

# Biomarkers for the Diagnosis, Prognosis, and Evaluation of Treatment Efficacy for Traumatic Brain Injury

Pramod K. Dash,\* Jing Zhao,\* Georgene Hergenroeder,<sup>†‡</sup> and Anthony N. Moore\*

\*Department of Neurobiology and Anatomy, <sup>†</sup>Department of Neurosurgery, and the <sup>‡</sup>Vivian L. Smith Center for Neurologic Research, The University of Texas Medical School, Houston, Texas 77225

**Summary:** Traumatic brain injury (TBI) remains a serious health concern, and TBI is one of the leading causes of death and disability, especially among young adults. Although preventive education, increased usage of safety devices, and TBI management have dramatically increased the potential for surviving a brain injury, there is still a need to develop reliable methods to diagnose TBI, the secondary pathologies associated with TBI, and predicting the outcomes of TBI. Biomarkers (changes of amount or activity in a biomolecule that reflect injury or disease) have shown promise in the diagnosis of several conditions, including cancer,

heart failure, infection, and genetic disorders. A variety of proteins, small molecules, and lipid products have been proposed as potential biomarkers of brain damage from TBI. Although some of these changes have been reported to correlate with mortality and outcome, further research is required to identify prognostic biomarkers. This need is punctuated in mild injuries that cannot be readily detected using current techniques, as well as in defining patient risk for developing TBI-associated secondary injuries. **Key Words:** Blast injury, diffused axonal injury, intracranial pressure, loss of consciousness, post-concussive symptoms, PTSD.

## TRAUMATIC BRAIN INJURY: A GLOBAL HEALTH PROBLEM

The human brain is highly vulnerable to injuries that can cause profound cognitive and neurobehavioral dysfunction, compromising quality of life. TBI can result from shearing forces caused by a sudden change in acceleration to deceleration (e.g., vehicular accidents), from impacts (e.g., falls, blunt force trauma), as a result of rapid changes in pressure (e.g., blast exposure), or by penetration by high-velocity projectiles (e.g., bullets, blast/shrapnel). Considered a “silent epidemic” in the United States, TBI has its greatest impact on young men and women. These patients (mean, 29.5 years) pose a tremendous burden to families and society in terms of years of lost productivity, increased demands on the healthcare system, and reduction in healthy life spans. Approximately 5.3 million in the United States alone are living with disabilities resulting from TBI.

One of the primary causes of death after a trauma is brain injury. In less developed countries, traffic accidents are the most significant cause of brain injury, with most accident-related fatalities being linked to brain damage. According to a World Health Organization, World Bank report titled,

“The Global Burden of Disease,” traffic injuries are expected to become the third highest disease burden by the year 2020. For example, every motor vehicle injury that results in death in the United States, 13 people sustain injuries severe enough to require hospitalization, according to the World Health Organization report titled, “The Injury Pyramid.” The majority of injuries from these types of brain trauma are caused by sudden changes in inertia (rapid acceleration and deceleration) leading to distortion of the brain tissue within the skull. A primary feature of this type of injury is axonal damage, often referred to as diffuse axonal injury.

In this century, brain injuries resulting from blasts have emerged as the major cause for TBIs among military personnel. The number of TBI casualties, sustained by Coalition Forces engaged in the wars in Afghanistan, Operation Enduring Freedom, and Operation Iraqi Freedom during the past 5 years, indicates that TBI will continue to be a major threat to military personnel in the 21st century.<sup>1,2</sup> According to the Joint Theater Trauma Registry, compiled by the U.S. Army Institute of Surgical Research, 22% of wounded soldiers from Iraq and Afghanistan have injuries of the head, face, or neck, which has substantially increased in comparison with the casualties suffered in the Vietnam War.<sup>3</sup> At least some of this increase in TBI is due to increased survivability, resulting from improvements in body armor and helmet design, and rapid medical intervention. However, knowl-

Address correspondence and reprint requests to: Pramod Dash, Department of Neurobiology and Anatomy, The University of Texas Medical School, P.O. Box 20708, Houston, TX 77225. E-mail: p.dash@uth.tmc.edu.

edge of the pathophysiology of TBI resulting from blasts is in an infant stage. Recent studies are beginning to show there may be a number of differences between acceleration and deceleration from blast-induced TBI in terms of neurochemical changes, cell death, and outcome.

Although TBI resulting from vehicular accidents is on the decline, as a result of enforced seat-belt laws and other safety laws, penetrating brain injury from gunshots, knives, and blast/shrapnel is on the rise. This type of injury often causes cavitations resulting from the transfer of kinetic energy from the high-power projectile into the soft brain tissue. Depending on the path of the projectile, penetrating injury can cause profound deficits in specific sensory, motor, or cognitive domains, and is often associated with post-traumatic epilepsy. In the case of shrapnel injuries, persons may have mixed pathologies caused not only by the penetration of the projectile, but as a result of the blast overpressure. These types of mixed injuries are most commonly observed in soldiers exposed to improvised explosive devices.

The incidence of TBI can be reduced by preventive education programs (such as drunk driving prevention), and the severity of the primary injury can be lessened through the use of improved protective equipment (such as the use of helmets for athletes and body armor for military personnel). However, an effective clinical therapy to treat human brain injury is not yet available.

### **PRIMARY AND SECONDARY INJURIES ARE KEY FEATURES OF TBI**

The pathology of TBI reflects the initial insult, resulting from mechanical damage to neural and vascular structures (the primary injury), and the evolution of a cascade of secondary events that impair function, damage structures, and promote further cell death (the secondary injury).<sup>4</sup> The primary injury results from the deformation of grey and white matter leading to the distortion and/or disruption of cell membranes and release of intracellular contents. In human TBI, there is a heterogeneity (i.e., focal, multifocal, or diffuse) that is associated with the primary insult. The evolution of secondary damage represents a cascade of biochemical and molecular events that collectively mediate cell damage. The extent or severity of secondary damage is at least partly dependent on the proximity and/or connectivity of the affected tissue to the areas of primary damage, and is also dependent on the relative vulnerability of the different cell types from which it is composed.

Some of the earliest findings in the injured brain include damage to axons, excessive neuronal activity (reflected in part from unregulated glutamate release), widespread changes in neurotransmitter levels, altered cerebral blood flow, hemorrhage, and disruption of the

blood-brain barrier. Coincident with these events are physiologic disturbances including hypotension, hypoxemia, elevated intracranial pressure, decreased cerebral perfusion pressure, edema, ischemia, and silent seizures (in many cases).

### **TBI BIOMARKERS**

A biomarker is an indicator of a specific biological or disease state that can be measured using samples taken from either the affected tissue or peripheral body fluids. These markers can be altered enzymatic activity, changes in protein expression, or post-translational modification, altered gene expression, protein, or lipid metabolites, or a combination of these changes. As a consequence, a variety of strategies have been used for biomarker discovery including transcriptional profiling, proteomic and metabolomic approaches.

The two most commonly used approaches for biomarker discovery are “top-down” and “bottom-up” methodologies. In a top-down approach, potential biomarkers are evaluated based on their known involvement in the disease state. For instance, the cell death that occurs after TBI in the core of the primary injury is largely necrotic in nature. As necrosis is associated with calpain activation, specific protein degradation profiles will be generated (both in terms of the resultant peptide fragments and the time course of their appearance), giving rise to putative biomarkers of necrosis.<sup>5</sup> These markers would be distinct from those biomarkers generated as a result of apoptotic cell death, because apoptosis is associated with activation of caspases and is typically more delayed in progression.<sup>6,7</sup> Although the “top-down” approach has been used to discover biomarkers associated with TBI, many of these markers were first identified in the injured brain, and then their presence in a more accessible sample source (e.g., blood, urine, and so on) was examined. If the biomarker is absent (or present in very low amounts) in the peripheral bodily fluids, its usefulness for diagnostic purposes may be lessened. A “bottom-up” approach examines changes in the composition of a tissue or bodily fluid, and then attempts to establish links between these changes and pathology. Although a “bottom-up” approach using a sample source, such as serum for example, may overcome the potential problem of biomarker detectability; this approach often identifies general markers that are not solely indicative of the condition of interest. As an example of using serum samples from severely injured TBI patients and healthy volunteer controls, C-reactive protein and serum amyloid A have been reported to be capable of distinguishing between these two groups.<sup>8</sup> However, both of these proteins are general indicators of injury/infection, making them less likely to distinguish brain injury patients from persons with other bodily injuries. On the other hand, if

brain injury alone is suspected, or if these markers were used in combination with other biomarkers, a rise in the levels of these proteins in serum may further support a brain injury diagnosis.

### Protein biomarkers of TBI

To date, the majority of TBI biomarker research has focused on protein profiling. However, as the human genome is estimated to contain ~23,000 genes, and as many as 50% of the translated proteins are considered to be low in abundance, surveying the entire proteome is a daunting endeavor. For instance, the Human Proteome Organization collaborative initiative has identified only 889 abundant serum proteins (using two-dimensional gel mass spectrometry) with a confidence level of at least 95%.<sup>9</sup> This suggests that <10% of the “core” serum proteins (estimated to contain at least 10,000 proteins) are effectively being sampled. To enhance the probability of detecting low-abundance protein biomarkers, a number of methods have been used including fractionation, immunodepletion of abundant proteins, the use of subproteome (e.g., inflammatory proteome, glycoproteome, phosphoproteome) arrays, and isolation techniques, such as combinatorial ligand libraries. The advantages and disadvantages of these approaches have been reviewed elsewhere.<sup>10–12</sup>

### S100B

The most extensively studied TBI biomarker is S100B. Changes in the expression of this marker have been shown to correlate with injury magnitude, survivability, and neurologic outcome.<sup>13</sup> S100B is a low molecular weight (10.5 kDa) calcium-binding protein that is primarily expressed and secreted by astrocytes, constituting 1 to 1.5  $\mu\text{g}/\text{mg}$  of all soluble protein. S100B can be found in very low levels in human CSF and serum, and normal levels of this protein have been strongly correlated with the absence of intracranial injury.<sup>14</sup> After brain injury, S100B is released into the CSF and serum. The serum half-life of S100B is less than 60 min and can be readily detected soon after injury. Increased levels of this protein have been proposed as biomarkers of poor outcome. For example, Vos et al.<sup>15</sup> demonstrated that in patients with severe TBI, serum S100B concentrations >1.13 ng/mL were associated with increased mortality (100% sensitivity; 41% specificity) and morbidity (88% sensitivity; 43% specificity). The low basal levels of S100B in human serum allow increases in the concentrations of this protein to be very sensitive indicators of brain injury. However, several recent observations concerning S100B have tempered the original enthusiasm regarding the usefulness of this protein as a biomarker. First, because S100B does not cross the intact blood brain barrier, there is a poor correlation between its levels in the CSF and those detected in the serum. This has raised concerns as to whether the serum levels of this protein actually correlate with the degree of brain dam-

age or are more reflective of blood brain barrier (BBB) disruption. Secondly, S100B has been reported to be released into the serum after experimental ischemic injury to the liver, kidney, and gut;<sup>16</sup> its levels have been found to have increased after extracranial trauma<sup>14</sup> and burns,<sup>17</sup> and it has been suggested to be a biomarker of melanoma.<sup>18</sup> Finally, conflicting reports have been published regarding the usefulness of this marker in predicting outcome after pediatric TBI.<sup>19–21</sup>

