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Diagnostic Performance of GFAP, UCH-L1, and MAP-2 Within 30 and 60 Minutes of Traumatic Brain Injury

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Abstract

IMPORTANCE Data on the performance of traumatic brain injury (TBI) biomarkers within minutes of injury are lacking.

OBJECTIVES To examine the performance of glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), and microtubule-associated protein 2 (MAP-2) within 30 and 60 minutes of TBI in identifying intracranial lesions on computed tomography (CT) scan, need for neurosurgical intervention (NSI), and clinically important early outcomes (CIEO).

DESIGN, SETTING, AND PARTICIPANTS This cohort study is a biomarker analysis of a multicenter prehospital TBI cohort from the Prehospital Tranexamic Acid Use for TBI clinical trial conducted across 20 centers and 39 emergency medical systems in North America from May 2015 to March 2017. Prehospital hemodynamically stable adult patients with traumatic injury and suspected moderate to severe TBI were included. Blood samples were measured for GFAP, UCH-L1, and MAP-2. Data were analyzed from December 1, 2023, to March 15, 2024.

MAIN OUTCOMES AND MEASURES The presence of CT lesions, diffuse injury severity on CT, NSI within 24 hours of injury, and CIEO (composite outcome including early death, neurosurgery, or prolonged mechanical ventilation ≥7 days) within 7 days of injury.

RESULTS Of 966 patients enrolled, 804 patients (mean [SD] age, 41 [19] years; 418 [74.2%] male) had blood samples, including 563 within 60 minutes and 375 within 30 minutes of injury. Among patients with blood drawn within 30 minutes of injury, 212 patients (56.5%) had CT lesions, 61 patients (16.3%) had NSI, and 112 patients (30.0%) had CIEO. Among those with blood drawn within 60 minutes, 316 patients (56.1%) had CT lesions, 95 patients (16.9%) had NSI, and 172 patients (30.6%) had CIEO. All biomarkers showed significant elevations with worsening diffuse injury on CT within 30 and 60 minutes of injury. Among blood samples taken within 30 minutes, GFAP had the highest area under the receiver operating characteristic curve (AUC) to detect CT lesions, at 0.88 (95% CI, 0.85-0.92), followed by MAP-2 (AUC, 0.78; 95% CI, 0.73-0.83) and UCH-L1 (AUC, 0.75; 95% CI, 0.70-0.80). Among blood samples taken within 60 minutes, AUCs for CT lesions were 0.89 (95% CI, 0.86-0.92) for GFAP, 0.76 (95% CI, 0.72-0.80) for MAP-2, and 0.73 (95% CI, 0.69-0.77) for UCH-L1. Among blood samples taken within 30 minutes, AUCs for NSI were 0.78 (95% CI, 0.72-0.84) for GFAP, 0.75 (95% CI, 0.68-0.81) for MAP-2, and 0.69 (95% CI, 0.63-0.75) for UCH-L1; and for CIEO, AUCs were 0.89 (95% CI, 0.85-0.93) for GFAP, 0.83 (95% CI, 0.78-0.87) for MAP-2, and 0.77 (95% CI, 0.72-0.82) for UCH-L1. Combining the biomarkers was no better than GFAP alone for all outcomes. At GFAP of 30 pg/mL within 30 minutes, sensitivity for CT lesions was 98.1% (95% CI, 94.9%-99.4%) and specificity was 34.4% (95% CI, 27.2%-42.2%). GFAP levels greater than 6200 pg/mL were associated with high risk of NSI and CIEO.

(continued)

Key Points

Question How do biomarkers glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1, and microtubule-associated protein 2 measured within 30 and 60 minutes perform in identifying intracranial lesions on computed tomography, need for neurosurgical intervention, and clinically important early outcomes among patients with traumatic brain injury (TBI)?

Findings In this cohort study including 804 patients with TBI, all 3 biomarkers were significantly elevated in blood within 30 and 60 minutes of TBI and were associated with CT lesions, diffuse injury severity on CT scan, 24-hour neurosurgical intervention, and clinically important early outcomes, including early death, neurosurgery, and prolonged mechanical ventilation over 7 days. GFAP had the strongest independent association, with sensitivities of 98%-99% and specificities of 18%-36% for estimating outcomes.

Meaning This cohort study found that GFAP was associated with acute outcomes with high diagnostic accuracy within 30 minutes of TBI and sets the precedent for early use of GFAP in future clinical and research efforts.

+ Invited Commentary

+ Supplemental content

Author affiliations and article information are listed at the end of this article.

Abstract (continued)

CONCLUSIONS AND RELEVANCE In this cohort study of prehospital patients with TBI, GFAP, UCH-L1, and MAP-2 measured within 30 and 60 minutes of injury were significantly associated with traumatic intracranial lesions and diffuse injury severity on CT scan, 24-hour NSI, and 7-day CIEO. GFAP was the strongest independent marker associated with all outcomes. This study sets a precedent for the early utility of GFAP in the first 30 minutes from injury in future clinical and research endeavors.

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Introduction

Despite the volume of traumatic brain injury (TBI) biomarker research over the last 2 decades, researchers have yet to examine the performance of biomarkers at very early time points after injury. Several large-scale studies have measured TBI biomarkers over the first 24 hours of injury,[1-4] but only a handful of studies have examined them within 2 to 4 hours of TBI, particularly for identifying intracranial lesions on computed tomography (CT) scans and need for neurosurgical intervention.[5-14] In a study examining the time course of glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) within 4 hours of injury, both GFAP and UCH-L1 were elevated within an hour of injury, even as early as 15 minutes.[8] Biomarker information obtained this early after injury could inform decision-making in various settings, including prehospital transport decisions to trauma centers, military transport to medical facilities from theater, sports-related brain injuries on the sidelines, and triaging patients for future therapeutic clinical trials for TBI.

