Clinical Neurology and Neurosurgery 171 (2018) 6–20

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

Profiling biomarkers of traumatic axonal injury: From mouse to man

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ARTICLE INFO

Keywords:
Traumatic brain injury
Traumatic axonal injury
Biomarkers
Neurofilaments
Myelin basic protein
Tau
Amyloid
Spectrin breakdown products

ABSTRACT

Traumatic brain injury (TBI) poses a major public health problem on a global scale. Its burden results from high mortality and significant morbidity in survivors. This stems, in part, from an ongoing inadequacy in diagnostic and prognostic indicators despite significant technological advances. Traumatic axonal injury (TAI) is a key driver of the ongoing pathological process following TBI, causing chronic neurological deficits and disability. The science underpinning biomarkers of TAI has been a subject of many reviews in recent literature. However, in this review we provide a comprehensive account of biomarkers from animal models to clinical studies, bridging the gap between experimental science and clinical medicine. We have discussed pathogenesis, temporal kinetics, relationships to neuro-imaging, and, most importantly, clinical applicability in order to provide a holistic perspective of how this could improve TBI diagnosis and predict clinical outcome in a real-life setting. We conclude that early and reliable identification of axonal injury post-TBI with the help of body fluid biomarkers could enhance current care of TBI patients by (i) increasing speed and accuracy of diagnosis, (ii) providing invaluable prognostic information, (iii) allow efficient allocation of rehabilitation services, and (iv) provide potential therapeutic targets. The optimal model for assessing TAI is likely to involve multiple components, including several blood biomarkers and neuro-imaging modalities, at different time points.

1. Introduction

Traumatic brain injury (TBI) is a global public health problem. It is amongst the leading causes of mortality in young people in developed countries [1], and many survivors of TBI suffer from chronic, persistent disabilities. In the United States (US) alone, 1.7 million individuals sustain a TBI every year, causing 52,000 deaths, and contributing to approximately 30% of all injury-related deaths [2]. In Europe, the annual incidence of TBI is estimated to be around 235 cases per 100,000 across 23 different European countries, with an average mortality of 15 per 100,000 [3]. Furthermore, the incidence of TBI continues to rise globally, and is predicted by the World Health Organisation (WHO) to become one of the leading causes of death and disability by 2020. Approximately 5.3 million victims of TBI in the US [4], and 7.7 million in the European Union [3], live with disabilities resulting from the initial traumatic injury. Indeed, TBI consists of both an acute insult and delayed changes resulting in chronic disability. The clinical consequences of the continuing pathological process is reflected by a range of neurological, cognitive, and neuropsychiatric deficits [5,6], with a devastating impact on the patient's quality of life and considerable cost to the healthcare system as a result of its chronic and heterogeneous nature. Cognitive and neuropsychiatric dysfunction post TBI is diverse, including attentional deficits [7], memory impairment [8], executive dysfunction [9], defective emotional recognition [10], agitation [11], depression [12], and language difficulties [13].

Amongst the key components of TBI pathophysiology is traumatic axonal injury (TAI), sometimes referred to as diffuse axonal injury (DAI) [14]. TAI is thought to contribute to the long-term manifestations of TBI and its understanding could be vital for predicting outcomes. TAI can affect large white matter tracts of the brain, which play a key role in

Abbreviations: Aβ, amyloid beta protein; AD, Alzheimer's disease; APP, amyloid precursor protein; cmTBI, complicated mild traumatic brain injury; CSF, cerebrospinal fluid; CT, computed tomography; C-tau, C-terminal tau fragment; CTE, chronic traumatic encephalopathy; DAI, diffuse axonal injury; DTI, diffusion tensor imaging; DTT, diffusion tensor tractography; FA, fractional anisotropy; GCS, Glasgow coma scale; GFAP, glial fibrillary acidic protein; GOS, Glasgow outcome scale; GOSE, extended Glasgow outcome scale; HARDI, high angular resolution diffusion imaging; ICP, intracranial pressure; MAP, microtubule associated protein; MBP, myelin basic protein; MRI, magnetic resonance imaging; NF-H, heavy neurofilament chain; NF-L, light neurofilament chain; pNF-H, phosphorylated heavy neurofilament chain; P-tau, hyperphosphorylated tau; PTSD, post-traumatic stress disorder; RPCQ, rivermead post concussion symptoms questionnaire; SBDP, spectrin breakdown products; SNTF, spectrin N-terminal fragment; SWI, susceptibility weighted imaging; TAI, traumatic axonal injury; TBI, traumatic brain injury; T-tau, total tau; VABS, vineland adaptive behaviour scales; WHO, World Health Organisation

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https://doi.org/10.1016/j.clineuro.2018.05.017
Received 27 January 2018; Received in revised form 5 May 2018; Accepted 14 May 2018
Available online 18 May 2018

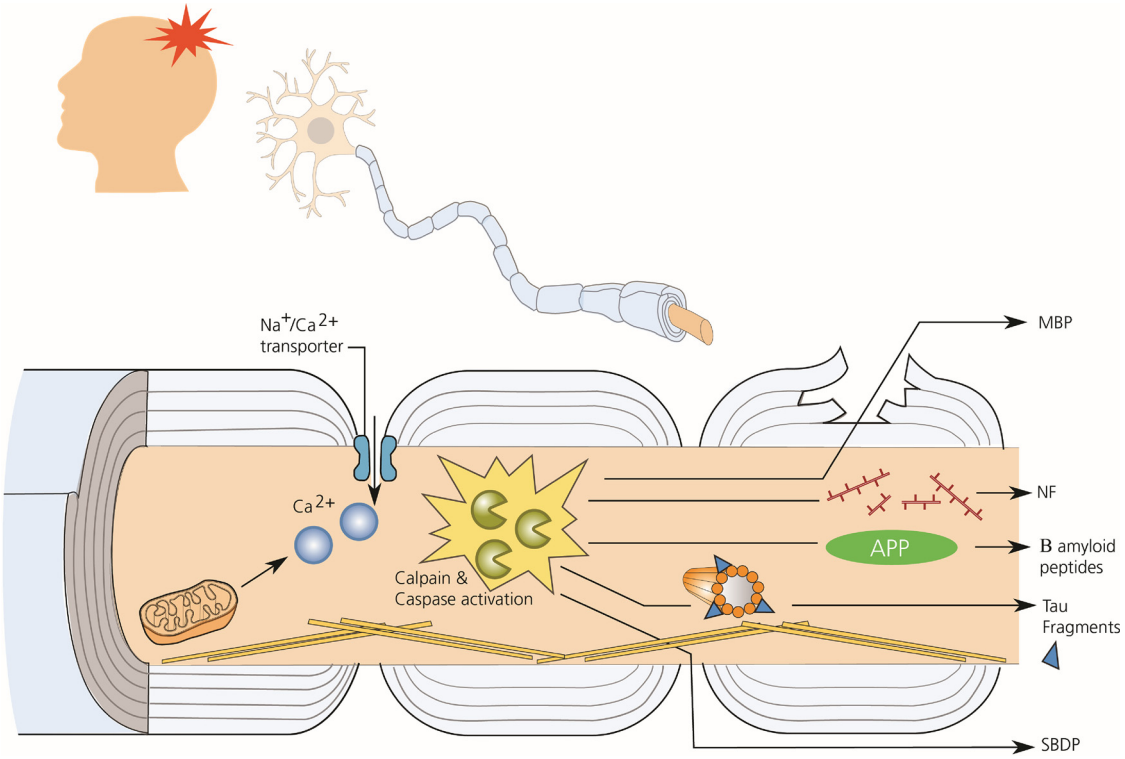

Fig. 1. Pathogenesis of TBI and the generation of biomarkers. Acceleration/ deceleration forces during TBI results in axonal undulations demonstrated above. Intra-axonal calcium concentrations then rise due to (i) influx from extracellular sources via transporters and due to mechanoporation, and (ii) intracellularly from mitochondria under oxidative stress following injury. Increase in intracellular calcium then causes activation of calpains and caspases, which then contribute to breakdown of: (i) myelin sheath, releasing MBP, (ii) neurofilaments, releasing NF chains of varying weights, (iii) APP, releasing B amyloid peptides of various weights, (iv) tau, a microtubule associated protein, into various fragments, and (v) spectrin, a cytoskeletal protein contributing to axonal morphology, into a collection of products known as SBDP. Abbreviations: APP- amyloid precursor protein, MBP- myelin basic protein, NF- neurofilaments, SBDP- spectrin breakdown products, TBI- traumatic brain injury.

communication between neuroanatomically distinct regions, facilitating connectivity and the formation of large-scale networks. Whilst axonal injury has long been appreciated on neuropathological examination of post TBI brains through observation of signs such as axonal 'retraction bulbs', varicosities, and white matter damage [14–16], understanding its functional effect on networks in the brain *in vivo* has yet to be fully explored. However, more recent advances in network science and imaging have enabled visualization and analysis of the large-scale structural and functional connectivity of the brain. The 'default mode network' and 'salience network' are examples of networks in the brain that have been extensively studied in the normal healthy population, which provides a reference point for comparisons in pathological disease states [17]. By studying the level of activity in such networks, the severity and specific distributions of DAI have been elegantly demonstrated to correlate with several cognitive impairments seen post-TBI, including attention [18], memory [19], and executive function [20] (for review see Sharp et al [17]).

