Association between plasma GFAP concentrations and MRI abnormalities in patients with CT-negative traumatic brain injury in the TRACK-TBI cohort: a prospective multicentre study

John K Yue, Esther L Yuh*, Frederick K Korley*, Ethan A Winkler, Xiaoying Sun, Ross C Puffer, Hansen Deng, Winward Choy, Ankush Chandra, Sabrina R Taylor, Adam R Ferguson, J Russell Huie, Miri Rabinowitz, Ava M Puccio, Pratik Mukherjee, Mary J Vassar, Kevin K W Wang, Ramon Diaz-Arrastia, David O Okonkwo, Sonia Jain, Geoffrey T Manley, and the TRACK-TBI Investigators†*

Summary

Background After traumatic brain injury (TBI), plasma concentration of glial fibrillary acidic protein (GFAP) correlates with intracranial injury visible on CT scan. Some patients with suspected TBI with normal CT findings show pathology on MRI. We assessed the discriminative ability of GFAP to identify MRI abnormalities in patients with normal CT findings.

Methods TRACK-TBI is a prospective cohort study that enrolled patients with TBI who had a clinically indicated head CT scan within 24 h of injury at 18 level 1 trauma centres in the USA. For this analysis, we included patients with normal CT findings (Glasgow Coma Scale score 13–15) who consented to venepuncture within 24 h post injury and who had an MRI scan 7–18 days post injury. We compared MRI findings in these patients with those of orthopaedic trauma controls and healthy controls recruited from the study sites. Plasma GFAP concentrations (pg/mL) were measured using a prototype assay on a point-of-care platform. We used receiver operating characteristic (ROC) analysis to evaluate the discriminative ability of GFAP for positive MRI scans in patients with negative CT scans over 24 h (time between injury and venepuncture). The primary outcome was the area under the ROC curve (AUC) for GFAP in patients with CT-negative and MRI-positive findings versus patients with CT-negative and MRI-negative findings within 24 h of injury. The Dunn Kruskal–Wallis test was used to compare GFAP concentrations between MRI lesion types with Benjamini–Hochberg correction for multiple comparisons. This study is registered with ClinicalTrials.gov, number NCT02119182.

Findings Between Feb 26, 2014, and June 15, 2018, we recruited 450 patients with normal head CT scans (of whom 330 had negative MRI scans and 120 had positive MRI scans), 122 orthopaedic trauma controls, and 209 healthy controls. AUC for GFAP in patients with CT-negative and MRI-positive findings versus patients with CT-negative and MRI-negative findings was 0·777 (95% CI 0·726–0·829) over 24 h. Median plasma GFAP concentration was highest in patients with CT-negative and MRI-positive findings (414·4 pg/mL, 25–75th percentile 139·3–813·4), followed by patients with CT-negative and MRI-negative findings (74·0 pg/mL, 17·5–214·4), orthopaedic trauma controls (13·1 pg/mL, 6·9–20·0), and healthy controls (8·0 pg/mL, 3·0–14·0; all comparisons between patients with CT-negative MRI-positive findings and other groups p<0·0001).

Interpretation Analysis of blood GFAP concentrations using prototype assays on a point-of-care platform within 24 h of injury might improve detection of TBI and identify patients who might need subsequent MRI and follow-up.

Funding National Institute of Neurological Disorders and Stroke and US Department of Defense.

Lancet Neurol 2019; 18: 953–61

Published **Online** August 23, 2019 http://dx.doi.org/10.1016/S1474-4422(19)30282-0

See **Comment** page 908

*Contributed equally

†TRACK-TBI Investigators are listed at the end of the paper

Department of Neurological Surgery (J K Yue MD, E A Winkler MD, W Choy MD, A Chandra MS, S R Taylor PhD, A R Ferguson PhD, J R Huie PhD, M J Vassar MS, Prof G T Manley MD) **and Department of Radiology** (E L Yuh MD, Prof P Mukherjee MD), **University of California San Francisco, San Francisco, CA, USA; Brain and Spinal Injury Center, Zuckerberg San Francisco General Hospital, San Francisco, CA, USA** (J K Yue, E L Yuh, E A Winkler, W Choy, A Chandra, S R Taylor, A R Ferguson, J R Huie, P Mukherjee, M J Vassar, Prof G T Manley); **Department of Emergency Medicine, University of Michigan, Ann Arbor, MI, USA** (F K Korley MD); **Department of Family Medicine and Public Health, University of California San Diego, San Diego, CA, USA** (X Sun MS, Prof S Jain PhD); **Department of Neurological Surgery, Mayo Clinic, Rochester, MN, USA** (R C Puffer MD); **Department of Neurological Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA** (R C Puffer, H Deng MD, M Rabinowitz PhD, A M Puccio PhD, Prof D O Okonkwo MD); **Department of Psychiatry and Neurosciences, McKnight Brain Institute, University of Florida, Gainesville, FL, USA**

Introduction

Traumatic brain injury (TBI) is a complex disorder that comprises a spectrum of intracranial pathologies, many of which present diagnostic challenges. Historically, diagnosis of TBI is established according to the initial Glasgow Coma Scale (GCS) score (range 3–15: mild, 13–15; moderate, 9–12; severe, 3–8), which is generally followed by a CT scan of the brain.[1] However, heterogeneity in the presentation of TBI is a barrier to precise assessment of injury severity, optimal treatment of the insult, and for predicting clinical outcomes. To overcome these barriers, identification of a robust blood biomarker—one that is reliably elevated during the acute phase of the insult—could improve screening, diagnosis, and follow-up of patients with TBI of different severities and pathologies. Research over the past two decades has identified several promising candidates, including glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase L1 (UCH-L1), S100 calcium-binding protein B (S100B), and neuron-specific enolase (NSE). Studies characterising GFAP and UCH-L1 have

(K K W Wang PhD); **and Department of Neurology, Center for Neurodegeneration and Repair, University of Pennsylvania, Philadelphia, PA, USA** (Prof R Diaz-Arrastia MD)

Correspondence to: Prof Geoffrey T Manley, Department of Neurological Surgery, University of California San Francisco, San Francisco, CA 94110, USA
manleyg@neurosurg.ucsf.edu

Research in context

Evidence before this study

We searched PubMed for studies on plasma glial fibrillary acidic protein (GFAP) and MRI in patients with traumatic brain injury (TBI) published in English up to Nov 1, 2018, with the search terms "glial fibrillary acidic protein" AND "magnetic resonance imaging" OR "MRI", AND "traumatic brain injury" OR "concussion" in the publication title or abstract. To date, a single prospective study has investigated the discriminatory ability of GFAP and three other biomarkers in blood samples taken within 48 h of injury in 274 patients with mild TBI (Glasgow Coma Scale [GCS] 13–15; 28 patients with negative CT and positive MRI; 69 patients with positive CT and positive MRI; 177 patients with negative CT and negative MRI) and 49 healthy controls, using a benchtop research assay. The area under the receiver operating characteristic curve (AUC) for patients with negative CT and positive MRI versus those with negative CT and negative MRI was 0·740. However, 48 h from injury is outside the window for diagnosis of acute injury. Furthermore, a benchtop assay was used for this study, which requires a research setting.