### Glial fibrillary acidic protein

The glial fibrillary acidic protein (GFAP) is a monomeric intermediate filament protein expressed by astrocytes. It is a brain-specific protein that is released after TBI.<sup>15,22–24</sup> Increases in serum GFAP levels after TBI have been shown to be predictive of elevated intracranial pressure (ICP), reduced mean arterial pressure, low cerebral perfusion pressure, poor Glasgow outcome scores (GOS), and increased mortality. For example, GFAP serum concentrations >1.5 ng/mL were found to be predictive of death (85% sensitivity; 52% specificity) or poor outcome (GOS at 6 months; 80% sensitivity; 59% specificity).<sup>15</sup> Consistent with its brain-specific expression, GFAP levels were found to be normal in multi-trauma patients that did not have brain injury.<sup>24</sup>

### Neuron-specific enolase

The neuron-specific enolase (NSE) is one of the five isozymes of the glycolytic enzyme, enolase. NSE was initially found to be expressed in neurons, but has subsequently been identified in neuroendocrine cells, oligodendrocytes, thrombocytes, and erythrocytes. NSE has a serum half-life of approximately 24 hours and can be detected within 6 hours of injury. The serum level for NSE (normally <12.5 ng/mL) has been reported to increase after TBI, the levels of which have been correlated with the severity of the injury.<sup>25–27</sup> At serum levels >21.7 ng/mL, NSE has been demonstrated to be a sensitive indicator of mortality (85% sensitivity) and poor outcome (80% sensitivity). However, inadequate sensitivity and specificity have limited the use of NSE regarding the neuropsychological outcome (55% sensitivity; 77.8% specificity) and predicting the presence of intracranial lesions (77% sensitivity; 52% specificity).<sup>15,28,29</sup> Used in isolation, elevated serum NSE levels do not necessarily indicate the presence of brain trauma, as this protein has also been suggested to be a marker for small cell lung cancer, neuroendocrine bladder tumors, ischemic stroke, and neuroblastomas.

### Myelin basic protein

The myelin basic protein (MBP) is a major protein component of myelin, and is released into the CSF and blood after white matter injury. Normal serum levels of MBP are very low, typically <0.3 ng/mL. In a study of pediatric brain injury, high serum MBP levels were found to correlate with worse outcome.<sup>30</sup> Interestingly,

this same study demonstrated that MPB levels were not increased in children with hypoxic-ischemic encephalopathy. This is consistent with previous observations that ischemic injury does not cause disruptions of white matter.

### Fatty acid binding proteins

The fatty acid binding proteins (FABPs) are expressed in multiple tissues, with the exception of brain type-FABP (B-FABP), which is expressed exclusively in the brain.<sup>31</sup> In a study to evaluate the clinical usefulness of B-FABP as a biomarker of brain injury, Pelsers et al.<sup>32</sup> reported that B-FABP could be detected in the serum of 68% of the mild TBI patients examined. No B-FABP could be detected in the serum of healthy volunteer controls. Although FABP is not highly sensitive, it proved to be more accurate in identifying mild TBI patients as compared with S100B (increase of 45% of mild TBI patients) or NSE (increase of 61% of mild TBI patients).

### Proteolytic breakdown products

One of the key pathological features of TBI is the unregulated breakdown of cellular proteins by protease families such as calpain and caspase. Recently, BDPs of the structural protein  $\alpha$ -II spectrin and the dendritically enriched microtubule-binding protein tau have been shown to correlate with injury severity in rodent models of TBI, and in human TBI patients.<sup>33,34</sup> For example, the levels of calpain-cleaved  $\alpha$ -II spectrin BDPs, but not the level of S100B, in the ipsilateral cortex and CSF of rats subjected to cortical impact injury were found to correlate with lesion size.<sup>35</sup> In humans, the levels of spectrin BDPs were initially found to be upregulated in the CSF of patients with severe TBI, but decreased at between 6 to 96 hours in those with a better outcome.<sup>36</sup> Measurement of cleaved tau (C-tau) levels in the serum of brain injured rats also revealed a significant increase in these BDPs by 6-h post-injury.<sup>33</sup> Consistent with this, the initial post-injury CSF levels of C-tau were found to be elevated in patients with severe TBI, correlated with clinical outcome (92% sensitivity; 94% specificity), and predicted elevated ICP (78% sensitivity; 79% specificity).<sup>37</sup>

### Phosphorylated neurofilament

A recent addition to the growing number of neuron-specific protein markers of injury is phosphorylated neurofilament H. Using a rodent model of TBI, Anderson et al.<sup>38</sup> demonstrated that phosphorylated neurofilament H could be detected early (within 6-h post-injury) in the blood of animals with brain injuries. Peak levels (detected 24-48-h post-injury) were found to correlate with the volume of the injured cortex. The sensitivity of this marker in detecting human brain injury has not yet been investigated.

### Ubiquitin C-terminal hydrolase

The ubiquitin C-terminal hydrolase is enriched in neurons; therefore it has been proposed to be a putative biomarker for TBI. To test this possibility, Papa et al.<sup>39</sup> carried out a prospective study to determine the CSF levels of ubiquitin C-terminal hydrolase in patients who had severe brain injury and in uninjured controls. They found that ubiquitin C-terminal hydrolase levels were significantly increased in the CSF of brain-injured patients, and that the magnitude of this increase correlated with mortality, post-injury complications, and outcome tested 6 months post-discharge.

## INFLAMMATORY MARKERS

Increased serum and/or CSF concentrations of pro- and anti-inflammatory cytokines, chemokines, and acute phase reactant proteins have been observed after TBI. The importance of inflammation on the progression of TBI-associated pathologies has been the focus of numerous studies.<sup>40-43</sup> More recently, the expression of these factors has been appreciated as potential markers of injury, and studies have been carried out to test if their levels correlate with outcome or can serve as surrogate markers of treatment efficacy. Acute phase proteins (e.g., C-reactive protein, amyloid A, pro-inflammatory cytokines [e.g., IL-1, TNF- $\alpha$ , IL-6], and anti-inflammatory cytokines [IL-10, transforming growth factor beta, or TGF- $\beta$ ] and chemokines [e.g., ICAM-1, macrophage inflammatory protein (MIP)-1, MIP-2]) have been reported to change in response to TBI. The CSF and/or serum levels of a number of these markers have been correlated with injury, and in some cases with outcome. For example, it has been reported that the acute phase reactant proteins C-reactive protein and serum amyloid A are rapidly increased in the serum of brain trauma patients.<sup>8</sup> Using a multiplex approach to simultaneously evaluate a panel of chemokines and cytokines, Buttram et al.<sup>44</sup> reported that severe TBI in children was associated with significant increases in the CSF levels of interleukin (IL)-1 $\beta$ , IL-6, IL-12p70, IL-10, IL-8, and MIP-1 $\alpha$ . Because the injury to other organs can increase the serum level of these markers, these markers by themselves may not provide high specificity. However, the temporal changes in the serum levels of these cytokines may be different after TBI and injury to other organs. Furthermore, in certain situations in which selective brain injury is suspected (e.g., inflicted TBI), these markers can be helpful in diagnosing TBI.<sup>45</sup>

### Interleukin-1

Since the earliest study suggesting that IL-1 may contribute to the development of astroglial scars after brain injury,<sup>46</sup> IL-1 has been extensively studied after TBI. For example, Singhal et al.<sup>47</sup> evaluated the levels of IL-1 and

IL-6 in the CSF of severe brain injury patients and found peak IL-1 levels correlated with 3-month outcomes. Both in adult and pediatric TBI patients, high CSF IL-1 levels have been shown to indicate a poor GOS.<sup>48</sup>

### Tumor necrosis factor

Tumor necrosis factor (TNF)- $\alpha$  as a pro-inflammatory factor has been shown to play an important role in the immune response. A major immune function of TNF- $\alpha$  is stimulating neutrophil and monocyte recruitment to the site of injury to phagocyte debris. In the CNS, TNF- $\alpha$  is mainly secreted by microglia but can also be released by other cells including astrocytes, neurons, and endothelial cells.<sup>49–52</sup> Increased TNF- $\alpha$  concentrations in serum and CSF have been reported in human TBI cases.<sup>53–55</sup> In a prospective study to examine the correlation between serum TNF- $\alpha$  levels and mortality, Crespo et al.,<sup>53</sup> found that although initial TNF- $\alpha$  levels were significantly increased in the serum of severely injured patients, this increase did not correlate with mortality.

### Interleukin-6

A number of studies performed in animals and humans with TBI have reported changes in CSF and serum levels of the cytokine IL-6. The CSF levels of IL-6 reach a maximum between days 3 and 6 in TBI subjects,<sup>56</sup> with the severity of injury being related to the degree of intracranial IL-6 gradient (venous to arterial ratio) at the time of admission.<sup>57</sup> An *in vivo* microdialysis study has shown that increased parenchymal IL-6 levels correlate with survival and GOS suggesting that IL-6 is an endogenous neuroprotective cytokine.<sup>58</sup> Furthermore, increased CSF levels of IL-6 have been reported to correlate with improved outcome in pediatric TBI.<sup>59</sup> Interestingly, induced hypothermia has no effect on IL-6 levels in children, a finding that is in contrast to that observed in adult TBI patients in which IL-6 levels were found to decrease after therapeutic hypothermia.<sup>60</sup>

### Anti-inflammatory cytokines

The anti-inflammatory cytokines (e.g., IL-10, TGF- $\beta$ ) counteract the detrimental effects of pro-inflammatory molecules such as IL-1, TNF- $\alpha$ , and IFN- $\gamma$ ,<sup>61</sup> and have been reported to also be increased as a result of TBI. For example, the CSF levels of IL-10 increase within the first 24 h of TBI. This rise in CSF IL-10 is associated with a corresponding decrease in TNF- $\alpha$  levels, consistent with its anti-inflammatory function.<sup>62</sup> Increase in IL-10 levels has been shown to correlate with mortality in pediatric TBI.<sup>63</sup> TGF- $\beta$  is a member of a large super family of growth factors that are important for a number of biological functions including cell growth and differentiation, angiogenesis, immune function, extracellular matrix production, cell chemotaxis, and apoptosis. There are approximately 25 members in this family, which includes activins, inhibins, bone morphogenetic proteins,

growth and differentiation factors, and glial-derived neurotrophic factor.<sup>64</sup> After TBI, the CSF levels of TGF- $\beta$  are rapidly increased (peaking by 24-h post-injury), and they remain at this level for >3 weeks.<sup>65</sup>

### Chemokines

The chemokines play a vital role in TBI-associated brain inflammation. They recruit peripheral immune cells into the injured brain, where these cells release a number of cytokines and also provide scavenging functions. For example, increased expression of ICAM-1 is critical for neutrophils to traverse across the BBB. It appears that the degree of BBB compromise correlates with serum ICAM levels after TBI.<sup>66</sup> Monocyte chemo-attractant protein-1 is important for the recruitment of monocytes into the brain. Using rodent TBI models, it has been reported that expression of monocyte chemo-attractant protein-1 is enhanced in the brain by 4-h post-injury.<sup>67</sup> However, its diagnostic value for the detection of human TBI has not been examined.

