

# Biomarkers in traumatic brain injury: a review

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## ABSTRACT

Biomarkers allow physiological processes to be monitored, in both health and injury. Multiple attempts have been made to use biomarkers in traumatic brain injury (TBI). Identification of such biomarkers could allow improved understanding of the pathological processes involved in TBI, diagnosis, prognostication and development of novel therapies. This review article aims to cover both established and emerging TBI biomarkers along with their benefits and limitations. It then discusses the potential value of TBI biomarkers to military, civilian and sporting populations and the future hopes for developing a role for biomarkers in head injury management.

## INTRODUCTION

Traumatic brain injury (TBI) is defined as ‘a blow or a jolt to the head or a penetrating head injury that disrupts brain functioning’.<sup>1</sup> Every 90 s in the UK somebody is admitted to a hospital with a brain injury and approximately one million people are living with the lasting consequences of TBI.<sup>2</sup> It is the leading civilian cause of traumatic death in the under-35 age group in England and Wales<sup>3</sup> and, perhaps controversially, mild TBI has been described as ‘the signature (injury) of the modern conflicts’<sup>4</sup> when considering the military population. TBI remains a complex subject given its unpredictable and variable nature creating obstacles for classification, study design and management in this cohort of patients.

Public interest in TBI has risen since the death of Jules Bianchi this year and the life-changing injury sustained by Michael Schumacher in 2013. Awareness of TBI in sports is also increasing with the large number of successful lawsuits involving American footballers and the death of young rugby player Ben Robinson in 2011 due to second impact syndrome.

## THE ROLE OF BIOMARKERS

Biomarkers of neuronal injury have been sought after since the 1950s and interest in them has increased significantly over the past 25 years<sup>5</sup> (Figure 1). A biomarker can be defined as a naturally occurring characteristic that can be objectively measured and interpreted as an indicator of biological processes or responses to therapeutic interventions.<sup>6</sup> Several review articles concerning the use of TBI biomarkers have been published since the 1980s<sup>5 7–10</sup> and collectively their conclusions are similar. All reviews until now champion the need for further research into biomarkers and the high priority this should be given.

To understand the clinical relevance of biomarkers, it must be understood how they can be interpreted. Each type of biomarker, whether physical

## Key messages

- ▶ Traumatic brain injury (TBI) is an increasingly survivable phenomenon of conflict.
- ▶ Biomarkers are developing as a tool in the management of TBI and are therefore relevant to this cohort of injured personnel.
- ▶ Promising advances have been made in the use of biomarkers in the diagnosis of mild TBI.

or biological, is a surrogate for a relevant clinical endpoint.<sup>11</sup> A clinical endpoint is defined as a ‘characteristic or variable that reflects how a patient feels, functions or survives’.<sup>6</sup> Ideally, a biomarker of TBI should reflect the level of neuronal injury correlating to brain function and outcome in a linear fashion.

An example of a commonly used biomarker is that of prostate-specific antigen, a glycoprotein produced by the prostate and used to detect and monitor prostate cancer and assess therapeutic interventions. Similarly, biomarkers of neuronal injury may one day aid in early diagnosis of TBI (particularly mild TBI), improve prognostication, monitor ongoing pathological processes and measure the efficacy of treatments.<sup>5 10 12</sup> There is also a collective desire that these biomarkers will become reliable enough to detect mild TBI, a diagnosis that is currently complex, often missed and not without potentially serious consequences.

In 1983, while researching TBI biomarkers found in cerebrospinal fluid (CSF), Bakay and Ward<sup>13</sup> described the ideal characteristics for a biomarker of brain injury (Table 1). Studies so far have yet to identify a biomarker that satisfies all of these criteria and it is likely that a combination of markers is more likely to have a stronger clinical relevance.