Added value of this study

To our knowledge, our study is the largest study to date (450 patients with negative CT scans, of whom 330 had negative MRI scans and 120 had positive MRI scans) to investigate the discriminatory ability of plasma GFAP obtained within 24 h of injury for MRI-positive lesions versus MRI-negative lesions in patients with CT-negative TBI with GCS score 13–15. Furthermore, GFAP was measured using a prototype assay on the handheld i-STAT point-of-care instrument capable of generating quantitative GFAP concentrations within 15 min. The i-STAT instrument is CE marked and has approval from the US Food and Drug Administration for the quantitative measurement of various analytes in point-of-care or clinical laboratory settings. This is also the first study to include both orthopaedic trauma controls (n=122) and healthy controls (n=209) to assess the differential discriminatory ability of GFAP across time intervals within the first 24 h, to evaluate negative predictive value thresholds, and to assess the ability of GFAP to distinguish types of MRI pathology in patients with normal CT findings.

Implications of all the available evidence

Day-of-injury plasma GFAP concentrations measured using a prototype assay on a point-of-care platform showed good discriminatory ability for CT-negative MRI-positive lesions versus CT-negative MRI-negative lesions, with the highest AUC (0·852) at 9–16 h post injury, and thus could aid in diagnosis of TBI in patients with negative CT scans. Plasma GFAP concentrations also correlate with MRI lesion types. These results indicate that the diagnostic utility of GFAP extends beyond CT visible pathology and might help to identify patients with more subtle injury. In summary, analysis of GFAP concentrations within 24 h of injury might improve detection of TBI and help identify which patients might need subsequent MRI and follow-up.

shown good diagnostic and prognostic value, which prompted US Food and Drug Administration approval[2] as the first biomarkers to aid in the assessment of patients aged 18 years or older with suspected TBI and a GCS score of 13–15 being considered for standard of care imaging (mild TBI).[3–9]

GFAP is a monomeric intermediate filament protein expressed almost uniquely in astrocytes, serving as a specific marker of astrocyte injury. UCH-L1 is a degradation enzyme highly and specifically expressed in neurons and has served as a histological marker. GFAP and UCH-L1 are released into the systemic circulation following TBI and have been found to be elevated in the serum during the acute phase of TBI.[5,7,10] Studies have shown that serum GFAP concentrations in patients with severe TBI are consistently higher than the reference range, correlate with neurological outcomes at 6 months and 1 year,[6,8] and are elevated in patients who died or had worse outcomes compared with those who were alive or had better outcomes.[11] Evidence across multiple studies shows elevated GFAP and UCH-L1 concentrations after TBI, and GFAP comparatively outperforms UCH-L1 in detection of intracranial lesions on CT.[3,5,12] Furthermore, in two large prospective studies, GFAP differentiated between patients with mild TBI and uninjured controls as well as between patients with mild or moderate TBI and non-TBI trauma patients.[12,13]

In patients with TBI, elevated GFAP and UCH-L1 concentrations are known to discriminate between patients with versus those without intracranial lesions on head CT.[12,13] These findings prompted the pivotal ALERT-TBI trial, which led to the FDA clearance of the first TBI blood-based biomarkers in the setting of preventing unnecessary CT radiation.[14] Additionally, Metting and colleagues[7] reported that elevated serum GFAP was associated with neurological abnormalities on brain MRI. A study has shown that nearly 30% of patients with TBI with negative initial head CT show injuries detectable on MRI, predominantly as gliding contusions and axonal shear injury—pathologies without significant haemorrhage but that nevertheless correlate with a range of clinical deficits.[15] These CT-occult injuries can cause chronic sequelae and impairment, and pose unique challenges to TBI diagnosis and severity stratification.[16]

A validated biomarker does not yet exist for CT-occult intracranial lesions visible on MRI. Thus, we assessed the diagnostic value of plasma GFAP using a prototype assay on a point-of-care platform for identification of traumatic intracranial pathology on MRI, despite a normal head CT, in patients with mild TBI (GCS score 13–15). A decision was made to focus on GFAP over UCH-L1 because of better performance in CT-based studies.[3,5,12] A blood-based biomarker capable of identifying patients with these CT-occult injuries might enable improved timing and

accuracy of diagnosis, guide treatment and surveillance strategies, and improve clinical trials by permitting more precise stratification of injury types and enrichment of study populations.

Methods

Study population

Patients with TBI were identified and enrolled in the prospective Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) study in accordance with previously published methods.[17,18] Patients presenting at 18 participating level 1 US trauma centres were enrolled from Feb 26, 2014, to June 15, 2018. Written consent was obtained from all patients or their legal representatives before enrolment. In cases when a waiver of consent was applied for emergency data capture or venepuncture, consent in person was pursued at the earliest subsequent time. Eligibility included presentation within 24 h of injury with head trauma warranting clinical evaluation with a non-contrast head CT in the emergency department on the basis of practice guidelines.[19] Demographic history, comorbidities, and clinical course of the injury, including GCS score, trauma mechanism, and loss of consciousness, were obtained at initial assessment by a treating physician, then confirmed by study staff when blood samples were collected. For the current analysis, we included patients with mild TBI (GCS 13–15) who consented to venepuncture within 24 h of injury and who had an MRI 7–18 days post injury.

Patients with isolated orthopaedic trauma were identified and enrolled using the same process as that for patients with TBI. Patients were eligible for inclusion as orthopaedic trauma controls if they presented with isolated trauma to their limbs, pelvis, or ribs, and had an Abbreviated Injury Scale score of less than 4 for those body regions. Patients were excluded from being orthopaedic trauma controls if they had loss of consciousness, disturbance of consciousness, post-traumatic amnesia or retrograde amnesia, or other clinical findings suggestive of a head injury. Potential orthopaedic trauma controls who had a head CT scan were also excluded.

Healthy non-injured controls were recruited either via a relationship with a TRACK-TBI participant or through public advertisement within TRACK-TBI institutions, and were able to provide informed consent. Healthy controls were excluded if they had a history of TBI, concussion, or any traumatic injury causing polytrauma in the 12 months before enrolment in the study.

This study received approval from the institutional review board of record at the University of California, San Francisco (Committee on Human Research). Reporting adhered to the STROBE statement.

