

Review Article

Thorough overview of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein as tandem biomarkers recently cleared by US Food and Drug Administration for the evaluation of intracranial injuries among patients with traumatic brain injury

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Traumatic brain injury (TBI) is a major cause of mortality and morbidity affecting all ages. It remains to be a diagnostic and therapeutic challenge, in which, to date, there is no Food and Drug Administration-approved drug for treating patients suffering from TBI. The heterogeneity of the disease and the associated complex pathophysiology make it difficult to assess the level of the trauma and to predict the clinical outcome. Current injury severity assessment relies primarily on the Glasgow Coma Scale score or through neuroimaging, including magnetic resonance imaging and computed tomography scans. Nevertheless, such approaches have certain limitations when it comes to accuracy and cost efficiency, as well as exposing patients to unnecessary radiation. Consequently, extensive research work has been carried out to improve the diagnostic accuracy of TBI, especially in mild injuries, because they are often difficult to diagnose. The need for accurate and objective diagnostic measures led to the discovery of biomarkers significantly associated with TBI. Among the most well-characterized biomarkers are ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein. The current review presents an overview regarding the structure and function of these distinctive protein biomarkers, along with their clinical significance that led to their approval by the US Food and Drug Administration to evaluate mild TBI in patients.

Key words: Biomarker, brain injury, diagnostic marker, GFAP, UCH-L1

INTRODUCTION

TRAUMATIC BRAIN INJURY (TBI) remains a leading cause of mortality and neurological disability worldwide affecting children and adults. In the latest surveillance

report issued by the Centers for Disease Control and Prevention, the number of TBI-related emergency department visits, hospitalizations, and deaths in 2014 reached 2.87 million in the USA, 53% higher than the casualties reported in 2006.¹ Despite that, to date, no drug has been approved by the US Food and Drug Administration (FDA) for the treatment of patients suffering from TBI. In fact, over the past three decades, more than 30 clinical trials of drugs that showed promising beneficial effects in preclinical and phase I/II have failed to make it to phase III.² Among the significant challenges encountered in this regard are the complex pathophysiology of TBI and the poorly understood heterogeneity of the injury along with its clinical characteristics.

The severity of TBI, occurring due to a blow or jolt to the head, ranges from mild to moderate–severe and can be assessed by different classification systems, including the Glasgow Coma Scale (GCS) score. Clinical trials usually

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enroll patients with severe TBI, that is, GCS score of 8 or less³; however, the impairments resulting from a TBI are also frequent after moderate and mild TBI (mTBI). In addition to the injury severity, pathoanatomic classification is another major system that has been deployed in brain injuries describing the anatomical feature or the location of the injury type to be treated. As a consequence of TBI, lesions and abnormalities can occur, such as contusion and focal and diffuse patterns of axonal injury that can be assessed through neuroimaging including magnetic resonance imaging (MRI) and computed tomography (CT) scan.⁴ Both the classification system and the current imaging techniques present certain limitations in the diagnosis of TBI. For instance, several factors, irrelevant to the brain injury, can influence the scale, including the misinterpretation of the guidelines by the clinicians.⁵ In addition, CT scans expose patients to potentially harmful ionizing radiation, raising health-care costs.^{6,7} Accordingly, accurate diagnosis complementing clinical and imaging assessment is required. Biochemical markers, identified in body fluids, are considered as an objective and rapid measure that can confirm the diagnosis of TBI long after the injury. Furthermore, recent studies showed that TBI biomarkers are capable of assessing the severity of the injury and indicating patient prognosis even in mTBI, which sometimes can be difficult to diagnose by other neurological means.^{8,9} The most studied biomarkers cover a wide range of cell-specific proteins such as S100 calcium-binding protein B (S100B), neuron-specific enolase (NSE), Tau, neurofilament-light, ubiquitin C-terminal hydrolase-L1 (UCH-L1), and glial fibrillary acidic protein (GFAP) proteins. The levels of these biomarkers in biofluids, whether measured alone or in combination, present a potential indicator of injury severity and a predictor for positive CT scan in TBI subjects.^{10,11} Blood tests simultaneously measuring the levels of UCH-L1 and GFAP have recently been approved by the FDA to evaluate concussion in adults. The UCH-L1 biomarker complements GFAP as each is produced by a different type of cell and measures distinctive molecular events.¹² This review presents the latest advances in biomarker discovery and the clinical significance of GFAP and UCH-L1 proteins in the diagnosis and prognosis of TBI.

BIOCHEMICAL MARKERS OF BRAIN DAMAGE: UCH-L1 AND GFAP

CELLULAR DAMAGE, RESULTING from brain injury, leads to the release of cell-type-specific proteins into biofluids such as cerebral spinal fluid (CSF), serum, plasma, or blood. There are several characteristics that allow a biofluid marker to be clinically significant, amongst which is the availability of the protein in the above-mentioned

fluids and the ability to readily determine and quantify it. Additionally, the biomarker should increase significantly in the acute phase post-TBI as compared to control subjects, should be brain-specific, and should be highly sensitive, reflecting the severity of the TBI.⁹ Several biomarkers have been identified as indicators of TBI pathophysiological events including necrosis (SBDP150, SBDP145, and SNTF), apoptosis (SBDP120), neuronal cell body injury (UCH-L1 and NSE), astrogliosis/astroglia injury (GFAP), and inflammation (interleukin-6 and autoantibodies) and neurodegeneration (Tau, pTau), which can have temporal profile as shown in Figure 1.¹³ Recent clinical trials investigated novel neuronal and glial proteins and the reliability of utilizing their expression as an indicator of TBI progression.^{14–16} Among the promising biomarkers are UCH-L1 and GFAP as clinically validated early time biomarkers for TBI, as shown in Figure 1.

