HHS Public Access

Author manuscript

Exp Neurol. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Exp Neurol. 2016 January ; 275(0 3): 334–352. doi:10.1016/j.expneurol.2015.05.004.

Current status of fluid biomarkers in mild traumatic brain injury

Jacqueline R. Kulbe and **James W. Geddes***

Spinal Cord and Brain Injury Research Center, College of Medicine, University of Kentucky, Lexington, KY, 40536-0509, USA

Department of Anatomy and Neurobiology, College of Medicine, University of Kentucky, Lexington, KY, 40536-0509, USA

Abstract

Mild traumatic brain injury (mTBI) affects millions of people annually and is difficult to diagnose. Mild injury is insensitive to conventional imaging techniques and diagnoses are often made using subjective criteria such as self-reported symptoms. Many people who sustain a mTBI develop persistent post-concussive symptoms. Athletes and military personnel are at great risk for repeat injury which can result in second impact syndrome or chronic traumatic encephalopathy. An objective and quantifiable measure, such as a serum biomarker, is needed to aid in mTBI diagnosis, prognosis, return to play/duty assessments, and would further elucidate mTBI pathophysiology. The majority of TBI biomarker research focuses on severe TBI with few studies specific to mild injury. Most studies use a hypothesis-driven approach, screening biofluids for markers known to be associated with TBI pathophysiology. This approach has yielded limited success in identifying markers that can be used clinically, additional candidate biomarkers are needed. Innovative and unbiased methods such as proteomics, microRNA arrays, urinary screens, autoantibody identification and phage display would complement more traditional approaches to aid in the discovery of novel mTBI biomarkers.

Keywords

Mild traumatic brain injury; Biomarkers; Serum; Cerebral spinal fluid; Biofluid; Novel; Discovery; Unbiased

Mild traumatic brain injury (mTBI) affects millions of people annually and is difficult to diagnose. It is associated with a number of sequelae, such as post-concussive syndrome, second-impact syndrome, and chronic traumatic encephalopathy, all of which can result in extensive morbidity. An objective and quantifiable measure, such as a biomarker, is needed to aid in mTBI diagnosis, prognosis, return to play/duty assessments, and would help to further elucidate mTBI pathophysiology. Research of TBI biomarkers in biofluids including CSF and serum has largely focused on moderate-to-severe injuries, targeting proteins that are present at high levels in affected cells and compartments, using a hypothesis-driven approach to discovery. There has been considerable progress in this area, although relatively

*Corresponding author at: Spinal Cord and Brain Injury Research Center, B483 BBSRB, 741 S. Limestone Street, University of Kentucky, Lexington, KY 40536-0509., Fax: +1 859 257 5737. jgeddes@uky.edu (J.W. Geddes).

few studies have targeted mTBI and a clinically useful biomarker has not yet been identified. This review aims to summarize the research on potential mTBI fluid biomarkers and identifies the need for novel mTBI biomarkers.

Mild traumatic brain injury (mTBI), also often referred to as concussion, accounts for the majority of TBI in the United States. 1.4 million TBIs are reported annually (Bruns and Jagoda, 2009), and 70–90% are estimated to be mild (Holm et al., 2005). This is a gross underestimate, however, since mTBI often goes unreported, particularly in sports and military communities (Jordan, 2013; Marion et al., 2011). Sports-related TBIs alone are estimated to be as high as 1.6 to 3.8 million annually (Langlois et al., 2006). According to medical records, 179,000 military service personnel sustained a TBI during the conflicts in Iraq and Afghanistan (Marion et al., 2011). This number is potentially higher; a RAND corporation survey identified that 19.5% of members surveyed reported a probable TBI (RAND Corporation, 2008).

Mild TBI lacks a consensus definition and frequently relies on subjective, often self-reported symptoms to make the diagnosis. In general, mTBI is defined as loss of consciousness (LOC) <30 minutes, post traumatic amnesia (PTA) <24 hours, Glasgow Coma Scale (GCS) 13–15, or transient changes in mental status or neurologic function. In addition, some definitions require negative radiology findings, while others exclude a GCS of 13 (Rosenbaum and Lipton, 2012), likely due to its higher rate of complications and intracranial lesions (Stein, 2001; Williams et al., 1990) Many of these criteria can be difficult to assess in intoxicated patients, children, and people with pre-existing neurologic conditions (Saatman et al., 2008), and GCS is particularly poor at assessing mild injuries (Jagoda et al., 2009; Saatman et al., 2008). In military populations, recognition of mTBI can be further complicated by delay in diagnosis, often by months or years, and is made based on the patient's memory of events in combination with clinical judgment (Pogoda et al., 2014). A more objective measure is needed to aid in the diagnosis of mTBI.

While computed tomography (CT) is a more objective measure, it lacks sensitivity in mTBI. It is used to diagnose intracranial lesions requiring neurosurgical intervention, the presence of which would typically preclude an mTBI diagnosis. In 15% of GCS 14–15 patients identified as having intracranial injuries, only 1% required neurosurgical intervention (Jagoda et al., 2009). Magnetic Resonance Imaging (MRI) is more sensitive than CT in identifying mild brain injury, and advanced imaging techniques such as diffusion tensor imaging (DTI) are able to identify white matter tract damage (Bigler, 2013). Despite increased sensitivity, current clinical guidelines make no recommendations regarding MRI in the diagnosis of mTBI (Jagoda et al., 2009). Radiologic imaging also has drawbacks. CT exposes the patient to radiation, which is concerning to the pediatric population because children are more susceptible to the effects of radiation (Chen et al., 2014; Pearce et al., 2012). Additionally, MRI has many contraindications, including imbedded metallic objects such as the shrapnel or bullet fragments that are often found in injured military personnel. Furthermore, MRI is expensive and limited in some environments, e.g. rural communities or combat deployment. Therefore, other objective and quantifiable methods, such as biofluid biomarkers, are needed to aid in the diagnosis of mTBI, and would be particularly useful in the acute stages of injury.

Many patients recover fully from mTBI, however, others go on to develop post-concussive syndrome (PCS), a potentially debilitating syndrome that consists of physical symptoms (headache, dizziness, fatigue), cognitive disturbances (impaired concentration and memory), or emotional problems including depression and anxiety (Arciniegas et al., 2005; Ryan and Warden, 2003), and can lead to an increased risk for suicide or development of psychiatric illness (Carroll et al., 2014; Carroll et al., 2004). While these symptoms often resolve within 2 weeks, some patients can experience persistent symptoms for months to years (Arciniegas et al., 2005; Carroll et al., 2004, 2014; Holm et al., 2005; Ryan and Warden, 2003). Development of PCS is multifactorial, encompassing pre-injury factors (age, gender, personality), injury factors (mechanism, location) and post-injury factors (medication, hormones, plasticity) (Begaz et al., 2006). PCS itself is difficult to diagnose as symptoms overlap with other disorders that can occur independently of brain injury, such as depression, substance abuse, and post-traumatic stress disorder (PTSD). The difficulty is compounded in populations, such as the military (Hoge et al., 2008, 2009; Stein and McAllister, 2009), with high rates of these disorders (Seal et al., 2007). There is currently no accurate method for predicting which mTBI patients will go on to develop persistent PCS. A number of studies have been conducted that assess the ability of clinical symptoms (headache, LOC, PTA, vomiting) or imaging (CT and MRI) to predict PCS type symptoms. However, none have been successful enough to affect clinical decision making (Berger, 2006). A better method for predicting PCS is needed, and a prognostic biomarker, measured over time or in the post-acute to chronic stage, would aid in outcome predictions and assessments. For multifaceted processes such as PCS, such a prognostic biomarker will likely be used in combination with other clinical factors (Begaz et al., 2006; Berger, 2006).

In addition to PCS, individuals that sustain repetitive mild traumatic brain injuries are at risk for development of chronic traumatic encephalopathy (CTE) or second impact syndrome (SIS). These sequelae are of particular concern to athletes and military personnel where high incidences of mTBI put them at risk for repeat injury. CTE is a neurodegenerative disorder with features of Alzheimer's disease that results in dementia and parkinsonism (Doolan et al., 2012; McKee et al., 2009). SIS, which can be fatal, occurs when a second concussion occurs before symptoms from a previous concussion have resolved (Doolan et al., 2012; Jordan, 2013). Therefore, when assessing mTBI in patients at risk for repetitive injury, it is extremely important to accurately determine when it is safe for the individual to return to play or duty. In the sports community there are currently a variety of return to play guidelines. In general they focus on rest and rehabilitation, and a stepwise protocol that increases physical activity as long as the player remains asymptomatic. Most importantly, players are only allowed to return to play if they are asymptomatic with normal neuropsychological testing (Doolan et al., 2012; 2006; McCrory et al., 2013). However, initial symptoms of mTBI can be subtle and patients may not self-report symptoms accurately so relying on symptom resolution as a criteria for return to play can be problematic. Additionally, while neuropsychological testing can be done by the treating physician it should, ideally, be done by a neuropsychologist (McCrory et al., 2013). A biomarker, whose levels could be monitored quantitatively post-injury, would aid in return to play/duty assessments, lessening the patient's risk of SIS or development of CTE.

Similarly, a biomarker would aid in return to academic assessments. Physical, as well as cognitive exertion can aggravate mTBI symptoms and prolong recovery. Therefore, appropriate assessment of academic re-entry is critical, especially in children and adolescents where recovery is often prolonged (Baker et al., 2014). Additionally, a biomarker would aid in return to work predictions. Return to work is an important indicator of recovery and individuals employed post-injury report better health and quality of life. Identification of individuals at risk for delayed return to work could help identify those who could benefit from additional intervention and rehabilitation (Cancelliere et al., 2014).

The need for quantitative and objective biomarkers of mTBI has been emphasized in recent NIH and military workshops (Manley et al., 2010; Marion et al., 2011; Saatman et al., 2008). Bakay and Ward (1983) propose that the ideal brain injury biomarker be present in high quantities and specific to the brain, released only after irreversible damage, released in time-locked sequence with injury, have a concentration that is correlated with severity of injury, and be clinically relevant. However, it is unlikely that a single biomarker will perfectly fit each of these criteria. This is particularly true in the post-acute phase where pathology is more likely to reflect regenerative or neuroplastic processes (Ottens et al., 2014) and in prognostic predictions for multifactorial disorders such as PCS (Begaz et al., 2006; Berger, 2006).

The Biomarkers Definitions Working Group more broadly defines a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (Biomarkers Definitions Working, 2001). The ideal peripheral biomarker will be measured non-invasively such as in an easily accessible biofluid, e.g. serum or urine. Furthermore, a panel of multiple biomarkers would likely have greater sensitivity and specificity than a single marker alone (Jeter et al., 2013a; Marion et al., 2011; Yokobori et al., 2013). Serum biomarkers are currently used clinically to diagnose other pathologies, e.g. troponin in myocardial infarction, brain natriuretic peptide in congestive heart failure, and amylase/lipase in pancreatitis. Therefore, a clinically validated serum biomarker holds great potential for the diagnosis of mTBI as well as outcome predictions, return to play/duty assessments, and therapeutic efficacy evaluations. Furthermore, identification of novel biomarkers would help to further elucidate the pathophysiology of mTBI.

The temporal profile of a biomarker is also important. For biomarkers to be suitable for evaluation of treatment efficacy, it will be essential to develop a panel of biomarkers representing distinct phases of injury and recovery. Based on the timing of various components of the secondary injury cascade (Fig. 1), a biomarker for an early postinjury timepoint may not be appropriate for a later time point. Immediately following TBI, axon stretching and diffuse axonal injury can result in mechanoporation of cellular membranes, including axonal membranes, and plasmalemmal leakage of cellular proteins into the CSF and serum (Farkas et al., 2006; Greer et al., 2013). Glial membranes may also be damaged, resulting in protein release to biofluids (Papa et al., 2012a; Yang et al., 2013b). Released proteins may include proteolytic fragments produced by the activation of calpains and caspases (Liu et al., 2006; Pineda et al., 2004; Siman et al., 2004, 2013). An injury-triggered inflammatory response can result in elevated levels of pro-inflammatory cytokines (Svetlov

et al., 2009; Yang et al., 2013a). The temporal profile of each of the above events differs (Fig. 1), and therefore the timing of biofluid collection following the injury is an important variable in the interpretation of results from different studies. Biomarkers at much later time points may also be helpful in the diagnosis of postconcussive syndrome, second impact syndrome, and chronic traumatic encelphalopathy. In Table 1, the timing of sample collection is indicated for investigations of mTBI biomarkers.

Most biomarker research has focused on proteins abundant in cells impacted by TBI, including neurons and glia, with the majority of studies evaluating moderate-to-severe TBI as summarized in recent reviews (Dash et al., 2010; Kochanek et al., 2008; Svetlov et al., 2009; Yokobori et al., 2013). TBI consists of a primary injury induced by an external force such as direct impact, acceleration/deceleration, or blast. The primary injury is followed by a secondary injury cascade in which there is excitotoxicity (Maas et al., 2008), free radical generation and lipid peroxidation (Hall and Braughler, 1993; Hall et al., 2010), astrocyte swelling and loss (Floyd and Lyeth, 2007), mitochondrial dysfunction, axonal degeneration, and neuronal death. There is a large inflammatory response and alterations in metabolism and blood flow (Maas et al., 2008). Additionally, microglia proliferate, migrate to the site of injury, and release cytokines (Kreutzberg, 1996). Therefore, the hypothesis-driven approach focuses on identifying proteins abundant in astroglia (S100B, Glial Fibrillary Acidic Protein), neurons (Neuron Specific Enolase, Ubiquitin C-Terminal Hydrolase-L1), oligodendrocytes (Myelin Basic Protein), neuronal cytoskeletal proteins (Spectrin Break Down Products, Tau, Neurofilament), inflammatory cytokines, metabolites, and oxidized lipids (Dash et al., 2010; Giacoppo et al., 2012; Kobeissy et al., 2008; Kochanek et al., 2008; Pineda et al., 2004; Sandler et al., 2010; Svetlov et al., 2009; Yokobori et al., 2013).

The hypothesis-driven approach outlined has been successful in identifying candidate biomarkers for severe TBI. However, current clinical guidelines on managing severe TBI do not include the use of biomarkers (Brain Trauma et al., 2007; Kochanek et al., 2012). These biomarkers are now being investigated in mTBI. The following summarizes the existing research on evaluating potential mTBI fluid biomarkers. This review attempts to limit itself to mTBI, but in order to be comprehensive some mild injury studies that also investigate more severe injuries must be included. These studies are identified and the mTBI data is summarized. However, not all of these studies present their mTBI independent of more severe injuries. This is noted in the analysis of such studies. This review concludes that new strategies such as unbiased methods are needed for the discovery of novel biomarkers and that novel biomarkers would benefit the field of mTBI. Examples of unbiased approaches are presented with the recommendation that similar and additional approaches can be used with mild injury.

Biofluids for mTBI biomarkers

Blood (serum and plasma) is a preferred biofluid for biomarker discover because of the ease of access, ease of processing, relatively homogenous samples, and the large amount of normative data available (Lundblad, 2003). However, because it comes into contact with all tissues and organs, blood can present difficulties in identifying the source of a protein and can also present challenges in terms of sensitivity and dynamic range (Good et al., 2007).

Most mTBI studies have evaluated potential biomarkers in serum (Table 1). The process of clotting removes fibrinogen and other proteins, either directly or indirectly, and may add other proteins secreted from cells during blood coagulation (Lundblad, 2003). There is not strong evidence to demonstrate a preference for either EDTA plasma or serum for biomarker detection and measurement, but it is important to recognize that there can be differences in detectability and variability for individual biomarkers (Alsaif et al., 2012; O'Neal et al., 2014).

CSF provides a window into changes in the CNS, and contains relatively low protein levels relative to volume making detection of released proteins or fragments easier than with serum (Romeo et al., 2005). While individuals with severe TBI typically receive a ventriculostomy catheter for collection of CSF, obtaining CSF from individuals with mTBI and healthy controls is more difficult as CSF samples are not typically obtained following mTBI (Good et al., 2007).

Other potential sources of biofluids for markers of brain injury include saliva and urine, which can be obtained with non-invasive techniques. However, a disadvantage is both biofluids are compositionally dependent on flow rates. Saliva samples have been utilized to measure cortisol levels in response to stress following TBI (Bay et al., 2005; Bohnen et al., 1992), but is otherwise largely unexplored as a potential source of TBI biomarkers. Urine has begun to be evaluated for potential biomarkers and is discussed in greater detail later in this review.

The hypothesis-driven approach

Based on the success of serum troponin-T as a biomarker for myocardial damage, (Mair et al., 1992), a search began for biomarkers of brain injury. Putative candidates were identified based on their relatively high abundance in cells or cellular compartments known to be affected in TBI, and protein concentrations in blood or CSF were evaluated.

Early TBI biomarker candidates were lactate dehydrogenase and creatine kinase BB. However, these did not have sufficient specificity, sensitivity, or selectivity (Ingebrigtsen and Romner, 2003; Raabe et al., 1999). More promising was the beta form of S-100, S-100B, a calcium binding protein produced in astrocytes (Ingebrigtsen and Romner, 2003; Ingebrigtsen et al., 1995; Raabe et al., 1999). Other candidates examined included neuron-specific enolase and myelin basic protein, as markers of neuron and axonal damage, respectively (Kochanek et al., 2008). Also evaluated as putative biomarkers are intermediate filament proteins in astrocytes (glial fibrillary acidic protein (Eng et al., 1971)) and neurons (neurofilaments). Ubiquitin C-terminal hydrolase L1 was discovered by two-dimensional gel electrophoresis as a neuron-specific protein, also known as protein gene product (PGP) 9.5, abundant in the neuronal cytoplasm (Doran et al., 1983). This initial study suggested its potential utility in the detection of neuronal damage. Spectrin (also known as brain fodrin), is a component of the membrane cytoskeleton abundant in axons and presynaptic terminals. Spectrin was identified as a substrate for calpain 1 (Siman et al., 1984) and subsequently shown to be degraded in vivo by excitotoxic insults (Siman and Noszek, 1988). Antibodies against spectrin breakdown products identified vulnerable neurons following cerebral

ischemia (Roberts-Lewis et al., 1994) and experimental traumatic brain injury (Pike et al., 1998; Saatman et al., 1996). Fragments of spectrin were identified in CSF following brain injury in rats (Pike et al., 2001; Siman et al., 2004) and humans (Farkas et al., 2005). More recently, improvements in detection have enabled evaluation of spectrin breakdown products in serum as potential biomarkers of TBI (Berger et al., 2012; Siman et al., 2013). These and other putative TBI biomarkers are discussed below. Other molecules known to be affected by TBI pathophysiology such as metabolites, lipids, and cytokines have also been targeted as potential TBI biomarkers (Tables 1 and 2).