## SMALL MOLECULE BIOMARKERS

Metabolites of neurotransmitters, second messengers, ions, and glycolytic intermediates have a rich history as potential biomarkers for primary and secondary injury.<sup>68–71</sup> The first such marker to be tested was cAMP when Rudman and colleagues<sup>72</sup> measured the levels of cAMP in the CSF as a putative biomarker for the depth of coma. The concentration of cAMP in CSF was found to correlate with the grade of coma with a coefficient of  $-0.8$ .<sup>73</sup> Since that time, several “top-down” studies have been carried out to evaluate the levels of small molecules for their ability to diagnose TBI, or to correlate with its severity. For example, Clifton et al.<sup>74</sup> demonstrated that serum norepinephrine levels, although normal in TBI patients with a Glasgow coma score (GCS) of 14, dramatically increased in comatose patients. Interestingly, in polytrauma patients, norepinephrine was increased regardless of GCS. The concentration of metabolites of dopamine (homovanillic acid) and serotonin (5-hydroxyindolacetic acid) have also been reported to increase in the CSF of TBI patients,<sup>75,76</sup> with elevations in 5-hydroxyindolacetic acid being reliable indicators of trauma.

In addition to altered neurotransmitter metabolism, a number of studies have shown that TBI alters the levels of glucose, lactate, pyruvate, glycerol, and glutamate, both in the CSF and in microdialysates obtained from the injured brain. For example, lactate accumulation in the CSF after craniocerebral injury has been shown to correlate with injury severity.<sup>77</sup> Furthermore, clinical hypothermia reduces lactate levels,<sup>78</sup> suggesting that this small molecule biomarker may be able to serve as a surrogate marker of treatment effectiveness. N-acetylas-

partate (NAA), a derivative of aspartate, is present in high levels in the brain (NAA comprises up to 0.1% of the wet weight of the brain).<sup>79</sup> In the brain, NAA is abundantly present in neurons and their processes, and its decrease is considered a marker for injury. NAA can be detected as a single intense peak at 2.02 ppm by proton MR spectroscopy. For example, Yeo et al.<sup>80</sup> have measured the ratios of NAA/creatine and choline/creatine in the anterior and posterior halves of the supraventricular region of the brain in pediatric TBI patients. Their findings indicate that each ratio predicts aspects of neuropsychological deficits indicating the usefulness of measuring region/structure-specific biomarker measurements.

### LIPID METABOLITES AS BIOMARKERS

The brain has high levels of fatty acids, yet relatively low levels of antioxidant enzymes, making it susceptible to oxidative damage. Lipid peroxidation, which leads to changes in the biophysical properties of cell membranes and the functions of membrane-bound proteins, is one of the principal consequences of oxidative damage after TBI. As such, lipid peroxidation products have drawn attention as putative biomarkers of injury.

#### F2-isoprostane

The F2-isoprostanes represent a group of prostaglandin-like compounds produced from arachidonic acid as a result of peroxidation. Elevated F2-isoprostane levels have been reported in various human diseases, including cardiovascular diseases such as ischemia and atherosclerosis, lung diseases such as asthma and chronic obstructive pulmonary disease, and neurological diseases such as Alzheimer's disease and multiple sclerosis.<sup>81</sup> Multiple studies have shown that brain injuries increase the levels of F2-isoprostane both in the CSF and the serum of TBI patients.<sup>82–84</sup> For example, Varma et al.<sup>85</sup> demonstrated that in children who had severe TBI, the level of F2-isoprostane was elevated by 6-fold compared with that observed in uninjured controls. This marker was found to correlate well with the appearance of NSE, further supporting its use as an indicator of brain damage. In adults, a gender bias was found in that males had approximately twice the levels of CSF F2-isoprostane than females, and only males had decreased levels of this lipid metabolite in response to hypothermia.<sup>86</sup> This disparity in lipid peroxidation has been proposed to be a potential reason for the greater neuroprotection seen in females than in males. Although F2-isoprostane levels have been called the "gold standard" of lipid peroxidation, determination of its levels requires specialized equipment, thereby limiting its clinical usefulness.

#### 4-Hydroxynonenal

The 4-hydroxynonenal (4-HNE), an endogenous end product of lipid peroxidation, is another well accepted

lipid peroxidation marker. Although being an amphiphilic compound, 4-HNE is more concentrated in membranes where it reacts with membrane-bound proteins and phospholipids, compromising their functions.<sup>87</sup> Elevated 4-HNE level has been observed in both cortical and hippocampal tissues early after TBI in animal models,<sup>88,89</sup> and reduced 4-HNE level has been found in parallel with neuroprotective effects in animals treated with antioxidants.<sup>90</sup> Although in TBI-related studies, 4-HNE levels were evaluated in brain tissue, studies examining this compound in CSF and plasma have been carried out for other neurologic disorders such as Alzheimer's disease, amyotrophic lateral sclerosis, and stroke.<sup>91–93</sup>

### BIOMARKERS FOR MILD TBI

Although approximately 80% of all TBI cases are believed to be mild, proper diagnosis of these patients remains a challenge. Current diagnosis of mild traumatic brain injuries (MTBIs) resulting from a blast is largely based on the Military Acute Concussion Evaluation (MACE) (see [www.DVBICE.org](http://www.DVBICE.org)). In the presence of the loss of consciousness or post-traumatic amnesia, a MTBI can be readily diagnosed. However, it is challenging to properly diagnose these patients who have an altered consciousness, but no loss of consciousness, post-traumatic amnesia, or without a positive head computed tomographic (CT) scan. The Military Acute Concussion Evaluation can provide positive diagnosis when administered <12 hours after injury. It can be erroneous when administered at a later time point. Soldiers engaged in Operation Enduring Freedom and Operation Iraqi Freedom are constantly exposed to blasts of different degrees (i.e, some close and some at a distance). It is often difficult for these soldiers to recall that they have been exposed to a blast if tested days or months after the initial event. Post-concussive symptoms including headaches, sleep disturbances, nausea, impaired attention, and memory problems are hallmarks of MTBI. Approximately 20% of patients with these symptoms remain unemployed at 1-year post-injury.<sup>94</sup> As the consequences of MTBI can be long-lasting, there is an urgent need to identify biomarkers that can be used to assist in the diagnosis of this condition.

A number of studies have examined the serum levels of S100B as a diagnostic marker of MTBI. Similar to that observed at higher injury magnitudes, the serum concentration of this marker is transiently increased after MTBI.<sup>14</sup> For example, a clinical study evaluating serum S100B levels in patients with mild head injuries found that patients with intracranial injuries (as determined by using head CT scans) had significant elevations in S100B levels compared with patients who did not have such injuries.<sup>95</sup> Consistent with this evaluation, a recent re-

evaluation of six prospective clinical trials involving more than 2000 MTBI patients indicated that S100B has a sensitivity of 98.2% in identifying the presence of brain injury.<sup>96</sup> Although the sensitivity of this biomarker can be high, it has low specificity.<sup>97,98</sup> For example, in a prospective cohort study, Nygren et al.<sup>99</sup> demonstrates that while S100B levels were significantly increased in the serum of MTBI patients compared with uninjured controls, these levels could not be used to differentiate between TBI and orthopedic injury groups.

In contrast to the high sensitivity but low specificity seen with S100B after MTBI, NSE has the opposite problem as a biomarker of MTBI. Ingebrigtsen and Romner<sup>100</sup> evaluated the diagnostic potential of NSE after MTBI and found that although this marker had high specificity, it was not very sensitive. This is likely due to the relatively small increase in serum NSE levels found as a result of MTBI.<sup>101</sup> One of the few other biomarkers that have been clinically tested in MTBI patients is tau. To test the diagnostic accuracy of C-tau in identifying MTBI patients with intracranial injuries, Kavalci et al.<sup>102</sup> examined serum tau levels from 88 MTBI patients and correlated these changes with the presence or absence of intracranial lesions on their head CT. No significant difference in tau protein levels was detected between the two groups. In addition, no relationship has been found between initial serum C-tau levels and 3-month outcome.<sup>103</sup>

### BIOMARKERS FOR DIFFERENTIALLY DIAGNOSING MILD TBI VS PTSD

Post-traumatic stress disorder (PTSD) is an anxiety disorder characterized by a cluster of symptoms summarized as avoidance, intrusive thoughts/re-experiencing, and hyperarousal. These symptoms are evident after a person is reminded of a traumatic event by a personal “trigger” and this causes extreme distress. PTSD and MTBI patients share a number of similarities in cognitive dysfunction including deficits in attention and difficulties with episodic and working memory.<sup>104,105</sup> These similarities in symptomatology make an accurate diagnosis between MTBI and PTSD difficult. A few MRI studies have reported that patients with PTSD have smaller hippocampal volumes, which could be used for the diagnosis of PTSD.<sup>106,107</sup> However, it has been argued that smaller hippocampal volume may reflect a pre-existing vulnerability rather than resulting from the traumatic event.<sup>108</sup> It is believed that PTSD symptoms result from a hypoactive medial prefrontal cortex and a hyperactive amygdala, which is supported by experimental findings in animals and humans.<sup>109</sup> However, comparative studies are needed to determine if this imaging marker is uniquely associated with PTSD, but not with MTBI or chronic stress.