Blood-based biomarkers GFAP and UCH-L1 were approved by the US Food and Drug Administration (FDA) for clinical use in adult patients with mild TBI with Glasgow Coma Scale (GCS) score of 13 to 15 to help determine the need for CT scan within 12 hours of injury in 2018 and 2021, respectively.[15,16] GFAP is an astroglial biomarker of injury and is found in the astroglial skeleton of both white and gray brain matter.[17] UCH-L1 is a neuronal brain injury biomarker found in high abundance in neurons.[18] Microtubule-associated protein 2 (MAP-2) is a relatively novel biomarker of human TBI that is localized mainly in neuronal cell bodies and dendrites and is a component of the neuron microtubule system that modulates microtubule organization and stabilizes the cytoskeleton.[19-21] Following severe TBI, MAP-2 measured within 6 hours of injury is associated with TBI severity and early 2-week mortality[22] and has been shown to improve the 6-month neuroprognostic performance of validated clinical models.[23]

The Prehospital Tranexamic Acid (TXA) for TBI trial was a large multicenter trial that obtained blood samples from participants with suspected TBI with GCS score 3 to 12 within 4 hours of injury and provided an opportunity to examine blood-based biomarkers at very early time points after injury.[24] Our current study sought to examine the performance of GFAP, UCH-L1, and MAP-2 within 30 minutes and 60 minutes of injury in (1) identifying patients with traumatic intracranial lesions on initial CT scan and examining the association with CT injury severity, (2) identifying patients requiring neurosurgical intervention within 24 hours of injury, (3) identifying patients having clinically important early outcomes (CIEO) from TBI within 7 days of injury, and (4) examining threshold concentrations of biomarkers to inform TBI risk stratification for potential clinical and research purposes.

Methods

This cohort study was approved by the institutional review board for the Resuscitation Outcomes Consortium Clinical Trials Center at the University of Washington, with contingent approval provided by individual sites. Participants were enrolled under US regulations for Exception From Informed

Consent Requirements for Emergency Research (21CFR50.24) and the Canadian Tri-Council Policy Statement 2 (Ethical Conduct for Research Involving Humans). Consent for continued participation was obtained from the participant or their legally authorized representative at the earliest feasible opportunity. An opportunity for opt out was provided according to the requirements set forth by each site's local regulatory board. This study is reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Design and Setting

This is a biomarker analysis of a multicenter prehospital TBI cohort from the Prehospital TXA Use for Traumatic Brain Injury clinical trial, a multicenter double-blinded, randomized clinical trial performed across 20 centers and 39 emergency medical systems in the United States and Canada (ClinicalTrials.gov identifier: NCT01990768). The trial was designed to examine the efficacy and safety of prehospital administration of TXA compared with placebo in patients with suspected moderate to severe TBI. The full trial protocol has been published elsewhere.[24]

Population

Between May 2015 and March 2017, hemodynamically stable (systolic blood pressure >90 mm Hg) patients aged older than 15 years with suspected TBI with a prehospital GCS score of 3 to 12 (prior to sedatives) and at least 1 reactive pupil were eligible and were randomized to 1 of 3 groups: placebo, 2 g TXA bolus plus a placebo infusion over 8 hours, or 1 g TXA bolus plus 1 g TXA infusion over 8 hours. Although eligible patients were randomized to 1 of 3 groups, our group recently demonstrated that TXA had no effect on the levels of TBI-related biomarkers GFAP, UCH-L1, or MAP-2,[25] so the entire cohort of patients in the trial was included in this analysis.

Race and ethnicity were identified through the electronic medical record. Classification was in accordance with National Institute of Neurological Disorders and Stroke definitions to ensure research is inclusive of racial and ethnic minority groups. Race was categorized as American Indian or Alaskan Native, Asian, Black or African American, Native Hawaiian or Pacific Islander, White, more than 1 race, and unknown; ethnicity was categorized as Hispanic or not Hispanic. Race and ethnicity were included in analysis per National Institutes of Health guidelines.

Blood Sampling

Initial blood samples were obtained from each patient with suspected TBI as soon as possible on arrival at the emergency department within 4 hours of injury. Samples were collected in serum separator tubes and then centrifuged, aliquoted for serum, placed in bar-coded aliquot containers, and frozen at −80 °C. They were batch shipped by the clinical research staff to the Donald D. Trunkey for Civilian and Combat Casualty Care, Oregon Health & Science University, for storage until they were transported to a central laboratory for biomarker analysis.

Outcome Measures

The primary outcome measure was the presence of an acute traumatic intracranial lesion visualized on CT scan and included such findings as extra-axial lesions (epidural or subdural hematomas), subarachnoid hemorrhage, intra-axial lesions (cortical and noncortical parenchymal contusions or hemorrhages), cerebral edema, traumatic axonal injury, midline shift of intracranial contents, or any signs of brain herniation. Only initial head CT scans performed on arrival at the hospital were used for this analysis. Deidentified digital images of head CTs were transferred to the Oregon Health & Science University image repository and reviewed centrally by a neuroradiologist-trained technician and audited by a neuroradiologist; 10% of scans were audited to verify accurate and consistent measurements. Marshall classification was used to group CT lesion into 6 categories based on morphological anomalies (Diffuse Injury I-IV, evacuated mass lesions, and nonevacuated mass lesions).[26]

The secondary outcome measure was the need for neurosurgical intervention within 24 hours of injury, including craniotomy, craniectomy, and placement of intracranial neuromonitoring or drainage devices. Early identification of patients requiring neurosurgical intervention is imperative so patients can be quickly transported or transferred to facilities that provide definitive neurosurgical care. A delay in this care can be detrimental.