S100B

S100B is a calcium binding protein, highly abundant in astroglia and also associated with neurons (Michetti et al., 2012). It is the most extensively studied biomarker in all severities of TBI, with well over 300 studies to date. S100B is the closest a biomarker for mTBI has come to clinical use. In neuroimaging guidelines for adults with mTBI, the American College of Emergency Physicians/Centers for Disease Control and Prevention state that “in mild TBI patients without significant extra-cranial injuries and a serum S-100B level less than 0.1μg/L measured within four hours of injury, consideration can be given to not performing a CT” while noting that measuring S100B levels is not an FDA approved test for clinical use (Jagoda et al., 2009). 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, considering it a “moderate quality, strong recommendation” (Unden et al., 2013). However, although acknowledging that S100B can be a sensitive indicator of brain injury, whether it adds value to clinical guidelines for mTBI diagnosis has been questioned (Kontogeorgis, 2013).

S100B is not specific to the brain. It is also found in Schwann cells, chondrocytes, adipocytes, and exocrine cells (Haimoto et al., 1987). S100B is also not specific to brain injury; serum levels are elevated after ischemic-reperfusion injury of abdominal organs (Pelinka et al., 2004a), myocardial ischemia (Cai et al., 2011; Mazzini et al., 2005) and following non-cranial trauma (Anderson et al., 2001). It is elevated in patients with mood disorders (Schroeter et al., 2013) and is currently being investigated as a biomarker of melanoma (Gogas et al., 2009; Tandler et al., 2012). Furthermore, S100B levels vary in the pediatric population based on age (Astrand et al., 2011). Whether S100B is correlated with injured brain tissue or passes through a damaged blood brain barrier has been questioned (Koh and Lee, 2014). Aside from its putative diagnostic value, S100B may promote neurogenesis and recovery (Kleindienst and Bullock, 2006).

Despite its lack of specificity, S100B continues to be investigated as a potential biomarker of TBI. Its ability to identify TBI patients and predict intracranial lesions has been covered by the previously mentioned reviews as well as reviews of mild injury (Begaz et al., 2006; Jeter et al., 2013a; Mondello et al., 2014; Zetterberg et al., 2013). Because of the vast number of studies evaluating S100B as a potential biomarker for various TBI severities, we have focused on S100B’s role in outcome predictions following mTBI (Table 1).

Several studies examined S100B levels in the first few hours after mTBI as a possible predictor of PCS at later time points. Ingebrigtsen et al. (2000) found no significant correlation between a high serum S100B concentration (>0.20 μg/L, <3 h post-injury) and PCS symptoms or duration of sick leave at three months post-injury. In a prospective study, Bazarian et al. (2006) also found no correlation between serum S100B (<6 h) and PCS three months post-injury. De Kruijk et al. (2002) assessed post-traumatic complaints (PTC) six months post-injury, in patients who presented with GCS 14–15, and found that elevated serum S100B (>0.30 μg/L, <6 h) was significantly associated with forgetfulness. Presenting clinical symptoms such as dizziness, headache, nausea and vomiting were also significantly associated with PTC. Savola and Hillbom (2003) evaluated PCS symptoms present for greater than one month following mTBI. At a cutoff of 0.20 μg/L for serum S100B levels normalized for time of injury, sensitivity was 92% and specificity was 41% for predicting PCS. However, multivariate analysis also identified skull fracture, dizziness, headache and age as risk factors for PCS. Topolovec-Vranic and colleagues used the Rivermead Post-Concussive Symptoms Questionnaire (RPQ), neurocognitive testing, and postural stability measures to evaluate mTBI patients at 3 days, 1 week and 6 weeks post-injury. A lower Galveston Orientation and Amnesia Test in the emergency room, higher RPQ score on day 3, and an elevated S100B (>0.1 μg/L, <4 h) level predicted poor outcome 1 week post-injury with an area under the curve (AUC) of 0.877. However, once S100B was removed the AUC was 0.865, and S100B alone was a poor predictor of outcome 1 week post-injury (Topolovec-Vranic et al., 2011). In summary, the predictive value of acute serum S100B levels for the persistence of TBI symptoms characteristic of PCS is inconclusive.

Cognitive impairment has also been assessed in relation to S100B levels. Stapert et al. (2005) found that in GCS 14–15 patients, elevated levels of serum S100B (>0.22 μg/L, <6 h) did not correlate with decreased cognitive speed or impaired memory upon neuropsychological testing 7–21 days post-injury. de Boussard et al. (2005) did not observe a correlation between elevated serum S100B (>0.15 μg/L, <24 h) or S100A1B (>0.85 μg/L, <24 h) in GCS 14–15 patients with cognitive impairment at three months post-injury. In contrast to these findings, Muller et al. (2009) reported that GSC 13–15 patients with elevated serum S100B (>0.14 μg/L, <12 h) performed significantly worse on neuropsychological testing at baseline and six months post-injury. However, this was also true for patients with a GCS of 13–14 or a positive CT/MRI scan. None of these factors influenced the time trend of recovery. This study's contrasting results are likely due to including patients with more severe injuries (GCS 13) and a greater number of intracranial lesions.

Additional relationships between S100B and outcomes have been explored. High serum S100B levels (>0.15 μg/L, <3 h) in GCS 15 patients predicted failure to return to work or activities one week post-injury with 80.0% sensitivity and 74.4% specificity, although 99% of the mTBI patients returned to work within one month (Stranjalis et al., 2004). Very high serum S100B (>0.48 μg/L, <6 h) in patients presenting with a wide range of initial TBI severities (GCS 4–15) was predictive of Glasgow Outcome Scale Extended (GOSE) scores of <5 (severe disability) at one month post-injury, with 90% sensitivity and 83% specificity (Townend et al., 2002).

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.

Glial fibrillary acidic protein (GFAP)

GFAP is an intermediate filament protein associated with the astroglial cytoskeleton (Eng et al., 1971). It is specific to the nervous system, and increased GFAP immunoreactivity is used as an indicator of brain injury following experimental models of mTBI (Bolton and Saatman, 2014; Hylin et al., 2013). GFAP was first successfully measured in human blood in 1999, with serum GFAP levels elevated in 12 of 25 patients with severe TBI (Missler et al., 1999).

Using a mouse weight drop model to evaluate two levels of mTBI, one with hemorrhage (complicated mTBI) and one without (uncomplicated mTBI), Yang et al. (2013b) found that serum GFAP was significantly elevated above sham in both injury models at 90min and 6 h post-injury, but had returned to normal by 24 h.

In a small study of nine patients, Kou et al. (2013) found serum GFAP (<24 h) significantly elevated in mTBI patients. Levels were further significantly increased in patients with hemorrhage, but did not correlate with white matter damage. However, the small sample size in this study precludes general conclusions. In a larger study, the ability of serum GFAP (<4 h) to predict intracranial lesions on CT was compared to that of S100B (Papa et al., 2014). Although patients consisted of GCS 9–15, only 3 of the 209 TBI patients had a GCS < 13 and 10% were identified as having intracranial lesions. Both S100B and GFAP were significantly elevated in TBI and further elevated in patients with intracranial lesions. For GCS 14–15 patients, the AUC for identifying intracranial lesions was 0.82 for GFAP and 0.77 for S100B. In the presence of extra-cranial injuries using a cutoff level of 0.067ng/mL, GFAP was 100% sensitive and 55% specific for predicting intracranial lesions. A cutoff of 0.20ng/mL for S100B also yielded 100% sensitivity but only 5% specificity. The authors conclude that GFAP outperforms S100B in identifying intracranial lesions in mild and moderate TBI, even in the presence of extra-cranial injuries.

Papa et al. (2012a) investigated GFAP breakdown products (GFAP-BDP (<4 h) in serum of four groups: mTBI (GCS 13–15), moderate TBI (GCS 9–12,) trauma controls, and non-trauma controls. GFAP-BDP were significantly elevated in the mTBI patients compared to the non-trauma controls, and when GCS 15 patients were assessed independently, the AUC was 0.88 for identifying brain injury. GCS 15 patients also had significantly higher GFAP-BDP levels than the trauma controls, and GFAP-BDP was further elevated in GCS 15 patients with CT positive lesions. While this study suggests GFAP-BDP may be a potential marker of mTBI, most of the statistical analysis was performed using all patient data (GCS 9–15) or for the GCS 15 patients only. Additionally, a relatively high percentage (19.8%) of GCS 15 patients receiving CT scans were positive for an intracranial lesion. This is much greater than the 5–6% of GCS 15 patients with intracranial lesions in other studies (Holm et

al., 2005; Jagoda et al., 2009; Miller et al., 1996). Also of interest is that a significant increase in GFAP-BDP was observed in trauma controls. The source of this elevated serum GFAP is uncertain.

In a study that included a range of TBI severities from mild to severe, plasma GFAP-BDP (<24 h) was able to distinguish mild injury from moderate-severe injury with an AUC of 0.87 (Okonkwo et al., 2013). However, control patients were not included, there was no analysis of mild versus moderate patients, and the majority of statistics were analyzed using all injury levels.

Overall, serum GFAP and its breakdown products appear to be increased in mTBI and may represent a more sensitive marker than S100B inidentifying intracranial lesions. However, additional studies focusing specifically on mTBI (GCS 13–15), which include the appropriate controls and statistical comparisons, are needed for validation.

Neuron specific enolase (NSE)

NSE is a glycolytic protein located in the cytoplasm of neurons (Vinores et al., 1984). Unlike its name suggests, NSE is not neuronal specific as it is also found in neuroendocrine cells, oligodendrocytes, thrombocytes, and erythrocytes (Dash et al., 2010; Schmechel et al., 1978). Serum levels are increased during cardiopulmonary bypass (Johnsson et al., 2000), trauma, shock and ischemic-reperfusion injury (Pelinka et al., 2005; Pelinka et al., 2004b). 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 order to avoid false-positives, NSE should be measured in serum with very low levels of free hemoglobin (Tolan et al., 2013). Despite this lack of specificity and difficulty in measuring accurately, numerous studies have investigated NSE as a potential biomarker of mTBI.

Using a mouse weight drop model, Yang et al. (2013b) found serum NSE significantly elevated at 90 minutes in mTBI both with and without hemorrhage, but at 6 h and 24 h it only remained significantly elevated in the hemorrhagic group. Using the same mTBI model with hemorrhage parameters, this group combined mTBI with a period of hypoxia. At 24 hours, serum NSE was significantly elevated in mTBI and even further elevated in mTBI +hypoxia (Yang et al., 2013a). In a study focusing on cerebrovascular inflammation in a rat model of mild blast injury (mbTBI), Abdul-Muneer et al. (2013) found serum NSE significantly elevated over control at 6 h and 24 h post-injury.

In humans, early studies showed serum NSE to be elevated in mTBI patients (Pelsers et al., 2004; Skogseid et al., 1992), although subsequent reports indicate a lack of clinical relevance despite elevated levels. 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). In assessing the ability of serum NSE (<3 h) to predict intracranial lesions, Wolf et al. (2013) used a cutoff value of 14.7 μg/L to obtain a sensitivity of 56%, a specificity of 77%, and an AUC of 0.64. AUC reached 0.88 when NSE levels were combined with S100B, nausea, amnesia, unconsciousness, and an age >60, leading the authors to conclude that NSE levels

alone were of little prognostic value. Acutely, Mussack et al. (2002) found no significant differences in serum NSE (~24.3min) between intoxicated mTBI patients and controls. In patients with a variety of TBI severities, there was a correlation between serum NSE level (<24 h) and mild injury GCS scores (>13) (Meric et al., 2010). However, there was not a significant difference in NSE levels between mTBI and trauma controls, or NSE and outcome (GOS score). In a study of professional hockey players, there was not a significant change in serum NSE levels between those obtained preseason and following concussion (Shahim et al., 2014).

In contrast to the above studies, NSE exhibited potential as a TBI biomarker in a pediatric population (<13 years of age) with varying severities of inflicted (iTBI) and non-inflicted (nTBI) (Berger et al., 2005). Serum NSE was significantly elevated in both iTBI and nTBI patients but did not correlate with the GCS score. Using a cutoff value of 11.36ng/mL to identify TBI, the AUC was 0.85, sensitivity was 71% and specificity was 64%. When combined with S100B levels (cutoff of (0.017 ng/mL), this was improved to AUC 0.87 and the sensitivity and specificity to 80% and 73%, respectively. In a small number of patients (n=12) with GCS 15, normal CT, no LOC, and no seizure, 89% had elevated NSE levels. While this study appears to show potential for NSE as a biomarker of pediatric TBI, particularly when combined with S100B, mTBI patients were not analyzed separately, and the lack of correlation between NSE and severity conflicts with other data (Meric et al., 2010; Woertgen et al., 2001). The authors acknowledge this, indicating that the lack of correlation may result from fluid resuscitation causing dilution in severe TBI patients as well as difficulty in obtaining GCS scores in infants, intubated, sedated, and paralyzed patients. In a follow-up study, serum and/or CSF was analyzed in well-appearing infants (<1 yoa) with inflicted iTBI. This study specifically targeted “well-appearing infants” with non-specific symptoms such as vomiting, seizure, lethargy and fussiness. A cutoff value of 11.77ng/mL gave an AUC of 0.70, a sensitivity of 69% and a specificity of 70% for identifying iTBI. Sensitivity and specificity levels were altered when combined with the additional marker myelin basic protein. An increase in either protein resulted in 79% sensitivity and a 70% specificity. An increase in both markers was 100% specific, but only 31% sensitive. The authors conclude that NSE may be useful as a screening test in infants that are at risk for iTBI (Berger et al., 2006).

NSE has also been investigated as a potential marker of outcome following mTBI. In GCS 14–15 patients, De Kruijk et al. (2002) found that six months post-injury, an elevated serum NSE (>0.10 μg/L, <6 h) was significantly associated with headache, although clinical symptoms at time of admission were also associated with post-traumatic complaints. In studies assessing one year outcomes, a correlation between serum NSE levels (<10 h) and PTSD was not found (Sojka et al., 2006; Stalnacke et al., 2007). Additionally, there was no significant correlation between NSE and patients accepting a consultation for further management of injury, or between NSE and consultation patients with decreased cognitive function. Topolovec-Vranic et al. (2011) used multiple measures to assess outcome after mTBI. At six weeks post-injury, female gender, LOC, higher RPQ score on day 3, and elevated NSE levels (>14.6 μg/L, <4 h) predicted poor outcome with an AUC of 0.895.

However, once NSE was removed, the AUC was still 0.873, and NSE alone was a poor predictor of outcome at 6 weeks with an AUC of only 0.629.

In summary, while serum NSE may be elevated in mTBI, its location in erythrocytes makes it difficult to utilize for diagnosing mTBI or predicting outcome in adults. Berger and colleagues propose that further investigation of NSE in the pediatric population is warranted (Berger et al., 2005, 2006).

Ubiquitin C-terminal hydrolase-L1 (UCHL1)

UCHL1 is located in the cytoplasm of neurons (Doran et al., 1983; Jackson and Thompson, 1981) and makes up 1–2% of total soluble protein in the brain (Jeter et al., 2013a). However, it is also located in neurons of the peripheral nervous system, particularly the neuromuscular junction (Chen et al., 2010), as well as cells of the diffuse neuroendocrine system (Thompson et al., 1983). UCHL1 has also been identified in aortic endothelial and smooth muscle cells (Takami et al., 2007) and tumors (Campbell et al., 2003). It is involved in the addition or removal of ubiquitin from proteins destined for the proteasome pathway and is important for the removal of excessive, oxidized, or misfolded proteins in both physiologic and pathologic conditions such as neurodegenerative disorders (Mondello et al., 2014; Yokobori et al., 2013).

Papa et al. (2012b) analyzed serum UCHL1 (<4 h) in mild TBI (n = 86), moderate TBI (n =10), trauma controls, and non-trauma controls. For GCS 15 patients, serum UCHL1 was significantly elevated above non-trauma controls with an AUC of 0.87. GCS 15 patients also had significantly higher UCHL1 levels than the trauma controls, and levels were further elevated in GCS 15 patients with CT positive lesions and GCS 15 patients requiring neurosurgical intervention (NSI). While this study does suggest UCHL1 may be a potential marker of mTBI, most of the statistics in this study were either calculated using all patient data (GCS 9–15) or for the GCS 15 patients only. Additionally, 5% of GCS 15 patients (4/77) required neurosurgical intervention, which is greater than the 1% of GCS 14–15 patients requiring neurosurgical intervention in other studies (Holm et al., 2005; Jagoda et al., 2009).

In a much smaller study (n=9), serum UCHL1 (<6 h) was significantly elevated in mTBI patients (<6 h) (Kou et al., 2013). However, UCHL1 levels did not correlate with white matter damage. In a study of various TBI severity levels, serum UCHL1 (<24 h) could distinguish mTBI patients with intracranial lesions from those without intracranial lesions with an AUC of 0.713 (Diaz-Arrastia et al., 2014). In mTBI patients, however, there was no correlation between UCLH1 and recovery (GOSE >8) six months post-injury. While there was a significant increase in UCHL1 levels in moderate/severe TBI when compared to mTBI, mTBI patients were not compared to controls.

In a study of high school football players, 15 athletes had serum drawn before and after two games (Puvenna et al., 2014). There was not a significant difference in UCHL1 levels between negative controls and mTBI positive individuals (<6 h) regardless of positive or negative CT findings. Additionally, there was no correlation between serum UCHL1 and the number of sub-concussed head hits. UCHL1 and S100B, used as markers of brain damage

and blood brain barrier disruption, respectively, were both elevated post-game. However, only S100B, not UCHL1, was correlated with number of sub-concussive head hits and the elevation in UCHL1 did not correlate with increases in S100B. The authors suggest the post-game UCHL1 elevations could be due to neuromuscular junction release. A small (mTBI=11) pediatric study, encompassing multiple levels of injury also found no significant elevations of UCHL1 in serum (<24 h) of mTBI patients (Berger et al., 2012).

In summary, there are contrasting data regarding use of UCHL1 as a serum biomarker of mTBI. Some studies suggest itmay hold promise for diagnosing mTBI or identifying intracranial lesions, while others did not find a significant correlation with injury. Release from non-CNS sources, including the neuromuscular junction, may contribute to elevated serum levels.

Myelin basic protein (MBP)

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, 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 diffuse axonal injury (DAI), a characteristic of mTBI (Sharp and Ham, 2011; Shenton et al., 2012), MBP has been identified as a potential biomarker of mTBI.

