Biomarkers for the Clinical Differential Diagnosis in Traumatic Brain Injury—A Systematic Review

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SUMMARY

Rapid triage and decision-making in the treatment of traumatic brain injury (TBI) present challenging dilemma in “resource poor” environments such as the battlefield and developing areas of the world. There is an urgent need for additional tools to guide treatment of TBI. The aim of this review is to establish the possible use of diagnostic TBI biomarkers in (1) identifying diffuse and focal brain injury and (2) assess their potential for determining outcome, intracranial pressure (ICP), and responses to therapy. At present, there is insufficient literature to support a role for diagnostic biomarkers in distinguishing focal and diffuse injury or for accurate determination of raised ICP. Presently, neurofilament (NF), S100 β , glial fibrillary acidic protein (GFAP), and ubiquitin carboxyl terminal hydrolase-L1 (UCH-L1) seemed to have the best potential as diagnostic biomarkers for distinguishing focal and diffuse injury, whereas C-tau, neuron-specific enolase (NSE), S100 β , GFAP, and spectrin breakdown products (SBDPs) appear to be candidates for ICP reflective biomarkers. With the combinations of different pathophysiology related to each biomarker, a multibiomarker analysis seems to be effective and would likely increase diagnostic accuracy. There is limited research focusing on the differential diagnostic properties of biomarkers in TBI. This fact warrants the need for greater efforts to innovate sensitive and reliable biomarkers. We advocate awareness and inclusion of the differentiation of injury type and ICP elevation in further studies with brain injury biomarkers.

Introduction

Traumatic brain injury (TBI) continues to take a dramatic toll on patients and those around them. In the United States, an estimated 1.7 million people suffer a TBI each year [1]. About 50,000 people die annually and at least 5.3 million live with severe disabilities related to TBI [2]. Worldwide, TBI is recognized as the leading cause of mortality and morbidity in young adults [3,4].

The important factors determining prognosis of TBI patients are severity of the primary brain injury and degree of subsequent secondary damage. Theoretically, we cannot avoid the primary insult. We focus our attention on preventing the aforementioned secondary damage. Thus, much TBI research has focused on early diagnosis and injury characterization so that surgical treatment, including evacuation of mass lesions, can be optimized.

The diagnosis of TBI has been traditionally depended on neuroimaging, specifically computed tomography (CT). Moreover, surgical decisions are usually guided by CT in conjunction with intracranial pressure (ICP) monitoring, according to recent TBI guidelines [5–10]. However, TBI patients not only in the battlefield and undeveloped areas, but also in the acute, prehospital setting have limited availability to these diagnostic tools, which are essential for deciding which patients to take to the operating room. Thus, the ability of protein biomarkers to tease out and

identify focal and diffuse brain injury or the potential to diagnose intracranial hypertension would make these markers extremely valuable. At present, their potential for guiding the differential diagnosis has not been well defined.

We present a systematic review of the literature using MESH search terms in PubMed, MEDLINE, and the Cochrane Database to identify all articles published with data regarding the use of biomarkers in differential diagnosis.

The aim of this review is as follows: (1) to summarize the pathological foundation of each biomarker and (2) to rank the utility of each biomarker for diagnosing focal and diffuse brain injury as well as intracranial hypertension in TBI patients.

What is the “Ideal Biomarker” in TBI

Typically, brain biomarkers are proteins that can be repeatedly and safely measured from biofluids. They provide clinicians with specific information about the relevant organs, namely the brain and spinal cord. It is important to understand characteristics that make a brain biomarker useful. The *ideal* biomarker will afford (1) specificity: it will be uniquely present in the central nervous system and accurately reflect the extent of brain damage, (2) sensitivity: it would be highly abundant and easily detectable, and (3) measure therapeutic efficacy: biomarker levels will reflect success-

ful therapeutic intervention. With these concepts in mind, we will focus on protein structural biomarkers measured from the blood and the cerebral spinal fluid (CSF). The markers covered in the following review will include neurofilaments (NFs), cleaved-tau (C-tau), microtubule-associated protein 2 (MAP2), myelin basic protein (MBP), neuron-specific enolase (NSE), S100 β , and glial fibrillary acidic protein (GFAP), ubiquitin carboxyl terminal hydrolase L1 (UCHL1), and alpha-II spectrin breakdown products (SBDPs; see Table 1).