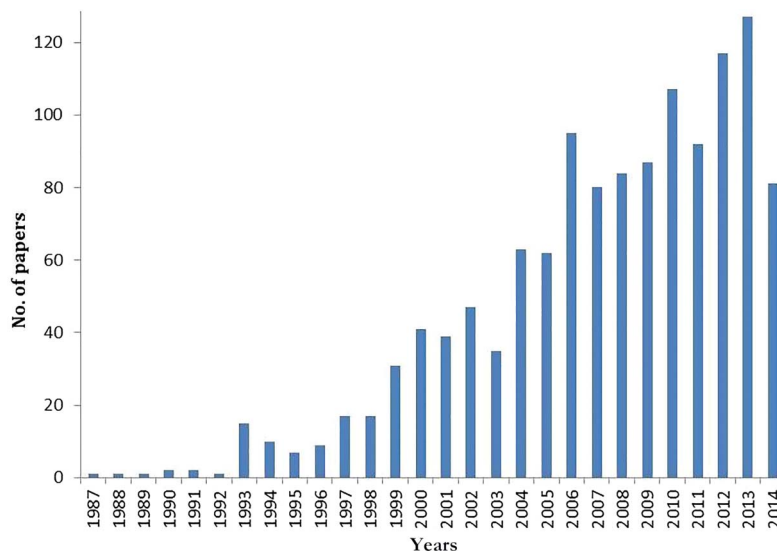
## POTENTIAL TBI BIOMARKERS Lactate dehydrogenase

Cerebral lactate dehydrogenase (LDH) was first described by Robinson in 1965<sup>14</sup> in a paper outlining its properties sampled from human frontal cortex. The isotype of LDH found in greatest abundance in the central nervous system (CNS), however, can also be found in the heart, kidney and erythrocytes.<sup>15</sup> It is for this reason that systemic trauma as well as isolated neuronal injury can cause a rise in serum LDH.

Initial studies in the 1970s found a positive correlation between LDH, severity of head injury and clinical outcome. However, this was disputed by Bakay and Ward in 1983 where they concluded

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**Figure 1** Number of brain biomarkers papers published by year. Pubmed search.



LDH could not be used as a reliable marker of neuronal injury given its lack of specificity and sensitivity in the context of systemic trauma.<sup>13</sup> A 2002 review highlighted the ‘methodological weakness’ of the initial 1970s papers given these studies were conducted prior to the introduction of the GCS or CT scanning.<sup>8</sup>

### Creatine kinase

There are three identifiable isotypes of creatine kinase, with creatine kinase of the brain (CK-BB) located in the astrocytes of the CNS. Although lower concentrations of CK-BB can also be found in the abdominal organs, it is not evident in erythrocytes making serum levels physiologically low. Both serum and CSF concentrations of CK-BB have been shown to increase following TBI, with levels peaking in the acute phase of injury before returning to normal.<sup>16 17</sup> Levels have also been shown to rise significantly in CSF following hypoxic brain injury in cardiac arrest<sup>18</sup> highlighting the potential for CK-BB release secondary to cerebral hypoperfusion due to systemic trauma. Ingebrigtsen and Romner also concluded that CK-BB has low specificity and sensitivity for TBI.<sup>8</sup>

### S-100 $\beta$ proteins

S-100 $\beta$  has been recognised as a ‘promising, non-proprietary brain injury biomarker’<sup>9</sup> and has even been suggested as the ‘CRP of the brain’.<sup>19</sup> It was first described in 1965, the name S-100 $\beta$  being derived from the protein characteristics; it is

100% soluble in saturated ammonium sulfate at neutral pH.<sup>20</sup> Three types of S-100 proteins are formed from variants of their two distinct subunits,  $\alpha$  and  $\beta$ . The types found predominantly in the cytosol of CNS glial cells are commonly referred to as the S-100 $\beta$  proteins. They have negligible concentration levels in other cells and are metabolised by the kidney prior to excretion in the urine.