Ubiquitin C-terminal hydrolase-L1

Ubiquitin C-terminal hydrolase-L1 is a cytoplasmic deubiquitinating enzyme that is specific to neurons, exclusively in the cytoplasm, and highly abundant constituting up to 1–2% of total proteins in the brain. Moreover, UCH-L1, being an element of the axonal skeleton, plays a role in axonal transport.¹⁷ During normal and neuropathological situations (i.e. neurodegenerative disorders), UCH-L1 removes excessive, misfolded, or oxidized proteins, thereby regulating brain protein metabolism by controlling the proteasome pathway.¹⁸ In addition to UCH-L1, other isoforms in the class of UCH exist, including UCH-L3, UCH-L5, and BRCA-associated protein-1; however, only UCH-L1 is abundant in the brain.^{19,20}

Several factors can alter the structure and function of UCH-L1, including reactive lipid species, genetic mutations, and post-translational modification.^{21,22} Reactive lipids such as prostaglandins and isoprostanes, accumulating post-stroke, and other brain injuries, can covalently modify cysteine residues on specific proteins.²³ Likewise, the inactivation of UCH-L1 might occur due to familial point mutations occurring at certain gene coding regions, resulting in enhanced neurotoxicity associated with familial Parkinson's disease (PD) and other neurodegenerative disorders.²⁴ Post-translational modification as well plays a crucial role in the alteration of UCH-L1 through different means. For example, oxidative stress, which is significantly correlated with numerous neurological diseases, including TBI, results in protein oxidation and/or nitration. It has been shown that in Alzheimer's disease (AD) and PD, UCH-L1 acts as a major target of oxidation, resulting in carbonyl formation, methionine oxidation, and cysteine oxidation.²⁵ Moreover,

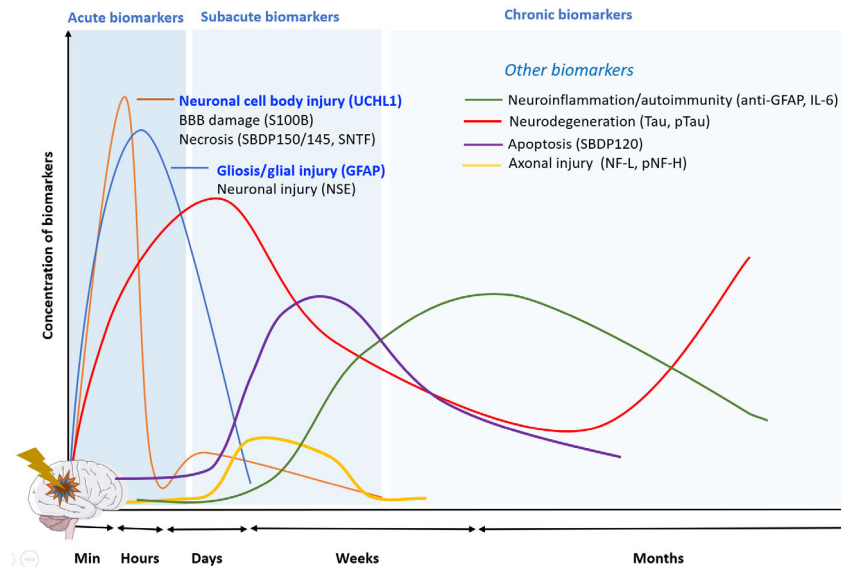


Fig. 1. Ubiquitin C-terminal hydrolase-L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) proteins have been reported as promising biomarkers for traumatic brain injury at early time points, and received approval from the US Food and Drug Administration. BBB, blood–brain barrier; IL-6, interleukin-6; NFL, neurofilament light chain; NSE, neuron-specific enolase; p-NF-H, phosphorylated neurofilament heavy subunit.

the conversion of UCH-L1 from its cytosolic form to its membrane-associated form, implicated in alpha-synuclein association and alpha-synuclein dysfunction, seems to be induced through O-glycosylation and farnesylation.²² Remarkably, reduced levels of cytosolic UCH-L1 have been observed in AD and associated with the formation of UCH-L1 immunoreactive Tau tangles.²⁶

Glial fibrillary acidic protein

Glial fibrillary acidic protein is a monomeric intermediate filament protein representing the main component of the astroglial cytoskeleton.²⁷ It is a highly specific marker for the central nervous system²⁸ found in glial cells in both gray and white brain matter.^{29,30} The main function of GFAP is to maintain the cytoskeletal structure of glial cells and their mechanical strength; in addition to supporting the blood–brain barrier and the neighboring neurons.³¹ Interestingly, upon the activation of astrocytes, GFAP plays a crucial role in promoting the morphological changes acquired, including thickening and elongation. Accordingly, in astrogliosis, the increase in size and number of glial cells leads to a remarkable increase in the expression level of GFAP. Furthermore, in the case of astrocytic death, GFAP is released into biofluids, acting as an indicator of brain injury and other degenerative diseases, such as AD and PD.^{32–34}

Glial fibrillary acidic protein also can be subjected to mutations and numerous post-translational modifications.

Mutations are suggested to result in gain-of-function, primarily occurring in the coding regions of the *GFAP* gene and less often in the promoter regions.³⁵ Nevertheless, the mutated version of the *GFAP* gene is associated with aggregate formation, resulting in astrocytic inclusions often observed in brains of patients with Alexander disease.³⁶ Glial fibrillary acidic protein is a key element in the signaling pathway involved in intermediate filament assembly, highly regulated by protein kinases. The N-terminal domain of GFAP includes numerous phosphorylation sites that can be targeted, in which elevated phosphorylation of such sites inhibits the polymerization of GFAP and hence disrupts the filament assembly.^{37,38} It is also suggested that the phosphorylation of GFAP plays a role in the neuronal–glial cross-talk due to its involvement in the pathway associated with the G-protein-coupled mGluR receptor.³⁸ Likewise, lysine residues in GFAP are prone to differential acetylation, observed mainly in the spinal cord of amyotrophic lateral sclerosis patients; however, the effect of such modification on the structure and function of GFAP is not fully understood.³⁹ Furthermore, it has been reported that GFAP is highly vulnerable to proteolysis, at both the C- and N-terminal, resulting in GFAP breakdown products (BDPs) that appear to be glia-toxic.^{40,41} Such BDPs are observed significantly in TBI, spinal cord injury, and AD,^{40,42,43} in which the GFAP cleavage is mediated by calpain, predominantly, and caspases, leading to the disruption of intermediate filament elongation.⁴⁰