Neurofilaments

Neurofilament is a major cytoskeletal component in axons. The NF heteropolymer consists of light chain (NFL, 68 kDa), medium chain (NFM, 150 kDa), heavy chain (NFH, 190–210 kDa), and alpha-internexin polypeptides [11]. NF subunits are localized in the soma and flow in axonal transport, so that they accumulate in disconnected axons [12]. After injury, calcium influx activates calcineurin altering the phosphorylation state and the repelling forces of the NF sidearms. Such forces lead to the compaction of NF and marked local loss of cytoskeletal integrity [13]. Subsequently, activated calpain and caspase-3 mediate the dephosphorylation and proteolysis of phosphorylated NFs (pNFs). In this detached, dephosphorylated state, NFs collapse into tightly packed bundles in the centers of axons [14]. These modifications reduce the stability of pNFs, the calibers of axons, and the number of microtubules thus promoting pNF degradation.

Zurek *et al.* [15] reported the efficacy of serum NFH measurement in predicting the injury type and outcome in children with TBI. In this study, levels of NFH were significantly higher in patients with diffuse axonal injury on initial CT scan [15]. In another study, Vajtr *et al.* [16] compared serum NFH concentrations between diffuse axonal injury (DAI) and focal injury showing that the median serum NFH was higher in DAI compared with focal TBI during all 10 days after admission.

The above citation clarifies NFH as a promising biomarker in distinguishing DAI from focal injury. However, the cutoff value for this aim has not been established. Also, there is no current human or basic research correlating NFs levels with ICP elevation.

Cleaved-Tau (C-Tau)

Tau is an intracellular, microtubule-associated protein (MAPT) with a molecular mass of 48 kDa to 67 kDa that is highly enriched in axons. As a microtubule-binding phosphoprotein, tau protein stably assembles axonal microtubule bundles and participates in anterograde axoplasmic transport [17,18]. Under normal conditions, axonal MAP-tau is below the level of detection using immunostaining. After axonal injury, activated calpain depolymerizes microtubules in both perikarya and neurites [19]. Aggregated, hyperphosphorylated tau then forms into filamentous inclusions termed neurofibrillary tangles. These “tau tangles” are a pathologic hallmark of axonal injury. During injury, MAP-tau is cleaved by calpain-1 and caspase-3 into polypeptides with molecular masses of 17 kDa and 50 kDa, respectively. These fragments are referred to as cleaved-tau (C-tau). A large quantity of C-tau from damaged microtubules diffuses into the CSF and serum through the compromised blood–brain barrier (BBB) [20–22]. C-tau pos-

sesses many desirable characteristics of a biochemical marker, including low molecular weight, high organ specificity, and high solubility [19].

In a study of severe TBI in humans, the presence of C-tau in the CSF was a highly sensitive indicator of axonal injury. In DAI patients, its levels were shown to be increased 500- to 1000-fold at 1 h postinjury, soaring to 40,000-fold at 24 h and steadily declining within 3 days [20,21]. The levels of C-tau in serum increased because of a partial or transient opening of the compromised BBB, which allowed C-tau to diffuse [23]. Because the clearance rates and half-lives of biomarkers also influence their final concentrations in plasma, the C-tau level in plasma was only one-tenth of that in the CSF [18]. Therefore, C-tau in the CSF is a more sensitive biomarker of DAI than in the serum.

C-tau has been explored for other nonspecific measures. There are several articles relating C-tau concentration with patient outcome. Ost *et al.* [17] clarified that initial CSF total tau level correlated with 1-year outcome in 39 severe TBI patients. The correlation was found between initial CSF total tau and Glasgow Outcome Scale Extended (GOSE) levels ($R = 0.42$, $P < 0.001$). A CSF total tau level >2126 pg/mL on days 2–3 discriminated between dead and alive (sensitivity of 100% and a specificity of 81%) [17].

Furthermore, Liliang *et al.* [24] used the receiver operating characteristic (ROC) curve to demonstrate that a tau protein level ≥ 114.5 pg/mL yielded 88% sensitivity and 94% specificity for predicting poor outcome, thus showing that serum tau protein level may serve as an indicator for the prediction of outcome following severe TBI.

On the other hand, in mild TBI, serum C-tau failed to predict outcome or the presence of lesional pathology on CT [25]. There are few studies comparing the difference in C-tau levels between diffuse and focal injury. One study confirmed the detection of serum C-tau within the first 10 h after head injury was associated with an increased risk of intracranial lesion on head CT scan [26]. However, the clinical utility of serum C-tau as screening test for CT diagnosis appears to be limited, with a sensitivity of only 53% (95% CI 36–59%) [26]. This lack of sensitivity could be explained by the fact that C-tau protein is located only in neuronal axons. It is reasonable to postulate that C-tau is more likely to be released after DAI specifically and to a lesser extent after other types of injuries such as extra-axial hemorrhage without DAI.

