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Blood biomarkers for brain injury: What are we measuring?

Keisuke Kawata[a,b,1], **Charles Y. Liu**[c], **Steven F. Merkel**[d,2], **Servio H. Ramirez**[d,3], **Ryan T. Tierney**[a,4], and **Dianne Langford**[b,*,5]

[a]Department of Kinesiology, Temple University, Philadelphia, PA, USA

[b]Department of Neuroscience, Temple University School of Medicine, Philadelphia, PA, USA

[c]Department of Neurological Surgery, Keck School of Medicine, University of Southern California, 1520 San Pablo St. #3800, Los Angeles, CA 90033, USA

[d]Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA, USA

Abstract

Accurate diagnosis for mild traumatic brain injury (mTBI) remains challenging, as prognosis and return-to-play/work decisions are based largely on patient reports. Numerous investigations have identified and characterized cellular factors in the blood as potential biomarkers for TBI, in the hope that these factors may be used to gauge the severity of brain injury. None of these potential biomarkers have advanced to use in the clinical setting. Some of the most extensively studied blood biomarkers for TBI include S100β, neuron-specific enolase, glial fibrillary acidic protein, and Tau. Understanding the biological function of each of these factors may be imperative to achieve progress in the field. We address the basic question: what are we measuring? This review will discuss blood biomarkers in terms of cellular origin, normal and pathological function, and possible reasons for increased blood levels. Considerations in the selection, evaluation, and validation of potential biomarkers will also be addressed, along with mechanisms that allow brain-derived proteins to enter the bloodstream after TBI. Lastly, we will highlight perspectives and implications for repetitive neurotrauma in the field of blood biomarkers for brain injury.

Keywords

Blood biomarker; Concussion; Mild TBI; Blood-brain barrier

[*]Corresponding author at: Temple University School of Medicine, Medical Education and Research Building, Department of Neuroscience, 3500 North Broad Street, Philadelphia, PA 19140, USA. tdl@temple.edu (D. Langford). .
[1]1800 N. Broad St., 251 Pearson Hall, Philadelphia, PA 19122, USA.
[2]Medical Education Research Building room 880A, 3500 N. Broad St., Philadelphia, PA 19140, USA.
[3]Medical Education Research Building room 844, 3500 N. Broad St., Philadelphia, PA 19140, USA.
[4]1800 N. Broad St., 259 Pearson Hall, Philadelphia, PA 19122, USA.
[5]Medical Education Research Building room 750, 3500 N. Broad St., Philadelphia, PA 19140, USA.
keisuke.kawata@temple.edu (K. Kawata), chasliu@cheme.caltech.edu (C.Y. Liu), tue39636@temple.edu (S.F. Merkel), servio@temple.edu (S.H. Ramirez), rtierney@temple.edu (R.T. Tierney).

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1. Introduction

In the United States, every year approximately 1.7 million people sustain a traumatic brain injury (TBI) with the majority (80% or 1.4 million) of these injuries falling into the category of concussion or mild TBI (mTBI) (Mondello et al., 2014). Although the amount of research investigating mTBI has increased substantially in the last 5 years, clinicians still rely on subjective measurements such as self-reported symptoms to diagnosis concussion. In order to manage both the acute and chronic effects of mTBI, a better panel of tests is needed to accurately diagnose concussion, define a threshold between mTBI and moderate TBI (with a discreet clinical presentation), and gauge recovery for return-to-play/work post-injury. Furthermore, monitoring individuals who sustain multiple concussions and understanding the cumulative effects of mTBI is a critical goal for biomedical researchers engaged in the pursuit of better clinical care for “at risk” individuals including athletes and military service personnel.

Previous studies have shown that a history of TBI is associated with increased risk for sustaining future concussions (Guskiewicz et al., 2003; Laurer et al., 2001). Moreover, returning to the field too soon after a concussion, without allowing adequate time for recovery, is associated with worse long-term outcome. Thus, there may be a window of increased vulnerability during which sustaining subsequent head impacts may cause greater injury than may have been incurred if the injury from the first concussion had resolved (McCrory et al., 2012). Notably, discovering a biomarker that can identify when this period of vulnerability has passed and when the effects of the original concussion have subsided would have immense utility in battlefield triage and sports medicine.

Clinical application of blood biomarkers for mTBI is not only cost effective and minimally invasive, but may also aide in identifying patients who require referrals for neuroradiological assessment or follow-up examination. To date, highly sensitive and specific blood biomarkers for mTBI do not exist. The search for reliable serological markers of mTBI that are easily obtainable in the blood has yielded numerous promising candidates (Table 1). Although there are a number of excellent reviews, book chapters, and even a special issue summarizing current knowledge and identifying future directions in the field (Brody et al., 2015; Dashnaw et al., 2012; Di Battista et al., 2013; Giza and Difiori, 2011; Giza and Hovda, 2001, 2014; Jeter et al., 2013; Kovesdi et al., 2010; Ling et al., 2015; Lorente, 2015; Neher et al., 2014; Papa et al., 2015; Stoicea et al., 2016; Zetterberg and Blennow, 2015), rarely are potential blood biomarkers for mTBI discussed in terms of cellular origin and function. In continuing to validate existing serological biomarkers, it is important to understand their fundamental biological functions within the body and to explore the clinical implications of their increased presence in the blood. Furthermore, the presence of central nervous system (CNS) proteins in the blood may provide insights into the degree of neural damage, blood-brain barrier (BBB) compromise, neuro-adaptation following mTBI, and the physiological mechanisms that restore homeostasis in the brain parenchyma after injury.

This review will address a basic question in the field of blood biomarker research: what are we measuring? Several of the most widely studied blood biomarkers for brain injury will be

discussed in the context of protein origin, implications for presence in the circulation, and the biological significance for the change in circulating levels.

2. Origin of the protein, detection, and increased presence in the blood

A number of important factors must be considered regarding the application of potential blood biomarkers in the diagnosis and prognosis of mTBI. Addressing each of these questions may improve the selection, evaluation, and the validation process for current and future candidate blood biomarkers. Some of the key points addressed in this review include:

1) What is the origin of the protein of interest? Is the protein expressed only in the CNS or does it also originate from sources in the periphery? Understanding the origin of the protein can help to establish a range of baseline values for its presence in the blood.

2) Why is a protein of interest different from baseline values or significantly elevated in the blood post-mTBI? An initial increase in serum levels of a CNS protein may signify a release from injured cells, whereas elevated levels at later time points may likely be a result of upregulation.

3) Do cells of the BBB transport brain-derived proteins into the blood to eliminate waste generated by neurotrauma, or is the integrity of the BBB transiently compromised by mTBI? Furthermore, within what time frame may BBB integrity be restored after a single concussion, and to what extent do multiple concussions affect the BBB?

4) Do physical activities like sports-play and strenuous exercise affect blood levels of the protein of interest when being measured?

5) Is the biomarker specific to damage associated with neurotrauma such as axonal distortion, glial cell activation, and/or cell death within the neurovascular unit? Furthermore, in the context of sports-related injury, is the biomarker uniquely affected by TBI and distinct from other injuries commonly sustained by athletes (i.e. orthopedic trauma)?

6) When does a protein of interest reach its peak concentration in the blood? Defining when a potential biomarker for mTBI emerges from the brain and enters the blood is crucial for clinicians to know in order to test for the presence of a serological marker within the appropriate timeframe. Numerous reports have characterized the cascade of events leading to axonal degeneration after TBI, finding that the process begins within hours and can persist for weeks after an injury (Dashnaw et al., 2012; Volman and Ng, 2013). Therefore, understanding the physiological mechanisms that influence the efflux of a protein of interest from the brain parenchyma into the blood may help to reveal when such a test should be administered following an injury.

3. How do brain-derived proteins reach the bloodstream?

Many studies using samples obtained from TBI patients have shown that brain-derived proteins enter the blood. However, the physiological activity that allows neuronal and glial factors to reach the peripheral circulation remains largely undetermined. There are two general hypotheses for how brain-derived proteins enter the bloodstream. First, damage to the neurovascular unit (NVU) after TBI, particularly at the capillary level, may allow CNS proteins to cross the BBB. Alternatively, the glymphatic system, a means for bulk fluid movement in the brain, may clear brain-derived proteins from the CNS after injury (Iliff et al., 2013a). Importantly, understanding each of these processes in greater detail may assist in answering the question, “what are we measuring?”

3.1. Blood-brain barrier

The BBB is composed of numerous cellular components including brain microvascular endothelial cells (BMVEC), astrocytes, and pericytes, which collectively function to support the metabolic requirements of neurons in the CNS. Together, neurons, associated glia, and cells of the BBB form the NVU. TBI may not only damage neurons, but also astrocytes, microglia, oligodendrocytes, and the cerebral endothelium; in short, the entire NVU. Notably, neurovascular damage may be a result of either primary or secondary injury processes.

Primary injury may cause mechanical deformation of the BBB endothelium by shear stress forces, (Rodriguez-Baeza et al., 2003; Vajtr et al., 2009) disrupting the stability of specialized tight junction (TJ) complexes and compromising barrier integrity. In health, TJ complexes produce a physical barrier, connecting BMVECs to one another and establishing distinct compartments that separate blood products from those in the brain parenchyma (Fig. 1A). During primary injury, mechanical trauma may trigger cytoskeletal rearrangements throughout the NVU, compromising TJ stability and leading to critical axonal strain. These processes may lead to the release of neuronal and glial factors along with BBB damage, enhancing intra- and para-cellular BMVEC permeability, (Chodobski et al., 2011) to allow for the early detection of TBI biomarkers in the circulation (Fig. 1B).