Hence there is a need for additional clinical tools in the diagnosis and prognosis of TAI. By understanding the mechanisms underlying TAI, specific proteins released during axonal injury can be characterised and measured, serving as biomarkers.

This can potentially aid patient healthcare by (i) increasing speed of diagnosis in the acute setting owing to accessibility of potential fluid biomarker assays, (ii) improving accuracy of diagnosis since the short half life of several biomarkers mean that they reflect a specific part of the TBI process, (iii) providing more certainty to the outlook of victims on their future quality of life, (iv) focusing rehabilitation services to those with poorer prognostic indicators, and (v) providing potential therapeutic targets (see review by Hill et al [21]). Such biomarkers may exist in several bodily fluids including CSF, blood, and saliva [22]. Extensive research has been carried on the use of biomarkers in TBI, but there has been little appreciation for their use in the context of TAI.

From a clinical perspective, biomarkers can be any quantifiable product serving as a marker of insult. This definition, however, does not appreciate the direct pathophysiological link between the site and nature of injury, and biomarker measured. For instance, there are several biomarkers that may reflect TAI but cannot be realized conceptually from a pathological perspective. Neuron specific enolase (NSE), S-100B, and glial fibrillary acidic protein (GFAP) are all examples of promising biomarkers for TBI [23]. GFAP, as the name suggests, is of glial origin and shown to rise acutely in severe TBI, peaking during the first few days post injury before gradually decreasing, and demonstrated to predict clinical outcome [24]. This was corroborated by further evidence of the use of both serum S-100B, a calcium binding protein found in Schwann cells and glia, and GFAP as successful diagnostic indicators of TBI severity [25]. NSE, an enzyme found in the neuronal soma, along with S-100B and myelin basic protein (MBP), could be used to predict outcomes in paediatric TBI [26]. Whilst all of these biomarkers demonstrate clinical validity, their origins mean that they share no direct conceptual link with the axon itself. Indeed, evaluation of biomarkers in this fashion results in a broad classification of direct and indirect biomarkers of axonal injury. In this review we will focus on direct biomarkers of axonal injury because it facilitates an explanation of effect-causal relationships, and signifies the meeting point of basic scientific understanding and translation to the clinical setting. For a more general account of the use of biomarkers in TBI, please see the review by Kawata et al [27].

2. Methods

A multi-database (PubMed, Embase, Web of Science) literature review was performed between January 1980 and June 2017 for biomarkers of TAI. Search terms included combinations of: “neurofilament”, “NF”, “NF-H”, “NF-L”, “myelin basic protein”, “MBP”, “tau protein”, “tau fragments”, “amyloid”, “amyloid peptides”, “spectrin breakdown products”, “spectrin”, “SBDP”, “traumatic brain injury”, and “traumatic axonal injury”. All abstracts were reviewed for relevance to the chosen topic. Animal studies were assessed and included in background sections. Clinical studies were evaluated with respect to: (i) biomarkers, (ii) body fluids, (iii) kinetics (iv) outcome measures, and (v) findings. Pertinent findings were collated and each biomarker evaluated in the following format: (i) background, (ii) clinical studies, and (iii) kinetics.

3. Pathogenesis

Understanding of the pathogenesis of TAI at a cellular level underpins the rationale of using biomarkers (see Fig. 1). The axon is a slender projection from the neuronal soma that can extend over vast distances and ultimately facilitates the immense connectivity of the nervous system. Structural stability is provided by proteins such as neurofilaments, actin, and spectrin, whilst axonal transport occurs via microtubules, supported by microtubule associated proteins (MAP) such as tau protein.

There are several angles to consider in order to understand the pathophysiological processes underlying brain response to head trauma: (i) pattern of damage, (ii) cellular mechanisms of injury, (iii) histopathological correlates of injury, and (iv) timescales of events.

3.1. Cellular mechanism of injury

Axonal injury can be divided into primary and secondary axotomy, each resulting from distinct processes. Primary axotomy is mechanical breakage of the axon resulting from forces transmitted from traumatic impact. The mechanism involves initial disruption of the axonal transport system, causing axonal retraction, and eventually leading to axonal breakage, or axotomy [30]. However, primary axotomy is not considered a major component of the pathological changes seen in TAI [14,31].

Secondary axotomy, in contrast, is delayed, extensive, considerably more complex, and likely to result in the clinical manifestations seen in TAI. Rotational acceleration of the brain can cause stretching of white matter axons, leading to a dysregulation in sodium and potassium influx and efflux, respectively, culminating in an increase in intracellular calcium concentration [14,32]. The source of calcium influx is multiple, both from intracellular organelles and extracellular fluid, and has pleiotropic effects within the neuron. One effect involves stimulation of two systems: calpain-mediated necrosis and caspase-mediated apoptosis [33,34]. Animal models demonstrate that each has characteristic peaks of proteolytic activity [35,36] but calpain-mediated proteolysis predominates in the initial phase of severe TBI and shown to result in biomarker release during this phase when sampled in human CSF [37].

Proteolytic activity results in disruption of the axonal cytoskeleton and degradation of structural proteins, such as spectrin [38], neurofilaments [39], tau protein [40], and several others. Another effect of the raised intracellular calcium concentration is the disruption of mitochondrial membrane stability, thus altering neuronal metabolism [41] that is already under the strain of increased metabolic activity. Ultimately, a simple mechanical trigger results in a cascade of structural and metabolic insults. The subsequent axonal damage stimulates glutamate release that results in unregulated NMDA receptor-mediated excitation of surrounding neurons, causing further damage and finally resulting in secondary axotomy [42].

3.2. Histopathological correlates

The two main histopathological findings associated with axonal injury are axonal ‘retraction bulbs’ and varicosities [14,43]. Axonal bulbs are thought to represent the culmination of processes that occur during primary axotomy. Evidence shows that bulb formation occurs due to interruption in the axonal transport system and subsequent accumulation of amyloid beta precursor protein at axonal endings [43]. This underlies the use of beta-amyloid as a histological marker of axonal damage [44].

Whilst periodic axonal swellings, commonly referred to as varicosities, are also widely recognized as a hallmark of axonal injury, the intracellular structural correlate that manifests in this finding had not been clearly elucidated until recently. The process of TAI was visually captured using transmission electron microscopy and immunohistochemical methods on an *in vitro* model of axonal injury [43]. This model demonstrated that acute injury manifests as axonal undulations within two minutes, and later forms varicosities over the course of a few hours. Interestingly, this breakage was only partial as less than a third of microtubules in these focal regions were affected. Given the key role of microtubules in axonal transport, it follows that a degree of transport can still occur uninterrupted. The more complex mechanical forces inflicted on the brain during real-life TBI and the anatomical milieu in which axons reside are likely to affect the rate and location of formation of these swellings. Indeed, this is evidenced by the identification of a combination of axonal undulations, swellings, and bulbs in human acute severe TBI samples, suggesting the presence of additional factors that require further studies.

4. Biomarkers of axonal injury

Although there is a scarcity of studies evaluating TAI specifically with the use of sensitive imaging methods such as diffusion tensor imaging and its correlation with biomarkers of TAI, there is still extensive research on the use of biomarkers in TBI. The following is a review of the most prominent biomarkers arising from axonal origin: neurofilaments, MBP, tau protein, amyloid protein, and spectrin breakdown products (SBDP). The fact that these biomarkers are accepted to arise directly from axons suggests that their elevation in TBI is likely to be a reflection of the axonal component of TBI pathology and thus an indirect reference to TAI. Therefore, we will review the literature on both TAI and TBI where relevant.