The use of C-tau to make the diagnosis of intracranial hypertension was addressed by Zemlan *et al.* [20] demonstrating that initial CSF C-tau levels were a significant predictor of ICP elevation. The ability of initial C-tau levels to discriminate between patients with normal ICP (<30 mmHg) versus patients with elevated ICP (>30 mmHg) was assessed. Patients with normal ICP demonstrated significantly lower C-tau levels compared to patients with elevated ICP ($P < 0.014$). Moreover, they calculated 1500 ng/mL to be an optimal C-tau cutoff value for predicting normal ICP, with a 78% sensitivity and a 79% specificity [20].

Microtubule-Associated Protein 2 (MAP2)

Microtubule-associated protein 2 (MAP2) is primarily expressed in the nervous system and is one of the most abundant proteins in

Table 1 Potential biomarkers in serum/CSF for differential diagnosis in human TBI

Candidate biomarkers for differential diagnosis	Marker origin	Attributes	Literature focused on the differentiation of diffuse and focal TBI	Literatures compared with ICP elevation	Other literatures, relating mortality, morbidity, or other topic
Neurofilament proteins (NFL, NFM, NFH)	Cytoskeletal component	Axonal injury markers	Zurek <i>et al.</i> [15] (serum NFH was significantly higher in DAI) Vajtr <i>et al.</i> [16] (serum NFH was higher in DAI than focal injury)	N/A	Zurek and Fedora. [50] (serum)
Cleaved-tau(C-Tau)	Assembles axonal microtubule bundles	Axonal injury markers	N/A	Zemlan <i>et al.</i> [20] (CSF C-tau threshold 1,500 ng/mL for ICP>30; 78% sensitivity/79% specificity)	Liliang <i>et al.</i> [24] Shaw <i>et al.</i> [26] (serum C-tau W/Wo CT abnormality) Ost <i>et al.</i> [17] (CSF) Ma <i>et al.</i> [25] (serum, in mild TBI) Liliang <i>et al.</i> [24] (serum) Mondello <i>et al.</i> [32] (serum MAP2)
Microtubule-associated protein 2 (MAP2)	Dendrites	Dendritic injury	N/A	N/A	Berger <i>et al.</i> [36,37] (serum/CSF MBP and inflicted TBI) Su <i>et al.</i> [35] (CSF MBP and AHT)
Myelin basic protein (MBP)	Oligodendrocytes/Schwann cells	Demyelination	N/A	N/A	Berger <i>et al.</i> [38] Yamazaki <i>et al.</i> [40] (serum) Thomas <i>et al.</i> [39] (serum)
Neuron-specific enolase (NSE)	Cytoplasm of neurons, platelets, red blood cells	Neural damage marker	Herrman <i>et al.</i> [53] (different release pattern of serum NSE in >48 h from TBI)	Stein <i>et al.</i> [54] (CSF NSE levels were correlated with increasing "Pressure [ICP>20] times Time Dose" value in late phase [>12 h after injury])	Ross <i>et al.</i> [52] (serum and CSF NSE) Zurek and Fedora. [50] (serum) Bohmer <i>et al.</i> [51] (CSF) Chabok <i>et al.</i> [49] (serum in DAI) Vos <i>et al.</i> [69] (serum) Yamazaki <i>et al.</i> [40] (serum)
S100 β	Glia, astrocytes, fat, bone marrow, skeletal muscle	CNS injury marker benchmark	Raabe <i>et al.</i> [61] (serum) Herrman <i>et al.</i> [53] (serum) Pelinka <i>et al.</i> [59,70] (serum) Vajtr <i>et al.</i> [16] (serum)	Hayakata <i>et al.</i> [64] (CSF) positive correlation ($r^2 = 0.729$, $P < 0.0001$) between peak CSF S100B concentration and ICP value Kirchhoff <i>et al.</i> [64] (CSF) CSF S100B to predict intracranial hypertension which	Raabe <i>et al.</i> [57] (serum) Stein <i>et al.</i> [112] (serum) Zurek and Fedora. [50] (serum) Bohmer <i>et al.</i> [51] (CSF) Goyal <i>et al.</i> [58]

(continued)

Table 1 (Continued)