Secondary injury processes such as altered cerebral metabolism, fluctuations in cerebral blood flow, brain edema, elevated intracranial pressure, and immune activation may also increase BBB permeability after TBI. For example, expression of pro-inflammatory cytokines, matrix metalloproteases, and reactive oxygen species is known to impair TJ stability and exacerbate neuronal dysfunction, potentially leading to greater efflux of brain-derived proteins into the bloodstream (Fig. 1B). Understanding how neurovascular damage, diffuse axonal injury (DAI), and inflammation evolve over time after a concussion may help investigators to identify when blood levels of candidate biomarkers can be expected to vary from baseline values. This data may be used to correlate blood levels with prognosis or direct decision-making towards specific treatment paradigms.

3.2. Glymphatic system

Recent studies using two-photon imaging show that cerebrospinal fluid (CSF) can enter the interstitial space surrounding brain cells through narrow gaps between the arteriole endothelium and basal lamina (Fig. 2). As CSF flows into this space, it mixes with interstitial fluid (ISF) throughout the brain. When cells in the NVU are damaged, they release cytosolic and membrane-bound proteins into the CSF and ISF (Fig. 2). Brain-derived factors suspended in this fluid can be driven into the paravenous space by astrocytic water systems, and cerebral arterial pulse pressure (Iliff et al., 2013a, 2014, 2012, 2013b). Collected cytosolic proteins may follow two pathways in the glymphatic system. They may be reabsorbed into the subarachnoid CSF, or they may be transported into the peripheral bloodstream through arachnoid villi of the dural sinuses and deep cervical lymph nodes, (Aspelund et al., 2015) then partially drained into the subclavian veins via lymphatic ducts (Plog et al., 2015).

Using a mouse model, studies by Iliff et al. showed that by knocking out the astrocytic water channel aquaporin-4 and then inducing experimental TBI, one could increase the accumulation of amyloid-β and hyperphosphorylated Tau in the ipsilateral cortex, (Iliff et al., 2014) suggesting that glial water efflux systems in the brain may clear these factors out of the parenchyma after injury. Plog and colleagues also examined the relationship between the glymphatic system and the efflux of TBI-induced brain-derived proteins, finding that when the glymphatic system was blocked, experimental TBI failed to increase serum levels of S100β, NSE or GFAP (Plog et al., 2015). Currently, the glymphatic system is one of the most attractive topics in the field of TBI biomarkers; however, further validation is needed in human cohorts. There are many questions that need to be addressed, including the role of the glymphatic system in protein ratios in the blood and the CSF, the effects of exercise and activity on glymphatic system efficiency, and the potential consequences of glymphatic system impairment caused by injury.

4. Serological biomarkers for brain injury

4.1. S100β

4.1.1. Cell origin and normal function—S100β is one of the most extensively studied biomarkers for TBI. It is a brain-enriched member of the S-100 family of low molecular weight (10.5 kDa) calcium-, copper-, and zinc ion-binding proteins that regulate intracellular calcium levels. The homodimeric S100β protein consists of two β subunits and is preferentially expressed by astrocytes (Jeter et al., 2013) and to a lesser extent by neurons, microglia, and oligodendrocytes (Richter-Landsberg and Heinrich, 1995; Steiner et al., 2008). By modulating second messenger calcium signaling, S100β is involved in various activities including cellular differentiation and motility (Schafer and Heizmann, 1996). S100β is an intracellular protein, but can be released into the extracellular space (Fig. 3A). Regardless of its location, the effects of S100β appear to be concentration-dependent. For example, it is protective and trophic at low concentrations, but toxic and pro-apoptotic at high concentrations. Interestingly, in health, S100β co-localizes and interacts with Tau to promote neurite outgrowth (Baudier et al., 1992; Fujii et al., 1986). However, extracellular S100β is a ligand for the cell-surface receptor for advanced glycation end products (RAGE),

which is mainly expressed on the neuronal plasma membrane (Fig. 3B). Notably, increased extracellular S100β binds to neuronal RAGE receptors leading to hyperphosphorylation of Tau through the activation of c-Jun N-terminal kinase (JNK) signal transduction and the upregulation of Dickkopf-1 (DKK-1) by transcription factors, c-Jun and AP-1. Over-expression of DKK-1 induces abnormal phosphorylation of glycogen synthase kinase 3β (GSK-3β) and β-catenin degradation, which stimulates hyperphosphorylation of Tau, an initial step in neurofibrillary Tau tangle formation (Fig. 3B) (Esposito et al., 2008). In fact, evidence has shown that increased levels of S100β in the brain directly correlate with the loss of neuronal connections in neurodegenerative disease (Mrak et al., 1996; Sheng et al., 1994; Van Eldik and Griffin, 1994).

S100β was previously thought to be brain specific, however, evidence has shown that it is expressed outside of the brain by pulmonary alveolar cells, cardiomyocytes, chondrocytes, and adipocytes (Diaz-Romero et al., 2014). Fluctuations in serum S100β have been reported in a variety of pathological conditions, including musculoskeletal injury, cardiac arrest, obesity, and bone fracture (Van Eldik and Griffin, 1994; Rothermundt et al., 2003). While S100β is enriched in the brain, taking the diverse functions of S100β into account is important for understanding the implications of its increased presence in the blood after TBI. Of note, previous reports have shown that TBI triggers neuroinflammation, astrocyte activation, increased production of S100β, and its translocation to the extracellular matrix, stimulating cellular damage and degeneration in the CNS (Fig. 3).

4.1.2. Presence in blood—In health, S100β is detected at very low levels in human serum (0.05 ng/ml) with no age or gender effects on baseline levels (Jeter et al., 2013; Biberthaler et al., 2001, 2000; Wiesmann et al., 1998). While S100β is the most extensively studied TBI biomarker, how it reaches the peripheral circulation remains unclear. Despite the lack of consistent and direct mechanistic evidence, increased levels of S100β, which are typically viewed as a marker for activated astrocytes, are also regarded by many as a surrogate marker for diminished BBB integrity (Puvenna et al., 2014; Marchi et al., 2013; Kanner et al., 2003; Kapural et al., 2002). Since S100β does not cross an intact BBB, elevated serum levels of S100β following TBI have been attributed to BBB permeability. Blyth et al. addressed this hypothesis by showing that abnormal CSF-serum albumin quotients (QA), indicative of BBB disruption, and serum S100β concentrations were strongly correlated in severe-TBI patients (Blyth et al., 2011, 2009). Furthermore, Huang et al. induced BBB disruption using a magnetic bead extraction method coupled with CSF-serum QA, and reported that elevations in serum S100β were associated with increased BBB permeability (Huang et al., 2013). More recently, Winter et al. assessed BBB disruption in 14 TBI patients post-injury using dynamic contrast-enhanced (DCE)-MRI and single-photon emission computed tomography (SPECT). The DCE-MRI and SPECT data showed an excellent correlation in detection of BBB damage. However, when these data were assessed in the context of serum S100β levels, no significant correlations were found (Winter et al., 2015). Thus, their findings showed evidence of post-traumatic BBB damage with no correlation to serum S100β levels.

As stated, normal levels of S100β are approximately 0.05 ng/ml. Previous reports have shown that this level can increase to nearly 5 ng/ml after severe TBI. Increased serum levels

of S100β have been observed within 24 h of severe TBI and strongly correlate with a prognosis of mortality (Goyal et al., 2013; Korfias et al., 2007; Raabe et al., 1999; Rainey et al., 2009; Vos et al., 2004; Pelinka et al., 2004a; Di Battista et al., 2015). Similarly, S100β has been useful in predicting whether a patient would regain consciousness or remain unconscious 3–6 months post-injury. The average peak value of serum S100β ranges from 0.2 to 1.5 ng/ml in patients with positive outcomes, and between 1.1 and 4.9 ng/ml in those with poor outcomes. Cutoff values predicting unfavorable outcomes were between 2.0 and 2.5 ng/ml (Kovesdi et al., 2010). The superiority of S100β as a prognostic biomarker for mortality and unfavorable outcomes was supported by a recent study using a multi-marker approach that included GFAP, NSE, brain-derived neurotrophic factor, and monocyte chemoattractant protein-1. While the combination of these markers discriminated mortality and outcome measures, S100β alone could predict poor prognosis after TBI equally well as the multi-marker panel (Di Battista et al., 2015).

