TISSUE AND FLUID BASED INDICATORS FOR TRAUMATIC BRAIN INJURY (REVIEW)

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Abstract

Traumatic brain injury (TBI) is among the leading causes of death and disability worldwide. While it has a heterogeneous nature and can present with a wide range of pathological findings, at times there are little to no morphological findings to be observed during the conventional neuroimaging techniques, especially in the cases of mild TBI. Motivated by the high incidence of TBI, the research interest in biomarkers for TBI has increased tremendously in recent years. Multiple molecules have been studied in the context of their diagnostic and prognostic value in hopes to optimize the diagnosis, evaluation, treatment and clinical outcome of TBI. This review presents the current understanding of different fluid and tissue based indicators of TBI, highlighting their potential utility in the management of brain injury.

Keywords: *TBI, DAI, brain injury, biomarkers*

INTRODUCTION

Traumatic brain injury (TBI) is one of the leading causes of death and disability, especially in the younger population, with an estimated 64-74 million people sustaining a TBI each year [1, 2]. Although mild TBI rarely reaches a fatal outcome, it is the most common grade of TBI and often labelled with the term "concussion"[3]. TBI has a heterogeneous nature and can present with a wide range of pathological findings (swelling, intracranial haemorrhages, contusions, thrombosis, etc.) but at times there are little to no morphological findings that can be observed during the neuroimaging of the patient [4, 5]. This proves the milder spectrum of traumatic brain injury challenging to diagnose by the use of conventional diagnostic procedures. In 1982, Adams et al. introduce the term diffuse axonal injury (DAI) which later is described as the presence of microscopic axonal damage in the white matter of the brain, caused by mechanical forces [6]. Subsequently there were three different grades of DAI established, based on their microscopic morphology [7]. The term DAI implicates that there is diffuse distribution of the traumatic findings, which is usually not the case [8]. Distribution of DAI has a predisposition for white matter tracts in the midline of the brain - corpus callosum, internal capsule, cerebral peduncles, brainstem and grey-white junction of the cerebral cortex [4, 7, 9]. Supposedly, the only way DAI could be diagnosed and graded correctly is by a microscopic examination of brain tissue (biopsy) [7, 10], which is highly unlikely to be performed during the clinical evaluation of living individuals. Various notable biochemical indicators of DAI have been investigated and could potentially be utilised to identify DAI in a clinical setting.

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Hence, the aim of the current literature review is to identify all relevant and promising diagnostic markers of DAI with strong evidence of their potential of implementation into the diagnosis of DAI in patients who suffered TBI.

Mechanism of injury and pathophysiology of DAI

Without the existence of structural abnormalities in the cranial cavity, evident on computed tomography, traumatic axonal damage is considered the primary cause of post-traumatic loss of consciousness [3, 11]. Diffuse traumatic axonal injury is considered to be the result of rotational or acceleration-deceleration injury during which straining and shearing forces are in action causing rapid deformation and damage of the axonal exoskeleton of the neurons [8, 12, 13, 14, 15]. The damage to the axons does not always result in their immediate destruction. Animal studies have demonstrated that often there is no axon breaking immediately after brain trauma, with the myelin of the axons staying intact [14, 15, 16, 17]. Following the traumatic brain injury, axonal degeneration is identified as a transition from axonal transport disruption to axonal swelling, followed by secondary disconnection and demyelination [4, 9]. These alterations in the axonal cytoskeleton disrupt the axoplasmic transport processes, resulting in the progressive accumulation of transport products and alterations in neuronal homeostasis. The initial impact of the brain causes focal perturbation in the axon, resulting in a change in axonal transport and an accumulation of the β-amyloid precursor protein (β-APP), a transmembrane glycoprotein widely expressed in the membranes of the central nervous system, which can be detected usually within two hours and as early as 30 minutes after sustaining TBI [18, 19, 20, 21, 22]. The damage to the cytoskeleton may lead to disruption of the calcium homeostasis, which activates a cascade of reactions in the damaged axons leading to destruction of the cell membrane [11, 23, 24]. The neuro-inflammatory response is another key part in the mechanism of injury of axons [24, 25]. Traumatic brain injury associated with DAI macroscopically may present with focal findings such as injuries to the soft tissues of the head, skull, subarachnoid haemorrhage or contusions in the cortex. These findings are non-specific and thus not necessary for the diagnosis of DAI. Haemorrhagic tears located in the brain's deep white matter tracts such as the brainstem tracts, internal capsule, corpus callosum, superior cerebellar peduncles are indicative of diffuse axonal injury [8]. Nevertheless, often there are little to no macroscopic findings in the brain tissue. Thus, a microscopic examination of the brain tissue is required when diagnosing DAI [26, 27]. Subsequently there were three different grades of DAI established, based on their microscopic morphology (Table 1) [7].