A recent review in 2013 concluded that S-100 $\beta$  can distinguish between an injured patient and a non-injured patient<sup>21</sup> and several studies have correlated levels of S-100 $\beta$  to both injury severity and outcome after TBI.<sup>9 22–25</sup> Serum samples of S-100 $\beta$  have been found to accurately predict acute mortality, whereas CSF levels are associated with outcomes and overall mortality.<sup>26</sup> This may be explained by the expressions of S-100 $\beta$  in extracranial tissues such as adipocytes or chondrocytes, leading to increased levels that are observed in patients with polytrauma without brain injury.<sup>26</sup>

While studies have shown S-100 $\beta$  to be useful after severe injury, the evidence in mild injury is less convincing.<sup>24 27</sup> Serum S-100 $\beta$  can be used as a marker of blood–brain barrier (BBB) disruption and can be ‘favourably compared’ with the CSF–serum albumin quotient, the gold standard for assessment of BBB permeability.<sup>28</sup> Levels of S-100 $\beta$  therefore remain dependent on the integrity of the BBB and plasma levels may correlate poorly to levels within the brain itself.<sup>29</sup> This makes S-100 $\beta$  less reliable in minor TBI, where there is a lower level of BBB disruption.<sup>30</sup>

The drawback with S-100 $\beta$  is its short half-life; only the most severe TBI will have a raised level beyond an hour following injury and hence its use for measurements as a marker of ongoing disease processes is limited.<sup>31</sup>

### Neuron-specific enolase

Enolases are glycolytic enzymes comprising three different subunits ( $\alpha$ ,  $\beta$ ,  $\gamma$ ), originally described across four different animal species in the 1960s.<sup>20</sup> The two most stable forms are isoforms  $\gamma\gamma$  and  $\alpha\gamma$ , which are referred to as neuron-specific enolase (NSE) due to the fact that they are restricted to the cytoplasm of neurons, peripheral neuroendocrine tissue and tumours of the amine uptake and degradation system.

The potential value of NSE is evident as its action is directly related to neuronal activity rather than glial or Schwann cells.<sup>8</sup> Although suggested to correlate with GCS when used as a

**Table 1** Bakay and Ward’s criteria for the ideal characteristics for brain injury biomarkers<sup>14</sup>

Detection and laboratory characteristics	High specificity for brain tissue High sensitivity for brain injury Rapidly appear in serum Have reliable assays for immediate analysis
Pathophysiological characteristics and clinical application	Only be released after irreversible destruction of brain tissue Be released in time-locked sequence with injury Have a low age and sex variability Have clinical relevance

stand-alone biomarker,<sup>32</sup> the role of NSE is mostly recognised in conjunction with additional markers.<sup>21 33</sup> In mild TBI, combining S-100 $\beta$  with NSE added value in the early prognosis of patients<sup>34</sup> and as part of a biomarker panel can be found in the CSF for up to 3 days post-injury in severe TBI.<sup>35</sup>

NSE proteins can also be found in erythrocytes and platelets<sup>9</sup> making the process of haemolysis a significant source of cross-contamination when measured in trauma.<sup>36</sup> This was demonstrated in a 2003 study in which a similar increase in plasma NSE in both TBI and non-TBI trauma cohorts was demonstrated.<sup>37</sup> Another drawback of NSE is its slow elimination from plasma leading to difficulties in distinguishing between primary and secondary insults to the brain.<sup>26</sup> This does make acute levels difficult to interpret and perhaps does not reflect 'real-time' pathological processes when measured alone.

### Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is also found within the glial cells of the CNS, a filament protein first described by Eng in 1971.<sup>38</sup> The usefulness of GFAP as an indicator of CNS pathology has been reported in numerous conditions, including cerebral infarction, preterm neurological abnormality, encephalopathy and TBI.<sup>8</sup> It can differentiate injury severity in a manner similar to the Marshall scoring system for CT scans, as well as those patients who had raised intracranial pressure (ICP) or reduced cerebral perfusion pressure.<sup>22</sup> GFAP levels are higher in patients with mass lesions compared with diffuse injury,<sup>39</sup> arguably a redundant characteristic in hospitals where CT scanners are readily available but could be of potential value in the prehospital setting.