A number of other studies have shown an increase in serum S100β levels after concussion (Kiechle et al., 2014; Shahim et al., 2014) and even subconcussive head impacts. These reports have produced similar results, finding that mean S100β levels are 0.113 ± 0.052 ng/ml 1 h post-gameplay compared to pre-game means of 0.061 ± 0.019 ng/ml (Puvenna et al., 2014; Marchi et al., 2013). However, poor association with other prognostic parameters raised questions regarding whether or not the increase in S100β was due to CNS-damage. Another recent study attempted to distinguish concussion-related elevations in serum S100β from the effects of exercise in contact sport athletes (Kiechle et al., 2014). First, pre-season baseline S100β levels were measured in 46 athletes. Then, 30 of the 46 athletes were subjected to exhaustive exercise intervention. Notably, there was no difference between baseline and post-exercise S100β levels, suggesting that serum S100β fluctuations may be specific to concussion (Kiechle et al., 2014). Another study reported significantly higher levels of serum S100β at 3-h post-concussion (mean, 0.099 ± 0.008 ng/ml) compared to post-exercise (mean, 0.070 ± 0.03 ng/ml) and baseline levels (mean, 0.058 ± 0.006 ng/ml). This increase disappeared by 2 days post-concussion, indicating that there may be a finite period within which blood samples must be obtained after mTBI (Kiechle et al., 2014; Shahim et al., 2014). Moreover, Dorminy et al. examined the effects of soccer ball heading on blood levels of S100β. In this study, approximately 16 collegiate soccer players were asked to complete 5 soccer ball headers at 25, 35 or 45 miles per hour (Dorminy et al., 2015). Results showed no differences in blood levels of S100β either before or immediately after headers, regardless of ball velocity.

Although these reports have produced consistent data, some data sets have failed to differentiate athletes with concussion from extraneous exercise groups (Anderson et al., 2001; Otto et al., 2000; Dang et al., 2014). This may be a result of significant increases in S100β levels after exhaustive sports training, as additional studies have detected elevated S100β concentrations in the serum and saliva of professional athletes and age-matched controls post-exercise (Michetti et al., 2011; Stalnacke et al., 2003). Furthermore, results from several investigations have failed to differentiate concussion from orthopedic injury (Anderson et al., 2001; Otto et al., 2000; Dang et al., 2014). Ohrt-Nissen et al. examined S100β levels in groups with an isolated head injury, those with multi-trauma (including a head injury), and those with trauma but no head injury. This study reported significantly

higher S100β levels in the multi-trauma with head injury patients (mean, 1.68 ng/ml; range, 0.71–6.10 ng/mL) compared to the isolated head injury group (mean, 0.47 ng/ml; range, 0.24–1.11 ng/ml) and the trauma but no head injury group (mean, 0.49 ng/ml; range, 0.10–2.16 ng/ml), suggesting that S100β fluctuations were influenced by an extra-cranial source (Ohrt-Nissen et al., 2011).

Non-CNS sources of S100β complicate its use as a brain specific marker. For example, adipocytes can produce and secrete S100β (Goncalves et al., 2010), especially during fasting, which can contribute to serum S100β levels without affecting CSF S100β levels (Netto et al., 2006). The high content of S100β in adipocytes and the wide distribution of these cells throughout the body suggests that S100β released from muscles and other tissues may at least in part alter blood levels of S100β (Goncalves et al., 2008). However, other studies have found no correlation between the amount of body fat and serum S100β. Furthermore, S100β levels do not appear to fluctuate in patients with kidney, lung, bladder, or liver cancers, sarcoma, or epilepsy (Pham et al., 2010), supporting the idea that elevations in serum S100β may be indicative of brain injury. For future studies, one important fact that must be taken into consideration is that the estimated half-life of S100β after mTBI is approximately 97 min (Townend et al., 2006). Therefore, longitudinal measures of S100β after TBI may be useful in gauging injury severity, referrals to neuroimaging, recovery post-TBI, or predicting adverse outcomes.

4.2. Neuron-specific enolase

4.2.1. Cell origin and normal function—Enolase is a crucial catabolic enzyme that converts 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway for ATP production (McAleese et al., 1988; Woertgen et al., 1999). It can be comprised of α, β, or γ subunits, however, γ-γ homodimers, a.k.a. neuron-specific enolase (NSE), are predominantly found in neurons and neuroendocrine cells (Rider and Taylor, 1975). NSE is a cytosolic protein that participates in axonal transport, and its expression levels can fluctuate depending on energy demand within a cell. Furthermore, when axons are injured, NSE is upregulated to maintain homeostasis. Based on its cellular origin and function, NSE is believed to be a surrogate marker of neuronal damage. Post-mortem analysis have shown that NSE selectively labels injured axons in the corpus callosum of patients sustaining fatal DAI, while, NSE is nearly undetectable in non-injured axons from both patient and control subjects (Ogata and Tsuganezawa, 1999).

4.2.2. Presence in blood—In health, NSE is largely confined to neurons, however, baseline serum levels (10 ng/ml) originate from red blood cells (Schoerkhuber et al., 1999; Planche et al., 2010). Sudden increases in serum NSE have been reported after various types of neurological damage including TBI (Cheng et al., 2014; Anand and Stead, 2005), ischemic stroke (Anand and Stead, 2005), and cerebral hemorrhage (Oertel et al., 2006). Recent investigations have suggested that following TBI elevated serum levels of NSE may be influenced by the glymphatic system rather than BBB injury. Previous studies have shown that animals with intact glymphatic function exhibit significant increases in serum NSE after experimental TBI, while those with glymphatic suppression failed to display a similar increase (Plog et al., 2015). Interestingly, both control and glymphatic suppressed

animals experienced equivalent BBB dysfunction following TBI, supporting the hypothesis that NSE reaches the bloodstream via the glymphatic system.

Mean serum NSE levels in control subjects without neurological disease have been reported to be approximately 10 ng/ml with no age or gender effects (Casmiro et al., 2005; Nygaard et al., 1998). Multiple studies have shown that serum NSE levels spike after moderate to severe head injury (Skogseid et al., 1992; Dauberschmidt et al., 1983) with post-TBI levels greater than 21.7 ng/ml. Moreover, NSE levels were strongly predictive of either death (sensitivity 85%) or poor outcome (sensitivity 80%) 6 months post-injury (Vos et al., 2004). Similarly, in 90 pediatric patients who experienced closed-head TBI, serum NSE predicted poor outcomes with 86% sensitivity and 74% specificity (Guzel et al., 2008; Bandyopadhyay et al., 2005). Severe and moderate TBI patient serum NSE levels (81.3 and 54.52 ng/ml, respectively) have also been shown to strongly correlate with typical neurological exams used to assess the degree of brain injury (Guzel et al., 2008; Meric et al., 2010). Although NSE has been extensively studied after mTBI, results indicate that serum NSE in patients with concussion does not significantly differ from control levels (de Kruijk et al., 2001; Geyer et al., 2009). Most notably, Shahim et al. reported that in 35 concussed ice hockey players, there was no difference in serum NSE from pre-season baseline levels to post-concussion values, (Shahim et al., 2014) suggesting that current assays to detect NSE may not be sensitive enough to observe serum fluctuations after mTBI. However, increased serum levels of NSE have also been reported after hypoperfusion, liver and kidney damage, femur fracture, and migraine, potentially limiting its utility as a biomarker for TBI (Pelinka et al., 2005, 2004b; Yilmaz et al., 2011).

Previous research has shown that long-term neurological impairment is more common in athletes who sustain repetitive TBI (Laurer et al., 2001; Dashnaw et al., 2012; Echemendia et al., 2001; Matser et al., 1999). For instance, serum NSE levels remained significantly higher (median, 11 ng/ml; range, 2.3–41 ng/ml) in 44 boxers following 2 months of rest when compared to healthy control non-boxers (median, 4.8 ng/ml; range, 0.78–27 ng/ml), suggesting that athletes exposed to very frequent, repetitive head trauma may experience prolonged neuronal decay (Zetterberg et al., 2009). Interestingly, given that the half-life of serum NSE is 24–48 h with peak serum levels occurring within 6-h post-TBI, these findings also suggest sustained release of NSE into the peripheral circulation after repetitive TBI, even in the absence of recent head trauma (Jeter et al., 2013; Zetterberg et al., 2009).

Additional studies are required to validate serum NSE as a biomarker for cumulative neuronal damage after repetitive mTBI. Furthermore, future studies may succeed in qualifying NSE as an initial screening tool for mortality and/or poor neurological outcome in moderate to severe TBI patients. However, more comprehensive approaches are necessary to increase the specificity of serum NSE levels for TBI as opposed to internal organ and/or orthopedic injuries. Moreover, the American Academy of Neurology has endorsed level B evidence to support the use of serum NSE for the prognosis of poor outcome after global cerebral hypoperfusion in patients requiring CPR. However, limited availability has delayed the general application of this test to inform clinical decision-making after global cerebral hypoperfusion.

4.3. Glial fibrillary acidic protein

4.3.1. Cell origin and normal function—Glial fibrillary acidic protein (GFAP) is the principle structural protein of cytoskeletal intermediate filaments expressed by astrocytes, the most abundant cell type in the brain. Ten different GFAP isoforms have been identified to date. Although GFAP-α is astrocyte-specific and the most abundant isoform, GFAP-β is enriched in non-myelinating Schwann cells in the peripheral nervous system, while GFAP-γ has been detected in the bone marrow and spleen (Yang and Wang, 2015). Therefore, studies have shown that GFAP can be detected in non-CNS tissues at low levels.

GFAP is a key intermediate filament protein responsible for supporting structural integrity of the astrocytic cytoskeleton in response to traumatic mechanical forces (Eng et al., 2000). Emerging evidence suggests that in response to neurotrauma, astrocytes proliferate, increase in size, expand their processes, and develop a more tortuous arborization (Fig. 4). These morphological changes are accompanied by an increase in GFAP expression, generally referred to as astrocyte activation or astrogliosis (Eddleston and Mucke, 1993). Post-injury astrogliosis aids in glial scar formation, preventing further deterioration of the neuronal complex (Jeong et al., 2014). Not only do expression levels of GFAP increase in response to injury (Gao et al., 2013), but intracellular GFAP translocates into the extracellular space and extracellular GFAP levels have been shown to correlate with TBI severity (Fig. 4) (Di Pietro et al., 2015).