Berger et al. (2005) evaluated serum MBP in the pediatric population. In children with multiple severities of inflicted and non-inflicted TBI, serial serum samples were measured, and initial MBP levels were not different from controls. However, there was a difference in peak MBP levels between patients with and without intracranial hemorrhage. Using a cutoff value of 0.3ng/mL to identify intracranial hemorrhage, the AUC was 0.69, sensitivity was 44% and specificity was 96%. Importantly in a small number of patients (n=12) with GCS 15, normal CT, no LOC, and no seizure, 0% of patients had an elevated MBP. Similarto NSE, the authors found no correlation between MBP and GCS score and conclude that MBP is unlikely to be a useful screening tooling for TBI because MBP levels do not peak promptly. In their follow-up study of iTBI in infants, MBP was 36% sensitive and 100% specific for identifying iTBI at a cutoff value of 0.30ng/mL (Berger et al., 2006). When combined with the additional marker NSE, an increase in either protein resulted in 79% sensitivity and a 70% specificity. An increase in both markers was 100% specific, but only 31% sensitive. The authors conclude that MBP may be useful as a screening test in infants at risk for iTBI.

Similar to NSE, MPB may be of value as a biomarker for pediatric TBI including iTBI. 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. MBP also shows promise as an indicator of intracranial hemorrhage. However, most studies evaluating MBP as a potential TBI biomarker have examined more severe injuries, and those examining mTBI have had relatively small sample sizes.

Spectrin break down products (SBDP)

αII-Spectrin is a cytoskeletal protein abundant in axons and pre-synaptic terminals of neurons (Weiss et al., 2009). It is cleaved to spectrin break down products (SBDP) during cellular insults and cell death (Siman and Noszek, 1988; Wang et al., 1998). Calpain, involved in necrosis, cleaves spectrin to 150 and 145 kDa fragments (SBDP150 and SBDP145), while caspase-3, involved in apoptosis can also produce a 150 kDa band along witha 120 kd breadown product SBDP120 (Wang et al., 1996, 1998). However, αII-spectrin is not specific to the brain and is a target during apoptosis of other cells such as lymphocytes and hematopoietic cells (Wang et al., 1998). Although studies are few to date, serum detection of spectrin breakdown products is emerging as a promising biomarker of mTBI.

In a controlled cortical impact (CCI) rat model of mild and severe TBI, Ringger et al. (2004) found that SBDP were significantly elevated in the CSF of mTBI rats 2 h, 6 h, and 24 h post-injury. To date, serum SBDPs have not been evaluated in experimental mTBI models.

In a human study, Siman et al. (2013) evaluated the N-terminal αII-spectrin fragment (SNTF) producedby calpain cleavage. In plasma (<24 h) of patients with negative CT scans, SNTF was elevated in a subset of mTBI and orthopedic injury patients. Elevated SNTF levels were associated with diffuse axonal injury as assessed by diffusion tensor imaging, as well as with impaired cognitive function. Elevated plasma SNTF in CT-negative mTBI patients was 100% sensitive and 75% specific for predicting impaired cognitive performance three months post-injury. In a subsequent study, SNTF was evaluated in serum of concussed hockey players (Siman et al., 2014). Significant elevations above average pre-season baseline levels were observed 12–144 h post-concussion, and levels returned to baseline by the time post-concussive symptoms had resolved and players were allowed to return to play. Furthermore, players whose symptoms took longer than six days to resolve had significantly higher levels of SNFT 12–36 h post-concussion than players whose symptoms resolved more quickly. The AUC for diagnosing concussions with persistent post-concussive symptoms was 0.87. Tau measured at 12 h was also found to be significantly elevated in players whose symptoms took greater than six days to resolve, but multivariate analysis combining SNTF and tau did not improve the diagnostic accuracy above SNTF alone. In contrast, Berger et al. (2012) evaluated serum SBDP (<24 h) in a small (mTBI= 11) pediatric population and found no statistical difference between mTBI and control patients.

Overall, current data regarding serum spectrin breakdown products as biomarkers of mTBI is promising yet data is limited.

Tau

Tau is a microtubule-associated protein abundant in axons, but can also be found in the liver, kidney, and testes (Morris et al., 2011). Tau is phosphorylated under both physiologic and pathologic conditions (Schwab et al., 1994), but is hyper-phosphorylated in neurofibrillary tangles characteristic of Alzheimer's and CTE (Morris et al., 2011). Similar to αII-spectrin, tau can be proteolytically cleaved by caspases and calpains to generate a 17kDa fragment (c-tau) (Canu et al., 1998).

In pediatric patients (<14 yoa) with GCS 14–15, Guzel et al. (2010) found serum tau was significantly elevated in mTBI, although there was no difference in levels between patients with positive versus negative CT. In a cohort of GCS 13–15 patients that also included children, there was also no correlation between serum tau levels (<24 h) and positive CT (Kavalci et al., 2007). A significant increase in serum tau (<10 h) was observed in mTBI patients at high risk for intracranial lesions (GCS 13, worsening headache, seizure, vomiting, >60 years of age) (Bulut et al., 2006). However, this is of limited clinical value as these patients would likely receive CT scans based on their risk factors.

In a study of professional hockey players in which NSE was also evaluated post-mTBI (Shahim et al., 2014), plasma total tau levels were also measured using an ultra-sensitive assay, and found to be significantly increased in concussed players when compared to preseason controls, with levels remaining significantly elevated for at least 6 days. Importantly, the concentration of tau one hour post-injury was correlated with the number of days it took for symptoms to resolve, and high levels at 6 days correlated with persistent PCS.

Tau has also been investigated as a prognostic marker in mTBI, although with negative results. Serum cleaved-tau (<6 h) did not correlate with PCS at three months following mTBI (Bazarian et al., 2006; Ma et al., 2008), or (<10 h) at six months postinjury (Bulut et al., 2006).

In summary, the utility of serum tau or cleaved-tau as a biomarker for mTBI is inconsistent. Some studies suggest it is elevated in mTBI and correlates with post-injury symptoms, while others find it cannot predict CT positive lesions or outcome. Increased sensitivity of tau assays (Rubenstein et al., 2014; Shahim et al., 2014) could clarify utility of tau as a biomarker for mTBI.

Neurofilaments (NF)

NFs are intermediate filament proteins found in axons and dendrites of neurons (Gatson et al., 2014). They are CNS specific and are composed of light (NFL), medium (NFM) or heavy chains (NFH). NFH can be extensively phosphorylated (pNFH), which protects neurofilaments from degeneration. Biofluid NFH levels are increased in a variety of CNS disorders such as multiple sclerosis, Alzheimer’s disease, and amyotrophic lateral sclerosis (Petzold, 2005). Few studies have investigated biofluid NF in mTBI.

Mild CCI in rats produced no increase in serum pNFH at any time point examined (1 h–7 d) (Anderson et al., 2008). NFH was included in a panel of serum biomarkers in experimental blast TBI studies as discussed below for Reverse Phase Protein Microarray.

However, in a human mTBI study, Gatson et al. (2014) investigated serum levels of pNFH 1 and 3 days post-injury, an important time frame since mTBI patients often delay seeking medical attention. At both time points, pNFH was significantly elevated above controls. One day post-injury pNFH distinguished mTBI from controls, a cutoff value of 110.5pg/ml provided a sensitivity and specificity of 100%. pNFH levels were also significantly elevated in CT positive patients versus CT negative patients, and were inversely correlated with GCS

score. Three days post-injury, a cutoff value of 77.5 pg/ml gave a sensitivity of 100% and a specificity of 96.43%.

The high sensitivity and specificity of pNFHto identify mild traumatic brain injury 24–72 h post-injury is encouraging, but currently limited to a single human mTBI study.

Metabolites

Metabolic dysregulation is known to occur in moderate and severe TBI (Maas et al., 2008) and metabolic changes have also been observed after mTBI (Gross et al., 1996; Vagnozzi et al., 2010). Metabolites are oftenmonitored using imaging techniques suchaspositron emission tomography (PET) and proton magnetic resonance spectroscopy (Jeter et al., 2013a). Few studies have investigated metabolites in the biofluids of mTBI patients.

Jeter et al. (2012) used chromatography and mass spectrometry to assess L-arginine and its metabolic products in the plasma (<24 h) of mTBI patients, but did not observe changes as compared to controls. The same group also assessed branched chain amino acids (BCAA) in plasma (<24) of mTBI patients (Jeter et al., 2013b). BCAAs and several of their metabolites were significantly reduced in the mTBI patients. However, this was not specific to brain injury as plasma levels of BCAAs are also decreased in orthopedic trauma patients. The lack of specificity, decreased concentrations and need for assessment via mass spectrometry make BCAA an unlikely mTBI biomarker.

Lipid peroxidation

Reactive oxygen species and reactive nitrogen species generated during the secondary injury cascade can induce lipid peroxidation (Hall and Braughler, 1993). The brain is highly enriched in a number of lipids susceptible to peroxidation such as arachidonic acid, linoleic acid, linolenic acid, and docosahexaenoic acid. Lipid peroxidation disrupts membrane architecture and generates neurotoxic aldehydes that can bind proteins and impair their function (Hall et al., 2010). Products of lipid peroxidation such as isoprostanes, neuroprostanes, and isofuranes are elevated in CSF and serum of severe TBI patients (Bayir et al., 2002; Corcoran et al., 2011; Seifman et al., 2008; Varma et al., 2003) and increases in lipid peroxidation are seen in the tissue of rodents following mTBI (Caner et al., 2004; Inci et al., 1998; Shultz et al., 2013). Currently there are no published studies focused on detection of lipid peroxidation in biofluids of mTBI, although 4-hydroxynonenal (HNE) has been found to be elevated in rodent serum following mild blast injury (see below) using reverse phase protein microarray (Ahmed et al., 2013).

Inflammatory markers, multiplex antibody array, and reverse phase protein microarray

TBI results in an inflammatory response (Agoston et al., 2009; Kreutzberg, 1996; Zetterberg et al., 2013). Multiplex antibody array technologies investigate multiple inflammatory markers concurrently. Antibody arrays have been developed that are large-scale, stable and reproducible, and can be used in the detection of very low abundance proteins such as cytokines (Agoston et al., 2009). Commercial multiplex antibody arrays simultaneous screen for multiple cytokines, using low sample volumes. They are available either as ELISA sandwich arrays or as flow cytometry beads (Lash et al., 2006; Martins et al., 2002).

Although these arrays allow for a broad evaluation of markers, they screen for targets of pre-selected antibodies are therefore considered a hypothesis-driven approach.

In two separate experiments using a mouse weight drop model of mTBI, one of which combined mTBI with hypoxia, Yang et al. (2013a, b) used a multiplex assay to evaluate serum chemokines and cytokines. Interleukin-6 (IL-6), keratinocyte-derived chemokine, macrophage inflammatory protein-1α, and macrophage-derived chemokine were all significantly elevated at various time points. For serum IL-6 (6 h), the AUC was 0.86, and a cutoff value of 172 pg/mL gave an 80% sensitivity and an 80% specificity for predicting mTBI. In another mouse study, a mild closed head injury was combined with stress to simulate PTSD. Thirty three days post-injury, plasma IL-17A was significantly elevated in the mTBI/no stress group (Ojo et al., 2014).

In a small pediatric study (n=16), Berger et al. (2009) used five multiplex bead assays to investigate 44 markers in the serum of well-appearing infants with mild iTBI. Nine markers showed significant increases (matrix metallopeptidase-9, hepatocyte growth factor, fibrinogen, IL-6) or decreases (Intracellular cell adehesion molecule-1 ICAM, vascular cellular adhesion molecule VCAM, eotaxin, tumor necrosis factor receptor 2, IL-12). VCAM and IL-6, the two markers that showed the greatest change were 87% sensitive and 90% specific for identifying mild iTBI. Since VCAM was decreased and unlikely to easily be used as a biomarker, IL-6 and MMP-9 were also analyzed and gave a sensitivity of 81% and specificity up to 94%.

Reverse phase protein microarray (RPPM) is a method in which protein from tissue or biofluid is printed onto an array using serial dilutions to allow for quantification of protein. These arrays are then screened with antibodies (Agoston et al., 2009). RPPM is an extremely sensitive, high throughput technique and can be used to screen large numbers of samples using very small quantities (Gyorgy et al., 2010). Similar to multiplex antibody arrays, RPPM allows for a broad evaluation of markers using pre-selected antibodies.

Ina rat weight drop model of mTBI, Rostami et al. (2012) used RPPM to evaluate serum biomarker levels. S100B, MBP, and tau were significantly elevated 1–14 days post-injury, while NFH was elevated 1–3 days post-injury. Kamnaksh et al. (2012) used RPPM to evaluate GFAP, NSE, NFH, and vascular endothelial growth factor (VEGF) in the plasma (2 h and 22 d) of rats exposed to either single or multiple mild blasts. All four proteins were significantly elevated in at least one condition or time point. In a similar study, Ahmed et al. (2013) used RPPM to analyze thirteen plasma proteins 42 days after single or multiple mild blasts. HNE, ceruloplasmin, von Willebrand factor vWF, chemokine receptor 5, hypoxia-inducible factor-1α, formyl peptide receptor 1, p38 mitogen-activated protein kinase, MMP-8, VEGF, GFAP, MBP, and NFH were all significantly elevated in at least one condition. Gyorgy et al. (2011) used RPPM to evaluate S100B, NSE, MBP, and NFH in the serum (6 h, 24 h, 72 h, 2 weeks) of pigs exposed to different severities of blast. In mbTBI, S100B and MBP were significantly elevated at all four time points; NSE was slightly elevated at 6 h with levels returning to control by 2 weeks, and NFH showed a unique temporal profile with mbTBI levels peaking late (72 h–2 weeks) compared to severe levels which peaked at 6 hours.

In summary, multiplex technology and RPPM identified modulation of inflammatory markers, especially interleukins and matrix metallopeptidases, in multiple TBI studies. Alterations in vascular permeability were also seen in multiple studies as assessed by the markers VEGF, vWF, and ICAM. Other markers, such as the hypoxia/oxidative stress markers HNE and ceruloplasmin, were elevated under more than one injury condition. With the exception of Berger et al. (2009), these studies have been limited to animal models.

Cytokine multiplex antibody arrays are important in understanding the inflammatory response that occurs following mTBI, and RPPM allows for simultaneous evaluation of multiple samples, proteins, time points, and injury parameters. However, it can be difficult to distinguish between cranial vs. non-cranial sources of inflammatory markers. Evaluating panels of biomarkers may provide signature patterns that are not evident from evaluation of single molecules.

The unbiased approach

The above hypothesis-driven biomarkers have largely been evaluated in moderate to severe TBI. For mTBI, diagnosis is more challenging because of the less severe symptoms and typically negative conventional imaging results. As discussed above, evaluation of existing biomarkers reveals some promising candidates to assist in the diagnosis of mTBI, although many challenges remain. Several studies included multiple injury levels and did not analyze the mild injury data independently. Mild traumatic brain injuries are heterogeneous, and there is a lack of standardization in methodology, sample sizes, appropriate control groups, and patient characteristics (Berger, 2006; Mondello et al., 2014). In terms of prognosis, outcomes measures are often inappropriate and tests for evaluation are not standardized (Berger, 2006). Large multi-center and longitudinal studies using homogenous populations, appropriate controls, testing metrics and statistical analysis can improve the limitations clinical studies face (Berger, 2006; Mondello et al., 2014). A previous review concluded that “no biomarker has consistently demonstrated the ability to predict postconcussive syndrome after mTBI” (Begaz et al., 2006) and a separate study of serum biomarkers for mTBI found “the discriminative power of the biomarkers alone was limited (Topolovec-Vranic et al., 2011). As there is not yet a fully satisfactory biomarker for mTBI, research into the identification of additional biomarkers should continue (Mondello et al., 2014).

To identify novel biomarkers, unbiased discovery approaches have begun to be utilized. In the unbiased approach novel biomarkers are screened for, initially without regard to pathophysiology or the use of pre-selected antibodies. MicroRNA arrays and proteomics are two well-known unbiased techniques (Table 3). Phage display represents another promising approach, although it has not yet been applied to mTBI.

MicroRNAs

MicroRNAs are small, non-coding RNAs approximately 22 nucleotides in length (Ambros, 2001). They serve as post-transcriptional regulators of gene expression and are involved in mRNA degradation and repression (Lagos-Quintana et al., 2001). MicroRNAs show tissue specificity (Babak et al., 2004; Lagos-Quintana et al., 2002) and plasma microRNAs can be elevated after tissue injury (Laterza et al., 2009). There is interest in identification of

circulating miRNAs to diagnose disease, especially cancer. However, there are challenges with fluid selection, RNA extraction, platform variation, qPCR validation, and sequencing (De Guire et al., 2013). MicroRNAs are beginning to be investigated as biomarkers of mTBI.

Balakathiresan et al. (2012) exposed rats to repetitive mild blast injury using short (2 h) or long inter-injury intervals (24 h) and evaluated microRNAs in serum and CSF three hours or 24 hours post-blast. Five microRNAS (miR-let7i, miR-122, miR-340-5p, miR-200b, and miR-874) were elevated in multiple conditions. Upon further validation, serum and CSF levels of miR-let7i were significantly up-regulated in the short interval group only. Bioinformatics analysis revealed miR-let-7i may regulate inflammatory cytokines, S100B and UCHL1.

Redell et al. (2010) assessed microRNA levels in plasma (<24 h) of patients with mild or severe TBI. Levels of miR-16 and miR-92a were significantly elevated in mTBI, which starkly contrasted with decreased levels observed in severe injuries. miR-16 is associated with cell proliferation, cell cycle progression and apoptosis, while miR-92a is a negative regulator of angiogenesis. However, these microRNAs only offered fair diagnostic accuracy for mTBI (miR-16's AUC was 0.82 and miR-92a's AUC was 0.78). Additionally, there was no significant difference in miR-16 or miR-92a levels between mTBI and orthopedic injury patients.

Using a much longer time point, Pasinetti et al. (2012) evaluated peripheral blood mononuclear cells (PBMCs) in 18 veterans of the Iraq and Afghanistan conflicts, half of which had sustained an mTBI. For the mTBI patients, the time since last deployment ranged from 1.2 to 6.6 years. Thirteen small non-coding RNAs were significantly lower in the mTBI patients, and a panel of three small nucleolar RNAs (HBII-289, ENSG199411, and U35A) was able to identify mTBI with 82% selectivity and 78% specificity. While these offer a potential diagnostic for mTBI years after the injury, replication/validation is needed with larger sample sizes.

