REVIEW

Serum Biomarkers Of Hypoxic-Ischemic Brain Injury

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Summary

Brain injury is a multifaceted condition arising from nonspecific damage to nervous tissue. The resulting cognitive developmental impairments reverberate through patients' lives, affecting their families, and even the broader economic landscape. The significance of early brain injury detection lies in its potential to stave off severe consequences and enhance the effectiveness of tailored therapeutic interventions. While established methods like neuroimaging and neurophysiology serve as valuable diagnostic tools, their demanding nature restricts their accessibility, particularly in scenarios such as small hospitals, nocturnal or weekend shifts, and cases involving unstable patients. Hence, there is a pressing need for more accessible and efficient diagnostic avenues. Among the spectrum of brain injuries, hypoxic-ischemic encephalopathy stands out as a predominant affliction in the pediatric population. Diagnosing brain injuries in newborns presents challenges due to the subjective nature of assessments like Apgar scores and the inherent uncertainty in neurological examinations. In this context, methods like magnetic resonance and ultrasound hold recommendations for more accurate diagnosis. Recognizing the potential of serum biomarkers derived from blood samples, this paper underscores their promise as a more expedient and resource-efficient means of assessing brain injuries. The review compiles current insights into serum biomarkers, drawing from experiments conducted on animal models as well as human brain pathologies. The authors aim to elucidate specific characteristics, temporal profiles, and the available corpus of experimental and clinical data for serum biomarkers specific to brain injuries. These include neuron-specific enolase (NSE), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), S100 calcium-binding protein beta (S100B), glial fibrillary acidic protein (GFAP), and high-mobility-group-protein-box-1 (HMGB1). This comprehensive endeavor contributes to advancing the understanding of brain injury diagnostics and potential avenues for therapeutic intervention.

Key words

Brain injury • Serum biomarkers • Neuron-specific enolase • Ubiquitin carboxy-terminal hydrolase L1 • S100 calcium-binding protein beta • Glial fibrillary acidic protein • High-mobility-group-protein-box-1

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Introduction

Brain injury has a heterogeneous origin and could occur during a lifetime, even in antepartum development. Primary brain injury affects directly brain tissue (e.g. trauma, infarction, hemorrhage), and secondary brain injury is brain damage caused by an indirect condition of systemic collapse (e.g. hypoxia, cardiovascular arrest, polytrauma). Primary and secondary causes of brain injury affect both neuronal and

glial cells of brain tissue. Brain injury results in neuronal and glial damage causing lasting deficits. Structural and functional changes could be assessed with different diagnostic approaches. The time management is critical for the final extent of the neuronal tissue impairment. Clinical examination and neuroimaging are, however, possess several drawbacks making them unsatisfactory for the urgent and precise diagnosis of the brain injury [1]. Therefore, easily accessible, specific and sensitive biomarkers of the neuronal injury are highly desired.

Biomarkers of the brain injury

According to the Food and Drug Administration (FDA), biomarkers are classified as a "characteristic that serves as an objective indicator of normal biological processes, pathogenic processes, or response to an exposure or intervention, including therapeutic interventions" [2]. Biomarker is a helpful diagnostic tool that represents an objective measurement of impaired tissue and response to therapeutic interventions. Biomarker represents a biological principle that could be evaluated by a different diagnostic method such as neuroimaging, neurophysiologic method or biochemistry testing. Analysis and interpretation of biomarkers is a key to clinical diagnosis. An ideal biomarker is easily obtained and objectively measured as an indicator of physiological response to insult and therapeutic interventions. Several modalities of biomarkers of brain injury are established. These biomarkers are used already in everyday clinical practice as a tool for patient investigation such as neuroimaging methods (Table 1), others are more experimental (functional neuroimaging methods) or demanding such as neurophysiological methods which are both time challenging and need to be realized by an experienced specialist. An early diagnosis of the brain injury is essential for subsequent neuroprotective treatment and prognosis. Although neuroimaging methods such as MRI and CT provide good spatial resolution they are time-demanding methods such as MRI and CT, and need to be performed in a specialized radiology unit with highly experienced radiologists. Also, the clinical status of compromised patients could avoid an MRI scan due to their unstable conditions. Utilizing biological materials (tissue/liquid), molecular techniques involve comparisons to norms or repetitive assessments to unveil dynamic changes. These techniques encompass biomarkers obtained from sources like cord blood, peripheral blood, cerebrospinal fluid, urine, or saliva. Serum, extracted from blood, is employed for molecular diagnostics. Notably, serum protein biomarkers hold promise as a triage tool for neuroimaging in individuals diagnosed with TBI [3].

