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Current status of fluid biomarkers in mild traumatic brain injury

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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.

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 nbiomarker, 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 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)

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.

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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), which 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; 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