INITIAL PROTEOMICS DISCOVERY

IN THE EARLY 1980s, Jackson *et al.* were the first to report UCH-L1 as a human brain-specific protein, of approximately 27 kDa molecular weight, using high-resolution 2D polyacrylamide gel electrophoresis.⁴⁴ Later, UCH-L1, as a TBI marker, was originally identified by Kobeissy *et al.* in a proteomics study in a rat TBI model in the laboratory of Wang and Hayes in 2006.⁴⁵ Using the mass spectrometry–proteomic approach and western blot assays, the differential expression of several cytoplasmic neuroproteins, including UCH-L1, was shown to be upregulated with the incidence of TBI. After that, the identification of UCH-L1 was investigated in biofluids of TBI subjects, including CSF and blood, and within 24 h post-injury to assess the biomarker profiles associated with the injury, suggesting that UCH-L1 is among the candidate TBI markers detected in biofluids.^{45–49} Likewise, GFAP has been well characterized in the past decades, achieving the status of astroglia-specific marker. The first isolation of this protein dates back to 1969, by Eng *et al.*, who described it as “plaque protein” after its extraction from cerebral tissues of patients suffering from multiple sclerosis.⁵⁰ Interestingly, GFAP was then identified as a major component present in patients with fibrous gliosis, characterized by fibrous astrocytes and demyelinated neurons.²⁷ As astrocytosis is considered among the cascade of events occurring after injuries and in several neurodegenerative diseases, it was believed that GFAP can be a promising diagnostic biomarker for astroglial pathology associated with neurological disorders⁵¹ and TBI.²⁸ More importantly, GFAP BDPs were reported in severe TBI⁵² and mild-to-moderate TBI,⁵³ and have been associated with injury severity, intracranial lesions, and mortality. Accordingly, the detection of enhanced levels of GFAP BDPs can be a potential marker for measuring brain injury. Preclinical and clinical studies considering the promise of UCH-L1 and GFAP as diagnostic biomarkers for TBI are discussed in the next section.

APPLICATION IN ANIMAL MODELS

AS MENTIONED EARLIER, the initial identification of UCH-L1 in the context of TBI was in a rat model of controlled cortical impact (CCI) in which the authors estimated a two-fold increase in the expression of this protein in the cortex at 48 h post-injury.⁴⁵ Interestingly, another study evaluated the expression of UCH-L1 in the non-invasive rat model of closed-head projectile concussive impact demonstrating mTBI and reported upregulation of this protein in the cortical tissue.⁵⁴ As the size of UCH-L1 is relatively small, it was suggested that it can readily cross the blood–

brain barrier following injury and can hence be detected in CSF and blood.⁵⁵ Accordingly, several studies were then carried out in order to investigate the levels of UCH-L1 in biofluids after brain injuries. Liu *et al.*, in a rat CCI model, showed that UCH-L1 was detectable in the CSF within 0.5–2 h after the injury, and persisted up to 24 h, with a similar elevation profile obtained in the rats’ serum.⁴⁷ Likewise, the release of UCH-L1 into biofluids was validated in other models of TBI including controlled blast overpressure exposure,⁵⁹ penetrating ballistic brain injury (PBB),⁴⁰ and fluid percussion injury (FPI).⁶⁰

Similarly, GFAP, either as an intact (50 kDa) protein or as its subsequent breakdown products (BDPs) (44–38 kDa), is released into biofluids shortly after TBI. In the PBB rat model, Zoltewicz *et al.* showed that GFAP expression increased significantly in the injured cortex at day 7 after the injury, and in CSF acutely at day 1 post-TBI, in which the increase reflected the injury severity.⁴⁰ In another study, the expression of GFAP was measured to assess the neurotoxicity in rats.⁵⁶ The authors revealed that GFAP increased in CSF and was upregulated in the hippocampus and cortex beginning 24 h post-kainic acid injection, reaching the peak at 48 h. Furthermore, elevations in GFAP levels were reported in blast TBI at the acute phase (within 24 h) in CSF⁵⁷ and serum.⁵⁸ Recently, Lafrenaye *et al.* assessed serum GFAP levels in a pig model of mTBI, and correlated the increase in the circulating biomarker with the axonal injury and histological features of glia. The authors concluded that in diffuse injury, monitoring serum biomarkers can provide clinical relevance regarding the underlying acute pathophysiology following mild injuries.⁵⁹

CLINICAL STUDIES

THE PROMISE OF UCH-L1 and GFAP in preclinical studies proposing their use as specific biomarkers for TBI was further validated and confirmed through clinical trials; these are illustrated in Table 1. Ubiquitin C-terminal hydrolase-L1 was first investigated in CSF and serum of patients with severe TBI, including pediatric patients, compared to uninjured subjects. The studies reported a significant increase in UCH-L1 levels in the acute phase (within 24 h) and an association between the obtained concentration and the injury severity.^{60–64} In addition, Papa *et al.* reported a marked increase in serum UCH-L1 in patients with mild and moderate TBI in which the biomarker levels were detectable in the serum within 1 h post-injury and was associated with measures of injury severity (including GCS score), CT lesions, and neurological intervention.⁶⁵ Likewise, several studies reported that the elevation of serum GFAP levels in patients with severe TBI is correlated with

Table 1. Key clinical studies or trials of blood ubiquitin C-terminal hydrolase-L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) in traumatic brain injury (TBI)