Proteomics

Traditionally, the field of proteomics encompasses high-throughput techniques such as gel electrophoresis, mass spectrometry (LC-MS, CE-MS, SELDI-MS, TOF-MS), antibody arrays, and high-throughput immunoblotting, which are then combined with bioinformatics (Agoston et al., 2009; Denslow et al., 2003; Ottens et al., 2006, 2007; Wang et al., 2004, 2005). As with any technique, there are some limitations to these methodologies. For example, 2D gel electrophoresis is most relevant for medium sized proteins and has low sensitivity toward integral membrane proteins (Denslow et al., 2003; Zurbig and Jahn, 2012). Also, artifacts can arise due to other medical conditions, sample collection, and sample storage (Diamandis, 2004). Overall, proteomics is a powerful approach to identifying novel biomarkers of TBI and mTBI. To date, TBI proteomics has largely been applied to models such as degenerating neuron cultures, brain tissue, or moderate-severe injuries (Cadosch et al., 2010; Conti et al., 2004; Cortes et al., 2012; Guingab-Cagmat et al., 2012; Hanrieder et al., 2009; Haqqani et al., 2007; Haskins et al., 2005; Hergenroeder et al.,

2008; Jenkins et al., 2002; Kobeissy et al., 2006; Lakshmanan et al., 2010; Liu et al., 2006; Ottens et al., 2010).

These approaches are useful for mTBI biomarker discovery, provided the findings are further validated in mTBI biofluids. This approach was used by Siman et al. (2004) who began with an in vitro model of degenerating neurons. In a necrosis model, 2D-PAGE-MS identified the release of 56 proteins. Importantly, further validation via western blot showed that 14-3-3ζ, 14-3-3(3, α-spectrin, and tau were elevated in the CSFof rats exposed to mild fluid percussion injury. Subsequent studies examining tau and α-spectrin in mTBI studies are discussed above.

Studies that begin their proteomic screens in the biofluids of mTBI subjects are the most likely to be successful in the identification of mTBI biomarkers. Such studies are discussed below.

Crawford et al. (2012) used CCI mouse models of mild and severe brain injury. Plasma (24 h, 1 month) was analyzed using LC-MS, and 30 proteins were identified as being significantly modulated after injury. At 24 h there was no overlap between mild and severe injury groups. Additionally, in the mTBI group there was no overlap between the 24 h and 1 month post-injury time points. Bioinformatics analysis of proteins modulated after injury identified functions known to be associated with TBI such as the acute phase response, oxidative stress, and lipid metabolism. This study nicely demonstrates how protein profiles vary based on injury parameters.

In a human study, Gao et al. (2014) evaluated serum (~12 h) from infants that had sustained a mild abusive head injury (AHI) with an acute extra-axial hemorrhage. Beginning with 2D-DIGE combined with LC-MS, the two spots with the highest spot change between control and injury were identified as serum amyloid A (SAA). Upon validation with western blot, SAA was significantly higher in mild AHI than in controls, with a very high AUC of 0.96. Further analysis of multiple injury levels via western blot and ELISA continued to show SAA levels elevated above controls, but there was not a difference in SAA levels among the injury severities. SAA is an acute phase reactant, making it a non-specific marker of inflammation. However, the authors did not see an association between SAA and fractures and also screened for other sources of inflammation such as liver injury. The authors conclude that SAA, with its likely long half-life, is a candidate biomarker to identify children with mild AHI that would benefit from radiologic evaluation.

Phage display

Phage display is a method to select peptides, proteins or antibodies with specific binding properties. It is most widely used to investigate protein-protein interactions, receptor- and antibody-binding sites, and for selecting antibodies against a range of antigens (Bradbury, 2010). It uses bacteriophages (viruses that infect bacteria) in which DNA encoding peptides or proteins is inserted into the gene encoding a coat protein of a filamentous phage such as M1 3 phage. Libraries of bacteriophages expressing 10^9 random hexapeptide or dodecapeptide sequences are commercially available. Following identification of phages which selectively bind to a sample of interest, the inserted DNA sequence is translated to

obtain the peptide sequence of the foreign protein/peptide expressed on the tip of the phage. This peptide can then be synthesized and used in pull-down assays to attract and bind proteins of interest. The captured proteins can then be identified by mass spectrometry.

Phage display has been successfully utilized to identify serum biomarkers in non-CNS disorders (Wang et al., 2011; Weng et al., 2012; Zhang et al., 2011) and to identify autoantibodies in multiple sclerosis (Somers et al., 2008), but has not previously been applied to other CNS disorders including TBI. Phage display represents a potential, unbiased, strategy to identify novel serum biomarkers of mTBI.

Novel approaches

Biomarkers in urine

Urine is easily obtained, particularly in the post-acute phase where it could be self-collected. However, quantitation of urinary biomarkers is problematic as they can very dependent upon flow rates. Urinary S100B measurement revealed elevations in children and adults with severe TBI and strong correlations with serum S100B levels (Berger and Kochanek, 2006; Hallen et al., 2010; Rodriguez-Rodriguez et al., 2012). However, a separate study of pediatric TBI found similar S100B levels in urine from individuals with cranial and extracranial injury, questioning the suitability of urinary S100B as a TBI biomarker (Pickering et al., 2008). A preliminary study suggests elevations in urinary trypsin inhibitor with brain injury, although the sample size was very small with five head injury patients (GCS 4–13) and five control subjects (Sakai et al., 2003).

Urine was evaluated in a recent proteomics study of moderate to severe TBI, with subprofiles of 2476 discriminant variables correlating with injury severity (Ottens et al., 2014). Urine was chosen because of its likelihood to contain small metabolic brain injury byproducts consistent with brain barrier efflux, which is important in the post-acute time period when the blood brain barrier is no longer disrupted. Mass spectrometry was used to screen urine collected from severe TBI patients ~17 days post-injury in order to identify potential biomarkers that could aid in assessment of rehabilitation. A “TBI urinary signature” was identified and accurately classified TBI subjects and controls. This signature correlated with measures of injury severity and behavioral and neurocognitive function such as GCS, Patient Competency Scale (PCRS), and Frontal Systems Behavioral Scale (FSBC). Furthermore, identified proteins of the signature were biologically relevant, and involved in processes such as outgrowth and guidance, the extracellular matrix, the post-synaptic density, and neuroplasticity. To our knowledge, urinary biomarkers have not been examined following mTBI.

Autoantibodies

Following mild traumatic brain injury the blood brain barrier is compromised and brain specific proteins can escape to trigger an immune response and development of autoantibodies. Autoantibodies against brain proteins have been implicated in a variety of neurologic disorders such as multiple sclerosis, Alzheimer’s disease, stroke, and epilepsy (Zhang et al., 2014).

Zhang et al. (2014) collected serum from severe TBI patients 0–10 days post-injury and screened it against human brain lysate via western blot to identify autoantibodies. Mass spectrometry revealed autoantibodies directed against GFAP and its breakdown products. Autoantibodies were able to distinguish post-acute severe TBI patients from controls with an AUC of 0.78. Patients who achieved a GCS score of 9–13 within 24 h of injury had significantly lower levels than those with GCS scores remaining below 8, levels negatively correlated with GOSE at time of discharge, and negatively correlated with poor outcome 6 months post-injury.

Used in mild TBI this method has the potential to identify novel autoantibodies and their antigens, either of which could serve as potential biomarkers. Autoantibodies are long lasting and could be particularly important for diagnosing post-acute or chronic mTBI.

Conclusions

Millions of people are affected by mTBI and its sequelae, but mTBI is difficult to diagnose. It lacks a consensus definition, relies on subjective self-reported symptoms, and is insensitive to commonly available imaging modalities. An objective and quantifiable measure of mTBI, such as a serum biomarker, is needed. Suitable biomarkers would aid in diagnosis, prognosis, return to play/duty assessments, therapeutic evaluations, and provide additional knowledge regarding mTBI pathophysiology. Most studies to date have employed a hypothesis-driven approach to evaluate potential mTBI biomarkers; focusing on proteins present at high levels in affected cells.

The hypothesis-driven approach has produced several potential biomarker candidates. Of these, S100b has been the most thoroughly investigated. Low serum S100B levels may provide reassurance regarding the relatively mild nature of the injury when combined with other diagnostics, while high levels are associated with slower recovery and worse outcomes. The major drawback to S100B as a mTBI biomarker is the non-CNS sources contributing a lack of specificity. Other putative mTBI biomarkers have been evaluated much less extensively than S100B. GFAP and its breakdown products show promise in mTBI studies with good sensitivity, although there are also concerns regarding specificity. Specificity is also problematic for NSE, as release from erythrocytes can contribute to serum elevations. MBP exhibits a delayed elevation following mTBI and appears particularly useful in the post-acute phase, although it appears to have relatively weak sensitivity. Spectrin breakdown products show promise as mTBI biomarkers based on the limited data currently available. Results for tau protein along with cleaved tau as mTBI biomarkers is inconsistent, recent improvements in the sensitivity of tau assays may help clarify tau's utility as a mTBI biomarker. For NFH, the limited data available appears promising. For identification of novel biomarkers, proteomics identified a unique post-acute urinary signature in severe TBI patients. Phage display has identified serum biomarkers for non-CNS disorders, but has not yet been applied to mTBI. These unbiased approaches and use of unconventional biomarker biofluids such as urine could complement current approaches to identify biomarkers of mTBI.

Acknowledgments

The author's research was supported by NIH grants R21 NS084088 and RO1 NS062993.

Abbreviations

AHI	Abusive Head Injury
AUC	Area Under The Curve
BCAA	Branched Chain Amino Acids
CCI	Controlled Cortical Impact
CCR5	Chemokine Receptor 5
CRMP	Collapsin Response Mediator Protein Family
CTE	Chronic Traumatic Encephalopathy
GCS	Glasgow Coma Scale
GFAP	Glial Fibrillary Acidic Protein
GFAP-BDP	Glial Fibrillary Acidic Protein Breakdown Product
FPR1	Formyl Peptide Receptor 1
GAP43	Growth Associated Protein 43
GOSE	Glasgow Outcome Scale Extended
HGF	Hepatocyte Growth Factor
HIF-1α	Hypoxia-Inducible Factor-1α
HNE	4-Hydroxynonenal
ICAM-1	Intracellular Adhesion Molecule-1
IL-6	Interleukin-6
IL-12	Interleukin-12
IL-17A	Interleukin-17A
iTBI	Inflicted TBI
KC	Keratinocyte-Derived Chemokine
LOC	Loss of Consciousness
MAP2	Microtubule Associated Protein 2
MBP	Myelin Basic Protein
MIP-1α	Macrophage Inflammatory Protein-1α
MDC	Macrophage-Derived Chemokine
MMP-8	Matrix Metallopeptidase-8
MMP-9	Matrix Metallopeptidase-9

mTBI	Mild Traumatic Brain Injury
NF	Neurofilament
NSE	Neuron Specific Enolase
NSI	Neurosurgical Intervention
PBMC	Peripheral Blood Mononuclear Cell
PCS	Post-Concussive Syndrome
PTA	Post-Traumatic Amnesia
PTC	Post-Traumatic Complaints
RPPM	Reverse Phase Protein Microarray
RPQ	Rivermead Post-Concussive Symptoms Questionnaire
SAA	Serum Amyloid A
STNF	N-Terminal αII-Spectrin Fragment
SBDP	αII-Spectrin Break Down Products
SIS	Second Impact Syndrome
TNFR2	Tumor Necrosis Factor Receptor 2
UCHL1	Ubiquitin C-Terminal Hyrdrolase-L1
VCAM	Vascular Cellular Adhesion Molecule
VEGF	Vascular Endothelial Growth Factor
vWF	von Willebrand Factor
YOA	Years of Age

References

Abdul-Muneer PM, Schuetz H, Wang F, Skotak M, Jones J, Gorantla S, Zimmerman MC, Chandra N, Haorah J. Induction of oxidative and nitrosative damage leads to cerebrovascular inflammation in an animal model of mild traumatic brain injury induced by primary blast. Free Radic Biol Med. 2013; 60:282–291. [PubMed: 23466554]

Agoston DV, Gyorgy A, Eidelman O, Pollard HB. Proteomic biomarkers for blast neurotrauma: targeting cerebral edema, inflammation, and neuronal death cascades. J Neurotrauma. 2009; 26:901–911. [PubMed: 19397421]

Ahmed FA, Kamnaksh A, Kovesdi E, Long JB, Agoston DV. Long-term consequences of single and multiple mild blast exposure on select physiological parameters and blood-based biomarkers. Electrophoresis. 2013; 34:2229–2233. [PubMed: 23712899]

Alsaif M, Guest PC, Schwarz E, Reif A, Kittel-Schneider S, Spain M, Rahmoune H, Bahn S. Analysis of serum and plasma identifies differences in molecular coverage, measurement variability, and candidate biomarker selection. Proteomics Clin Appl. 2012; 6:297–303. [PubMed: 22641612]

Ambros V. microRNAs: tiny regulators with great potential. Cell. 2001; 107:823–826. [PubMed: 11779458]

Anderson RE, Hansson LO, Nilsson O, Dijlai-Merzoug R, Settergren G. High serum S100B levels for trauma patients without head injuries. Neurosurgery. 2001; 48:1255–1258. (discussion 1258–1260). [PubMed: 11383727]

Anderson KJ, Scheff SW, Miller KM, Roberts KN, Gilmer LK, Yang C, Shaw G. The phosphorylated axonal form of the neurofilament subunit NF-H (pNF-H) as a blood biomarker of traumatic brain injury. J Neurotrauma. 2008; 25:1079–1085. [PubMed: 18729720]

Arciniegas DB, Anderson CA, Topkoff J, McAllister TW. Mild traumatic brain injury: a neuropsychiatric approach to diagnosis, evaluation, and treatment. Neuropsychiatr Dis Treat. 2005; 1:311–327. [PubMed: 18568112]

Astrand R, Romner B, Lanke J, Unden J. Reference values for venous and capillary S100B in children. Clin Chim Acta. 2011; 412:2190–2193. [PubMed: 21864523]

Babak T, Zhang W, Morris Q, Blencowe BJ, Hughes TR. Probing microRNAs with microarrays: tissue specificity and functional inference. RNA. 2004; 10:1813–1819. [PubMed: 15496526]

Bakay RA, Ward AA Jr. Enzymatic changes in serum and cerebrospinal fluid in neurological injury. J Neurosurg. 1983; 58:27–37. [PubMed: 6847906]

Baker JG, Rieger BP, McAvoy K, Leddy JJ, Master CL, Lana SJ, Willer BS. Principles for return to learn after concussion. Int J Clin Pract. 2014; 68:1286–1288. [PubMed: 25348379]

Balakathiresan N, Bhomia M, Chandran R, Chavko M, McCarron RM, Maheshwari RK. MicroRNA let-7i is a promising serum biomarker for blast-induced traumatic brain injury. J Neurotrauma. 2012; 29:1379–1387. [PubMed: 22352906]

Barbarese E, Barry C, Chou CH, Goldstein DJ, Nakos GA, Hyde-DeRuyscher R, Scheld K, Carson JH. Expression and localization of myelin basic protein in oligodendrocytes and transfected fibroblasts. J Neurochem. 1988; 51:1737–1745. [PubMed: 2460587]

Bay E, Hagerty B, Williams RA, Kirsch N. Chronic stress, salivary cortisol response, interpersonal relatedness, and depression among community-dwelling survivors of traumatic brain injury. J Neurosci Nurs. 2005; 37:4–14. [PubMed: 15794439]

Bayir H, Kagan VE, Tyurina YY, Tyurin V, Ruppel RA, Adelson PD, Graham SH, Janesko K, Clark RS, Kochanek PM. Assessment of antioxidant reserves and oxidative stress in cerebrospinal fluid after severe traumatic brain injury in infants and children. Pediatr Res. 2002; 51:571–578. [PubMed: 11978879]

Bazarian JJ, Zemlan FP, Mookerjee S, Stigbrand T. Serum S-100B and cleavedtau are poor predictors of long-term outcome after mild traumatic brain injury. Brain Inj. 2006; 20:759–765. [PubMed: 16809208]

Begaz T, Kyriacou DN, Segal J, Bazarian JJ. Serum biochemical markers for post-concussion syndrome in patients with mild traumatic brain injury. J Neurotrauma. 2006; 23:1201–1210. [PubMed: 16928178]

Berger RP. The use of serum biomarkers to predict outcome after traumatic brain injury in adults and children. J Head Trauma Rehabil. 2006; 21:315–333. [PubMed: 16915008]

Berger RP, Kochanek PM. Urinary S100B concentrations are increased after brain injury in children: A preliminary study. Pediatr Crit Care Med. 2006; 7:557–561. [PubMed: 17006387]

Berger RP, Adelson PD, Pierce MC, Dulani T, Cassidy LD, Kochanek PM. Serum neuron-specific enolase, S100B, and myelin basic protein concentrations after inflicted and noninflicted traumatic brain injury in children. J Neurosurg. 2005; 103:61–68. [PubMed: 16122007]

Berger RP, Dulani T, Adelson PD, Leventhal JM, Richichi R, Kochanek PM. Identification of inflicted traumatic brain injury in well-appearing infants using serum and cerebrospinal markers: a possible screening tool. Pediatrics. 2006; 117:325–332. [PubMed: 16452350]

Berger RP, Ta'asan S, Rand A, Lokshin A, Kochanek P. Multiplex assessment of serum biomarker concentrations in well-appearing children with inflicted traumatic brain injury. Pediatr Res. 2009; 65:97–102. [PubMed: 18787505]

Berger RP, Hayes RL, Richichi R, Beers SR, Wang KK. Serum concentrations of ubiquitin C-terminal hydrolase-L1 and alphaII-spectrin breakdown product 145 kDa correlate with outcome after pediatric TBI. J Neurotrauma. 2012; 29:162–167. [PubMed: 22022780]

Bigler ED. Neuroimaging biomarkers in mild traumatic brain injury (mTBI). Neuropsychol Rev. 2013; 23:169–209. [PubMed: 23974873]

Biomarkers Definitions Working, G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001; 69:89–95. [PubMed: 11240971]

Boggs JM. Myelin basic protein: a multifunctional protein. Cell Mol Life Sci. 2006; 63:1945–1961. [PubMed: 16794783]

Bohnen N, Jolles J, Twijnstra A, Mellink R, Sulon J. Coping styles, cortisol reactivity, and performance in a vigilance task of patients with persistent postconcussive symptoms after a mild head injury. Int J Neurosci. 1992; 64:97–105. [PubMed: 1342053]

Bolton AN, Saatman KE. Regional neurodegeneration and gliosis are amplified by mild traumatic brain injury repeated at 24-hour intervals. J Neuropathol Exp Neurol. 2014; 73:933–947. [PubMed: 25232942]

Brain Trauma, F., American Association of Neurological, S., Congress of Neurological, S. Guidelines for the management of severe traumatic brain injury. J Neurotrauma. 2007; 24(Suppl. 1):S1–S106.