Candidate biomarkers for differential diagnosis	Marker origin	Attributes	Literature focused on the differentiation of diffuse and focal TBI	Literatures compared with ICP elevation	Other literatures, relating mortality, morbidity, or other topic
Glial fibrillary acidic protein (GFAP)	Glia	Gliosis/glia cell injury	<p>Pelinka et al. [71] (serum)</p> <p>J Neurosurgery (serum)</p> <p>Mondello et al. [74] (serum)</p> <p>Mondello et al. [87] (serum)</p> <p>serum GFAP levels were higher in patients with mass lesion than in patients with diffuse injury.</p>	<p>exhibiting a mean ICP >15 mmHg</p> <p>Stein et al. [54] ICH was found to be associated with serum S100β levels</p> <p>Raabe et al. [57] (serum)</p> <p>significant correlation between the peak value of serum S100β and the highest ICP value</p> <p>Pelinka et al. [59,71] (serum)</p> <p>serum S100B was lower in ICP <25 than ICP > or = 25</p> <p>Pelinka et al. [59] (serum)</p> <p>Pelinka et al. [71] (serum)</p> <p>serum GFAP concentrations were significantly elevated in patients with ICP >25 mmHg</p>	<p>(serum and CSF)</p> <p>Pelinka et al. [59,71] (serum)</p> <p>Vos et al. [70] (serum)</p> <p>Chatfield et al. [60] (serum)</p> <p>Zurek and Fedora. [50] (serum)</p> <p>Czeiter et al. [72] (serum, CSF)</p> <p>Nylen et al. [73] (serum)</p> <p>Pelinka et al. [59] (serum)</p> <p>Pelinka et al. [71] (serum)</p> <p>Vos et al. [70] (serum)</p>
Ubiquitin C-terminal hydrolase-L1 (UCH-L1)	Neuronal cell body	Neuronal cell body injury	<p>Mondello et al. [74] (serum)</p> <p>Mondello et al. [87] (serum)</p> <p>(J Neurotrauma)</p>	N/A	<p>Berger et al. [85] (serum)</p> <p>Mondello et al. [86] (serum, CSF)</p> <p>Papa et al. [76] (CSF)</p> <p>Papa et al. [82] (serum)</p> <p>Czeiter et al. [72] (serum, CSF)</p> <p>Brophy et al. [84] (serum, CSF)</p> <p>Mondello et al. [105] (CSF)</p> <p>Berger et al. [85] (serum)</p>
SBDP150/SBDP145	Axon (calpain-generated)	Acute necrosis	Pineda et al. [106] (CSF)	ICP > 25 mmHg Brophy et al. [107] (CSF)	

(continued)

Table 1 (Continued)

Candidate biomarkers for differential diagnosis	Marker origin	Attributes	Literature focused on the differentiation of diffuse and focal TBI	Literatures compared with ICP elevation	Other literatures, relating mortality, morbidity, or other topic
SBDP120	Axon (caspase-3-generated)	Delayed apoptosis	N/A	Brophy <i>et al.</i> [107] (CSF)	(serum) Czeiter <i>et al.</i> [72] (serum, CSF) Cardali and Maugeri [111] (CSF) Pineda <i>et al.</i> [104] Cardali and Maugeri [111] (CSF)

CSF, cerebral spinal fluid; CT, computed tomography; DAJ, diffuse axonal injury; ICP, intracranial pressure; SBDP, spectrin breakdown product; TBI, traumatic brain injury.

the brain [27,28]. MAP2 interacts with microtubules through its tubulin-binding domain, which mainly associates with the acidic region on the C-terminal of tubulin. MAP2 is thought to be a dendrite-specific protein [29]. It is thought to be a good candidate biomarker for a dendritic injury [30,31].

Recently, Mondello *et al.* [32] reported the outcome predicting potential of this dendritic marker. Their group demonstrated serum concentrations of MAP2 to be evaluated in 16 patients with severe TBI. MAP2 levels correlated with the GOSE (Glasgow Outcome Scale Extended) and Levels of Cognitive Functioning Scale (LCFS) measured at 6 months after injury [32]. Unfortunately, the use of MAP2 for guiding the differential diagnosis has not been addressed in the literature.

Myelin Basic Protein

Myelin basic protein is one of the most abundant proteins in white matter, representing 30% protein content of myelin. It possesses clusters of positively charged amino acid residues, and it forms the compact myelin sheath [33,34]. MBP consists of four major isoforms of 21.5 kDa, 18.5 kDa, 17 kDa, and 14 kDa. In rats, intact MBP diminished significantly in the contused cortex as early as 2 h after TBI, reaching its lowest level (a 75-fold reduction) at 48 h [34]. MBP returned to basal levels at 3–7 days [33]. Correspondingly, MBP's *N*-terminal degradation products, 8 kDa and 10 kDa, began to accumulate in the rat cortex at 2 h after TBI, peaked at 1–2 days, returning to basal levels at 6–7 days when necrotic tissue had been scavenged by microglia and infiltrating macrophages [33].

