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Introduction

Path Toward Blood Test for Concussion

Currently, concussion (which is also known as mild traumatic brain injury) is largely a clinical diagnosis based on injury history, neurologic examination, neuropsychological testing, and, at times, neuroimaging. Early and tailored management of athletes following a concussion can provide them with the best opportunity to avoid further injury. Brain-specific biomarkers measured through a simple blood test could complement the clinical evaluation of concussion and potentially guide management decisions [1–5]. The pursuit of these elusive markers has been most intense over the last decade [6–8]. Previously, human trials examined only moderate to severe TBI but are now expanding to include injuries on the milder end of the TBI spectrum, such as concussion, and subconcussive injuries and the effects of head acceleration events.

The degree of brain injury depends on the primary mechanism/magnitude of injury, secondary insults, and the patient's genetic and molecular response. Following the initial injury, cellular responses and neurochemical and metabolic cascades contribute to secondary injury which may evolve over the ensuing hours and days. These secondary insults can be mediated through physiologic events which decrease the supply of oxygen and energy to the brain tissue or through a cascade of cytotoxic events mediated by molecular and cellular processes. The

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release of brain injury biomarkers is not a static process. Studying the time course of a biomarker is critical to optimizing timing and clinical use. The time course (or temporal profile) may be affected by source of the sample, lesion type (mass lesion versus diffuse injury) and location, concomitant extracranial injuries (fractures, solid organ injuries), secondary insults, and individual patient physiology. Biomarkers could reflect these secondary insults as well as blood-brain barrier disruption. Complicating the release of biomarkers is the potential for extracranial sources of release after trauma. In cases when the biomarkers are released from tissues other than the brain, caution must be taken when interpreting results. For instance, S100 β can be released from soft tissues, cartilage, and bone after trauma and may not accurately reflect brain injury [9, 10]. Furthermore, individual physiology and pre-existing disease states, such as kidney or liver disease, may alter the metabolism or clearance of a given biomarker.

Key features that would make concussion biomarkers clinically useful include the following: (1) a high sensitivity (come from the brain) and specificity (low or undetectable in blood in non-injury states) for brain injury; (2) the ability to stratify patients by severity of injury (concentration of the biomarker should increase with worsening injury); (3) the timely appearance in accessible biological fluid such as serum, saliva, or urine; (4) a well-defined time course; (5) the ability to monitor injury and response to treatment; (6) the ability to predict functional outcome; and (7) be easily measured [8, 11].

To follow is a review of the most widely studied proteomic biomarkers for mild TBI and concussion in humans. Proteomic biomarkers are often represented by their neuroanatomic location in the central nervous system including astroglia (GFAP, S100 β) and neuronal cells, with specific areas of the neuron such as the cell body (UCH-L1) and axon (Tau, neurofilament) (Fig. 13.1). Furthermore, a novel group of promising transcriptomic biomarkers called microRNAs will also be discussed.

Proteomic Biomarkers

Glial Fibrillary Acidic Protein (GFAP) and Ubiquitin C-Terminal Hydrolase (UCH-L1)

GFAP and UCH-L1 for Mild-to-Moderate Traumatic Brain Injury

Glial fibrillary acidic protein (GFAP) is a protein found in the astroglial skeleton of both white and gray brain matter and is used as a histological marker for glial cells. Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is a protein in neurons that is involved in the addition and removal of ubiquitin from proteins that are destined for metabolism and is used as a histological marker for neurons [12, 13].

The specificity of GFAP and UCH-L1 for detecting brain injury has been examined in a number of studies. GFAP and UCH-L1 have been shown to distinguish mild and moderate TBI patients from orthopedic controls and motor vehicle crash controls as well as from those TBI patients with negative computed tomography (CT) scans [10, 14, 15]. In these studies, trauma control patients were exposed to