4.1. Neurofilaments

4.1.1. Background

Neurons of the CNS contain type IV intermediate filaments, also known as neurofilaments (NF). These structures are approximately 10 nm in diameter, and are composed of an assembly of three chains: a light chain (NF-L), an intermediate chain (NF-M), and a heavy chain (NF-H), weighing 68 kDa, 150 kDa, and 190 to 210 kDa, respectively [45]. It follows from the pathogenesis of TAI that NF chains may be released after axonal damage and could serve as biomarkers of axonal injury. This is supported by evidence of their release in the context of axonal damage in multiple sclerosis and can be measured in various biological fluids [46], and altered expression in zebrafish models of TBI [47]. Phosphorylation of NF-H is specific to axons, forming the basis of interest in the use of phosphorylated NF-H (pNF-H) as a biomarker of axonal injury.

Both animal and human models of TBI support the involvement of NF in axonal injury. Intraneuronal redistribution of NF-H and NF-L, and colocalisation with beta-amyloid protein, has been demonstrated in animal models of TBI [44]. Similarly, a rat model of impact acceleration TBI correlated DTI measures of white matter damage with reduced levels of NF-L on histopathological examination in selected regions of the brain [48]. These findings are also reflected by serum levels of pNF-H in

early models of TBI in rats, which demonstrate a rise in serum pNF-H levels that also correlates with severity of injury [49]. More recently, serum and CSF levels of pNF-H were shown to correlate with severity of mechanical impact in a Marmarou impact acceleration model in rats [50]. Given existing evidence of this brain injury model correlating with TAI in particular [51], these findings reinforce a more specific role of NF-H in axonal injury. Also, post-mortem histopathological examination of the corpus callosum in human TBI brains showed reduced levels of NF-H and NF-L proteins, and an increase in calpain-mediated spectrin breakdown products [52], implying calpain-mediated proteolysis of neurofilaments in TAI. Taken together, there is considerable evidence from histopathological, biological fluid, and radiological perspectives in animal studies to support the use of NF as a clinical biomarker of axonal injury.

4.1.2. Clinical studies (see Tables 1 and 2)

NF was also utilised as a marker of TAI in clinical studies. One retrospective study [53] assessed the use of serum NF-L as a prognostic biomarker in TBI patients. Serum NF-L significantly enhanced predictive capability when combined with other clinical and radiological parameters. A more recent study [4] demonstrated that serum NF-L distinguishes between controls and severe TBI cohorts and correlates with 12 month GOS. Consistent with the previous study, it significantly enhanced the predictive capacity of existing models consisting of other clinical parameters [55]. Also, an ultra-sensitive detection method, the Simoa platform [56], with a lower detection limit for NF-L than the conventional ELISA assay was used. It is possible that the limitations of the ELISA assay in the first study [53] contributed to the lack of a significant correlation between NF-L and TAI. This is supported by a recent study [57], which clearly demonstrated that NF-L could distinguish between controls and severe TBI groups, with a significant correlation with DTI based measures of axonal white matter injury. Taken together, there is evidence that serum NF-L can be used for diagnostic purposes, as part of a prognostic model of TBI, and as an indicator of severity of TAI, but further studies are required with appropriate radiological modalities and more sensitive detection assays to assess the role of NF-L in TAI more definitively.

Phosphorylated NF-H (pNF-H), similarly to NF-L, shows potential for use as a biomarker of axonal injury. Serum pNF-H levels measured on the second to fourth days post TBI were significantly higher in patients with a poorer outcome at 6 months. Initial serum pNF-H was higher in patients with evidence of TAI on initial CT scans [58]. Similarly, serum pNF-H is significantly higher in adults with mild TBI with positive compared to negative CT scans [59]. Serum pNF-H at 24 h post TBI could be used as a predictive marker of death at 6 months and levels at 72 h post TBI could distinguish between unfavourable and favourable GOS at 6 months [61],. Interestingly, median pNF-H was higher in the TAI patients at all time points over a ten day period compared to the focal group, but there was no difference between patients with expansive contusions and those without such lesions [62]. This supports the theoretical assumption that a biomarker originating from the axonal cytoskeleton would be further raised in TAI and highlights the danger of assuming that biomarkers of axonal origin studied in the context of TBI are necessarily similar to what would be seen with TAI. Nonetheless, it is not unreasonable to postulate that whilst absolute levels may differ according to severity of axonal injury, the underlying trajectory may be similar.

4.1.3. Kinetics (see Table 3)

Serum NF-L and pNF-H appear to follow similar trajectories in clinical studies. Shahim et al. [54] recorded an acute rise in NF-L over the first 12 days post TBI with a peak at 12 days and a return to baseline at 1 year follow up. However, it is unclear whether NF-L continues to rise beyond 12 days and when exactly it returns to baseline. Animal studies suggest that NF-L may continue to rise up to 3 weeks post injury [63] but this requires corroboration in human studies. Rat models of TBI reported a peak in serum pNF-H at 2 days, followed by a decline over several days [64]. In human TBI, serum pNF-H rises acutely from 4 to 10 days post TBI but, again, its return to baseline is less clear [62]. Given the early evidence of temporal differences in accumulation of the three NF chains in a swine model of TBI [39], further studies are necessary to clearly elucidate the kinetics of NF-L and pNF-H.

4.2. Myelin basic protein (MBP)

4.2.1. Background

White matter axons are surrounded by a myelin sheath produced by oligodendrocytes centrally and Schwann cells peripherally. Whilst myelin is mainly formed by both lipid and protein components, MBP forms approximately 30% of the protein component. MBP has long been associated with axonal injury and degeneration in several animal models [65,66]. Furthermore, the diagnostic and prognostic potential of serum MBP in head injury patients has been explored at a very early stage [67]. CSF levels of MBP have been extensively studied in various neurological pathologies in the paediatric population and shown to correlate with clinical parameters [68,69]. Also, one early study [70] showed that CSF levels of MBP measured in the first week post TBI correlated with GOS at 7 days, 3 months, and 6 months post injury. A rat model of TBI demonstrated the proteolytic cleavage of MBP into various smaller fragments detected by mass spectrometry [71], supporting their potential use as biomarkers of axonal injury. Thus the theoretical, clinical, and experimental background of MBP indicates its potential utility as a biomarker of axonal injury.

4.2.2. Clinical studies (see Tables 1 and 2)

Zhang and colleagues [60] demonstrated that MBP, along with other biomarkers, can be used to differentiate between controls and severe TBI patients, survivors and non-survivors, and unfavourable and favourable GOS at 6 months. Su et al. [72] demonstrated that CSF-MBP could be used as diagnostic and prognostic indicator in paediatric victims of severe TBI by its ability to distinguish between controls and severe TBI groups and between abusive and non-abusive trauma. This latter finding is significant as it suggests the potential for the use of MBP from a forensic perspective, and corroborates earlier findings with serum MBP [73]. Since myelination is a physiological process occurring from infancy to adolescence [74], results from paediatric studies cannot be applied to adults as they may differ. Essentially, there is a lack of recent studies focusing on the role of serum MBP in traumatic axonal injury. This may be a result of the fact that MBP necessitates a severely compromised blood brain barrier to be released into the serum in larger amounts [75], and more sensitive detection assays have not been used to study MBP as of yet.

4.2.3. Kinetics (see Table 3)

Serum MBP also rises in the acute phase of TBI, with detection as soon as 1.5–8 h post injury [76] but remains elevated for up to two weeks [67]. However, more studies are required to elucidate the exact trajectory of MBP and its proteolytic processing in patients with TAI. In conclusion, whilst MBP has been identified at a very early stage as a potential axonal biomarker, little attempt has been made to use more sensitive detection assays and to correlate serum levels with TAI in the clinical setting.

4.3. Tau

4.3.1. Background

Tau protein is a MAP found in axons [77], weighing 48–68 kDa, and playing multiple roles in maintaining the intraneuronal mileu including axonal transport, cell signaling, and crosslinking microtubule networks [78,79]. The latter function is of importance in traumatic axonal injury: tau ultimately mediates the viscoelastic response to the rotational forces that trigger axonal injury discussed earlier [14,80,81]. Indeed,

Table 1
detailing findings from recent studies of biomarkers of axonal injury in patients with TBI.