A number of neurochemicals have been identified as potential biomarkers for PTSD.<sup>110,111</sup> Many of these changes result from altered function of the hypothalamic-pituitary-adrenal (HPA) axis. For example, it has been reported that high levels of plasma norepinephrine are related to increased risk of PTSD. When faced with a stressful situation, increases in catecholamine levels elevate heart rate, blood pressure, and blood glucose. Shortly after being exposed to a trauma, individuals who develop PTSD have higher heart rates than those who do not.<sup>112,113</sup> Measurement of neuropeptide Y indicates that its serum levels are higher in veterans without PTSD as compared with veterans with PTSD. These results suggest that that neuropeptide Y levels may represent a biomarker for resilience to PTSD (a process of adapting well when faced with trauma, serious threats, and so on) rather than a diagnostic marker.<sup>114</sup> Finally, dehydroepiandrosterone and dehydroepiandrosterone/cortisol ratios have been linked to PTSD. However, it is unclear if this ratio is a marker for symptom severity or if it is related to symptom improvement.<sup>115</sup> Because TBI can alter the function of the hypothalamic-pituitary-adrenal axis, it remains to be determined if the degree and/or a combination of these changes can be used to differentiate people with mild TBI from those with PTSD.

### BIOMARKERS FOR PENETRATING TBI

TBI resulting from high-velocity projectiles (e.g., bullets, shrapnel) is on the rise both among civilians and the military. A recent retrospective study at the National Naval and Walter Reed Army Medical Centers has indicated 56% of severe military TBI casualties were caused by penetrating injuries resulting from either embedded fragments driven into the brain by an explosive blast event (72%) or from bullets from assault weapons (23%).<sup>116</sup> Yao et al.<sup>117</sup> have used a high throughput immunoblotting technique to search for biomarkers for penetrating ballistic-like brain injuries. Using a total of 998 well-characterized monoclonal antibodies, these investigators have identified 18 proteins whose tissue levels are altered in a penetrating ballistic-like brain injury model. The most interesting of these is a precursor protein of endothelial monocyte-activating polypeptide II. The levels of this protein were increased after penetrating brain injury and decreased after middle cerebral artery occlusion, a stroke model. This indicates that different types of brain injury can potentially be diagnosed with appropriate biomarkers or a biomarker signature.

### BIOMARKERS FOR SECONDARY PATHOLOGIES

Secondary injury encompasses a number of pathological processes that can continue to evolve during a period of

days, weeks, or even months after the primary insult. These secondary pathological processes include breakdown of the BBB, neuroinflammation, altered ionic homeostasis, cerebral edema (fluid-mediated swelling), increased ICP, impaired metabolism, hypotension, ischemia, and cell death. Because these pathologies evolve in time after the primary injury, they may provide a window of opportunity for therapeutic intervention. Although a number of researchers have focused on identifying biomarkers to diagnose injury severity and survivability, only a few groups have focused on identifying prognostic biomarkers for these secondary pathologies. The discovery of biomarkers for these secondary injuries would greatly aid in the design of management strategies for individual patients.

### Intracranial pressure

One of the primary considerations of clinicians caring for TBI patients is controlling elevated ICP. Sustained elevated ICP measurements  $>20$  mmHg have been linked to increased morbidity and mortality in TBI patients.<sup>118</sup> Elevated ICP can lead to further brain tissue damage via cerebral hypoperfusion, secondary ischemia, and brain tissue herniation, and may eventually result in death.<sup>119</sup> In a prospective study comparing the levels of C-tau between severe TBI and neurologic and non-neurologic controls, Zemlan et al.<sup>37</sup> reported that serum C-tau levels could be used to differentiate those TBI patients with an ICP  $<30$  mmHg from those with an ICP  $>30$  mmHg. The serum levels of GFAP and S100B have also been proposed as markers capable of differentiating between TBI patients based on their ICP status. Pelinka and colleagues,<sup>120</sup> evaluating serum GFAP and S100B levels in 92 TBI patients, found that these proteins were significantly elevated in patients with ICP  $>25$  mmHg. Using a “bottom-up” approach, it has been found that the serum concentration of retinol binding protein 4, measured between 24- and 36-h post injury, is predictive of subsequent increase of intracranial pressure (86% sensitivity; 88% specificity).<sup>8</sup> However, the transient nature and relatively small magnitude of this change likely limits its clinical usefulness. Ceruloplasmin, also called iron(II):oxygen oxidoreductase, is the major copper carrier protein in the blood and plays an important role in copper and iron metabolism.<sup>121</sup> Consistent with its role as an acute phase reactant, it has been found that ceruloplasmin levels are significantly increased in severe TBI patients (GCS  $<8$ ) by 3 days post-injury. However, prior to this delayed increase, ceruloplasmin levels during the first 24 h after injury were found to be significantly reduced in patients who subsequently developed high ICP (i.e., ICP  $\geq 25$  mmHg).<sup>122</sup> This decrease was found to have prognostic accuracy in identifying these patients compared with TBI patients whose ICP  $\leq 20$  mmHg throughout the study period (cut-off, 140  $\mu\text{g/mL}$ ; 87% sensitivity; 73% specificity). Likewise, low total serum

copper ( $<1.32$   $\mu\text{g/mL}$ ) within the first 24-h of injury was also found to be predictive of high ICP (86% sensitivity; 73% specificity). The combination of serum ceruloplasmin and serum copper (cut-offs, 140 $\mu\text{g/mL}$  and 1.50  $\mu\text{g/mL}$ , respectively) increased the specificity to 100% while maintaining a sensitivity of 67%.<sup>122</sup>

### Blood brain barrier compromise

Loss of BBB integrity allows for the unrestricted entry of blood-borne materials into the brain and has been linked to exacerbated inflammation, increased ICP, altered homeostasis, and other pathological consequences of TBI.<sup>123</sup> Because S100B does not cross the intact BBB, its levels in the serum (or a serum/CSF ratio) have been proposed to be an indicator of barrier integrity. To investigate this possibility, Kirchhoff et al.<sup>124</sup> evaluated S100B in the serum of severe TBI patients to determine if its levels correlated with the presence of albumin in the CSF. This study revealed that there was a positive correlation between serum S100B levels and the degree of albumin leakage at various time points after injury.

### Secondary ischemia

Secondary or delayed ischemia is another prominent pathology that influences outcome after TBI.<sup>125</sup> Delayed ischemia can result from multiple processes, including intracranial hypertension resulting from elevated ICP, compromised cerebral pressure autoregulation, vasospasm, and/or hemorrhaging from damaged vasculature. These processes result in reduced blood flow to the injured tissue that is insufficient to meet the tissue's metabolic needs. Cardiolipin is a phospholipid that is highly enriched in the inner membrane of mitochondria, and under periods of high oxidative stress, it is oxidized and is believed to contribute to mitochondrial dysfunction and cell death. Although limited research has been done on the consequences of TBI on cardiolipin levels, there is increasing evidence that the presence of anticardiolipin antibodies may be an independent risk factor for stroke.<sup>126,127</sup> If TBI results in the production of anticardiolipin (or other antiphospholipid) antibodies, these may serve as biomarkers for identifying those patients at risk for developing post-TBI ischemic events.

### Post-traumatic epilepsy

TBI is a frequent cause of acquired epilepsy.<sup>128</sup> Seizure activity after TBI has been categorized as immediate (i.e., within 24 h); early (i.e., within 1 week) and late (i.e.,  $>1$ -week post-injury).<sup>129</sup> Post-traumatic epilepsy occurs in  $\leq 50\%$  of severe TBI patients, with the incidence being highest after penetrating brain injury.<sup>130–132</sup> The Brain Trauma Foundation guidelines recommend anticonvulsants to decrease the incidence of early post-traumatic seizures, but because of adverse effects and no demonstrated improvement on outcomes, anticonvulsants are not recommended for preventing late post-trau-



matic seizures.<sup>133</sup> Electroencephalography, CT, and MRI (hemosiderin deposits and gliotic scarring<sup>134</sup>) may be used to try to predict seizures, but these methods are inadequate. Currently we do not have a method of preventing the development of late post-traumatic seizures. The apolipoprotein E epsilon 4 allele has been associated with post-traumatic seizures.<sup>135</sup> Reductions of hippocampal NAA, a marker for viable neurons, have been shown in temporal lobe epilepsy and mesial temporal lobe sclerosis.<sup>136</sup> Collapsin response-mediated protein-2, and proteins involved in axonal outgrowth, path finding, and maintaining neuronal polarity have been demonstrated to be down-regulated in hippocampal tissue of patients with mesial temporal lobe epilepsy.<sup>137</sup> All of these have also been associated with other neurodegenerative disorders, such as Alzheimer's disease. Identifying biomarkers that are predictive of post-traumatic seizure development and response to medication would greatly aid in the treatment of TBI.

### BIOMARKERS FOR PREDICTING OUTCOME

Some of the most disruptive symptoms experienced by TBI patients who survive their injuries are persistent memory and behavioral problems. In addition to overt memory disturbances, brain injury is associated with increased rates of depression, sleep disturbances, mood changes, impulsiveness, and an inability to extinguish fearful memories. These memory and behavioral problems can interfere with post-TBI recovery and rehabilitation. The identification of biomarkers that can be used to predict these clinical outcomes would be invaluable to identify those patients likely to benefit from a rehabilitation regimen, to inform and counsel family members regarding the prognosis, and to encourage the patient (or caretaker of the patient) to be vigilant for reporting future symptoms. This is especially important for mild TBI patients who are discharged quickly because their injuries are typically not life-threatening. Outcome prediction after MTBI is dependent on proper diagnosis of the condition, especially the patients without an overt brain injury. However, recent studies are beginning to show that a significant number of these patients develop psychological and behavioral problems that can impact their family and work life.

Although several studies have used the GOS to assess the long-term association of a biomarker change with outcome, the dichotomous nature of reporting (e.g., good vs poor) makes it difficult to assess what aspect of outcome is being correlated. Nevertheless, studies are beginning to show that biomarkers (either alone or in combination) can be used to predict favorable or poor outcome. For example, high initial serum levels of S100B, NSE, GFAP, and tau have all been linked to poor outcome 6 months later.<sup>15,37,138,139</sup> Bandyopadhyay et

al.<sup>140</sup> have reported that NSE at a cut-off value of 21.2 ng/mL has 86% sensitivity and 74% specificity for poor outcome in pediatric patients. However, it is yet to be determined if these markers can be used to identify those patients with specific pathologies (e.g., memory disturbances) or if it can be used as surrogate markers to evaluate the effectiveness of a rehabilitation program.