Studies have shown that GFAP is a better predictor of severe disability and vegetative states in comparison with predicting good outcomes<sup>22 40</sup> and has been found to strongly predict death at 6 months.<sup>39</sup> So far GFAP shows good potential to predict outcome after severe TBI, but has not been adequately studied in mild and moderate injuries.<sup>9</sup>

### Myelin basic protein

Specific to myelin, this protein is found in growing oligodendroglial cells, bound to the cell membrane and is released into the serum on damage to the brain or during demyelination. In combination with NSE, myelin basic protein (MBP) may be useful in screening for inflicted TBI in children.<sup>33</sup> Although reports have demonstrated excellent specificity for TBI, it has limited sensitivity<sup>23</sup> and has more potential when measured alongside NSE levels;<sup>33</sup> it is due to this lack of clinical sensitivity that interest in MBP as a marker has lessened in recent years in comparison with NSE, S-100 $\beta$  and GFAP.<sup>21</sup>

### Spectrin breakdown products

During necrotic cell death and apoptotic cell death, cysteine proteases, named calpain and caspase-3, cleave components of the axonal cytoskeleton<sup>41</sup> resulting in signature molecular weight breakdown products (spectrin breakdown products (SBDPs)). Following brain injury (and ischaemia), calpains and caspases become hyperactivated and SBDPs have therefore been suggested as possible biomarkers of TBI.<sup>9</sup> SBDPs were first isolated from TBI-induced rat brains in 1998<sup>42</sup> and have since been isolated in human CSF of patients with TBI.<sup>43–47</sup>

Since their discovery, however, several problems with SBDPs have been highlighted. First, it has been recognised that SBDPs are not neuronally specific and serum levels may reflect multi-organ damage in trauma.<sup>9</sup> Also, in 2010, Li *et al*<sup>10</sup> suggested that SBDPs cannot be accurately measured in CSF contaminated by

blood, given that some proteins found in erythrocytes are similar to those found in the neuronal cytoskeleton; as a large number of TBI cases involve traumatic subarachnoid haemorrhage, this may severely reduce the value of SBDPs.

### Microtubule-associated proteins

Microtubule-associated proteins (MAPs) exist in many different cell types regulating the stability of microtubules. A breakdown product of MAP-tau, found exclusively in neuronal axons and dendrites, has been isolated from brain-injured rats and named c-tau.<sup>48 49</sup> In 2006, a study demonstrated higher levels of post-traumatic CSF c-tau were associated with a poorer clinical outcome following severe TBI.<sup>40</sup> However, no statistically significant correlation has been found between levels of c-tau and outcome following mild TBI.<sup>27</sup> MAP-2 has been used in humans as a marker of hypoxia and ischaemia<sup>50</sup> and there is some evidence that it may be a potential marker for higher cognitive functioning, as patients with better recovery following severe TBI demonstrate chronic release of MAP-2 thought to be due to neuroplasticity.<sup>51</sup>

### Neurofilaments

Consisting of three chains, light (L), medium (M) and heavy (H), neurofilaments make up part of the axonal cytoskeleton. Their tail sections can be phosphorylated with increasing levels of phosphorylation proportional to axonal diameter and velocity of axonal transport.<sup>10</sup> Following axonal injury, the influx of calcium alters the phosphorylation state and there is loss of cytoskeletal structure and subsequent proteolysis. Neurofilament-L has been shown in CSF to be sensitive and specific to TBI,<sup>52</sup> however, serum detection of neurofilament-H is considered a more likely biomarker candidate.<sup>53</sup>

### Neuroinflammatory cytokine markers

Previously thought to be 'immune privileged', the CNS does in fact demonstrate features of inflammation in response to injury, infection or disease. Lucas *et al*<sup>54</sup> produced a review in 2006 that provided a detailed account of the role of inflammation following varying CNS insults.

Inflammatory proteins, such as the interleukins (IL-6, IL-8 and IL-10), are increased in CSF in response to severe TBI; however, studies into their use in mild TBI are minimal.<sup>50</sup> Hayakata *et al* studied both serum and CSF levels of inflammatory mediators in patients with severe TBI with and without additional (extracranial) injuries. They concluded that CSF anti-inflammatory mediators may be useful indicators of the severity of brain damage in relation to ICP and overall prognosis in severe TBI.<sup>55</sup> Both this study and a 2005 study showed that serum concentrations of these cytokines were less accurate than CSF levels, especially in the context of polytrauma.<sup>55 56</sup> More recently, Hergenroeder *et al*<sup>57</sup> have published reports showing that serum IL-6 may be a marker for elevated ICP in isolated head injuries, however, again of less value in patients with polytrauma.

