

# Biomarkers and prognostication in traumatic brain injury

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## Abstract

A number of patients who suffer from mild head injury later on develop significant disabilities. Biomarkers help identify and quantify the extent of injury and help predict the possible functional outcome of the patients. There are promising candidate biomarkers for axonal injury (Tau) and astrocytic damage (glial fibrillary acidic protein and S100 $\beta$ ) in traumatic brain injury. However, the biological significance of these markers cannot be confidently declared due to lack of studies with adequate sample size.

**Key words:** Biomarkers, prognostication, traumatic brain injury

## INTRODUCTION

In recent years, there is an enhanced interest in mild head injury. A number of patients who suffer from mild head injury later on develop significant disabilities. It is necessary to know the extent of injury suffered by the brain in the initial phase. Biomarkers help identify and quantify the extent of injury and help predict the possible functional outcome of the patients. Accurate biochemical tests of axonal, neuronal and astroglial injury would be helpful to indicate whether head trauma caused an injury to the brain and to define when the injury has resolved. Studies have shown that returning to work too soon after a concussion without allowing time for recovery worsens the long-term outcome.

The advantages of blood biomarkers are:

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- They are cost-effective
- They involve minimally invasive sample collection
- They can provide a reference for neuroimaging referrals
- They may be used to identify various types of parenchymal brain injury and/or blood-brain barrier (BBB) damage
- Fluctuations in blood levels of biomarkers may help classify injury severity.

Some important questions that need to be addressed in evaluation of the biomarkers are:

- What is the origin of the protein of interest?
- Do cells of the BBB transport brain-derived proteins into the blood?
- Do physical activities such as sports-play and strenuous exercise affect blood levels of the protein of interest?
- Is the biomarker specific to damage associated with neurotrauma such as axonal distortion, glial cell activation and/or cell death within the neurovascular unit?
- When does a protein of interest reach its peak concentration in the blood?

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Mild head injury does not produce gross pathology that can be visualised on a computed tomography (CT) scan but causes neurological symptoms which resolve in about a week to 2 weeks' time. However, about 15% of patients have persistent cognitive dysfunction.<sup>[1]</sup> Although axonal disconnection occurs rarely at the time of injury, the rapid stretching of axons causes an unregulated outflux of  $K^+$  and influx of  $Na^{+2}$  which, in turn, causes an increase in intra-axonal  $Ca^{2+}$  concentrations.  $Ca^{2+}$  stimulates protease calpain, which causes proteolysis of cytoskeleton.  $Ca^{2+}$  also stimulates n-methyl-d-aspartate receptors and the associated injury.<sup>[3]</sup>

In addition to these ionic disturbances, ultrastructural studies of axons show mechanical breakage and buckling of microtubules at the time of injury, which can trigger progressive microtubule disassembly.<sup>[4]</sup> Studies using advanced magnetic resonance imaging (MRI) techniques, such as diffusion tensor imaging, show that the extent of white matter abnormalities after mild traumatic brain injury (TBI) correlates with the severity of postconcussion cognitive problems.

Biomarkers can be classified into those identified in cerebrospinal fluid (CSF) and those that are identified in the blood.

## CEREBROSPINAL FLUID BIOMARKERS

CSF composition reflects the changes that take place in the brain. CSF biomarkers of brain injury include proteins that indicate BBB integrity and neuroinflammation, as well as axonal, neuronal and astroglial damage.

### Markers of blood–brain barrier integrity

The CSF: serum albumin ratio is a standard biomarker of BBB function. An increase in this ratio indicates BBB damage, which is found in patients with various central nervous system (CNS) disorders.<sup>[5]</sup> Neuroinflammatory response to severe TBI increases CSF: serum ratio. The ratio is increased in severe TBI<sup>[6]</sup> and not in mild TBI.<sup>[7]</sup>

### Markers of neuroinflammation

In general, levels of inflammatory proteins, such as interleukin-6 (IL-6), IL-8 and IL-10, are increased in CSF in response to severe TBI.<sup>[8-10]</sup> The magnitude of the rise correlates with the patient's outcome, and in some studies also with the extent of BBB dysfunction, as shown by the CSF: serum albumin ratio. However, one of the fallacies with these markers is that they are not brain specific. They may be liberated in the peripheral tissues and slowly seep into the CSF.

