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Review

Biomedical research brings mTBI biomarkers a step closer to the bedside – implementation in clinical practice

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Abstract. Research in the field of TBI (traumatic brain injury) has long been focused on severe brain injury, while the number of mild injuries far overweigh severe injuries. Mild head injuries constitute up to 95% of all traumatic head injuries. The purpose of this work is to identify mTBI (mild traumatic brain injury) patients who are unlikely to benefit from CT (computed tomography) scanning. Biomarkers capable of clearly discriminating between CT-positive and CT-negative subjects are needed. Biomarkers hold the potential to document whether a concussion occurred, especially when the history is unclear and neurocognitive sequelae persist. Recently, following advances in proteomics analysis, investigators have introduced ubiquitin C-terminal hydrolase-L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) as two promising brain injury biomarkers. The authors provide an update on the current knowledge of TBI biomarkers, especially protein biomarkers for neuronal cell body injury (UCH-L1) and astroglial injury (GFAP, S100B), and a focused literature review dealing with implementation of mTBI biomarkers in clinical practice.

Key words: Mild traumatic brain injury — mTBI biomarkers — GFAP — UCH-L1 — S100B

Abbreviations: AD, Alzheimer disease; AUC, area under curve; BTI, brain trauma indicator; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; CTE, chronic traumatic encephalopathy; DAI, diffuse axonal injury; DTI, diffusion tensor imaging; FDA, Food and drug administration; GCS, Glasgow coma scale; GFAP, glial fibrillary acidic protein; GOS, Glasgow outcome scale; IVH, intraventricular haematoma; MBP, myelin basic protein; MRI, magnetic resonance imaging; mTBI, mild traumatic brain injury; NfL, neurofilament light chain; NPV, negative predictive value; NSE, neuron specific enolase; p-NF, phosphorylated neurofilament; p-NfH, phosphorylated neurofilament heavy chain; p-tau, phosphorylated tau; p-tau 181, tau phosphorylated on threonine 181; PTSD, post-traumatic stress disorder; RBC, red blood cell; SBDP, spectrin break down products; SNTF, spectrin N-terminal fragment; sTBI, severe traumatic brain injury; TAI, traumatic axonal injury; TBI, traumatic brain injury; t-tau, total tau; UCH-L1, ubiquitin C-terminal hydrolase-L1.

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Introduction

More than 50 million people worldwide sustain a traumatic brain injury annually. Traumatic brain injuries are stratified as severe, moderate or mild based on a Glasgow Coma Scale (GCS) score of 3–8, 9–12 or 13–15, respectively. Up to 90% of all traumatic brain injuries are classified as mild traumatic brain injuries (mTBI). The prevalence of CT-detected intracranial injury is typically less than 10% (Bazarian et al. 2018). The diagnosis of TBI in the acute setting is based on neurological examination and neuroimaging tools such as computed tomography scanning and magnetic resonance imaging (MRI). However, CT scanning has low sensitivity for detecting diffuse brain damage and confers exposure to radiation. MRI can provide information on the extent of diffuse injuries, but its widespread application is restricted by cost, the limited availability of MRI in many centers, and the difficulty of performing it in physiologically unstable patients. In particular, the recognition of diffuse axonal injury (DAI)/traumatic axonal injury (TAI) is even more difficult and standard neuroimaging techniques may not detect TBI. Diffusion tensor imaging (DTI) is a promising neuroimaging technique that may help to identify axonal injury after mTBI (Bazarian et al. 2007; Huang et al. 2009).

MTBI, despite negative CT scan, causes rapid-onset neurophysiological and neurological dysfunction that resolves spontaneously. However, 15% of individuals with mTBI develop persistent cognitive dysfunction (Zetterberg et al. 2013). MTBI typically affects the frontal and temporal lobes of the brain, which are associated with executive function, learning and memory. Each mTBI subsequently causes greater cognitive deterioration and longer recovery time (Huan et al. 2018).

Table 1. Clinical decision rules – Canadian CT head rules* – for predicting intracranial injury after mTBI. Computed tomography is only required for patients with minor head injury with any 1 of the following findings: Patients with minor head injury who present with a GCS score of 13 to 15 after witnessed loss of consciousness, amnesia, or confusion.

High Risk for Neurosurgical Intervention
1. GCS score lower than 15 at 2 h after injury
2. Suspected open or depressed skull fracture
3. Any sign of basal skull fracture†
4. Two or more episodes of vomiting
5. 65 years or older
Medium Risk for Brain Injury Detection by CT Imaging
6. Amnesia before impact of 30 or more minutes
7. Dangerous mechanism‡

* The rule is not applicable if the patient did not experience trauma, has a GCS score lower than 13, is younger than 16 years, is taking warfarin or has a bleeding disorder, or has an obvious open skull fracture – in this case, CT imaging is routinely recommended unless otherwise contraindicated. † signs of of basal skull fracture include hemotympanum, racoon eyes, cerebrospinal fluid, otorrhea or rhinorrhea, Battle's sign; ‡ dangerous mechanism is a pedestrian struck by a motor vehicle, an occupant ejected from a motor vehicle, or a fall from an elevation of 3 or more feet or 5 stairs. (Adapted from Stiell et al. 2005).

Biomarkers in mild traumatic brain injury

Clinical decision rules

In severe TBI (sTBI) computed tomography is directly indicated. In minor head injuries Clinical decision rules are used. The most relevant of these is Canadian CT Head Rule (CCHR). The CCHR high-risk criteria have sensitivity of 99% to 100% with specificity of 48% to 77% for injury requiring neurosurgical intervention. Other rules such as New Orleans criteria (NOC), National Emergency X-Radiography Utilization Study II (NEXUS II), Neurotraumatology Committee of the World Federation of Neurosurgical Societies, Scandinavian, and Scottish Intercollegiate Guidelines Network produce similar sensitivities for injury requiring neurosurgical intervention but with lower and more variable specificity values. The most widely researched decision rule is the CCHR, which has consistently shown high sensitivity for identifying injury requiring neurosurgical intervention with an acceptable specificity to allow considered use of cranial computed tomography. No other decision rule has been as widely validated or demonstrated as acceptable results, but its exclusion criteria make it difficult to apply universally (Harnan et al. 2011). Clinical decision rules – CCHR – for predicting intracranial injury after mTBI are presented in Table 1.

The validity of the CCHR in cases of minor TBI was tested in Lamba study. A total of 101 patients met the inclusion criteria. 62 subjects fulfilled the CCHR criteria. Out of 62 subjects who fulfilled the CCHR criteria, 46 (74.1%) were reported to have normal CT scans, while 16 had either haemorrhages ($n = 12$) or contusions ($n = 4$). All the subjects who didn't fulfil the CCHR ($n = 39$), were reported to have normal CT scans. The CCHR has 100% sensitivity as a screening tool for patients requiring CT brains in case of TBI though the specificity is found to be rather low (45.8%) (Lamba et al. 2021). Adding a biomarker to the clinical decision rules significantly increases diagnostic performance for predicting intracranial injury.