In human studies of adult and pediatric TBI patients, serum and CSF MBP has been studied as a biomarker for nonaccidental pediatric TBI [35] or inflicted traumatic brain injury [36,37] and outcome estimation [38–40].

However, there is still no evidence for its use as a diagnostic biomarker for focal and diffuse TBI or intracranial hypertension, which is far more common. Clearly, more studies are needed.

Neuron-Specific Enolase

Neuron-specific enolase, a 78-kD dimeric γ -isoenzyme of the glycolytic enzyme enolase, is localized prominently in the cytoplasm of neurons and participates in slow axoplasmic transport [41,42]. NSE is not normally secreted into extracellular fluids by intact neurons. However, when axons are damaged, NSE is upregulated in an attempt to maintain homeostasis [43]. Due to leakage and upregulation, NSE can subsequently be detected in cytoplasm surrounding the disrupted axons [44]. This upregulation of NSE, among other proteins, and increased energy demand has been evidenced by an increased glucose metabolism with a concomitant decline in extracellular glucose in pericontusional domains of injured brains during the first week postinjury [43]. Given its location and abundance, NSE in body fluids should possess relatively high specificity and sensitivity for axonal injury [45,46]. Accordingly, NSE seems to have excellent theoretical potential as a long-term prognostic biomarker and as a therapeutic monitor in the neurological intensive care [47,48].

At present, there have been several studies relating NSE as an outcome predictive biomarker [40,49–52]. In contrast, there are few human studies relating to differential diagnosis.

In 2012, Chabok *et al.* [49] focused on DAI and studied the relation between serum NSE and outcome in 28 patients with this pattern of injury. They found that with DAI, an increased serum concentration of NSE within the first 3 days after injury was associated with poor outcome. This article did not, however, explore the difference of NSE biokinetics between focal and diffuse pathophysiology.

Another study investigated the release patterns of NSE and S100 β after TBI in 66 patients via serum concentration and their association with intracranial pathologic changes as demonstrated by CT [53]. Release patterns of S100 β and NSE differed in patients with primary cortical contusions, DAI, and suspected cerebral edema without focal mass lesions. In patients with cortical contusions, NSE was highest on first measurement at about 1 day after injury, decreasing from this peak over 4 days. On the other hand, S100 β slowly increased over the 4-day study period. All serum concentrations of NSE and S100 β were significantly correlated with contusion volume, but the first sample taken was the most valid indicator of TBI severity. In DAI patients and patients with signs of elevated ICP, the peak serum levels of NSE and S100 β were day 3. This report concluded that the release patterns of NSE and S100 β may mirror different pathophysiological consequences of traumatic brain injury [53]. Unfortunately, this study could not differentiate between focal and diffuse TBI from the initial NSE measurement on day 1.

Further information regarding the ability of NSE to detect ICP elevation comes from Stein *et al.* compared CSF S100 β and NSE levels with the percentage of time patients spent with an elevated ICP (>20 mmHg). They analyzed both the early phase (<12 h after injury) and late phase (>12 h after injury) [54]. Intracranial hypertension was found to be associated with early and late S100 β levels when measured as percent of time with ICP >20 mmHg, whereas late increasing NSE levels were only weakly correlated with elevated ICP [54].

S100 β

S100 β is a small, dimeric calcium-binding protein that has been extensively studied as a TBI biomarker. The S100 β proteins are located in the cytoplasm and nucleus of a wide range of cells and are involved in the regulation of a number of cellular processes, such as cell cycle progression and differentiation. Among cells expressing S100 β , glial and neural cells of the CNS are the most abundant, thus it can be detected in serum following TBI [55]. Interestingly, this protein also plays a significant role in the stabilization of tau and MAP2 [56]. Currently, there are several publications discussing S100 β as a potential prognostic biomarker [50,51,57–60].

In 1998, Raabe *et al.* first described the correlation between computed tomography findings and serum NSE and S100 β following severe head injury. In this study, admission CT scans of 44 patients and their initial levels of S100 β and NSE were compared between the different categories of CT findings. A significant correlation was seen between the volume of contusion

visible on CT scan and serum S100 β concentration [61]. Furthermore, a follow-up study by the same group established a strong correlation between S100 β values and computed tomographic findings [57].

However, in the other studies, serum levels of S100 β have failed to efficiently identify intracranial pathology on imaging studies [37,55,62]. These findings agree with a study by Morochovic *et al.* [63], which showed that S100 β is an unreliable screening tool for detecting intracranial pathology in adults and children.

In 2004, Pelinka *et al.* [59] prospectively compared the serum S100 β concentration between focal and diffuse injury, classified with the Marshall classification. In this study of 92 TBI patients, there was a significant difference of serum S100 β concentration between the category “diffuse injury II” and “nonevacuated mass lesion” [59].