### Markers of acute axonal injury

The two best-established CSF biomarkers of axonal injury are total tau protein and neurofilament light polypeptide (NFL). Tau protein is highly expressed

in thin, nonmyelinated axons of cortical interneurons, whereas NFL is most abundant in the large-calibre myelinated axons that project into deeper brain layers and the spinal cord. The consensus in the literature is that total tau protein levels in ventricular CSF correlate with lesion size and clinical outcome in patients with TBI; high levels are an indication of more severe injury.<sup>[11,12]</sup> Neurofilaments are composed of neuron-specific intermediate filaments. Each intermediate filament consists of one light subunit (NFL) plus either a medium subunit or a heavy subunit (NFH), arranged head to tail. High levels of phosphorylated NFH have been demonstrated in the ventricular CSF of patients with severe TBI. The degree of rise in NFL is larger than tau in TBI. NFL in CSF seems to be the most sensitive biomarker of axonal injury.<sup>[13]</sup>

### Markers of acute neuronal injury

Neuron-specific enolase (NSE) is a glycolytic enzyme mostly present in neuronal cell body. NSE levels in CSF correlate with mortality in TBI, Glasgow Coma Scale and Glasgow Outcome Scale.<sup>[14]</sup> Since NSE is also abundantly present in erythrocytes, its levels are highly sensitive to haemolysis. Therefore, contamination of CSF sample with blood might greatly influence the NSE measurement.

### Markers of acute astroglial injury

S100 $\beta$  is an intracellular protein but can be released into the extracellular space. Since S100 $\beta$  does not cross an intact BBB, elevated serum levels of S100 $\beta$  following TBI have been attributed to BBB permeability. The effects of S100 $\beta$  appear to be concentration dependent. It is protective and trophic at low concentrations but toxic and pro-apoptotic at high concentrations.

S100 $\beta$  has been thought to be specific for astroglia but has been detected in oligodendrocytes and adipocytes. Patients with mild TBI have slightly elevated levels of S100 $\beta$  after the injury, but the increase is not as much pronounced as tau and NFL. Similar results have been reported for glial fibrillary acidic protein (GFAP), a CNS-specific protein that is almost exclusively expressed in astroglia. They are slightly elevated after mild TBI, but the CSF levels of this protein, when added to the clinical data, improved the power of outcome prediction.<sup>[15]</sup>

### Markers of amyloid-related processes

Amyloid precursor protein (APP) accumulates in neurons and axons after brain trauma that causes axonal damage. APP accumulates in TBI after about 2–3 h of injury. In addition, intra-axonal accumulation of  $A\beta$  is common in TBI patients. Aggregation of  $A\beta$  is associated with the development of Alzheimer's disease (AD).  $A\beta$  is released into the tissue surrounding damaged axons, where it leads to plaque formation. Ventricular CSF levels of  $A\beta_{40}$  and  $A\beta_{42}$  increase during the 1<sup>st</sup> week after

head trauma in patients with severe TBI.<sup>[16]</sup> However, samples of CSF from lumbar puncture did not show changes in A $\beta$ 40 and A $\beta$ 42. Thus, the A $\beta$  levels in CSF samples obtained by lumbar puncture are less sensitive to the effects of mild TBI.

## BLOOD BIOMARKERS

The proteins that are expressed in brain are also detectable in low concentrations in the blood. The low concentration of the biomarkers in peripheral blood is a technical challenge to the use of most standard immunoassays. However, the number of biomarkers of brain injury in peripheral blood is steadily increasing as the analytical tools for their detection become ever more sensitive.