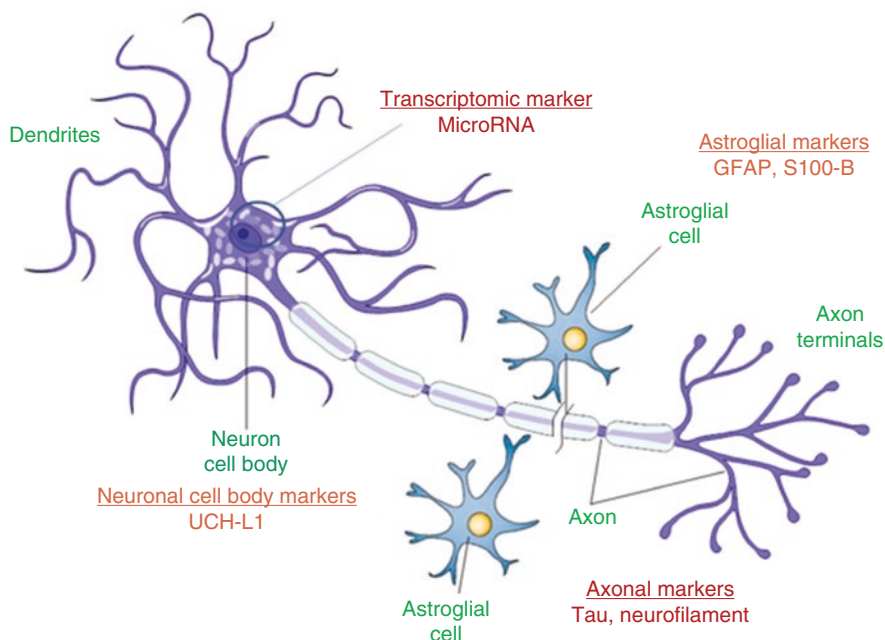


Fig. 13.1 Proteomic biomarkers are often represented by their neuroanatomic location in the central nervous system including astroglia (GFAP, S100 β) and neuronal cells, with specific areas of the neuron such as the cell body (UCH-L1) and axon (Tau, neurofilament). A novel group of promising TBI markers include transcriptomic biomarkers called microRNAs

significant trauma including the acceleration-deceleration vectors of motor vehicle collisions and substantial falls. Both GFAP and UCH-L1 showed a graded response to the severity of injury from uninjured to orthopedic trauma, to mild and moderate TBI. However, GFAP appears to be the most brain-specific in the setting of poly-trauma with substantial extracranial injuries and fractures [2, 3, 9, 10, 14–16].

The temporal profiles of GFAP and UCH-L1 over a week following a mild traumatic brain injury have been clearly described in a large cohort of emergency department trauma patients. GFAP consistently identified concussion over 7 days. GFAP also detected with good accuracy traumatic intracranial lesions on head computed tomography (CT) and neurosurgical intervention over a week [15]. GFAP was detectable in serum within an hour of concussion and remained elevated for several days after rendering it a promising contender for clinical use for concussion diagnosis within a week of injury [15]. In contrast, UCH-L1 rose rapidly within 30 min of injury and peaked at 8 h after injury and decreased steadily over 48 h with small peaks and troughs over 7 days – making UCH-L1 a very early marker of concussion [15].

Over the last decade, glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal Hydrolase (UCH-L1) have been found in several distinct studies to detect traumatic intracranial CT scan lesions and predict neurosurgical intervention

in adults with mild to moderate TBI [10, 14, 15, 17–20]. More recently, these findings have been replicated in children [2, 3, 21, 22]. In early 2018, GFAP and UCH-L1 were FDA approved for clinical use in adult patients with mild to moderate TBI to help determine the need for CT scan within 12 h of injury [23]. The approval was based on the ability to find lesions on a CT scan but was not approved to diagnose a concussion. Moreover, it was not approved for use in children.

GFAP and UCH-L1 for Concussion and Subconcussive Brain Injury

Computed tomography (CT) is the standard imaging modality for assessing damage in TBI during the acute phase of injury. CT scan can detect macroscopic traumatic lesions such as skull fractures, intracranial hematomas, contusions, subarachnoid hemorrhages, and swelling. However, the more subtle injuries associated with mild TBI are often not demonstrated by this imaging modality. This discrepancy is evidenced by the lack of CT abnormalities in patients with cognitive, physical, and behavioral dysfunction following a mild TBI. Therefore, CT does not have sufficient sensitivity to detect damage incurred in mild TBI. This group of TBI patients represents the greatest challenge to accurate diagnosis and outcome prediction. Metting et al. found that patients with axonal injury on MRI, but not CT, had elevated GFAP levels. Similarly, Yue et al. assessed GFAP in mild TBI patients with a negative CT scan and found GFAP was able to detect MRI lesions with an area under the curve of 0.78 despite the CT scan not showing any lesions [24]. UCH-L1 was not examined in either of these studies.