4.3.2. Presence in blood—The rise in serum GFAP levels post-injury are believed to be a result of its release from astrocytes damaged by either mechanical deformation or regional necrosis. Therefore, increases in serum GFAP may be a surrogate marker for astrocytic injury. Furthermore, it is unclear whether damage to the BBB and diminished barrier integrity (Brunkhorst et al., 2010) following TBI or the upregulation of GFAP after injury drive the increase in blood levels (Sofroniew, 2009). In healthy individuals, blood levels of GFAP do not typically exceed the lower detection limits in most assays (detection limit: 0.012 ng/ml) (Foerch et al., 2012; Jung et al., 2007; Missler et al., 1999). Additional studies have reported GFAP levels ranging from 0.03–0.07 ng/ml, while others have failed to measure detectable levels (Foerch et al., 2012; Jung et al., 2007; Missler et al., 1999). Conversely, the highest serum GFAP levels are often observed during the first few hours to days after TBI (Missler et al., 1999). In patients with severe TBI, serum GFAP levels have been shown to increase significantly (4.52 ± 8.69 ng/ml) compared to healthy controls (0.061 ± 0.044 ng/ml), and predict mortality, recovery, outcome, and intracranial lesion (Pelinka et al., 2004c; Vos et al., 2010; Lumpkins et al., 2008; Nylen et al., 2006). Interestingly, Pelinka et al. proposed that serum GFAP levels may identify specific types of brain damage. For instance, GFAP levels were significantly higher in patients with focal brain injury compared to those with DAI (Pelinka et al., 2004a; Pelinka et al., 2004c). These findings were corroborated by Mondello et al. who showed that GFAP levels were significantly higher in patients with focal mass lesions (2.95 ± 0.48 ng/ml) compared to patients with DAI (0.74 ± 0.11 ng/ml) (Mondello et al., 2011). However, when the DAI group was further divided into levels: I–II (mild) and III–IV (moderate to severe), analyses showed that serum GFAP was significantly higher in levels III–IV than I–II, potentially confounding its utility in distinguishing types of brain injury.

Only recently have studies focused on concussion/mTBI cohorts evaluated serum GFAP levels. These reports have shown significantly higher serum GFAP levels among mTBI patients with abnormal computer tomography (CT) (1.20 2.65 ng/ml) compared to those with normal CT (0.05 ±0.17 ng/ml) ± (Metting et al., 2012). Similarly, Papa et al. demonstrated that serum GFAP levels were significantly elevated in mTBI patients with intracranial lesions compared to those without, and moreover, could predict patients who required neurosurgery (Papa et al., 2012a, 2014). Furthermore, regarding the specificity of GFAP as a TBI biomarker, a number of validation studies have examined the effects of extracerebral injuries on serum GFAP, finding no significant difference in GFAP levels between TBI patients and those suffering TBI with additional injuries (i.e. polytrauma) (Nylen et al., 2006). Studies have also shown that serum GFAP levels remain normal in polytrauma patients without TBI (Pelinka et al., 2004a) and patients with orthopedic injuries (Papa et al., 2012a, 2014). Collectively, these data identify serum GFAP as a relatively specific marker for astrocytic injury, astrogliosis, or BBB damage after TBI. However, further validation studies in concussion cohorts with longitudinal measures are warranted. Furthermore, the exact half-life of serum GFAP and the influence of plasma proteases on its stability remain unclear and need further investigation (Foerch et al., 2012).

4.4. Tau

4.4.1. Cell origin and normal function—The microtubule binding protein Tau is predominantly expressed by neurons and preferentially localized within axons (Binder et al., 1985). Tau facilitates axonal trafficking and neuronal signaling by binding tubulin subunits, stabilizing microtubular networks, and crosslinking microtubule bundles to establish neuronal viscoelastic properties (Ebneth et al., 1998; Terwel et al., 2002). Viscoelasticity in the brain enables stretching and retraction against mechanical forces due to the flexibility of microtubule bundles. Unlike focal brain injury, which is typically caused by a direct impact to the head resulting in cerebral contusions and hematomas (Gennarelli, 1993), diffuse brain injury is caused by inertial forces (e.g., stretch, twist, and retraction) that occur during rapid head rotation (Johnson et al., 2013; Lipton et al., 2009). Tau appears to mediate the viscoelastic response to these forces; however, inertial stress beyond threshold limits can disrupt microtubule networks, leading to DAI (Johnson et al., 2013; Lipton et al., 2009; Browne et al., 2011). A recent study by Ahmadzadeh et al. evaluated Tau viscoelasticity in the context of mechanical strain, by applying high and low strain rates to a micromechanical model of axonal microtubules cross-linked by Tau proteins. Interestingly, at lower strain rates, mechanical forces were mitigated by extension of the Tau proteins, which allowed microtubules to slide relative to one another without damaging axonal structure. Conversely, higher strain rates disrupted Tau and transferred the mechanical load directly to microtubule bundles, resulting in breakdown and dissociation of the axonal microtubule network (Fig. 5) (Ahmadzadeh et al., 2014). Likewise, in vivo studies using a rodent model of experimental TBI found that the density of Tau fragments in the cortex and hippocampus rose with increasing severity of brain injury (Gabbita et al., 2005). These findings not only support the hypothesis that axonal injury depends on the magnitude and severity of TBI, but also that Tau serves as a cytoskeletal shock-absorber and a potential biomarker for DAI in response to mechanical loading in the brain.

Since Tau is naturally unfolded, it is highly sensitive to endogenous proteolysis, especially when dislodged from microtubules due to mechanical stress or hyperphosphorylation (Wang et al., 2010). There are at least 79 putative serine/threonine phosphorylation sites on the longest isoform of Tau (441 amino acids). For review, please see Buée et al. (Buee et al., 2000). The majority of these sites lie outside of the microtubule-binding domain, except for six serine residues that participate in binding tubulin. Phosphorylation of these residues is not always sufficient to prevent Tau binding to tubulin (Biernat et al., 1993), however, phosphorylation at sites, such as s262, is commonly observed in neurofibrillary tangles in neurodegenerative diseases (Biernat et al., 1993). Proline-directed protein kinase family members, including mitogen-activated kinase (MAPK), glycogen synthase kinase-3 beta (GSK3-β), cyclin-dependent kinases (CDK), integrin linked kinases (ILK) and stress-activated protein kinases (SAPK) are most frequently associated with Tau phosphorylation (Lee et al., 2011). These kinases are counterbalanced by Tau phosphatases belonging to the phosphoprotein phosphatase (PPP) and protein tyrosine phosphatase (PTEN) families. Together, these enzymes regulate Tau hyperphosphorylation, which is a phenomena commonly observed in various neurodegenerative conditions including Alzheimer's disease and chronic traumatic encephalopathy (Martin et al., 2013). Notably, aberrant Tau phosphorylation can interfere with its ability to bind and stabilize microtubule networks (Fig. 5A), opening unbound Tau to enzymatic modification.

Abnormally phosphorylated Tau is subject to proteolytic cleavage by at least six different proteases (Fig. 5B), some of which generate neurotrophic fragments beneficial to neurons, and others that produce neurotoxic species resistant to proteasomal/autophagosomal degradation (Chesser et al., 2013). Ca^{2+}-activated calpains and thrombins can cleave Tau at multiple sites, generating a variety of fragments. However, whether these Tau cleavage products are neuro-protective or degenerative remains unclear (Tompa et al., 2004; Cuerrier et al., 2005; Arai et al., 2005). Puromycin-sensitive aminopeptidase (PSA) and high temperature requirement serine protease A1 (HTRA1) are proteases that assist in clearing soluble Tau through proteolytic degradation (Fig. 5B). Cathepsins are lysosomal proteases that can be released into the cytoplasm under pathological conditions and produce Tau fragments highly susceptible to abnormal phosphorylation (Kenessey et al., 1997). Caspases are a class of cysteine proteases also capable of cleaving Tau at multiple sites (Fig. 5B) including Asp 421, which has previously been shown to produce a truncated Tau fragment that accumulates in neurofibrillary tangles (Gamblin et al., 2003; Rissman et al., 2004). Although caspases 1, 3, 7 and 8 can cleave Tau at Asp 421 (Wang et al., 2010), cleavage by caspase 6 in particular is implicated in initiating fibril formation (Gamblin et al., 2003; Rissman et al., 2004). Neurotoxic Tau fragments can aggregate to form insoluble neurofibrillary tangles. Recent studies have demonstrated that these tangles do not necessarily activate apoptotic mechanisms (Spires-Jones et al., 2008, 2009), but rather induce cellular dysfunction by creating a chronic energy deficit at the mitochondrial level, where N-terminal fragments consisting of Tau amino acids 22–46 enter the mitochondria and interfere with the production of ATP (Atlante et al., 2008). Furthermore, evidence indicates that serum levels of neurotoxic C-terminal Tau fragments (c-Tau: Fig. 5B) are significantly elevated after mTBI (Shahim et al., 2015).