Biomarkers of neuronal degeneration reflect damage to the neuronal tissue after the brain injury. Ideal serum neuronal biomarker should be elevated early after the insult, easily crosses the brain-blood barrier (small to middle molecular weight molecules), and possess reasonable stability in peripheral blood for detection. The brain injury triggers cascades of pathophysiological changes in neuronal tissue which can lead to a wide spectrum of effects in both neuronal and glial cells including the damage of cytoskeleton. Thus, it leads to the release of structural proteins from affected cells into the extracellular fluid and to the blood (Table 2), including astrocytic protein (S100 calcium-binding protein B – S100b, glial fibrillary acidic protein – GFAP, myelin basic protein (MBP)) and neuronal protein (neuron specific enolase – NSE, ubiquitin carboxy-terminal hydrolase L1 – UCHL1, neurofilament heavy chain (NF-H), neurofilament light chain (NfL) [16,17,18]. Neuronal biomarkers are presently the subject of extensive investigation within the realm of neurological clinical research. Diverse configurations of these biomarkers are tailored to specific neurological disorders. Notably, NfL is regarded as a biomarker indicative of neuroaxonal injury, predominantly applied in the investigation of neurodegenerative conditions such as multiple sclerosis and Alzheimer's disease [18]. Cell death-associated molecular patterns (CDAMP), damage-associated molecular-pattern (DAMP), and excitotoxic molecules, separately and in combination, triggers neuroinflammation which results in increased extracellular levels of markers of inflammation (high-mobility-group-protein-box-1 – HMGB1, tumor necrosis factor-alpha (TNF-α), interferon-gamma (INF-γ), interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 13 (IL-13) [17,19]. Nuclear factor erythroid 2-related factor 2 (Nrf2) functions as a pivotal nuclear factor that governs genes regulated by the antioxidant response element (ARE), playing a crucial role as a key regulator of endogenous inducible defense systems within the body. In response to acute oxidative stress or inflammation, there is a significant upregulation of Nrf2 expressions in neurons following brain injury. This has led to the proposition that Nrf2, when present in circulation, could potentially serve as a biochemical marker for acute brain injuries.

Table 1. Methods and samples used to evaluate of the brain injury.

Neuroimaging methods	Functional neuroimaging methods	Neurophysiological methods	Molecular methods from biological material (ELISA)
Ultrasound (US) [4] Computed Tomography (CT) [5] Magnetic Resonance Imaging (MRI) [5]	Functional MRI (fMRI) [6] Diffusion Weighted MRI (DW-MRI) [7] Proton magnetic resonance spectroscopy (1H-MRS) [7] Magnetoencephalography Functional Near-Infrared Spectroscopy (fNIRS) [8]	Electroencephalography (EEG) – conventional [9] aEEG (amplitude integrated) [10] qEEG (quantitative) [11] Evoked potentials (EP) [12] Visual EP (VEP) [12] Somato-sensory EP (SSEP) [13] Brain Stem Auditory EP (BAEP) [14]	Serum (peripheral blood) Serum (cord blood) Cerebrospinal fluid (CSF) Urine Saliva [15]

Table 2. List of molecules considered as serum biomarkers of the brain injury.

Astrocytic protein	Neuronal proteins	Inflammation protein
S100 calcium binding protein B (S100b) [16] Glial fibrillary acidic protein (GFAP) [16] Myelin basic protein (MBP) [16]	Neuron specific enolase (NSE) [17] Ubiquitin carboxy-terminal hydrolase L1 (UCHL1) [17] Neurofilament heavy chain (NF-H) [16] Neurofilament light chain (NfL) [18]	High-mobility-group-protein-box-1 (HMGB1) [19] Tumor necrosis factor alpha (TNF-α) [17] Interferon-gamma (INF-γ) [17] Interleukin 1 (IL-1) [17] Interleukin 6 (IL-6) Interleukin 8 (IL-8) [17] Interleukin 10 (IL-10) [17] Interleukin 13 (IL-13) [17] Nuclear Factor Erythroid 2-related Factor (Nrf2) [20]