Bruns JJ Jr, Jagoda AS. Mild traumatic brain injury. Mt Sinai J Med. 2009; 76:129–137. [PubMed: 19306376]

Bulut M, Koksal O, Dogan S, Bolca N, Ozguc H, Korfali E, Ilcol YO, Parklak M. Tau protein as a serum marker of brain damage in mild traumatic brain injury: preliminary results. Adv Ther. 2006; 23:12–22. [PubMed: 16644603]

Cadosch D, Thyer M, Gautschi OP, Lochnit G, Frey SP, Zellweger R, Filgueira L, Skirving AP. Functional and proteomic analysis of serum and cerebrospinal fluid derived from patients with traumatic brain injury: a pilot study. ANZ J Surg. 2010; 80:542–547. [PubMed: 20795970]

Cai XY, Lu L, Wang YN, Jin C, Zhang RY, Zhang Q, Chen QJ, Shen WF. Association of increased S100B, S100A6 and S100P in serum levels with acute coronary syndrome and also with the severity of myocardial infarction in cardiac tissue of rat models with ischemia-reperfusion injury. Atherosclerosis. 2011; 217:536–542. [PubMed: 21663912]

Campbell LK, Thomas JR, Lamps LW, Smoller BR, Folpe AL. Protein gene product 9.5 (PGP 9.5) is not a specific marker of neural and nerve sheath tumors: an immunohistochemical study of 95 mesenchymal neoplasms. Mod Pathol. 2003; 16:963–969. [PubMed: 14559978]

Cancelliere C, Kristman VL, Cassidy JD, Hincapie CA, Cote P, Boyle E, Carroll LJ, Stalnacke BM, Nygren-de Boussard C, Borg J. Systematic review of return to work after mild traumatic brain injury: results of the International Collaboration on Mild Traumatic Brain Injury Prognosis. Arch Phys Med Rehabil. 2014; 95:S201–S209. [PubMed: 24581906]

Caner H, Can A, Atalay B, Erdogan B, Albayrak AH, Kilinc K, Bavbek M, Altinors N. Early effects of mild brain trauma on the cytoskeletal proteins neurofilament160 and MAP2, and the preventive effects of mexilitine. Acta Neurochir (Wien). 2004; 146:611–621. (discussion 621). [PubMed: 15168230]

Canu N, Dus L, Barbato C, Ciotti MT, Brancolini C, Rinaldi AM, Novak M, Cattaneo A, Bradbury A, Calissano P. Tau cleavage and dephosphorylation in cerebellar granule neurons undergoing apoptosis. J Neurosci. 1998; 18:7061–7074. [PubMed: 9736630]

Carroll LJ, Cassidy JD, Peloso PM, Borg J, von Holst H, Holm L, Paniak C, Pepin M, Injury, W.H.O.C.C.T.F.o.M.T.B. Prognosis for mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med. 2004; 43(Suppl):84–105. [PubMed: 15083873]

Carroll LJ, Cassidy JD, Cancelliere C, Cote P, Hincapie CA, Kristman VL, Holm LW, Borg J, Nygren-de Boussard C, Hartvigsen J. Systematic review of the prognosis after mild traumatic brain injury in adults: cognitive, psychiatric, and mortality outcomes: results of the International Collaboration on Mild Traumatic Brain Injury Prognosis. Arch Phys Med Rehabil. 2014; 95:S152–S173. [PubMed: 24581903]

Chen F, Sugiura Y, Myers KG, Liu Y, Lin W. Ubiquitin carboxyl-terminal hydrolase L1 is required for maintaining the structure and function of the neuromuscular junction. Proc Natl Acad Sci U S A. 2010; 107:1636–1641. [PubMed: 20080621]

Chen JX, Kachniarz B, Gilani S, Shin JJ. Risk of malignancy associated with head and neck CT in children: A systematic review. Otolaryngol Head Neck Surg. 2014; 151:554–566. [PubMed: 25052516]

Conti A, Sanchez-Ruiz Y, Bachi A, Beretta L, Grandi E, Beltramo M, Alessio M. Proteome study of human cerebrospinal fluid following traumatic brain injury indicates fibrin(ogen) degradation products as trauma-associated markers. J Neurotrauma. 2004; 21:854–863. [PubMed: 15307898]

Corcoran TB, Mas E, Barden AE, Durand T, Galano JM, Roberts LJ, Phillips M, Ho KM, Mori TA. Are isofurans and neuroprostanes increased after subarachnoid hemorrhage and traumatic brain injury? Antioxid Redox Signal. 2011; 15:2663–2667. [PubMed: 21702684]

Cortes DF, Landis MK, Ottens AK. High-capacity peptide-centric platform to decode the proteomic response to brain injury. Electrophoresis. 2012; 33:3712–3719. [PubMed: 23160985]

Crawford F, Crynen G, Reed J, Mouzon B, Bishop A, Katz B, Ferguson S, Phillips J, Ganapathi V, Mathura V, Roses A, Mullan M. Identification of plasma biomarkers of TBI outcome using proteomic approaches in an APOE mouse model. J Neurotrauma. 2012; 29:246–260. [PubMed: 21895520]

Dash PK, Zhao J, Hergenroeder G, Moore AN. Biomarkers for the diagnosis, prognosis, and evaluation of treatment efficacy for traumatic brain injury. Neurotherapeutics. 2010; 7:100–114. [PubMed: 20129502]

de Boussard CN, Lundin A, Karlstedt D, Edman G, Bartfai A, Borg J. S100 and cognitive impairment after mild traumatic brain injury. J Rehabil Med. 2005; 37:53–57. [PubMed: 15788333]

De Guire V, Robitaille R, Tetreault N, Guerin R, Menard C, Bambace N, Sapieha P. Circulating miRNAs as sensitive and specific biomarkers for the diagnosis and monitoring of human diseases: promises and challenges. Clin Biochem. 2013; 46:846–860. [PubMed: 23562576]

de Kruijk JR, Leffers P, Menheere PP, Meerhoff S, Twijnstra A. S-100B and neuron-specific enolase in serum of mild traumatic brain injury patients. A comparison with health controls. Acta Neurol Scand. 2001; 103:175–179. [PubMed: 11240565]

De Kruijk JR, Leffers P, Menheere PP, Meerhoff S, Rutten J, Twijnstra A. Prediction of post-traumatic complaints after mild traumatic brain injury: early symptoms and biochemical markers. J Neurol Neurosurg Psychiatry. 2002; 73:727–732. [PubMed: 12438478]

Denslow N, Michel ME, Temple MD, Hsu CY, Saatman K, Hayes RL. Application of proteomics technology to the field of neurotrauma. J Neurotrauma. 2003; 20:401–407. [PubMed: 12803973]

Diamandis EP. Analysis of serum proteomic patterns for early cancer diagnosis: drawing attention to potential problems. J Natl Cancer Inst. 2004; 96:353–356. [PubMed: 14996856]

Diaz-Arrastia R, Wang KK, Papa L, Sorani MD, Yue JK, Puccio AM, McMahon PJ, Inoue T, Yuh EL, Lingsma HF, Maas AI, Valadka AB, Okonkwo DO, Manley GT, Track-Tbi I, Casey IS, Cheong M, Cooper SR, Dams-O'Connor K, Gordon WA, Hricik AJ, Menon DK, Mukherjee P, Schnyer DM, Sinha TK, Vassar MJ. Acute biomarkers of traumatic brain injury: relationship between plasma levels of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein. J Neurotrauma. 2014; 31:19–25. [PubMed: 23865516]

Doolan AW, Day DD, Maerlender AC, Goforth M, Gunnar Brolinson P. A review of return to play issues and sports-related concussion. Ann Biomed Eng. 2012; 40:106–113. [PubMed: 21997390]

Doran JF, Jackson P, Kynoch PA, Thompson RJ. Isolation of PGP 9.5, a new human neurone-specific protein detected by high-resolution two-dimensional electrophoresis. J Neurochem. 1983; 40:1542–1547. [PubMed: 6343558]

Eng LF, Vanderhaeghen JJ, Bignami A, Gerstl B. An acidic protein isolated from fibrous astrocytes. Brain Res. 1971; 28:351–354. [PubMed: 5113526]

Farkas O, Polgar B, Szekeres-Bartho J, Doczi T, Povlishock JT, Buki A. Spectrin breakdown products in the cerebrospinal fluid in severe head injury–preliminary observations. Acta Neurochir (Wien). 2005; 147:855–861. [PubMed: 15924207]

Farkas O, Lifshitz J, Povlishock JT. Mechanoporation induced by diffuse traumatic brain injury: an irreversible or reversible response to injury? J Neurosci. 2006; 26:3130–3140. [PubMed: 16554464]

Floyd CL, Lyeth BG. Astroglia: important mediators of traumatic brain injury. Prog Brain Res. 2007; 161:61–79. [PubMed: 17618970]

Gao W, Lu C, Kochanek PM, Berger RP. Serum amyloid A is increased in children with abusive head trauma: a gel-based proteomic analysis. Pediatr Res. 2014; 76:280–286. [PubMed: 24941216]

Gatson JW, Barillas J, Hynan LS, Diaz-Arrastia R, Wolf SE, Minei JP. Detection of neurofilament-H in serum as a diagnostic tool to predict injury severity in patients who have suffered mild traumatic brain injury. J Neurosurg. 2014; 121:1232–1238. [PubMed: 25192482]

Giacoppo S, Bramanti P, Barresi M, Celi D, Foti Cuzzola V, Palella E, Marino S. Predictive biomarkers of recovery in traumatic brain injury. Neurocrit Care. 2012; 16:470–477. [PubMed: 22528282]

Gogas H, Eggermont AM, Hauschild A, Hersey P, Mohr P, Schadendorf D, Spatz A, Dummer R. Biomarkers in melanoma. Ann Oncol. 2009; 20(Suppl. 6):vi8–vi13. [PubMed: 19617299]

Good DM, Thongboonkerd V, Novak J, Bascands JL, Schanstra JP, Coon JJ, Dominiczak A, Mischak H. Body fluid proteomics for biomarker discovery: lessons from the past hold the key to success in the future. J Proteome Res. 2007; 6:4549–4555. [PubMed: 17970587]

Greer JE, Hanell A, McGinn MJ, Povlishock JT. Mild traumatic brain injury in the mouse induces axotomy primarily within the axon initial segment. Acta Neuropathol. 2013; 126:59–74. [PubMed: 23595276]

Gross H, Kling A, Henry G, Herndon C, Lavretsky H. Local cerebral glucose metabolism in patients with long-term behavioral and cognitive deficits following mild traumatic brain injury. J Neuropsychiatry Clin Neurosci. 1996; 8:324–334. [PubMed: 8854305]

Guingab-Cagmat JD, Newsom K, Vakulenko A, Cagmat EB, Kobeissy FH, Zoltewicz S, Wang KK, Anagli J. In vitro MS-based proteomic analysis and absolute quantification of neuronal-glial injury biomarkers in cell culture system. Electrophoresis. 2012; 33:3786–3797. [PubMed: 23161537]

Guzel A, Karasalihoglu S, Aylanc H, Temizoz O, Hicdonmez T. Validity of serum tau protein levels in pediatric patients with minor head trauma. Am J Emerg Med. 2010; 28:399–403. [PubMed: 20466216]

Gyorgy AB, Walker J, Wingo D, Eidelman O, Pollard HB, Molnar A, Agoston DV. Reverse phase protein microarray technology in traumatic brain injury. J Neurosci Methods. 2010; 192:96–101. [PubMed: 20674607]

Gyorgy A, Ling G, Wingo D, Walker J, Tong L, Parks S, Januszkiewicz A, Baumann R, Agoston DV. Time-dependent changes in serum biomarker levels after blast traumatic brain injury. J Neurotrauma. 2011; 28:1121–1126. [PubMed: 21428721]

Haimoto H, Hosoda S, Kato K. Differential distribution of immunoreactive S100-alpha and S100-beta proteins in normal nonnervous human tissues. Lab Invest. 1987; 57:489–498. [PubMed: 3316838]

Hall ED, Braughler JM. Free radicals in CNS injury. Res Publ Assoc Res Nerv Ment Dis. 1993; 71:81–105. [PubMed: 8380240]

Hall ED, Vaishnav RA, Mustafa AG. Antioxidant therapies for traumatic brain injury. Neurotherapeutics. 2010; 7:51–61. [PubMed: 20129497]

Hallen M, Karlsson M, Carlhed R, Hallgren T, Bergenheim M. S-100B in serum and urine after traumatic head injury in children. J Trauma. 2010; 69:284–289. [PubMed: 20734463]

Hanrieder J, Wetterhall M, Enblad P, Hillered L, Bergquist J. Temporally resolved differential proteomic analysis of human ventricular CSF for monitoring traumatic brain injury biomarker candidates. J Neurosci Methods. 2009; 177:469–478. [PubMed: 19263575]

Haqqani AS, Hutchison JS, Ward R, Stanimirovic DB. Biomarkers and diagnosis; protein biomarkers in serum of pediatric patients with severe traumatic brain injury identified by ICAT-LC-MS/MS. J Neurotrauma. 2007; 24:54–74. [PubMed: 17263670]

Haskins WE, Kobeissy FH, Wolper RA, Ottens AK, Kitlen JW, McClung SH, O'Steen BE, Chow MM, Pineda JA, Denslow ND, Hayes RL, Wang KK. Rapid discovery of putative protein biomarkers of traumatic brain injury by SDS-PAGE-capillary liquid chromatography-tandem mass spectrometry. J Neurotrauma. 2005; 22:629–644. [PubMed: 15941373]

Hergenroeder G, Redell JB, Moore AN, Dubinsky WP, Funk RT, Crommett J, Clifton GL, Levine R, Valadka A, Dash PK. Identification of serum biomarkers in brain-injured adults: potential for predicting elevated intracranial pressure. J Neurotrauma. 2008; 25:79–93. [PubMed: 18260791]

Herring SA, Bergfeld JA, Boland A, Boyajian-O'Neill LA, Cantu RC, Hershman E, Indelicato P, Jaffe R, Kibler WB, McKeag DB, Pallay R, Putukian M. Concussion (mild traumatic brain injury) and the team physician: a consensus statement. Med Sci Sports Exerc. 2006; 38:395–399. [PubMed: 16531912]

Hoge CW, McGurk D, Thomas JL, Cox AL, Engel CC, Castro CA. Mild traumatic brain injury in U.S. Soldiers returning from Iraq. N Engl J Med. 2008; 358:453–463. [PubMed: 18234750]

Hoge CW, Goldberg HM, Castro CA. Care of war veterans with mild traumatic brain injury–flawed perspectives. N Engl J Med. 2009; 360:1588–1591. [PubMed: 19369664]

Holm L, Cassidy JD, Carroll LJ, Borg J, eurotrauma Task Force onMild Traumatic Brain Injury of the, W.H.O.C.C. Summary of the WHO Collaborating Centre for Neurotrauma Task Force on Mild Traumatic Brain Injury. J Rehabil Med. 2005; 37:137–141. [PubMed: 16040469]

Hylin MJ, Orsi SA, Zhao J, Bockhorst K, Perez A, Moore AN, Dash PK. Behavioral and histopathological alterations resulting from mild fluid percussion injury. J Neurotrauma. 2013; 30:702–715. [PubMed: 23301501]

Inci S, Ozcan OE, Kilinc K. Time-level relationship for lipid peroxidation and the protective effect of alpha-tocopherol in experimental mild and severe brain injury. Neurosurgery. 1998; 43:330–335. (discussion 335–336). [PubMed: 9696087]

Ingebrigtsen T, Romner B. Biochemical serum markers for brain damage: a short review with emphasis on clinical utility in mild head injury. Restor Neurol Neurosci. 2003; 21:171–176. [PubMed: 14530579]

Ingebrigtsen T, Romner B, Kongstad P, Langbakk B. Increased serum concentrations of protein S-100 after minor head injury: a biochemical serum marker with prognostic value? J Neurol Neurosurg Psychiatry. 1995; 59:103–104. [PubMed: 7608699]

Ingebrigtsen T, Romner B, Marup-Jensen S, Dons M, Lundqvist C, Bellner J, Alling C, Borgesen SE. The clinical value of serum S-100 protein measurements in minor head injury: a Scandinavian multicentre study. Brain Inj. 2000; 14:1047–1055. [PubMed: 11147577]

Jackson P, Thompson RJ. The demonstration of new human brain-specific proteins by high-resolution two-dimensional polyacrylamide gel electrophoresis. J Neurol Sci. 1981; 49:429–438. [PubMed: 7217993]

Jagoda AS, Bazarian JJ, Bruns JJ Jr, Cantrill SV, Gean AD, Howard PK, Ghajar J, Riggio S, Wright DW, Wears RL, Bakshy A, Burgess P, Wald MM, Whitson RR. Clinical policy: neuroimaging and decisionmaking in adult mild traumatic brain injury in the acute setting. J Emerg Nurs. 2009; 35:e5–e40. [PubMed: 19285163]

Jenkins LW, Peters GW, Dixon CE, Zhang X, Clark RS, Skinner JC, Marion DW, Adelson PD, Kochanek PM. Conventional and functional proteomics using large format two-dimensional gel electrophoresis 24 hours after controlled cortical impact in postnatal day 17 rats. J Neurotrauma. 2002; 19:715–740. [PubMed: 12165133]

Jeter CB, Hergenroeder GW, Ward NH 3rd, Moore AN, Dash PK. Human traumatic brain injury alters circulating L-arginine and its metabolite levels: possible link to cerebral blood flow, extracellular matrix remodeling, and energy status. J Neurotrauma. 2012; 29:119–127. [PubMed: 21942884]

Jeter CB, Hergenroeder GW, Hylin MJ, Redell JB, Moore AN, Dash PK. Biomarkers for the diagnosis and prognosis of mild traumatic brain injury/concussion. J Neurotrauma. 2013a; 30:657–670. [PubMed: 23062081]

Jeter CB, Hergenroeder GW, Ward NH 3rd, Moore AN, Dash PK. Human mild traumatic brain injury decreases circulating branched-chain amino acids and their metabolite levels. J Neurotrauma. 2013b; 30:671–679. [PubMed: 23560894]

Johnsson P, Blomquist S, Luhrs C, Malmkvist G, Alling C, Solem JO, Stahl E. Neuron-specific enolase increases in plasma during and immediately after extracorporeal circulation. Ann Thorac Surg. 2000; 69:750–754. [PubMed: 10750755]

Jordan BD. The clinical spectrum of sport-related traumatic brain injury. Nat Rev Neurol. 2013; 9:222–230. [PubMed: 23478462]

Kamnaksh A, Kwon SK, Kovesdi E, Ahmed F, Barry ES, Grunberg NE, Long J, Agoston D. Neurobehavioral, cellular, and molecular consequences of single and multiple mild blast exposure. Electrophoresis. 2012; 33:3680–3692. [PubMed: 23161523]

Kavalci C, Pekdemir M, Durukan P, Ilhan N, Yildiz M, Serhatlioglu S, Seckin D. The value of serum tau protein for the diagnosis of intracranial injury in minor head trauma. Am J Emerg Med. 2007; 25:391–395. [PubMed: 17499655]

Kleindienst A, Bullock RM. A critical analysis of the role of the neurotrophic protein S100B in acute brain injury. J Neurotrauma. 2006; 23:1185–1200. [PubMed: 16928177]