4.4.2. Presence in blood—Several studies have detected extracellular Tau species in the blood after TBI, identifying T-Tau and C-Tau as the most common species. Shahim et al. reported that serum levels of these species were elevated in professional hockey players after concussion, and furthermore found that A-Tau levels were associated with the duration of post-concussion symptoms (Shahim et al., 2014, 2015). Enzyme-linked immunosorbent assays (ELISA) with cut-off values of 12 pg/ml were initially used to investigate serum Tau levels. Bulut et al. found that serum T-Tau levels were increased in mTBI patients (188 ± 210 pg/ml) compared to healthy controls (86 ± 48 pg/ml); however, the difference was statistically non-significant ($p = 0.445$) (Bulut et al., 2006). Alternatively, correlational analyses between CT imaging and serum T-Tau levels among mTBI patients revealed that T-Tau successfully differentiated patients with intracranial injury (307 ± 246 pg/ml) from those without intracranial injury (77 ± 61 pg/ml). On the contrary, Kavalci et al. reported no difference in serum T-Tau levels again between mTBI patients with intracranial lesions (mean: 18.39 pg/ml; range: 2.19–714.47 pg/ml) and those without (mean: 16.29 pg/ml; range: 2.12–215.97 pg/ml) (Kavalci et al., 2007). Since demographics and emergency room admission times were similar in these studies, the discrepancy between serum T-Tau levels (307 vs. 18.39 pg/ml) among mTBI patients CT positive for intracranial injury may be due, in part, to the different assays utilized in each study.

In order to address such discrepancies and overcome the low sensitivity of Tau protein assays, a novel immunoassay using digital array technology was recently developed, yielding cutoff values of 0.02 pg/ml (Randall et al., 2013). Using this assay, a significant increase in serum T-Tau was detected in concussed athletes (mean: 10.0 pg/ml; range: 2.0–22.7 pg/ml) when compared to preseason baseline levels (mean: 4.5 pg/ml; range: 0.006–22.7 pg/ml). Furthermore, serum T-Tau levels 1 h post-concussion were significantly correlated with the resolution of post-concussion symptoms and return-to-play. Conversely, serum S100β and NSE levels failed to show such association (Shahim et al., 2014), suggesting the superiority of Tau proteins to diagnose and monitor concussed athletes. Interestingly, serum T-Tau levels were also significantly higher in boxers sustaining repetitive subconcussive blows (2.46 ± 5.10 pg/ml) compared to baseline values (0.79 ± 0.961 pg/ml) (Neselius et al., 2013), suggesting that serum Tau levels may be used to assess cumulative axonal damage following repetitive TBI. Additional Tau fragments have also been found to be significantly elevated in the serum of athletes after concussion compared to preseason baseline values. And while serum A-Tau levels remained constant after concussion, A-Tau levels have been shown to positively correlate with persistent symptoms lasting more than 10 days (Shahim et al., 2015).

Novel imaging and cell-based assays to assess Tau levels in the brain and blood are currently under development. Positron emission tomography (PET) imaging is now commonly used to assess Tau pathology in the brain using radiolabeled ligands that bind to the Tau protein. Furthermore, a cell-based assay using fluorescence resonance energy transfer (FRET) technology can measure the ability of Tau to promote aggregation, an initial step in neurofibrillary tangle formation commonly observed in neurodegenerative disease (Cook et al., 2015). This approach is particularly attractive given the ability of Tau to seed neighboring cells with aggregation prone species as shown in a Tau-transgenic mouse model

(Holmes et al., 2014). Future studies to explore Tau aggregates as TBI biomarkers for repetitive injury and accurate diagnosis are warranted.

5. Emerging blood biomarkers for TBI

In addition to the markers already discussed, continuing efforts have yielded a number of promising new candidates (Table 1). For example, spectrin is an axon-enriched cytoskeletal protein and the precursor of two novel TBI biomarkers, αII-Spectrin N-Terminal Fragment (SNTF) and αII-Spectrin Breakdown Product 150 (SBDP150). STNF is a calpain-induced cleavage product of spectrin first discovered and characterized by Siman et al. as a surrogate marker for necrotic neurodegeneration occurring subsequent to axonal injury (Siman et al., 2004, 1984; Siman and Noszek, 1988). Recent studies have shown that serum STNF levels are elevated from 12 to 36 h post-concussion. These levels are not only distinctly high when compared to baseline, but may also be useful as a prognostic marker for distinguishing athletes who develop post-concussion syndrome lasting longer than 6 days from those returning to gameplay within that time (Siman et al., 2015). Moreover, when tested in combination with diffusion tensor imaging after mTBI, high serum SNTF levels were significantly associated with structural white matter abnormalities in the corpus callosum and uncinate fasciculus. Likewise, elevated serum SBDP150 levels were strongly associated with intracranial damage observed by CT scan and Glasgow Coma Scale (GCS) scores (Papa et al., 2012b). Furthermore, serum SBDP150 could be used to distinguish mTBI patients from those with orthopedic injury (Papa et al., 2012b). Similar to NSE, spectrin is highly expressed by red blood cells. Therefore, although SBDP150 may be useful in identifying mTBI from orthopedic injury, serum STNF and SBDP150 levels may be affected by cardiovascular injury or hematopoietic disease.

Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is an enzyme highly expressed in the brain, testes, and ovaries that hydrolyzes a precursor protein to generate the monomers used in ubiquitination. In the brain, UCH-L1 is primarily expressed by neurons and is therefore an excellent marker for neuronal damage. In 2010, Papa et al. demonstrated that cerebrospinal fluid UCH-L1 levels closely reflected GCS scores in severe TBI patients (Papa et al., 2010). Similarly, serum UCH-L1 levels outperformed NSE, S100β, and myelin basic protein (an additional TBI biomarker candidate; see Table 1) in discriminating moderate/severe TBI from mTBI and orthopedic injury, by showing significant correlations between UCH-L1 and Glasgow Outcome Scale scores (Berger et al., 2012; Diaz-Arrastia et al., 2014). While the effects of sport-related concussion on UCH-L1 remain unclear, several recent studies have identified significant elevations in serum UCH-L1 levels in individuals with non-sport-related concussion (e.g., motor vehicle accidents, falls) compared to healthy controls (Papa et al., 2012a; Li et al., 2015; Kou et al., 2013).

A new panel of blood biomarkers was recently tested to accurately discriminate patients with mTBI from healthy controls and patients with orthopedic injury. The panel included copeptin (a fragment of a precursor peptide also consisting of arginine vasopressin and neurophysin II produced by hypothalamic neurons), galectin-3 (a pro-inflammatory lectin family member that plays an important role in cell–cell adhesion and leukocyte-endothelial interactions), matrix metalloproteinases-9 (a collagenase enzyme involved in degrading the

extracellular matrix), and occludin (an integral tetraspanin highly expressed at epithelial tight junctions including those of the BBB). Significant increases were detected in both mTBI and orthopedic injury patients for all 4 markers when compared to healthy controls with no differences between injury groups. However, additional analyses revealed that a combination of galectin-3 and occludin could distinguish mTBI patients from those with orthopedic injury, suggesting that this multi-marker approach may have diagnostic value for mTBI (Shan et al., 2016). Alternatively, a panel of inflammatory markers including substance P, soluble CD40 ligand, tissue inhibitor of matrix metalloproteinases-1, and malondialdehyde, albeit not specific to the brain, has also been proposed as candidate biomarkers for gauging the severity of brain damage in TBI (Lorente, 2015). Many of these markers have shown compelling associations between serum concentrations and the extent of brain injury; however, these reports are limited, as these inflammatory markers have only been evaluated in animal models and cases of severe TBI in humans. Thus, future studies examining the sensitivity and specificity of these candidates in mTBI and in comparison to alternative traumas (i.e. orthopedic injury) are warranted.

Another novel approach has been to validate microRNAs (miRNAs) as blood biomarkers for TBI. miRNAs are small non-coding RNAs expressed by cells throughout the body and found in extracellular vesicles carried in the blood that modulate gene expression at the post-transcriptional level (Fire et al., 1998). Although the exact biological function of miRNAs is not fully understood, recent reports indicate that miRNAs may be useful in evaluating the burden of brain damage in TBI. For example, Redell et al. demonstrated that plasma levels of miR-16, miR-92a, and miR-765 were able to identify severe TBI patients from healthy controls with area under the curve (AUC) values of 0.89, 0.82, and 0.86, respectively. Furthermore, when these markers were combined, excellent diagnostic accuracy (100% specificity and 100% sensitivity) was found (Redell et al., 2010). In the same study, miR-92a and miR-16 were able to specifically identify patients with mTBI from healthy controls with AUC values of 0.78 and 0.82, respectively (Redell et al., 2010). In addition, miR-93, miR-191, and miR-499 successfully distinguished mTBI patients from healthy controls with AUC values of 1.00, 0.74, and 0.82, respectively (Yang et al., 2016). Taken together, while the biological significance of miRNAs warrants further investigation, data obtained from the studies above suggest that serum miRNA levels may have diagnostic value in TBI.

6. Perspectives, future directions, and conclusions

As concussion awareness grows, establishing objective measurements to gauge the severity of an injury is critical to promote the best clinical outcomes. In practice, efforts are being taken to standardize evaluations and return-to-play protocols. However, the value of an easily obtainable blood biomarker is clear, as evidenced by the utility of HbA1c in the management of diabetes. Blood biomarkers for TBI are attractive for several reasons: 1) they are cost effective; 2) they require minimally invasive sample collection; 3) they can provide a reference for neuroimaging referrals; 4) they may be used to identify various types of parenchymal brain injury and/or BBB damage; and 5) fluctuations in blood levels may help to classify injury severity and indicate the resolution of brain damage. These benefits may allow for accurate diagnosis and immediate triage in response to concussive and

subconcussive brain injury and contribute significantly to clinical prognosis, return-to-play/work decisions, and the need for hospitalization in more severe cases.