Biomarker	Biological fluid	Injury	Findings	Reference
pNF-H	Blood	Paediatric TBI	• Serum pNF-H significantly increased when DAI on initial CT • Days 2 to 4 serum pNF-H significantly increased in GOS = 1 • Ratio of days 2 to 6 serum pNF-H and day 1 pNF-H significantly different between GOS = 1 and GOS > 1	[56]
		DAI and focal TBI	• Median serum pNF-H higher in DAI than focal TBI for 1 to 10 days post admission	[59]
		Mild TBI	• Significantly higher serum pNF-H on day 1 and 3 compared to control • Significantly higher in CT + mild TBI patients • Significant Inverse correlation between pNF-H and GCS	[57]
		Severe TBI	• All markers significantly higher in TBI than controls • All markers significantly higher in non-survivors than survivors, and unfavourable than favourable GOS score	[69]
		TBI	• 24 h serum pNF-H predicts death at 6 months • 72 h serum pNF-H predicts unfavourable outcome	[58]
NF-L	Blood	TBI	• NF-L combined with core parameters significantly enhanced outcome prediction at 6-12 months	[51]
		Severe TBI	• 24 h NF-L level separates severe TBI from control, and survivors from non-survivors, and predicts 12 month outcome • Significant correlation with CT Marshall score and pupil reactivity, but not GCS	[52]
		Severe TBI/ DAI	• Serum NF-L distinguishes TBI from controls • Higher serum NF-L correlates with lower FA	[55]
MBP	Blood	Severe TBI	• Serum levels remain high for 2 weeks post injury • Day 2 to 6 serum MBP higher in poor outcome than good outcome	[64]
		Paediatric TBI	• Time to peak significantly lower with non-abusive TBI • Time to peak correlated with GOS, VABS, and IQ at 6 months	[71]
	CSF	Paediatric severe TBI	• Higher levels for 5 days after injury • Mean concentration lower if < 1 year old, and in abusive head trauma	[70]
		Severe TBI	• All markers significantly higher in TBI than controls • All markers significantly higher in non-survivors than survivors, and unfavourable than favourable GOS score	[69]
Tau	Blood	Mild TBI	• Weak correlation between 6 hour C-tau and 3 month RPCQ and post concussive syndrome • Non significant predictor of 3 month post concussive syndrome	[89]
			• Higher serum tau in TBI than controls (non significant) • Could not discriminate post-concussive syndrome	[90]
			• C-tau has no significant correlation with head CT scan result or post-concussive syndrome	[91]
			• Significantly higher serum total tau post-concussion vs pre-season	[94]
			• No difference in pre- and post-season serum tau-A • Serum tau-C significantly higher post- vs pre-season • Serum tau-A correlates with severity of injury and return to play time	[83]
		Self-reported TBI	• Most participants sustained injury at least 18 months prior • Significant positive correlation between T-tau levels and frequency of TBI, post-concussion syndrome symptoms, and PTSD symptoms • No correlation with severity of depression post TBI	[95]
		TBI	• Serum tau at < 24 h and Day 30 post-TBI could separate cmTBI cohort from controls, with optimal accuracy at < 24 h • Significant correlation with post-traumatic amnesia and with radiological measures of injury severity	[96]
	CSF	Head injury; DAI	• Significantly higher CSF C-tau levels in closed head injury vs neurological controls • Association between clinical status (GCS) and CSF c-tau levels	[86]
		Severe TBI	• Day 1 CSF C-tau higher in TBI than controls, and correlates with discharge GCS and GOS • Initial C-tau distinguishes favourable/ unfavourable GOS and normal/ raised ICP	[87]
			• Tau significantly higher in TBI than controls (dementia and headache) • Normal tau levels when CSF collected > 43d post trauma • Significant negative correlation between time elapsed since head trauma and CSF collection • No correlation between tau and initial GCS or patient age	[117]
			• Higher CSF tau in TBI than NPH controls • Correlation between initial CSF tau and 1 year GOSE • Distinguishes good vs bad GOSE, and mortality	[88]

(*continued on next page*)

Table 1 (*continued*)

Biomarker	Biological fluid	Injury	Findings	Reference
Aβ peptides	Blood	Severe TBI	• Measurements taken during first week post TBI • Comparing TBI patients with controls: plasma Aβ42 significantly higher • Comparing survivors and non-survivors: plasma Aβ42 significantly lower	[119]
		TBI in young military personnel	• TBI cohort sustained injury on average 20.6 months prior • Significantly increased Aβ40 and reduced Aβ42/Aβ40 ratio in TBI compared to controls • Aβ42 increased in TBI but non-significant finding	[123]
		TBI	• Day 30 serum Aβ42 separates controls from cmTBI • Significant correlation between Day 30 serum Aβ42 and clinical outcome at 6 months • No difference between severity of injury identified radiologically or post-traumatic amnesia	[96]
	CSF	Severe TBI	• Significantly lower levels of Aβ40, higher levels of Aβ42, and lower Aβ40/Aβ42 ratio in TBI compared to controls	[115]
			• Aβ42 significantly lower than controls (headache and dementia) at all time points • No correlation between time since trauma and CSF collection time or with initial GCS • Significant correlation with patient age • Significant correlation between Aβ42 and GOS outcomes	[117]
			• Collected within 72 h of TBI; higher levels of Aβ oligomers in patients with better outcome • Significant correlation between CSF Aβ oligomers and GOS score and disability rating	[118]
			• Measurements taken during first week post TBI • Comparing TBI patients with controls: CSF Aβ42 significantly lower • Comparing survivors and non survivors: CSF Aβ42 significantly higher	[119]
αII SBDP	Blood	Mild TBI	• Day 1 αII SNTF correlates with white matter changes in selected CNS tracts, impaired cognition at 3 months post injury, and recovery from cognitive deficits	[130]
			• Mean serum αII SNTF significantly elevated at 1, 12, 36, 144 h post-concussion compared with preseason baseline, but unchanged after concussion-free training • Serum αII SNTF returned to baseline at the time of return to play • 36 h serum αII SNTF distinguishes between < 6 and > 6 days return to play	[131]
	CSF	Severe TBI	• αII SBDP significantly increased compared to controls at many time points • 12 h SBDP150/145 levels correlate with severity of injury, CT results, and outcome at 6 months	[129]
			• Median AUC and maximum concentration for SBDP150 and SBDP145 significantly greater in patients with static or worsening GCS scores than improving GCS scores at 24 h post injury • Positive correlation between number of hours of raised ICP and AUC for all αII SBDP	[35]

Abbreviations: Aβ- amyloid beta protein, A-tau- tau fragment, AUC- area under the curve, cmTBI- complicated mild traumatic brain injury, CNS- central nervous system, CSF- cerebrospinal fluid, CT- computed tomography, C-tau- C-terminal tau fragment, DAI- diffuse axonal injury, FA- fractional anisotropy, GCS- Glasgow coma scale, GOS- Glasgow outcome scale, GOSE- extended Glasgow outcome scale, ICP- intracranial pressure, IQ- intelligence quotient, MBP- myelin basic protein, NF-L- light neurofilament chain, pNF-H- phosphorylated heavy neurofilament chain, NPH- normal pressure hydrocephalus, RPCQ- Rivermead Post Concussion Symptoms Questionnaire, SBDP- spectrin breakdown products, SNTF- spectrin N-terminal fragment, TBI- traumatic brain injury, T-tau- total tau, VABS- Vineland Adaptive Behaviour Scales.

once the mechanical buffering capacity of tau is exceeded, axonal stretch can destroy the organisation of microtubules [82] and set off the cascade of events culminating in secondary axonal injury. The phosphorylation and proteolytic cleavage of tau protein following TBI is a complex process involving several enzymes that can generate a range of products that can aid neuronal function or possess neurotoxic properties [83]. Neurotoxic fragments may form a key component of neurofibrillary tangles seen in several neurodegenerative diseases [84], and the C-terminal fragment of tau (C-tau) is one example of a neurotoxic fragment seen in traumatic brain injury [85]. Recent evidence [86] that hyperphosphorylated tau (P-tau) accumulates in several brain regions associated with working memory in a mouse TBI model indicates that it may also play a role in long term cognitive deficits seen in TBI, highlighting its potential as a prognostic biomarker. There is also some evidence for the use of tau protein as a blood biomarker of chronic traumatic encephalopathy, a neurodegenerative condition occurring as a consequence of repetitive mild TBI, in sportsmen [87].