The tertiary outcome measure was CIEO within 7 days of injury, a composite outcome of early death within 7 days of injury secondary to TBI, the need for neurosurgical intervention within 7 days of injury, and mechanical ventilation for more than 7 days in patients with TBI.[27] Having 1 or any combination of these 3 outcomes would constitute a clinically important 7-day outcome. This end point was adapted from seminal work done in patients with TBI and GCS 13 to 15 at risk for clinically significant TBI and includes early death, intubation, and neurosurgical intervention.[28-30] Not only are these outcomes applicable to the full spectrum of TBI, they are particularly relevant very early after injury for risk stratification when TBI severity is not always clinically apparent and the GCS score can be inexact. These outcomes were analyzed as a together as a composite and individually. Threshold concentrations of biomarkers were explored to examine levels of biomarkers as very early indicators of injury severity to improve injury classification and inform clinical management and as potential inclusion criteria for future clinical trials very early after injury.

Biomarker Analysis

Serum GFAP, UCH-L1, and MAP-2 levels were measured in duplicate using a validated enzyme-linked immunosorbent assay platform (Banyan Biomarkers). Serum MAP-2 levels were measured using a standard sandwich enzyme-linked immunosorbent assay protocol reported previously.[22] For GFAP, the lower limit of quantification (LLOQ) was 3 pg/mL, the upper limit of quantification (ULOQ) was 320 pg/mL, and lower limit of detection (LoD) was 3 pg/mL. For UCH-L1, the LLOQ was 14 pg/mL, the ULOQ was 2560 pg/mL, and the LoD was 6 pg/mL. For MAP-2, the LLOQ was 26 pg/mL, the ULOQ was 1846 pg/mL, and the LoD was 9 pg/mL. Samples below the reportable range were analyzed as half of the LoD. Any samples yielding a signal over the quantification or calibrator range were diluted and reassayed.

Statistical Analysis

Descriptive statistics included means, medians, and proportions along with 95% CIs and IQRs. Biomarker data were expressed as medians and IQRs. Data were assessed for homoscedasticity and distribution and logarithmic transformations conducted accordingly. Group comparisons were performed using analysis of variance, and the χ^2 test. Receiver operating characteristics curves were created to explore the ability of the biomarkers to identify intracranial lesions on CT scan, 24-hour neurosurgical intervention, and 7-day CIEO. Combinations of the biomarkers were assessed using logistic regression analysis. Estimates of the areas under these curves (AUCs) were obtained. Classification performance was assessed by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% CIs.[31] Optimal thresholds for biomarker levels were selected using Youden Index. Analyses were performed using the statistical software package SPSS version 29.0 (IBM). Sample size was based on available data from the trial and the biomarkers and provided by the Data Coordinating Center. *P* values were 2-sided, and statistical significance was set at $P \leq .05$. Data were analyzed from December 1, 2023, to March 15, 2024.

Results

There were 966 patients enrolled in the original study, and 804 patients (mean [SD] age, 41 [19] years; 418 [74.2%] male) had blood samples drawn and biomarker data available for analysis. Patient characteristics are shown in **Table 1**. By race and ethnicity, 3 patients (0.5%) were American Indian or Alaskan Native, 18 patients (3.2%) were Asian, 68 patients (12.1%) were Black or African American, 2 patients (0.4%) were Native Hawaiian or Pacific Islander, 400 patients (71%) were White, 2

patients (0.4%) were more than 1 race, and 70 patients (12.4%) had unknown race; 78 patients (13.9%) were of Hispanic ethnicity. The median (IQR) injury severity score was 17 (6-26), and 546 injuries (97.0%) were from blunt mechanisms. There were 563 patients who had samples drawn within 60 minutes of injury and a subset of 375 patients who had samples drawn within 30 minutes

Table 1. Characteristics of Patients With Blood Samples Drawn Within 60 Minutes of Injury

Characteristics	Patients, No. (%)[a]				
	Blood samples within 30 min of injury (n = 375)		Blood samples within 60 min of injury (n = 563)		
	Without lesions on CT (n = 163)	With lesions on CT (n = 212)	Without lesions on CT (n = 247)	With lesions on CT (n = 316)	Total (n = 563)
Age, mean (SD), y	40 (17)	42 (19)	40 (18)	42 (19)	41 (19)
Gender					
Female	45 (27.6)	48 (22.6)	65 (26.3)	80 (25.3)	145 (25.8)
Male	118 (72.4)	164 (77.4)	182 (73.7)	236 (74.7)	418 (74.2)
Race					
American Indian or Alaskan Native	1 (0.6)	1 (0.5)	1 (0.4)	2 (0.6)	3 (0.5)
Asian	5 (3.1)	6 (2.8)	7 (2.8)	11 (3.5)	18 (3.2)
Black or African American	26 (16.0)	23 (10.8)	41 (16.6)	27 (8.5)	68 (12.1)
Native Hawaiian or Pacific Islander	0	1 (0.5)	0	2 (0.6)	2 (0.4)
White	111 (68.1)	157 (74.1)	166 (67.2)	234 (74.1)	400 (71.0)
≥1 Race	0 (0)	2 (0.9)	0 (0)	2 (0.6)	2 (0.4)
Unknown	20 (12.3)	22 (10.4)	32 (13.0)	38 (12.0)	70 (12.4)
Hispanic ethnicity	31 (19.0)	23 (10.8)	45 (18.2)	33 (10.4)	78 (13.9)
Prehospital GCS score					
3-8	62 (38.0)	126 (59.4)	84 (34.0)	195 (61.7)	279 (49.6)
9-12	94 (57.7)	83 (39.2)	148 (59.9)	118 (37.3)	266 (47.2)
13-15	7 (4.3)	3 (1.4)	15 (6.1)	3 (0.9)	18 (3.2)
ED GCS score					
3-8	76 (46.6)	146 (68.9)	101 (41)	222 (70)	323 (57)
9-12	45 (27.6)	35 (16.5)	65 (26)	51 (16)	116 (21)
13-15	42 (25.8)	31 (14.6)	81 (33)	43 (14)	124 (22)
Prehospital advanced airway management	53 (32.5)	121 (57.1)	76 (30.8)	185 (58.5)	261 (46.4)
Blunt mechanism of injury	161 (98.8)	201 (94.8)	244 (98.8)	302 (95.6)	546 (97.0)
Mechanism of injury					
Motor vehicle crash	51 (31.5)	70 (33.2)	87 (35.7)	101 (32.1)	188 (33.6)
Pedestrian struck by vehicle	21 (13.0)	31 (14.7)	27 (11.1)	42 (13.3)	69 (12.3)
Bicycle struck by vehicle	7 (4.3)	16 (7.6)	11 (4.5)	19 (6.0)	30 (5.4)
Motorcycle or motorized vehicle	13 (8.0)	26 (12.3)	20 (8.2)	38 (12.1)	58 (10.4)
Suicide	2 (1.2)	9 (4.3)	3 (1.2)	11 (3.5)	14 (2.5)
Assault	12 (7.4)	17 (8.1)	16 (6.6)	25 (7.9)	41 (7.3)
Fall (ground level)	37 (22.8)	18 (8.5)	48 (19.7)	37 (11.7)	85 (15.2)
Fall (>1 m)	15 (9.3)	19 (9.0)	28 (11.5)	34 (10.8)	62 (11.1)
Other	5 (3.1)	6 (2.8)	7 (2.8)	9 (2.8)	16 (2.8)
ISS, median (IQR) (n = 554)	5 (1-13)	22 (14-29)	5 (1-14)	22 (17-30)	17 (6-26)
NSG intervention within 24 h	2 (1.2)	59 (27.8)	3 (1.2)	92 (29.1)	95 (16.9)
Clinically important early outcome					
Any	2 (1.2)	110 (51.9)	3 (1.2)	169 (53.5)	172 (30.6)
TBI mortality within 7 d	0	31 (15.3)	0	47 (15.5)	47 (8.6)
NSG intervention within 7 d	2 (1.2)	64 (30.2)	3 (1.2)	99 (31.3)	102 (18.1)
Using mechanic ventilation >7 d	0	81 (39.5)	0	127 (41.4)	127 (23.5)