In 2012, Vajtr *et al.* reported the possibility of S100 β as a differential diagnostic biomarker to distinguish focal and diffuse injury. In this study of 38 TBI patients, the median serum S100 β concentration was higher in patients with focal mass lesions ($1.72 \pm 0.4 \mu\text{g/L}$ vs. $0.37 \pm 0.1 \mu\text{g/L}$, $P < 0.05$) compared to patients with DAI during 10 days of hospitalization [16]. These recent results indicate that serum S100 β can be useful in detecting focal injury in the acute phase.

In relation to the detection of intracranial hypertension, Raabe *et al.* [57] showed that there was a significant correlation between the peak value of serum S100 β and the highest ICP value ($r = 0.55$; $P < 0.001$). Kirchhoff also demonstrated the efficacy of CSF S100 β in predicting intracranial hypertension, which he defined as a mean ICP greater than 15 mmHg [64]. Similarly, Pelinka *et al.* [59] demonstrated that serum S100 β was lower with an ICP < 25 as compared to an ICP ≥ 25 . Lastly, Hayakata *et al.* [65] illustrated a positive correlation ($r^2 = 0.729$, $P < 0.0001$) between peak CSF S100 β concentration and ICP value.

Moreover, as mentioned previously, Stein *et al.* [54] compared CSF S100 β and the percent of time spent with an ICP >20 mmHg in early and late phases, the time point division being at 12 h. Intracranial hypertension was correlated with S100 β levels both in the early and late phase ($P < 0.01$). On the other hand, NSE value was only weakly associated with ICP elevation in late phase [54]. Conclusively, S100 β appears to be a more accurate biomarker for predicting intracranial hypertension than NSE, particularly in the early phase postinjury.

Glial Fibrillary Acidic Protein (GFAP)

Glial fibrillary acidic protein (GFAP) is a monomeric intermediate filament protein found in the astroglial skeleton [66]. It was first isolated by Eng *et al.* [66] in 1971. GFAP is found exclusively in CNS and therefore has recently received attention as a possible glial injury biomarker. Indeed, current evidence indicates that serum GFAP might be a useful marker for various types of brain damage, including neurodegenerative disorders and stroke [67]. Interestingly, in one stroke study, GFAP concentration was used to differentiate between intracranial hemorrhage and ischemic stroke patients [68]. In 1999, Missler *et al.* [69] demonstrated GFAP to be increased in the serum of patients suffering from severe head trauma. More recently, this protein has been confirmed as a specific marker for CNS damage [59,70,71]. GFAP has

been demonstrated to be a potentially useful biomarker in predicting clinical outcome [50,59,70,72,73].

There are several articles comparing GFAP concentrations between focal and diffuse injury. In 2004, Pelinka et al. [59,71] compared serum GFAP concentration among the types of Marshall CT classification, where they clarified that serum GFAP was significantly higher in focal nonevacuated mass lesion than in DAI.

Mondello et al. also compared serum GFAP levels between diffuse and focal TBI in 81 patients. GFAP levels in the first 24 h postinjury were significantly higher in patients with focal mass lesion (Marshall V–VI) compared to patients with diffuse injury (Marshall I–IV; 2.95 ± 0.48 ng/mL vs. 0.74 ± 0.11 ng/mL, $P = 0.003$). Additionally, they compared serum GFAP in diffuse injury I–II, diffuse injury III–IV, and focal mass lesion. The average levels of GFAP were 0.56 ± 0.12 ng/mL, 1 ± 0.2 ng/mL, and 2.95 ± 0.48 ng/mL, respectively, with significant difference demonstrated between all groups. The authors showed that serum GFAP levels were higher in patients with mass lesions than in patients with diffuse injury. Mondello et al. [74] concluded the different patterns of biomarker release that imply mass lesional injury seem to result in more damage to glia, the neural support system. The above evidence suggests that GFAP may be an appropriate and sensitive candidate for the diagnosis of focal brain injury, such as contusion or intracerebral hemorrhage.

Regarding utility of biomarkers, for the prediction of intracranial hypertension, there has been minimal support in the literature, for biomarkers in general, and for GFAP in particular. Pelinka et al. [59,71] reported that serum GFAP concentrations were significantly elevated in patients with ICP >25 mmHg. However, the cutoff value of GFAP for detecting ICP elevation remains unclear.

Ubiquitin C-Terminal Hydrolase-L1 (UCH-L1)

Ubiquitin C-terminal hydrolase-L1, also called neuronal-specific protein gene product (PGP 9.3), is highly abundant in the neuronal soma [75,76].