Comparison of S100B to two clinical decision rules – CCHR and NOC – for predicting traumatic intracranial injuries after mTBI was performed. The diagnostic performance of S100B (calcium binding protein) for predicting intracranial injury on head CT was compared to both the CCHR and NOC. Area under receiver operator character-

istic curves (AUC) was used and multivariable analysis was used to create a new decision rule based on a combination of S100B and decision rule-related variables. S100B had the highest negative predictive value (97.3%), positive predictive value (7.21%), specificity (33.6%) and positive likelihood ratio (1.3), and the lowest negative likelihood ratio (0.5). The proportion of mTBI subjects with potentially avoidable head CT scans was highest using S100B (37.7%). The addition of S100B to both clinical decision rules significantly increased AUC. A novel decision rule adding S100B to three decision rule-related variables significantly improved prediction ($p < 0.05$). Serum S100B outperformed clinical decision rules for identifying m TBI patients with intracranial injury. Incorporating clinical variables with S100B maximized intracranial injury prediction, but requires validation in an independent cohort (Jones et al. 2017).

Fluid biomarkers of TBI

A biomarker is an indicator of a specific biological or disease state that can be measured using samples taken from either the affected tissue or peripheral body fluids. In other organ disease and injury, the measurement of organ-specific markers is routinely used clinically as a rapid diagnostic tool. So far, in TBI, there has been no such definitive biomarker (Lee et al. 2015).

TBI biomarker attributes

In order for a biofluid-based TBI protein biomarker to be clinically useful, ideally it should have as many of the following attributes as possible (adapted from Wang et al. 2018):

1. The protein biomarker levels should be readily measured in accessible biofluid such as cerebrospinal fluid (CSF), serum, plasma and/or whole blood in TBI patients.
2. The biomarker levels must be elevated in various forms and/or severities of human TBI in the acute phase (3 h to 24 h post-injury), when compared to normal control.
3. The biomarker must have low background or basal biofluid levels in general non-injured healthy control population.
4. The biomarker detected in biofluid after TBI should be derived from or originated from the injured brain as the major source.
5. The biomarker levels in the above stated biofluids should be readily determined and quantified using sandwich ELISA or similar immunoassays with at least two assay formats or platforms.
6. There should be one or more available assay platform for such a biomarker with test-retest reliability and reproducibility, that meet assay analytical performance requirements acceptable to USA FDA (Food and Drug Administration), and other regulatory authorities.
7. The biomarker should be translational in nature with demonstrated evidence that there are similar to biofluid profiles in at least two different animal models of TBI (e.g. rodent control cortical impact, fluid percussion injury, closed head injury, penetrating ballistic brain injury or blast overpressure-wave brain injury).
8. The biomarker should be sensitive to the severity of TBI as defined by GCS, CT abnormality.
9. The biomarker should allow for repeated detections in one of the above-mentioned biofluids within a 48 h window following brain injury.
10. The biomarker should have initial acute levels (within first 48 h post-injury) that correlate with currently available and commonly accepted TBI patient outcome indices such as Glasgow outcome scale (GOS) or GOS-extended (GOS-E).
11. The post-TBI biofluid levels of the biomarker should be responsive to therapeutic treatments.

No single biomarker reflects all known pathophysiological mechanisms of TBI, particularly given their dynamic trajectories over time. In addition, the "majority of TBI biomarker research has focused on diagnostic biomarkers of acute TBI within the first 24 h after injury" (Kenney et al. 2021), and few candidates have been identified for the diagnosis of subacute (up to 1 week post-injury) or chronic sequelae after TBI (3 months to years).

More recently, the development of ultrasensitive assay techniques has generated interest in a new set of protein biomarkers as diagnostic and prognostic aids in TBI. These include glial fibrillary acid protein, ubiquitin C-terminal hydrolase, neurofilament light and total tau (t-tau). Each of these biomarkers has distinctive features and different temporal dynamics, and may provide complementary information about overall injury burden and potentially to specific tissue compartments at different time point's post-TBI. All of these have shown promise in recognizing those patients who have visible traumatic abnormalities in conventional imaging (CT/MRI), or in aiding in outcome prediction.

Candidates for mTBI biomarkers

Since collection of peripheral blood samples is considerably easier than collection of CSF in routine clinical practice, many candidate CSF biomarkers of mTBI have also been assessed in peripheral blood. The low concentration of potential biomarkers in peripheral blood is a technical limitation to the use of most standard immunoassays (Zetterberg et al. 2013). Evidence shows that biofluid (CSF, blood) levels of most acute TBI markers will return to baseline levels within a matter of days following TBI, especially for those who suffered from mild brain injury. Yet subacute and chronic effects of TBI can persist for months following the initial injury event (Wang et al. 2021).

Mirroring the different pathophysiologic processes occurring in TBI, a panel of TBI biofluid-based protein biomarkers has now been identified.

One of the most established approaches to developing fluid biomarkers for TBI is identifying proteins abundant in brain cells, such as:

astroglia: S100B protein, glial fibrillary acidic protein (GFAP);

neurons: neuron-specific enolase (NSE), ubiquitin C-terminal hydrolase-L1 (UCH-L1);

axonal cytoskeleton: tau, phosphorylated tau (p-tau), amyloid β (Aβ), neurofilament light chain (NfL), phosphorylated neurofilament heavy chain (p-NfH) and spectrin N-terminal fragment (SNTF);

oligodendrocytes: myelin basic protein (MBP).

The second approach is to study inflammatory cytokines, metabolites and oxidized lipids or to perform autoimmune profiling of novel TBI biomarkers associated with the pathophysiology of brain injury (Bogoslovsky et al. 2016).

Biomarkers of TBI diagnosis – biomarkers in the acute setting

The majority of TBI biofluid biomarker research has focused on diagnostic blood biomarkers of acute TBI, within the first 24 h after injury.

The first combination TBI biomarkers to receive FDA approval in acute TBI are GFAP and UCH-L1 with the Banyan Brain Trauma Indicator (BTI).

Research has shown that the BTI test has high sensitivity and a high negative predictive value for predicting traumatic intracranial injuries on head CT scan acutely after TBI, and for distinguishing CT-positive, more severely injured, from CT-negative, mTBI patients. The BTI test is not approved for the diagnosis of TBI; rather, its indication is to identify TBI patients with intracranial lesion that may require surgical intervention (Wilde et al. 2012).

GFAP is an intermediate filament protein associated with the astroglial cytoskeleton. It is specific to the nervous system, and increased GFAP immunoreactivity is used as an indicator of brain injury following experimental models of mTBI. GFAP was first successfully measured in human blood in 1999, with serum GFAP levels elevated in 12 of 25 patients with sTBI (Kulbe et al. 2016). Over the course of 1 week, GFAP demonstrated a diagnostic range of areas under the curve for detecting mild and moderate TBI of 0.73 to 0.94. For detecting intracranial lesions on CT, the diagnostic ranges of areas under the curve were 0.8 to 0.97. GFAP performed consistently in detecting mild and moderate TBI, CT lesions and neurosurgical intervention across 7 days (Papa et al. 2016).