A significantly understudied group in whom biomarkers are rarely examined are individuals who experience head trauma without symptoms of concussion. They are often classified as having “no injury” when, in fact, they may represent milder forms of concussion that do not elicit the typical signs or symptoms associated with concussion. Such injuries have been referred to as subconcussive injuries or head acceleration events. Emerging data have demonstrated that significant alterations in brain function can occur in the absence of clinically obvious symptoms following even a single head trauma [25–27]. The issue of subconcussive trauma has been a particular concern in military personnel [28] and in athletes, as repetitive subconcussive impacts have the potential for long-term deleterious effects [27, 29, 30]. To address this deficiency, a recent study evaluated how GFAP and UCH-L1 behave in subconcussive trauma in a large cohort of children and adult trauma patients presenting to three-level I trauma centers with a Glasgow Coma Scale (GCS) score of 15 and a normal mental status. The biomarkers were measured at 20 distinct time-points in patients with concussive, subconcussive, and non-concussive trauma (Fig. 13.2). Although blood levels of both GFAP and UCH-L1 showed incremental increases from body trauma (lowest levels), to head trauma without concussion (higher levels than body trauma), to concussion (highest levels), GFAP was much better in distinguishing between the groups than UCH-L1. UCH-L1 was expressed at much higher levels than GFAP in those with non-concussive trauma, particularly in children, suggesting that UCH-L1 is either not completely brain-specific or ultrasensitive to subtle impacts [22]. In athletes, UCH-L1 has shown elevations in both concussive

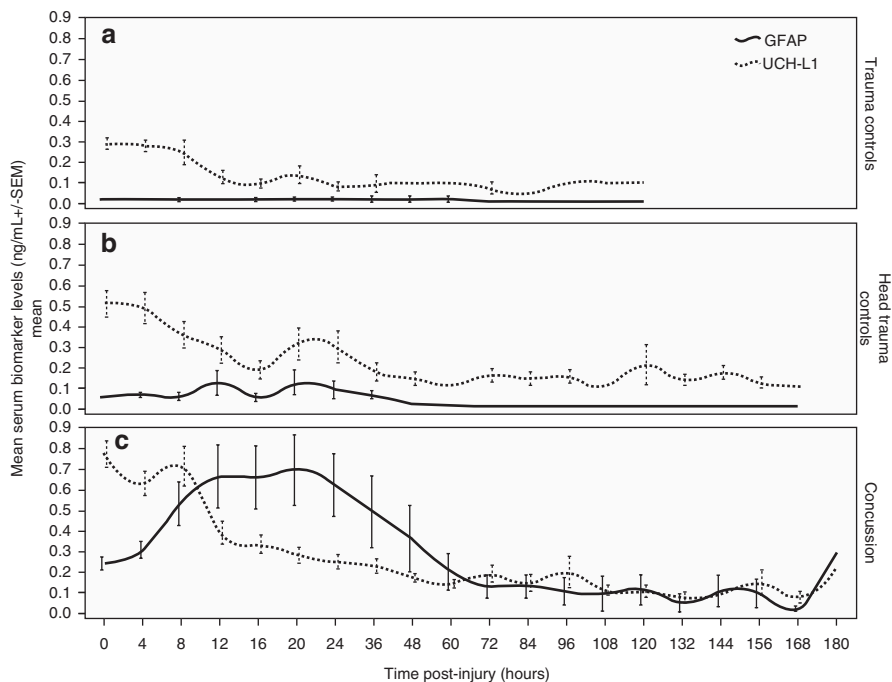


Fig. 13.2 Temporal profile of GFAP and UCH-L1 in three groups of trauma patients. (a) Temporal profile of GFAP and UCH-L1 in body trauma control patients. Means with error bars representing SEM. (b) Temporal profile of GFAP and UCH-L1 in head trauma control patients. Means with error bars representing SEM. (c) Temporal profile of GFAP and UCH-L1 in trauma patients with concussion. Means with error bars representing SEM. GFAP, glialfibrillary acidic protein; UCH-L1, ubiquitin C-terminal hydrolase. (Taken from Papa et al. [22])

[31] and subconcussive trauma [32]. However, these results are not consistent in all studies [33, 34].

Other Potential Proteomic Biofluid Biomarkers of Concussion

A systematic review of biomarkers in sports-related concussion showed that there have been at least eleven different biomarkers assessed in athletes [5]. Besides GFAP and UCH-L1, other potential biomarkers include S100 β , neuron-specific enolase, tau, neurofilament, amyloid beta, and brain-derived neurotrophic factor. Some correlate with the number of hits to the head (soccer), acceleration/deceleration forces (jumps, collisions, and falls), post-concussive symptoms, trauma to the body versus the head, and dynamics of injury [5]. Some of these and other novel markers are discussed below.