Sample collection and biomarker analysis

Blood samples were collected within 24 h of injury. All samples were dated and time stamped to compare with time of injury. The National Institute of Neurological Disorders and Stroke (NINDS) Common Data Elements (CDE) Biospecimens and Biomarkers Working Group consensus recommendations for plasma preparation were followed.[20] Plasma volumes of 500 µL were prepared for each patient and frozen at –80°C for future batch processing. Samples were batch-shipped in temperature-controlled overnight express freight containers to the TRACK-TBI Biospecimens Repository at the University of Pittsburgh Medical Center (Pittsburgh, PA, USA). All samples were deidentified using a unique study ID specific to site and patient. Study personnel from each site entered data into a central database maintained by the TRACK-TBI Clinical Core. These samples were part of the TRACK-TBI phase 1 biomarker cohort, which included the first 1375 enrolled patients with baseline blood-based biomarker data (figure 1).

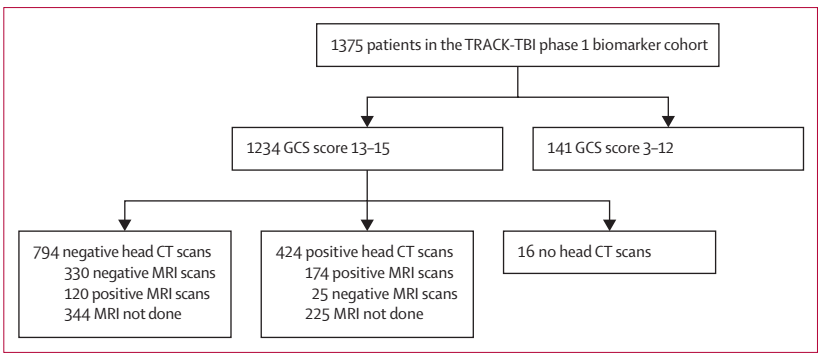

Figure 1: **Study profile**
TRACK-TBI=Transforming Research and Clinical Knowledge in Traumatic Brain Injury. GCS=Glasgow Coma Scale.

Sample analysis for GFAP was done by a single laboratory (Abbott, Abbott Park, IL, USA) in blinded fashion using a prototype immunoassay on the i-STAT (Abbott, Abbott Park, IL, USA) point-of-care platform. This assay generates quantitative GFAP concentrations within 15 min. The GFAP assay used a monoclonal antibody for capture and a monoclonal antibody–alkaline phosphatase conjugate for detection of GFAP and GFAP breakdown products. The GFAP calibrators ranged from 0 pg/mL to 50 000 pg/mL. The limit of detection and limit of quantitation, determined using Clinical and Laboratory Standards Institute (CLSI) protocol EP17-A2, are less than 15 pg/mL and less than 25 pg/mL, respectively.[21] Within-laboratory precision of 2·8% to 14·2% coefficient of variation was demonstrated over a concentration range of 15 000 pg/mL to 40 pg/mL. Evaluation of assay linearity demonstrates less than 10% deviation from linearity from 50 000 pg/mL to less than 25 pg/mL, as defined by CLSI protocol EP6-A.34.[21] Before initiation of the current study, the performance of the prototype GFAP immunoassay on the i-STAT point-of-care platform was compared with the Banyan GFAP benchtop analytic platform (Banyan, Alachua, FL, USA) in a subsample (n=22) of patients and was found to have a statistically significant correlation (Spearman's r=0·963). Sample analysis methods

	Overall (n=450)*	Negative CT and negative MRI (n=330)	Negative CT and positive MRI (n=120)	p value†
Age (years)	36·3 (15·0)	35·5 (15·1)	38·4 (14·8)	
Sex				0·740
Male	285 (63%)	207 (63%)	78 (65%)	
Female	165 (37%)	123 (37%)	42 (35%)	
Race				0·048
White	328 (74%)	231 (71%)	97 (81%)	
African-American or African	85 (19%)	71 (22%)	14 (12%)	
Other	33 (7%)	24 (7%)	9 (8%)	
Education level (years)	13·7 (2·8)	13·5 (2·7)	14·4 (2·9)	
Psychiatric history				0·192
No	352 (79%)	252 (77%)	100 (83%)	
Yes	95 (21%)	75 (23%)	20 (17%)	
Previous TBI				0·094
No	294 (65%)	208 (63%)	86 (72%)	
Yes	156 (35%)	122 (37%)	34 (28%)	
Mechanism of injury				0·193
Road traffic accident	304 (68%)	224 (68%)	80 (67%)	
Incidental fall	89 (20%)	59 (18%)	30 (25%)	
Violence or assault	19 (4%)	15 (5%)	4 (3%)	
Other	38 (8%)	32 (10%)	6 (5%)	
Hypotension on admission				0·666
No	375 (84%)	277 (84%)	98 (82%)	
Yes	7 (2%)	6 (2%)	1 (1%)	
Unknown	67 (15%)	47 (14%)	20 (17%)	
Hypoxia on admission				0·760
No	377 (84%)	279 (85%)	98 (82%)	
Yes	3 (1%)	2 (1%)	1 (1%)	
Unknown	69 (15%)	49 (15%)	20 (17%)	
Presenting emergency department GCS score				0·004
13	9 (2%)	6 (2%)	3 (3%)	
14	65 (5%)	37 (11%)	28 (24%)	
15	368 (83%)	282 (87%)	86 (75%)	
Loss of consciousness				0·020
No	56 (12%)	50 (15%)	6 (5%)	
Yes	353 (79%)	249 (76%)	104 (87%)	
Suspected	22 (5%)	16 (5%)	6 (5%)	
Unknown	18 (4%)	14 (4%)	4 (3%)	

(Table 1 continues on next page)

See Online for appendix

For the **TRACK-TBI MRI protocol** see https://tracktbi.ucsf.edu/researchers

for UCH-L1, S100B, and NSE are included in the appendix (p 1).

CT imaging evaluation and analysis

Initial head CT scans were deidentified and uploaded to a central imaging database at the Laboratory of NeuroImaging (University of Southern California, Los Angeles, CA, USA), and independently assessed by a board-certified neuroradiologist in accordance with the NINDS CDE Neuroimaging Working Group consensus recommendations.[22] The central board-certified neuroradiologist was masked to the identity and clinical information associated with each CT scan. The result of each review was uploaded to the TRACK-TBI clinical database under the respective patient's record. CT scans were read as positive if there was any evidence of acute intracranial pathology consistent with TBI (eg, contusion, subarachnoid haemorrhage, subdural haematoma).

MRI methods and analysis

MRI was obtained at 7–18 days. Image sequences included T1, T2, FLAIR, and T2*. The MRI protocol was standardised across all sites and General Electric, Siemens, and Phillips MRI platforms. Baseline phantom scans were done at all centres to quantify differences between magnets and correct geometric variances across scanners. Structural MRI abnormalities were quantified according to CDE standards and definitions[22] by three board-certified neuroradiologists masked to the identity and clinical history of the patient. MRI scans were read as positive if there was any evidence of acute intracranial pathology consistent with TBI (eg, contusion, traumatic axonal injury, diffuse axonal injury). Analysis of inter-rater reliability showed a κ statistic of 0·91 for agreement for positive versus negative traumatic intracranial findings on MRI scans.