UCH-L1 is a neuronal brain injury biomarker found in high abundance in neurons. Previously has been used as a histological biomarker. UCH-L1 is a small (25 kDa) neuronal protease involved in either the addition or removal of ubiquitin from proteins that are destined for metabolism *via* the ATP-dependent proteasome pathway; it is abundantly expressed in the brain (1–5% of total soluble brain protein). UCH-L1 is released into the extracellular space as a consequence of cell destruction under diverse pathological conditions affecting the brain. Previous clinical studies have demonstrated increased UCH-L1 levels in cerebrospinal fluid and in serum in sTBI patients and that the magnitude of this increase correlated with injury severity, CT finding and patient outcome. Recently, a study was completed investigating UCH-L1 in adults with mild and moderate TBI showing increased UCH-L1 levels in mTBI patients compared to uninjured controls and that UCH-L1 was able to detect intracranial lesions on CT with an area under the curve of 0.73 (Papa et al. 2012). Based on these encouraging results and the fact that UCH-L1 is specific to neurons and its high specificity and abundance in the CNS, it appears to be an excellent candidate biomarker for the brain injury clinical studies (Kou et al. 2013). UCH-L1 performed best in detecting mild and moderate TBI, CT lesions in the early postinjury period. Over the course of 1 week UCH-L1 demonstrated diagnostic range of 0.3 to 0.67. For detecting intracranial lesions on CT the diagnostic range of areas under the curve were 0.31 to 0.77 for UCH-L1. UCH-L1 performed best in detecting mild and moderate TBI, CT lesions and neurosurgical intervention in the early postinjury period (Papa et al. 2016). UCH-L1 is detectable as early as 1 h after TBI, peaks at 8 h, and then declines slowly over 48 h after injury (Wilde et al. 2012).

S100B is a calcium binding protein. It is the most extensively studied biomarker in all severities of TBI. S100B is not specific to the brain. It is also found in Schwann cells, chondrocytes, adipocytes, and exocrine cells. S100B has been implemented into clinical practice in Scandinavia where guidelines for the initial management of minimal, mild, and moderate head injury recommend that GCS 14–15 patients with no risk factors and a serum S100B < 0.10 μg/l measured within six hours of injury be discharged without a CT scan (Kulbe et al. 2016). Based on the results of S100B kinetics studies, guidelines have specifically indicated a time window within 3 to 6 h post-injury for S100B to detect intracranial lesions (Mondello et al. 2021). In summary, low serum S100B levels in the first few hours following injury, when combined with other diagnostic measures, provide reassurance regarding the relatively 'mild' nature of the brain injury, but are not by themselves diagnostic. With more severe brain injuries, greater serum S100B levels tend to be associated with slower recovery and worse outcomes, although high S100B levels may be due to causes other than or in addition to brain injury (Kulbe et al. 2016).

NSE is a glycolytic protein located in the cytoplasm of neurons (Vinores et al. 1984). Contrary to its name, NSE is

not specific to neurons, as it is also found in neuroendocrine cells, oligodendrocytes, thrombocytes, and erythrocytes (Dash et al. 2010). Serum levels are increased during cardiopulmonary bypass (Johnsson et al. 2000), trauma, shock and ischemic-reperfusion injury (Pelinka et al. 2004b; 2005). Due to the high concentration of NSE in erythrocytes, even invisible hemolysis can increase levels in serum samples, and NSE can only be evaluated accurately in non-hemolyzed samples that have been stored properly (Ramont et al. 2005). In a study of 104 mTBI patients and 92 healthy controls, serum NSE (<6 h) was significantly elevated in mTBI patients, but the overlap with controls was deemed too considerable for NSE to be of diagnostic value (de Kruijk et al. 2001).

Alpha-II spectrin is the major structural component of the axonal cytoskeleton. Levels of SBDPs (spectrin breakdown products) in cerebrospinal fluid have been shown to rise in adults with sTBI and they have shown a significant relationship with the severity of injury and clinical outcome. Serum SBDPs have been measured in TBI patients and levels were significantly greater in subjects with moderate and sTBI than in control patients. However, this relationship was not demonstrated in patient with mTBI (Jones et al. 2017). The calpain-derived αII-**spectrin N-terminal fragment (SNTF)** accumulates in axons after traumatic injury and increases in human blood after mild traumatic brain injury in relation to white matter abnormalities and persistent cognitive dysfunction. In ice hockey players, compared with preseason levels, serum SNTF increased at 1 h after concussion and remained significantly elevated from 12 h to 6 days, before declining to preseason baseline (Siman et al. 2015).

Biomarkers of disease prognosis

There is a need for biomarkers to facilitate return-to-play/work/school/duty decisions for mTBI and to predict who may experience prolonged post-concussive symptoms. The use of prognostic biomarkers is less mature than that of diagnostic biomarkers. It is easier to validate short-term prognostic biomarkers because of their closer temporal association to the outcome of interest. Prognostic biomarkers in TBI can be divided into two categories: those that are measured early after injury and predict evolving TBI sequelae, and those that are measured in the chronic phases and predict long-term outcomes, such as the development of neurocognitive or movement disorders (Wilde et al. 2022)

Neurofilaments are components of the neuronal cytoskeleton. Following TBI, calcium influx into the cell triggers a phosphorylation cascade that contributes to axonal injury. Elevated levels of hyperphosphorylated neurofilaments have been found in the CSF of patients with sTBI compared with controls. Similarly, p-NF levels in venous blood have been shown to correlate with the severity of TBI in children. Comparisons in patients with mTBI and healthy controls were performed. They demonstrated that mTBI patients exhibited a significant increase in the serum levels of p-NF on days 1 ($p < 0.001$) and 3 ($p < 0.001$) following injury and the area under the curve of the receiver operating characteristic curve analysis for p-NF in mTBI was 100% at both 24–72 h post injury. Early work in animals demonstrated a serum rise in p-NF 6 h after injury, with levels peaking at 24–48 h before they gradually returned to baseline. This 6-h lag between the onset of injury and the rise in blood levels of p-NF may limit the usefulness of this biomarker as an aid to diagnosis in the acute setting. However, it may be a useful biomarker when used for prognostic purposes (Jones et al. 2020). It is also considered a marker of delayed axonal injury (Wang et al. 2021).

NfL is identified by several studies as a promising prognostic biomarker in TBI of all severities. Elevated levels of both plasma and exosomal NfL is associated with multiple (≥3) mTBIs and remote neurobehavioral symptoms in service members and veterans enrolled in the Chronic Effects of Neurotrauma Consortium longitudinal study. Serum NfL correlates with persistent post-concussive symptoms with an AUC of 0.81 in Swedish hockey players. Among civilian TBI survivors ($n = 230$), serum NfL correlates with initial injury severity and 5-year functional outcomes, as well as with imaging measures of atrophy and axonal injury, attesting not only to its predictive ability, but also multimodal validation. Brain-derived exosomal NfL is associated with decreased cognitive function in elderly veterans with remote TBI symptoms.

MBP is a component of oligodendrocytes of the central nervous system and Schwann cells of the peripheral nervous system (Barbarese et al. 1988). It is the second most abundant protein in CNS myelin (Boggs et al.2006) and is found in the CSF of patients with demyelinating diseases such as multiple sclerosis (Lamers et al. 2003). Since oligodendrocyte/white matter damage occurs during DAI, a characteristic of mTBI (Sharp et al. 2011), MBP has been identified as a potential biomarker of mTBI. MBP is unlikely to be a useful screening tooling for TBI because MBP levels do not peak promptly. Since MBP peaks in serum between 48 h and 72 h post-injury and can remain elevated for up to two weeks (Berger et al. 2005), it may be of greatest value in post-acute mTBI. Thus, it can be considered a delayed demyelination marker (Wang et al. 2018).