Kobeissy FH, Ottens AK, Zhang Z, Liu MC, Denslow ND, Dave JR, Tortella FC, Hayes RL, Wang KK. Novel differential neuroproteomics analysis of traumatic brain injury in rats. Mol Cell Proteomics. 2006; 5:1887–1898. [PubMed: 16801361]

Kobeissy FH, Sadasivan S, Oli MW, Robinson G, Larner SF, Zhang Z, Hayes RL, Wang KK. Neuroproteomics and systems biology-based discovery of protein biomarkers for traumatic brain injury and clinical validation. Proteomics Clin Appl. 2008; 2:1467–1483. [PubMed: 21136795]

Kochanek PM, Berger RP, Bayir H, Wagner AK, Jenkins LW, Clark RS. Biomarkers of primary and evolving damage in traumatic and ischemic brain injury: diagnosis, prognosis, probing mechanisms, and therapeutic decision making. Curr Opin Crit Care. 2008; 14:135–141. [PubMed: 18388674]

Kochanek PM, Carney N, Adelson PD, Ashwal S, Bell MJ, Bratton S, Carson S, Chesnut RM, Ghajar J, Goldstein B, Grant GA, Kissoon N, Peterson K, Selden NR, Tasker RC, Tong KA, Vavilala MS, Wainwright MS, Warden CR, American Academy of Pediatrics-Section on Neurological, S., American Association of Neurological Surgeons/Congress of Neurological, S., Child Neurology, S., European Society of, P., Neonatal Intensive, C., Neurocritical Care, S., Pediatric Neurocritical Care Research, G., Society of Critical Care, M., Paediatric Intensive Care Society, U.K., Society for Neuroscience in, A., Critical, C., World Federation of Pediatric, Critical Care, S. Guidelines for the acute medical management of severe traumatic brain injury in infants, children, and adolescents–second edition. Pediatr Crit Care Med. 2012; 13(Suppl. 1):S1–S82. [PubMed: 22217782]

Koh SX, Lee JK. S100B as a marker for brain damage and blood-brain barrier disruption following exercise. Sports Med. 2014; 44:369–385. [PubMed: 24194479]

Kontogeorgis D. BET 3: Can protein S100B add to current clinical guidelines in adult minor head injury? Emerg Med J. 2013; 30:597–599. [PubMed: 23765768]

Kou Z, Gattu R, Kobeissy F, Welch RD, O'Neil BJ, Woodard JL, Ayaz SI, Kulek A, Kas-Shamoun R, Mika V, Zuk C, Tomasello F, Mondello S. Combining biochemical and imaging markers to improve diagnosis and characterization of mild traumatic brain injury in the acute setting: results from a pilot study. PLoS One. 2013; 8:e80296. [PubMed: 24260364]

Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 1996; 19:312–318. [PubMed: 8843599]

Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science. 2001; 294:853–858. [PubMed: 11679670]

Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr Biol. 2002; 12:735–739. [PubMed: 12007417]

Lakshmanan R, Loo JA, Drake T, Leblanc J, Ytterberg AJ, McArthur DL, Etchepare M, Vespa PM. Metabolic crisis after traumatic brain injury is associated with a novel microdialysis proteome. Neurocrit Care. 2010; 12:324–336. [PubMed: 20225002]

Lamers KJ, Vos P, Verbeek MM, Rosmalen F, van Geel WJ, van Engelen BG. Protein S-100B, neuron-specific enolase (NSE), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP) in cerebrospinal fluid (CSF) and blood of neurological patients. Brain Res Bull. 2003; 61:261–264. [PubMed: 12909296]

Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. J Head Trauma Rehabil. 2006; 21:375–378. [PubMed: 16983222]

Lash GE, Scaife PJ, Innes BA, Otun HA, Robson SC, Searle RF, Bulmer JN. Comparison of three multiplex cytokine analysis systems: Luminex, SearchLight and FAST Quant. J Immunol Methods. 2006; 309:205–208. [PubMed: 16436279]

Laterza OF, Lim L, Garrett-Engele PW, Vlasakova K, Muniappa N, Tanaka WK, Johnson JM, Sina JF, Fare TL, Sistare FD, Glaab WE. Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury. Clin Chem. 2009; 55:1977–1983. [PubMed: 19745058]

Liu MC, Akle V, Zheng W, Dave JR, Tortella FC, Hayes RL, Wang KK. Comparing calpain- and caspase-3-mediated degradation patterns in traumatic brain injury by differential proteome analysis. Biochem J. 2006; 394:715–725. [PubMed: 16351572]

Lundblad R. Considerations for the use of blood plasma and serum for proteomic analysis. Internet J Genomics Proteomics 1 (online only). 2003

Ma M, Lindsell CJ, Rosenberry CM, Shaw GJ, Zemlan FP. Serum cleaved tau does not predict postconcussion syndrome after mild traumatic brain injury. Am J Emerg Med. 2008; 26:763–768. [PubMed: 18774039]

Maas AI, Stocchetti N, Bullock R. Moderate and severe traumatic brain injury in adults. Lancet Neurol. 2008; 7:728–741. [PubMed: 18635021]

Mair J, Dienstl F, Puschendorf B. Cardiac troponin T in the diagnosis of myocardial injury. Crit Rev Clin Lab Sci. 1992; 29:31–57. [PubMed: 1388708]

Manley GT, Diaz-Arrastia R, Brophy M, Engel D, Goodman C, Gwinn K, Veenstra TD, Ling G, Ottens AK, Tortella F, Hayes RL. Common data elements for traumatic brain injury: recommendations from the biospecimens and biomarkers working group. Arch Phys Med Rehabil. 2010; 91:1667–1672. [PubMed: 21044710]

Marion DW, Curley KC, Schwab K, Hicks RR, m, T.B.I.D.W. Proceedings of the military mTBI Diagnostics Workshop, St. Pete Beach, August 2010. J Neurotrauma. 2011; 28:517–526. [PubMed: 21265587]

Martins TB, Pasi BM, Pickering JW, Jaskowski TD, Litwin CM, Hill HR. Determination of cytokine responses using a multiplexed fluorescent microsphere immunoassay. Am J Clin Pathol. 2002; 118:346–353. [PubMed: 12219776]

Mazzini GS, Schaf DV, Oliveira AR, Goncalves CA, Bello-Klein A, Bordignon S, Bruch RS, Campos GF, Vassallo DV, Souza DO, Portela LV. The ischemic rat heart releases S100B. Life Sci. 2005; 77:882–889. [PubMed: 15921704]

McCrory P, Meeuwisse WH, Aubry M, Cantu RC, Dvorak J, Echemendia RJ, Engebretsen L, Johnston K, Kutcher JS, Raftery M, Sills A, Benson BW, Davis GA, Ellenbogen R, Guskiewicz KM, Herring SA, Iverson GL, Jordan BD, Kissick J, McCrea M, McIntosh AS, Maddocks D, Makdissi M, Purcell L, Putukian M, Schneider K, Tator CH, Turner M. Consensus statement on concussion in sport: the 4th International Conference on Concussion in Sport, Zurich, November 2012. J Athl Train. 2013; 48:554–575. [PubMed: 23855364]

McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, Santini VE, Lee HS, Kubilus CA, Stern RA. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. J Neuropathol Exp Neurol. 2009; 68:709–735. [PubMed: 19535999]

Meric E, Gunduz A, Turedi S, Cakir E, Yandi M. The prognostic value of neuron-specific enolase in head trauma patients. J Emerg Med. 2010; 38:297–301. [PubMed: 18499387]

Michetti F, Corvino V, Geloso MC, Lattanzi W, Bernardini C, Serpero L, Gazzolo D. The S100B protein in biological fluids: more than a lifelong biomarker of brain distress. J Neurochem. 2012; 120:644–659. [PubMed: 22145907]

Miller EC, Derlet RW, Kinser D. Minor head trauma: Is computed tomography always necessary? Ann Emerg Med. 1996; 27:290–294. [PubMed: 8599485]

Missler U, Wiesmann M, Wittmann G, Magerkurth O, Hagenstrom H. Measurement of glial fibrillary acidic protein in human blood: analytical method and preliminary clinical results. Clin Chem. 1999; 45:138–141. [PubMed: 9895354]

Mondello S, Muller U, Jeromin A, Streeter J, Hayes RL, Wang KK. Blood-based diagnostics of traumatic brain injuries. Expert Rev Mol Diagn. 2011; 11:65–78. [PubMed: 21171922]

Mondello S, Schmid K, Berger RP, Kobeissy F, Italiano D, Jeromin A, Hayes RL, Tortella FC, Buki A. The challenge of mild traumatic brain injury: role of biochemical markers in diagnosis of brain damage. Med Res Rev. 2014; 34:503–531. [PubMed: 23813922]

Morris M, Maeda S, Vossel K, Mucke L. The many faces of tau. Neuron. 2011; 70:410–426. [PubMed: 21555069]

Muller K, Ingebrigtsen T, Wilsgaard T, Wikran G, Fagerheim T, Romner B, Waterloo K. Prediction of time trends in recovery of cognitive function after mild head injury. Neurosurgery. 2009; 64:698–704. [PubMed: 19349827]

Mussack T, Biberthaler P, Kanz KG, Heckl U, Gruber R, Linsenmaier U, Mutschler W, Jochum M. Immediate S-100B and neuron-specific enolase plasma measurements for rapid evaluation of

primary brain damage in alcohol-intoxicated, minor head-injured patients. Shock. 2002; 18:395–400. [PubMed: 12412616]

Ojo JO, Greenberg MB, Leary P, Mouzon B, Bachmeier C, Mullan M, Diamond DM, Crawford F. Neurobehavioral, neuropathological and biochemical profiles in a novel mouse model of co-morbid post-traumatic stress disorder and mild traumatic brain injury. Front Behav Neurosci. 2014; 8:213. [PubMed: 25002839]

Okonkwo DO, Yue JK, Puccio AM, Panczykowski DM, Inoue T, McMahon PJ, Sorani MD, Yuh EL, Lingsma HF, Maas AI, Valadka AB, Manley GT, Transforming, R., Clinical Knowledge in Traumatic Brain Injury, I. GFAP-BDP as an acute diagnostic marker in traumatic brain injury: results from the prospective transforming research and clinical knowledge in traumatic brain injury study. J Neurotrauma. 2013; 30:1490–1497. [PubMed: 23489259]

O'Neal WK, Anderson W, Basta PV, Carretta EE, Doerschuk CM, Barr RG, Bleecker ER, Christenson SA, Curtis JL, Han MK, Hansel NN, Kanner RE, Kleerup EC, Martinez FJ, Miller BE, Peters SP, Rennard SI, Scholand MB, Tal-Singer R, Woodruff PG, Couper DJ, Davis SM, Investigators S. Comparison of serum, EDTA plasma and P100 plasma for luminex-based biomarker multiplex assays in patients with chronic obstructive pulmonary disease in the SPIROMICS study. J Transl Med. 2014; 12:9. [PubMed: 24397870]

Ottens AK, Kobeissy FH, Golden EC, Zhang Z, Haskins WE, Chen SS, Hayes RL, Wang KK, Denslow ND. Neuroproteomics in neurotrauma. Mass Spectrom Rev. 2006; 25:380–408. [PubMed: 16498609]

Ottens AK, Kobeissy FH, Fuller BF, Liu MC, Oli MW, Hayes RL, Wang KK. Novel neuroproteomic approaches to studying traumatic brain injury. Prog Brain Res. 2007; 161:401–418. [PubMed: 17618994]

Ottens AK, Bustamante L, Golden EC, Yao C, Hayes RL, Wang KK, Tortella FC, Dave JR. Neuroproteomics: a biochemical means to discriminate the extent and modality of brain injury. J Neurotrauma. 2010; 27:1837–1852. [PubMed: 20698760]

Ottens AK, Stafflinger JE, Griffin HE, Kunz RD, Cifu DX, Niemeier JP. Post-acute brain injury urinary signature: a new resource for molecular diagnostics. J Neurotrauma. 2014; 31:782–788. [PubMed: 24372380]

Papa L, Lewis LM, Falk JL, Zhang Z, Silvestri S, Giordano P, Brophy GM, Demery JA, Dixit NK, Ferguson I, Liu MC, Mo J, Akinyi L, Schmid K, Mondello S, Robertson CS, Tortella FC, Hayes RL, Wang KK. Elevated levels of serum glial fibrillary acidic protein breakdown products in mild and moderate traumatic brain injury are associated with intracranial lesions and neurosurgical intervention. Ann Emerg Med. 2012a; 59:471–483. [PubMed: 22071014]

Papa L, Lewis LM, Silvestri S, Falk JL, Giordano P, Brophy GM, Demery JA, Liu MC, Mo J, Akinyi L, Mondello S, Schmid K, Robertson CS, Tortella FC, Hayes RL, Wang KK. Serum levels of ubiquitin C-terminal hydrolase distinguish mild traumatic brain injury from trauma controls and are elevated in mild and moderate traumatic brain injury patients with intracranial lesions and neurosurgical intervention. J Trauma Acute Care Surg. 2012b; 72:1335–1344. [PubMed: 22673263]

Papa L, Silvestri S, Brophy GM, Giordano P, Falk JL, Braga CF, Tan CN, Ameli NJ, Demery JA, Dixit NK, Mendes ME, Hayes RL, Wang KK, Robertson CS. GFAP Out-performs S100B in detecting traumatic intracranial lesions on CT in trauma patients with mild traumatic brain injury and those with extracranial lesions. J Neurotrauma. 2014; 31:1815–1822. [PubMed: 24903744]

Pasinetti GM, Ho L, Dooley C, Abbi B, Lange G. Select non-coding RNA in blood components provide novel clinically accessible biological surrogates for improved identification of traumatic brain injury in OEF/OIF Veterans. Am J Neurodegener Dis. 2012; 1:88–98. [PubMed: 22737634]

Pearce MS, Salotti JA, Little MP, McHugh K, Lee C, Kim KP, Howe NL, Ronckers CM, Rajaraman P, Sir Craft AW, Parker L, Berrington de Gonzalez A. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study. Lancet. 2012; 380:499–505. [PubMed: 22681860]

Pelinka LE, Harada N, Szalay L, Jafarmadar M, Redl H, Bahrami S. Release of S100B differs during ischemia and reperfusion of the liver, the gut, and the kidney in rats. Shock. 2004a; 21:72–76. [PubMed: 14676687]

Pelinka LE, Jafarmadar M, Redl H, Bahrami S. Neuron-specific-enolase is increased in plasma after hemorrhagic shock and after bilateral femur fracture without traumatic brain injury in the rat. Shock. 2004b; 22:88–91. [PubMed: 15201708]

Pelinka LE, Hertz H, Mauritz W, Harada N, Jafarmadar M, Albrecht M, Redl H, Bahrami S. Nonspecific increase of systemic neuron-specific enolase after trauma: clinical and experimental findings. Shock. 2005; 24:119–123. [PubMed: 16044081]

Pelsers MM, Hanhoff T, Van der Voort D, Arts B, Peters M, Ponds R, Honig A, Rudzinski W, Spener F, de Kruijk JR, Twijnstra A, Hermens WT, Menheere PP, Glatz JF. Brain- and heart-type fatty acid-binding proteins in the brain: tissue distribution and clinical utility. Clin Chem. 2004; 50:1568–1575. [PubMed: 15217991]

Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci. 2005; 233:183–198. [PubMed: 15896809]

Pickering A, Carter J, Hanning I, Townend W. Emergency department measurement of urinary S100B in children following head injury: can extracranial injury confound findings? Emerg Med J. 2008; 25:88–89. [PubMed: 18212143]

Pike BR, Zhao X, Newcomb JK, Posmantur RM, Wang KK, Hayes RL. Regional calpain and caspase-3 proteolysis of alpha-spectrin after traumatic brain injury. Neuroreport. 1998; 9:2437–2442. [PubMed: 9721910]

Pike BR, Flint J, Dutta S, Johnson E, Wang KK, Hayes RL. Accumulation of non-erythroid alpha II-spectrin and calpain-cleaved alpha II-spectrin breakdown products in cerebrospinal fluid after traumatic brain injury in rats. J Neurochem. 2001; 78:1297–1306. [PubMed: 11579138]

Pineda JA, Wang KK, Hayes RL. Biomarkers of proteolytic damage following traumatic brain injury. Brain Pathol. 2004; 14:202–209. [PubMed: 15193033]

Pogoda TK, Iverson KM, Meterko M, Baker E, Hendricks AM, Stolzmann KL, Krengel M, Charns MP, Amara J, Kimerling R, Lew HL. Concordance of clinician judgment of mild traumatic brain injury history with a diagnostic standard. J Rehabil Res Dev. 2014; 51:363–375. [PubMed: 25019660]

Puvenna V, Brennan C, Shaw G, Yang C, Marchi N, Bazarian JJ, Merchant-Borna K, Janigro D. Significance of ubiquitin carboxy-terminal hydrolase L1 elevations in athletes after sub-concussive head hits. PLoS One. 2014; 9:e96296. [PubMed: 24806476]

Raabe A, Grolms C, Sorge O, Zimmermann M, Seifert V. Serum S-100B protein in severe head injury. Neurosurgery. 1999; 45:477–483. [PubMed: 10493369]

Ramont L, Thoannes H, Volondat A, Chastang F, Millet MC, Maquart FX. Effects of hemolysis and storage condition on neuron-specific enolase (NSE) in cerebrospinal fluid and serum: implications in clinical practice. Clin Chem Lab Med. 2005; 43:1215–1217. [PubMed: 16232088]

RAND Corporation. Invisible wounds – mental health and cognitive care needs of America's returning veterans. 2008.

