

Advancing Biomarker Research for Traumatic Brain Injury (TBI)

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In the early portion of the 21st Century, after decades of neglect and an almost complete lack of concern, concussions and head trauma (and their aftermath) have come to dominate the public consciousness. With the conflicts in Afghanistan and Iraq, and the alarming injury rates and subsequent deaths linked to contact sports, particularly American football, the onus has been placed on researchers to discover new and novel ways to address this significant and growing problem. There are estimates that every 21 seconds in the United States someone sustains a Traumatic Brain Injury (TBI) with 50,000 attributable deaths each year. These numbers account for one-third of all injury-related deaths in the U.S. and more than 1 million people seeking medical care for TBI annually. More than 5 million Americans are living with TBI-related disabilities and the United States spends an estimated \$56 billion per year treating the after-effects of this condition [4].

TBI can result from a multitude of causes, the most common being motor vehicle accidents, falls and/or assaults, exposure to explosions (i.e. soldiers serving in combat) and penetrating head trauma (gunshot wounds etc.). The terms mild TBI (mTBI) and concussion have often been used interchangeably and the traditional, societal perception of these injuries has been that they are insipid, or even inconsequential in nature. However, our growing body of knowledge is beginning to

paint a very different, and decidedly starker, picture. TBI, even of the mTBI variety, can cause significant damage to both white matter and axons [38] and repeated episodes of mTBI and/or subconcussive trauma can induce significant worsening of clinical symptoms that strongly resemble both Parkinson's and Alzheimer's disease among others.

Severe TBIs tend to be more straightforward than less severe forms such as mTBI. These less severe injuries have confounded accurate, concise definition and are incredibly difficult to diagnose. Clinicians and researchers have struggled to agree on a single, standard definition of mTBI. This is most likely due to the vast amount of diversity and lack of specificity in symptomology. All-too-often, those who have suffered an acute mTBI present with symptoms that mimic a variety of other conditions, some even psychological in nature. This heterogeneity in symptomology and the fact that many patients with mTBI have symptoms that closely mimic other disorders make many mTBIs almost impossible to accurately detect. Most mTBI patients recover well and do not suffer from lingering symptoms. However, many who have suffered an mTBI experience worsening symptoms that continue to progress and become more severe manifestations of emotional, psychological and physical trauma. Estimates are that 15% of patients with mTBI develop persistent cognitive dysfunction [28]. Currently, the American Congress of Rehabilitation Medicine defines an mTBI as, a traumatically induced physiological disruption of brain function, as manifested by at least one of the following: loss of consciousness, any loss of memory for events immediately before or after the event, focal neurologic deficit that may or may not be transient, but where the severity of the injury does not exceed a

loss of consciousness of approximately 30 min or less, an initial Glasgow Coma Scale (GCS) score of 13–15 and post-traumatic amnesia not greater than 24 h [10].

Concussions and mTBI have been called a “silent epidemic” [9] as both concussions and subconcussive events are often undiagnosed or unreported altogether. Mild traumatic brain injuries are also alarmingly common. It has been estimated that more than 1.5 million Americans sustain mTBI without a subsequent loss of consciousness and without the need for hospitalization. An equal or greater number sustain an injury that impairs consciousness but is not severe enough to require long-term hospitalization. Mild Traumatic brain injury affects up to 10 million people globally and accounts for 70-90% of all TBI cases [10].

Blasts, vehicle crashes, and other mechanisms place soldiers, athletes and us all at risk for TBI. A mild TBI or mTBI is characterized by mechanical injury to the head with brief loss of consciousness or altered mental status [35]. Surveys of soldiers who have recently returned from active duty indicate that up to 16% reported that a loss of consciousness was associated with their injuries [11]. Soldiers with TBI, particularly those who had lost consciousness with their injury are more likely to meet criteria for Posttraumatic Stress Disorder (PTSD) and major depression and poor general health. They also report more lost workdays, medical visits and a higher number of post concussive symptoms than do soldiers that experienced other types of injuries [11, 35]. Athletes suffer repetitive concussive and sub concussive brain trauma as well, and as a result they suffer executive dysfunction, memory impairment, depression and suicidality, apathy, poor impulse control, and eventually dementia [3]. It is important to note that most individuals with mTBI recover within 3-6 months of the injury. However, a subset will develop persistent neuropsychiatric symptoms [6, 17]. Unfortunately, most individuals with mTBI do not seek out and thus do not receive rehabilitation and thus many become permanently disabled [35]. Although it is difficult to detect a mTBI simply using a clinical examination, there are studies now which suggest that early identification and treatment of the symptoms can improve outcomes [36, 27]. It is therefore imperative to devote effort to the identification of novel biomarkers that will enhance early TBI detection, management and therapeutic response that could be tailored to the soldiers, athletes or the community.

Important to mTBI diagnosis and management is to determine an optimal combination of clinical indicators or biomarkers that could detect injury early with both high specificity and sensitivity and with limited invasiveness. In spite of the availability of a number of gene products considered as promising biomarkers (S100B and Neuron Specific Enolase (NSE)), it is recognized that their combined use with the available clinical information is still insufficient for early diagnosis and for guiding individualized therapeutic interventions and predicting outcomes [30]. However, there is growing interest in using proteomic approaches to identify serum autoantibodies recognizing trauma-associated antigens (TrAA), as well as serum microvesicles called exosomes and their content, as serological biomarkers [40, 21, 34, 23, 33, 13, 14]. This interest stems from the notion that these blood components are considered “sensors” of molecular events associated with pathology [20, 32, 33].

