	0	3 (0.5)
14	40 (12.3)	0	40 (6.8)
15	275 (84.6)	259 (100)	534 (91.4)
Mechanism of injury, No. (%)			
Motor vehicle crash	155 (47.7)	146 (56.4)	301 (51.5)
Fall	68 (20.9)	35 (13.5)	103 (17.6)
Motorcycle	40 (12.3)	17 (6.6)	57 (9.8)
Pedestrian struck	13 (4.0)	9 (3.5)	22 (3.8)
Bicycle struck by vehicle	13 (4.0)	10 (3.9)	23 (3.9)
Fall off bicycle	7 (2.2)	1 (0.4)	8 (1.4)
Assault	13 (4.0)	6 (2.3)	19 (3.3)
Sports injury	3 (0.9)	9 (3.5)	12 (2.1)
Other motorized vehicle	4 (1.2)	2 (0.8)	6 (1.0)
Other	9 (2.8)	24 (9.3)	33 (5.7)
Loss of consciousness, No. (%)	267 (82.2)	0	267 (45.7)
Amnesia, No. (%)	136 (41.8)	0	136 (23.3)
Admitted to hospital, No. (%)	130 (40.0)	59 (22.8)	189 (32.4)
Intoxicated with alcohol or drugs, No. (%)	30 (9.2)	8 (3.1)	38 (6.5)
Head CT performed, No. (%)	315 (96.9)	97 (37.5)	412 (70.5)
Intracranial lesions on head CT, No. (%)	35 (10.8)	0	35 (6.0)

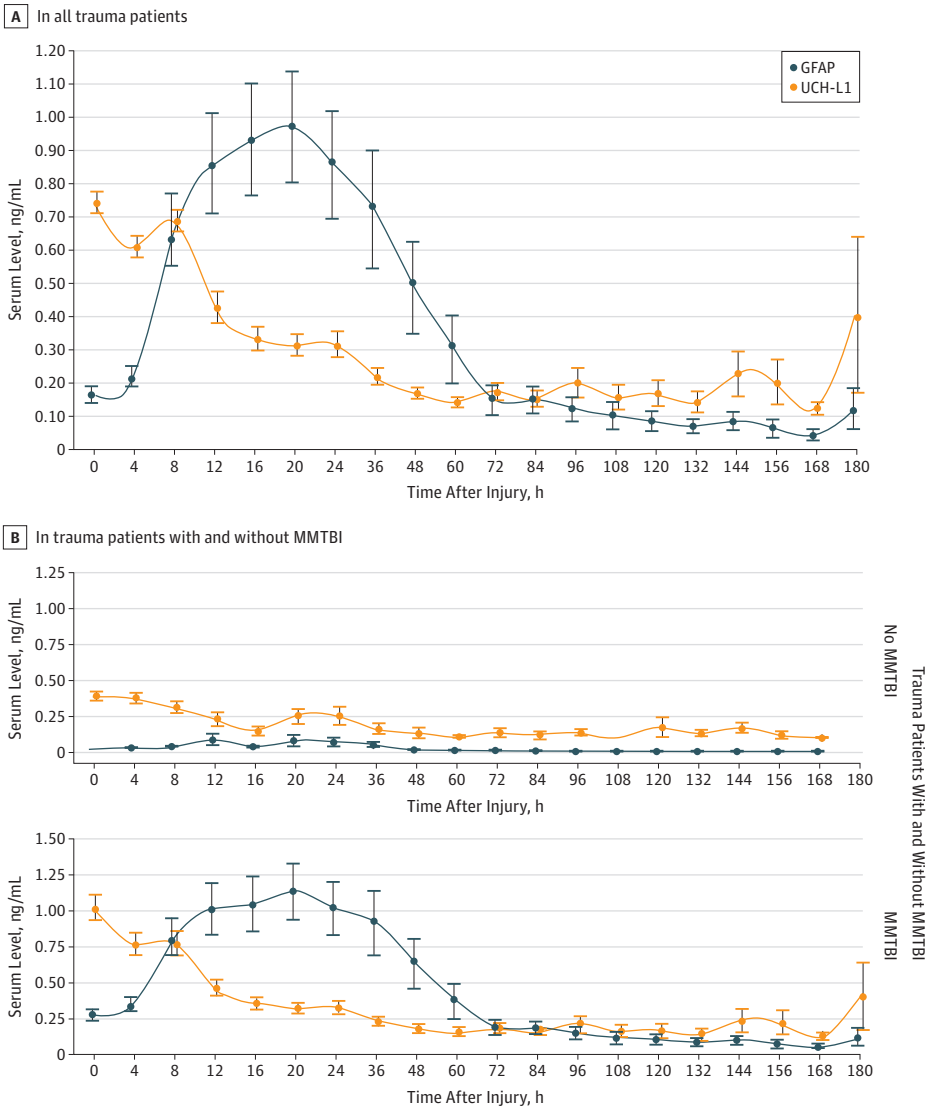
Abbreviations: CT, computed tomography; ED, emergency department; GCS, Glasgow Coma Scale; MMTBI, mild to moderate traumatic brain injury.