Statistical analysis

Demographics and clinical characteristics of the study cohort were summarised using descriptive statistics. Between-group comparisons were calculated using the Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables. Plasma GFAP concentrations were summarised using medians with 25–75th percentiles, and compared between groups using the Wilcoxon rank sum test. The area under the receiver operating characteristic (ROC) curve (AUC) was used to determine the ability of GFAP, UCH-L1, S100B, and NSE to identify patients with positive versus negative MRI findings in patients with negative CT findings. The primary outcome for this analysis was the AUC for GFAP within 24 h of injury. AUCs of less than 0·7 were considered poor, 0·7–0·8 fair, 0·8–0·9 good, and 0·9–1·0 excellent. AUCs were also calculated by time between injury and venepuncture (0–8 h, 9–16 h, 17–24 h) to evaluate the optimal GFAP sampling time. Sensitivity and specificity were calculated for the GFAP cutoffs selected on the basis of the criteria of adjusted negative predictive value (NPV) reaching levels of 0·96, 0·94, 0·92, 0·90, 0·85, and 0·80 using the κ-fold cross-validation method. The prevalence rate for the adjusted NPV was estimated from the sample at 0·27, and 1000 bootstrap samples were run to produce the median as the optimal cutoffs. Subgroup analyses for plasma GFAP concentrations of different lesion types present on MRI in patients with CT-negative scans were done, including isolated traumatic axonal injury (defined as 1–3 foci of axonal shear), diffuse axonal injury (defined as >3 foci of axonal shear), isolated contusion, and mixed lesions (>1 lesion type).[22] The Dunn Kruskal–Wallis test was used

for comparison among different MRI lesion types with a Benjamini–Hochberg correction for multiple comparisons.[23] Statistical significance was set at p<0·05. All analyses were done by the TRACK-TBI Statistical Core at the University of California, San Diego (San Diego, CA, USA) using R version 3.5.1. This study is registered with ClinicalTrials.gov, number NCT02119182.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 1375 patients in the TRACK-TBI phase 1 biomarker cohort, 794 had negative CT findings and a GCS score of 13–15. 450 of these patients had an MRI scan within 7–18 days, of whom 330 (73%) had negative MRI findings and 120 (27%) had positive MRI findings (figure 1). Demographic and clinical characteristics are shown in table 1. Non-TBI controls with biomarker data in the phase 1 cohort included 122 orthopaedic trauma controls and 209 healthy controls.

The mean time between injury and venepuncture was 12·1 h (SD 6·9). Plasma GFAP conscentrations in patients with negative CT findings ranged from 0 to 4095·1 pg/mL. Median GFAP concentration was higher in patients with negative CT and positive MRI findings than in those with negative CT and negative MRI findings (414·4 pg/mL [25–75th percentile 139·3–813·4] versus 74·0 pg/mL [17·5–214·4], respectively; p<0·0001). By comparison, median GFAP concentrations were 786·0 pg/mL (25–75th percentile 357·0–1863·3) in patients with positive CT scans, 13·1 pg/mL (6·9–20·0) in orthopaedic trauma controls, and 8·0 pg/mL (3·0–14·0) in healthy controls (table 2). The AUC for GFAP to discriminate between patients with CT-negative and MRI-positive findings versus patients with CT-negative and MRI-negative findings was 0·777 (95% CI 0·726–0–829) within 24 h of injury (figure 2). By time of venepuncture, median GFAP concentrations were 79·4 pg/mL (25–75th percentile 21·2–306·6) for 0–8 h post injury (n=166), 139·3 pg/mL (29·4–350·1) for 9–16 h post injury (n=123), and 133·3 pg/mL (31·7–417·3) for 17–24 h post injury (n=151). AUCs were 0·719 (95% CI 0·623–0·815) for 0–8 h post injury, 0·852 (0·781–0·923) for 9–16 h post injury, and 0·788 (0·694–0·883) for 17–24 h post injury (figure 2). By comparison, the overall AUC for UCH-L1 to discriminate between patients with CT-negative and MRI-positive findings versus patients with CT-negative and MRI-negative findings was 0·590 (95% CI 0·530–0·650; appendix p 2), and by time of venepuncture was 0·616 for 0–8 h post injury, 0·624 for 9–16 h post injury, and 0·570 for 17–24 h post injury (appendix p 3). Overall AUCs were lower for S100B (0·562, 95% CI 0·498–0·625) and NSE (0·505, 0·442–0·569; appendix p 4).

	Overall (n=450)*	Negative CT and negative MRI (n=330)	Negative CT and positive MRI (n=120)	p value†
(Continued from previous page)				
Post-traumatic amnesia				<0·0001
No	96 (21%)	85 (26%)	11 (9%)	
Yes	288 (64%)	206 (63%)	82 (68%)	
Suspected	7 (2%)	6 (2%)	1 (1%)	
Unknown	58 (13%)	32 (10%)	26 (22%)	
Emergency department disposition				0·031
Discharge	237 (53%)	186 (56%)	51 (43%)	
Admission to hospital ward	173 (38%)	116 (35%)	57 (48%)	
Admission to intensive care unit	40 (9%)	28 (8%)	12 (10%)	

Data are mean (SD) or n (%). TBI=traumatic brain injury. GCS=Glasgow Coma Scale. *Characteristics of the overall sample (n=450). †P values were calculated comparing patients with negative CT and negative MRI findings (n=330) versus patients with negative CT and positive MRI findings (n=120) using Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables.

Table 1: **Demographic and clinical characteristics of the study participants from the TRACK-TBI phase 1 biomarker cohort**

	Number of patients	Plasma GFAP concentration (pg/mL)			p value
		Mean (SD)	Median (25–75th percentile)	Range	
Positive CT	199	1400·9 (1598·6)	786·0 (357·0–1863·3)	0–9409·7	<0·0001*
Negative CT	450	308·0 (530·5)	110·3 (22·7–352·3)	0–4095·1	..
Negative CT and positive MRI	120	692·2 (827·6)	414·4 (139·3–813·4)	5·2–4095·1	<0·0001†
Negative CT and negative MRI	330	168·3 (250·9)	74·0 (17·5–214·4)	0–1864·5	..
Orthopaedic trauma controls	122	23·7 (37·2)	13·1 (6·9–20·0)	0–216·8	<0·0001‡§
Healthy controls	209	11·0 (12·7)	8·0 (3·0–14·0)	0–98·0	<0·0001‡§

GFAP=glial fibrillary acidic protein. P values were calculated from the Wilcoxon rank sum test for the comparisons, which compares the distributions of the two groups. *Compared with patients with negative CT. †Compared with patients with negative CT and negative MRI findings. ‡Compared with patients with negative CT and positive MRI findings. §Compared with patients with negative CT and negative MRI findings.