Redell JB, Moore AN, Ward NH 3rd, Hergenroeder GW, Dash PK. Human traumatic brain injury alters plasma microRNA levels. J Neurotrauma. 2010; 27:2147–2156. [PubMed: 20883153]

Ringger NC, O'Steen BE, Brabham JG, Silver X, Pineda J, Wang KK, Hayes RL, Papa L. A novel marker for traumatic brain injury: CSF alphaII-spectrin breakdown product levels. J Neurotrauma. 2004; 21:1443–1456. [PubMed: 15672634]

Roberts-Lewis JM, Savage MJ, Marcy VR, Pinsker LR, Siman R. Immunolocalization of calpain I-mediated spectrin degradation to vulnerable neurons in the ischemic gerbil brain. J Neurosci. 1994; 14:3934–3944. [PubMed: 8207497]

Rodriguez-Rodriguez A, Egea-Guerrero JJ, Leon-Justel A, Gordillo-Escobar E, Revuelto-Rey J, Vilches-Arenas A, Carrillo-Vico A, Dominguez-Roldan JM, Murillo-Cabezas F, Guerrero JM. Role of S100B protein in urine and serum as an early predictor of mortality after severe traumatic brain injury in adults. Clin Chim Acta. 2012; 414:228–233. [PubMed: 23031665]

Romeo MJ, Espina V, Lowenthal M, Espina BH, Petricoin EF 3rd, Liotta LA. CSF proteome: a protein repository for potential biomarker identification. Expert Rev Proteomics. 2005; 2:57–70. [PubMed: 15966853]

Rosenbaum SB, Lipton ML. Embracing chaos: the scope and importance of clinical and pathological heterogeneity in mTBI. Brain Imaging Behav. 2012; 6:255–282. [PubMed: 22549452]

Rostami E, Davidsson J, Ng KC, Lu J, Gyorgy A, Walker J, Wingo D, Plantman S, Bellander BM, Agoston DV, Risling M. A model for mild traumatic brain injury that induces limited transient memory impairment and increased levels of axon related serum biomarkers. Front Neurol. 2012; 3:115. [PubMed: 22837752]

Rubenstein R, Chang B, Davies P, Wagner AMD, Robertson CS, Wang KK. A novel, ultrasensitive assay for Tau: Potential for assessing traumatic brain injury in tissues and biofluids. J Neurotrauma. 2014

Ryan LM, Warden DL. Post concussion syndrome. Int Rev Psychiatry. 2003; 15:310–316. [PubMed: 15276952]

Saatman KE, Bozyczko-Coyne D, Marcy V, Siman R, McIntosh TK. Prolonged calpain-mediated spectrin breakdown occurs regionally following experimental brain injury in the rat. J Neuropathol Exp Neurol. 1996; 55:850–860. [PubMed: 8965100]

Saatman KE, Duhaime AC, Bullock R, Maas AI, Valadka A, Manley GT, Workshop Scientific, T., Advisory Panel, M. Classification of traumatic brain injury for targeted therapies. J Neurotrauma. 2008; 25:719–738. [PubMed: 18627252]

Sakai K, Okudera H, Hongo K. Significant elevation of urinary trypsin inhibitor in patients with brain contusion–a preliminary report. J Clin Neurosci. 2003; 10:677–679. [PubMed: 14592616]

Sandler SJ, Figaji AA, Adelson PD. Clinical applications of biomarkers in pediatric traumatic brain injury. Childs Nerv Syst. 2010; 26:205–213. [PubMed: 19902222]

Savola O, Hillbom M. Early predictors of post-concussion symptoms in patients with mild head injury. Eur J Neurol. 2003; 10:175–181. [PubMed: 12603294]

Schmechel D, Marangos PJ, Brightman M. Neurone-specific enolase is a molecular marker for peripheral and central neuroendocrine cells. Nature. 1978; 276:834–836. [PubMed: 31568]

Schroeter ML, Sacher J, Steiner J, Schoenknecht P, Mueller K. Serum S100B represents a new biomarker for mood disorders. Curr Drug Targets. 2013; 14:1237–1248. [PubMed: 23701298]

Schwab C, Bondada V, Sparks DL, Cahan LD, Geddes JW. Postmortem changes in the levels and localization of microtubule-associated proteins (tau, MAP2 and MAP1B) in the rat and human hippocampus. Hippocampus. 1994; 4:210–225. [PubMed: 7951696]

Seal KH, Bertenthal D, Miner CR, Sen S, Marmar C. Bringing the war back home: mental health disorders among 103,788 US veterans returning from Iraq and Afghanistan seen at Department of Veterans Affairs facilities. Arch Intern Med. 2007; 167:476–482. [PubMed: 17353495]

Seifman MA, Adamides AA, Nguyen PN, Vallance SA, Cooper DJ, Kossmann T, Rosenfeld JV, Morganti-Kossmann MC. Endogenous melatonin increases in cerebrospinal fluid of patients after severe traumatic brain injury and correlates with oxidative stress and metabolic disarray. J Cereb Blood Flow Metab. 2008; 28:684–696. [PubMed: 18183032]

Shahim P, Tegner Y, Wilson DH, Randall J, Skillback T, Pazooki D, Kallberg B, Blennow K, Zetterberg H. Blood biomarkers for brain injury in concussed professional ice hockey players. JAMA Neurol. 2014; 71:684–692. [PubMed: 24627036]

Sharp DJ, Ham TE. Investigating white matter injury after mild traumatic brain injury. Curr Opin Neurol. 2011; 24:558–563. [PubMed: 21986682]

Shenton ME, Hamoda HM, Schneiderman JS, Bouix S, Pasternak O, Rathi Y, Vu MA, Purohit MP, Helmer K, Koerte I, Lin AP, Westin CF, Kikinis R, Kubicki M, Stern RA, Zafonte R. A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. Brain Imaging Behav. 2012; 6:137–192. [PubMed: 22438191]

Shultz SR, Bao F, Weaver LC, Cain DP, Brown A. Treatment with an anti-CD11d integrin antibody reduces neuroinflammation and improves outcome in a rat model of repeated concussion. J Neuroinflammation. 2013; 10:26. [PubMed: 23414334]

Siman R, Noszek JC. Excitatory amino acids activate calpain I and induce structural protein breakdown in vivo. Neuron. 1988; 1:279–287. [PubMed: 2856162]

Siman R, Baudry M, Lynch G. Brain fodrin: substrate for calpain I, an endogenous calcium-activated protease. Proc Natl Acad Sci U S A. 1984; 81:3572–3576. [PubMed: 6328521]

Siman R, McIntosh TK, Soltesz KM, Chen Z, Neumar RW, Roberts VL. Proteins released from degenerating neurons are surrogate markers for acute brain damage. Neurobiol Dis. 2004; 16:311–320. [PubMed: 15193288]

Siman R, Giovannone N, Hanten G, Wilde EA, McCauley SR, Hunter JV, Li X, Levin HS, Smith DH. Evidence that the blood biomarker SNTF predicts brain imaging changes and persistent cognitive dysfunction in mild TBI patients. Front Neurol. 2013; 4:190. [PubMed: 24302918]

Siman R, Shahim P, Tegner Y, Blennow K, Zetterberg H, Smith DHD. Serum SNTF increases in concussed professional Ice hockey players and relates to the severity of post-concussion symptoms. J Neurotrauma. 2014

Skogseid IM, Nordby HK, Urdal P, Paus E, Lilleaas F. Increased serum creatine kinase BB and neuron specific enolase following head injury indicates brain damage. Acta Neurochir (Wien). 1992; 115:106–111. [PubMed: 1605077]

Sojka P, Stalnacke BM, Bjornstig U, Karlsson K. One-year follow-up of patients with mild traumatic brain injury: occurrence of post-traumatic stress-related symptoms at follow-up and serum levels of cortisol, S-100B and neuron-specific enolase in acute phase. Brain Inj. 2006; 20:613–620. [PubMed: 16754286]

Stalnacke BM, Bjornstig U, Karlsson K, Sojka P. One-year follow-up of mild traumatic brain injury: post-concussion symptoms, disabilities and life satisfaction in relation to serum levels of S-100B and neurone-specific enolase in acute phase. J Rehabil Med. 2005; 37:300–305. [PubMed: 16208863]

Stalnacke BM, Elgh E, Sojka P. One-year follow-up of mild traumatic brain injury: cognition, disability and life satisfaction of patients seeking consultation. J Rehabil Med. 2007; 39:405–411. [PubMed: 17549333]

Stapert S, de Kruijk J, Houx P, Menheere P, Twijnstra A, Jolles J. S-100B concentration is not related to neurocognitive performance in the first month after mild traumatic brain injury. Eur Neurol. 2005; 53:22–26. [PubMed: 15677870]

Stein SC. Minor head injury: 13 is an unlucky number. J Trauma. 2001; 50:759–760. [PubMed: 11303182]

Stein MB, McAllister TW. Exploring the convergence of posttraumatic stress disorder and mild traumatic brain injury. Am J Psychiatry. 2009; 166:768–776. [PubMed: 19448186]

Stranjalis G, Korfias S, Papapetrou C, Kouyialis A, Boviatsis E, Psachoulia C, Sakas DE. Elevated serum S-100B protein as a predictor of failure to short-term return to work or activities after mild head injury. J Neurotrauma. 2004; 21:1070–1075. [PubMed: 15319006]

Svetlov SI, Larner SF, Kirk DR, Atkinson J, Hayes RL, Wang KK. Biomarkers of blast-induced neurotrauma: profiling molecular and cellular mechanisms of blast brain injury. J Neurotrauma. 2009; 26:913–921. [PubMed: 19422293]

Takami Y, Nakagami H, Morishita R, Katsuya T, Cui TX, Ichikawa T, Saito Y, Hayashi H, Kikuchi Y, Nishikawa T, Baba Y, Yasuda O, Rakugi H, Ogihara T, Kaneda Y. Ubiquitin carboxyl-terminal hydrolase L1, a novel deubiquitinating enzyme in the vasculature, attenuates NF-kappaB activation. Arterioscler Thromb Vasc Biol. 2007; 27:2184–2190. [PubMed: 17690318]

Tandler N, Mosch B, Pietzsch J. Protein and non-protein biomarkers in melanoma: a critical update. Amino Acids. 2012; 43:2203–2230. [PubMed: 23053020]

Thompson RJ, Doran JF, Jackson P, Dhillon AP, Rode J. PGP 9.5–a new marker for vertebrate neurons and neuroendocrine cells. Brain Res. 1983; 278:224–228. [PubMed: 6640310]

Tolan NV, Vidal-Folch N, Algeciras-Schimnich A, Singh RJ, Grebe SK. Individualized correction of neuron-specific enolase (NSE) measurement in hemolyzed serum samples. Clin Chim Acta. 2013; 424:216–221. [PubMed: 23778024]

Topolovec-Vranic J, Pollmann-Mudryj MA, Ouchterlony D, Klein D, Spence J, Romaschin A, Rhind S, Tien HC, Baker AJ. The value of serum biomarkers in prediction models of outcome after mild traumatic brain injury. J Trauma. 2011; 71:S478–S486. [PubMed: 22072007]

Townend WJ, Guy MJ, Pani MA, Martin B, Yates DW. Head injury outcome prediction in the emergency department: a role for protein S-100B? J Neurol Neurosurg Psychiatry. 2002; 73:542–546. [PubMed: 12397148]

Tsai YD, Liliang PC, Cho CL, Chen JS, Lu K, Liang CL, Wang KW. Delayed neurovascular inflammation after mild traumatic brain injury in rats. Brain Inj. 2013; 27:361–365. [PubMed: 23438356]

Unden J, Ingebrigtsen T, Romner B, Scandinavian Neurotrauma, C. Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. BMC Med. 2013; 11:50. [PubMed: 23432764]

Vagnozzi R, Signoretti S, Cristofori L, Alessandrini F, Floris R, Isgro E, Ria A, Marziali S, Zoccatelli G, Tavazzi B, Del Bolgia F, Sorge R, Broglio SP, McIntosh TK, Lazzarino G. Assessment of metabolic brain damage and recovery following mild traumatic brain injury: a multicentre, proton magnetic resonance spectroscopic study in concussed patients. Brain. 2010; 133:3232–3242. [PubMed: 20736189]

Varma S, Janesko KL, Wisniewski SR, Bayir H, Adelson PD, Thomas NJ, Kochanek PM. F2-isoprostane and neuron-specific enolase in cerebrospinal fluid after severe traumatic brain injury in infants and children. J Neurotrauma. 2003; 20:781–786. [PubMed: 12965056]

Vinores SA, Herman MM, Rubinstein LJ, Marangos PJ. Electron microscopic localization of neuron-specific enolase in rat and mouse brain. J Histochem Cytochem. 1984; 32:1295–1302. [PubMed: 6389693]

Wang KK, Nath R, Raser KJ, Hajimohammadreza I. Maitotoxin induces calpain activation in SH-SY5Y neuroblastoma cells and cerebrocortical cultures. Arch Biochem Biophys. 1996; 331:208–214. [PubMed: 8660700]

Wang KK, Posmantur R, Nath R, McGinnis K, Whitton M, Talanian RV, Glantz SB, Morrow JS. Simultaneous degradation of alphaII- and betaII-spectrin by caspase 3 (CPP32) in apoptotic cells. J Biol Chem. 1998; 273:22490–22497. [PubMed: 9712874]

Wang KK, Ottens A, Haskins W, Liu MC, Kobeissy F, Denslow N, Chen S, Hayes RL. Proteomics studies of traumatic brain injury. Int Rev Neurobiol. 2004; 61:215–240. [PubMed: 15482817]

Wang KK, Ottens AK, Liu MC, Lewis SB, Meegan C, Oli MW, Tortella FC, Hayes RL. Proteomic identification of biomarkers of traumatic brain injury. Expert Rev Proteomics. 2005; 2:603–614. [PubMed: 16097892]

Waterloo K, Ingebrigtsen T, Romner B. Neuropsychological function in patients with increased serum levels of protein S-100 after minor head injury. Acta Neurochir (Wien). 1997; 139:26–31. (discussion 31–22). [PubMed: 9059708]

Weiss ES, Wang KK, Allen JG, Blue ME, Nwakanma LU, Liu MC, Lange MS, Berrong J, Wilson MA, Gott VL, Troncoso JC, Hayes RL, Johnston MV, Baumgartner WA. Alpha II-spectrin breakdown products serve as novel markers of brain injury severity in a canine model of hypothermic circulatory arrest. Ann Thorac Surg. 2009; 88:543–550. [PubMed: 19632410]

Williams DH, Levin HS, Eisenberg HM. Mild head injury classification. Neurosurgery. 1990; 27:422–428. [PubMed: 2234336]

Woertgen C, Rothoerl RD, Brawanski A. Neuron-specific enolase serum levels after controlled cortical impact injury in the rat. J Neurotrauma. 2001; 18:569–573. [PubMed: 11393260]

Wolf H, Frantal S, Pajenda GS, Salameh O, Widhalm H, Hajdu S, Sarahrudi K. Predictive value of neuromarkers supported by a set of clinical criteria in patients with mild traumatic brain injury: S100B protein and neuron-specific enolase on trial: clinical article. J Neurosurg. 2013; 118:1298–1303. [PubMed: 23451906]

Yang SH, Gangidine M, Pritts TA, Goodman MD, Lentsch AB. Interleukin 6 mediates neuroinflammation and motor coordination deficits after mild traumatic brain injury and brief hypoxia in mice. Shock. 2013a; 40:471–475. [PubMed: 24088994]

Yang SH, Gustafson J, Gangidine M, Stepien D, Schuster R, Pritts TA, Goodman MD, Remick DG, Lentsch AB. A murine model of mild traumatic brain injury exhibiting cognitive and motor deficits. J Surg Res. 2013b; 184:981–988. [PubMed: 23622728]

Yokobori S, Hosein K, Burks S, Sharma I, Gajavelli S, Bullock R. Biomarkers for the clinical differential diagnosis in traumatic brain injury–a systematic review. CNS Neurosci Ther. 2013; 19:556–565. [PubMed: 23710877]

Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat Rev Neurol. 2013; 9:201–210. [PubMed: 23399646]

Zhang Z, Zoltewicz JS, Mondello S, Newsom KJ, Yang Z, Yang B, Kobeissy F, Guingab J, Glushakova O, Robicsek S, Heaton S, Buki A, Hannay J, Gold MS, Rubenstein R, Lu XC, Dave JR, Schmid K, Tortella F, Robertson CS, Wang KK. Human traumatic brain injury induces autoantibody response against glial fibrillary acidic protein and its breakdown products. PLoS One. 2014; 9:e92698. [PubMed: 24667434]

Zurbig P, Jahn H. Use of proteomic methods in the analysis of human body fluids in Alzheimer research. Electrophoresis. 2012; 33:3617–3630. [PubMed: 23160951]

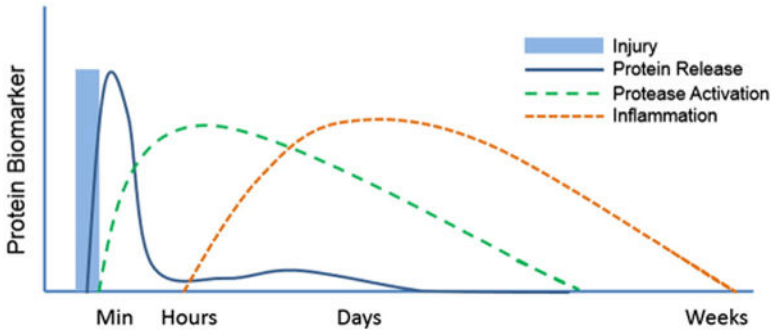

Fig. 1.

Temporal profile of biomarker-related events following mTBI. This illustration represents an approximation of the time course of events following mTBI [modified from (Mondello et al., 2011)].