[a] Percentages may not add up to 100% due to rounding.

at each time point after injury (eTable in the Supplement). GFAP demonstrated a range of AUCs between 0.73 (95% CI, 0.69-0.77) and 0.94 (95% CI, 0.78-1.00), and UCH-L1 demonstrated AUCs between 0.30 (95% CI, 0.02-0.58) and 0.67 (95% CI, 0.53-0.81). When GFAP and UCH-L1 were combined, the AUC ranged from 0.64 (95% CI, 0.35-0.92) to 0.89 (95% CI, 0.79-0.99) and closely mimicked the pattern of GFAP. GFAP outperformed UCH-L1 at all time points. The combination of GFAP and UCH-L1 marginally outperformed GFAP alone at enrollment and 8, 36, 60, and 168 hours; however, the differences were not statistically significant.

In patients with traumatic intracranial lesions on CT, GFAP levels were significantly elevated (median, 0.588 ng/mL; IQR, 0.140-2.014 ng/mL; range, 0.008-8.078 ng/mL) compared with those without lesions (median, 0.033 ng/mL; IQR, 0.008-0.189 ng/mL; range, 0.008-7.785 ng/mL) ($P < .001$). Similarly, UCH-L1 was significantly higher in those with lesions (median, 0.319 ng/mL; IQR, 0.131-0.811 ng/mL; range, 0.045-9.000 ng/mL) than those without lesions (median, 0.250 ng/mL; IQR, 0.106-0.586 ng/mL; range, 0.045-9.000 ng/mL) ($P < .001$). In **Figure 3**A, the time courses of GFAP and UCH-L1 are compared in patients with intracranial lesions on initial head CT (CT positive) and those without intracranial lesions (CT negative). Concentrations of GFAP were significantly higher ($P < .05$) in patients with intracranial lesions than in those without lesions at every time point after injury over 7 days except at 168 hours after injury. Concentrations of UCH-L1 were significantly higher in patients with intracranial lesions at enrollment and 4, 8, 12, 16, 24, and 48 hours after injury but not at any later time points. The ability of GFAP and UCH-L1 to detect traumatic intracranial lesions on CT was assessed over 7 days by calculating the AUC at each time point after injury (**Table 2**). GFAP demonstrated a range of AUCs between 0.80 (95% CI, 0.67-0.92) and 0.97 (95% CI, 0.93-1.00), and UCH-L1 demonstrated AUCs between 0.31 (95% CI, 0-0.63) and 0.77 (95% CI, 0.68-0.85). When GFAP and UCH-L1 were combined, the AUC ranged from 0.75 (95% CI, 0.33-1.00) to 0.97 (95% CI, 0.93-1.00) and corresponded closely with GFAP. GFAP

Figure 2. Temporal Profiles of Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin C-Terminal Hydrolase L1 (UCH-L1)