Biomarker	Study design	Patient population	Levels in controls	Levels in TBI patients	Outcomes	Clinical significance	Ref
CSF and Serum UCH-L1	<ul style="list-style-type: none"> Severe TBI (GCS \leq 8) Acute phase (lower 7 days) Samples collected every 6 h up to 7 days post-TBI 	<ul style="list-style-type: none"> CSF controls, $n = 24$ Serum controls, $n = 167$ sTBI, $n = 95$ 	<ul style="list-style-type: none"> CSF, 7.6 ng/mL (\pm 2.78) Serum, 0.12 ng/mL (\pm 0.02) 	<ul style="list-style-type: none"> Mean CSF level = 66.21 ng/mL (\pm 9.72) Mean serum level = 1.02 ng/mL (\pm 0.26) 	<ul style="list-style-type: none"> Increased CSF and serum UCH-L1 all time intervals after injury ($P < 0.001$) Within 12 h post-injury, CSF and serum UCH-L1 levels in patients with GCS 3–5 were higher than patients with GCS 5–8 ($P = 0.07$ and $P = 0.02$, respectively; Mann-Whitney U-test) Within 6 h post-injury, CSF levels of UCH-L1 for non-survivors was significantly higher than those of survivors (CSF 292.1 \pm 47.17 ng/mL versus 67.16 \pm 22.32 ng/mL; $P = 0.01$, Mann-Whitney U-test), as well as those levels over the duration of the study (CSF 97.51 \pm 10.93 ng/mL versus 34.33 \pm 3.2 ng/mL, respectively, $P < 0.001$). Serum levels of UCH-L1 for survivors were also significantly higher than those of non-survivors within the first 6 h (serum 8.42 \pm 2.58 ng/mL versus 1.00 \pm 0.66 ng/mL, $P = 0.01$), as well as throughout the study (1.62 \pm 0.33 ng/mL versus 0.23 \pm 0.03 ng/mL; $P < 0.001$), respectively 	<ol style="list-style-type: none"> Serum levels of UCH-L1 have potential clinical utility in diagnosing TBI, including correlating to injury severity and survival outcome UCH-L1 levels in CSF and serum appear to distinguish severe TBI survivors versus non-survivors within the study, with non-survivors having significantly higher and more persistent levels of serum and CSF UCH-L1 Cumulative serum UCH-L1 level $>$ 5.22 ng/mL predicted death (odds ratio 4.8) 	60
<ul style="list-style-type: none"> Mild and moderate TBI patients with blunt head trauma (within 4 h of injury) with GCS 9–15 	<ul style="list-style-type: none"> Mean in all controls = 0.083 ng/mL (\pm 0.005) Mean in all TBI groups = 0.955 ng/mL (\pm 0.248) 	<ul style="list-style-type: none"> Classification performance for detecting intracranial lesions on CT at a UCH-L1 cut-off level of 0.09 ng/mL yielded a sensitivity of 100% (95% CI, 88–100), a specificity of 21% (95% CI, 13–32), and a negative predictive value of 100% (76–100) 	65				

Table 1. (Continued)

Biomarker	Study design	Patient population	Levels in controls	Levels in TBI patients	Outcomes	Clinical significance	Ref
Plasma GFAP	<ul style="list-style-type: none"> TBI across the full injury spectrum GCS 3–15 Blood samples collected within 24 h post-injury All subjects underwent head CT scan 	Orthopedic controls, $n = 122$ TBI, $n = 1359$, of which 810 CT– and 549 CT+	Median 13 pg/mL; IQR, 7–20	Median 336 pg/mL; IQR, 69–1196	<ul style="list-style-type: none"> those without CT lesions (CT negative) ($P < 0.001$) UCHL1 in patients who had a neurosurgical intervention was significantly higher than those who received no such intervention ($P < 0.001$) Significantly higher GFAP levels in TBI patients compared to orthopedic trauma controls ($P < 0.001$) Significantly higher GFAP levels in subjects with a positive head CT (median 1358 pg/mL; IQR, 472–3803) compared with those with a negative head CT (median 116 pg/mL; IQR, 26–397), and orthopedic trauma control subjects (median 13 pg/mL; IQR, 7–20) ($P < 0.001$) GFAP levels were associated with the severity of the presenting GCS, with subjects in the severe to moderate range (GCS 3–12) having over 10-fold higher GFAP levels than those with GCS 13–15 	<ol style="list-style-type: none"> AUC of GFAP for predicting lesion on CT scan was 0.853 (95% CI 0.833–0.874) Using a predetermined cut-off value of 22 pg/mL, the GFAP point-of-care platform prototype assay had a sensitivity of 0.987 (95% CI, 0.962–1.000) and NPV of 0.988 (0.959–1.000), supporting a potential clinical role in ruling out the need for a CT scan in patients with a history of TBI 	73
Plasma GFAP	<ul style="list-style-type: none"> TBI patients with GCS 13–15 and normal CT findings Blood samples collected within 24 h of injury Subjects underwent MRI 7–18 days post-injury 	<ul style="list-style-type: none"> Healthy controls, $n = 209$ Orthopedic trauma subjects, $n = 122$ TBI, $n = 45$ 	<ul style="list-style-type: none"> Mean GFAP concentration in healthy controls 11 pg/mL Mean GFAP concentration in trauma controls 23.7 pg/mL 	<ul style="list-style-type: none"> Mean GFAP concentration in healthy controls 308 pg/mL 	<ul style="list-style-type: none"> 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; $P < 0.0001$) 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) ($P = 0.0002$) 	<ol style="list-style-type: none"> 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 AUCs for discriminating patients with negative CT findings with diffuse axonal injury from patients with CT-negative and MRI-negative findings, and from orthopedic trauma controls, were considered excellent (i.e., 0.9–1.0), at 0.903 (95% CI, 0.935–1.000) and 0.976 (0.828–0.977), respectively 	75
Serum GFAP	<ul style="list-style-type: none"> TBI of any severity Samples obtained within 24 h post-injury CT scan was carried out 	<ul style="list-style-type: none"> sTBI, $n = 601$ mTBI, $n = 222$ Mild TBI (GCS 13–14), $n = 457$ Mild TBI (GCS 15), $n = 1494$ 	N/A	Median value: <ul style="list-style-type: none"> sTBI = 21.32 ng/mL mTBI = 11.31 ng/mL Mild TBI (GCS 13–14) = 4.91 ng/mL Mild TBI (GCS 15) = 0.87 ng/mL 	<ul style="list-style-type: none"> Median values of GFAP displayed a clear association with injury severity (Spearman's Rho [95% CI] = –0.52) GFAP levels were higher in patients with CT+ compared to those that are CT– 	<ol style="list-style-type: none"> The AUC for GFAP to predict the presence of CT abnormalities is 0.89 [95% CI: 0.87–0.90] GFAP showed the highest discriminative ability in predicting abnormalities on MRI imaging performed within 3 weeks of injury in CT– patients (c-statistic 0.76; 95% CI, 0.67–0.85) 	74

Table 1. (Continued)