Ubiquitin carboxyl terminal hydrolase-L1 is involved in either the addition or removal of ubiquitin from proteins that are destined for metabolism via the ATP-dependent proteasome pathway [77]. Impairments in UCHL-1 function have been associated with familial Parkinson's disease [78]. In primary cultures, proteasome inhibitors have been shown to arrest neurite outgrowth and degeneration [79,80]. In focal cerebral ischemia and spinal cord injury, increased protein aggregates and decreased proteasome activity have been observed, respectively [81,82]. These data suggest that UCH-L1 might play an important role in the removal of excessive, oxidized, or misfolded proteins during both normal and neuropathological conditions such as neurodegenerative disorders including TBI [76,83–85].

Recently, Mondello et al. reported a case-control study in which 95 severe TBI subjects were enrolled. They studied the temporal profile of CSF and serum UCH-L1 levels over 7 days and concluded serum and CSF levels of UCH-L1 appear to have poten-

tial clinical utility in diagnosing TBI with strong correlation to injury severity and survival outcome [86].

To distinguish diffuse and focal TBI, UCH-L1 was studied in two previous studies. Mondello et al. [74] reported that serum UCH-L1 levels were higher in patients with diffuse injury, thus different from GFAP levels, which was higher in patients with mass lesions as mentioned above.

With these different patterns of biokinetics, they advocated the "glial neuronal ratio" (GNR) as a novel indicator to differentiate focal and diffuse injury [87]. The GNR was based on the ratio between serum GFAP and UCH-L1 concentrations and was significantly higher in the focal mass lesion group compared with the diffuse injury group (1.77 vs. 0.48, $P = 0.003$). Furthermore, ROC curve analysis illustrated that the GNR accurately discriminated between types of injury (area under the curve [AUC] = 0.72; $P = 0.003$). GNR was more accurate when measured earlier (≤ 12 h after injury) rather than later (AUC = 0.80; $P = 0.002$) [87]. Conclusively, these results show that the combination of UCH L1 and GFAP can be helpful in distinguishing focal and diffuse TBI in acute diagnosis. These two articles concluded that the sensitivity to intracranial hypertension and brain ischemia is different between neuronal (UCH L1) and glial (GFAP) cells, hence different injuries caused different release pattern of the biomarkers.

α -II Spectrin Breakdown Product (SBDP) 150, 145, and 120

The α -II spectrin protein forms part of the axolemmal cytoskeleton anchoring to ankyrin, which links axolemma constituents and the presynaptic terminal [88,89]. The spectrin protein stabilizes the nodal and paranodal structure of myelinated axons [89]. It is a major substrate for calpain-1 and calpain-2 and caspase-3, which are involved in cellular necrosis and apoptosis, respectively [90]. Thus, to distinguish necrotic and apoptotic pathophysiology, the calpain-specific 150- and 145-kDa SBDPs can be used as biomarkers of necrotic neuronal death and the caspase-3-specific 120-kDa SBDP as an apoptotic biomarker [91–101]. This separation has been widely discussed, for experimental and clinical TBI research, for injury diagnosis, injury severity gradation, and outcome prediction. [75,87,102–104]. For example, Mondello et al. [105] assessed the clinical utility of quantitative assessments of SBDPs, using SBDP 145 produced by calpain and SBDP 120 produced by caspase-3, in CSF as markers of brain damage and outcome after severe TBI. They analyzed 40 adult severe TBI patients. Mean CSF levels of SBDPs were significantly higher in TBI patients than in controls at all time points examined. SBDP 145 provided accurate diagnoses at all time points examined, while SBDP 120, the apoptotic biomarker, was more accurate 24 h after injury. Both types of SBDP levels were significantly higher in patients who died than in those who survived. SBDP 145 levels (>6 ng/mL) and SBDP 120 levels (>17.55 ng/mL) strongly predicted death (odds ratio 5.9 for SBDP 145 and 18.34 for SBDP 120) [105]. From these data, SBDP 145, the calpain-mediated necrotic biomarker, seems to be more accurate in predicting outcome of severe TBI.

As described, SBDPs have been applied for outcome prediction in human TBI. However, there is limited evidence in the literature relating injury type and SBDP concentration.

Pineda *et al.* [106] compared initial CT diagnosis with Marshall grade and SBDPs. Patients with Marshall classification category I–II (diffuse injury) and III–IV/presence of mass (focal) lesion were compared. The CSF SBDPs 150, 145, and 120 were higher in diffuse injury than focal group, yet this was only significant in SBDP 150 at 48 h postinjury [106]. The difference was not significant for the SBDP 145 or the SBDP 120 at any time point [106]. Because of the different release peaks between SBDPs 150, 145, and 120, necrotic biomarkers SBDP 150 and 145 seem to be more accurate in acute diagnosis than the apoptotic marker SBDP 120.