Abbreviations: GCS, Glasgow Coma Scale; ISS, Injury Severity Score; NSG, neurosurgical; TBI, traumatic brain injury.

[a] Due to rounding, percentages may not add up to 100.

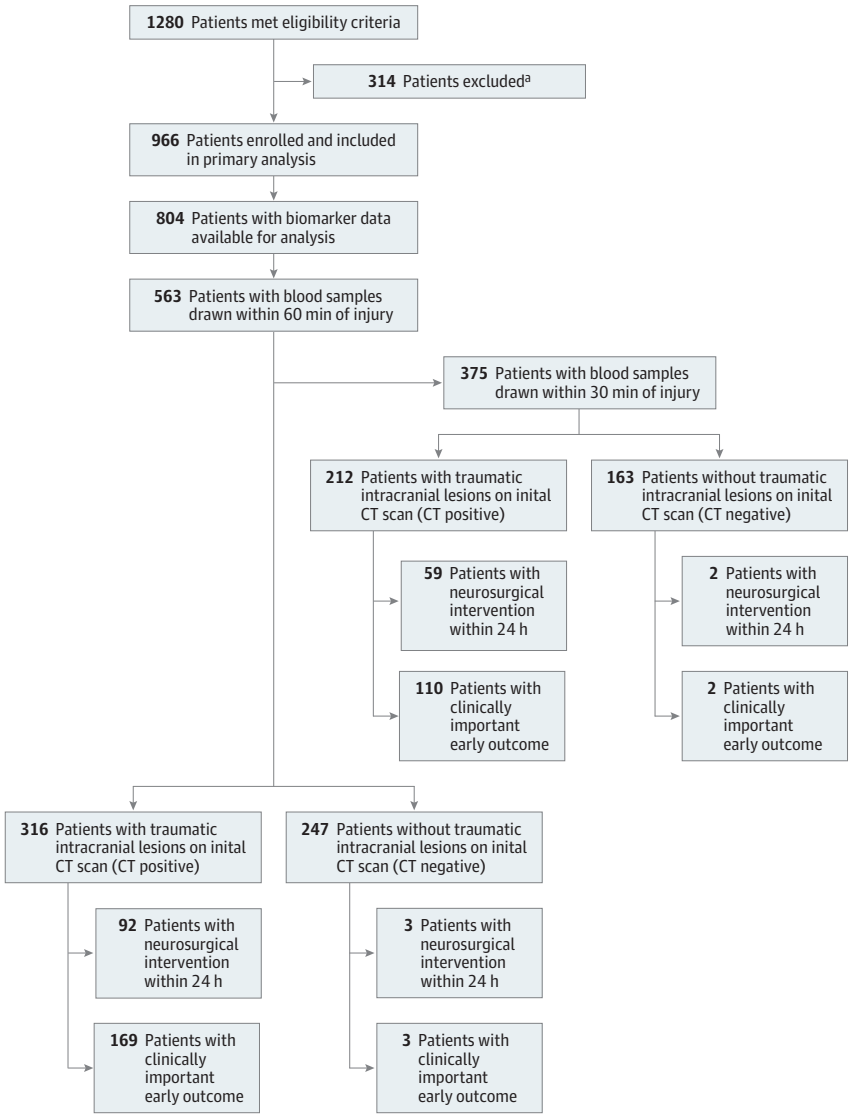
of injury (**Figure 1**). Of patients with samples drawn within 60 minutes, 316 patients (56.1%) had traumatic intracranial lesions on their initial CT scan, 95 patients (16.9%) had neurosurgical intervention within 24 hours of injury, and 172 patients (30.6%) had CIEO within 7 days of injury. Of patients with samples drawn within 30 minutes of injury, 212 patients (56.5%) had CT lesions, 61 patients (16.3%) had neurosurgical intervention within 24 hours, and 112 patients (30.0%) had CIEO (Figure 1). Three patients with no lesions on initial CT scan underwent a neurosurgical intervention within 24 hours (eTable 1 in Supplement 1).