Biomarker	Study design	Patient population	Levels in controls	Levels in TBI patients	Outcomes	Clinical significance	Ref
Serum GFAP	<ul style="list-style-type: none"> Severe TBI with abnormal head CT scan Serum specimens were collected on admission and then daily for the first 5 days Patient outcome was assessed at 6 months post injury with GOS and further grouped into death versus survival and unfavorable versus favorable 	Control, $n = 135$ TBI, $n = 67$	Not mentioned	At admission, -1.7 ng/mL	<ul style="list-style-type: none"> Serum GFAP levels over the study period were significantly higher in patients who died within 6 months after injury versus those who were alive, and higher in those with unfavorable outcomes versus favorable outcomes 	<ol style="list-style-type: none"> Good predictive ability of serum GFAP at the time of admission, with AUCs of 0.761 (95% CI, 0.606–0.917) for death and 0.823 (95% CI, 0.700–0.947) for unfavorable outcome For predicting death, using the cut-off value of 1.690 ng/mL, serum GFAP on admission had a sensitivity of 84.6% and specificity of 69.2%, with a PPV of 64.7% and an NPV of 87.1% For the prediction of the unfavorable outcome at 6 months post injury, admission GFAP (optimal cut-off value, 1.559 ng/mL) had a sensitivity of 85.3%, the specificity of 77.4%, PPV of 80.6%, and NPV of 82.8% 	69
Serum GFAP	<ul style="list-style-type: none"> Mild or moderate TBI (GCS 9–15) Blood samples were obtained within 4 h post-injury Trauma patients underwent standard CT scan of the head according to the judgment of the treating physician 	<ul style="list-style-type: none"> Trauma patients without mild/moderate TBI, $n = 188$ Mild/moderate TBI, $n = 209$ 	Not mentioned	With intracranial lesion, -0.72 ng/mL Mild/moderate TBI, -0.03 ng/mL	<ul style="list-style-type: none"> Levels of serum GFAP were significantly higher in those with intracranial lesions on CT scan (CT positive) versus those without CT lesions (CT negative) ($P < 0.001$) Levels of GFAP were significantly higher in those with intracranial lesions, compared with any of the extracranial lesions (scalp/facial hematoma and facial fractures) ($P < 0.05$) 	<ol style="list-style-type: none"> AUC for discriminating between CT scan-positive and CT scan-negative intracranial lesions was 0.84 (95% CI, 0.73–0.95) Classification performance for detecting intracranial lesions on CT at a GFAP cut-off level of 0.067 ng/mL yielded a sensitivity of 100% (95% CI, 63–100) and a specificity of 55% (95% CI, 43–66) 	72
Serum UCH-L1 and GFAP	<ul style="list-style-type: none"> Severe TBI (GCS ≤ 8) Blood drawn on admission Participants were followed up until death or completion of 6 months after head trauma 	Control, $n = 102$ TBI, $n = 102$	UCH-L1 = 247.7 ± 80.7 pg/mL GFAP = 2.3 ± 0.8 pg/mL;	UCH-L1 = 2931.6 ± 1542.3 pg/mL GFAP = 11.6 ± 4.6 pg/mL	<ul style="list-style-type: none"> UCH-L1 and GFAP concentrations were significantly higher in patients than in controls ($P < 0.001$) UCH-L1 and GFAP levels were significantly higher in patients with unfavorable outcome than those with favorable outcome ($P < 0.001$) 	No statistical significance in improving the predictive value of GOS score for prediction of long-term clinical outcome of sTBI	76
Serum UCH-L1 and GFAP	<ul style="list-style-type: none"> Mild/moderate TBI (GCS 9–15) Samples obtained within 6 h post-injury Patients underwent emergency head CT 	TBI, $n = 251$	N/A	GFAP median = 10.3 pg/mL UCH-L1 median = 65.8 pg/mL	<ul style="list-style-type: none"> Median values for UCH-L1 were higher among CT-positive patients (132.3 pg/mL) compared to those who were CT-negative (56.2 pg/mL) Median values for GFAP were higher among CT-positive patients (110.5 pg/mL) compared to those who were CT-negative (7.8 pg/mL) 	<ol style="list-style-type: none"> Determining negative head CTs in patients: UCH-L1 was 100% sensitive and 39% (95% CI, 33%–46%) specific at a value ≥ 40 pg/mL (specificity was 40%; 95% CI, 33%–47% when using a cut-off of 41 pg/mL) GFAP was 100% sensitive and 0% specific at a cut-off of 0 pg/mL, indicating that using the GFAP value associated with 100% sensitivity 	77

Table 1. (Continued)

Biomarker	Study design	Patient population	Levels in controls	Levels in TBI patients	Outcomes	Clinical significance	Ref
Serum UCH-L1 and GFAP	<ul style="list-style-type: none"> • Pediatric TBI (acute) • Mean (SD) age of cases was 3.8 (3.7) years • GCS 3–15 • Sample collected as soon as possible after arrival to the hospital • Outcome was assessed at hospital discharge and/or at a scheduled follow-up clinic visit 	Control, <i>n</i> = 40 sTBI, <i>n</i> = 19 Moderate TBI, <i>n</i> = 6 Mild TBI, <i>n</i> = 20	Median (IQR) UCH-L1 = 0.09 (0.03–0.11) ng/mL Median (IQR) GFAP = 0.01 (0.00–0.05) ng/mL	Median (IQR) UCH-L1 = 0.23 (0.12–0.55) ng/mL Median (IQR) GFAP = 0.48 (0.12–1.67) ng/mL	<ul style="list-style-type: none"> • Serum GFAP and UCH-L1 were significantly higher in cases versus controls (<i>P</i> < 0.0001) • Significant trend for increasing concentration of GFAP and UCH-L1 across severity groups/categories was found (<i>P</i> < 0.0001) • UCH-L1 concentrations were significantly higher in patients with ICI compared with those with both a negative CT (<i>P</i> = 0.004) or skull fracture (<i>P</i> = 0.02); GFAP did not show statistically significant difference between groups • Serum GFAP and UCH-L1 levels were significantly higher in children with unfavorable outcome than in those with favorable outcome (median GFAP, 1.12 versus 0.27 ng/mL, <i>P</i> = 0.013; median UCH-L1, 0.92 versus 0.18 ng/mL, <i>P</i> = 0.0005) 	<ol style="list-style-type: none"> 1. Diagnostic accuracy for differentiating cases and controls was good for both biomarkers: <ul style="list-style-type: none"> 2. AUCs 0.89 (95% CI, 0.82–0.96) for GFAP and 0.86 (95% CI, 0.78–0.94) for UCH-L1 3. The sensitivity of GFAP and UCH-L1 was high (89% and 100%, respectively), although the specificity was moderate to low (63% and 20%, respectively) 4. UCH-L1 cut-off point of 0.09 ng/mL was derived yielding a sensitivity of 93% and a specificity of 25% for the detection of ICI (AUC 0.81 [95% CI, 0.68–0.93], <i>P</i> = 0.0008) 5. The diagnostic accuracy of serum GFAP and UCH-L1 for the prediction of unfavorable outcome were 0.76 (95% CI, 0.60–0.92) and 0.86 (95% CI, 0.72–1.00), respectively 6. A cut-off of 16.97 ng/mL for GFAP and 2.22 ng/mL for UCH-L1 yielded a diagnostic specificity of 100%, while sensitivities were 9% and 27%, respectively 7. The combination of the two markers did not provide a higher level of predictive power compared to UCH-L1 alone 	78
			Control, <i>n</i> = 81 TBI, <i>n</i> = 324	Not mentioned	Median GFAP levels (lower and upper quartiles) at admission = 0.23 ng/mL (0.00 and 0.83 ng/mL) UCH-L1 levels at admission = 0.50 ng/mL (0.40 and 0.70 ng/mL)	<ul style="list-style-type: none"> • Levels of GFAP and UCH-L1 at admission significantly correlated with GCS scores (Spearman <i>r</i> = 20.426 [<i>P</i> = 0.001] and 20.294 [<i>P</i> = 0.001], respectively) 	<ol style="list-style-type: none"> 1. Levels of GFAP and UCH-L1 and the GFAP/UCH-L1 ratio were found to adequately discriminate between the mentioned severity classes at admission: AUC 0.729 (95% CI, 0.577–0.847), 0.701 (95% CI, 0.563–0.806), and 0.707 (95% CI, 0.553–0.820), respectively 2. Level of GFAP and GFAP/UCH-L1 ratio were found to adequately discriminate any CT scan pathology for all injury severity classes as measured with Marshall grading (Marshall I versus II–V), whereas levels of