A recent investigation conducted by Yan *et al.* has demonstrated that heightened serum levels of Nrf2 in traumatic brain injury (TBI) patients exhibit independent correlations with clinical severity. Additionally, these elevated Nrf2 levels emerged as independent predictors of outcomes, including 180-day mortality, overall survival, and unfavorable prognosis. Nevertheless, further studies are warranted to substantiate and validate these significant findings [20]. In recent times, a proliferation of literature has emerged elucidating the involvement of microRNAs (miRNAs) in the context of brain injury. The intricate posttranslational modifications coupled with a wide spectrum of functional implications intensify the significance of miRNAs in both the physiological and pathological aspects of brain function. However, additional clinical analysis and interpretation of the gathered data remain imperative [21]. For those inclined toward delving into this group of biomarkers, a comprehensive review authored by Watson *et al.* offers an exceptional resource [22].

The concentration of molecular biomarkers in blood serum following brain injury is significantly influenced by factors such as the permeability of the blood-brain barrier, which becomes enhanced subsequent to neuronal insult primarily as a consequence of neuroinflammation. Additionally, systemic clearance and protease activities contribute to this intricate balance. Furthermore, the temporal patterns exhibited by these biomarkers mirror the distinct underlying pathophysiological mechanisms governing their emergence.

The categorization of neuronal biomarkers into diagnostic, prognostic, or predictive roles presents

a complex challenge, attributable to the heterogeneity in data originating from existing clinical studies.

Biomarkers of the brain injury in newborns

A compromised newborn with severe hypoxic brain injury is a special diagnostic case in medicine. Moderate to severe hypoxic-ischemic encephalopathy can develop after the hypoxic insult in the perinatal period (antenatal, peripartal, and early postnatal period). Perinatal brain injury remains a leading cause of long-term neurological development disability and thus advances in its diagnosis and treatment are desired. Therapeutic hypothermia remains the main and most efficient treatment procedure in newborn hypoxic-ischemic injury [23].

Obstetrics guidelines recommend intrapartum cardiotocography (fetal heart beating) [24]. Innovative methods such as intrapartum ultrasound are now largely investigated, nevertheless it is not considered as a standard of care [25]. The only classification system of the newborn physical status is Apgar score. Apgar score provides a standardized assessment for newborns after delivery. The Apgar score is composed of 5 clinical markers: (1) color; (2) heart rate; (3) reflexes; (4) muscle tone; and (5) respiration. Each of these components is given a score of 0, 1, or 2. Thus, the Apgar score targets clinical signs of neonatal depression, such as cyanosis or pallor, bradycardia, depressed reflex response to stimulation, hypotonia, and apnea or gasping respirations. The score is reported at 1 min and 5 min and at 10 min after birth, and at 5-minute intervals until 20 min for infants with a score less than 7 [26]. However, the Apgar score is not a specific diagnostic algorithm to state the diagnosis of hypoxic-ischemic injury, even antepartum fetal heart rate does not improve the diagnostic decision [27]. Hypoxic newborn is usually in a very severe and unstable clinical condition which limits the use of more specific neuroimaging methods (such as CT, MRI). Neurophysiological methods such as EEG require a specialist which is typically not available during night shift or weekends. Clinical trials in hypoxic newborns are also more complicated than those in adults. Although there is available data on serum biomarkers of brain injury in the literature, limited data exists for the pediatric population [28]. Most information thus comes from animal models. Albeit, they mimic most important factors of the human condition (hypoxia ischemia) the information obtained have to be confirmed by clinical trials [29].

Molecular serum biomarkers of brain injury

Molecular biomarkers are typically proteins which under physiological situations are either not expressed or are localized predominantly within the brain tissue and do not leak to the blood in considerable levels. Thus, according to two major places of cellular origin, biomarkers can be further divided into those of neural or glial origin (Fig. 1).