Grade	***Histological findings***
Grade 1	Axonal injury in the white matter of the cerebral hemispheres, corpus callosum, brain stem and the cerebellum occasionally
Grade 2	Findings from Grade 1 plus additional focal lesion in the corpus callosum is present
Grade 3	Findings present in grade 1 and 2 plus additional focal lesion in the dorsolateral quadrant or quadrants of the rostral brain stem

Table 1 Grading of DAI based on histological findings as defined by Adams et al.

Diagnosing DAI with immunohistochemistry

Multiple structural proteins, such as TAU, S-100, glial fibrillary acidic protein (GFAP), Neuron-specific enolase (NSE), have been assessed through immunohistochemistry [28, 29, 30, 31, 32]. A study by Hausmann et al. shows that elevated immunostaining of GFAP following traumatic brain injury requires survival time of at least 1 day [33]. Smith et al. conclude that TAU immunohistoreactivity does not increase considerably after a fatal traumatic brain injury [31]. A study by Krohn et al. shows that there is no significant change in S100 and NSE immunohistoreactivity up to 2 hours after injury and even after that period, the changes are subtle

[29]. On the other hand, immunohistostaining for beta-amyloid precursor protein (β-APP) has solidified as an effective tool for detecting traumatic axonal injury in forensic cases [19, 34]. Positive β-APP immunohistoreactivity have been reported as soon as 35 minutes after injury [21], although studies show that the most common interval required for expression is 2 to 4 hours after injury [20, 35]. Two different immunostaining patterns of β-APP have be recognized – "axonal bulbs" and "varicose" axons. It is considered that the axonal bulbs pattern are prevalent in cases of traumatic brain injury, while the varicose axons are more common in ischemic injury [36, 37].

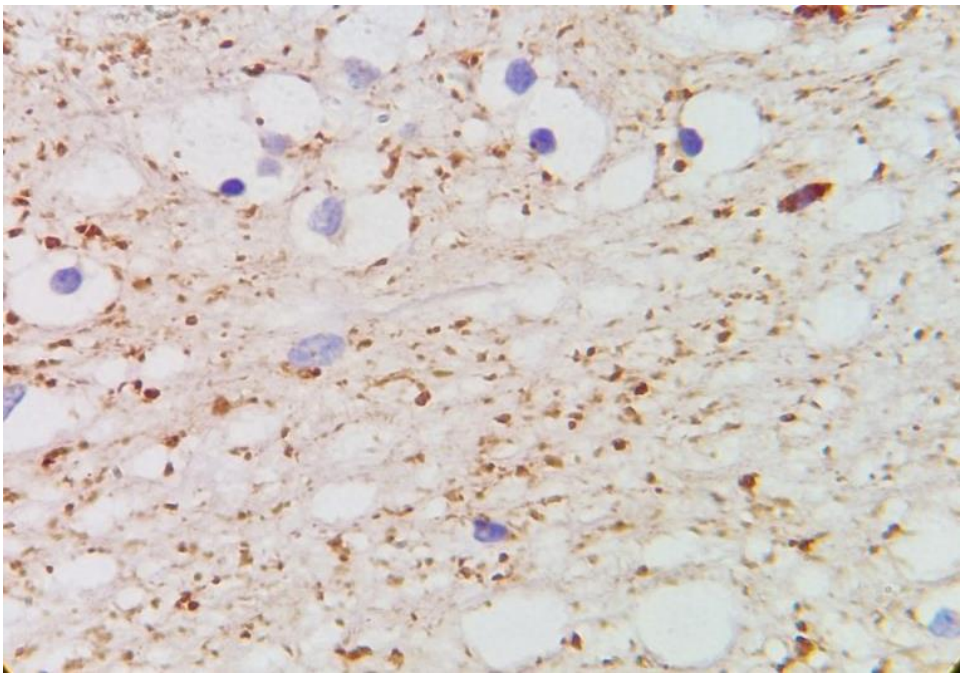

Figure 1 β-APP Immunostaining of brain tissue from a forensic case of TBI, from the Department of Forensic medicine, University Multi-profile Hospital for Active Treatment "St. George" Plovdiv, with multiple dark-brown bulb-like structures ("axonal bulbs"). Original magnification x100

Fluid indicators for DAI

Multiple molecules in CSF and blood have been the subject of study in the past decades. Among them several show potential as biomarkers for TBI. We have summarized them based on their structural origin (Fig. 2), highlighting their advantages and disadvantages as potential TBI biomarkers.