Despite significant progress in the study of blood biomarkers for brain injury, there is still a dire need for analytical tests to help guide clinical treatment following TBI. Clearly, the most widely studied biomarkers for brain injury require further assessment, while the search for new biomarkers must continue. Previous research has largely focused on neuronal and glial factors as biomarker candidates due to the axonal damage and glial activation known to occur in TBI. However, biomedical researchers may have to consider factors expressed by other cell types in the NVU to identify novel blood biomarker targets. Candidate biomarkers should not be limited to soluble factors only, since potential biomarkers may be expressed in different biological formats such as those shed in membrane-bound forms. For example, proteins of interest may be enveloped inside of microvesicles released as a result of TBI rather than in a soluble state (Colombo et al., 2012).

Damaged and injured cells undergo exocytosis, whereby segments of the plasma membrane are released as microvesicle particles. Notably, previous research has shown that blebbing of the axolemma occurs after DAI, releasing microvesicles into the brain parenchyma (Dashnaw et al., 2012; Lai and Breakefield, 2012). After these axonal particles are released, they may enter the blood-stream where they can be isolated along with other microvesicles, which are produced in a variety of sizes (Colombo et al., 2012; Lai and Breakefield, 2012). Moreover, technological advancements to help stratify various-sized microvesicles may reveal discrete populations of these membrane-bound particles to improve correlations with the severity of TBI. Therefore, refining our current medical understanding of the biological functions of microvesicle release after TBI will allow the identification of proteins and other factors (i.e. micro-RNAs) that these vesicles may encapsulate as a distinct compartment in the plasma. Finally, characterizing the composition of molecular targets packaged inside of microvesicles after DAI (or other aspects of secondary injury) may help to identify a “signature” microvesicle (or “panel in a particle” biomarker) to improve specificity for concussion as opposed to the release of microvesicles from other neuropathologies that cause neuronal degeneration, such as stroke or Alzheimer’s disease.

As advocacy programs and media outlets continue to draw attention to the repercussions of repetitive concussive and subconcussive head impacts, which are commonly sustained by athletes and military personnel, TBI researchers must propose more robust studies to monitor the pathophysiological changes that occur in multiply concussed individuals. These studies may aid in validating TBI biomarkers that can detect various degrees of brain injury, and inform clinical prognosis and return-to play/work decisions for multiply concussed patients. Furthermore, discovering a biomarker that is both sensitive and specific for predicting future complications or identifying patients who may be at risk for developing chronic traumatic encephalopathy (CTE) or other devastating neurodegenerative diseases would have unsurpassed value for battlefield and sports medicine clinicians interested in safeguarding patient health. In this context, novel surrogate markers of tauopathy have been proposed as potential biomarkers for repetitive brain injury. These surrogate markers are believed to reflect the amount of abnormal Tau present in the brain and CSF. In particular, the LIM-domain only protein PINCH has gained some recent attention (Adiga et al., 2014;

Ozdemir et al., 2013), but it remains to be seen if this protein truly correlates with the levels of abnormal Tau in the brain and CSF, and/or if it can be used to predict cumulative damage after repetitive TBI.

After reviewing several of the most extensively studied blood biomarkers, it is clear that there are promising biomarker candidates for axonal injury (Tau) and astrocytic damage (GFAP and S100β). However, the majority of TBI studies lack a large enough sample size to confidently assess the biological significance of the change in blood levels of these biomarkers. Therefore, a large-scale, multicenter longitudinal approach is needed to explore blood marker utility in diagnosing concussion, recovery after injury or predicting responses to future injuries.

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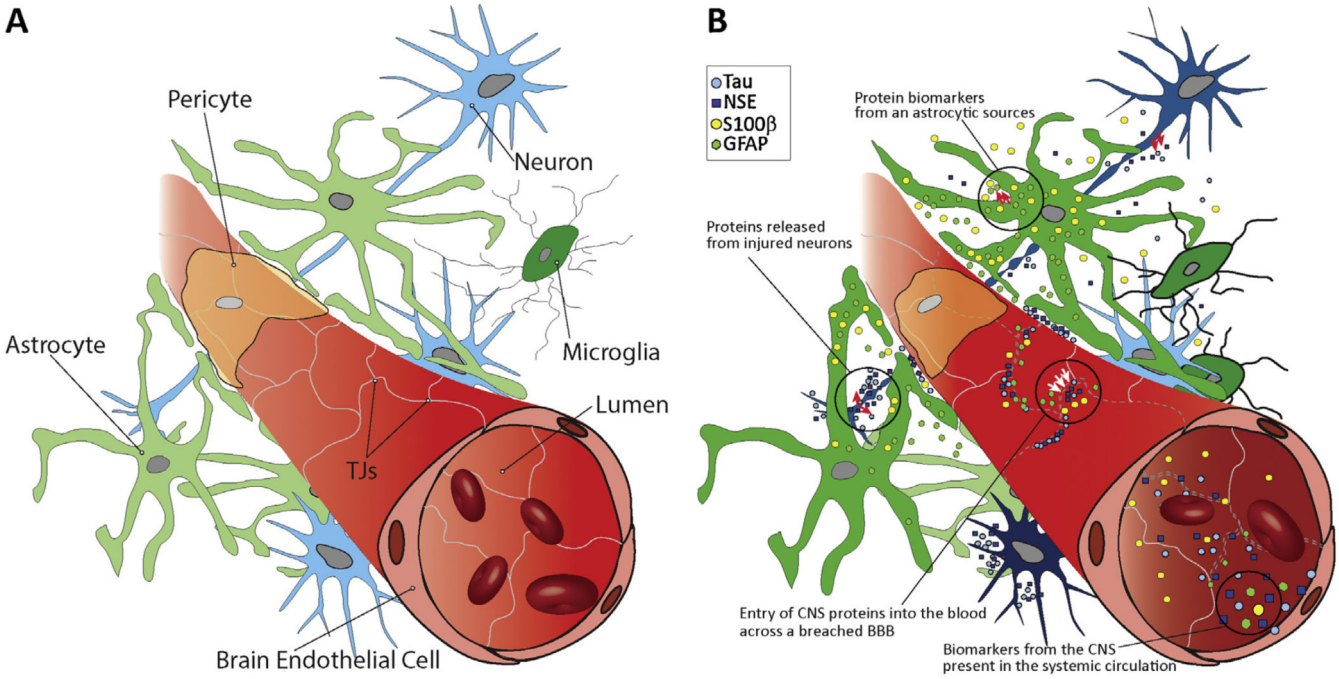

Fig. 1.

Schematic representation of changes in the NVU post-TBI. (A) Under homeostatic conditions, the NVU comprised of neurons, astrocytes, microglia, pericytes, and endothelial cells is properly maintained. Tight junctions (TJs-shown as solid lines) of the BBB are intact and greatly restrict paracellular movement of solutes between the blood and CNS. (B) Traumatic injury alters the status of cells throughout the NVU. Note, activated astrocytes (enlarged processes), diffuse axonal injury to neurons (depicted as undulations in the axon), morphological changes to microglia (retracted processes, larger cell body), and loss of BBB integrity (TJs-shown as dashed lines) are depicted. The consequence of both primary and secondary injury following TBI results in the release of CNS proteins (red arrows) from either activated or damaged cells. These factors then enter the systemic circulation at points where the BBB is compromised. NVU, neurovascular unit; BBB, blood-brain barrier; TJs, tight junctions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