### BIOMARKERS FOR POLYTRAUMA

In many cases, human brain injury occurs as a part of polytrauma. The Veterans Health Administration defines polytrauma as "injury to the brain in addition to other body parts or systems resulting in physical, cognitive, psychological, or psychosocial impairments and functional disability."<sup>141</sup> People involved in vehicular accidents, soldiers exposed to improvised explosive devices, and (to a lesser extent) athletes involved in contact sports often suffer from polytrauma. Multiple organ failure (MOF) and acute respiratory distress syndrome are the leading causes of death after polytrauma. "One-hit" and "two-hit" models have been proposed for MOF after severe and mild to moderate polytrauma.<sup>142,143</sup> A severe traumatic injury is believed to elicit a systemic inflammatory response that can give rise to MOF, the one-hit model. After a less severe trauma, organs remain at a vulnerable state (possibly due to a milder inflammatory response), and a second insult can precipitate MOF, the two-hit model. Surgical procedures that are often performed on polytrauma patients can represent a second hit and can give rise to MOF. Similar to the two-hit model for MOF, the brain is highly vulnerable to a second insult such as ischemia, hypoperfusion, and repeat concussive injury. Biomarkers whose levels are indicative of the duration of vulnerability would be useful for managing polytrauma patients. This is also important for determining how long a soldier or an athlete should remain inactive to avoid a second hit. The serum levels of inflammatory biomarkers (e.g., IL-1, IL-6, TNF $\alpha$ , sTNF-R p55, sTNF-R p75) have been measured in polytrauma patients,<sup>144-146</sup> with the levels of IL-6 shown to correlate with injury severity score, acute respiratory distress syndrome, and MOF. Furthermore, the initial serum levels (days 0-1) of sTNF-R p55 and sTNF-R p75 have good predictive value (0.75 and 0.72, respectively) for the development of late MOF.<sup>146</sup>

Diagnosis of TBI in polytrauma patients using current biomarkers can be challenging. As previously described, several of the putative biomarkers of injury suffer from a lack of specificity in that they can be released into the serum by conditions not due to brain injury. For example, S100B has been identified as a highly sensitive marker of brain damage. However, the levels of this protein are also increased in conditions such as ischemia to the liver, kidney, and gut,<sup>16</sup> after extracranial trauma<sup>14</sup>

**Table 1.** Summary of TBI-Related Biomarkers and their Utility

Biomarker	Diagnostic Value (Cut-off: Sensitivity/Specificity)	Sample Source	Comments	References
$\alpha$ -II-Spectrin BDPs	↑ In TBI; correlate with lesion size, injury severity	CSF, brain tissue, serum	Brain enriched Indicates mode of death	35, 34, 153, 154
$\alpha$ -Synuclein	↑ In TBI	Brain tissue	Needs validation in humans	156, 157
C-tau	↑ C-tau correlated with outcome; ↑ C-tau correlated with ↑ ICP 1600 ng/mL: GOS (92%/94%); 1500 ng/mL: ICP (78%/79%)	CSF, serum	Specific to CNS Poor diagnostic value for mild TBI	37
3',5'-cAM/2',3'-cAMP	CSF level correlated with coma grade	CSF	Second messenger	72
Ceruloplasmin/Cu	↓ In TBI patients with elevated ICP	Serum	Copper can be rapidly assessed	122
CK-BB	BBB disruption necessary for serum penetration; no correlation with contusion volume	CSF, serum	Short half-life, rapid elimination	27, 155
CRP & SAA	General injury markers	Serum	Rapidly induced. May indicate polytrauma	8
CRMP-2	↓ In mesial temporal lobe of epilepsy patients, associated with Alzheimer's	Brain tissue	May be a marker for post-traumatic epilepsy	137
FABP	Identifying mild TBI (↑)	Serum/plasma, brain tissue	High accuracy for mild TBI	32
F2-Isoprostane	↑ In multiple diseases	CSF, serum	Marker of oxidative damage	81-84
4-HNE	↑ In TBI brain tissue	Brain tissue	Marker of lipid peroxidation	88, 89
5-HIAA	↑ In CSF; indicator of trauma	CSF	Marker of altered neurotransmission	75, 76
GFAP	Predictive of ↑ ICP, ↓ MAP, ↓ CPP, mortality & GOS 1.5 ng/mL: Death (85%/52%) GOS (80%/59%)	CSF, serum	Specific to CNS May not be sensitive enough for mild TBI	15
HVA	↑ In CSF	CSF	Marker of altered neurotransmission	75
ICAM	↑ Correlates with ↑ BBB compromise	CSF, serum	Marker for neurovascular dysfunction	66
IL-1 $\beta$	↑ Correlates with worse outcome	CSF	Development of astroglial scars	46, 47, 48
IL-6	↑ In CSF in severe TBI Conflicting results. ↑ IL-6 correlates with improved outcome; ↑ peak IL-6 in nonsurvivors	CSF, serum, intracranial dialysate	Marker for multi-organ failure	58, 59
IL-8	↑ In CSF in severe TBI	CSF, serum	Marker of inflammation	44
IL-10	↑ CSF IL-10 associated with ↓ TNF- $\alpha$ & mortality	CSF, serum	Anti-inflammatory marker	62, 63
IL-12p70	↑ In CSF in severe TBI	CSF, serum	Marker of inflammation	44
Lactate	Correlates with injury severity	CSF	Marker for altered brain metabolism	77
Magnesium	↓ Mg over initial 5 days related to injury severity	Serum		152
MBP	↑ Correlates with worse outcome in children	CSF, serum	Marker for white matter injury	30
MCP-1	↑ In rodent brain 4-h post-injury	Brain tissue	Recruitment of monocytes into brain; not tested in humans	67
MIP-1 $\alpha$	↑ In CSF in severe TBI	CSF	Marker for inflammatory cell invasion	44
NAA	↓ Injury marker; ratio NAA/Cre & Cho/Cre predictive of deficits		Marker for detecting regional injury by magnetic resonance spectroscopy	79, 80

(Table continues)

Table 1. Continued

Biomarker	Diagnostic Value (Cut-off: Sensitivity/Specificity)	Sample Source	Comments	References
Phospho-neurofilament	↑ In TBI	Serum	Needs to be validated in humans	38
NSE	Neuropsychological outcome intracranial lesions 21.7 ng/mL: mortality (85%/80%) outcome (55%/78%) 15.3 ng/mL: intracranial lesions (77%/52%)	CSF, serum, brain tissue	Putative marker for small cell lung cancer, neuroendocrine bladder tumors, stroke, and neuroblastoma	28, 29, 138
NE	↑ In comatose patients; ↑ polytrauma	Serum	Marker of altered neurotransmission and stress	74
S100B	Mortality/morbidity; Mild TBI ↑ in patients with intracranial injury 1.13 ng/mL (100%/41%) 1.13 ng/mL (88%/43%) 0.10 μ/L (95%/31%)	CSF, serum	Not selectively specific to TBI; can be used as a marker of BBB compromise	15, 95, 139
TGF-β	↑ After TBI, remains elevated ~ 3 weeks	CSF	Cell growth and differentiation, angiogenesis, immune function, apoptosis	65
TNF-α	↑ In severe TBI; ↑ did not correlate with mortality	CSF, serum	Marker of inflammation	53
UCH-L1	↑ In CSF, correlated with mortality, complications and 6 month outcome	CSF	Brain enriched marker	39

↓ = decreased/downregulated; ↑ = increased/upregulated; BBB = blood brain barrier; BDPs = proteolytic breakdown products; B-FABP = brain type-fatty acid binding proteins; C-tau = cleaved tau; Cho/Cre = choline/creatine; CK-BB = creatine kinase-BB; CPP = cerebral perfusion pressure; CRMP-2 = collapsin response mediated protein-2; CRP = C-reactive protein; FABP = fatty acid binding proteins; GCS = Glasgow coma score; GDNF = glial derived neurotrophic factor; GFAP = glial fibrillary acidic protein; GOS = Glasgow outcome scale; 5-HIAA = 5-hydroxy indol acetic acid; HNE = 4-Hydroxynonenal; HVA = homovanillic acid; ICAM = intercellular adhesion molecule 1; ICP = intracranial pressure; IL = interleukin; MAP = mean arterial pressure; MBP = myelin basic protein; MCP-1 = monocyte chemo-attractant protein-1; MIP = macrophage inflammatory protein; NAA = N-acetylaspartate; NE = norepinephrine; NSE = neuron-specific enolase; phospho-NFH = phosphorylated neurofilament H; TBI = traumatic brain injury; TGF-β = transforming growth factor-beta; TNF-α = tumor necrosis factor-alpha; UCH-L1 = ubiquitin C-terminal hydrolase.

and burns.<sup>17</sup> Based on these reports, S100B may not have strong diagnostic value in the context of multi-trauma. da Rocha et al.<sup>147</sup> evaluated serum levels of S100B in TBI patients either with or without trauma to other organs. They found that S100B remained a strong predictor of mortality after TBI, irrespective of multi-trauma.

Although it appears that S100B may have prognostic value in identifying those patients for whom survival is doubtful, it remains a challenge to identify mild brain trauma in the context of other bodily injuries. For example, GFAP, although highly specific to brain injury and therefore less likely to be influenced by other bodily injuries,<sup>24</sup> has not been proven to be sensitive enough to detect mild TBI. Serum levels of BDPs of brain-specific proteins may be useful in diagnosing TBI in polytrauma patients. A single biomarker may not be able to diagnose polytrauma nor indicate which organs are injured. However, changes in multiple proteins may provide the sensitivity and specificity required to diagnose different types of polytrauma. For example, a combination of biomarkers from multiple disease pathways (troponin I [myocardial cell damage], N-

terminal prohormone brain natriuretic peptide [left ventricular dysfunction], C-reactive protein [inflammation], and cystatin C [renal failure]) improved risk stratification for death from cardiovascular causes among elderly men.<sup>148</sup> Therefore, continued research is required to identify specific and sensitive biological markers of brain damage when used in combination with markers of other organ damage (e.g., troponin and creatine phosphokinase isoenzyme MB [CPK-MB] for heart; aspartate, and alanine transaminases for liver; lipases for pancreas; blood urea nitrogen and creatinine for kidney), which can assist the physician in identifying the organs damaged as a result of polytrauma.