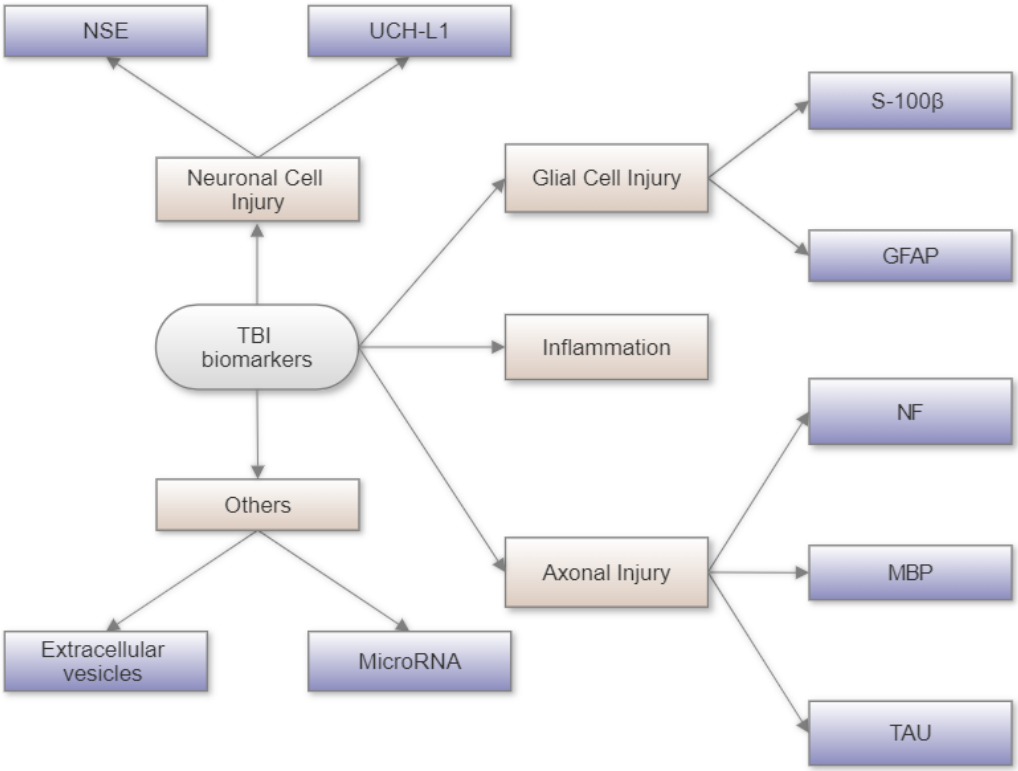

Figure 2 Graphic representation of the structural origin of promising biomarkers of TBI

Neuron-specific enolase (NSE) is an enzyme that is primarily found in neurons, but also in neuroendocrine cells. It is involved in energy production within cells, specifically in the glycolysis pathway [38]. In medicine, NSE levels are often measured in the blood or cerebrospinal fluid (CSF) to help diagnose and monitor various neurological conditions, such as traumatic brain injury, stroke, and certain cancers, including neuroblastoma, small cell lung cancer, and Merkel cell carcinoma. Studies show elevated levels of NSE in CSF, serum and plasma as early as the first 12 hours after TBI [39], but the levels could also me elevated in cases of haemolysis and the presence of other injuries [40].

Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), is an enzyme that plays an important role in the regulation of protein turnover and degradation in cells. It is primarily found in neurons and is believed to be involved in the maintenance of neuronal function and survival [41]. Research has also suggested that UCH-L1 may have potential as a biomarker for traumatic brain injury (TBI), as elevated levels of UCH-L1 have been found in the blood and cerebrospinal fluid of individuals who have sustained a TBI in the first 6 to 24 hours after the injury [39]. This has led to interest in developing diagnostic tests for TBI that can detect UCH-L1 levels. Although this marker has proven to be useful it shows elevated levels in conditions other than TBI such as stroke and Alzheimer's disease [41]. It can also decline rapidly after injury and thus is not reliable as an indicator of injury severity and outcome [42]. In summary, while UCH-L1 is a promising biomarker for TBI, its use should be considered in conjunction with other clinical and imaging measures.

S-100 is a calcium-binding protein with a low molecular weight released by astrocytes following brain injury. It has been the subject of many investigations. Elevated levels of this protein has been found in the first 24-48 hours after injury [39, 43, 44]. It appears that serum levels of S-100 correlate with the severity of the injury and neurophysiological dysfunction [44]. It appears to be valuable indicator of brain lesion but it is not specific to the central nervous system. High serum levels of S100β have been noted in patients with bone fractures and thoracic contusions [45].