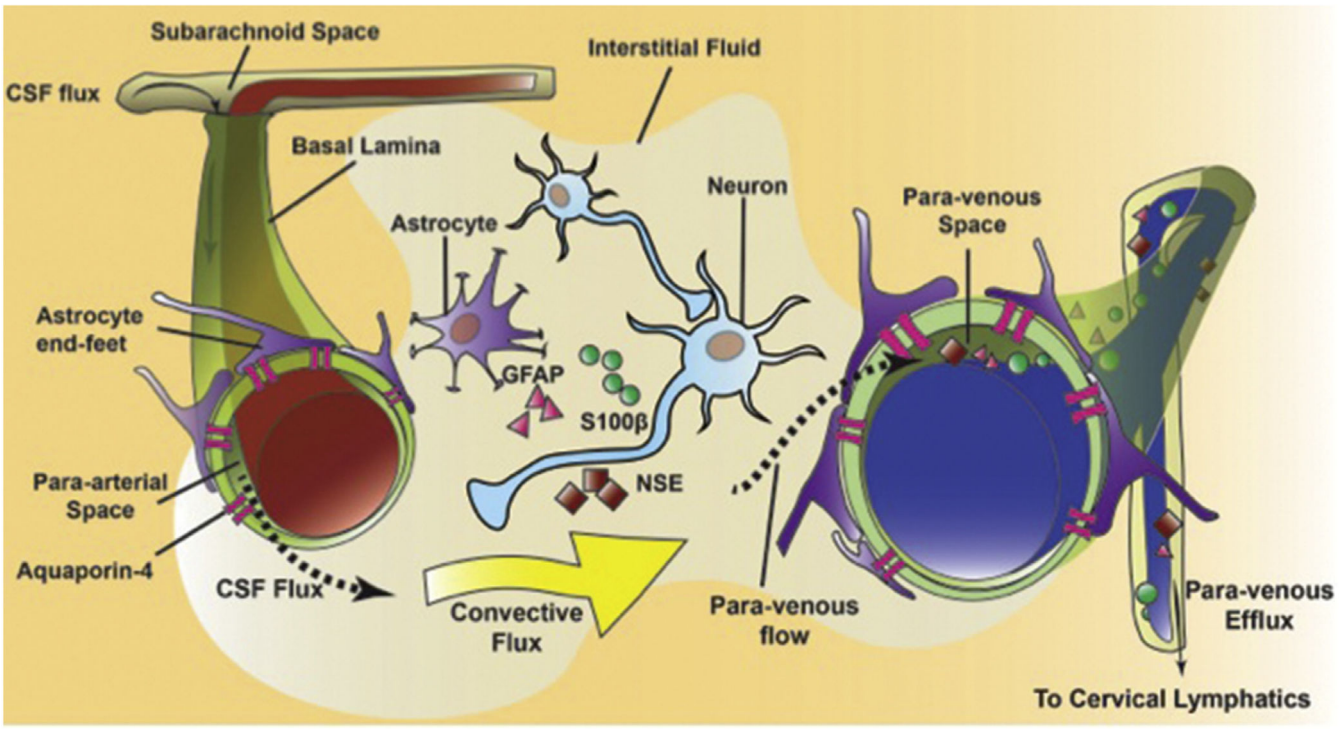

Fig. 2.

Glymphatic system and the clearance of brain-derived proteins from the CNS post-TBI. Factors such as GFAP, S100β, and NSE are released by parenchymal brain cells as a result of traumatic injury and enter the interstitial fluid where they are driven via convective flux generated by arterial pulse pressure and astrocytic water systems like aquaporin-4 into the para-venous space. From the para-venous space, these brain-derived proteins may be reabsorbed into the subarachnoid cerebrospinal fluid or be cleared from the CNS through cervical lymphatics leading to the peripheral circulation. CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NSE, neuron-specific enolase.

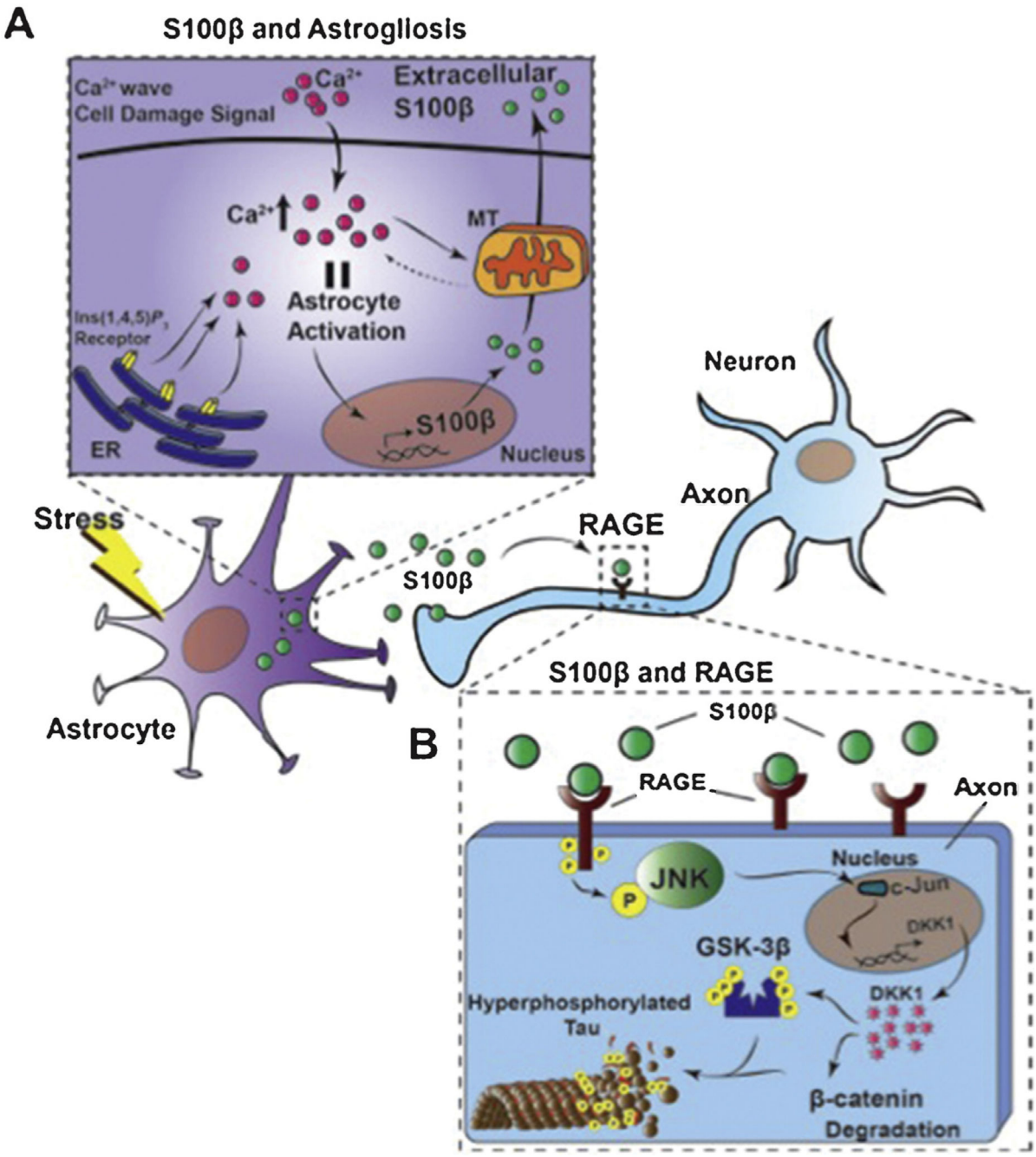

Fig. 3.

Extracellular S100β promotes neuronal cell death through RAGE receptors. Stress caused by TBI stimulates astrocyte-mediated release of S100β into the extracellular space, where it binds neuronal RAGE receptors leading to axonal degeneration. (A) Cell damage activates Ca^{2+} second messenger signaling cascades and extracellular Ca^{2+} waves that collectively raise intracellular Ca^{2+} levels consistent with astrogliosis. Astrocyte activation induces transcription of S100β, mitochondrial damage, and the translocation of S100β into the extracellular space. (B) Extracellular S100β binds to neuronal RAGE receptors and activates

JNK, ultimately leading to the upregulation of DKK1, which stimulates β-catenin degradation and Tau hyperphosphorylation by GSK-3β. Ca^{2+}, calcium; ER, endoplasmic reticulum; Ins P_3 receptor, inositol triphosphate receptor; MT, mitochondria; RAGE, cell-surface receptor for advanced glycation end products; JNK, c-jun kinase; GSK-3β, glycogen synthase kinase 3 beta; DKK1, Dickkopf-1.