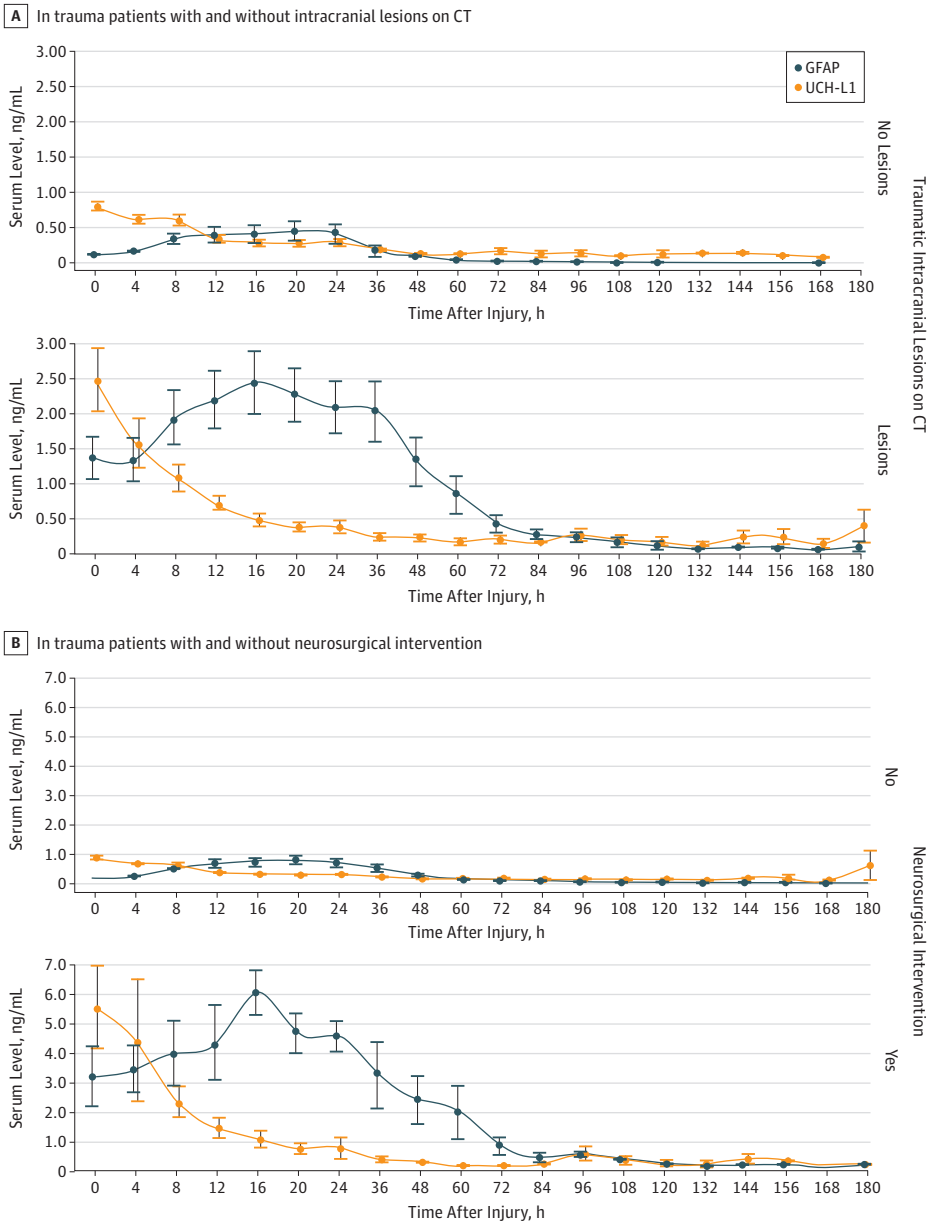


A-B, Shown are the means, with error bars representing the SEMs. B, In trauma patients who had clinical evidence of mild to moderate traumatic brain injury (MMTBI) compared with those who experienced trauma but had no evidence of MMTBI.

outperformed UCH-L1 at all time points. The combination of GFAP and UCH-L1 marginally outperformed GFAP alone at 12, 24, 108, 132, and 144 hours; however, the differences were not statistically significant.