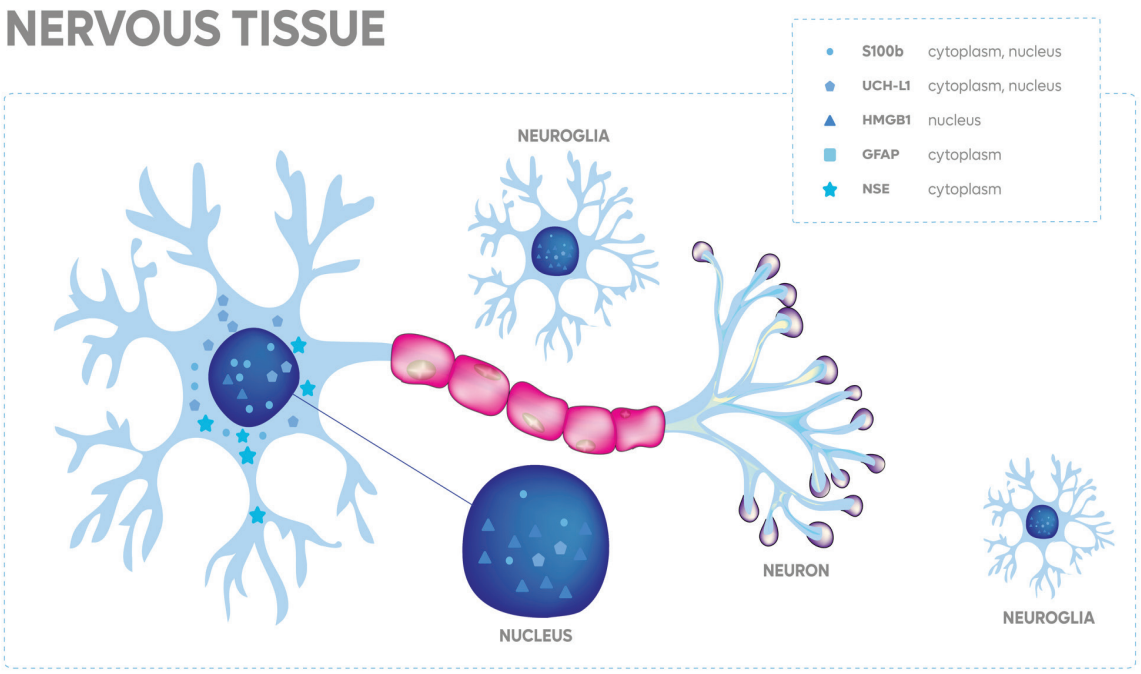

Fig. 1. Cell specific localization of proteins which are considered as the serum biomarkers of brain injury.

In the following section we will provide more detailed information for both neuronal and glial specific proteins found in the serum following the brain injury. In between those of neuronal origin we will cover – neuron-specific enolase (NSE) and ubiquitin carboxy-terminal hydrolase L1 (UCHL1). Concerning those of the glial origin – S100 calcium-binding protein beta (S100B) and glial fibrillary acidic protein (GFAP) will be described. In addition to those with clear cellular origin, molecules related to inflammation are also considered to be promising biomarkers of tissue injury in general and under specific conditions also for the brain injury. High-mobility-group-protein-box-1 (HMGB1) will be thus also explained. Since these biomarkers (related to the neuroinflammation) are not tissue specific they possess good potential, however, likely in combination with more specific biomarkers (Fig. 2).

PROTEIN	SYMBOL	MOLECULAR WEIGHT (kDa)	HALFLIFE (HRS)
S100b	●	11	0,5–1,5
UCH-L1	⬟	26	7–9
HMGB1	▲	25	17 minutes
GFAP	■	50	24–48
NSE	★	78	48–72

PROTEIN	SYMBOL	BRAIN	EXTRACRANIAL DISTRIBUTION	CELL DISTRIBUTION
S100b	●	yes (astrocyte)	yes	cytoplasma, nucleus
UCH-L1	⬟	yes (neuron)	no	cytoplasma, nucleus
HMG1B	▲	yes	yes	nucleus
GFAP	■	yes (astrocyte)	no	cytoplasm
NSE	★	yes (neuron)	yes	cytoplasm

Fig. 2. Molecular and physiological characteristics of selected biomarkers of the brain injury. Data derived from [30,31,32,33,34,35,36].