Table 2: **Plasma GFAP concentrations by imaging modality and findings**

The association between GFAP and traumatic intracranial abnormality on MRI was dose dependent. Of 90 patients with GFAP concentrations in the lowest quintile, seven (8%) had abnormal MRI findings whereas 58 (64%) of 90 with GFAP concentrations in the highest quintile had abnormal MRI findings (appendix p 5). In the combined cohort of healthy controls and orthopaedic trauma controls, the 99th percentile of GFAP concentrations was 157·2 pg/mL. Of the 450 patients with negative CT scans, 192 (43%) had GFAP concentrations greater than the 99th percentile of healthy controls and orthopaedic trauma controls. Of the 192 patients with negative CT scans with GFAP greater than the 99th percentile of healthy controls and orthopaedic trauma controls, 89 (46%) had an abnormal MRI. GFAP cutoff thresholds were selected on the basis of the criteria of adjusted NPV reaching levels of 0·96, 0·94, 0·92, 0·90, 0·85, and 0·80,

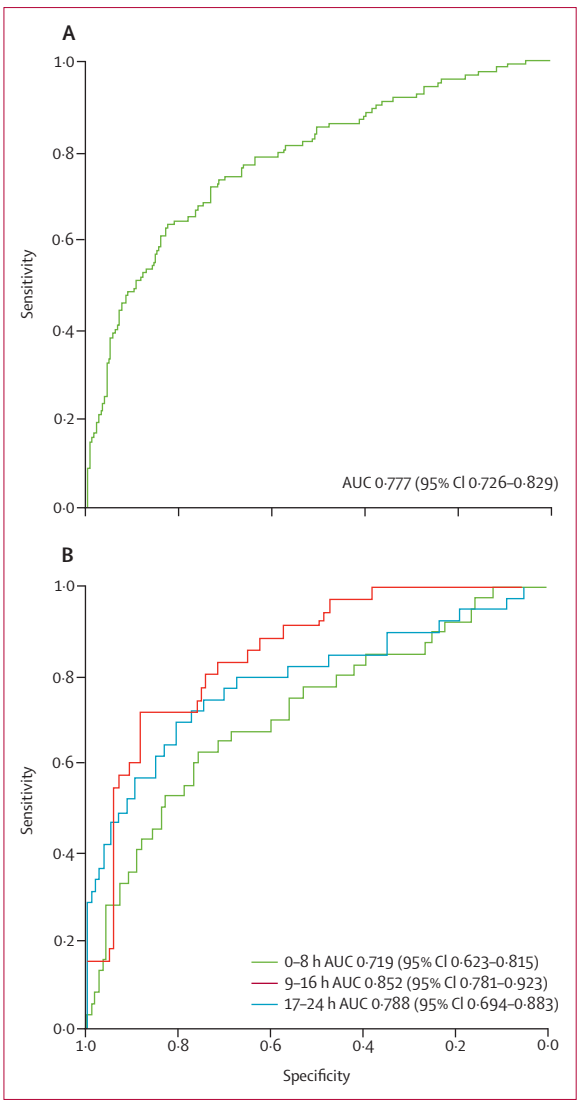

***Figure 2*: Receiver operating characteristic curves for GFAP in patients with CT-negative and MRI-positive findings versus patients with CT-negative and MRI-negative findings within 24 h of injury (A) and by time of venepuncture post injury (B)**

(A) Plasma GFAP concentration using a point-of-care platform shows fair discrimination (AUC 0·777) between patients with MRI-positive findings and patients with MRI-negative findings in patients with CT-negative traumatic brain injury. (B) GFAP at 9–16 h post injury shows improved discrimination to good (AUC 0·852), suggesting temporal effects of GFAP release or accumulation in the systemic circulation after traumatic brain injury and time for optimal or repeat measurement. GFAP=glial fibrillary acidic protein. AUC=area under the curve.

and their respective sensitivity, specificity, and positive predictive value (PPV) are shown in table 3.

Regarding MRI lesion types in the 120 patients with negative CT and positive MRI findings, 65 (54%) were isolated traumatic axonal injury, 21 (18%) were isolated diffuse axonal injury, and two (2%) were isolated contusions, while 14 (12%) were pure extra-axial lesions and 18 (15%) were mixed lesions. Comparisons across different lesion types showed that patients with diffuse axonal injury (>3 foci of axonal shear injury) had significantly higher plasma GFAP concentrations (median 1120·2 pg/mL, 25–75th percentile 638·6–1915·0) than did patients with traumatic axonal injury (1–3 foci of axonal shear; 315·2 pg/mL, 74·3–545·2; p=0·0002; figure 3). Patients with diffuse axonal injury also had higher GFAP concentrations—although differences were not significant—than did patients with extra-axial lesions (406·1 pg/mL, 123·0–910·1; p=0·082; figure 3), isolated contusions (150·15 pg/mL, 82·5–217·8, p=0·062), and mixed lesions (621·7 pg/mL, 443·9–1147·1; p=0·448). The AUCs for discriminating patients with negative CT findings with diffuse axonal injury from patients with CT-negative and MRI-negative findings, and from orthopaedic trauma controls, were considered excellent (ie, 0·9–1·0), at 0·903 (95% CI 0·935–1·000) and 0·976 (0·828–0·977), respectively.

Discussion

In this study, plasma GFAP biomarker concentrations in the acute phase after head trauma identified patients with a suspected TBI and normal head CT who had detectable pathology on MRI, confirming the diagnosis of TBI. Consistent with our previous results, 27% of patients with a normal CT had positive findings on an MRI, demonstrating the diagnostic utility of GFAP. GFAP outperformed UCH-L1, S100B, and NSE for detection of intracranial MRI abnormalities. Plasma GFAP concentrations also correlated with MRI lesion types and distinguished patients with diffuse axonal injury compared with patients with other lesion types.