Table 1. (Continued)

Biomarker	Study design	Patient population	Levels in controls	Levels in TBI patients	Outcomes	Clinical significance	Ref
Serum UCH-L1 and GFAP	<ul style="list-style-type: none"> Mild/moderate TBI (GCS 9–15) Repeated blood sampling undertaken at 4, 8, 12, 16, 20, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, and 180 h after injury Trauma patients underwent standard CT scan of the head based on the clinical judgment of the treating physician 	<p>Trauma patients without TBI, $n = 259$</p> <p>Trauma patients with moderate TBI, $n = 7$</p> <p>Trauma patients with mTBI; $n = 318$</p>	<p>UCH-L1: median, 0.171 ng/mL; IQR, 0.100–0.417 ng/mL; range, 0.045–4.241 ng/mL</p> <p>GFAP: median, 0.008 ng/mL; IQR, 0.008–0.030 ng/mL; range, 0.008–0.030 ng/mL</p>	<p>UCH-L1: median, 0.258 ng/mL; IQR, 0.109–0.627 ng/mL; range, 0.045–9.000 ng/mL</p> <p>GFAP: median, 0.112 ng/mL; IQR, 0.030–0.462 ng/mL; range, 0.008–8.078 ng/mL</p>	<ul style="list-style-type: none"> UCH-L1 and GFAP levels were significantly higher compared with the trauma controls ($P < 0.001$) 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) ($P < 0.001$) 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) ($P < 0.001$) In patients requiring neurosurgical intervention, 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) ($P < 0.001$). 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) ($P < 0.001$) 	<p>UCH-L1 reached only poor prediction capability at admission: AUC 0.739 (95% CI, 0.646–0.815), 0.621 (95% CI, 0.522–0.716), and 0.727 (95% CI, 0.626–0.804) for GFAP, UCH-L1, and GFAP/UCH-L1 ratio, respectively</p> <ol style="list-style-type: none"> The ability of GFAP and UCH-L1 to distinguish trauma patients with and without mild/moderate TBI was assessed over 7 days. GFAP showed a range of AUCs between 0.73 (95% CI, 0.69–0.77) and 0.94 (95% CI, 0.78–1.00) UCH-L1 showed AUCs between 0.30 (95% CI, 0.02–0.58) and 0.67 (95% CI, 0.53–0.81) GFAP and UCH-L1 combined, AUCs ranged from 0.64 (95% CI, 0.35–0.92) to 0.89 (95% CI, 0.79–0.99) 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: GFAP showed a range between 0.80 (95% CI, 0.67–0.92) and 0.97 (95% CI, 0.93–1.00) UCH-L1 showed a range between 0.31 (95% CI, 0–0.63) and 0.77 (95% CI, 0.68–0.85) GFAP and UCH-L1 combined: ranged from 0.75 (95% CI, 0.33–1.00) to 0.97 (95% CI, 0.93–1.00) 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: GFAP showed a range of 0.91 (95% CI, 0.79–1.00) and 1.00 (95% CI, 1.00–1.00) UCH-L1 showed a range between 0.50 (95% CI, 0–1.00) and 0.92 (95% CI, 0.85–1.00) 	80

Table 1. (Continued)

Biomarker	Study design	Patient population	Levels in controls	Levels in TBI patients	Outcomes	Clinical significance	Ref
Serum UCH-L1 and GFAP	<ul style="list-style-type: none"> • Suspected non-penetrating TBI, GCS 9–15 • Blood sampling within 12 h of injury • Patients underwent non-contrast head CT scanning within 12 h of injury 	TBI, n = 1959	N/A	GCS 13–15; GFAP: CT+ median ~135 pg/mL; CT- ~60 pg/mL; UCH-L1: CT+ median ~600 pg/mL; CT- ~500 pg/mL	<ul style="list-style-type: none"> • GFAP and UCH-L1 concentrations were significantly higher among patients who were CT-positive versus those who were CT-negative (median GFAP 135.0 pg/mL versus 22.2 pg/mL; P < 0.0001; median UCH-L1 604.8 pg/mL versus 261.0 pg/mL; P < 0.0001) 	<p>12. GFAP and UCH-L1 combined, AUC ranged from 0.50 (95% CI, 0–1.00) to 1.00 (95% CI, 1.00–1.00). Serum GFAP was the strongest predictor of having both intracranial lesion on CT (odds ratio, 3.45; 95% CI, 2.69–4.43) and neurosurgical intervention (odds ratio, 2.57; 95% CI, 2.04–3.21)</p> <p>Serum GFAP and UCH-L1 based test for acute CT-detected intracranial injury had sensitivity 0.976 (95% CI, 0.931–0.995) with specificity 0.364 (0.342–0.387) and NPV 0.996 (0.987–0.999)</p>	81