Neuron specific enolase (NSE)

NSE is the neuronal form of the intracytoplasmic glycolytic enzyme (isoenzyme homodimer, gamma enolase). Enolase, in vertebrates expressed by different genes, is present as enolase α (ubiquitous), enolase β (muscle-specific), and enolase γ which is neuron-specific. NSE, which occurs as γγ- and αγ-dimer, is a late event in neural differentiation, is found almost exclusively in neurons and cells of neuroendocrine origin, and is measurable in blood and cerebrospinal fluid [36]. NSE is released into the blood following the brain injury from neurons into the extracellular space and bloodstream. Levels of NSE in peripheral blood thus can serve as markers of the extent of the brain injury. The molecular weight of the dimeric form of NSE is 78 kDa [32]. The biological half-life of NSE in body fluids is approximately 48-72 h [32]. A positive correlation between NSE levels and infarct volume has been reported in acute ischemic stroke patients. Zaheer *et al.* have found a positive correlation between the concentration of NSE and infarct volume determined by computed tomography 1 day after stroke, and a strong negative correlation between the Glasgow coma scale (GCS) at presentation and concentration of NSE on day 1. Thus the serum levels of NSE in the first days after the stroke predict stroke severity and early functional outcome [38]. NSE has been evaluated also in other conditions where neuronal damage has been reported. Serum levels of NSE have been shown to provide quantitative measures of brain damage and to improve the diagnosis in intracerebral hemorrhage, seizures, and comatose patients after cardiopulmonary resuscitation for cardiac arrest, and traumatic brain injury. However, since the NSE is of a more general origin its levels are also elevated in various neuroendocrine tumors, lung cancer, neuroblastoma, seminoma, or melanoma, for the review see Isgrò *et al.* [37]. The clinical significance is emphasized when both NSE and S100B markers are

evaluated together. NSE and S100B were at the same time analyzed in acute ischemic and intracerebral hemorrhage. High serum NSE and S100B were associated with poor outcomes in ischemic stroke. These findings suggest the high potential of simultaneous evaluation of NSE and S100B as reliable prognostic markers for acute stroke [39].

S100B (S100 calcium-binding protein beta)

S100B is a low molecular weight (10.5 kDa) calcium-binding protein that is primarily expressed and secreted by astrocytes. S100B can be found in very low levels in human CSF and serum, and normal levels of this protein have been strongly correlated with the absence of intracranial injury [40]. After brain injury, S100B is immediately released from damaged glial cells and can be detected in the blood as fast as 30 min after the trauma and is eliminated from the organism by the kidney. The serum half-life of S100B ranges between 30 to 90 min. S100B becomes detectable promptly following a brain injury. During the initial 24 to 48 h after the injury, serum concentrations of S100B exhibit rapid fluctuations. However, a definitive and universally standardized timeframe for sample collection remains elusive [41]. Besides brain tissue, S100B expression is found in the adipose tissue, skin, or skeletal muscles [42]. Release into the serum after brain injury could theoretically be the result of several factors including impaired blood-brain barrier integrity [43]. Therefore, some authors suggest S100B as a candidate peripheral biomarker of blood-brain barrier (BBB) permeability and/or CNS injury. For more information see an excellent review from Michetti *et al.* [44]. Although S100B is currently regarded as a reliable biomarker of acute brain injury it has to be emphasized that during traumatic brain injury, an extraneural origin (adipose tissue) should be considered in the interpretation of the results since elevated levels of S100B has been detected also in patients after the trauma in the absence of head injuries [45]. S100B released from extracerebral sources, however, has a faster clearance compared to S100B released from the brain [46]. Adding serum protein (e.g. S100B) protein blood level to current recommendations could therefore reduce the need for CT examination and save costs [47].

UCH-L1 (Ubiquitin carboxy-terminal hydrolase L1)

The ubiquitin C-terminal hydrolases (UCH-L1, UCH-L3, UCH37, and BAP1) form a subfamily among the deubiquitinating enzymes that are capable of removing ubiquitin from their protein substrates. Expression of UCH-L1 is highly specific to neurons and to cells of the diffuse neuroendocrine system. Although UCH-L1 protein expression is specific to neurons and testis/ovary tissue, it has been also found to be expressed in lung-tumor cells [48]. UCH-L1 is a relatively small protein (molecular weight of 25 kDa) that catalyzes hydrolysis of C-terminal esters and amides of ubiquitin. The half-life of UCH-L1 in cerebrospinal fluid and serum spans between 7 to 9 h, respectively [33]. At the cellular level, UCH-L1 is expressed and fills the cytoplasm of neurons throughout the brain. Since UCH-L1 has restricted distribution in other tissues, it has led to the suggestion to use UCH-L1 as a neuron-specific biomarker of brain injury [49]. On 14 February 2018, the FDA authorized the marketing of the first blood test to evaluate concussion in adults based on peripheral levels of UCH-L1 and GFAP. Since then several trademarks using this principle appeared on the market. Levels of UCH-L1 and GFAP after mild traumatic brain injury/concussion can help predict which patients may have intracranial lesions visible by CT scan and which will not [50,51].