4.3.2. Clinical studies (see Tables 1 and 2)

It has been established at an early stage that C-tau is a specific marker of TBI when raised levels were found in the CSF of head injury patients but not in other neurological pathologies or controls [88]. The same group [89] then measured initial levels of C-tau in the CSF of patients with severe TBI and compared it with clinical outcome measured by GOS at discharge. Not only was C-tau significantly correlated with clinical outcome, but was also superior to GCS in predicting GOS at discharge. Whilst this early study lends some credibility to the use of C-tau as a biomarker, it is not informative of the long-term outcome of this patient cohort, which is a key aim of using biomarkers. However, a later study [90] found that levels of tau protein in ventricular CSF on days 2–3 post TBI distinguished between patients with favourable and unfavourable 1 year outcomes measured by GOSE (Extended Glasgow Outcome Scale). Thus there appears to be a differential role between tau and products of tau cleavage in predicting outcome in TBI patients.

Evidence on the use of serum levels of tau protein and its fragments as biomarkers in TBI have been mixed. Early studies [91,92] failed to elucidate a significant correlation between serum tau or C-tau and clinical symptoms following mild TBI, although serum tau protein levels were higher in patients compared to controls [92]. A later study [93] measured C-tau in the blood of mild TBI patients but also found no significant correlation with initial head CT or self-reported outcome measures. Larger sample size and more sensitive imaging methods may reveal more about the role of C-tau in traumatic axonal injury. It is possible that CSF levels are more apt than serum levels for its use as a biomarker, and that it is better suited to prognostic indication in severe TBI rather than mild TBI. However, it is difficult to reconcile this proposition with existing knowledge regarding the generation of C-tau in the underlying pathogeneic process. Indeed, with the advent of more sensitive immunoassays for detection of tau proteins [94,95], more positive results are emerging. A multicenter study [96] demonstrated that professional ice hockey players had significantly higher levels of serum tau protein at all sampling times after concussion, extending as far as 144 h post injury, than their preseason baseline. Also, 144-h serum tau levels correlated with severity of post concussion syndrome, and could be used to predict return to play. The fact that serum tau was superior to neuron specific enolase and S-100B as a biomarker of concussion in this study could be an indication of the importance of axonal pathology in the constellation of symptoms seen in mild traumatic brain

Table 2
detailing clinical use of biomarkers of axonal injury following TBI, all findings include blood biomarkers except where indicated.

Time since injury	Injury	Biomarker	Clinical Indication	Statistics	References
1 h	Mild TBI (sport-related concussion)	T-tau	Diagnosing sport related concussion (by comparing post concussion 1 h r T-tau with post friendly game T-tau)	*AUC*: 0.80 *95% CI*: 0.65-0.94	[94]
			Diagnosing post concussive syndrome lasting more than 6 days	*AUC*: 0.91 *95% CI*: 0.81-1.00	
		Tau	Diagnosing sport-related concussion	*AUC*: 0.74 *95% CI*: 0.61-0.86	[131]
< 6 h	Severe TBI	pNF-H	Prediction of unfavourable outcome at 6 months post injury	*Cut-off*: 833.3 pg/mL *Sensitivity*: 83.3% *Specificity*: 61.1% *AUC*: 0.759 *P-value*: 0.056	[69]
			Prediction of death at 6 months post injury	*Cut-off*: 940.4 pg/mL *Sensitivity*: 75.9% *Specificity*: 74.4% *AUC*: 0.815 *P-value*: 0.202	
		MBP	Prediction of unfavourable outcome at 6 months post injury	*Cut-off*: 15.6 µg/mL *Sensitivity*: 70.8% *Specificity*: 66.7% *AUC*: 0.781 *P-value*: 0.135	[69]
			Prediction of death at 6 months post injury	*Cut-off*: 16.9 µg/mL *Sensitivity*: 69.0% *Specificity*: 80.8% *AUC*: 0.805 *P-value*: 0.176	
		Tau	Prediction of unfavourable outcome at 6 months post injury	*Cut-off*: 303.2 pg/mL *Sensitivity*: 79.3% *Specificity*: 79.3% *AUC*: 0.808 *P-value*: 0.212	[69]
			Prediction of death at 6 months post injury	*Cut-off*: 282.9 pg/mL *Sensitivity*: 81.2% *Specificity*: 72.2% *AUC*: 0.782 *P-value*: 0.119	
12-36 h	Mild TBI	SNTF	Diagnosing post-concussive syndrome lasting longer than 6 days	*AUC*: 0.87 *95% CI*: 0.79-0.96	[131]
< 24 h	TBI	Tau	Distinguishing cmTBI from controls	*AUC*: 0.9013	[96]
24 h	Mild TBI	pNF-H	Categorising CT- and CT + mild TBI patients	*Cut off*: 110.5 pg/mL *Sensitivity*: 100% *Specificity*: 100% *P-value*: 0.0001*	[57]
	Severe TBI	NF-L	Separates survivors from non-survivors	*Cut off*: 411 pg/mL *Sensitivity*: 71% *Specificity*: 88% *LR+*: 6.0	[52]
			Separating patients with favourable (GOS 1-3) and unfavourable (GOS 4-5) outcome	*Cut-off*: 216 pg/mL *YI*: 0.38 *Sensitivity*: 83% *Specificity*: 56% *LR+*: 1.9	
			Separating severe TBI patients from controls	*Cut off*: 24.0 pg/mL *YI*: 0.96 *Sensitivity*: 97% *Specificity*: 96% *LR+*: 23.0	
	GCS = / < 13	pNF-H	Prediction of death at 6 months post injury	*Cut-off*: 240 pg/mL *AUC*: 0.930	[58]
36 h	Mild TBI	SNTF	Diagnosing sport-related concussion	*AUC*: 0.76 *95% CI*: 0.63-0.90	[131]
			Diagnosing sport-related concussion that delayed return to play for at least 6 days	*AUC*: 0.85 *95% CI*: 0.73-0.97	
48 h	Paediatric TBI	pNF-H	Prediction of unfavourable outcome (GOS = 1)	*Cut off*: 117.1 µg/L *Sensitivity*: 71.4% *Specificity*: 83.8% *AUC*: 0.764 *P-value*: 0.028*	[56]

(*continued on next page*)

Table 2 (*continued*)

Time since injury	Injury	Biomarker	Clinical Indication	Statistics	References
48-72 h	Severe TBI	T-tau (CSF)	Prediction of mortality	*Cut off*: 2126 pg/mL *Sensitivity*: 100% *Specificity*: 81.5% *AUC*: 0.934	[88]
			Discriminating between good (GOSE 1-4) and bad (GOSE 5-8) outcomes at 1 year	*Cut off*: 702 pg/mL *Sensitivity*: 83.3% *Specificity*: 69% *AUC*: 0.814	
< 72 h	Severe TBI	Aβ oligomers (CSF)	Prediction of poor outcome at 6 months	*AUC*: 0.8750 *95% CI*: 0.698-1.053	[118]
72 h	Mild TBI	pNF-H	Categorising CT- and CT + mild TBI patients	*Cut off*: 77.5 pg/mL *Sensitivity*: 100% *Specificity*: 96.43% *P-value*: 0.0001*	[57]
	GCS = / < 13		Prediction of unfavourable outcome (vegetative state or death) at 6 months post injury	*Cut-off*: 80 pg/mL *AUC*:?	[58]
144 h (Day 6)	Mild TBI	T-tau	Diagnosing post concussive syndrome lasting more than 6 days	*AUC*: 0.76 *95% CI*: 0.58-0.94	[94]
Day 30	TBI	Tau Aβ42	Distinguishing cmTBI from controls	*AUC*: 0.8225 *AUC*: 0.8362	[96]
> 18 months	Self-reported TBI	T-tau	Identification of self-reported TBI	*AUC*: 0.74 *95% CI*: 0.61-0.68 *P-value*: 0.007	[95]
			Identification of sustaining 3 or more TBI	*AUC*: 0.73 *95% CI*: 0.61-0.86 *P-value*: 0.003	
			Identification of medically validated TBI	*AUC*: 0.69 *95% CI*: 0.51-0.89 *P-value*: 0.007	
Time independent	Severe TBI	Aβ42 (CSF)	Prediction of poor outcome (GOS1-3)	*Cut off*: 230 pg/mL *Sensitivity*: 100% *Specificity*: 82%	[117]

Abbreviations: Aβ- amyloid beta protein, AUC- area under curve, CI- confidence interval, cmTBI- complicated mild traumatic brain injury, CSF- cerebrospinal fluid, CT- computed tomography, GCS- Glasgow coma scale, GOS- Glasgow outcome scale, GOSE- extended Glasgow outcome scale, LR- likelihood ratio, MBP- myelin basic protein, NF-L- light neurofilament chain, pNF-H- phosphorylated heavy neurofilament chain, SNTF- spectrin N-terminal fragment, TBI- traumatic brain injury, T-tau- total tau, YI- Youden's index.