### Ubiquitin C-terminal hydrolase L-1

Ubiquitin C-terminal hydrolase L-1 (UCH-L1) is a protein found in the neuronal cell body. Already investigated for its role in neurodegenerative diseases, it has recently been studied in relation to TBI. A 2010 paper used a rat model to demonstrate elevated levels of UCH-L1 in both CSF and serum samples following controlled cortical injury and middle cerebral artery occlusion.<sup>58</sup> In human studies, there has also been shown to be a significant correlation between UCH-L1 concentrations and

both neuroradiological findings and clinical outcome.<sup>39–59</sup> There is increasing evidence that UCH-L1 is one of only a few markers that has been found to identify minor TBI as well as more severe injury.<sup>26</sup>

Importantly, UCH-L1 also appears to be able to distinguish between patients with TBI and uninjured patients at 6 h when GCS is altered secondary to drugs and alcohol.<sup>26</sup> Papa *et al*<sup>60</sup> also showed that UCH-L1 levels can distinguish between patients with TBI demonstrating a GCS of 15 and control groups.

### Soluble urokinase plasminogen activator receptor

Urokinase plasminogen activator receptor (uPAR) is a cell surface protein that aids in plasmin proteolysis. It has been previously detected in CNS-infiltrating macrophages and the soluble form (soluble uPAR (suPAR)) has been detected in the CSF of patients with known inflammatory CNS pathologies such as neoplasia and prion disease.<sup>61</sup> A recent study from 2014 demonstrated correlation between elevated suPAR levels, GCS and prognosis when used in TBI. The paper concluded that suPAR has high diagnostic specificity and sensitivity to differentiate survivable TBI and non-survivable TBI and prognosis for survivors was worse in those with higher levels.<sup>62</sup> While this is an encouraging paper, issues again arise with specificity to TBI as suPAR is also found in extracranial tissues and has been implicated and studied for use as a biomarker in gastrointestinal cancers, intrinsic kidney disease and postcardiac arrest. Further research will be able to define the role of suPAR in TBI perhaps evolving from its current use in predicting survivability.

### POTENTIAL ROLE OF TBI BIOMARKERS

Biomarkers have the opportunity to allow us to possibly improve our diagnosis of TBI, improve our management by allowing us to monitor the disease processes, understand more the pathological processes involved and even target possible novel therapies. Much of the work with biomarkers has followed a typical pattern however. After an initial period of optimism, there is, then, a realisation that although an individual biomarker may be of some use, there are often multiple confounding factors. This is in part due to the nature of TBI. It is not a single pathological process as every injury is a unique mix of primary and secondary injuries producing different biomarkers depending on how each pathological process is involved.

Trying to understand the time courses by which the biomarkers become measurable in CSF is one problem, but to identify them in plasma is even more complex. The question of the time course of biomarkers in plasma is complicated by the concern that rather than measuring the amount of TBI, the biomarker has instead become a measure of the BBB injury. The downfall of many of the early TBI biomarkers was the extraneuronal production of biomarkers, particularly in patients with polytrauma. Unless a truly pathognomonic biomarker for TBI is found, this is likely to remain the primary drawback for detection of TBI in the context of the multiply injured patient.

So, where in particular could biomarkers be of value? In austere environments, where it is not possible to perform a CT scan or it is necessary to sedate a patient for other injuries, the potential for a biomarker to assess injury and give indication of worsening injury is huge, but this probably remains some way off. Biomarkers may also allow us to measure injury which is not evident on initial CT scans and hence allow observation of such patients to be arranged or modification of clinical management. A recent concern has been with regard to second impact syndrome, a process where a secondary injury to the brain

within a short period seems to have a particularly deleterious course.