AUC, area under the receiver operating characteristic curve; CI, confidence interval; CSF, cerebrospinal fluid; CT, computed tomography; ICI, intracranial injury; GCS, Glasgow Coma Scale; IQR, interquartile range; M/M, moderate/mild; MRI, magnetic resonance imaging; mTBI, mild TBI; N/A, not applicable; NPV, negative predictive value; PPV, positive predictive value; SD, standard deviation; sTBI, severe TBI.

injury severity and clinical outcomes.^{28,66–69} The GFAP blood levels were shown to predict cerebral hypoxia, which is a secondary insult occurring after brain injury, in patients with severe TBI.⁷⁰ The value of GFAP as a brain biomarker has also been established in patients with moderate and mTBI.^{53,71,72} Interestingly, along with GFAP levels, its corresponding BDPs can be of clinical significance. Papa *et al.* documented that GFAP BDPs can be detected in the serum within 1 h post-injury in patients with moderate and mBI where the elevated levels obtained were associated with intracranial lesions and neurosurgical intervention.⁵³ Similarly, another study reported that plasma GFAP BDP levels can distinguish the presence and severity of CT scans, thereby acting as a diagnostic biomarker in TBI.⁷¹

Most recently, the analytic phase I of the USA-based multicenter TRACK-TBI study (with 1,375 TBI subjects with a full range of severity) further shows that Abbott's i-STAT prototype GFAP assay has acute TBI diagnostic accuracy that matches previous studies.⁷³ Interestingly, in this study, GFAP showed a high discriminative ability to predict intracranial abnormalities on CT scan in patients with TBI (GCS 3–15), substantially outperforming serum S100B biomarker measured in these patients. Furthermore, Yue *et al.* also showed that GFAP, but not UCH-L1, is capable of detecting MRI abnormalities among patients with TBI that are CT-negative.⁷⁹ In parallel, the European Commission-funded multicenter CENTER-TBI study with 2,867 patients with <24 h post-injury, Czeiter *et al.* found that GFAP achieved the highest discrimination for predicting CT abnormalities (area under the receiver operating characteristic curve [AUC], 0.89) with a 99% likelihood of better discriminating CT-positive patients than clinical characteristics used in contemporary decision rules. Similarly, in patients with mTBI, GFAP also showed slightly improved diagnostic value, from AUC 0.84 to 0.89.⁷⁴

Despite the fact that UCH-L1 and GFAP alone display significant prognostic and diagnostic markers of TBI, several studies examined them together and showed that their combination would result in enhanced sensitivity and specificity for TBI diagnosis.^{12,49,76–78,82} In a case-control study, serum levels of UCH-L1 and GFAP were significantly elevated in patients with severe TBI compared to control subjects providing informative data about injury severity and outcome post-injury.⁴⁹ The study revealed the correlation between the elevations of serum biomarkers with GCS and CT findings in which GFAP levels were higher in patients with mass lesions and UCH-L1 levels were higher in patients with diffuse injury.⁴⁹ Moreover, in a pilot study undertaken on patients with mTBI, it was reported that UCH-L1 and GFAP biomarkers, along with advanced MRI

imaging techniques, could improve the diagnosis of the injury. Glial fibrillary acidic protein is capable of serving as a clinical screening tool for intracranial bleeding, whereas UCH-L1 complements MRI in injury detection.⁸³

Furthermore, Posti *et al.* reported a strong relation between GFAP and UCH-L1 plasma levels with the severity of TBI in the first week post-injury, supporting the promise of such biomarkers in the acute-phase diagnostics of TBI.⁷⁹ In a large cohort study ($n = 584$), Papa *et al.* assessed the diagnostic accuracy of UCH-L1 and GFAP over time and showed that GFAP can detect mild to moderate TBI, CT lesions, and neurological intervention across 7 days after the injury; however, UCH-L1 performed best in the early post-injury period (Table 1).⁸⁰ In another study, Papa *et al.* evaluated the combination of GFAP and UCH-L1 to detect concussion in both children and adults. It was shown that GFAP protein outperformed UCH-L1 in detecting concussion in both children and adults, whereas UCH-L1 was expressed at much higher levels than GFAP in those with non-concussive trauma, which is suggestive of previous subconcussive brain injury.⁸²

Interestingly, Bazarian *et al.* investigated the utility of serum UCH-L1- and GFAP-based tests for predicting the absence of intracranial injuries on head CT.⁸¹ The study undertaken on 1,959 patients with mild to moderate TBI (GCS 9–15) showed that such biomarkers are highly sensitive and have clinical potential in ruling out the need for CT scan at emergency departments. Within 12 h post-injury, levels of UCH-L1 and GFAP were significantly higher among those who were CT-positive compared with patients who were CT-negative ($P < 0.0001$), in which the median UCH-L1 was 604.8 pg/mL versus 261.0 pg/mL and the median of GFAP being 135.0 pg/mL versus 22.2 pg/mL. For detection of intracranial injury, the test based on levels of serum UCH-L1 and GFAP had a sensitivity of 0.976 (95% confidence interval [CI], 0.931–0.995), negative predictive value (NPV) of 0.996 (0.987–0.999), and positive predictive value (PPV) of 0.095 (0.079–0.112). The CT scan was positive when the test was negative in only three (<1%) of 1,959 patients. The test was 1.0 (0.631–1.00) sensitive and 0.344 (0.323–0.365) specific with 1.0 (0.995–1.00) NPV and 0.006 (0.003–0.012) PPV for detecting neurologically manageable lesions ($n = 8$). Furthermore, sensitivity analysis comparing the diagnostic accuracy of the test to each biomarker individually among 1,790 patients having quantitative values for both GFAP and UCH-L1 proteins demonstrated that the combination of both proteins outperformed each marker separately, but that the diagnostic improvement over GFAP alone was not significant.⁸¹ Accordingly, the results of this study were used to support the request to the FDA for the approval of the use of UCH-

L1 and GFAP as indicators to help avoid unnecessary neuroimaging in patients suffering from mTBI.