In patients requiring neurosurgical intervention, overall GFAP levels were significantly elevated (median, 1.847 ng/mL; IQR, 0.418-4.421 ng/mL; range, 0.119-8.078 ng/mL) compared with those not requiring such interventions (median, 0.054 ng/mL; IQR, 0.008-0.297 ng/mL; range, 0.008-7.973 ng/mL) ($P < .001$). Similarly, UCH-L1 was significantly higher in those requiring neurosurgical intervention (median, 0.508 ng/mL; IQR, 0.224-1.341 ng/mL; range, 0.100-9.000 ng/mL) than in those not requiring intervention (median, 0.250 ng/mL; IQR, 0.106-0.593 ng/mL; range, 0.045-9.000 ng/mL) ($P < .001$). In Figure 3B, the temporal profiles of GFAP and UCH-L1 are compared in patients having a neurosurgical intervention and in those not having a neurosurgical intervention. Concentrations of GFAP were significantly higher ($P < .05$) with a neurosurgical intervention at every time point after injury over 7 days except at 168 and 180 hours after injury. Concentrations of UCH-L1 were significantly higher in patients with a neurosurgical intervention at enrollment and 4, 8, 12, 16, 20, 24, 36, 48, and 96 hours after injury but not at any other time points. The association between GFAP and UCH-L1 and having a neurosurgical intervention was assessed over 7 days by calculating the AUC at each time point after injury (**Table 3**). GFAP demonstrated a range of AUCs between 0.91 (95% CI, 0.79-1.00) and 1.00 (95% CI, 1.00-1.00), and UCH-L1 demonstrated AUCs between 0.50 (95% CI, 0-1.00) and 0.92 (95% CI, 0.85-1.00). When GFAP and UCH-L1 were combined, the AUC ranged from 0.50 (95% CI, 0-1.00) to 1.00 (95% CI, 1.00-1.00) and corresponded closely with GFAP. GFAP outperformed UCH-L1 at all time points. The combination of GFAP and UCH-L1 outperformed GFAP alone at the earliest time points after injury,

Figure 3. Temporal Profiles of Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin C-Terminal Hydrolase L1 (UCH-L1)



A, In trauma patients who had computed tomography(CT) performed. Those with traumatic intracranial lesions on CT are compared with those without intracranial lesions on CT.
B, In trauma patients who had a neurosurgical intervention. Those having a neurosurgical intervention are compared with those not having a neurosurgical intervention.

including at enrollment and 4, 8, 12, 16, 20, 36, and 84 hours; however, the differences were not statistically significant.

When we adjusted for age, sex, and GCS score, the strongest predictor of having an intracranial lesion on CT was the level of serum GFAP (odds ratio, 3.45; 95% CI, 2.69-4.43). Correspondingly, the strongest predictor of having a neurosurgical intervention was also the level of serum GFAP (odds ratio, 2.57; 95% CI, 2.04-3.21).

Discussion

This prospective study assessed the temporal profiles and diagnostic accuracy at 20 distinct time points over 7 days of the glial and neuronal biomarkers GFAP and UCH-L1 in a large cohort of trauma patients seen at a single level I trauma center. This study is among the first and largest studies to assess the temporal profiles of these 2 biomarkers in a trauma population with and without mild TBI. The performance of GFAP and UCH-L1 alone and in combination was compared in patients with and without TBI and in those with traumatic intracranial lesions to capture the setting in which a blood test for TBI biomarkers would be used clinically. For this reason, uninjured healthy control patients were not included in this analysis. Concentrations of these 2 biomarkers have already been well characterized in uninjured healthy control patients.[6,7] This study has important clinical implications for when these TBI biomarkers should be used by health care professionals after

Table 2. AUC for Distinguishing Between Trauma Patients With and Without Intracranial Lesions on Head CT[a]

	AUC (95% CI)		
Time After Injury, h	GFAP	UCH-L1	Combination of GFAP and UCH-L1
Enrollment	0.86 (0.79-0.93)	0.77 (0.68-0.85)	0.82 (0.73-0.91)
4	0.84 (0.75-0.92)	0.73 (0.63-0.83)	0.84 (0.75-0.92)
8	0.81 (0.70-0.91)	0.67 (0.55-0.79)	0.82 (0.72-0.91)
12	0.82 (0.71-0.93)	0.71 (0.59-0.83)	0.83 (0.72-0.93)
16	0.81 (0.68-9.31)	0.70 (0.58-0.82)	0.80 (0.68-0.93)
20	0.80 (0.67-0.92)	0.61 (0.47-0.74)	0.80 (0.68-0.92)
24	0.82 (0.70-0.95)	0.64 (0.52-0.77)	0.83 (0.70-0.95)
36	0.97 (0.92-1.00)	0.61 (0.46-0.76)	0.97 (0.92-1.00)
48	0.96 (0.90-1.00)	0.67 (0.49-0.85)	0.96 (0.90-1.00)
60	0.97 (0.93-1.00)	0.60 (0.39-0.80)	0.97 (0.93-1.00)
72	0.95 (0.88-1.00)	0.63 (0.43-0.84)	0.95 (0.88-1.00)
84	0.94 (0.86-1.00)	0.65 (0.43-0.87)	0.94 (0.85-1.00)
96	0.91 (0.78-1.00)	0.63 (0.39-0.87)	0.90 (0.75-1.00)
108	0.90 (0.71-1.00)	0.61 (0.27-0.96)	0.92 (0.75-1.00)
120	0.91 (0.75-1.00)	0.59 (0.26-0.91)	0.88 (0.67-1.00)
132	0.94 (0.78-1.00)	0.31 (0-0.63)	1.00 (1.00-1.00)
144	0.94 (0.81-1.00)	0.44 (0.11-0.78)	0.96 (0.86-1.00)
156	0.93 (0.76-1.00)	0.57 (0.21-0.94)	0.86 (0.60-1.00)
168	0.84 (0.53-1.00)	0.75 (0.39-1.00)	0.75 (0.33-1.00)