Traumatic Intracranial Lesions on Initial CT Scan

Concentrations of GFAP, UCH-L1, and MAP-2 were plotted against the Marshall CT Classification system and all 3 biomarkers showed significant incremental elevations with worsening diffuse injury on CT within both 30 and 60 minutes of injury (eFigures 1-3 in Supplement 1). There were no evacuated mass lesions represented, as patients were within 30 to 60 minutes of injury.

AUCs were calculated for GFAP, UCH-L1, and MAP-2 for identifying traumatic intracranial lesions on CT scan in samples taken within 30 and 60 minutes of injury (**Table 2**; eFigure 4 in Supplement 1).

Figure 1. Flowchart of Screened and Enrolled Patients



CT indicates computed tomography.

[a] Reasons cited in detail in primary study report by Rowell et al.[24]

GFAP demonstrated the best performance for samples taken within 30 minutes of injury, with an AUC of 0.88 (95% CI, 0.85-0.92), followed by MAP-2 (AUC, 0.78; 95% CI, 0.73-0.83) and UCH-L1 (AUC, 0.75; 95% CI, 0.70-0.80). Various combinations of the 3 biomarkers were no better than GFAP alone (Table 2) (eFigure 4 in Supplement 1). Results were similar for samples taken within 60 minutes of injury (eFigure 4 in Supplement 1), and GFAP remained an independent marker of CT lesions (Table 2).

Neurosurgical Intervention Within 24 Hours of Injury

For samples taken within 30 minutes of injury, GFAP alone had the highest association with having 24-hour neurosurgical intervention, with an AUC of 0.78 (95% CI, 0.72-0.84), followed by MAP-2 (AUC, 0.75; 95% CI, 0.68-0.81) and UCH-L1 (AUC, 0.69; 95% CI, 0.63-0.75) (Table 2) (eFigure 5 in Supplement 1). GFAP also had the strongest independent performance for samples taken within 60 minutes of injury (eFigure 5 in Supplement 1). There was no added benefit from incorporating MAP-2 or UCH-L1 to GFAP alone at either 30 or 60 minutes (Table 2).

CIEO Within 7 Days of Injury

For samples taken within 30 minutes of injury, GFAP alone had the highest association with CIEO, with an AUC of 0.89 (95% CI, 0.85-0.93), followed by MAP-2 (AUC, 0.83; 95% CI, 0.78-0.87) and UCH-L1 (AUC, 0.77; 95% CI, 0.72-0.82) (Table 2; eFigure 6 in Supplement 1). GFAP also had the strongest association compared with the other biomarkers collected within 60 minutes (eFigure 6 in Supplement 1). There was no added benefit to adding MAP-2 or UCH-L1 to GFAP (Table 2). Individually, early mortality from TBI within 7 days, neurosurgery within 7 days, and mechanical ventilation in TBI more than 7 days each had significant associations with the biomarkers (Table 2), especially GFAP. Early mortality had a particularly significant association, with AUCs of 0.95 (95% CI, 0.93-0.98) for GFAP, 0.73 (95% CI, 0.63-0.82) for UCH-L1, and 0.85 (95% CI, 0.78-0.93) for MAP-2 in samples taken within 30 minutes and similarly within 60 minutes (Table 2).

Table 2. AUCs for GFAP, UCH-L1 and MAP-2 Individually and in Combination Measured Within 30 and 60 Minutes of Injury for Each Outcome

Blood draw time, min	AUC (95% CI)						
	GFAP	UCH-L1	MAP-2	GFAP and UCH-L1	GFAP and MAP-2	UCH-L1 and MAP-2	GFAP, UCH-L1, and MAP-2
Intracranial lesions on CT scan (primary outcome)							
≤30	0.88 (0.85-0.92)	0.75 (0.70-0.80)	0.78 (0.73-0.83)	0.88 (0.84-0.91)	0.87 (0.84-0.91)	0.78 (0.7X-0.83)	0.87 (0.83-0.90)
≤60	0.89 (0.86-0.92)	0.73 (0.69-0.77)	0.76 (0.72-0.80)	0.89 (0.86-0.92)	0.88 (0.85-0.91)	0.76 (0.72-0.80)	0.88 (0.85-0.91)
Neurosurgical intervention within 24 h (secondary outcome)							
≤30	0.78 (0.72-0.84)	0.69 (0.63-0.75)	0.75 (0.68-0.81)	0.71 (0.65-0.77)	0.77 (0.71-0.83)	0.74 (0.68-0.81)	0.75 (0.69-0.82)
≤60	0.78 (0.74-0.83)	0.69 (0.64-0.74)	0.74 (0.68-0.79)	0.71 (0.66-0.76)	0.77 (0.72-0.82)	0.72 (0.67-0.77)	0.72 (0.67-0.77)
Clinically important early outcome ≤7 d (tertiary outcome)[a]							
≤30	0.89 (0.85-0.93)	0.77 (0.72-0.82)	0.83 (0.78-0.87)	0.89 (0.86-0.93)	0.89 (0.86-0.93)	0.83 (0.78-0.88)	0.89 (0.86-0.93)
≤60	0.87 (0.84-0.90)	0.75 (0.71-0.79)	0.80 0.76-0.84)	0.87 (0.84-0.90)	0.87 (0.84-0.91)	0.81 (0.76-0.85)	0.87 (0.84-0.90)
Early mortality from TBI ≤7 d (tertiary outcome)							
≤30	0.95 (0.93-0.98)	0.73 (0.63-0.82)	0.85 (0.78-0.93)	0.95 (0.92-0.97)	0.95 (0.93-0.97)	0.83 (0.75-0.91)	0.94 (0.92-0.97)
≤60	0.92 (0.88-0.96)	0.74 (0.66-0.82)	0.83 (0.76-0.89)	0.91 (0.88-0.95)	0.92 (0.89-0.96)	0.81 (0.73-0.88)	0.91 (0.87-0.95)
Neurosurgical intervention ≤7 d (tertiary outcome)							
≤30	0.80 (0.74-0.85)	0.72 (0.66-0.78)	0.76 (0.69-0.82)	0.74 (0.68-0.80)	0.78 (0.72-0.84)	0.76 (0.70-0.82)	0.79 (0.74-0.85)
≤60	0.80 (0.75-0.84)	0.71 (0.66-0.76)	0.75 (0.70-0.80)	0.73 (0.68-0.78)	0.79 (0.74-0.84)	0.74 (0.69-0.79)	0.74 (0.70-0.79)
Mechanical ventilation >7 d with TBI (component of CIEO - tertiary outcome)							
≤30	0.92 (0.89-0.95)	0.79 (0.73-0.84)	0.82 (0.77-0.87)	0.92 (0.90-0.95)	0.92 (0.89-0.95)	0.83 (0.77-0.88)	0.92 (0.89-0.95)
≤60	0.88 (0.85-0.92)	0.76 (0.71-0.81)	0.80 (0.75-0.84)	0.88 (0.84-0.91)	0.89 (0.85-0.92)	0.79 (0.75-0.84)	0.88 (0.84-0.91)