Table 1

Biomarkers of mTBI: Hypothesis-drive based on pathophysiology

Marker	Injury Model	Source/Sensitivity	Diagnostic Value	Comments	Reference
S100B Prognosis	GCS 13–15	Serum	<0.1 μg/L within 4 h of injury consideration given to not performing CT	extra-cranial injuries must be absent	Jagoda et al. (2009)
	GCS 14–15: n=1958	Serum 0.1 μg/L	If undetectable within 3 h of injury can be discharged without a CT	Compilation of six studies	Unden et al. (2013)
	GCS 13–15: n=182 Hospital admitted <12 h	Serum 0.2 μg/L	Undetectable predicts negative CT scan.	Trend toward increased PCS symptoms if S100B is positive	Ingebrigtsen et al. (2000)
	GCS 13–15: n=35 ED <6 h	Serum 0.17 μg/L	Poor predictive value for 3 month PCS		Bazarian et al. (2006)
	GCS 14–15: n=107 ED <6 h	Serum 0.3 μg/L	Increased S100B associated with forgetfulness at 6 months	Presenting symptoms also associated with PTC	De Kruijk et al. (2002)
	GCS 13–15: n=172 ED <6 h	Serum 0.02 μg/L	92% sensitive and 41% specific for PCS	Skull fracture, age, and presenting symptoms also associated with PCS	Savola and Hillbom (2003)
	GCS 14–15: n=50 ED <6 h	Serum 0.22 μg/L	No correlation with cognitive speed or memory impairment		Stapert et al. (2005)
	GCS 14–15: n=97 ED <24 h	Serum 0.15 μg/L	No correlation with cognitive impairment	S100A1B also did not correlate with cognitive impairment	de Boussard et al. (2005)
	GCS 14–15: n=14 Hosp.Admit. <12 h	Serum sensitivity not indicated	S100B > 0.5 μg/L not correlated with cognitive functioning	One year Neuro-psychologicalfollow-up	Waterloo et al. (1997)
	GCS 13–15: n=59 ED <12 h	Serum 0.02 μg/L	S100B >0.14 μg/L associated with worse neuro-pschological outcomes at baseline and 6 months postinjury	GCS 13–14 and positive radiography also correlated with worse neuropsychological outcome	Muller et al. (2009)
	GCS 15: n=100 ED <3 h	Serum 0.15 μg/L	80% sensitive and 74.4% specific for predicting failure to return to work one week post-injury	99% return to work rate at one month	Stranjalis et al. (2004)
	TBI = 148 GCS 13–15: n=112 ED <6 h	Serum 0.02 μg/L	GCS:13–15: >0.48 μg/L is 90% sensitive and 83% specific for predicting severe disability one month post-injury	0.48 μg/L cutoff very high, one month too short to assess recovery of severe disability	Townend et al. (2002)
	GCS 13–15: n=69 ~3 and 10 h postinjury	Serum sensitivity not indicated	Association between S100B and disability, dizziness, not PTSD	Severity not assessed by CT	Stalnacke et al. (2005)

Marker	Injury Model	Source/Sensitivity	Diagnostic Value	Comments	Reference
	GCS 13–15 Same group as Stalnacke et al. (2005)	Serum sensitivity not indicated	S100B levels in 2nd sample (10 h postinjury) associated with PTSD symptoms	Small number developed PTSD (n=12), assessment via Impact of Event Scale	Sojka et al. (2006)
	GCS 13–15: n=141 ≤4 h	Serum elevated if ≥0.10 μg/L	AUC of 0.639 for predicting poor outcome one week post-injury	Discriminative power of biomarkers alone was limited	Topolovec-Vranic et al. (2011)
GFAP Diagnosis	Mouse weight drop: mild injury 90 min, 6 h, 24 h	Serum sensitivity not indicated	Levels elevated at 90 min and 6 h post-injury	Levels normalized by 24 h	Yang et al. (2013b)
	GCS 15: n=9 ED ≤6 h then every 6 h to 24 h	Serum 0.008ng/mL	Levels elevated in mTBI, particularly with hemorrhage	Did not correlate with DTI	Kou et al. (2013)
	TBI = 209 GCS 13–15: n=206 ED <4 h	Serum ≥.03 ng/mL	For GCS 14–15: AUC of 0.82 for predicting intracranial lesion	3 moderate TBI patients 10% intracranial lesions	Papa et al. (2014)
GFAP-BDP Diagnosis	TBI = 108 GCS 13–15: n=97 ED <4 h	Serum 0.020ng/mL	GCS 13–15: Levels elevated in mTBI vs. non-trauma controls GCS 15: AUC of 0.88 for identifying mTBI vs. non-trauma controls, mTBI levels elevated vs. trauma controls, further elevated in mTBI with intracranial lesion	High number of GCS 15 patients with intracranial lesion, also elevated in non-TBI trauma controls, most of the statistics calculated using GCS 9–15 or GCS 15 patient data only	Papa et al. (2012a)
	GCS 13–15: n=179 ED <24 h	Plasma 01.ng/mL	AUC of 0.87 for distinguishing mTBI from moderate to severe TBI	No controls, most of the statistics calculated using all patient data	Okonkwo et al. (2013)
NSE Diagnosis NSE Prognosis	Mouse weight drop: mild injury 90 min, 6 h, 24 h	Serum sensitivity not indicated	Levels elevated in mTBI, elevated longer with hemorrhage		Yang et al. (2013b)
	Mouse weight drop: mild injury 24 h	Serum sensitivity not Indicated	Levels elevated in mTBI, further elevated in injury+hypoxia		Yang et al. (2013a)
	Rat: mild blast	Serum sensitivity not indicated	Levels elevated in mbTBI at 6 h and 24 h		Abdul-Muneer et al. (2013)
	TBI = 60 GCS 13–15: n=42 Hospital admitted <6 h	Serum elevated if ≥10 μg/L	Elevated >10 μg/L in 31% of mTBI; Statistically significant difference between mTBI and moderate-severe TBI		Skogseid et al. (1992)
	GCS 14–15: n=130 ED <6 h	Serum elevated if ≥10 μg/L	Levels elevated >10 μg/L in 51% of mTBI		Pelsers et al. (2004)
	GCS 14–15: n=104 ED <6 h	Serum 2 μg/L	Levels elevated in mTBI	Distribution of NSE concentration has substantial overlap with controls	de Kruijk et al. (2001)
	GCS 13–15: 107 ED <3 h	Serum sensitivity not indicated	>14.7 μg/L is 56% sensitive & 77% specific for predicting intracranial lesions, AUC of 0.64	Discriminative power of biomarkers alone was limited	Wolf et al. (2013)

Marker	Injury Model	Source/Sensitivity	Diagnostic Value	Comments	Reference
	GCS 13–15: n=109 ED (IQR 18–62.5 minutes)	Plasma 0.01 ng/mL	Not elevated in intoxicated mTBI patients	Acute time point	Mussack et al. (2002)
	GCS 14–15: n=20 ED <24 h	Serum sensitivity not indicated	Levels correlated with GCS score 14–15	GCS 14–15: No increase over trauma controls, Sensitivity and specificity calculated with mild-severe data	Meric et al. (2010)
	Concussed ice hockey players n=28 1–144 h	Serum sensitivity not indicated	No change in NSE between pre and post season levels	Not all players had pre-season blood analyzed	Shahim et al. (2014)
	post-injury or at return to play 100 TBI <13 yoa nTBI & iTBI GCS 13–15: n=56 For mTBI: ASAP, 12 and 24 h after injury For iTBI: time of diagnosis	Serum sensitivity not indicated	>11.36ng/mL is 71% sensitive and 64% specific for identifying TBI, AUC of 0.85 Levels elevated in 89% of GCS 15 patients with normal CT, no LOC and no seizure	Sensitivity and specificity calculations not specific to mTBI, NSE levels were not found to correlate with GCS	Berger et al. (2005)
	<1 yoa iTBI – well appearing, non-specific symptoms: n=14	Serum and/or CSF sensitivity not indicated	>11.77ng/mL is 69% sensitive and 70% specific, AUC of 0.70 for identifying iTBI;		Berger et al. (2006)
	ED: at time of care TBI=60 GCS 14–15: n=20 ED <24 h	Serum sensitivity not indicated	GCS 14–15:no correlation with outcome one month post-injury	Sensitivity and specificity calculated with mild-severe data	Meric et al. (2010)
	GCS 14–15 n=107 ED <6 h	Serum elevated if ≥ 10 μg/L	Associated with headache 6-months post-injury	Presenting symptoms also associated with PTC	De Kruijk et al. (2002)
	GCS 13–15 Same group as Stalnacke et al. (2005)	Serum sensitivity not indicated	No association with PTSD one year post-injury	Small number developed PTSD (n=12), assessment via Impact of Event Scale	Sojka et al. (2006)
	GCS 13–15	Serum sensitivity not indicated	One year post-injury, no correlation between acceptance of consultation for further management or cognitive function		Stalnacke et al. (2007) Stalnacke et al. (2005)
	GCS 13–15: n=141 ≤4 h	Serum elevated if ≥0.12 ng/mL	>14.6 μg/L has an AUC of 0.629 for predicting poor outcome six weeks post-injury	Discriminative power of biomarkers alone was limited	Topolovec-Vranic et al. (2011)
UCHL1 Diagnosis UCHL1 Prognosis	GCS 15: n=86 ED <4 h	Serum 0.030ng/mL	GCS 15: AUC of 0.87 for identifying mTBI vs. non-trauma controls, elevated in mTBI vs. trauma controls, further elevated in mTBI with intracranial lesion or neurosurgical intervention	Most statistics calculated using GCS 9–15 or GCS 15 patient data only, high number of GCS 15 patients requiring neurosurgical intervention	Papa et al. (2012b)
	GCS 13–15 n=9 mTBI	Serum 0.10ng/mL	Levels elevated in mTBI,	Did not correlate with DTI	Kou et al. (2013)

Marker	Injury Model	Source/Sensitivity	Diagnostic Value	Comments	Reference
	ED ≤6 h then every 6 h to 24 h				
	206 TBI GCS 13–15: 83% ED <24 h	Plasma 0.03ng/mL	GCS 13–15: AUC of 0.713 for identifying intracranial lesion Significant difference between mTBI and moderate-severe TBI	GCS 13–15: no comparison to controls	Diaz-Arrastia et al. (2014)
	Sub-concussed football players: n=15; samples collected pre & post game ED <6 h	Serum 0.056ng/mL	Elevated in post-game sub-concussions, but not correlated with number of hits or blood brain barrier disruption.	Levels not elevated in mTBI positive controls	Puvenna et al. (2014)
	TBI = 39 <15 yoa GCS 13–15: n=11 <24 h following hospital presentation	Serum 0.025ng/mL	Not elevated in mTBI		Berger et al. (2012)
	206 TBI GCS 13–15: 83% ED <24 h	Plasma 0.03ng/mL	Levels unable to predict outcome at 6 months post-injury	Outcome analysis included all TBI levels	Diaz-Arrastia et al. (2014)
MBP Diagnosis	TBI = 100 <13 yoa nTBI & iTBI GCS 13–15: n=56 For mTBI: ASAP, 12 and 24 h after injury For iTBI: time of diagnosis	Serum sensitivity not indicated	>0.3ng/mL 44% sensitive and 96% specific, AUC of 0.69, for identifying intracranial hemorrhage in TBI Levels elevated in 0% of GCS 15 patients with normal CT, no LOC and no seizure	While, sensitivity and specificity calculations are not specific to mTBI, MBP levels were not found to correlate with GCS Levels are only elevated after stratification into +/− intracranial hemorrhage Levels do not peak promptly	Berger et al. (2005)
	<1 yoa iTBI – well appearing, non-specific symptoms: n=14 ED: at time of care	Serum and/or CSF sensitivity not indicated	>0.30ng/mL is 36% sensitive and 100% specific, AUC of 0.67, for identifying iTBI		Berger et al. (2006)
SBDP Diagnosis SBDP Prognosis	Rat: mild and severe CCI 2,6 and 24 h	CSF western blot	Levels elevated in mTBI 24 h post injury		Ringger et al. (2004)
	GCS 13–15: n=17 Negative CTED <24 h	Plasma SNTF 10 units	Levels elevated in mTBI and associated with DAI	Levels also elevated in orthopedic injury controls	Siman et al. (2013)
	Concussed ice hockey players n=28 1–144 h post-injury or at return to play	Serum SNTF 14 units	Levels increased in concussed players compared to pre-season baseline	Not all players had pre-season blood analyzed. Same group as Shahim et al. (2014)	Siman et al. (2014).
	TBI = 39 <15 yoa GCS 13–15: <24 h following hospital presentation	Serum 0.25ng/mL	Levels not elevated in mTBI		Berger et al. (2012)
	GCS 13–15: n=17 Negative CTED <24 h	Plasma SNTF 10 units	>10 units is100% sensitive and 75% specific for predicting impaired cognitive function three months post-injury	Also elevated in orthopedic injury controls. Calpain-cleaved αII-spectrin N-terminal fragment.	Siman et al. (2013)

Marker	Injury Model	Source/Sensitivity	Diagnostic Value	Comments	Reference
	Concussed ice hockey players n=28 1–144 h post-injury or at return to play	Serum SNTF 14 units	>14 units AUC of 0.86 identifying concussion that result in PCS > 6 days	Not all players had pre-season blood analyzed. Same group as Shahim et al. (2014)	Siman et al. (2014).
Tau Diagnosis Tau Prognosis	<14 yoa GCS 14–15 n=60 ED, time not indicated	Serum sensitivity not indicated	Levels elevated in mTBI	No difference in levels between mTBI with positive vs. negative CT scans	Guzel et al. (2010)
	GCS 13–15 n=88 ED <24 h	Serum 12pg/mL	Levels not correlated with mTBI patients with positive CT		Kavalci et al. (2007)
	GCS 13–15 n=48 ED <10 h	Serum 59pg/mL	Levels increased in high risk mTBI group	High risk mTBI group would already receive CT based on risk factors	Bulut et al. (2006)
	Concussed ice hockey players n=28 Post-injury: 1, 12, 36, 48, 144 h or at return to play	Plasma, t-tau 0.02pg/mL	Levels increased in concussed players compared to pre-season baseline,	Not all players had pre-season blood analyzed, Ultra-sensitive assay	Shahim et al. (2014)
	Concussed ice hockey players n=28 1–144 h post-injury or at return to play	Plasma, t-tau 0.02pg/mL	Acute and post-acute concentrations correlated with PCS	Not all players had pre-season blood analyzed, Ultra-sensitive assay	Shahim et al. (2014)
	GCS 13–15: n=35 ED <6 h	Serum, C-tau 30pg/well	No correlation with PCS 3 months post-injury	16 patients developed PCS	Bazarian et al. (2006); Ma et al. (2008)
	GCS 13–15 n=50 ED <12 h	Serum, C-tau 1.5ng/mL	No correlation with PCS 3 months post-injury	22 patients developed PCS	Bazarian et al. (2006); Ma et al. (2008)
	GCS 13–15 n=48 ED <10 h	Serum 59pg/mL	No correlation with PCS 6 months post-injury	8 patients developed PCS	Bulut et al. (2006)
NF (pNFH) Diagnosis	Rat: mild-severe CCI 1 h–7 days post-injury	Serum sensitivity not indicated	No significant elevation in mTBI		Anderson et al. (2008)
	GCS 13–15 n=34 Day 1 and Day 3 post-injury	Serum 0.029ng/mL	Day 1: >110.5pg/mL AUC of 1.0, 100% sensitive, 100% specific for identifying mTBI >1071pg/mL AUC of 0.825, 87.5% sensitive, 70% specific for distinguishing mTBI with CT+ vs CT− Day 3: >77.5pg/mL AUC of 0.995, 100% sensitive, 96% specific for identifying mTBI		Gatson et al. (2014)
L-arginine (metabolite) Diagnosis	TBI = 38 GCS 13–15: ED <24 h	Plasma LC-MS	No changes in mTBI		Jeter et al. (2012)

Marker	Injury Model	Source/Sensitivity	Diagnostic Value	Comments	Reference
BCAA (metabolite) Diagnosis	TBI = 38 GCS 13–15: n=18 ED <24 h	Plasma LC-MS	Reduced in mTBI	Also decreased in orthopedic trauma controls. Same group as Jeter et al. (2012)	Jeter et al. (2013b)
ICAM-1 Diagnosis	Rat weight drop: repetitive mild Pre-injury and 1–96 h post-injury	Serum sensitivity not indicated	Elevated in mTBI at 72 h and 96 h compared to baseline		Tsai et al. (2013)

Table 2

Additional hypothesis-driven methods of discovery.

Method	Injury Model	Sample Source	Diagnostic Value	Comments	Reference
Multiplex Array (cytokines)	Mouse weigh drop: mild +/− hemorrhage, mild +/−hypoxia	Serum	IL-6: AUC of 0.86, 80% sensitive and 80% specific for identifying mTBI, IL-6, KC, MIP-1α, MDC elevated		Yang et al. (2013a, b)
Multiplex Array (cytokines)	Mouse closed head: mild +/− stress	Plasma	IL-17A elevated in mTBI		Ojo et al. (2014)
Multiplex Array (cytokines)	iTBI (GCS 15) <1 yoa Well-appearing Non-specific symptoms	Serum	Increased: MMP-9, HGF, fibrinogen, IL-6 Decreased: ICAM, VCAM, eotaxin, TNFR2, IL-12, VCAM + IL-6: 87% sensitive and 90% specific for iTBI, MMP-9 + IL-6: 81% sensitivity and 94% specific for iTBI	Small study (n=16), not mTBI, VCAM unlikely to be used as biomarker due to decrease in levels	Berger et al. (2009)
RPPM	Rat weight drop: mild	Serum	S100B, MBP, tau, NFH elevated post-injury		Rostami et al. (2012)
RPPM	Rat: single and multiple mild blast	Plasma	GFAP, NSE, NFH, VEGF elevated post-injury	Elevated in at least one condition (single or multiple blast) and/or time point (2 h or 22 d)	Kamnaksh et al. (2012)
RPPM	Rat: single and multiple mild blast	Plasma	HNE, ceruloplasmin, vWF, CCR5, HIF-1α, FPR1, p38, MMP-8, VEGF, GFAP, MBP, NFH elevated post-injury	Elevated in at least one condition (single or multiple blast)	Ahmed et al. (2013)
RPPM	Pig: mild-severe blast	Serum	S100B, NSE, MBP, NFH elevated in mTBI	Elevated in at least one time point (6 h – 2 w), NFH unique temporal profile	Gyorgy et al. (2011)

Table 3

Unbiased mTBI biomarker discovery.

Method	Injury Model	Sample Source	Diagnostic Value	Comments	Reference
MicroRNA array	Rat: repetitive mild blast	Serum and/or CSF	miR-let7i, miR-122, mi-R340-5p, miR-200b, miR-874 elevated in multiple conditions (short/long repetitive blast interval, 3 h/24 h post-injury)	MicroRNAs associated with CNS conditions, miR-let7i further validated, miR-let7i possible regulator of cytokines, S100B and UCHL1	Balakathiresan et al. (2012)
MicroRNA array	GCS 13–15 Mild and Severe	Plasma	GCS 13–15: miR-16 & miR-92a elevated, miR-16 AUC of 0.82 and miR-92a AUC of 0.78 for diagnosing mTBI, no elevation vs. orthopedic injury	miR-16: cell proliferation, cell cycle progression and apoptosis miR-92a: negative regulator angiogenesis Mild and severe miRNA patterns different	Redell et al. (2010)
MicroRNA array	Veterans with mTBI	PBMC	13 miRNAs lower in mTBI, panel of HBII-289, ENSG199411 and U35A 82% selective and 78% specific for mTBI	mTBI veterans were 1.2–6.6 years post-deployment, small study (mTBI = 9)	Pasinetti et al. (2012)
Proteomics (LC-MS)	Mouse: mild and severe CCI	Plasma	30 proteins modulated in TBI, at 24 h no overlap between proteins of mild and severe injury, in mTBI no overlap between 24 h and 1 month	Proteins associated with acute phase response, oxidative stress, and lipid metabolism	Crawford et al. (2012)
Proteomics (2D-PAGE-MS) and western blot	Degenerating neurons, validation in rats exposed to mild fluid percussion injury	Culture and CSF	Degenerating neurons: elevations in tau, MAP2, spectrin, 14-3-3, CRMP2, CRMP4, GAP43 and creatine kinase CSF validation: elevations in 14-3-3, spectrin and tau	In-vitro method used for initial screening	Siman et al. (2004)
Proteomics (2D-DIGE, LC-MS), western blot, ELISA	Mild AHI <1 yoa	Serum	Identification of Serum Amyloid A, AUC of 0.96 for distinguishing mTBI from control on western blot	Validated increase with other severity levels, concentration did not correlate with injury severity, controlled for other sources	Gao et al. (2014)