Within the military, much has been written about blast injury causing TBI. Recent US studies have suggested that there has been a rapid rise in the incidence during the recent conflicts in the Iraq and Afghanistan.<sup>63</sup> It is postulated that the incidence of military head and neck injuries may increase further, as casualties who would have previously died from severe thoracic, abdominal or fatal head trauma are now surviving due to better protective equipment.<sup>64</sup> Although the mechanism remains unclear, explosive blast has been suggested as the causative factor for increasing brain injury within the military population, and mild TBI has been described as *'the signature of the modern conflicts'*.<sup>4</sup> This is a controversial statement given that blast TBI is not fully understood, and studies undertaken in other countries have suggested that the link between blast and TBI is not so clear. The hope is that biomarkers may provide evidence to allow the diagnosis of blast TBI to be confirmed and may also demonstrate the specific pathological processes involved and the timeline in which these develop.

There have been a number of studies looking at biomarker responses after blast exposure since the 1990s. A recent study suggested a potential biomarker specific to blast TBI—soluble cellular prion protein (PrPC). The hypothesis is that the primary blast wave can dislodge any extracellular PrPC and lead to a systemic rise in its concentration.<sup>65</sup> The hypothesis was demonstrable in rat models in a 2015 study and the authors concluded the PrPC could be a novel biomarker for detection of primary blast TBI in military personnel.

Within sport, many high-profile head injuries in rugby and football have raised concern about sports-related concussion and its long-term impact on sportsmen and women. These concerns have been around for a long time, more especially in American football players and boxers. In this cohort, biomarkers may also help to understand the significance of multiple head injuries over a longer period of time. In addition to its potential role in detection of blast TBI, PrPC has also been investigated as a promising marker for TBI in sports-related concussion.<sup>66</sup> Overall, a number of biomarkers have demonstrated a correlation with various forms of head injury across a selection of sports.<sup>67</sup> A recent systematic review of biomarkers in sports-concussion concluded by commenting that although *'there are no validated biomarkers for concussion as yet, there is potential for biomarkers to provide diagnostic, prognostic, and monitoring information post injury'*.<sup>67</sup>

### FUTURE OF BIOMARKERS

The recent 2014 review of biomarkers concluded positively. Forde *et al*<sup>68</sup> summarised that 'S100 $\beta$ , GFAP, TNF- $\alpha$  and MBP appear to have some use in determining the severity of TBI with GFAP and MBP proving to be the most specific for brain trauma'. The review, however, does conclude that no single biomarker alone has been proven to be of clinical use and future research should aim to identify a combination of sensitive and specific biomarkers to provide convincing prognostic TBI information.<sup>68</sup>

In the recent National Institute for Health and Care Excellence (NICE) head injury guideline update, the Guidelines Development Group (GDG) felt it was not yet appropriate to make a recommendation for the use of biomarkers to triage, diagnose or prognosticate TBI. However, the update states that biomarkers in future may be useful for the selection of patients requiring neuroimaging and reduce the need for excess CT scans and hospital admissions.<sup>69</sup> Prior to introducing the use of

biomarkers to everyday practice, the GDG also highlights the need for further research into cost effectiveness before widespread use across the NHS given cost implications. Each biomarker test currently costs £131.34 to process.<sup>69</sup>

Future studies will help to hone the use of known biomarkers and also identify potential new markers. As well as PrPC, there has been a recent description (2015) of another new biomarker—extracellular erizin, so far identified in samples taken from injured neurons in both live rat and human CSF.<sup>70</sup>

As well as direct application in management of head injury, a recent article in *Expert Review Neurotherapeutics* highlighted the potential use of biomarkers in research into neuroprotective therapies. The article, concerning losartan use post-TBI to reduce post-traumatic epilepsy, stressed the importance of developing reliable biomarkers to improve research into novel treatments.<sup>71</sup>

## CONCLUSION

There has been much enthusiasm towards further development of biomarkers despite the limitations identified so far. The common drawbacks of current markers include their ambiguity in the presence of multiple injuries and their detection and prognostication of mild TBI. It has been recognised that in future biomarkers are likely to be used as an adjunct, supplementing traditional examination and neuroimaging in the diagnosis and prognosis for patients with TBI. With applications across the military, civilian and sporting populations, there is intense interest in TBI biomarkers with an ever-increasing amount of research into their development and clinical application. The goal is, as ever, to improve our management of this common and often life-changing injury across its spectrum of pathology, progression and outcome.

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