Biomarkers are emerging diagnostic tools in TBI. Brain-derived circulating proteins in plasma are promising adjuncts that might confirm indication for neuroimaging and complement head CT scans in the assessment of TBI. GFAP is the first non-imaging-based FDA-approved biomarker with sufficient sensitivity to detect intracranial trauma on a CT scan.[4,7,14] Here, we extend these findings and show that GFAP can be used as a biomarker for traumatic intracranial lesions found on MRI but undetectable on CT. GFAP distinguishes patients with CT-occult, MRI-positive head trauma with an AUC of approximately 0·7–0·8. Our findings are consistent with a previous smaller study by Gill and colleagues[24] that reported a similar discriminative value in distinguishing between CT-negative findings with traumatic abnormalities on MRI versus CT-negative findings without MRI abnormalities using a benchtop single-molecule array platform. The absolute median plasma GFAP concentrations in the MRI-positive and MRI-negative groups in our study, however, were approximately two to three times lower than those in the study by Gill and colleagues. As supported by our data, different traumatic pathological findings, such as diffuse axonal injury, traumatic axonal injury, contusions, or extra-axial collections, show relative differences in absolute concentrations of GFAP in circulating plasma. Whether this discrepancy reflects

differences in traumatic intracranial pathology, timing of collection, methods (or a combination thereof) between the two studies, or differences in the assays used, remains to be seen. Further standardisation for TBI biomarker studies across different platforms is required. We chose to use a prototype assay in development that provided GFAP concentrations over a dynamic range of 0–50 000 pg/mL. Unlike conventional ELISA analysis, plasma GFAP concentrations can be quantified in as little as 15 min, providing real-time information to inform clinical decision making and need for ancillary diagnostic testing. Handheld devices also do not require patients to be transported away from clinical surveillance or to undergo diagnostic testing, which might be contraindicated in unstable traumatic injuries. Such devices might be particularly useful for the military and civilian trauma populations. Up to 27% of patients with mild TBI with negative CT imaging have pathological MRI findings related to their trauma, which are associated with long-term cognitive and neuropsychiatric sequelae.[7,15] MRI is not readily available at all centres and is more expensive than CT scanning. Point-of-care GFAP testing might, therefore, aid in diagnosis of TBI in the CT-negative population as well as screen for patients who might need MRI, additional assessment, or follow-up.

Our findings are not limited to patients with intracranial trauma on MRI. GFAP concentrations were also elevated in patients with TBI with negative CT and negative MRI when compared with both healthy non-injured controls and non-head-injured orthopaedic trauma controls, consistent with previous reports.[24] This finding suggests that head trauma does result in elevations in GFAP circulating in plasma without gross changes on structural MRI. One potential explanation is that microscopic cell injury, such as axonal retraction or cell death, might occur but remain undetectable on standard MRI. This has been consistently demonstrated in rodent TBI models.[25–28] For the present study, a 3T MRI was used for all imaging experiments. A previous study has shown increased sensitivity with 3T magnets for traumatic pathologies, especially for axonal injury.[29] Hence, GFAP has the potential to serve as a marker for future trials in diffuse axonal injury. Furthermore, our study shows that patients with GFAP concentrations more than 20 pg/mL (upper limit for orthopaedic trauma controls) but less than 140 pg/mL (lower limit for patients with negative CT and positive MRI) might have occult injury not visible on structural MRI but that might show up on resting state functional MRI or advanced microstructural imaging such as diffusion tensor imaging or tractography.

We also found that 64% of patients with GFAP in the highest quintile had positive MRI findings. This result suggests that GFAP elevations correlate with extent of brain injury even in patients with milder TBIs, and previous studies on the relatively modest prognostic value of GFAP in mild TBI outcomes might reflect that mild TBI outcome is more contingent on injury type, location, comorbidity, and premorbid risk rather than strictly injury severity. It also remains to be seen whether multiplexing plasma GFAP concentrations with other established blood-based TBI markers—such as UCHL-1, BDNF, tau, or phosphorylated tau—improves discriminatory ability with comparatively milder injuries and represents an important future direction.[5,7,14,30,31]

	Sensitivity	Specificity	NPV	PPV
4·40 pg/mL	1·000 (1·000–1·000)	0·024 (0·009–0·042)	1·000 (1·000–1·000)	0·271 (0·268–0·275)
12·95 pg/mL	0·958 (0·925–0·992)	0·188 (0·148–0·230)	0·925 (0·863–0·981)	0·300 (0·287–0·313)
25·15 pg/mL	0·908 (0·850–0·958)	0·333 (0·288–0·388)	0·910 (0·861–0·957)	0·332 (0·312–0·354)
71·95 pg/mL	0·825 (0·750–0·892)	0·494 (0·442–0·549)	0·888 (0·845–0·924)	0·373 (0·344–0·407)
282·70 pg/mL	0·642 (0·558–0·733)	0·803 (0·758–0·842)	0·861 (0·832–0·890)	0·543 (0·482–0·603)
848·75 pg/mL	0·233 (0·158–0·308)	0·964 (0·942–0·982)	0·775 (0·760–0·793)	0·698 (0·555–0·842)

The κ-fold cross-validation method was used to select the optimal cutoffs for predicting MRI-positive versus MRI-negative findings in patients with negative CT based on the criteria of adjusted NPV above the level of 0·96, 0·94, 0·92, and 0·90, 0·85, and 0·80, in accordance to data standards for clinical laboratory assays set by the manufacturer. The prevalence of positive MRI scans among patients with negative CT scans was estimated to be 0·27 on the basis of the sample rate to calculate the adjusted NPV. 1000 bootstraps were conducted to determine the optimal cutoffs using the median from each run. The optimal cutoff thresholds were then applied to the full data to calculate the corresponding sensitivity, specificity, NPV, and PPV. GFAP=glial fibrillary acidic protein. NPV=negative predictive value. PPV=positive predictive value.

***Table* 3: Cutoff concentrations of plasma GFAP to predict MRI-positive versus MRI-negative findings in patients with negative CT**