Tau also has promise as a prognostic biomarker. It is a microtubule associated binding protein that provides cytoskeletal support and facilitates axonal transport, as well as having many other physiological functions. It is also found in the liver, kidney and testes (Morris et al. 2011). Tau undergoes post-translational modifications, such as phosphorylation, necessary for its regular function. Abnormal phosphorylation, however, triggers microtubule-bound tau to be released. Hyperphosphorylated tau aggregates generate neurofibril-

lary tangles that are considered the pathological hallmark of tauopathies including Alzheimer disease, chronic traumatic encephalopathy, frontal-temporal dementia and others (Edwards et al. 2020).

Studies have reported elevated plasma t-tau and **p-tau**, as well as a ratio of p-tau over tau in sTBI patients 6–8 months after injury. T-tau in blood samples collected 1 h after sports-related concussion showed diagnostic accuracy for TBI. A recent study found that levels of tau, ß-amyloid-42, and IL-10 were higher in exosomes of military personnel who had experienced mTBIs than in personnel who had not. Among TBI patients, regression models show that post-concussive symptoms are most related to exosomal tau elevations, whereas exosomal IL-10 levels relate to PTSD (post-traumatic stress disorder) symptoms (Gill et al. 2018). In the CENC (Chronic Effect Neurotrauma Consortium) cohort ($n = 195$), experiencing multiple (≥3) mTBIs is associated with increased exosomal t-tau and p-tau as well as with late neurobehavioral symptoms.

Biomarkers of disease progression

There is substantial interest in the potential for traumatic brain injury to result in progressive neurological deterioration. Increasing evidence has suggested that TBI may also be a risk factor for the development of age-associated neurodegenerative disorders including Alzheimer disease (AD), Parkinson's disease, Amyotrophic lateral sclerosis and Multiple sclerosis.

It is also important to understand how a biomarker can be used to advance treatment for chronic somatosensory, neuropsychiatric, and cognitive deficits post-TBI (Wilde et al. 2022).

Traumatic brain injury has been suggested as a risk factor for tauopathies by triggering disease onset and facilitating its progression. Several studies indicate that TBI seems to be a risk factor to development of AD and chronic traumatic encephalopathy (CTE), because there is a relationship of TBI severity and propensity to development of these illnesses (Wilde et al. 2022). Predictive biomarker value in postconcussion symptoms and cognition deficit persistence is presented in Table 2.

In CTE, p-tau is found at first around the small vessels at the depths of the sulci in the cerebral cortex (stage I), then in the superficial layers of the adjacent cortex (stage II), later in the frontal, insular, temporal and parietal cortices, and amygdala, hippocampus, and entorhinal cortex (stage III), and finally p-tau pathology is found widespread in the entire brain (stage IV). The risk of CTE is also suggested to be increased when linked to the number of TBI events and the length of time contact sports athletes and military personnel are active.

In animal study just one day after the TBI induction, pathological tau in P301S TBI mice was detected primarily in the overall and ipsilateral area of the impacted side, compared to age-matched sham mice. This shows that TBI induces rapid acceleration of tau hyperphosphorylation (Edwards et al. 2020).

Majority of studies is based on tau phosphorylated on threonine 181 (p-tau 181). Higher plasma tau levels collected within the first 6 h after injury may be prognostic of prolonged recovery from acute sports concussions. Amyloid isoforms, including amyloid beta 40 and amyloid beta 42, are associated with axons and accumulate as early as 2–3 h after TBI as a result of injured axons. However, acute CSF levels of these proteins are increased only after severe and not after mTBI, making them less broadly useful as diagnotic biomarkers. This may be because of the microstructural organization of neurons being remote to capillaries and vessels, whereas astrocytes directly contact blood vessels with their end feet (Wilde et al. 2022).

CSF levels of tau protein molecules that have been proteolytically cleaved (c-tau) are significantly elevated following

Table 2. Predictive biomarker value in postconcussion symptoms and cognition deficit persistence

	Posttraumatic symptoms, cognition deficit
S100B protein	6 months post-injury forgetfulness, dizziness, headache, nausea and vomiting at level of biomarker >0.3μg/l, <6 h *(De Kruijk et al. 2002).* Several studies did not confirm correlation of elevated biomarker level with posttraumatic signs and cognition disturbances *(Kulbe et al. 2016).*
SBDP/SNTF	Correlation of levels SNTF with duration of post-concussion symptoms. Ice hockey players, whose symptoms took longer than six days to resolve, had significantly higher levels of SNTF 12–36 h post-concussion than players whose symptoms resolved more quickly *(Siman et al. 2013).*
NSE	6 months post-injury an elevated serum NSE >0.1μg/l, <6 h was significantly associated with headache *(De Kruijk et al. 2002).*
Tau	Worse performance in memory tests *(Bogoslovsky et al. 2016).*
GFAP	There was not strongly association between initial GFAP value and postconcussive symptoms in children, when evaluated 1 month post-injury *(Babcock et al. 2016).* Small number of studies evaluating GFAP and results of neuropsychological testing in mTBI.
UCH-L1	Inverse correlation of UCH-L1 and postconcussive symptoms *(Babcock et al. 2016).*

TBI and these levels correlate with clinical outcome. However, c-tau and t-tau protein levels in peripheral blood have been shown to be a poor predictor of traumatic lesions on CT and postconcussion syndrome (Jones et al. 2017).

Elevated levels of p-tau are seen in the brain in CTE for years following mTBI or repeated concussions as a significant tauopathic neurodegenerative disease, likely occurring as a result of repeated concussions (McKee et al. 2009; Omalu et al. 2010). Using the high sensitivity SIMOA platform (Quanterix), tau can be observed in the acute to subacute/chronic stage following mTBI (hockey players and military veterans). In parallel, an ultrasensitive rolling cycle amplification based ELISA format platform has been recently developed for both t-tau and p-tau assays. This found elevations of serum p-tau and t-tau from severe human TBI and in rodent repetitive mTBI in both the acute and subacute period (Rubenstein et al. 2015; Yang et al. 2015).

Rubenstein et al. (2017) demonstrated that p-tau plasma levels and p-tau/t-tau ratios outperformed t-tau as diagnostic and prognostic markers of TBI, and, compared with t-tau levels alone, p-tau levels and p-tau/t-tau ratios show more robust and sustained elevations among patients with chronic TBI.

While blood biomarkers such as GFAP and NfL have been widely explored in characterizing acute traumatic brain injury, their use in the chronic phase is limited. Given increasing evidence that these proteins may be markers of ongoing neurodegeneration in a range of diseases, Newcombe's study examined their relationship to imaging changes and functional outcome in the months to years following TBI. Two-hundred and three patients were recruited in two separate cohorts; 6 months post-injury ($n = 165$) and >5 years post-injury ($n = 38$; 12 of whom also provided data ~8 months post-TBI). The persistent elevation of GFAP and NfL at 8 months was significantly related to contemporaneous metrics of microstructural injury on DTI, as measured by mean diffusivity and fractional anisotropy in whole brain white matter, and mean diffusivity in cortical gray matter and deep gray matter. They confirm that patients with TBI show a greater predicted brain age difference than normal (suggesting accelerated brain aging in the TBI cohort). Critically, in patients where data were available at both 8 months and >5 years, we show that NfL levels at 8 months predicted white matter volume loss at >5 years, and indexed JD (Jacobian determinant) – a voxel-based measure of annual brain volume loss – between 8 months and 5 years. Finally, we show that late protein biomarker and imaging changes are potentially clinically relevant, since patients who worsened functionally between 8 months and >5 years showed a higher PAD and elevated levels of NfL compared to those who improved or remained stable (Newcombe et al. 2021). An overview of mTBI biomarkers is presented in Table 3.