GFAP (Glial fibrillary acidic protein)

GFAP is a polypeptide with a molecular weight of 49 kDa [34]. GFAP is an intermediate filament protein, found in the cytoskeleton of astrocytes. The filament is expressed in mature astrocytes throughout the gray and white matter of the brain, the cerebellum, the subventricular and subgranular zones, Mueller cells in the retina, in the periphery by Schwann cells, mature glial cells in the gut, hepatic stellate cells, and other non-neural cells. The isoform that is most abundantly expressed and most often analyzed in the literature is GFAPα [52]. The mechanisms underlying the release of GFAP and its breakdown products into the blood after the injury is rather complex and are a matter of continuing debate. It has been suggested that drainage is likely to result from a combination of CSF absorption *via* arachnoid villi, flow along the glymphatic system and the cervical lymph nodes, and exchange at the BBB which typically compromised after the acute brain injury (for more detail see an excellent review from Abdelhak *et al.* [52]). GFAP is detectable in blood one hour after injury, its peak 20-24 h, GFAP demonstrates a comparatively extended half-life, encompassing a range of 24 to 48 h [53]. GFAP in combination with other neuron-specific markers such as NSE or UCH-L1 seems to be a reliable

and applicable biomarker of mild traumatic brain injury and has been approved by FDA already [50].

HMGB1 (High-mobility-group-protein-box-1)

HMGB1 protein has been known as a highly conserved nuclear protein and is made up of 215 amino acids. In a homeostatic state, HMGB1 acts as a chaperon of DNA [54]. Clinically HMGB1 plays an important role in various types of diseases such as autoimmune, infectious, and inflammatory. HMGB1 is considered to be an alarmin or damage-associated molecular pattern molecule, which reaches extracellular space through active secretion or passive release immediately after tissue injury, thus triggering and mediating consequent inflammation and immune response [55]. After the cell injury, HMGB1 translocates to the cytoplasm from nuclei, and consequently to extracellular space. HMGB1 is also actively secreted by inflammatory cells like macrophages or monocytes and NK-cells during tissue injury [56]. The functions of HMGB1 could be divided on the basis of its localization (intracellular, extracellular space) and modifications. Different physiological functions of the HMGB1 protein are based on its redox forms [36]. Posttranslational modifications and different redox forms aggravate the interpretation of serum levels of HMGB1 [57]. HMGB1 reacts very early after an insult, well documented in several studies. Elevation of HMGB1 peaks after the mechanical brain trauma in plasma as early as tens of minutes to 6 h after the injury, however, in this study patients with isolated head injuries were excluded [58]. Systemic HMGB1 levels *in vivo* in mice model started at 8 h and increased substantially from 16 to 32 h after lipopolysaccharide administration [59]. After the brain injury, HMGB1 is quickly released from affected neurons through a mechanism mediated by the N-methyl D-aspartate receptor. The half-life of HMGB1 is contingent upon its redox state, with a calculated duration of 17 min in serum [60]. HMGB1 protein induces an inflammatory response in the experimental traumatic brain injury model by increasing the expression of TLR4 and RAGE receptors, which leads to brain edema and neuronal apoptosis [61]. Although HMGB1 is not specific for neuronal tissue injury, its sudden and extensive increase has been considered also in other neurological diseases including seizures and epilepsy [62]. A clinical study on pediatric epilepsy patients showed that levels of HMGB1 and TLR4 in the severe epilepsy group were significantly increased compared to both the control group and the mild epilepsy group. Moreover, patients in the mild epilepsy group also significantly differed from those in the control group, indicating that the overexpression of HMGB1 and TLR4 is related to the severity of epilepsy [63]. Serum levels of HMGB1 are negatively correlated with neurological function scores of epileptic patients but positively correlated with seizure frequency and the number of epileptiform discharges. This finding thus suggests that serum HMGB1 levels can be considered also as a prognostic marker for epileptogenesis [64].