Abbreviations: AUC, area under the curve; CT, computed tomography; GFAP, glial fibrillary acidic protein; UCH-L1, ubiquitin C-terminal hydrolase L1.

[a] Shown is the performance of GFAP and UCH-L1 alone and in combination.

Table 3. AUC for Distinguishing Between Patients With MMTBI Having and Not Having a Neurosurgical Intervention[a]

	AUC (95% CI)		
Time After Injury, h	GFAP	UCH-L1	Combination of GFAP and UCH-L1
Enrollment	0.94 (0.88-1.00)	0.92 (0.83-1.00)	0.99 (0.97-1.00)
4	0.99 (0.97-1.00)	0.90 (0.80-1.00)	0.98 (0.96-1.00)
8	0.93 (0.85-1.00)	0.92 (0.83-1.00)	0.94 (0.87-1.00)
12	0.92 (0.84-1.00)	0.92 (0.84-1.00)	0.93 (0.85-1.00)
16	0.98 (0.95-1.00)	0.92 (0.85-1.00)	0.99 (0.97-1.00)
20	0.96 (0.92-1.00)	0.86 (0.75-0.98)	0.97 (0.93-1.00)
24	0.98 (0.95-1.00)	0.80 (0.65-0.95)	0.98 (0.95-1.00)
36	0.92 (0.85-1.00)	0.82 (0.68-0.97)	0.93 (0.85-1.00)
48	0.96 (0.88-1.00)	0.87 (0.75-0.99)	0.96 (0.88-1.00)
60	0.94 (0.84-1.00)	0.67 (0.32-1.00)	0.93 (0.79-1.00)
72	0.96 (0.88-1.00)	0.70 (0.51-0.88)	0.95 (0.85-1.00)
84	0.91 (0.79-1.00)	0.80 (0.62-0.97)	0.92 (0.81-1.00)
96	1.00 (1.00-1.00)	0.89 (0.69-1.00)	1.00 (1.00-1.00)
108	1.00 (1.00-1.00)	0.90 (0.69-1.00)	1.00 (1.00-1.00)
120	1.00 (1.00-1.00)	0.77 (0.49-1.00)	1.00 (1.00-1.00)
132	1.00 (1.00-1.00)	0.72 (0.28-1.00)	1.00 (1.00-1.00)
144	1.00 (1.00-1.00)	0.74 (0.37-1.00)	0.96 (0.86-1.00)
156	1.00 (1.00-1.00)	0.89 (0.68-1.00)	0.94 (0.80-1.00)
168	1.00 (1.00-1.00)	0.86 (0.60-1.00)	1.00 (1.00-1.00)
180	1.00 (1.00-1.00)	0.50 (0-1.00)	0.50 (0-1.00)

Abbreviations: AUC, area under the curve; GFAP, glial fibrillary acidic protein; MMTBI, mild to moderate traumatic brain injury; UCH-L1, ubiquitin C-terminal hydrolase L1.

[a] Shown is the performance of GFAP and UCH-L1 alone and in combination.

trauma. Patients are seen at different times after their injury, and these data provide valuable information on the pattern of release of GFAP and UCH-L1. These data also demonstrate the diagnostic ability of these 2 biomarkers at 20 distinct time points over the course of 7 days to distinguish between those with and without TBI and those with and without intracranial lesions on CT. They also show the pattern of release in those having and not having a neurosurgical intervention.