Mondello et al. in 2021 conducted a systematic review and meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Comparative efficacy of mTBI biomarkers is shown in Table 4, also adding different recent sources.

Promising multimarker analysis

Two promising brain injury biomarkers have emerged for mTBI, namely, GFAP and UCH-L1.

The ALERT-TBI trial was designed to validate the ability of a biomarker test combining UCH-L1 and GFAP to predict CT-detected traumatic intracranial injuries within 12 h of TBI. A total of 1959 patients with TBI and valid head CT scan were included. Among all patients, both GFAP and UCH-L1 concentration were significantly higher among those who were CT positive compared with those who were CT negative. This study results showed that serum biomarker test combining GFAP and UCH-L1 had a sensitivity 97.6% and negative predictive value (NPV) 99.6% (Bazarian et al. 2018).

Current evidence indicates that both serum GFAP and UCH-L1 are detectable in serum in less than 1 h after a mTBI and can distinguish between patients with mTBI and other trauma patients without acute brain injury following the traumatic incident. GFAP and UCH-L1 levels are significantly elevated in patients with TBI with intracranial lesions on computed tomography and, in patients with mTBI, can distinguish between those with a normal and abnormal CT scan of the brain. The sensitivity of GFAP and UCH-L1 for detecting intracranial lesions on CT ranges between 94% to 100% in both children and adults. Notably, GFAP and UCH-L1 are elevated in patients with mTBI requiring neurosurgical intervention, and can predict with high sensitivity which patients with mTBI will require neurosurgery. The results of these studies suggest that both GFAP and UCH-L1 are specific for brain injury. Preliminary studies have shown benefit in combining these 2 biomarkers for predicting CT lesions. UCH-L1 is detectable within 1 h of injury. UCH-L1 rose rapidly and peaked at 8 h after injury, then declined rapidly over 48 h. GFAP peaked at 20 h after injury and slowly declined over 72 h (Papa et al. 2016).

How does this blood test compare with validated clinical decision rules published Papa et al. (2022)? 349 adult patients with suspected mTBI presenting within 4 h of injury, all of whom underwent a CT scan, were included in Papa's study. 314 (90%) had a GCS score of 15, and 23 (7%) had positive CT findings. The clinical decision rules included the CCHR, New Orleans Criteria, and National Emergency X-Radiography Utilization Study II criteria. Blood samples for measuring GFAP and UCH-L1 levels were drawn, but laboratory personnel were blinded to clinical results. In this cohort study, the CCHR, the NOC, and GFAP plus UCH-L1 biomarkers had equally high sensitivities, and the CCHR had

Table 3. Overview of mTBI biomarkers

	Biomarker origin	CNS specificity	Early posttraumatic serum detection	Correlation with mTBI	Correlation with serious TBI	Found in various disorders	Biomarker limitations
S100B protein	Astrocytes, oligodendrocytes, adipocytes, chondrocytes, peripheral tissue *Kulbe et al. 2016*	No (positivity in polytrauma) *Gan et al. 2019*	Detection 3–6 h post injury *Mondello et al. 2021*	Yes Low serum S100B levels in first few hours following injury provide reassurance regarding the relatively „mild" nature of the brain injury *Kulbe et al. 2016*	Yes *Kulbe et al.2016*)	Polytrauma, melanoma, myocardial ischemia, ischemic-reperfusion injury *Kulbe et al. 2016*	Not specific to the brain
GFAP	Astrocytes, ependymal cells *Papa et al. 2012*	Yes *Papa et al. 2012*	Yes (early detection in 4 h, peak concentration 12–20 h after injury *Papa et al. 2016*	Yes *Papa et al. 2016*	Yes *Papa et al. 2016*	No *Papa et al. 2012*	Cut-off values
MBP	Oligodendrocytes, Schwann.cells, peripherial system nerve cells *Kulbe et al. 2016*	Yes (+peripherial system nerve cells) *Zetterberg et al. 2013*	No (do not peak promptly) 48–72 h after injury *Kulbe et al. 2016*	Yes *Kulbe et al. 2016*	Yes *Kulbe et al. 2016*	Multiple sclerosis *Kulbe et al. 2016*	May be of greatest value in post-acute mTBI
Tau protein	Axons, liver, kidney, testes	No	No Better detected in CSF than in serum *Jones et al. 2017*	Inconsistent data *Jones et al. 2017*	Inconsistent data *Jones et al. 2017*	AD, CTE	More sensitive analysis methods are needed
NfL/ pNF-H	Myelinated axons, dendrites *Gatson et al. 2014*	Yes *Gatson et al. 2014*	Detection 6 h after injury (peak values in 24–48 h after injury *Jones et al. 2017*	Yes *Jones et al. 2017*	Yes *Jones et al. 2017*	Multiple sclerosis, AD, amyotrophic lateral sclerosis (ALS)	6-h lag between the onset of injury and the rise in blood levels may limit its usefulness in the acute setting *Kulbe et al. 2016*
SNTF	Axons, presynaptic neurons *Zetterberg et al. 2013*	No	Rise in 1 h after injury, stay stable 12 h–6 days *Siman et al. 2015*	Non significant elevation *Siman et al. 2014*	Yes *Siman et al. 2014*	–	Non significant elevation in mTBI
NSE	Neurons,oligodendrocytes, RBC, PLT, endocrine cells *Papa et al. 2015*	No *Papa et al. 2015*	Detected in first 6 h after injury, but values overlaped with controls *Kulbe et al. 2016*	No *Kulbe et al. 2016*	Yes (values in ventricular CSF correlated with GCS) *Jones et al. 2017*	Cardio-pulmonary bypass, shock state, ischemic-reperfusion injury, hemolysis *Kulbe et al. 2016*	False positivity in case of hemolysis *Papa et al. 2015*
UCH-L1	Neurons, peripheral neurons, neuromuscular junction, diffuse neuroendocrine system *Papa et. al. 2010*	Almost exclusively in brain *Papa et al. 2010*	Rise rapidly after injury, reach peak at 8 h *Papa et al. 2016*	Yes *Kulbe et al. 2016*	Yes *Kulbe et al. 2016*	–	Cut-off values

Table 4. Comparative efficacy of mTBI biomarkers for discriminating between TBI patients with intracranial lesions on CT (performance in detecting intracranial lesions on CT)