Abbreviations: AUC, area under the receiver operating characteristic curve; CT, computed tomography; GFAP, glial fibrillary acidic protein; MAP-2, microtubule-associated protein 2; TBI, traumatic brain injury; UCH-L1, ubiquitin carboxy-terminal hydrolase L1.

[a] Clinically important early outcome includes mortality within 7 days from a TBI, neurosurgical intervention within 7 days, or mechanical ventilation greater than 7 days after injury.

Classification Performance and Thresholds

Based on FDA-approved GFAP cutoff level for CT lesion detection of 30 pg/mL,[16] the sensitivity and specificity for detecting CT lesions were 98.1%(95% CI, 94.9%-99.4%) and 34.4% (95% CI, 27.2%-42.2%) for samples from within 30 minutes and 98.7% (95% CI, 96.6%-100%) and 36.4% (95% CI, 30.5%-42.8%) for samples from within 60 minutes, respectively (**Table 3**). For 24-hour neurosurgical intervention, the sensitivity and specificity were 98.4% (95% CI, 90.0%-99.9%) and 18.8% (95% CI, 14.7%-23.6%) for samples from within 30 minutes and 97.9% (95% CI, 91.9%-99.6%) and 19.7% (16.2-23.6) for samples from within 60 minutes (Table 3). For CIEO, the sensitivity and specificity were 99.1% (95% CI, 94.4%-100%) and 22.4% (95% CI, 17.6%-28.1%) for samples from within 30 minutes and 98.8% (95% CI, 95.4%-99.8%) and 23.5% (95% CI, 19.5%-28.1%) for samples from within 60 minutes (Table 3). Similar results were found in later samples from 60 minutes (eTable 2 in Supplement 1).

The optimal threshold calculated for GFAP using Youden Index for identifying patients with intracranial lesions on CT (**Figure 2**) was consistent with the FDA-approved level of 30 pg/mL. This

Table 3. Classification Performance of GFAP Concentration Cutoff at 30 pg/mL Measured Within 30 and 60 Minutes of Injury for Identifying Traumatic Intracranial Lesions on CT Scan, 24-Hour NSI, and CIEO Within 7 Days

Measure	Blood sample, % (95% CI)	
	Within 30 min of TBI	Within 60 min of TBI
Lesions on CT scan (primary outcome)		
GFAP ≥30 pg/mL, No.		
With lesions	208	312
Without lesions	107	157
GFAP <30 pg/mL, No.		
With lesions	4	4
Without lesions	56	90
Sensitivity	98.1 (94.9-99.4)	98.7 (96.6-100)
Specificity	34.4 (27.2-42.2)	36.4 (30.5-42.8)
PPV	66.0 (60.4-71.2)	66.5 (62.0-70.7)
NPV	93.3 (83.0-97.8)	95.7 (88.8-98.6)
NSI within 24 h (secondary outcome)		
GFAP ≥30 pg/mL, No.		
NSI	60	93
No NSI	255	376
GFAP <30 pg/mL, No.		
NSI	1	2
No NSI	59	92
Sensitivity	98.4 (90.0-99.9)	97.9 (91.9-99.6)
Specificity	18.8 (14.7-23.6)	19.7 (16.2-23.6)
PPV	19.0 (15.0-23.9)	19.8 (16.4-23.8)
NPV	98.3 (89.9-99.9)	97.9 (91.8-99.6)
CIEO (tertiary outcome)[a]		
GFAP ≥30 pg/mL, No.		
CIEO	111	170
No CIEO	204	299
GFAP <30 pg/mL, No.		
CIEO	1	2
No CIEO	59	92
Sensitivity	99.1 (94.4-100)	98.8 (95.4-99.8)
Specificity	22.4 (17.6-28.1)	23.5 (19.5-28.1)
PPV	35.2 (30.0-40.8)	36.2 (31.9-40.8)
NPV	98.3 (89.9-99.9)	97.9 (91.8-99.6)