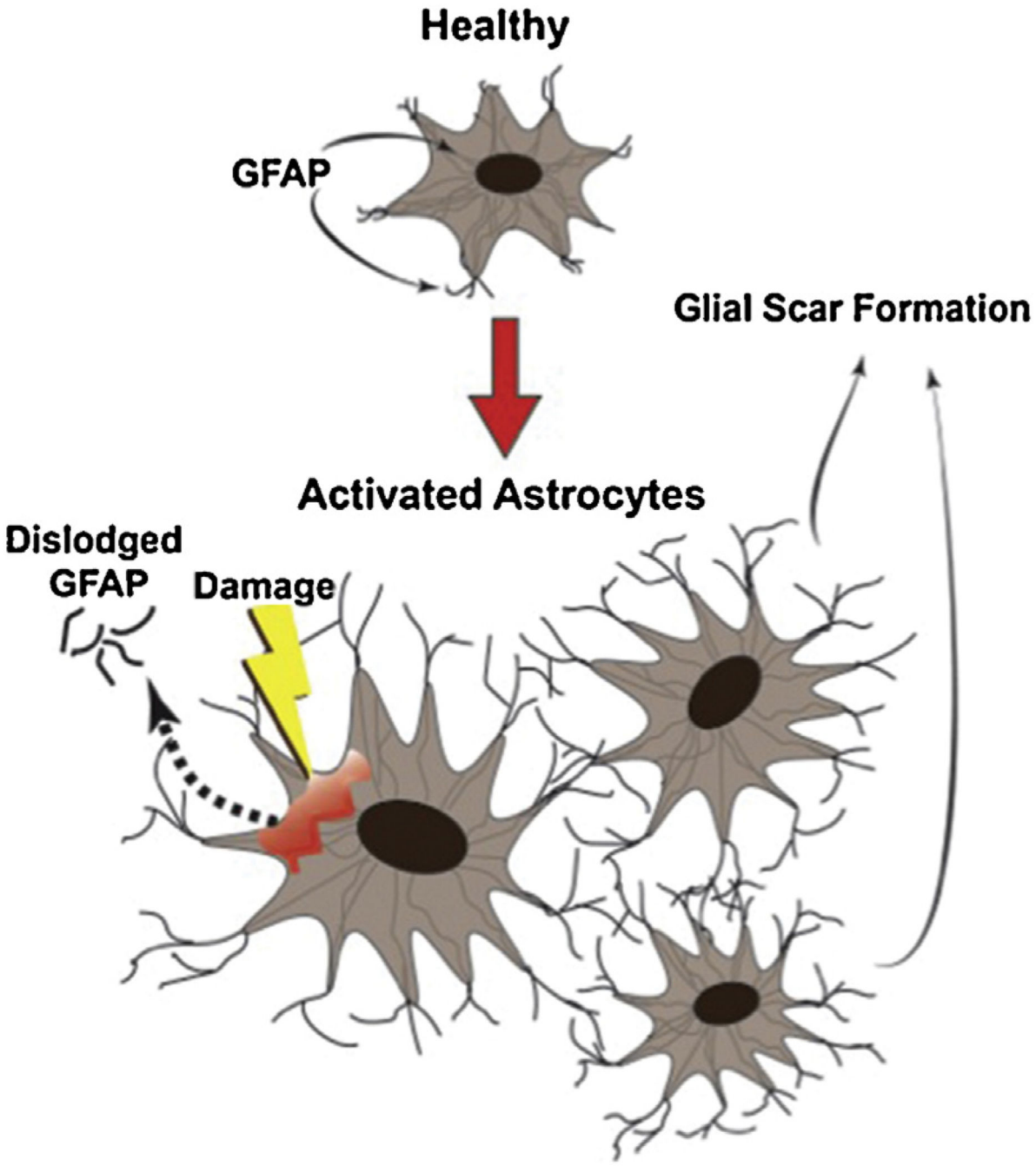

Fig. 4.

Reactive astrogliosis and glial scar formation. In health, astrocytes express GFAP as a cytoskeletal intermediate filament that supports quiescent cell morphology. However, in response to TBI, damaged astrocytes may shed dislodged GFAP into the brain parenchyma that makes its way to the peripheral circulation, raising serum concentrations. Furthermore, activated astrocytes over-express GFAP, alter their cellular morphology by developing more ramified processes, and proliferate, forming a glial scar at the site of injury. GFAP, glial fibrillary acidic protein.

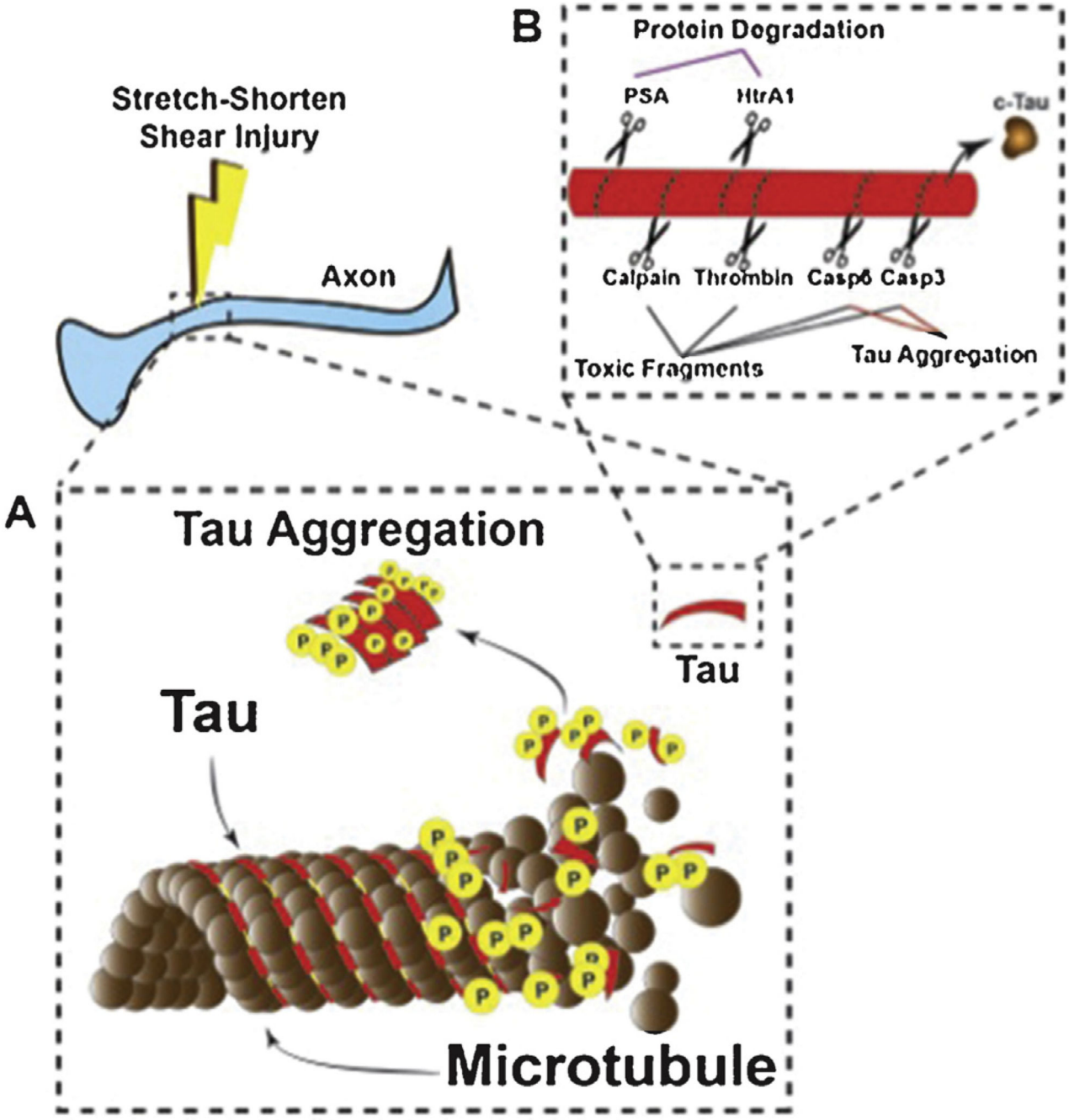

Fig. 5.

Tau hyperphosphorylation disrupts axonal microtubule networks and opens Tau to proteolytic cleavage. Stretch and shear forces in TBI can lead to diffuse axonal injury. These forces and the activation of cell damage signals that promote kinase-mediated hyperphosphorylation of Tau destabilize microtubule crosslinking in the axon. (A) Hyperphosphorylation of Tau interferes with its ability to bind tubulin, resulting in microtubule disassembly and the accumulation of Tau aggregates in the brain. (B) At least six different proteases may cleave Tau into fragments. Proteases like PSA and HtrA1 help to

degrade Tau to prevent the formation of aggregates, while others such as calpain, thrombin, Casp6, and Casp3 generate Tau cleavage products resistant to proteasomal or autophagosomal degradation. C-Tau is one of these products that may be released into the extracellular space and cleared from the brain parenchyma to be detected in the blood, or remain in the brain to form neurofibrillary tangles commonly observed in neurodegenerative disease. P, phosphate; PSA, puromycin-sensitive aminopeptidase; HtrA1, high temperature requirement serine protease A1; c-Tau, C-terminal fragment of Tau; Casp6, caspase 6; Casp3, caspase 3.

Table 1

Blood biomarker profiles.

Biomarker	Mechanism	Primary Source	Extracerebral Source	Peak Serum Levels after a Concussion
S100β	Astrogliosis	Astrocyte Oligodendrocyte	Adipocytes Chondrocytes Cardiomyocytes Alveolar cells	1–3 h
NSE	Neuronal Damage	Neuron	Neuroendocrine cells, tumors	~12h
GFAP	Glial Damage	Astrocyte	–	~24 h
T-Tau	Axonal Injury	Neuronal Axon	–	~1 h
A-Tau	Axonal Injury / proteolytic cleavage of T-tau	Neuronal Axon	–	~12 h
C-Tau	Axonal Injury / proteolytic cleavage of T-Tau	Neuronal Axon	–	–
OCLN	BBB dis-rup-tion	BBB	Tight Junction in: Intestine Kidney liver lung	~8 h
SNTF	Axonal Injury	Neuron	–	12–36h
UCH-L1	Neuronal Damage	Neuron	Lung tumors Testis/ovary	~6 h
Copeptin	Neuronal Damage	Hypothalamic Neuron	–	–
Galectin-3	Fibrosis Cancer Cardiovascular Disease	Non-Neuronal Cell	Lung Intestine Adipocyte	
MMP-9	Cancer Vascular Disease	Non-Neuronal Cell	Tonsil Lymph node Bone marrow	–
SP	Neuronal Damage	Sensory Neuron	Placenta Endothelial cell Inflammatory cell Immune cell	–
sCD40L	Platelet Activation	Platelet	–	–
TIMP-1	MMP Upregulation	Non-Neuronal Cell	Adipocyte Smooth muscle Cardiomyocyte Retina Pineal Gland	–
MDA	Oxidative Stress	Non-Neuronal Cell	Various Cell Membranes Adipocyte	–

NSE, neuron-specific enolase; GFAP, glial fibrillary acidic protein; T-Tau, total tau; A-Tau, A-disintegrin and metalloproteases 10-generated Tau fragment; C-Tau, cleaved Tau; BBB, blood-brain barrier; OCLN, occludin; SNTF, αII-spectrin N-terminal fragment; UCH-L1, ubiquitin carboxyl-terminal hydrolase L1; MMP, Matrix metallopeptidase; SP, substance P; sCD40L, soluble CD40 Ligand; TIMP, Tissue Inhibitors of Metalloproteinases; MDA, Malondialdehyde.