Biomarker	Sensitivity (%)	Specificity (%)	Thresholds	AUC	Reference
S100B	96	31	0.1–0.11 μg/l		*Mondello et al. 2021*
	80	74.4	0.15 μg/l		*Stranjalis et al. 2004*
	91	30	0.1 μg/l		*Seidenfaden et al. 2021*
	61	69	0.72 μg/l		*Seidenfaden et al. 2021*
GFAP	67–100	0–89	0–0.6 ng/ml		*Mondello et al. 2021*
	100	55	0.067 ng/ml		*Papa et al. 2014*
				0.93	*Gill et al. 2018*
	71	71	0.626 ng/ml		*Amoo et al. 2022*
NSE	56–100	7–77	9–14.7 μg/l		*Mondello et al. 2021*
	71	64	11.36 ng/ml	0.85	*Berger et al. 2005*
UCH-L1	100	21–39	0.029–0.04 ng/ml		*Mondello et al. 2021*
	100	21	0.09 ng/ml	0.73	*Papa et al. 2012*
Tau	50	75			*Mondello et al. 2021*
pNfL	100	100	110.5 pg/ml		*Gatson et al. 2014*
UCH-L1 combined with GFAP	97.6	36.4	327 pg/ml 22 pg/ml		*Bazarian et al. 2018*

the highest specificity. However, using different cutoff values reduced both sensitivity and specificity of GFAP plus UCH-L1. Use of GFAP significantly improved the performance of the clinical decision rules, independently of UCH-L1. Together, the CCHR and GFAP had the highest diagnostic performance (Papa et al. 2022).

Also the prognostic value of GFAP and UCH-L1 as day-of-injury predictors of functional outcome after traumatic brain injury was studied. Patients from the Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) observational cohort study were enrolled. They had day-of-injury plasma samples for measurement of GFAP and UCH-L1 and completed 6-month assessments for outcome due to traumatic brain injury with the Glasgow Outcome Scale-Extended. Data from 1696 participants with brain injury were available. The AUC of GFAP for predicting death at 6 months in all patients was 0.87 (95% CI 0.83–0.91), for unfavorable outcome was 0.86 (0.83–0.89), and for incomplete recovery was 0.62 (0.59–0.64). The corresponding AUCs for UCH-L1 were 0.89 (95% CI 0.86–0.92) for predicting death, 0.86 (0.84–0.89) for unfavorable outcome, and 0.61 (0.59–0.64) for incomplete recovery at 6 months. AUCs were higher for participants with traumatic brain injury and Glasgow Coma Scale score of 3–12 than for those with GCS score of 13–15 (Korley et al. 2022).

In a Mahan study of 118 trauma subjects who received clinically ordered head CT, blood samples were collected at 0–8 h (initial) and 12–32 h (delayed) postinjury and analyzed for GFAP, UCH-L1, and S100B concentrations. These were then compared in CT-negative and CT-positive subjects. Median GFAP, UCH-L1, and S100B concentrations were greater in CT-positive subjects at both timepoints compared with CT-negative subjects. In addition, median UCH-L1 and S100B concentrations were lower at the delayed timepoint, whereas median GFAP concentrations were increased. As predictors of a positive CT of the head, GFAP outperformed UCH-L1 and S100B at both timepoints (initial: 0.89 sensitivity, 0.62 specificity; delayed: 0.94 sensitivity, 0.67 specificity). GFAP alone also outperformed all possible combinations of biomarkers. GFAP, UCH-L1, and S100B demonstrated utility for rapid prediction of a CT-positive TBI within 0–8 h of injury. GFAP exhibited the greatest predictive power at 12–32 h. Furthermore, these results suggest that GFAP alone has greater utility for predicting a positive CT of the head than UCH-L1, S100B, or any combination of the 3 (Mahan et al. 2019).

In a study published by Whitehouse, 2869 patient were included. The intent was to identify lesion type using biomarkers. All severities of TBI (mild, moderate and severe) were included for analysis with a majority (n = 1946, 68%) having a mild injury (GCS 13-15). Patients with severe diffuse injury (Marshall III/IV) showed significantly higher levels of all measured biomarkers, with the exception of NfL, than patients with focal mass lesions (Marshall grades V/VI). Patients with either diffuse axonal injury + intraventricular haematoma (IVH) or subdural haematoma (SDH) + intraparenchymal haemorrhage (IPH) + traumatic subarachnoid haemorrhage (tSAH), had significantly higher biomarker concentrations than patients with extradural haematoma (EDH). The complex and heterogenous nature of TBI creates substantial overlap between pathoanatomical lesions, making it difficult for biomarkers to identify individual lesion types (Whitehouse et al. 2021).

Compared to healthy volunteers, plasma NfL, beta-synuclein, and GFAP were significantly increased after polytrauma. The markers demonstrated highly distinct time courses, with beta-synuclein and GFAP peaking early and NfL concentrations gradually elevating during the 10-day observation period. At the time of admission, all three markers differentiated well between trauma patients who sustained a TBI and those who did not, with the combination of GFAP and beta-synuclein showing superior discriminating potential (AUC: 0.93) compared to each marker alone (GFAP AUC: 0.89, beta-synuclein AUC: 0.79, and NfL AUC: 0.79). This finding promotes the idea of combining neurological markers reflecting structurally different injuries to the brain to obtain a fluid-biomarker-supported TBI diagnosis (Halbgebauer et al. 2022) These results are in line with a study on serum GFAP where discrimination between isolated TBI and non-TBI trauma patients revealed an AUC for GFAP of 0.89 (Laverse et al. 2020). This is the first longitudinal study on severe trauma patients including a plasma synaptic biomarker, demonstrating elevated levels of beta-synuclein, NfL, and GFAP in the time course after injury, with a high early predictive potential of outcomes (Halbgebauer et al. 2022).

How long do blood biomarker levels remain elevated after mTBI?

A few studies have examined biomarker levels several months or years after mTBI. Blood half-lives of most biomarkers (UCH-L1, S100B, GFAP) are short (less than 48 h).

An alternative to direct passage across a disrupted blood brain barrier was recently proposed. The so-called glymphatic system is, according to this hypothesis, responsible for the migration of S100B and GFAP from the injured brain into the peripheral blood. There are several important considerations that glymphatic drainage of astrocytic protein implies. First, it may explain secondary delayed biomarker surges after TBI (Shahim et al. 2020). Cerebral edema may trigger pathological changes in GFAP and S100B brain synthesis, resulting in more significant extravasation *via* the glymphatic pathway. Second, it may also explain the accumulation of tau protein in the CNS after severe trauma (Iliff et al. 2014).

TBI biomarkers as drug development tools

Some preclinical animal experimental therapeutic studies support the use of TBI biomarkers as therapeutic development tools. There is additional evidence that the post-TBI elevation of GFAP is severity-dependent (Zoltewicz et al. 2013). Large-scale therapeutic clinical trials are also beginning to incorporate blood-based biomarkers to assess the effect of an experimental drug (Wang et al. 2018). Most importantly, one experimental drug, Simvastatin, was found to suppress the levels of serum GFAP at 24 h in fluid percussion injury and penetrating ballistic brain injury models (Mountney et al. 2016). Another experimental drug, nicotinamide, also attenuated serum GFAP levels at 24 h in penetrating ballistic brain injury and control cortical impact models (Shear et al. 2016).

Current state of implementation mTBI biomarkers into clinical practice

To date, the adoption and implementation of mTBI biomarkers into clinical practice is limited.