Glial fibrillary acidic protein (GFAP) is primarily expressed in astrocytes, and is involved in maintaining their shape and function, hence it is highly specific for this cell type [38]. GFAP has been extensively studied as a potential biomarker for traumatic brain injury (TBI). When the brain

experiences trauma, such as a blow to the head, it can result in the disruption of the blood-brain barrier and the release of various proteins, including GFAP, into the bloodstream. Studies have shown that GFAP levels in the blood can be elevated following TBI specifically in the first 24-48 hours, and that the extent of this elevation is related to the severity of the injury [39, 46]. In particular, higher GFAP levels have been associated with more severe TBI and poorer outcomes [46, 47]. Measurement of GFAP levels in the blood has shown promise as a potential tool for the diagnosis and monitoring of TBI. For example, a rapid blood test for GFAP has been developed and is currently in use in some clinical settings to aid in the diagnosis of TBI [46].

Neurofilaments are a type of protein filament found in the neurons. They are a major component of the neuronal cytoskeleton and play an important role in maintaining the shape and mechanical properties of neurons [48]. Neurofilaments are composed of three different subunits: neurofilament light chain (NFL), neurofilament medium chain (NFM), and neurofilament heavy chain (NFH). These subunits can combine in various ways to form different types of neurofilaments with different properties [49]. Neurofilament light chain (NFL) is one of the three subunits that make up neurofilaments. NFL is the smallest and most abundant subunit. NFL is a useful biomarker for axonal injury and degeneration, as it is released into the cerebrospinal fluid (CSF) and blood when axonal damage occurs [50,51,52]. Therefore, measuring the levels of NFL in CSF or blood has been proposed as a diagnostic and prognostic tool for a variety of neurological conditions, including traumatic brain injury, stroke, multiple sclerosis, and Alzheimer's disease [53]. Studies have shown that elevated levels of NFL are associated with more severe and widespread neurodegeneration, and that the level of NFL in CSF or blood could be used to track disease progression over time [39]. NFL is considered to be a promising biomarker for TBI, as it has been found to be sensitive to detecting both acute and chronic brain injury [52].

Tau is another biomarker studied as a potential indicator of brain injury. Tau is a microtubule-associated protein that is predominantly expressed in neuronal axons. Cerebrospinal fluid and serum in TBI patients contain substantially elevated levels of cleaved Tau, according to studies [54], [55] Specifically, tau has been found to be a sensitive and specific biomarker for detecting repetitive head impacts, which can lead to chronic traumatic encephalopathy (CTE) and other neurodegenerative conditions [43]. Studies show elevated levels in CSF and plasma in the first 24 hours after injury [39, 56, 57].

Myelin Basic Protein (MBP) is a molecule found in oligodendrocytes in the central and peripheral neural system. TBI causing axonal damage can lead to release of MBP in the bloodstream, but studies show, that this occurs 1 to 3 days after the injury, which makes it unsuitable marker for early diagnosis of TBI [39, 58].

Inflammatory factors, such as TNF-α, IL-1, IL-6, IL-8, IL-10 have been excessively studied in correlation to TBI. Their levels both in the bloodstream and CSF can be found after glial activation due to the sustained TBI [25, 59, 60]. Among them IL-6 has been the most studied, proving elevated IL-6 6 to 8 hours after TBI [39, 61]. The disadvantage of the inflammatory biomarkers is the fact they aside from TBI, they can be elevated due to a wide variety of reasons, among which are sex, age and the presence of an infection or an infectious disease, which immediately makes them very unreliable as individual biomarkers of TBI [60].

Extracellular Vesicles (EVs) and MicroRNA: EVs and MicroRNA abnormalities is a novelty in the research field. EVs are membranous particles (vesicles) secreted by all types of brain cells [62]. Although they are present in healthy people, studies show elevation of their levels in patients with TBI [63]. The downside to those new molecules is that their structure is highly variable, which makes their isolation from raw biological fluids extremely difficult [38]. MicroRNA is a large group of small, endogenous non-coding RNAs which modulates protein synthesis [64]. Some of those MicroRNAs play a critical role in structuring the neural network in the brain. Various studies have shown, that TBI could lead to rise in MicroRNA abnormalities, but so could ischemic changes in the brain due to other non-traumatic causes [65].

CONCLUSION:

In this review we present the most commonly studied alternative markers of TBI and more specifically DAI. Its complex pathogenesis and pathophysiology in combination with the highly varying clinical course and outcome, makes DAI highly difficult to diagnose by using conventional methods. The implementation of appropriate highly specific for TBI fluid biomarkers could drastically improve the early diagnosis, treatment and outcome of TBI. Overall, the presented in the current review biomarkers show promise as tools for the diagnosis and monitoring of TBI. However, further research is necessary to evaluate their clinical utility, as well as to determine the optimal cut-off values and timing for their measurement in TBI patients.

CONFLICT OF INTERESTS:

There is no conflict of interest.

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