Temporal profiles of molecular biomarkers in the serum after the brain injury

Understanding the temporal dynamics of serum molecular biomarker levels associated with brain injuries holds the potential to facilitate precise diagnoses by healthcare practitioners. An extensively researched biomarker, S100B, demonstrates a rapid surge shortly after traumatic brain injury (TBI) and boasts a relatively short half-life of approximately one hour [65]. This kinetic behavior positions S100B as a potential diagnostic tool for excluding TBI in circumstances where neuroimaging methods are unavailable [66]. In contrast, sustained elevated levels of serum NSE are attributed to its neuron-specificity, rendering it valuable for predicting neurological outcomes following systemic hypoxic insults [67]. In a study involving a pediatric population, serum UCH-L1 exhibited a peak at 8 h post-brain injury, followed by a rapid decline [68].

The release of inflammation markers into the bloodstream from various peripheral sources contributes to the complexity of using HMGB1 for precise assessment of neuronal injury. However, in combination with more specific yet less sensitive biomarkers such as NSE, which elevates at later stages post-TBI, HMGB1 can offer valuable insights [69]. The observed serum peaks of the aforementioned biomarkers are illustrated in Figure 3.

Emerging findings from animal models suggest that S100B, along with NSE and GFAP, is transported into the bloodstream through glymphatic drainage, independent of blood-brain barrier (BBB) permeability [68]. Notably, larger molecular weight biomarkers experience reduced renal clearance, leading to prolonged serum elevation. Serum S100B, distinctively, demonstrates leakage after BBB disruption, further positioning it as a potential marker of BBB permeability, contrasting with NSE (Fig. 4) [70].

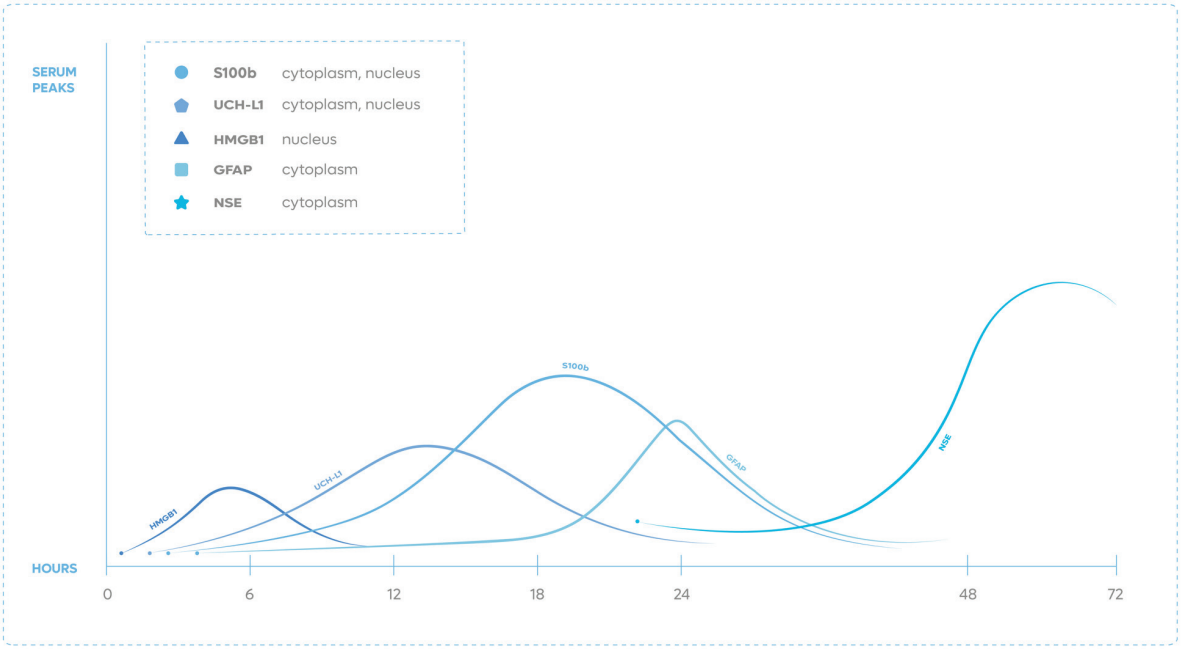

Fig. 3. Serum time profiles of selected molecular biomarkers of the brain injury (serum peaks) – information from text.