Implementation of S100B protein

The Nordic Radiation Protection co-operation wrote in 2012 that they were concerned about the rapidly increasing amount of CT examinations in Norway. In 2013 the Scandinavian Neurotrauma Committee (SNC) published updated guidelines for the initial management of minimal, mild and moderate traumatic head injuries that included serum analysis of protein S100B as a marker for brain tissue damage. In the updated guidelines it is estimated that the S100B test could eliminate the need for CT examination in approximately 30% of all patients, based on several studies that have shown a negative predictive value of 97–100% and specificity above 30% with a cutoff value at 0.10 μg/l.

A prospective cohort study was performed in Akershus University Hospital (AHUS), using data collected between from June 30th and December 15th, 2014. Patients with minimal, mild and moderate traumatic head injuries were included, and filled in forms recording the time, indication and result of any S100B sampling and/or head computer tomography examinations. Data from these forms were compared to information derived from the electronic patient records for patients with minimal, mild and moderate traumatic head injuries and related diagnoses, and with data from the laboratory for all patients that had undergone the S100B analysis within the same period. Of the 188 patients, 69 (36.7%) patients had a negative S100B test, defined as values less than 0.10 μg/l. Still, in 31 of these patients a CT examination was performed, therefore the number of CT examinations avoided based on the S100B screening was 38. This represented 8.2% of all minimal, mild and moderate traumatic head injuries patients potentially requiring a head CT ("moderate" to "mild" groups, and 21.1% of all directly discharged patients). There was no intracranial pathology found in any of the 31 patients where a CT scan was performed despite a negative S100B result, suggesting CT scanning was not necessary in these patients and supporting the proposed threshold for S100B plasma level of 0.10 μg/l.

As previously mentioned, the clinical usefulness of S100B also depends on the time it takes from the clinical evalua-

tion to the availability of a test result, which in the case of a positive S100B test will be the same as a delay in having an indicated CT examination. A near 2-h delay in the diagnosis and management of a traumatic brain haemorrhage may, in some cases, have severe or even fatal consequences. The implementation of the S100B and the updated SNC guidelines resulted in one third of the minimal, mild and moderate traumatic head injuries cases being discharged without further observation or CT examinations. Reassuringly, no readmissions or missed severe traumatic injuries to the brain were observed (Ananthaharan et al. 2018).

S100B plasma testing reduces processing time. Is turnaround time for plasma shorter than for serum?

Despite being already included in some guidelines, the implementation of S100B testing into standard care is still lacking. This might be explained by a turnaround time too long for serum S100B to be used in clinical decision-making in emergency settings. Plasma-based S100B testing compares favorably with serum-based testing, substantially reducing processing times, which is one of the prerequisites for integrating S100B level into management of TBI patients. The proposed new clinical decision algorithm for TBI management needs to be validated in further prospective large-scale studies. S100B concentrations in the peripheral blood of healthy individuals are low (<0.105 ng/ml), with elevated levels being associated with various pathologic conditions including TBI. Nevertheless, in clinical practice, S100B is mainly used as a protein tumor marker for follow-up of melanoma patients. Hence, in this elective setting, assay turnaround times are not decisive. Accordingly, all immunoassay test systems that are commercially available rely on serum for the analysis of S100B. Notably, this also applies to the measurement of S100B in cases of (suspected) TBI and mTBI, potentially constituting critical emergency situations. In order to evaluate whether S100B testing from serum can generally be replaced by plasma-based testing particularly in TBI, a methodical comparison was conducted in 136 matching samples from melanoma and TBI patients. Linear regression demonstrated a high level of agreement between the two testing modalities ($r^2 = 0.9978$). In summary, this study demonstrates the interchangeability of heparin-plasma- and serum-based S100B testing for TBI patients requiring rapid exclusion of organic brain damage. The analytical reliability of S100B testing from plasma was further confirmed by verification studies (Haselmann et al. 2021).

S100B cut-off

In emergency departments, the serum S100B is used as a supportive tool for initial in-hospital triage of adult patients with mTBI. S100B levels < 0.10 µg/l within 6 h of trauma is considered safe for ruling out traumatic intracranial lesions in adult patients with mTBI and its use reduces the number of cerebral CTs and lengths of stay in the emergency departments for patients with mTBI. The biomarker GFAP has been proposed as another candidate for triage and rapid risk-stratification in patients with TBI, but no cut-point for GFAP has been established. In Seidenfaden's study, prehospital and in-hospital blood samples were drawn from 566 adult patients with mild traumatic brain injury with GCS 14–15. The study measured serum S100B and GFAP concentrations, and compared their predictive utility to the reference standard – endpoint adjudication of the traumatic intracranial lesion based on medical records. The primary outcome was prehospital sensitivity of S100B in relation to the traumatic intracranial lesion. Traumatic intracranial lesions were found in 32/566 (5.6%) patients. The sensitivity of S100B > 0.10 µg/l was 100% (95%CI: 89.1;100.0) in prehospital samples and 100% (95% CI 89.1;100.0) in in-hospital samples. The specificity was 15.4% (95%CI: 12.4;18.7) in prehospital samples and 31.5% (27.5;35.6) in in-hospital samples. GFAP was only detected in less than 2% of cases with the assay used. The GFAP results of this study were hampered by the detection limit of the chosen GFAP assay at 0.045 ng/ml. When initiating the study, the GFAP assay was chosen to the best of knowledge at the time. In the meantime, Bazarian et al. (2018) suggested a GFAP cut-off at 0.022 ng/ml, suggesting that an assay with a lower detection limit should have been used for analysis. Thus, the GFAP results from the low-sensitivity assay used in the current study only identified patients with relatively high GFAP concentrations. Only 11 patients had GFAP concentrations above the assay cut-off of 0.045 ng/ml in one of the samples. If these patients had been the most severe cases, these values would have been interesting for triage and identification of high-risk patients. Unfortunately, they were not, and six of these 11 patients did not have an intracranial lesion. Early prehospital and in-hospital S100B levels <0.10 µg/l safely rules out traumatic intracranial lesions in adult patients with mild traumatic brain injury, but specificity is lower with early prehospital sampling than with in-hospital sampling (Seidenfaden et al. 2021). Amoo et al. (2022) reviewed total of 2939 citations, and 38 studies. Thirty-two studies reported data for S100B. At its conventional threshold of 0.1 µg/l, S100B had a pooled sensitivity of 91% (95%CI 87–94) and a specificity of 30% (95%CI 26–34). The optimal threshold in that study for S100B was 0.72 µg/l, with a sensitivity of 61% (95% CI 50–72) and a specificity of 69% (95% CI 64–74). The authors conclude that there is sufficient evidence to support the use of S100B as a screening tool in mTBI (Amoo et al. 2022). However, as a screening tool, sensitivity and negative predictive value should be prioritized over specificity. A sensitivity of 61% means that 4 out of 10 mTBI patients

managed using this test threshold would have a negative test result, despite brain pathology that would be clearly visible on CT scan. Since the results of S100B testing is used to determine the need for CT scans, CT scan would not be performed in these cases, and the patient would be discharged with the brain pathology undetected. The authors of this review caution against such rash notions of "optimization", which could harm patients, as well as hurting the entire field of TBI biomarker testing by making it, seem an unreliable and dangerous practice. We maintain that mTBI biomarker testing should be explored first and foremost as a screening tool with high sensitivity, even if this means that many test results end up being false positives.