In terms of when these biomarkers could potentially be used for clinical decision making, it appears (based on the AUC) that GFAP has a consistent ability to detect MMTBI and detect traumatic intracranial lesions on CT over 7 days after

injury, whereas the ability of UCH-L1 seems to be more limited to the earliest time points after injury. Both biomarkers have an excellent predictive value for predicting neurosurgical intervention early after injury. UCH-L1 performed best within 16 hours of injury. Determining whether a patient with an MMTBI will require a neurosurgical intervention is most important within 24 hours after injury to allow decisions such as transferring a patient to a trauma center or deciding whether someone should be admitted for observation.

We elected to study both mild and moderate injury because TBI on the mild end of the spectrum is heterogeneous. Initial GCS scores in the ED in this population can be inaccurate, and the classification of a TBI as mild or moderate can change based on neuroimaging results and the presence of factors that change mental status, such as intoxication, medications, and other injuries. A patient with a GCS score of 15 who has an acute bleed on CT scan can be classified as having a moderate injury. Conversely, a patient with a GCS score of 11 who has no evidence of intracranial injury on CT scan can be classified as having a mild injury. Although we studied patients with TBI with a GCS score of 9 to 15, 97.8% (318 of 325) of our patients with TBI had a GCS score of 13 to 15.

Both glial biomarker GFAP and neuronal biomarker UCH-L1 were detectible within 1 hour of injury. GFAP reached a peak at 20 hours after injury and steadily decreased over 72 hours but was still detectable at 7 days. In contrast, UCH-L1 rose more rapidly after injury than GFAP, reached a peak at 8 hours, and decreased steadily over 48 hours. Therefore, the window for detecting UCH-L1 in mild TBI is narrow because it rises and falls quickly after injury compared with GFAP. In the context of developing a point-of-care test, the early and rapid rise of UCH-L1 could be used to detect TBI immediately at the scene of injury in settings such as in the ambulance, on the playing field, or at the battlefield. The longer half-life of GFAP makes it a favorable biomarker to use in both the acute and subacute phases of injury because it is able to detect CT lesions for up to 7 days after injury. Although its rise is not as rapid as UCH-L1, it performs well for detecting mild TBI and CT lesions within 1 hour of injury.

It is common for patients who have had a concussion or mild TBI not to seek immediate medical attention. Therefore, understanding the behavior of these biomarkers over days after injury is important for detection of injury in those who may only seek medical care several days after injury. Patients with mild TBI or concussion, who would normally have been discharged from the ED, were captured in our sample because they were admitted for other injuries. For detecting MMTBI (trauma without MMTBI vs trauma with MMTBI), GFAP alone outperformed UCH-L1 alone at all time points, and the combination of the 2 biomarkers did not appreciably improve the performance of GFAP alone. Accordingly, when GFAP and UCH-L1 were combined to predict traumatic intracranial lesions on CT, the combination of GFAP and UCH-L1 marginally outperformed GFAP alone, but the differences were not significant. These findings are consistent with a recent study by Diaz-Arrastia et al[12] in which inclusion of UCH-L1 with GFAP did not improve on GFAP level alone in predicting intracranial lesions on CT in a cohort of patients with mild to severe TBI.

We recognize that there are limitations to this study. Our study addressed the severity of injury in the acute care setting and did not describe long-term outcome in these patients. The main outcomes used in this study reflect current standards of practice and accepted definitions of acute brain injury severity. However, future studies to better define the severity of concussion and mild TBI need to be pursued, particularly when neuroimaging is negative. Accordingly, we performed a subgroup analysis of milder cases of MMTBI without loss of consciousness (58 of 325 patients [or 17.8% of our total population]) and compared them with trauma patients without TBI. Overall levels of GFAP were significantly higher in patients with MMTBI with no loss of consciousness (milder injuries) compared with the trauma controls, which did not hold true for UCH-L1 (eFigure in the Supplement). Although this determination was not the objective of this study, it should be explored further in future studies.

All patients were seen at a single level I trauma center to assess their performance in a multiple-trauma setting. The setting at a single level I site may limit the generalizability to other centers, particularly community hospitals. However, the demographics of our population are comparable to other trauma centers across the country.