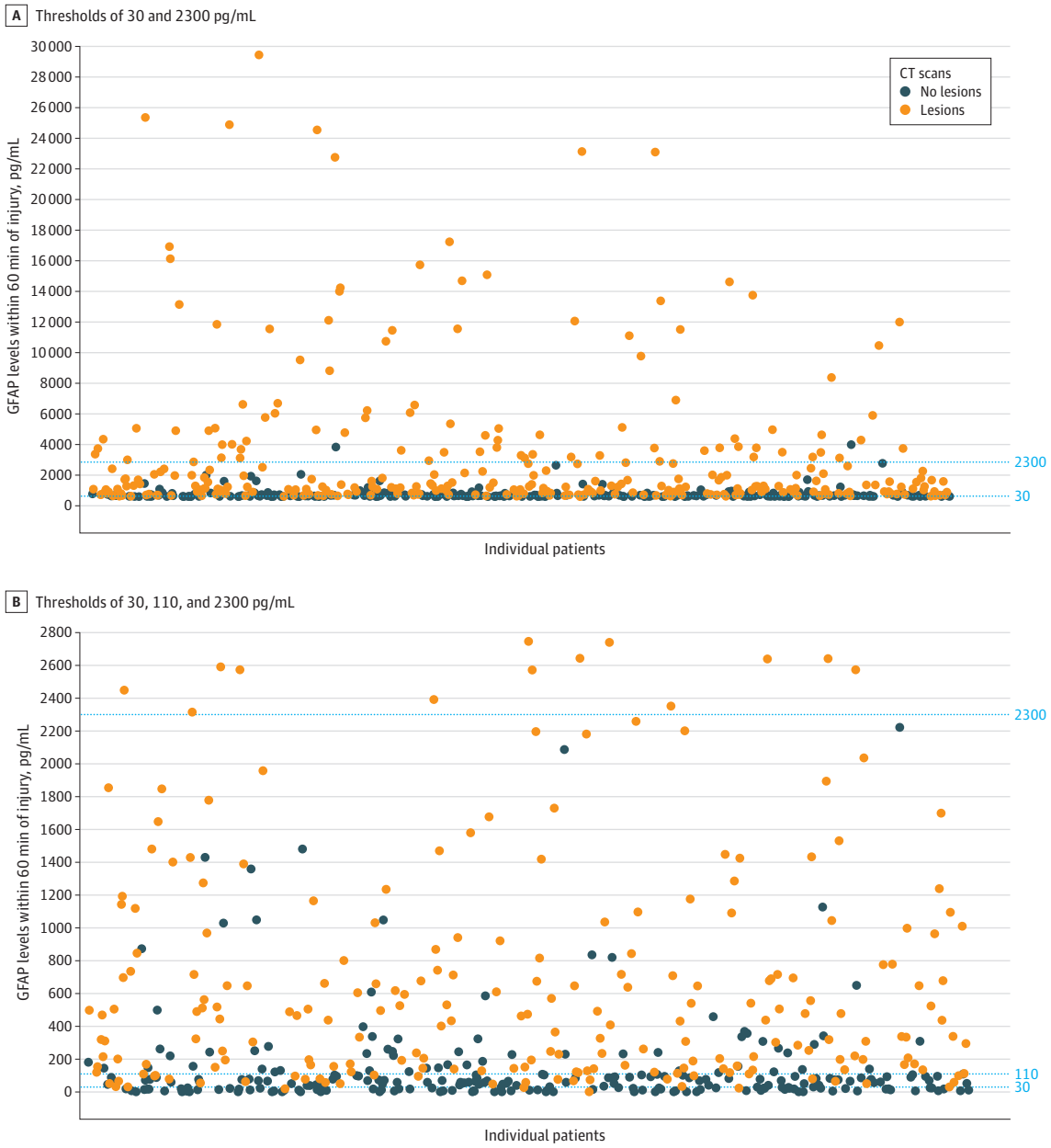
Abbreviations: CIEO, clinically important early outcomes; CT, computed tomography; GFAP, glial fibrillary acidic protein; NPV, negative predictive value; NSI, neurosurgical intervention; PPV, positive predictive value; TBI, traumatic brain injury.

[a] Includes mortality within 7 days from a TBI, neurosurgical intervention within 7 days, or mechanical ventilation greater than 7 days after injury.

level optimized the sensitivity and NPV (as a rule-out test) so patients below the threshold were highly unlikely to have lesions on CT (NPV, 95.7%; 95% CI, 88.8%-98.6%) (eTable 3 in Supplement 1). A threshold of 110 pg/mL optimized both sensitivity and specificity (eTable 3 in Supplement 1). A threshold of 2300 pg/mL optimized the specificity and PPV of GFAP (as a rule-in test) so that patients with GFAP levels above this threshold were highly likely to have lesions on CT scan (PPV, 97.1%; 95% CI, 91.3%-99.2%) (eTable 3 in Supplement 1). These thresholds yielded similar performances for samples drawn within 30 and 60 minutes of injury (eTable 3 in Supplement 1).

For 24-hour neurosurgical intervention, a threshold of 55 pg/mL optimized GFAP sensitivity, 500 pg/mL optimized both sensitivity and specificity, and 6200 pg/mL optimized the specificity for samples from within both 30 and 60 minutes of injury (eTable 3 and eFigure 7 in Supplement 1). For

Figure 2. Glial Fibrillary Acidic Protein (GFAP) Levels Within 60 Minutes of Injury and Presence of Lesions on Computed Tomography (CT) Scans



GFAP levels were calculated using Youden Index for identifying patients with intracranial lesions on CT.

CIEO, cutoffs were similar with thresholds of 55 pg/mL, 485 pg/mL, and 6200 pg/mL (eTable 3 and eFigure 8 in Supplement 1), indicating that patients with concentrations of GFAP greater than 6200 pg/mL were at highest risk of CIEO within 7 days of injury. For early mortality from TBI, the thresholds were 60 pg/mL, 1000 pg/mL, and 15 000 pg/mL, respectively (eTable 3 in Supplement 1), indicating that patients with GFAP levels greater than 15000 pg/mL were at highest risk of early mortality.

Discussion

Given the lack of data on the performance and accuracy of brain injury biomarkers very early after injury, this cohort study addresses a critical gap in the literature by using a large cohort of patients with TBI enrolled in a multicenter prehospital TBI clinical trial. Studies evaluating TBI biomarkers very early after injury are very challenging to conduct and the prehospital TXA trial provided a unique opportunity to obtain samples within minutes of injury. This study confirmed that validated biomarkers GFAP and UCH-L1 and the novel biomarker MAP-2 were significantly elevated within 30 and 60 minutes following TBI and were associated with presence of traumatic intracranial lesions on initial CT scan, need for neurosurgical intervention within 24 hours of injury, and CIEOs within 7 days of injury with very good performance characteristics. Additionally, biomarker concentrations were significantly associated with the severity of diffuse injury on initial CT and provided valuable information on the severity of the TBI much sooner after injury than previously reported. GFAP was a strong independent biomarker associated with injury, followed by MAP-2 and UCH-L1. Notably, MAP-2 showed considerable promise as a novel biomarker.