Table 3
detailing kinetic profile of biomarkers of axonal injury following TBI.

Biomarker	Injury/ model	Biological fluid	Kinetics	Reference
pNF-H	TBI- DAI and focal	Blood	Serum NF-H increases from day 4 to day 10 • 0.263 – 1.325 ng/L in DAI • 0.103 – 1.108 ng/L in focal	[59]
NF-L	TBI		Upward trend over course of 14 days	[51]
	Severe TBI		Significantly higher levels than control for first 12d, peak at 12d, and normalised over course of 1y	[52]
Aβ42	TBI	Blood	Significantly elevated for up to 90 days post TBI with a peak at 30 days	[96]
	Severe TBI		Significant elevation at all time points during first week post TBI	[119]
	Severe TBI	CSF	Significant reduction on Day 1 and 3, and Day 5–7 during initial week post-TBI	[119]
Tau	Mild TBI	Blood	Peak during first hour post concussion, significant decline in following 12 h, and non-significant reductions from 12 to 144 h	[94]
	TBI		Significantly elevated for up to 90 days post TBI with a peak at < 24 h followed by a decline over following 90 days	[96]
	Severe TBI	CSF	In two patients with poor outcome (GOS-2 and 3): peak between Day 5 and 15 post-TBI followed by decline, normalises by Day 43	[117]
C-tau	Severe TBI	CSF	Initial peak and decline over first 3 days	[87]
A-tau,	Mild TBI	Blood	Tau-A peaked at 12 h post concussion (non significant),	[83]
αII SBDP	Severe TBI	CSF	SBDP150 and SBDP145 peak at 6 h and remain elevated during first 24-72 h; SBDP120 elevated at all time points except 24 h post injury Half life of SBDP150, SBDP145, and SBDP120 was 24, 23, and 40 h respectively	[35]
			Marker elevation: SBDP150 up to 24 h, SBDP145 up to 72 h, and SBDP120 at all time points except 24 h post injury	[129]

Abbreviations: Aβ- amyloid beta protein, A-tau- tau fragment, CSF- cerebrospinal fluid, C-tau- C-terminal tau fragment, DAI- diffuse axonal injury, GOS- Glasgow outcome scale, NF-H- heavy neurofilament chain, NF-L- light neurofilament chain, pNF-H- phosphorylated heavy neurofilament chain, SBDP- spectrin breakdown products, TBI- traumatic brain injury.

injury. A later study by the same group [85], studied the use of tau fragments for the same purpose, and found that A-tau, another product of proteolytic cleavage of tau protein, correlated both with duration of clinical symptoms and predicting safe return to play.

Olivera et al. [97] assessed the relationship between plasma concentrations of T-tau and both TBI and its sequelae in military personnel. The study model involved a TBI cohort defined by self-reported measures of TBI, of which over a third had medical evidence of TBI, and matched controls. The majority of self-reported TBI participants had sustained injury at least 18 months prior. Significant positive correlations were found between T-tau concentrations and frequency of TBI, post-concussion syndrome symptoms, and post traumatic stress disorder symptoms but not severity of depression. Whilst these findings demonstrate the potential utility of T-tau as a biomarker of injury and neuro-cognitive deficit following TBI, the lack of medically diagnosed TBI in the TBI cohort suggests that further research is required for validation of the findings. Indeed, individuals of the self-reported TBI group with accompanying medical evidence of TBI displayed a significantly higher serum T-tau concentration than the self-reported group alone.

A more recent study [98], assessing GFAP, Aβ42, and tau protein, demonstrated a diagnostic and prognostic role for plasma levels of tau protein in patients with TBI. Both < 24 h and Day 30 post TBI serum levels were capable of separating complicated mild TBI (cmTBI) cohort from controls, with optimal accuracy at < 24 h. Whilst the other biomarkers measured also distinguished between these groups, the trajectory of plasma tau protein over 90 days also differed between cmTBI and moderate TBI cohorts, with the former group showing a steady decline from initial time points to 90 days and the latter group remaining equally elevated for up to 30 days before declining at 90 days. The prognostic utility of plasma tau was demonstrated by its correlation with both post-traumatic amnesia and radiological measures of injury severity.

Taken together, existing evidence on the use of tau protein is encouraging but further work is required to (i) compare CSF and serum levels of total tau and tau fragments during TBI, and observe any differences in their trajectories, (ii) use more sensitive imaging modalities for axonal injury such as MRI and DTI to assess correlation with tau protein, and (iii) elucidate the relationship between tau and long term neurocognitive outcomes in TAI assessed by neuropsychological tools. Future avenues include the use of tau as a therapeutic target, evidenced by one study [99] demonstrating a possible attenuation in the pathological effects of diffuse axonal injury in a rat model by reducing loss of tau protein, and other studies [100,101] blocking P-tau in animal TBI models to prevent pathological sequelae of the disease.

4.3.3. Kinetics (see Table 3)

Shahim et al [96] measured a peak in serum total tau concentrations at 1 h post concussion and a significant drop at 12 h followed by persistent elevation compared to baseline until 144 h post injury. One study [98] demonstrated an acute rise in tau protein < 24 h post injury and a decline over the following 90 days, but remained significantly elevated at all time points. This suggests that serum tau rises earlier than other axonal biomarkers of traumatic injury, which may be a reflection of its role as a mechanical buffer whose threshold is crossed to inflict axonal injury. Tau fragments show a different pattern [85] with A-tau peaking at 12 h post injury, although this was not significantly different to other time points up to 144 h, and C-tau was significantly elevated following injury compared to baseline but not demonstrating any visible peak. This delay between total tau and tau fragments may reflect underlying processes such as proteolytic cleavage and subsequent release into the bloodstream.

4.4. Amyloid protein

4.4.1. Background

Amyloid precursor protein (APP) accumulates predominantly as a result of disruption to axonal transport after trauma [102], although there is also extensive evidence of increased expression of the APP gene following TBI [103–105,102,106–108]. The two major isoforms of amyloid β (Aβ) yielded by proteolytic cleavage of APP are Aβ42 and Aβ40. Although Aβ peptides are regarded as a physiological component of human interstitial fluid [109], potentially released as a by-product of neuronal metabolism [110], cytotoxic properties have been attributed to the aggregation of Aβ into an insoluble form. The relevance of Aβ to axonal injury is exemplified by a recent study involving post-mortem examination of patients who had previously undergone surgical leucotomy for the treatment of schizophrenia and survived for a minimum of 40 years after the procedure [117]. The procedure involves the bilateral severance of axons in the prefrontal cortex, providing an excellent model of severe axonal damage in 'iatrogenic' TBI. Neuropathological examination demonstrated evidence of white matter damage and a combination of P-tau and Aβ accumulation in the grey matter surrounding the site of axonal injury, which was both specific to the site of injury and absent in matched, non-leucotomised controls. These findings are particularly relevant due to their similarities to pathological signs seen in CTE, and supports the current theory that CTE is a result of chronic axonal damage occurring post-TBI.

Aβ has been extensively studied as a marker of neuronal injury in several different contexts. Concentrations of Aβ are altered in severe brain injury in a wide range of biological environments including brain tissue and extracellular fluid [118–120]. Notably, CSF Aβ has been referred to as a 'state marker' of Alzheimer's disease due to its correlation with AD pathology [121,122]. Taken together, these studies demonstrate that Aβ peptides are promising candidates for use as biomarkers of axonal injury in TBI.