## FUTURE DIRECTIONS

Although several biomarkers of brain injury have been identified, as summarized in Table 1, continued research is required. These markers can take on many forms, with proteins, peptides, lipids, and small molecules having been shown to have diagnostic and prognostic value. However, several of these markers suffer from a lack of

specificity, often being induced or released into the serum in response to other diseases or bodily injuries. This lack of specificity has hampered the effort to identify markers of mild TBI, especially in the context of poly-trauma. A single biomarker may not have desired sensitivity and specificity for diagnosis nor for predicting outcome. Biomarkers, in combination with other clinical data, such as GCS and head CT scan, would maximize the diagnostic accuracy. Su and Liu<sup>149</sup> have provided a theoretical framework for the optimal, linear combination of biomarkers that maximizes the area under the receiver operator characteristic curve. Modifications to this linear combination approach (e.g., in which sensitivity is maximized instead of the area over a range of specificity) have also been described.<sup>150</sup> Another method that could be used to overcome this problem is by applying boosting algorithms.<sup>151</sup> Boosting occurs in stages, during each of which a weak predictor (e.g., a biomarker that has a low specificity) is trained with the data. The output of the weak learner is then weighted (proportional to the accuracy of the weak predictor) and added to the overall function. The data is then re-weighted so that patients who are misidentified are “boosted” in importance. New biomarkers are trained and added to attempt to correct the misclassifications. One advantage of this technique is that the biomarkers do not need to be biological in nature, but can be the result of neurologic examinations, imaging, physiological measurements, or other diagnostic tests. With continued research it may be possible to find biomarkers that not only predict secondary pathologies, such as elevated intracranial pressure, but also those that can identify patients at risk for adverse long-term outcomes, such as memory loss, depression, and post-traumatic stress disorder. Because these pathologies depend on different brain structures and cell types, it is likely that a single biomarker will not be able to accurately predict which patients are at risk for their occurrence. Future studies will be required to not only identify these biomarkers, but to develop a clinically useful test in which a panel of TBI-associated biomarkers (a biomarker signature) are evaluated and used to direct treatment.

**Acknowledgments:** The authors would like to thank Drs. Jordan Clark and Dan Johnson for their critical reading of the manuscript. We also thank a grant sponsored by the National Institutes of Health, The Mission Connect/TIRR Foundation (Grant No.s: NS35457, NS049160, MH072933, CDMRP: W81XWH-08-2-0134, and R38OT10585).

## REFERENCES

- Hoge CW, Castro CA, Messer SC, McGurk D, Cotting DI, Koffman RL. Combat duty in Iraq and Afghanistan, mental health problems, and barriers to care. *N Engl J Med* 2004;351:13–22.
- Okie S. Traumatic brain injury in the war zone. *N Engl J Med* 2005;352:2043–2047.
- Holcomb JB, Stansbury LG, Champion HR, Wade C, Bellamy RF. Understanding combat casualty care statistics. *J Trauma* 2006;60:397–401.
- Kochanek PM, Clark RS, Ruppel RA, et al. Biochemical, cellular, and molecular mechanisms in the evolution of secondary damage after severe traumatic brain injury in infants and children: Lessons learned from the bedside. *Pediatr Crit Care Med* 2000;1:4–19.
- Liu MC, Akle V, Zheng W, et al. Comparing calpain- and caspase-3-mediated degradation patterns in traumatic brain injury by differential proteome analysis. *Biochem J* 2006;394:715–725.
- Colicos MA, Dixon CE, Dash PK. Delayed, selective neuronal death following experimental cortical impact injury in rats: possible role in memory deficits. *Brain Res* 1996;739:111–119.
- Colicos MA, Dash PK. Apoptotic morphology of dentate gyrus granule cells following experimental cortical impact injury in rats: possible role in spatial memory deficits. *Brain Res* 1996;739:120–131.
- Hergenroeder G, Redell JB, Moore AN, et al. Identification of serum biomarkers in brain-injured adults: potential for predicting elevated intracranial pressure. *J Neurotrauma* 2008;25:79–93.
- States DJ, Omenn GS, Blackwell TW, et al. Challenges in deriving high-confidence protein identifications from data gathered by a HUP0 plasma proteome collaborative study. *Nat Biotechnol* 2006;24:333–338.
- Gao J, Garulacan LA, Storm SM, et al. Biomarker discovery in biological fluids. *Methods* 2005;35:291–302.
- Petricoin EE, Paweletz CP, Liotta LA. Clinical applications of proteomics: proteomic pattern diagnostics. *J Mammary Gland Biol Neoplasia* 2002;7:433–440.
- Haskins WE, Kobeissy FH, Wolper RA, et al. 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.
- Uden J, Astrand R, Waterloo K, et al. Clinical significance of serum S100B levels in neurointensive care. *Neurocrit Care* 2007;6:94–99.
- Savola O, Pyhtinen J, Leino TK, Siitonen S, Niemela O, Hillbom M. Effects of head and extracranial injuries on serum protein S100B levels in trauma patients. *J Trauma* 2004;56:1229–1234.
- Vos PE, Lamers KJ, Hendriks JC, et al. Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. *Neurology* 2004;62:1303–1310.
- 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* 2004;21:72–76.
- Anderson RE, Hansson LO, Nilsson O, Djalil-Merzoug R, Settergren G. High serum S100B levels for trauma patients without head injuries. *Neurosurgery* 2001;48:1255–1258.
- Torabian S, Kashani-Sabet M. Biomarkers for melanoma. *Curr Opin Oncol* 2005;17:167–171.
- Piazza O, Storti MP, Cotena S, et al. S100B is not a reliable prognostic index in pediatric TBI. *Pediatr Neurosurg* 2007;43:258–264.
- 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.
- Berger RP, Beers SR, Richichi R, Wiesman D, Adelson PD. Serum biomarker concentrations and outcome after pediatric traumatic brain injury. *J Neurotrauma* 2007;24:1793–1801.
- Missler U, Wiesmann M, Wittmann G, Magerkurth O, Hagenström H. Measurement of glial fibrillary acidic protein in human blood: analytical method and preliminary clinical results. *Clin Chem* 1999;45:138–141.
- Nylen K, Ost M, Csajbok LZ, et al. Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome. *J Neurol Sci* 2006;240:85–91.
- Pelinka LE, Kroepfl A, Schmidhammer R, et al. Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma. *J Trauma* 2004;57:1006–1012.
- Herrmann M, Jost S, Kutz S, et al. Temporal profile of release of neurobiochemical markers of brain damage after traumatic brain injury is associated with intracranial pathology as dem-

- onstrated in cranial computerized tomography. *J Neurotrauma* 2000;17:113–122.
26. Ross SA, Cunningham RT, Johnston CF, Rowlands BJ. Neuron-specific enolase as an aid to outcome prediction in head injury. *Br J Neurosurg* 1996;10:471–476.
  27. 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.
  28. Fridriksson T, Kini N, Walsh-Kelly C, Hennes H. Serum neuron-specific enolase as a predictor of intracranial lesions in children with head trauma: a pilot study. *Acad Emerg Med* 2000;7:816–820.
  29. Herrmann M, Curio N, Jost S, et al. Release of biochemical markers of damage to neuronal and glial brain tissue is associated with short and long term neuropsychological outcome after traumatic brain injury. *J Neurol Neurosurg Psychiatry* 2001;70:95–100.
  30. 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.
  31. Glatz JF, van der Vusse GJ. Cellular fatty acid-binding proteins: their function and physiological significance. *Prog Lipid Res* 1996;35:243–282.
  32. Pelsers MM, Hanhoff T, Van der Voort D, et al. Brain- and heart-type fatty acid-binding proteins in the brain: tissue distribution and clinical utility. *Clin Chem* 2004;50:1568–1575.
  33. Gabbita SP, Scheff SW, Menard RM, Roberts K, Fugaccia I, Zemlan FP. Cleaved-tau: a biomarker of neuronal damage after traumatic brain injury. *J Neurotrauma* 2005;22:83–94.
  34. Wang KK, Ottens AK, Liu MC, et al. Proteomic identification of biomarkers of traumatic brain injury. *Expert Rev Proteomics* 2005;2:603–614.
  35. Ringger NC, O'Steen BE, Brabham JG, et al. A novel marker for traumatic brain injury: CSF alphaII-spectrin breakdown product levels. *J Neurotrauma* 2004;21:1443–1456.
  36. Cardali S, Maugeri R. Detection of alphaII-spectrin and breakdown products in humans after severe traumatic brain injury. *J Neurosurg Sci* 2006;50:25–31.
  37. Zemlan FP, Jauch EC, Mulchahey JJ, et al. C-tau biomarker of neuronal damage in severe brain injured patients: association with elevated intracranial pressure and clinical outcome. *Brain Res* 2002;947:131–139.
  38. Anderson KJ, Scheff SW, Miller KM, et al. 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.
  39. Papa L, Akinyi L, Liu MC, et al. Ubiquitin C-terminal hydrolase is a novel biomarker in humans for severe traumatic brain injury. *Crit Care Med* 2009 Sep 1 [Epub ahead of print].
  40. Morganti-Kossmann MC, Rancan M, Otto VI, Stahel PF, Kossmann T. Role of cerebral inflammation after traumatic brain injury: a revisited concept. *Shock* 2001;16:165–177.
  41. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. *Nat Rev Neurosci* 2001;2:734–744.
  42. Kadhim HJ, Duchateau J, Sebire G. Cytokines and brain injury: invited review. *J Intensive Care Med* 2008;23:236–249.
  43. Whitney NP, Eidem TM, Peng H, Huang Y, Zheng JC. Inflammation mediates varying effects in neurogenesis: relevance to the pathogenesis of brain injury and neurodegenerative disorders. *J Neurochem* 2009;108:1343–1359.
  44. Buttram SD, Wisniewski SR, Jackson EK, et al. Multiplex assessment of cytokine and chemokine levels in cerebrospinal fluid following severe pediatric traumatic brain injury: effects of moderate hypothermia. *J Neurotrauma* 2007;24:1707–1717.
  45. 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.
  46. Giulian D, Lachman LB. Interleukin-1 stimulation of astroglial proliferation after brain injury. *Science* 1985;228:497–499.
  47. Singhal A, Baker AJ, Hare GM, Reinders FX, Schlichter LC, Moulton RJ. Association between cerebrospinal fluid interleukin-6 concentrations and outcome after severe human traumatic brain injury. *J Neurotrauma* 2002;19:929–937.
  48. Chiaretti A, Genovese O, Aloe L, et al. Interleukin 1beta and interleukin 6 relationship with paediatric head trauma severity and outcome. *Childs Nerv Syst* 2005;21:185–193.
  49. Brenner T, Yamin A, Abramsky O, Gallily R. Stimulation of tumor necrosis factor-alpha production by mycoplasmas and inhibition by dexamethasone in cultured astrocytes. *Brain Res* 1993;608:273–279.
  50. Sawada M, Kondo N, Suzumura A, Marunouchi T. Production of tumor necrosis factor-alpha by microglia and astrocytes in culture. *Brain Res* 1989;491:394–397.
  51. Mier JW, Vachino G, van der Meer JW, et al. Induction of circulating tumor necrosis factor (TNF alpha) as the mechanism for the febrile response to interleukin-2 (IL-2) in cancer patients. *J Clin Immunol* 1988;8:426–436.
  52. Shohami E, Gallily R, Mechoulam R, Bass R, Ben-Hur T. Cytokine production in the brain following closed head injury: dexanabinol (HU-211) is a novel TNF-alpha inhibitor and an effective neuroprotectant. *J Neuroimmunol* 1997;72:169–177.
  53. Crespo AR, Da Rocha AB, Jotz GP, et al. Increased serum sFas and TNFalpha following isolated severe head injury in males. *Brain Inj* 2007;21:441–447.
  54. Goodman JC, Robertson CS, Grossman RG, Narayan RK. Elevation of tumor necrosis factor in head injury. *J Neuroimmunol* 1990;30:213–217.
  55. Ross SA, Halliday MI, Campbell GC, Byrnes DP, Rowlands BJ. The presence of tumour necrosis factor in CSF and plasma after severe head injury. *Br J Neurosurg* 1994;8:419–425.
  56. Kossmann T, Hans V, Imhof HG, Trentz O, Morganti-Kossmann MC. Interleukin-6 released in human cerebrospinal fluid following traumatic brain injury may trigger nerve growth factor production in astrocytes. *Brain Res* 1996;713:143–152.
  57. Minambres E, Cemborain A, Sanchez-Velasco P, et al. Correlation between transcranial interleukin-6 gradient and outcome in patients with acute brain injury. *Crit Care Med* 2003;31:933–938.
  58. Winter CD, Pringle AK, Clough GF, Church MK. Raised parenchymal interleukin-6 levels correlate with improved outcome after traumatic brain injury. *Brain* 2004;127:315–320.
  59. Chiaretti A, Antonelli A, Mastrangelo A, et al. Interleukin-6 and nerve growth factor upregulation correlates with improved outcome in children with severe traumatic brain injury. *J Neurotrauma* 2008;25:225–234.
  60. Aibiki M, Maekawa S, Nishiyama T, Seki K, Yokono S. Activated cytokine production in patients with accidental hypothermia. *Resuscitation* 1999;41:263–268.
  61. Morganti-Kossmann MC, Rancan M, Stahel PF, Kossmann T. Inflammatory response in acute traumatic brain injury: a double-edged sword. *Curr Opin Crit Care* 2002;8:101–105.
  62. Csuka E, Morganti-Kossmann MC, Lenzlinger PM, Joller H, Trentz O, Kossmann T. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function. *J Neuroimmunol* 1999;101:211–221.
  63. Bell MJ, Kochanek PM, Doughty LA, et al. Comparison of the interleukin-6 and interleukin-10 response in children after severe traumatic brain injury or septic shock. *Acta Neurochir Suppl (Wien)* 1997;70:96–97.
  64. Attisano L, Wrana JL, Lopez-Casillas F, Massague J. TGF-beta receptors and actions. *Biochim Biophys Acta* 1994;1222:71–80.
  65. Morganti-Kossmann MC, Hans VH, Lenzlinger PM, et al. TGF-beta is elevated in the CSF of patients with severe traumatic brain injuries and parallels blood-brain barrier function. *J Neurotrauma* 1999;16:617–628.
  66. Pleines UE, Stover JF, Kossmann T, Trentz O, Morganti-Kossmann MC. Soluble ICAM-1 in CSF coincides with the extent of cerebral damage in patients with severe traumatic brain injury. *J Neurotrauma* 1998;15:399–409.
  67. Bye N, Habgood MD, Callaway JK, et al. Transient neuroprotection by minocycline following traumatic brain injury is associated with attenuated microglial activation but no changes in cell apoptosis or neutrophil infiltration. *Exp Neurol* 2007;204:220–233.
  68. Palmer AM, Marion DW, Botscheller ML, Bowen DM, DeKosky