It is standard practice in patients with suspected TBI with initially low GCS scores (3-12) to perform CT scan of the head as part of their initial clinical management. Although TBI biomarkers may not be necessary to decide whether a CT scan should be performed, there are reasons and opportunities to use TBI biomarkers in this population. Although GCS score is an important triage tool in determining TBI severity, its accuracy is limited in situations where neurologic function may be impaired by alcohol, illicit drugs, medications (eg, sedatives or neuromuscular blockers), emotional trauma, distraction by other injuries, or physiological compromise from hypoxia or hypotension. Although the patients enrolled in this study were all suspected of having a moderate to severe TBI, more than 40% had no evidence of traumatic intracranial injuries on initial CT scan of the head, and only 16% had a neurosurgical intervention within 24 hours. Thus, there is an opportunity for TBI biomarkers to impact risk stratification of patients with suspected TBI very early after injury, even in those with more severe injury. Moreover, there are many settings in which access to CT scans may be limited and clinical decision-making could benefit from a point of care test. An objective measure of injury available so early after injury could also inform early in-hospital care, such intensive care unit admission, need for neuromonitoring, and early treatment.

Given that GFAP had the strongest independent association of the 3 biomarkers evaluated, GFAP thresholds within 30 and 60 minutes of injury were explored to gauge the classification performance for the 3 outcomes. Creating cutoffs served to risk stratify patients by GFAP concentrations. Clinically, GFAP thresholds have the potential to drive prehospital transport decisions to trauma centers with neurosurgical capabilities, military transport to medical facilities located far from theater, sideline decisions at organized sports events, and rapid emergency department triage and treatment prior to neuroimaging.

From a research perspective, there is tremendous potential for GFAP to guide selection and inclusion of patients with TBI into future therapeutic clinical trials. For example, if a trial aimed to include only patients with TBI with intracranial lesions on CT (rule-in), levels of GFAP greater than 2300 pg/mL (with a specificity of 99% and PPV of 97%) could select for patients most likely to have traumatic intracranial lesions. Conversely, if a clinical trial aimed to include only patients with TBI without intracranial lesions (rule-out), a GFAP threshold of less than 30 pg/mL (with a sensitivity of 99% and NPV of 99%) could potentially select patients highly unlikely to have intracranial lesions on CT scan. This premise holds for selection of patients with need for neurosurgical intervention within

24 hours and CIEO using cutoff thresholds of 55 pg/mL and 6200 pg/mL as rule-out and rule-in criteria, respectively. If a treatment were aimed at patients with TBI at risk for neurosurgical intervention, early death, or prolonged mechanical ventilation within 7 days of injury, a minimum GFAP threshold of 6200 pg/mL could potentially identify these patients (rule-in) with a specificity of 99% and a PPV 90% within 30 minutes of injury. Moreover, patients with GFAP levels greater than 15 000 pg/mL were at the highest risk of early mortality.

These results are consistent with other studies that have measured biomarkers in subsets of milder TBI cohorts early after injury.[8,9,11,12,32] Both GFAP and UCH-L1 have been shown to be detectible within an hour of injury in patients with mild TBI.[8] It appears that the presence of these TBI biomarkers in blood within the first 30 minutes of injury is consistent across different severities of TBI, including those with GCS scores of 13 to 15 who are less likely to have intracranial lesions on CT. One caveat is that the prevalence of TBI in a cohort can affect the NPV and PPV of a diagnostic test and must be accounted for when interpreting results. In a cohort of patients with TBI and GCS of 3 to 12 in which the prevalence of CT lesions is 40% to 70%, the sensitivity and NPV of the biomarkers will likely be better than in cohorts with GCS of 13 to 15 (prevalence CT lesions of 10%-20%). Results from this cohort are therefore likely to exhibit better performance characteristics than cohorts with milder injuries.

Limitations

Despite the important findings of this study, we recognize that there are limitations to the analysis. A substantial proportion of the patients in this study was male (74%). Although the incidence of TBI is higher in males than females,[33] the limited representation of females needs to be recognized, as biomarker concentrations may differ in females, warranting further study.[34]

Although neurosurgical intervention is an important outcome for identifying patients needing neurosurgical care, we did not examine the specific clinical circumstances surrounding ICP monitoring, drainage devices, or specific neurosurgical procedures. Withdrawal from life-sustaining measures was not accounted for.

Although this trial had 3 treatment groups, our study group previously demonstrated that the 3 TXA treatment groups had no association with any of the levels of TBI-related biomarkers GFAP, UCH-L1, or MAP-2.[25] The assigned study infusion was initiated by emergency medical services in the field, and the blood draw was obtained immediately on hospital arrival.

Conclusions

In this cohort study of prehospital patients with TBI, concentrations of GFAP, UCH-L1, and MAP-2 measured within 30 and 60 minutes of injury were significantly associated with the presence of traumatic intracranial lesions on CT scan, degree of diffuse injury on CT scan, neurosurgical intervention within 24 hours of injury, and CIEO within 7 days of injury. GFAP had the strongest independent associations of the 3 biomarkers measured, followed by MAP-2 and UCH-L1. GFAP thresholds to optimize sensitivity and specificity provided an important overview of how GFAP could be applied clinically and in future clinical trials. These findings support the clinical value of TBI biomarkers measured in the first 30 minutes from injury and set the precedent for early use of these biomarkers in future clinical and research endeavors.

ARTICLE INFORMATION

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SUPPLEMENT 1.

eTable 1. Description of 3 Patients With an Initially Unremarkable CT Scan Who Required Subsequent Neurosurgical Intervention
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SUPPLEMENT 2.

Data Sharing Statement