Serum levels of S100B obtained within 12–36 h from TBI, taken from patients in neurointensive care units, correlate with patient outcomes (Thelin et al. 2013). These studies have been confirmed by a German research group showing a significant correlation of S100B concentrations in serum and GOS at 6 months. In addition, they also found that serum levels of S100B > 0.7 ng/ml correlate with 100% mortality (Kellerman et al. 2016).

Implementation of GFAP and UCH-L1

The full validation of the diagnostic and prognostic utilities of a TBI biomarker has been a slow process. This is in part due to the lack of clinically compatible platforms that can run such TBI biomarker assays (with the exception of S100B being available on the Roche Elecsys platform), and the lack of formal regulatory agency approval (e.g. FDA). Within the next five years, we anticipate that at least one major diagnostic company (e.g. Abbott i-STAT platform) will overcome these hurdles and make newer markers (e.g. UCH-L1 and GFAP) available for clinical uses. Another trend we are seeing is that an increasing number of diagnostic companies, both large and small, are entering the race by adding new POC (Point-of-care), or automated/semi-automated clinical lab-based assay platforms to TBI-based diagnostics (e.g., including BioMerieux, Phillips, Sysmex, BioDirection and Banyan Biomarkers). We also anticipate that the clinical utilities of additional new markers will be independently validated within this period (Wang et al. 2021).

In Amoo's review, nine studies reported data for GFAP. The "optimal" threshold for GFAP was determined to be 626 pg/ml, at which sensitivity was 71% (95%CI 41–91) and specificity was 71% (95%CI 43–90). Sensitivity of GFAP was maximized at a threshold of 22 pg/ml, which had a sensitivity of 93% (95%CI 73–99) and a specificity of 36% (95%CI 12–68) (Amoo et al. 2022). We maintain that, in the context of biomarkers used for screening, the clinically optimal threshold is the one with the maximized sensitivity, and that this is the threshold that should be used, at least in the initial stages of mTBI biomarker adoption.

In February 2018, the FDA approved the use of the Brain Trauma Indicator, a UCH-L1 and GFAP assay, for determining the clinical necessity of obtaining a head computed tomography scan in patients with mTBI. GFAP and UCH-L1 must be measured together in the United States to assess the need for a head CT scan (Korley et al. 2021). This is the first FDA-approved blood test to detect intracranial lesions after mild to moderate traumatic brain injury. The approval was based on data obtained from a prospective, multicenter ALERT-TBI clinical study by Bazarian and coworkers, discussed in the previous section. This study examined a total of 1947 adults with suspected mTBI, and data collection took place at 24 clinical sites (NCT01426919). 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 must be noted that the above-mentioned Banyan's Brain Trauma IndicatorTM was run on a semiautomated ELISA assay platform, which requires skilled technical personnel to operate, and takes several hours to run. Importantly, the Brain Trauma Indicator has not been commercialized, thus this UCH-L1/GFAP tandem test is still not widely available as a clinical diagnostic test in the clinical setting. 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-alpha. 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 licensing 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 (Wang et al. 2021).

The current guidance from the FDA

The FDA continues to study TBI. It's current guidance, issued in the spring of 2021, states that "none of the medical devices cleared or approved by FDA are intended to be used alone without the judgment of a health care provider trained to diagnose and treat TBI. The FDA has not cleared or approved any medical products that are intended to diagnose or treat TBI alone without other diagnostic tests or treatments managed by a health care provider."

MTBI biomarkers in children - a challenge

In children, several recent large-scale epidemiological studies have described a link between radiation exposure from

CT scans and the risk of future cancers (Pearce et al. 2012; Mathews et al. 2013; Miglioretti et al. 2013). This is why children can be admitted for inpatient observation, with cranial CT scans performed only on those with clinical deterioration. This approach reduces X-ray exposure, but it is more costly than utilizing cranial CT scans for the initial diagnosis (Norlund et al. 2006).

Considering the increased risk from ionizing radiation and the challenging clinical examination of children, a reliable brain biomarker would be important in managing mTBI in these patients. Although studies seem promising, with similar diagnostic performance to adult studies, more data is needed before the test can be recommended in guidelines. The lesions seen on CT in children with sTBI have low sensitivity in predicting outcomes. Therefore, novel objective methods are needed to improve or even replace clinical and radiological parameters that have been associated with outcomes in children with sTBI (Janigro et al. 2022).

For S100B and probably other biomarkers, cut-off levels and paediatric reference ranges had to be defined and used. For mTBI in children, a recent meta-analysis demonstrated the usefulness of serum S100B as a biomarker in the management of pediatric mTBI while emphasizing that a large multicenter study is missing for this population (Oris et al. 2018). Sampling should take place within 3 hours of trauma. Cut-off levels should be based on pediatric reference ranges, because S100B serum concentrations are high at the beginning of life, with mean values around 0.5 µg/l in the first weeks of life, reaching adult values after 18 months (Bouvier et al. 2011; Simon-Pimmel et al. 2017; Oris et al. 2018). In the Bouvier study, the S100B serum assay will be considered positive in children according to age: 0–9 months: >0.35 µg/l, 9–24 months: >0.23 µg/l and >24 months: >0.18 µg/l (Bouvier et al. 2011).

Discussion

Current assessments for acute TBI are limited to physical examination and imaging. A strategy to perform neuroimaging in all patients with head injury guarantees that no structural damage is overlooked, but this is costly and exposes many patients to unnecessary radiation. Clinical decision rules have been developed to select patients for CT scanning.

Early diagnosis of TBI by testing peripheral fluids such as blood or saliva has been the focus of many research efforts, leading to FDA approval for a bench-top assay for blood GFAP and UCH-L1 and a plasma point-of-care test for GFAP. The biomarker S100B has been included in clinical guidelines for mTBI in Europe. Despite these successes, several unresolved issues have been recognized, including the robustness of prior data, the presence of biomarkers in tissues beyond the central nervous system, and the time course of biomarkers in peripheral body fluids (Janigro et al. 2022).

Biomarkers such as GFAP, UCH-L1 and S100 calcium-binding protein B have shown predictive value as indicators of TBI and potential screening tools. Owing to analytical heterogeneity among laboratories, a direct comparison across studies is not always possible. Future side-by-side studies need to use predetermined cut-off values and reproducible, publicly available, measurement strategies.

For each marker, only some assay formats could differentiate TBI from the control. Also, different assays for the same biomarker reported divergent biomarker values for the same biosamples.

Conclusion

The majority of TBI biofluid biomarker research has focused on diagnostic blood biomarkers of acute TBI, within the first 24 h after injury. Adding a biomarker to the clinical decision rules significantly increases diagnostic performance for predicting intracranial injury. In the context of biomarkers used for intracranial injury screening, the clinically optimal threshold is the one with the maximized sensitivity. In clinical practice also assay turnaround time is decisive.

There is a need for biomarkers to facilitate return-to-play/work/school/duty decisions for mTBI and to predict who may experience prolonged post-concussive symptoms. Due to the variability of TBI marker assay in performance and reported values, standardization strategies are recommended when comparing reported biomarker levels across assay platforms.

Conflicts of interest. The authors declare no conflict of interest.

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