S100 β

S100 β is expressed in astrocytes and helps to regulate intracellular levels of calcium. It is considered a marker of astrocyte injury or death. Of note, it can also be found in cells that are not neuronal such as adipocytes, chondrocytes, and melanoma cells and, therefore, it is not brain-specific [35, 36]. Despite this, S100 β is one of the most extensively studied biomarkers for TBI [4, 5, 37].

A number of studies have found correlations between elevated serum levels of S100 β and CT abnormalities in adults and children [4, 38]. Elevated concentrations of S100 β in serum have been associated with increased incidence of post-concussive symptoms, problems with cognition, and traumatic abnormalities on MRI [39–43]. However, there are also a number of studies negating these findings [16, 44–46]. Similarly, several studies have shown that serum S100 β increases after concussive [31, 47, 48] and subconcussive brain injury [34, 49, 50]. However, a number of studies have shown a poor association with other prognostic parameters [33, 46, 51]. Peripheral sources of S100 β complicate its use as a brain-specific marker, particularly in the setting of polytrauma. S100 β has been shown to be elevated in injured patients with peripheral trauma who have had no direct head trauma [2, 10, 52]. Since many of these results have been inconsistently reproduced, the clinical value of S100 β in TBI, particularly mild TBI and concussion, is still controversial. Despite these inconsistent findings, S100 β has been approved for use by TBI patients in Europe.

Tau Protein

Tau is an intracellular, microtubule-associated protein that is amplified in axons and is involved with assembling axonal microtubule bundles and participating in anterograde axoplasmic transport [53]. Tau lesions are apparently related to axonal disruption such as in trauma or hypoxia [54, 55]. After release, it is proteolytically cleaved at the N- and C-terminals. In a study by Shaw et al., an elevated level of C-Tau was associated with a poor outcome at hospital discharge and with an increased chance of an intracranial injury on head CT [56]. However, these findings were not reproducible when C-Tau was measured in peripheral blood in mild TBI [57]. Two additional studies showed that C-Tau was a poor predictor of CT lesions and a poor predictor of post-concussive syndrome [44, 58]. Similarly, Bulut et al. found total Tau (T-Tau) differentiated patients with intracranial injury from those without intracranial injury [59]. However, they were not able to detect milder injuries.

In 2014, a study of professional hockey players showed that serum T-Tau outperformed S-100B and NSE in detecting concussion at 1 h after injury and that levels were significantly higher in post-concussion samples at all times compared with preseason levels [48]. T-Tau at 1 h after concussion also correlated with the number of days it took for concussion symptoms to resolve. Accordingly, T-Tau remained significantly elevated at 144 h in players with post-concussive symptoms (PCS) lasting more than 6 days versus players with PCS for less than 6 days [48].

Phosphorylated-Tau (P-Tau) is also being examined as a potential biomarker for brain trauma. Following TBI, axonal injury is coupled to Tau hyperphosphorylation, leading to microtubule instability and Tau-mediated neurodegeneration [60]. The

P-tau level has been shown to outperform the T-tau level in distinguishing CT positive from CT negative cases and identifying patients with poor outcome [61]. Moreover, several months after TBI, P-Tau has been shown to be elevated in TBI patients compared to healthy controls. The ratio between P-Tau and T-Tau has shown similar results [61]. High levels of total and phosphorylated Tau have been found in postmortem samples of TBI patients and athletes [62, 63]. Further study is needed to elucidate the role of T-Tau and P-Tau in detecting chronic encephalopathy.

Neurofilaments

Neurofilaments are heteropolymeric components of the neuron cytoskeleton that consist of a 68 kDa light neurofilament subunit (NF-L) backbone with either 160 kDa medium (NF-M) or 200 kDa heavy subunit (NF-H) side-arms [64]. Following TBI, calcium influx into the cell contributes to a cascade of events that activates calcineurin, a calcium-dependent phosphatase that dephosphorylates neurofilament side-arms, presumably contributing to axonal injury [65]. Phosphorylated NF-H has been found to be elevated in the CSF of adults and children with severe TBI [66, 67]. It remains significantly elevated after a few days in children with poor outcome and diffuse axonal injury (DAI) on initial CT scan [67]. Similarly, in a study by Vajtr et al. serum NF-H was much higher in patients with DAI over 10 days after admission with highest levels from day 4 to day 10 [68].