In addition to clinical variables available at the time of injury, the potential utility of quantifying serum biomarkers of structural damage or as mediators of cellular, biochemical, or molecular secondary injury cascades for predicting outcome after TBI has been investigated [15, 9]. S100B is a calcium-binding protein found in glial cells, predominantly astrocytes, and at physiologic concentrations has been shown to provide both neurotrophic and neuroprotective effects. Elevated presence of S100B in peripheral blood and/or Cerebral Spinal Fluid (CSF) may indicate neuronal damage and possible blood brain barrier disruption [12]. In acute TBI and post-acute scenarios, S100B has been documented to be elevated and as a result may lack specificity, better serving as a marker for polytrauma. NSE is a glycolytic enzyme that catalyzes the conversion of 2-phosphoglycerate to Phosphoenolpyruvate (PEP). The neuron specific isomer of enolase is found primarily in neuron cytoplasm. In TBI patients, NSE may indicate neuronal damage as NSE has been reported elevated in patients with mTBI. However, NSE lacks sensitivity and specificity with its levels shown to be elevated in non-trauma patients due to a variety of causes [28]. Additionally, an increase NSE level can result from hemolysis a common occurrence in trauma cases. There is also a poor correlation between NSE serum levels and Glasgow Outcome Scale (a longer term evaluative measure when compared with GCS) [7].

Together, S100B and NSE have been shown to be sensitive to cranial pathology after mTBI with strong negative predictive values [35]. In addition, elevated levels of these biomarkers have been linked to poor predictive outcomes up to 12 months post injury. Testing these biomarkers has not been adopted clinically because of their low levels, specificity and reports that non-cranial injuries also contribute to elevations in these two markers [1]. It is therefore imperative that proteome-profiling & immunoseroproteomics approaches, currently considered the most promising strategy for the identification of serum biomarkers [34, 33, 13, 14] be adapted and applied to TBI and mTBI. The application of such approaches in the context of soldiers, athletes or the general population has never been done before.

The list of potential biomarkers for TBI, though small, continues to grow with the top investigated targets, besides S100B and NSE, being Glial Fibrillary Acidic Protein (GFAP), Myelin Basic Protein (MBP), and Ubiquitin C-terminal Hydrolase-L1 (UCH-L1) [31]. GFAP is an intermediate filament protein, encoded by the *GFAP* gene, that is expressed in many cell types in the CNS, most particularly glial cells but also astrocytes and ependymal cells [8]. GFAP is a major component of the astrocyte cytoskeleton which is acutely elevated following mTBI [19, 39]. Presence of GFAP in CSF and peripheral blood in trauma patients may suggest astrocyte damage and possible blood brain barrier (BBB) disruption [26]. GFAP seems to have both high sensitivity and specificity but has not yet been shown to correlate with longer-term disruption. MBP, found in myelin and known to play a key role in myelination, is one of the most abundant CNS proteins [29]. MBP levels are elevated in trauma patients post TBI [24]. Higher MBP levels may suggest axonal injury and damage and may be related to poor outcome in trauma patients [2]. Detection of aberrant levels of serum MBP may take 48-72 hours and may be somewhat impractical for diagnostic purposes at this point. Cleaved tau (C-tau), a microtubule associated protein that is expressed in CNS axons, has been recorded post TBI [5]. A presence of C-tau in plasma may suggest hyperphosphorylation and formation of neurofibrillary tangles that have been seen in post mortem samples of patients with chronic traumatic encephalopathy (CTE) [18]. Although C-tau has been correlated with TBI, to date its presence was not indicative of intracranial lesions on CT scans of patients with head trauma. Furthermore, C-tau has not been proven to be an

accurate predictor of post concussive syndrome (PCS) and longer-term recovery [37, 18]. UCH-L1 is a deubiquitinating enzyme found in the cytoplasm of interneurons and is the newest proposed biomarker in TBI. Its presence may suggest neuronal damage and BBB disruption as its presence is abundant in neurons [25]. UCH-L1 is also known in the neuron literature as neuronal-specific protein gene product (PGP 9.3) and in studies performed in CSF, an inverse relationship exists as its presence increased as GCS decreased. This relationship indicates a direct association between UCH-L1 and trauma levels [25]. However, to date, little is known about UCH-L1 levels in trauma patients [37, 22, 31] making UCH-L1 a very promising target in TBI studies. Produced by calpain and caspase 3 alpha 2, Spectrin Breakdown Products (SBDP) are found in presynaptic terminals and axons [37]. SBDP presence in trauma patients, especially those with subarachnoid hemorrhage (SAH) may suggest cellular necrosis following brain injury [16]. Along with UCH-L1, SBDPs are promising proteins for head trauma analysis.

Despite the recent increase in interest and funding in the study of head trauma, the field is in its infancy. There are currently no widely accepted clinical biomarkers known to be indicative of head trauma. In spite of the availability of a number of gene products considered as promising biomarkers (described above), it is recognized that their combined use with the available clinical information is still insufficient for early diagnosis and for guiding individualized therapeutic interventions and predicting outcomes [30]. Much has been made of the urgent need to discover a novel diagnostic tool in order to alleviate the high costs of radiologic testing and expedite the treatment process. Compounding the difficulty in the diagnosis of concussion and the more mild forms of TBI, where there may exist no findings on a CT scan, is the fact that the etiology of the continuum from concussion to PCS to CTE is a mystery. What allows one trauma patient with a concussion or head injury to recover quickly and be largely asymptomatic while another, with similar acute presentation, suffering long term or permanent disability is unknown? We are only just beginning to understand the most severe end of the head trauma spectrum, the CTE exhibited by retired boxers and American football players. But we do not yet understand all of the factors that lead from one, or multiple, concussive events to the tragic disabilities exhibited by some of our greatest icons like Muhammad Ali and the brave men and women who served our country in the Armed services.

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