- ST. Increased transmitter amino acid concentration in human ventricular CSF after brain trauma. *Neuroreport* 1994;6:153–156.
69. Reinert M, Khaldi A, Zauner A, Dopperberg E, Choi S, Bullock R. High level of extracellular potassium and its correlates after severe head injury: relationship to high intracranial pressure. *J Neurosurg* 2000;93:800–807.
  70. 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.
  71. Goodman JC, Robertson CS. Microdialysis: is it ready for prime time? *Curr Opin Crit Care* 2009;15:110–117.
  72. Rudman D, Fleischer A, Kutner MH. Concentration of 3',5' cyclic adenosine monophosphate in ventricular cerebrospinal fluid of patients with prolonged coma after head trauma or intracranial hemorrhage. *N Engl J Med* 1976;295:635–638.
  73. Fleischer AS, Rudman DR, Fresh CB, Tindall GT. Concentration of 3',5' cyclic adenosine monophosphate in ventricular CSF of patients following severe head trauma. *J Neurosurg* 1977;47:517–524.
  74. Clifton GL, Ziegler MG, Grossman RG. Circulating catecholamines and sympathetic activity after head injury. *Neurosurgery* 1981;8:10–14.
  75. Porta M, Bareggi SR, Collice M, et al. Homovanillic acid and 5-hydroxyindole-acetic acid in the csf of patients after a severe head injury. II. Ventricular csf concentrations in acute brain post-traumatic syndromes. *Eur Neurol* 1975;13:545–554.
  76. Inagawa T, Ishikawa S, Uozumi T. Homovanillic acid and 5-hydroxyindoleacetic acid in the ventricular CSF of comatose patients with obstructive hydrocephalus. *J Neurosurg* 1980;52:635–641.
  77. Czernicki Z, Grochowski W. [Cerebrospinal fluid lactates following cranio-cerebral injuries]. *Neurol Neurochir Pol* 1976;10:651–653.
  78. Soukup J, Zauner A, Dopperberg EM, et al. Relationship between brain temperature, brain chemistry and oxygen delivery after severe human head injury: the effect of mild hypothermia. *Neurol Res* 2002;24:161–168.
  79. Narayanan S, De SN, Francis GS, et al. Axonal metabolic recovery in multiple sclerosis patients treated with interferon beta-1b. *J Neurol* 2001;248:979–986.
  80. Yeo RA, Phillips JP, Jung RE, Brown AJ, Campbell RC, Brooks WM. Magnetic resonance spectroscopy detects brain injury and predicts cognitive functioning in children with brain injuries. *J Neurotrauma* 2006;23:1427–1435.
  81. Montuschi P, Barnes PJ, Roberts LJ. Isoprostanes: markers and mediators of oxidative stress. *FASEB J* 2004;18:1791–1800.
  82. Bayir H, Adelson PD, Wisniewski SR, et al. Therapeutic hypothermia preserves antioxidant defenses after severe traumatic brain injury in infants and children. *Crit Care Med* 2009;37:689–695.
  83. Seifman MA, Adamides AA, Nguyen PN, et al. 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.
  84. Wagner AK, Bayir H, Ren D, Puccio A, Zafonte RD, Kochanek PM. Relationships between cerebrospinal fluid markers of excitotoxicity, ischemia, and oxidative damage after severe TBI: the impact of gender, age, and hypothermia. *J Neurotrauma* 2004;21:125–136.
  85. Varma S, Janesko KL, Wisniewski SR, et al. F2-isoprostane and neuron-specific enolase in cerebrospinal fluid after severe traumatic brain injury in infants and children. *J Neurotrauma* 2003;20:781–786.
  86. Bayir H, Marion DW, Puccio AM, et al. Marked gender effect on lipid peroxidation after severe traumatic brain injury in adult patients. *J Neurotrauma* 2004;21:1–8.
  87. Poli G, Schaur RJ, Siems WG, Leonarduzzi G. 4-hydroxynonenal: a membrane lipid oxidation product of medicinal interest. *Med Res Rev* 2008;28:569–631.
  88. Hall ED, Detloff MR, Johnson K, Kupina NC. Peroxynitrite-mediated protein nitration and lipid peroxidation in a mouse model of traumatic brain injury. *J Neurotrauma* 2004;21:9–20.
  89. Singh IN, Sullivan PG, Deng Y, Mbye LH, Hall ED. Time course of post-traumatic mitochondrial oxidative damage and dysfunction in a mouse model of focal traumatic brain injury: implications for neuroprotective therapy. *J Cereb Blood Flow Metab* 2006;26:1407–1418.
  90. Singh IN, Sullivan PG, Hall ED. Peroxynitrite-mediated oxidative damage to brain mitochondria: Protective effects of peroxynitrite scavengers. *J Neurosci Res* 2007;85:2216–2223.
  91. Markesbery WR, Carney JM. Oxidative alterations in Alzheimer's disease. *Brain Pathol* 1999;9:133–146.
  92. Smith RG, Henry YK, Mattson MP, Appel SH. Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. *Ann Neurol* 1998;44:696–699.
  93. Agre P. Molecular physiology of water transport: aquaporin nomenclature workshop. *Mammalian aquaporins. Biol Cell* 1997;89:255–257.
  94. Dikmen SS, Temkin NR, Machamer JE, Holubkov AL, Fraser RT, Winn HR. Employment following traumatic head injuries. *Arch Neurol* 1994;51:177–186.
  95. Muller K, Townend W, Biasca N, et al. S100B serum level predicts computed tomography findings after minor head injury. *J Trauma* 2007;62:1452–1456.
  96. Uden J, Romner B. A new objective method for CT triage after minor head injury—serum S100B. *Scand J Clin Lab Invest* 2009;69:13–17.
  97. Bazarian JJ, Beck C, Blyth B, von AN, Hasselblatt M. Impact of creatine kinase correction on the predictive value of S-100B after mild traumatic brain injury. *Restor Neurol Neurosci* 2006;24:163–172.
  98. Ingebrigtsen T, Romner B, Marup-Jensen S, et al. The clinical value of serum S-100 protein measurements in minor head injury: a Scandinavian multicentre study. *Brain Inj* 2000;14:1047–1055.
  99. Nygren De BC, Fredman P, Lundin A, Andersson K, Edman G, Borg J. S100 in mild traumatic brain injury. *Brain Inj* 2004;18:671–683.
  100. 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.
  101. de Kruijk JR, Leffers P, Menheere PP, Meerhoff S, Tuijnstra 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.
  102. Kavalci C, Pekdemir M, Durukan P, et al. The value of serum tau protein for the diagnosis of intracranial injury in minor head trauma. *Am J Emerg Med* 2007;25:391–395.
  103. Bazarian JJ, Zemlan FP, Mookerjee S, Stigbrand T. Serum S-100B and cleaved-tau are poor predictors of long-term outcome after mild traumatic brain injury. *Brain Inj* 2006;20:759–765.
  104. Vasterling JJ, Duke LM, Brailey K, Constans JI, Allain AN Jr., Sutker PB. Attention, learning, and memory performances and intellectual resources in Vietnam veterans: PTSD and no disorder comparisons. *Neuropsychology* 2002;16:5–14.
  105. Weiss SJ. Neurobiological alterations associated with traumatic stress. *Perspect Psychiatr Care* 2007;43:114–122.
  106. Kitayama N, Vaccarino V, Kutner M, Weiss P, Bremner JD. Magnetic resonance imaging (MRI) measurement of hippocampal volume in posttraumatic stress disorder: a meta-analysis. *J Affect Disord* 2005;88:79–86.
  107. Smith ME. Bilateral hippocampal volume reduction in adults with post-traumatic stress disorder: a meta-analysis of structural MRI studies. *Hippocampus* 2005;15:798–807.
  108. Gilbertson MW, Shenton ME, Ciszewski A, et al. Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat Neurosci* 2002;5:1242–1247.
  109. Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* 1992;106:274–285.
  110. Koren D, Hemel D, Klein E. Injury increases the risk for PTSD: an examination of potential neurobiological and psychological mediators. *CNS Spectr* 2006;11:616–624.
  111. Yehuda R, Flory JD, Southwick S, Charney DS. Developing an agenda for translational studies of resilience and vulnerability following trauma exposure. *Ann N Y Acad Sci* 2006;1071:379–396.
  112. Buckley TC, Kaloupek DG. A meta-analytic examination of basal cardiovascular activity in posttraumatic stress disorder. *Psychosom Med* 2001;63:585–594.