The number of samples available for analysis decreased over the course of the study, which reflects the challenge of obtaining samples over time in patients with less severe injuries because they are not hospitalized as long. However, there were many patients without TBI and patients with mild TBI who were captured in our longitudinal sample because they were admitted for other injuries. Important next steps will be to capture samples within minutes of injury.

Conclusions

In a cohort of trauma patients in whom most had mild TBI, GFAP and UCH-L1 exhibited distinct temporal profiles over the course of 7 days. Individually, GFAP outperformed UCH-L1 at all time points in diagnostic accuracy for detecting TBI, CT lesions, and neurosurgical intervention. The combination of GFAP and UCH-L1 outperformed GFAP alone at some time points, but these differences were not statistically significant. GFAP appears to detect MMTBI and traumatic intracranial lesions on CT and predict neurosurgical intervention consistently over 7 days after injury, whereas the ability of UCH-L1 seems to be more limited to the earliest time points after injury.

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REFERENCES

1. Papa L, Ramia MM, Edwards D, Johnson BD, Slobounov SM. Systematic review of clinical studies examining biomarkers of brain injury in athletes after sports-related concussion. *J Neurotrauma*. 2015;32(10):661-673.

2. Papa L. Exploring the role of biomarkers for the diagnosis and management of traumatic brain injury patients. In: Man TK, Flores RJ, eds. *Proteomics: Human Diseases and Protein Functions*. Rijeka, Croatia: InTech; 2012.

3. Papa L, Ramia MM, Kelly JM, Burks SS, Pawlowicz A, Berger RP. Systematic review of clinical research on biomarkers for pediatric traumatic brain injury. *J Neurotrauma*. 2013;30(5):324-338.

4. Eng LF, Vanderhaeghen JJ, Bignami A, Gerstl B. An acidic protein isolated from fibrous astrocytes. *Brain Res*. 1971;28(2):351-354.

5. Jackson P, Thompson RJ. The demonstration of new human brain-specific proteins by high-resolution two-dimensional polyacrylamide gel electrophoresis. *J Neurol Sci*. 1981;49(3):429-438.

6. Papa L, Lewis LM, Falk JL, et al. Elevated levels of serum glial fibrillary acidic protein breakdown products in mild and moderate traumatic brain injury are associated with intracranial lesions and neurosurgical intervention. *Ann Emerg Med*. 2012;59(6):471-483.

7. Papa L, Lewis LM, Silvestri S, et al. Serum levels of ubiquitin C-terminal hydrolase distinguish mild traumatic brain injury from trauma controls and are elevated in mild and moderate traumatic brain injury patients with intracranial lesions and neurosurgical intervention. *J Trauma Acute Care Surg*. 2012;72(5):1335-1344.

8. Papa L, Silvestri S, Brophy GM, et al. GFAP out-performs S100β in detecting traumatic intracranial lesions on computed tomography in trauma patients with mild traumatic brain injury and those with extracranial lesions. *J Neurotrauma*. 2014;31(22):1815-1822.

9. Papa L, Mittal MK, Ramirez J, et al. In children and youth with mild and moderate traumatic brain injury, glial fibrillary acidic protein out-performs S100β in detecting traumatic intracranial lesions on computed tomography. *J Neurotrauma*. 2016;33(1):58-64.

10. Papa L, Zonfrillo MR, Ramirez J, et al. Performance of glial fibrillary acidic protein in detecting traumatic intracranial lesions on computed tomography in children and youth with mild head trauma. *Acad Emerg Med*. 2015;22(11):1274-1282.

11. Metting Z, Wilczak N, Rodiger LA, Schaaf JM, van der Naalt J. GFAP and S100B in the acute phase of mild traumatic brain injury. *Neurology*. 2012;78(18):1428-1433.

12. Diaz-Arrastia R, Wang KK, Papa L, et al; TRACK-TBI Investigators. Acute biomarkers of traumatic brain injury: relationship between plasma levels of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein. *J Neurotrauma*. 2014;31(1):19-25.

13. Stiell IG, Clement CM, Rowe BH, et al. Comparison of the Canadian CT Head Rule and the New Orleans Criteria in patients with minor head injury. *JAMA*. 2005;294(12):1511-1518.