4.4.2. Clinical studies (see Tables 1 and 2)

The use of amyloid-related proteins, such as APP and Aβ, as clinical biomarkers of TBI is supported by several early studies demonstrating changes in CSF concentrations following TBI [123,124]. In addition, there was early evidence that CSF Aβ42 levels correlated significantly with clinical outcome following TBI, but no correlation with clinical status at admission [125,126]. Mondello et al [127] studied the relationship between severe TBI and Aβ42 levels measured in both CSF and plasma. Irrespective of biological fluid, Aβ42 levels distinguished severe TBI patients from controls, and survivors from non-survivors. However, an interesting difference in the dynamics between CSF and plasma measurements was demonstrated: Aβ42 was significantly lower in CSF and significantly higher in plasma when the TBI cohort was compared to controls, and when non-survivors were compared to survivors. Two theories may reconcile these findings: (i) reduced CSF levels may reflect amyloid plaque deposition, and (ii) compromised blood brain barrier resulting in release of Aβ42 into the bloodstream with a concomitant decrease in CSF. There is increasing evidence to support the latter hypothesis as blood brain barrier compromise has been linked to clinical outcome following TBI [128]. Neither CSF nor plasma levels of Aβ42 were capable of differentiating between focal lesions and diffuse injuries identified on CT, which is inconsistent with studies demonstrating raised extracellular fluid concentration of Aβ42 following diffuse brain injury on cerebral microdialysis [120,129]). This raises significant questions regarding the use of Aβ42 as a marker of traumatic axonal injury. It is possible that this simply reflects a biological environment dependent difference, as interstitial fluid sampling is thought to be a more focal representation of biomarker dynamics whilst CSF is considered more global [130]. Alternatively, it could be a result of the inadequacy of CT in identification of diffuse brain injury.

A cross-sectional study of young military personnel with a history of deployment-related TBI [131] evaluated the role of serum Aβ42 and

Aβ40 as markers of chronic neurological status. Participants with a positive history of TBI were assigned to the TBI cohort, with a mean period of 20.6 months since injury. Compared to matched controls, the TBI cohort demonstrated a significantly increased Aβ40 concentration and significantly reduced Aβ42/ Aβ40 ratio. Whilst Aβ42 was increased in the TBI cohort, this finding was non-significant. Considering Aβ as a key pathological feature of neurodegeneration, the relationship demonstrated between Aβ and TBI highlights the persistent neuronal injury that outlasts the acute traumatic insult. Furthermore, the increased incidence of depression, post-traumatic stress disorder, and post-concussion syndrome in the TBI cohort suggests a potential role for Aβ in the prediction or diagnosis of cognitive deficits following TBI, although no direct relationship was found in this study.

A recent study measured GFAP, tau, and Aβ42 proteins in the serum of TBI patients at < 24 h, 30 days, and 90 days post trauma [98]. Both the diagnostic and prognostic potential of Aβ42 was demonstrated by (i) the use of day 30 serum Aβ42 to separate controls from cmTBI, defined as GCS > 13 on admission with evidence of brain injury on CT scan, and (ii) a significant correlation between day 30 serum Aβ42 and clinical outcome at 6 months post injury measured by GOSE scores. However, Aβ42 could not distinguish between different severities of injury measured radiologically or the presence of post-traumatic amnesia. Taken together, Aβ peptides appear to be optimal for use in the sub-acute or chronic stages of TBI as a long-term prognostic indicator. More studies with sensitive neuroimaging modalities for detection of DAI are required to correlate Aβ more definitively with axonal injury in the clinical setting.

4.4.3. Kinetics (see Table 3)

One study [98] demonstrated that Aβ42 is elevated for up 90 days post TBI and peaks at 30 days, in contrast to more acute biomarkers. This conflicts with prior evidence [127] of a significant reduction in CSF Aβ42 at several time points, and a significant elevation in plasma Aβ42 at all time points during the initial week. More studies are required to elucidate the trajectory of different Aβ peptides during the initial phase of TBI, and find a coherent explanation for the differences in concentration and temporal changes between CSF, plasma, and interstitial fluid for each.

4.5. Spectrin breakdown products

4.5.1. Background

Spectrin is widely known as a structural protein that helps maintain cell shape and membrane integrity [132] and α-II spectrin fulfills this role as a component of the axolemmal cytoskeleton. The breakdown of α-II spectrin after axonal injury has been elucidated, and involves the calpain (calpain-1, calpain-2) and caspase (caspase-3) systems [34]. The protein is cross-linked and sequestered in the cristae of swollen mitochondria, as a consequence of the calcium influx in traumatic axonal injury, prior to cleavage by spectrin protease. This process is augmented by the cytochrome c release by the damaged mitochondria [133]. This results in the generation of spectrin breakdown products (SBDP). A recent study [134] demonstrated the involvement of spectrin in disrupting axonal transport mechanisms in a rat optic nerve stretch model, implicating its contribution to the secondary axotomy process described earlier. Also, another study [135] elucidated the breakdown of βII-spectrin under various conditions in *in vitro* cell culture and *in vivo* rat models in an attempt to recapitulate the neurotoxic environment created by TBI. Again, SBDP fragments, more specifically βSBDP, were found to correlate with calpain or caspase mediated proteolysis. Neuropathology of human and swine models of both mild and severe TBI demonstrate that SBDP does correlate with axonal injury, but suggests a more heterogeneous nature to axonal injury than the existing gold standard model with APP [136].

4.5.2. Clinical studies (see Tables 1 and 2)

There have been numerous studies on levels of SBDP in CSF of patients with TBI. Indeed, studies [37,137] have demonstrated raised levels of SBDP generated by calpain-mediated proteolysis (SBDP-150 and SBDP-145) in the CSF of patients with severe TBI. Furthermore, its diagnostic and prognostic potential is demonstrated by its correlation with severity of injury and 6 month GOS [137]. The role of serum spectrin N-terminal fragment (SNTF) as a biomarker of axonal injury and outcomes in mild TBI patients was demonstrated by a seminal paper [138]. Here, plasma SNTF samples at a median 14 h post injury were raised in a subset of mild TBI patients but not in controls. The key finding was that, when participants were stratified as SNTF positive or negative, the former group showed significant changes in the corpus callosum and uncinate fasciculus on DTI, which was performed within 96 h of injury. Furthermore, cognitive deficits were apparent from various neuropsychological tests at 4-days, 1-month, and 3-months post injury in the SNTF positive group. The same group [139] also demonstrated the application of SNTF to predicting post concussion symptoms in professional ice hockey players, further supporting its role as a prognostic indicator. The fact that initial serum SNTF correlates with both imaging and long term cognitive tests of axonal injury in patients with initial CT negative scans provides strong evidence that biomarkers are key in helping build a prognostic model for TBI that is currently lacking.

4.5.3. Kinetics (see Table 3)

CSF studies show that different SBDP fragments may have different temporal profiles. Brophy et al [37] effectively characterised the trajectory of both calpain (SBDP-150, SBDP-145) and caspase (SBDP-120) mediated SBDP in the CSF of severe TBI patients. Whilst all fragments increased within 6 h of injury, which correlates with in vitro models of calpain activity in TBI [140], SBDP-150 and -145 peak at 34 h and remain elevated for 24–72 h but SBDP-120 peaks at 56 h and remains elevated for up to 5 days. However, more studies are required to elucidate the trajectory of SBDP in serum following TBI. In summary, SBDP are promising when compared to other existing biomarkers of axonal injury as they (i) provide a window for observing the unfolding of different mechanisms of pathogenetic processes in TAI, (ii) serve as a prognostic indicator in TBI, and (iii) correlate with axonal injury in neuropathological specimens. One major drawback of SBDP, however, is the high expression of spectrin in erythrocytes [141], which impacts its specificity as a blood biomarker of axonal injury.