In a cohort of professional hockey players who underwent blood biomarker assessment at 1, 12, 36, and 144 h after concussion and at return-to-play, serum NF-L increased over time and returned to normal at return-to-play. Also, serum NF-L levels were higher in players with prolonged post-concussive symptoms [69]. In a group of amateur boxers, serum NF-L concentrations showed elevations 7–10 days after about and subsequently decreased following 3 months of rest. Levels were also significantly correlated with the number of hits to the head [69]. Moreover, NF-L has been shown to increase in adult soccer players following repetitive subconcussive head impacts compared to baseline levels, however, only after 24 h of the impacts [70]. In contrast, NF-L levels in blood taken at baseline and at 6- and 14-days post-concussion in contact sport athletes showed no differences between any of the pre-post timepoints [71].

Transcriptomic Biomarkers

MicroRNAs as the Next Generation of Biomarkers for Concussion

Initial exploration of TBI biomarkers began using animal models. These models have been helpful in providing histologic and pathophysiologic information on potential biomarkers. As a result, the selection of TBI biomarkers has been based on neuroanatomic location and on mechanisms of injury induced by trauma such as neuroinflammation and ischemia.

A novel set of biomarkers, called microRNAs (miRNA), are now being studied as the next generation of biomarkers for many diseases and disorders such as cancer, cardiovascular, and neurodegenerative diseases [72]. MiRNAs are small (19–28 nucleotides) endogenous RNA molecules that regulate protein synthesis at the post transcriptional level. MiRNAs can be detected in serum and can be an indicator of disease pathology in neuronal cells. MiRNAs are relatively abundant in biofluids such as cerebrospinal fluid, serum, and urine and are relatively stable at variable pH conditions, resistant to repeated freeze thaw and enzymatic degradation. Due to these properties, miRNA has advantages over protein-based markers. The utility of miRNAs as diagnostic markers of mild TBI or concussion has recently been explored [73–77]. In 2016, Bhomia et al. identified specific and sensitive miRNA-based biomarkers for mild and moderate TBI using real-time PCR methodology [74]. Samples from human subjects with mild to severe TBI were compared to trauma and normal controls and identified 10 miRNA signatures miR-151-5p, miR-328, miR-362-3p, miR-486, miR-505*, miR-451, miR-30d, miR-20a, miR-195, and miR-92a. Moreover, Johnson et al. identified 6 salivary miRNAs with overlapping CSF alterations (miR-182-5p, miR-221-3p, miR-26b-5p, miR-320c, miR-29c-3p, and miR-30e-5p) that distinguished children with TBI from healthy controls [75]. In a study by the same group, 52 children with concussion had 5 salivary miRNAs (miR-320c, miR-133a-5p, miR-769-5p, let-7a-3c, and miR-1307-3p) that were associated with prolonged post-concussive symptoms [76].

More recently, studies have been evaluating the role of microRNA in sports-related concussion. In one recent study, microRNA biomarkers measured pre- and post-season in collegiate football players were associated with worsening neurocognitive functioning over the course of a season in those with no concussions [78]. The study found significant elevations in circulating miRNA measured before the athletic season began and prior to any contact practices. All the players had significantly elevated levels compared to non-athlete controls ($p < 0.001$) suggesting residual circulating miRNA biomarkers from prior concussive and subconcussive impacts [78, 79]. Pre-season miRNA levels predicted baseline SAC scores with very good areas under the curve, the highest being miR-195 (0.90), miR-20a (0.89), miR-151-5p (0.86), miR-505* (0.85), and miR-9-3p (0.77). Athletes who demonstrated worsening neurocognitive function from pre- to post-season showed elevations in concentrations of miRNAs over the same period. The miRNAs with the most significant increases over the course of the season were miR-505*, miR-362-3p, miR-30d, miR-92a, and miR-486. Similarly, a study of saliva miRNA levels from 32 rugby players detected 5 miRNAs (miR-27b-3p, miR-142-3p, let-7i, miR-107, and miR-135b-5p) at 48 to 72 h after sports-related concussion that correlated with reaction time on IMPACT testing and predicted concussion better than other protein biomarkers [80].

Conclusion

TBI biomarkers measured through a simple blood test have the potential to provide invaluable information for the management of concussion by facilitating diagnosis and risk stratification; offering timely information about the pathophysiology of injury; monitoring recovery; and furnishing opportunities for drug target identification and surrogate measures for future clinical trials. In light of their timeliness, accuracy, and risk stratification potential, biofluid biomarkers with reliable sensitivity and specificity would be welcomed tools in treating concussion. This is especially so in settings limited by acute care resources such as in rural settings and non-hospital environments such as the playing field, battlefield, and primary care practices.

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