BBB is a barrier to the transfer of protein expressed in the brain into the blood. Impaired BBB integrity, as seen in severe TBI, can increase the levels of brain-derived proteins in the blood. Some potential biomarkers undergo proteolytic degradation and some of them might be cleared by liver and kidney. For these reasons, the blood concentrations of the biomarkers are bound to be less than that of CSF concentration.

### Markers of astroglial injury

Normal blood levels of S100 $\beta$  are approximately 0.05 ng/ml. These levels can increase to nearly 5 ng/ml after severe TBI. Increased serum levels of S100 $\beta$  have been observed within 24 h of severe TBI and strongly correlate with mortality.<sup>[17,18]</sup> The levels of S100 $\beta$  and GFAP in serum correlate with Glasgow Coma Scale scores and neuroradiological findings at hospital admission.<sup>[19]</sup> Since S100 $\beta$  can be raised from fractured bones or injured skeletal muscle, its reliability seems to be low.

GFAP is the principal structural protein of cytoskeletal intermediate filaments expressed by astrocytes. Serum GFAP levels rise post-injury as a result of its release from damaged astrocytes. Increases in serum GFAP act as a surrogate marker for astrocytic injury. Blood levels of GFAP are 0.012 ng/ml in health. They increase significantly ( $4.52 \pm 8.69$  ng/ml compared to healthy controls  $0.061 \pm 0.044$  ng/ml) in severe brain injury and predict mortality, recovery, outcome and intracranial lesion.<sup>[20-23]</sup> GFAP is not expressed by any extracerebral tissue. Therefore, it is a more reliable biomarker.

### Markers of axonal and neuronal injuries

Some of the important biomarkers in the blood are NSE, myelin basic protein (MBP) and hyperphosphorylated NFH. Enolase is a crucial catabolic enzyme that converts 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway for adenosine triphosphate production. NSE reaches the bloodstream through the lymphatic system. Serum NSE levels in normal

individuals are around 10 ng/ml. Values >21.7 ng/ml have been reported after TBI. Moreover, NSE levels have a high sensitivity to predict death (85%) or poor outcome (80%) 6 months after injury.<sup>[24]</sup> The increase in the levels of NSE in CSF observed in response to lysis of erythrocytes is a major limitation of this biomarker. MBP levels have been shown to be more specific markers of TBI than NSE levels (specificity 96% vs. 64%), but their sensitivity is less (44% vs. 71%). NHF levels increased over the 6 consecutive days in TBI patients who died.

### Markers of amyloid-related processes

Tau is predominantly expressed by neurons and preferentially localised within axons. Serum levels of tau protein could be measured by an ultrasensitive digital immunoassay technique with a lower limit of detection of 0.02 pg/ml. The values ranged from <10 pg/ml to 400 pg/ml in patients who were resuscitated after cardiac arrest. This sensitive assay method will be useful in assessing the severity of TBI. Serum T-Tau levels were increased in mild TBI patients ( $188 \pm 210$  pg/ml) compared to healthy controls ( $86 \pm 48$  pg/ml);<sup>[25]</sup> correlational analyses between CT imaging and serum T-Tau levels among mild TBI patients revealed that T-Tau successfully differentiated patients with intracranial injury ( $307 \pm 246$  pg/ml) from those without intracranial injury ( $77 \pm 61$  pg/ml).

### Emerging blood biomarkers

Spectrin degradation products from CSF samples of severe TBI patients correlated with the Glasgow Coma Scale scores and improved the prediction of outcomes. Levels of spectrin breakdown products in CSF were measured together with those of another potential marker, ubiquitin carboxyl-terminal hydrolase isoenzyme L1, a deubiquitinase that is highly expressed in neurons. The levels of these two markers contributed significantly to the outcome prediction model.<sup>[26]</sup>

## CONCLUSION

There are promising biomarkers for axonal injury (Tau) and astrocytic damage (GFAP and S100 $\beta$ ) in TBI. However, the biological significance of these markers cannot be confidently declared due to lack of studies with adequate sample size.

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### Conflicts of interest

There are no conflicts of interest.

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