In addition to that, several biomarkers, including UCHL-1 and GFAP, hold promise for a translational point-of-care (POC) application allowing for a rapid transferability to the clinical practice.⁷³ As published recently, POC devices for TBI biomarkers are currently in development.^{84,85} For instance, a detection method has been proposed by a research team in Arizona to measure the levels of four biomarkers, GFAP, NSE, S100B, and tumor necrosis factor- α .⁸⁶ The device is capable of detecting the concentrations of such biomarkers within 90 s by a gold disc electrode that measures a microliter volume-sized sample of blood. Moreover, Yue *et al.* reported that the i-STAT device can measure the plasma levels of GFAP within 24 h post-injury.⁷⁵ Interestingly, the device was able to discriminate between MRI-positive patients and MRI-negative patients with an AUC of 0.777 (95% CI, 0.726–0.829). Although the biomarker-based POC testing holds promise in the rapid diagnosis of mTBI, this new technology requires further development, optimization, and additional prospective studies to assure its specificity and sensitivity in evaluating concussions in patients with TBI.

FOOD AND DRUG ADMINISTRATION CLEARANCE LETTER AND AND FUTURE REGULATORY PATH

ON 14 FEBRUARY 2018, the FDA authorized the marketing of the first blood test to evaluate concussion in adults.^{87,88} The Brain Trauma Indicator™, developed by Banyan Biomarkers in partnership with the US Department of Defense, was reviewed and permitted in less than 6 months under the FDA Breakthrough Devices Program. The primary objective of such an assay is to prevent unnecessary neuroimaging (CT scan) and associated radiation exposure to patients. The Brain Trauma Indicator measures the levels of UCH-L1 and GFAP proteins released from the brain into the blood within 12 h post-injury and the test result can be available in 3–4 h. Levels of such biomarkers in the blood after mTBI can predict the presence of intracranial lesions in patients visible by CT scan. Accordingly, health-care professionals can decide whether a CT scan is needed or not. The FDA Commissioner Scott Gottlieb said, upon authorizing this test, “A blood-testing option for the evaluation of mTBI/concussion not only provides health-care professionals with a new tool but also sets the stage for a more modernized standard of care for testing of suspected cases. In addition, the availability of a blood test for mTBI/concussion will likely reduce the CT scans performed on patients with concussion each year, potentially saving our

health-care system the cost of often unnecessary neuroimaging tests.”⁸⁷

The approval was based on data obtained from a prospective, multicenter ALERT-TBI clinical study by Bazarian and coworkers, discussed in the previous section, including 1,947 adults included in the analysis with suspected mTBI at 24 clinical sites (NCT01426919).⁸¹ The FDA evaluated the product’s performance by comparing the patients’ blood samples with CT scan findings. Remarkably, the test predicted patients with intracranial lesions with 97.5% accuracy and patients without lesions (NPV) with 99.6%. The high accuracy of the test indicated its reliability in predicting the absence of intracranial lesions and, therefore, its utility in ruling out the need for CT scan in patients suffering from mTBI.

It is noted that the above-mentioned Banyan’s Brain Trauma Indicator™ was run on a semiautomated ELISA assay platform that requires skilled technical personnel to operate and takes several hours to run. Importantly, Brain Trauma Indicator has not been commercialized thus this UCH-L1/GFAP tandem test is still not widely available as clinical diagnostic test in clinical setting. In addition to that, several biomarkers, including UCHL-1 and GFAP, hold promises for a prototype point-of-care (POC) application allowing for a rapid transferability to the clinical practice⁷⁴. As published recently, POC devices for TBI-biomarkers are currently in development⁸⁷. For instance, a detection method has been proposed by a research team in Arizona to measure the levels of four biomarkers: GFAP, NSE, S100B, and tumour necrosis factor-alpha⁸⁸. The device is capable of detecting the concentrations of such biomarkers within 90 seconds via a gold disc electrode that measures a microliter volume-sized sample of blood. In the past few years, enabled by a license agreement with Banyan, Abbott Diagnostics has created their own prototype i-STAT Point-of-Care version of UCH-L1/GFAP diagnostic blood test for TBI.⁸⁹ Oknowkwo *et al.* and Wang *et al.* also reported CT abnormality prediction similarly to previously reported results based on day of injury plasma GFAP and UCH-L1 levels, respectively, using a large TRACK-TBI consortium study’s phase 1 analytic cohort of 1,375 TBI subjects (*submitted for publication*). Using the same cohorts, Yue *et al.* demonstrated that the prototype i-STAT-device determined plasma levels of GFAP within 24 hours post-injury can also discriminate between MRI-positive patients and MRI-negative patients with an area under the ROC curve of 0.777 [95% CI, 0.726 to 0.829].⁷⁹ Following these encouraging data, Abbott Diagnostic is now partnering with US department of Defense and TRACK-TBI consortium to conduct a multicenter pivotal clinical trial on their i-STAT Point-of-Care version of UCH-L1/GFAP tandem plasma tests on

mild TBI patients. Their primary goal is to show mild TBI diastolic performance equivlancy to the previous Banyan's test results. Upon the anticipated FDA clearance this i-STAT UCH-L1/GFAP test, it will be included in Abbott i-STAT clinical diagnostic test menu and become widely accessible in various clinical setting acrossing the USA and in other countries thereafter.

CONCLUSION

B IOMARKERS PRESENT AN accurate and objective diagnostic and prognostic tool implicated in several neurological diseases, including TBI. Among the most studied biomarkers implicated in brain injuries are UCH-L1 and GFAP, representing cell types that are dominant in the human brain. Promising findings from animal studies led to the assessment of the clinical significance of such markers in patients suffering from severe and mild to moderate TBI. The elevation of UCH-L1 and GFAP in biofluids was associated with injury severity and clinical outcomes. Later, the use of one diagnostic test with this tandem markers was authorized by the FDA to aid in the diagnosis and care of mTBI patients. Other clinical diagnostic platforms bearing UCH-L1/GFAP tests are expected to be cleared by FDA in the near future. Considering the remarkable significance of such markers in assessing and managing neurotrauma, more studies are needed to further examine their diagnostic value in other clinical practices.

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DISCLOSURE

Approval of the research protocol: NA

Informed consent: NA

Registry and the registration no. of the study/trial: NA

Animal studies: NA

Conflict of interest: K.K.W. is a shareholder of Banyan Biomarkers, Inc. a company interested in the commercialization of traumatic brain injury biomarkers as medical diagnostics. The other authors have no conflict of interest.

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