There is little evidence in the literature relating any correlation of intracranial hypertension with SBDP concentrations. In one study, Brophy *et al.* [107] found a positive correlation between patients with longer elevations of ICP (>25 mmHg) and CSF SBDPs.

In summary, SBDPs, especially CSF SBDP 150, may have a role as a differential diagnostic biomarker in the ability to distinguish focal and diffuse injury in the acute phase of TBI. Other than this, studies regarding SBDPs and differential diagnosis have shown largely negative results.

MicroRNA—A New Field of Biomarker Research in TBI

MicroRNA (miRNA) is a new promising biofluid biomarker for neurotrauma. miRNAs are small RNA (~22 nts) molecules that are expressed endogenously and play an important part in regulating gene expression. Redell *et al.* [107] compared the plasma miRNA profiles between severe TBI patients (Glasgow Coma Scale ≤ 8) and healthy volunteers. They concluded that decreases in the levels of miR-16 and miR-92a, and increased levels of miR-765, were good markers of severe TBI at 25–48 h after injury. These miRNAs thus seem to be good candidate biomarkers for differential diagnosis regarding injury type and intracranial hypertension. Further studies on this topic would help establish the efficacy of miRNAs on acute differential diagnosis. A simplified technique for miRNA measurement in biofluids could provide rapid diagnosis and allow for treatment decisions.

Limitations of Diagnostic Biomarkers—The Specificity and Biokinetics

As we have described above, several studies illustrated the diagnostic potential of these brain injury biomarkers. In reviewing biomarker data, one must keep a few limitations in mind. For instance, NSE initially held promise as a brain injury biomarker because originally it was believed to be strictly neuronal in origin. However, additional research showed that NSE was also present in red blood cells and platelets decreasing its diagnostic utility as a marker due to possible cross-contamination by hemolysis in blood samples [102]. Increases in NSE levels have been observed in multiple types of trauma with and without

TBI, limiting its ability to properly discriminate brain injury magnitude [103]. Assays of serum NSE together with S100 β have been reported to be valuable in prediction of TBI outcome [36,38].

One problem relating to the use of S100 β has been its low specificity. Although S100 β is glial specific and expressed primarily by astrocytes and Schwann cells, it is also found in several non-nervous system cells such as adipocytes (white and brown fat), chondrocytes, skin, glioblastoma, and melanoma cells [104]. The presence of multiple extracranial injuries can influence the levels of S100 β , yielding inadequate or even confusing results. Thus, S100 β has also been utilized for “theragnosis” general systemic trauma care. Nevertheless, there is a significant quantity of literature related to the efficacy of S100 β .

Another concern is the specific biokinetics in each biomarker. For example, MBP concentrations usually peak 48–72 h after TBI and the peak concentration seems to be a more accurate representation of TBI than the initial concentration [38,109]. This fact explains the difficulty with MBP in the initial diagnosis. Furthermore, the differences in half-life confound the interpretation of biomarker value.

For example, the half-life of GFAP is almost 7 days; consequently, we are unable to distinguish current ICP elevation and previously existing TBI, using GFAP concentration alone.

With all of this in mind, the best way to apply these biomarkers to differential diagnosis may be the combined, simultaneous estimation of multiple biomarkers, such as has been made with troponin-T and creatine phosphokinase isoenzymes M and B (CPK-MB) for diagnosis in myocardial infarction [110,111].

Conclusion

At present, there is insufficient literature to support a role for diagnostic biomarkers in distinguishing focal and diffuse injury or for accurate determination of raised ICP (Table 1). Presently, NF, S100 β , GFAP, and UCH-L1 seemed to have the best potential as diagnostic biomarkers for distinguishing focal and diffuse injury, whereas C-tau, NSE, S100 β , GFAP, and SBDPs appear to be candidates for ICP reflective biomarkers. With the combinations of different pathophysiology related to each biomarker, a multibiomarker analysis seems to be effective and would likely increase diagnostic accuracy. Importantly, we still need to identify cutoff values for differential diagnosis with each candidate marker.

In conclusion, there is limited research focusing on the differential diagnostic properties of biomarkers in TBI. This fact warrants the need for greater efforts to innovate sensitive and reliable biomarkers. We advocate awareness and inclusion of the differentiation of injury type and ICP elevation in further studies with brain injury biomarkers.

Conflict of Interest

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