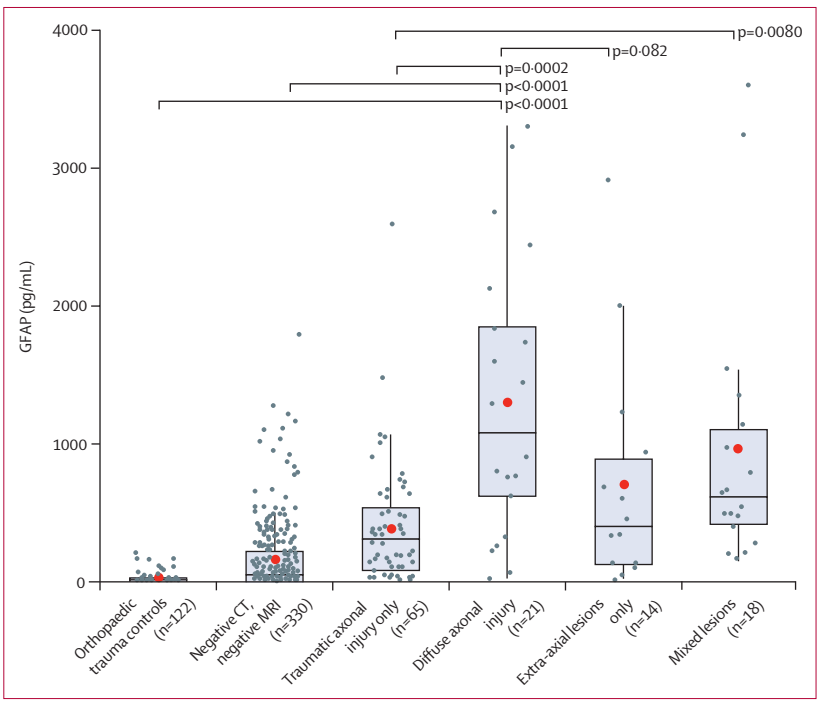

***Figure* 3: GFAP concentration by MRI pathology**

The red dot signifies mean plasma GFAP concentration while boxplots provide range, median, and 25–75th percentiles. Individual dot values are plotted for reference. The Dunn Kruskal–Wallis test for comparisons among different MRI lesion types with a Benjamini–Hochberg correction for multiple comparisons[23] showed that GFAP concentrations are significantly higher in patients with isolated diffuse axonal injury than in those with isolated traumatic axonal injury. Separate Wilcoxon rank sum tests also showed significantly higher GFAP concentrations in patients with isolated diffuse axonal injury than in patients with negative CT and negative MRI findings, and orthopaedic trauma controls. P values are displayed for relevant comparisons. Two patients with isolated intracerebral contusions (GFAP 14·9 pg/mL, 285·4 pg/mL) were not included as a boxplot. GFAP=glial fibrillary acidic protein.

The results of this study suggest a greater role for MRI in the diagnosis of TBI. Plasma GFAP concentrations might help to guide the judicious use of MRI and identify patients for more comprehensive follow-up. Furthermore, the diagnosis of TBI pathology by imaging is clinically important not only during acute care but also for follow-up, patient counselling, and qualification for disability or health-care benefits. Historically, many patients with mild TBI who had negative CT scans were deemed to have no injury. However, we previously found in the TRACK-TBI pilot study that approximately 30% of patients with mild TBI with negative initial head CT had a positive MRI and poor 3-month outcome measured by the Extended Glasgow Outcome Scale.[15] Analysis is underway to compare the prognostic utility of imaging and blood-based biomarkers using the comprehensive TRACK-TBI outcome assessment battery across cognitive, psychological, and functional domains.

To our knowledge, this study is the largest to date to investigate the diagnostic utility of GFAP to detect who in a CT-negative mild TBI population might have a positive finding of lesions consistent with TBI on MRI. However, it is not a medical device labelling study with prespecified cutoff points. Nonetheless, this study provides data needed to specify values of GFAP for diagnosing CT-occult lesions on MRI in future regulatory studies. We also found that the best performance for detecting MRI abnormalities occurred at 9–16 h after injury, which might limit the utility of this point-of-care test in the very acute setting. However, repeat testing using biomarkers can be done when clinically indicated in the acute care setting, and there might be value in repeating GFAP testing as an aid in diagnosis of TBI. We also did not specifically assess for GFAP differences in isolated mild TBI compared with mild TBI with polytrauma. We included a large number of orthopaedic trauma controls who were found to have low concentrations of GFAP compared with patients with mild TBI, indicating that GFAP is able to distinguish between these two clinically important groups.

Plasma GFAP biomarker concentrations obtained using a plasma prototype assay on a point-of-care platform can aid in diagnosis of TBI in patients with a GCS score of 13–15 and normal CT in the acute phase after head trauma. Plasma GFAP concentrations also correlate with MRI lesion types. These results indicate that the diagnostic utility of GFAP might extend beyond CT-visible pathology and might help to identify patients with more subtle injury. Analysis of blood GFAP concentrations within 24 h of injury might improve detection of TBI and assist in identification of patients who need a subsequent MRI and follow-up.

Contributors

JKY, ELY, FKK, RD-A, DOO, and GTM contributed to the literature search, figures, study design, data collection, data analysis, data interpretation, and writing of the report. EAW, RCP, HD, WC, AC, SRT, ARF, JRH, and MR contributed to the literature search, data analysis, data interpretation, and writing of the report. XS and SJ contributed to the study design, data analysis, data interpretation, and writing of the report. AMP, PM, MJV, and KKWW contributed to the literature search, study design, data analysis, data interpretation, and writing of the report.

The TRACK-TBI Investigators

In alphabetical order by last name: Opeolu M Adeoye (University of Cincinnati); Neeraj Badjatia (University of Maryland); Kim Boase (University of Washington); Yelena Bodien (Massachusetts General Hospital); M Ross Bullock (University of Miami); Randall Chesnut (University of Washington); John D Corrigan (Ohio State University); Karen Crawford (University of Southern California); Sureyya S Dikmen (University of Washington); Ann-Christine Duhaime (Mass General Hospital); Richard Ellenbogen (University of Washington); V Ramana Feeser (Virginia Commonwealth University); Brandon Foreman (University of Cincinnati); Raquel C Gardner (University of California, San Francisco); Etienne Gaudette (University of Southern California); Joseph Giacino (Spaulding Rehabilitation Hospital); Dana Goldman (University of Southern California); Luis Gonzalez (TIRR Memorial Hermann); Shankar Gopinath (Baylor College of Medicine); Rao Gullapalli (University of Maryland); J Claude Hemphill (University of California, San Francisco); Gillian Hotz (University of Miami); Joel Kramer (University of California, San Francisco); Natalie Kreitzer (University of Cincinnati); Harvey S Levin (Baylor College of Medicine); Christopher Lindsell (Vanderbilt University); Joan Machamer (University of Washington); Christopher Madden (UT Southwestern); Alastair Martin (University of California, San Francisco); Thomas W McAllister (Indiana University); Michael McCrea (Medical College of Wisconsin); Randall Merchant (Virginia Commonwealth University); Lindsay Nelson (Medical College of Wisconsin); Laura B Ngwenya (University of Cincinnati); Florence Noel (Baylor College of Medicine); Eva M Palacios (University of California, San Francisco); Daniel Perl (Uniformed Services University); Claudia Robertson (Baylor College of Medicine); Jonathan Rosand (Massachusetts General Hospital); Angelle Sander (Baylor College of Medicine); Gabriela G Satris (University of California, San Francisco); David M Schnyer (UT Austin); Seth A Seabury (University of Southern California); Mark Sherer (TIRR Memorial Hermann); Murray Stein (University of California, San Diego); Nancy R Temkin (University of Washington); Arthur Toga (University of Southern California); Alex B Valadka (Virginia Commonwealth University); Paul Vespa (University of California, Los Angeles); Ross Zafonte (Harvard Medical School).

Declaration of interests

We declare no competing interests.