113. Shalev AY, Sahar T, Freedman S, et al. A prospective study of heart rate response following trauma and the subsequent development of posttraumatic stress disorder. *Arch Gen Psychiatry* 1998;55:553–559.
114. Yehuda R, Brand SR, Golier JA, Yang RK. Clinical correlates of DHEA associated with post-traumatic stress disorder. *Acta Psychiatr Scand* 2006;114:187–193.
115. Rasmusson AM, Vasek J, Lipschitz DS, et al. An increased capacity for adrenal DHEA release is associated with decreased avoidance and negative mood symptoms in women with PTSD. *Neuropsychopharmacology* 2004;29:1546–1557.
116. Bell RS, Vo AH, Neal CJ, et al. Military traumatic brain and spinal column injury: a 5-year study of the impact blast and other military grade weaponry on the central nervous system. *J Trauma* 2009;66:S104–S111.
117. Yao C, Williams AJ, Ottens AK, et al. Detection of protein biomarkers using high-throughput immunoblotting following focal ischemic or penetrating ballistic-like brain injuries in rats. *Brain Inj* 2008;22:723–732.
118. Treggiari MM, Schutz N, Yanez ND, Romand JA. Role of intracranial pressure values and patterns in predicting outcome in traumatic brain injury: a systematic review. *Neurocrit Care* 2007;6:104–112.
119. Hlatky R, Valadka AB, Robertson CS. Intracranial hypertension and cerebral ischemia after severe traumatic brain injury. *Neurosurg Focus* 2003;14:e2.
120. Pelinka LE, Kroepfl A, Leixnering M, Buchinger W, Raabe A, Redl H. GFAP versus S100B in serum after traumatic brain injury: relationship to brain damage and outcome. *J Neurotrauma* 2004;21:1553–1561.
121. Hellman NE, Gitlin JD. Ceruloplasmin metabolism and function. *Annu Rev Nutr* 2002;22:439–458.
122. Dash PK, Redell JB, Hergenroeder GW, Zhao J, Clifton GL, Moore AN. Serum ceruloplasmin and copper are early biomarkers of elevated intracranial pressure. *J Neurosci Res* 2009(in press).
123. Habgood MD, Bye N, Dziegielewska KM, et al. Changes in blood-brain barrier permeability to large and small molecules following traumatic brain injury in mice. *Eur J Neurosci* 2007;25:231–238.
124. Kirchhoff C, Buhmann S, Braunstein V, et al. Cerebrospinal s100-B: a potential marker for progressive intracranial hemorrhage in patients with severe traumatic brain injury. *Eur J Med Res* 2008;13:511–516.
125. Signorini DF, Andrews PJ, Jones PA, Wardlaw JM, Miller JD. Adding insult to injury: the prognostic value of early secondary insults for survival after traumatic brain injury. *J Neurol Neurosurg Psychiatry* 1999;66:26–31.
126. Orefice G, Ames PR, Coppola M, Campanella G. Antiphospholipid antibodies and cerebrovascular disease. *Acta Neurol (Napoli)* 1993;15:303–310.
127. Brey RL. Antiphospholipid antibodies in young adults with stroke. *J Thromb Thrombolysis* 2005;20:105–112.
128. Agrawal A, Timothy J, Pandit L, Manju M. Post-traumatic epilepsy: an overview. *Clin Neurol Neurosurg* 2006;108:433–439.
129. Lowenstein DH. Epilepsy after head injury: an overview. *Epilepsia* 2009;50(suppl 2):4–9.
130. Temkin NR, Haglund MM, Winn HR. Causes, prevention, and treatment of post-traumatic epilepsy. *New Horiz* 1995;3:518–522.
131. Lowenstein DH. Epilepsy after head injury: an overview. *Epilepsia* 2009;50(suppl 2):4–9.
132. Annegers JF, Hauser WA, Coan SP, Rocca WA. A population-based study of seizures after traumatic brain injuries. *N Engl J Med* 1998;338:20–24.
133. Bratton SL, Chestnut RM, Ghajar J, et al. Guidelines for the management of severe traumatic brain injury. XIII. Antiseizure prophylaxis. *J Neurotrauma* 2007;24(suppl 1):S83–S86.
134. Kumar R, Gupta RK, Husain M, et al. Magnetization transfer MR imaging in patients with posttraumatic epilepsy. *AJNR Am J Neuroradiol* 2003;24:218–224.
135. Diaz-Arrastia R, Gong Y, Fair S, et al. Increased risk of late posttraumatic seizures associated with inheritance of APOE epsilon4 allele. *Arch Neurol* 2003;60:818–822.
136. Schuff N, Meyerhoff DJ, Mueller S, et al. N-acetylaspartate as a marker of neuronal injury in neurodegenerative disease. *Adv Exp Med Biol* 2006;576:241–262.
137. Czech T, Yang JW, Csaszar E, Kappler J, Baumgartner C, Lubec G. Reduction of hippocampal collapsin response mediated protein-2 in patients with mesial temporal lobe epilepsy. *Neurochem Res* 2004;29:2189–2196.
138. Raabe A, Grolms C, Keller M, Dohnert J, Sorge O, Seifert V. Correlation of computed tomography findings and serum brain damage markers following severe head injury. *Acta Neurochir (Wien)* 1998;140:787–791.
139. Liliang PC, Liang CL, Weng HC, et al. tau proteins in serum predict outcome after severe traumatic brain injury. *J Surg Res* 2009 Jan 10 [Epub ahead of print].
140. Bandyopadhyay S, Hennes H, Gorelick MH, Wells RG, Walsh-Kelly CM. Serum neuron-specific enolase as a predictor of short-term outcome in children with closed traumatic brain injury. *Acad Emerg Med* 2005;12:732–738.
141. Department of Veterans Affairs, Veterans Health Administration Directive, Polytrauma Rehabilitation Centers. Veterans Health Administration. Washington DC, 2005:2
142. Pape HC, Giannoudis P, Krettek C. The timing of fracture treatment in polytrauma patients: relevance of damage control orthopedic surgery. *Am J Surg* 2002;183:622–629.
143. Rotstein OD. Modeling the two-hit hypothesis for evaluating strategies to prevent organ injury after shock/resuscitation. *J Trauma* 2003;54:S203–S206.
144. Keel M, Mica L, Stover J, Stocker R, Trentz O, Harter L. Thio-pental-induced apoptosis in lymphocytes is independent of CD95 activation. *Anesthesiology* 2005;103:576–584.
145. Bose D, Tejwani NC. Evolving trends in the care of polytrauma patients. *Injury* 2006;37:20–28.
146. Maier B, Lefering R, Lehnert M, et al. Early versus late onset of multiple organ failure is associated with differing patterns of plasma cytokine biomarker expression and outcome after severe trauma. *Shock* 2007;28:668–674.
147. da Rocha AB, Schneider RF, de Freitas GR, et al. Role of serum S100B as a predictive marker of fatal outcome following isolated severe head injury or multitrauma in males. *Clin Chem Lab Med* 2006;44:1234–1242.
148. Zethelius B, Berglund L, Sundstrom J, et al. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. *N Engl J Med* 2008;358:2107–2116.
149. Su JQ, Liu J. Linear combinations of multiple diagnostic markers. *J Am Stat Assoc* 1993;88:1350–1355.
150. Liu A, Schisterman EF, Zhu Y. On linear combinations of biomarkers to improve diagnostic accuracy. *Stat Med* 2005;24:37–47.
151. Qu Y, Adam BL, Yasui Y, et al. Boosted decision tree analysis of surface-enhanced laser desorption/ionization mass spectral serum profiles discriminates prostate cancer from noncancer patients. *Clin Chem* 2002;48:1835–1843.
152. Kahraman S, Ozgurtas T, Kayali H, et al. Monitoring of serum ionized magnesium in neurosurgical intensive care unit: preliminary results. *Clin Chim Acta* 2003;334:211–215.
153. Ringger NC, O'Steen BE, Brabham JG, et al. A novel marker for traumatic brain injury: CSF alphaII-spectrin breakdown product levels. *J Neurotrauma* 2004;21:1443–1456.
154. Pineda JA, Lewis SB, Valadka AB, et al. Clinical significance of alpha II-spectrin breakdown products in cerebrospinal fluid after severe traumatic brain injury. *J Neurotrauma* 2007;24:354–366.
155. Schwartz JG, Baxan III C, Gage CL, et al. Serum creatine kinase isoenzyme BB is a poor index to the size of various brain lesions. *Clin Chem* 1989;35:651–654.
156. Smith DH, Uryu K, Saatman KE, et al. Protein accumulation in traumatic brain injury. *Neuromolecular Med* 2003;4:59–72.
157. Newell KL, Boyer P, Gomez-Tortosa E, et al. Alpha-synuclein immunoreactivity is present in axonal swellings in neuroaxonal dystrophy and acute traumatic brain injury. *J Neuropathol Exp Neurol* 1999;12:1263–1268.