5. Neuroimaging for TAI diagnosis

In the clinical setting, CT is the most rapid, safe, and accessible option for evaluating TBI [142], providing valuable information on focal injuries and intracranial bleeding that may necessitate immediate neurosurgical intervention [143,144]. Whilst CT can identify severe TAI through the presence of smaller haemorrhagic lesions, its ability to identify fine lesions associated with TAI is lacking [145]. Conventional MRI is more capable of detecting these subtler lesions and white matter damage [146] but is lacking in practical feasibility in the acute setting. Indeed, there is extensive evidence of post-mortem examination of TBI brains demonstrating signs of axonal injury that was undetected by prior neuroimaging [147–150]. Therefore, both CT and low resolution MRI are suboptimal for identification of the TAI spectrum [151,152]

Diffusion tensor magnetic resonance imaging (DTI) is a non-invasive and sensitive method of assessing white mater structure and integrity. DTI measures the direction of diffusion of water molecules, meaning it can exploit the unidirectional flow of water along normal white matter tracts to differentiate it from the disorganization seen in white matter pathology, measured by a reduction in fractional anisotropy (FA) [153]. Such changes on DTI have been correlated with traumatic axonal and white matter injury on histopathological studies in animal models of TBI [154–156,48,157] and with long-term cognitive deficits in human

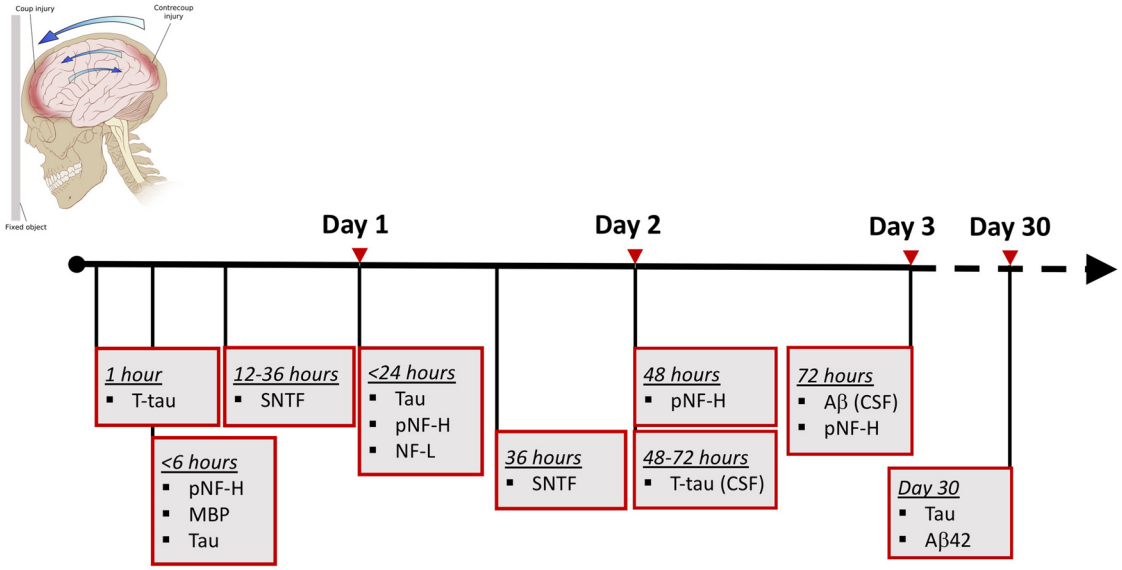

Fig. 2. Summarises timepoints following TBI at which serum biomarkers (unless stated as CSF) can yield useful clinical information. For details of whether this is of prognostic or diagnostic use, and which severity of TBI, see Table 3. Abbreviations: Aβ- amyloid beta protein, CSF- cerebrospinal fluid, MBP- myelin basic protein, NF-L- light neurofilament chain, pNF-H- phosphorylated heavy neurofilament chain, SNTF- spectrin N-terminal fragment, TBI- traumatic brain injury, T-tau- total tau.

TBI patients [158–162]. Furthermore, recent evidence demonstrated a correlation between levels of tau protein measured in cerebral microdialysis samples by sequential ELISA methods and FA measures on DTI in patients with severe TBI [163]. Drawbacks of DTI include difficulty of access in the acute phase of TBI and a limited number of times for usage [164]. Also, there is still a degree of uncertainty with interpreting FA since comparison of DTI and histological findings in a rat TBI model suggest that the reactive gliosis occurring with brain injury contributes to an increase in FA [165], which questions the fidelity of the relationship between white matter and FA.

Another imaging modality based on DTI is diffusion tensor tractography (DTT), which allows a three dimensional appreciation of central nervous system (CNS) tracts [166–168], and is of growing interest in the context of TAI [169]. Indeed, several studies have demonstrated its use in detecting lesions in many CNS tracts including the corticospinal tract, spinothalamic tract, medial lemniscus, fornix, and cingulum [170–175]. A recent case study [176] demonstrated the utility of DTT in identifying pathological correlates of delayed neurological symptoms following mild TBI. For example, identification of damage to spinothalamic tracts explained abnormal pain sensation experienced by the patient. The fact that DTT detected damage to various CNS tracts that correlated with clinical findings when MRI findings were negative underscores the insufficiency of existing imaging modalities and the efficacy of more advanced methods. Taken together with the prior pathophysiological discussion of TAI, this study demonstrates the use of tractography in identifying the specific neuroanatomical locations of secondary axonal injury that correspond to clinical findings. Whilst this is a huge step forward, there are several practical limitations with respect to incorporating such imaging modalities into a routine healthcare setting.

One major drawback of DTI based imaging is that it measures diffusion based on a Gaussian distribution model, when this is not always the case in the brain [177], and is limited in evaluating white matter with more complex fiber configurations [178]. High angular resolution diffusion imaging (HARDI) modalities have been designed to circumvent this [179]. A recent study [180] used HARDI to assess patients with mild traumatic brain injury. Despite imaging being performed on average 21.2 days post injury, a significant correlation between FA and clinical outcome at 3–6 months was found in a cohort of mild TBI patients that experienced less than 24 h post traumatic amnesia. Although the results are applicable to a limited cohort of TBI patients, the fact that imaging performed beyond the acute stage of injury could predict future outcomes is an encouraging prospect.

Susceptibility weighted imaging (SWI), is an imaging modality of increasing interest due to its ability to discern traumatic axonal pathology through identification of associated traumatic microbleeds [181], and showing superiority over other existing imaging methods [182,183]. Given the evidence of potential relationships between specific characteristics of traumatic microbleeds and clinical outcome in patients, this MRI sequence may be an effective modality for identifying TAI. Indeed, there is recent evidence demonstrating relationships between lesions in the mesencephalic region identified with SWI and clinical outcomes in patients with DAI [184]. Thus, continuing developments in imaging modalities and varied approaches to image analysis highlight the key role of neuroimaging in TAI.

6. Conclusion

Taken together, there are several angles to the clinical conundrum of TAI. An ideal model for diagnosing, managing, and predicting outcome of TAI would include several tools incorporating the strengths of each individual component for optimal function. Although large numbers would be required to deduce this information, international research groups such as CENTER-TBI [185] and TRACK-TBI [186] are already gathering large data sets with which to achieve this. Also, several studies have addressed the advantages of combining several different biomarkers to optimize prediction models rather than relying on a single biomarker [60,98]. The practical issues associated with the use of advanced imaging modalities such as DTI and DTT for assessing TAI in an acute clinical setting suggests that it would be better suited to a longer term assessment of outcome after the acute stage has passed. The ideal TAI model would use body fluid biomarkers for immediate triage of patients, providing an indicator of those that require further radiological assessment and follow up. Based on this indication, preliminary CT and/or MRI imaging could be arranged appropriately for the short term, and DTI or DTT for longer term prognosis.

Furthermore, biomarker measurements at different time points (see Fig. 2) could enhance prognostic information that would be of benefit to clinicians for planning further management and for victims in providing a more definitive picture of their condition. By correlating

biomarker measurements with neuroanatomical sites of axonal injury on neuroimaging, patients at risk of specific neuro-cognitive deficits could be notified, and arrangements made accordingly. The next step would be the use of biomarkers to monitor potential treatment strategies targeting pathways leading to their generation and thus making the transition from accurately informing patients of their condition to being able to offer therapeutic solutions.

In this review we have explored some of the existing major biomarkers of axonal injury and their role in TAI. There are several other biomarkers that have been extensively studied, such as GFAP, NSE, S-100B, and upcoming biomarkers such as various miRNAs (see review by Kawata et al [27]. Whilst they are all of relevance to TBI, we aimed to provide a concise review of biomarkers of axonal origin in traumatic injury to evaluate their role in TAI and also in TBI, since it is likely that their release is a result of axonal injury even when the diagnosis is less specifically labeled as TBI. This proposition is supported by evidence such as the relationship between extracellular concentrations of tau protein on cerebral microdialysis and FA on DTI in patients with severe TBI [163], and the inadequacy of routinely used neuroimaging modalities to detect axonal injury suggests that it is highly likely that a multifaceted approach will be required. However, there are still several issues regarding the discussed axonal biomarkers: (i) the temporal relationship of biomarker concentrations in extracellular fluid in cerebral microdialysis, CSF, and serum; (ii) their relationships with FA measures on DTI, and the reliability of a method of correlating this with TAI; (iii) the long-term trajectory of these biomarkers; and, (iv) the optimal model for diagnostic and prognostic purposes in TAI/TBI, and the combination of biomarkers and clinical parameters this would involve.

7. Declarations of interest

None.

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