Acknowledgments

This work was supported by the following grants: National Institute of Neurological Disorders (N1NDS) 1RC2NS069409-01, 3RC2NS069409-02S1, 5RC2NS069409-02, 1U01NS086090-01, 3U01NS086090-02S1, 3U01NS086090-02S2, 3U01NS086090-03S1, 5U01NS086090-02, 5U01NS086090-03; US Department of Defense (DOD) W81XWH-13-1-0441, US DOD W81XWH-14-2-0176 (to GTM). We thank the following contributors to the development of the TRACK-TBI database and repositories by organisation and in alphabetical order by last name: Peter Chiarelli, Garen Staglin (One Mind, Rutherford, CA, USA); Vibeke Brinck, Michael Jarrett (QuesGen Systems, Burlingame, CA, USA); and Sirimon O'Charoen (Thomson Reuters, San Francisco, CA, USA). We also acknowledge editorial and project management support from Amy J Markowitz.

References

1 Teasdale G, Maas A, Lecky F, Manley G, Stocchetti N, Murray G. The Glasgow Coma Scale at 40 years: standing the test of time. *Lancet Neurol* 2014; **13:** 844–54.

2 US Food and Drug Administration. Evaluation of automatic class III designation for Banyan Brain Trauma Indicator. https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170045.pdf (accessed July 1, 2019).

3 Posti JP, Takala RSK, Runtti H, et al. The levels of glial fibrillary acidic protein and ubiquitin C-terminal hydrolase-L1 during the first week after a traumatic brain injury: correlations with clinical and imaging findings. *Neurosurgery* 2016; **79:** 456–64.

4 McMahon PJ, Panczykowski DM, Yue JK, et al. Measurement of the glial fibrillary acidic protein and its breakdown products GFAP-BDP biomarker for the detection of traumatic brain injury compared to computed tomography and magnetic resonance imaging. *J Neurotrauma* 2015; **32:** 527–33.

5 Diaz-Arrastia R, Wang KKW, Papa L, et al. 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:** 19–25.

6 Lei J, Gao G, Feng J, et al. Glial fibrillary acidic protein as a biomarker in severe traumatic brain injury patients: a prospective cohort study. *Crit Care* 2015; **19:** 362.

7 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:** 1428–33.

8 Nylén K, Ost M, Csajbok LZ, et al. Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome. *J Neurol Sci* 2006; **240:** 85–91.

9 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:** 1815–22.

10 Okonkwo DO, Yue JK, Puccio AM, et al. GFAP-BDP as an acute diagnostic marker in traumatic brain injury: results from the prospective Transforming Research and Clinical Knowledge in Traumatic Brain Injury study. *J Neurotrauma* 2013; **30:** 1490–97.

11 Vos PE, Jacobs B, Andriessen TMJC, et al. GFAP and S100B are biomarkers of traumatic brain injury: an observational cohort study. *Neurology* 2010; **75:** 1786–93.

12 Papa L, Brophy GM, Welch RD, et al. 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. *JAMA Neurol* 2016; **73:** 551–60.

13 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:** 471–83.

14 Bazarian JJ, Biberthaler P, Welch RD, et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. *Lancet Neurol* 2018; **17:** 782–89.

15 Yuh EL, Mukherjee P, Lingsma HF, et al. Magnetic resonance imaging improves 3-month outcome prediction in mild traumatic brain injury. *Ann Neurol* 2013; **73**: 224–35.

16 Carlson AP, Ramirez P, Kennedy G, McLean AR, Murray-Krezan C, Stippler M. Low rate of delayed deterioration requiring surgical treatment in patients transferred to a tertiary care center for mild traumatic brain injury. *Neurosurg Focus* 2010; **29:** e3.

17 Manley GT, MacDonald CL, Markowitz A, et al. The Traumatic Brain Injury Endpoints Development (TED) Initiative: progress on a public-private regulatory collaboration to accelerate diagnosis and treatment of traumatic brain injury. *J Neurotrauma* 2017; published online March 31. DOI:10.1089/neu.2016.4729.

18 Yue JK, Vassar MJ, Lingsma HF, et al. Transforming research and clinical knowledge in traumatic brain injury pilot: multicenter implementation of the common data elements for traumatic brain injury. *J Neurotrauma* 2013; **30:** 1831–44.

19 Jagoda AS, Bazarian JJ, Bruns JJ Jr, et al. Clinical policy: neuroimaging and decisionmaking in adult mild traumatic brain injury in the acute setting. *J Emerg Nurs* 2009; **35:** e5–40.

20 Manley GT, Diaz-Arrastia R, Brophy M, et al. Common data elements for traumatic brain injury: recommendations from the biospecimens and biomarkers working group. *Arch Phys Med Rehabil* 2010; **91:** 1667–72.

21 Wayne PA, ed. CLSI. Evaluation of detection capability for clinical laboratory measurement procedures; approved guideline - second edition. 2012. https://clsi.org/standards/products/method-evaluation/documents/ep17/ (accessed July 1, 2019).

22 Duhaime AC, Gean AD, Haacke EM, et al. Common data elements in radiologic imaging of traumatic brain injury. *Arch Phys Med Rehabil* 2010; **91:** 1661–66.

23 Dunn OJ. Multiple comparisons using rank sums. *Technometrics* 1964; **6:** 241–52.

24 Gill J, Latour L, Diaz-Arrastia R, et al. Glial fibrillary acidic protein elevations relate to neuroimaging abnormalities after mild TBI. *Neurology* 2018; **91:** e1385–89.

25 Kane MJ, Angoa-Pérez M, Briggs DI, Viano DC, Kreipke CW, Kuhn DM. A mouse model of human repetitive mild traumatic brain injury. *J Neurosci Methods* 2012; **203:** 41–49.

26 Broussard JI, Acion L, De Jesús-Cortés H, et al. Repeated mild traumatic brain injury produces neuroinflammation, anxiety-like behaviour and impaired spatial memory in mice. *Brain Inj* 2018; **32:** 113–22.

27 Chen H, Desai A, Kim HY. Repetitive closed-head impact model of engineered rotational acceleration induces long-term cognitive impairments with persistent astrogliosis and microgliosis in mice. *J Neurotrauma* 2017; **34:** 2291–302.

28 Mondello S, Shear DA, Bramlett HM, et al. Insight into pre-clinical models of traumatic brain injury using circulating brain damage biomarkers: operation brain trauma therapy. *J Neurotrauma* 2016; **33:** 595–605.

29 Luccichenti G, Giugni E, Péran P, et al. 3 Tesla is twice as sensitive as 1.5 Tesla magnetic resonance imaging in the assessment of diffuse axonal injury in traumatic brain injury patients. *Funct Neurol* 2010; **25:** 109–14.

30 Korley FK, Yue JK, Wilson DH, et al. Performance evaluation of a multiplex assay for simultaneous detection of four clinically relevant traumatic brain injury biomarkers. *J Neurotrauma* 2018; published online July 23. DOI:10.1089/neu.2017.5623.

31 Rubenstein R, Chang B, Yue JK, et al. Comparing plasma phospho tau, total tau, and phospho tau-total tau ratio as acute and chronic traumatic brain injury biomarkers. *JAMA Neurol* 2017; **74:** 1063–72.