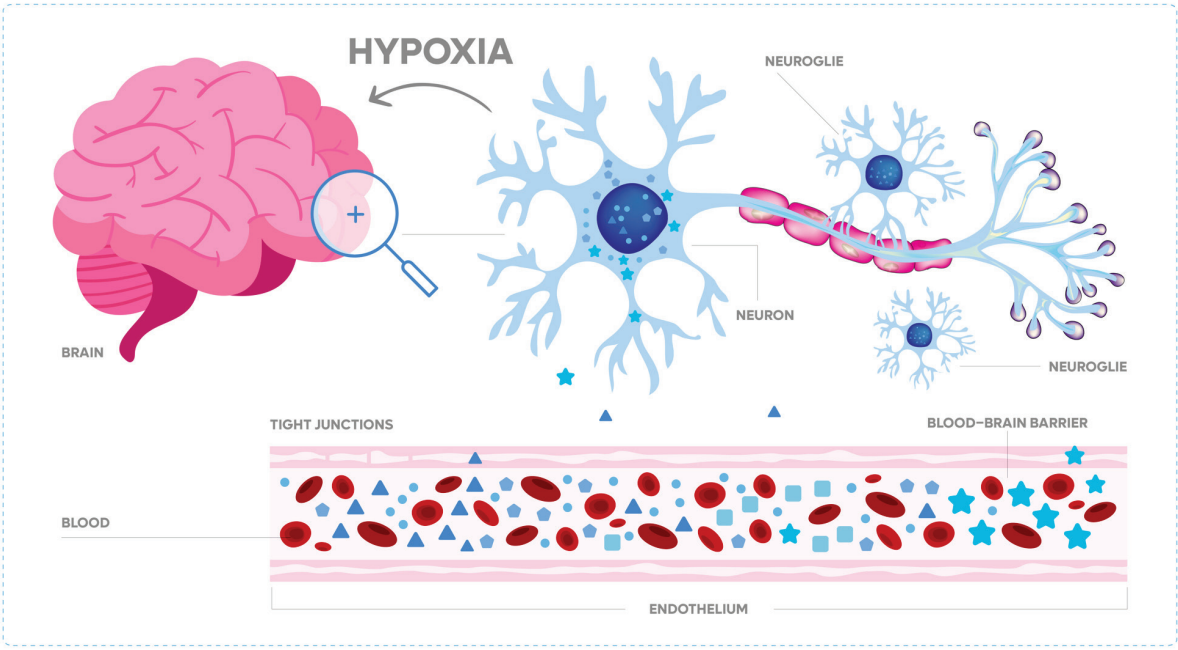

Fig. 4. Blood-brain barrier environment after hypoxic insult. Blood-brain barrier represents a specific milieu in the organism. Each symbol represents a different neuronal biomarker excreted into the interstitium after hypoxic insult. From the interstitium markers pass through the blood-brain barrier to the blood where they are detected.

Current application of molecular biomarkers of the brain injury in newborns

Clinical utilization of serum biomarkers is restricted in the present to traumatic brain injury by international guidelines. Emergency guidelines use clinical imaging (CT) and recently added blood biomarkers in acute assessment of traumatic brain injury. In 2018, the FDA approved the assessment of serum levels of both GFAP and UCH-L1 reliable for use in the emergency department to screen for traumatic intracranial abnormalities and facilitate urgent clinical decisions regarding acute head CT scanning. Scandinavian guidelines also recommended the use of S100B for traumatic brain injury [71]. However, the efficacy

in urgent pediatric care has to be confirmed by larger studies in the pediatric population. An innovative assessment and design of the new French study is now enrolling patients in Clermont-Ferrand, France [72]. The efficacy of biomarkers to assess the extent of brain injury after the hypoxic-ischemic insult in newborns is less documented in the literature. Hypoxic-ischemic brain injury in newborns is a special but still frequent and serious acute condition in pediatric emergency medicine. Early and right diagnosis including estimation of the extent of the brain injury is therefore critical in decision making for immediate treatment by therapeutic hypothermia.

Higher serum IL-6 and G-CSF at birth in hypoxic-ischemic patients were associated with the development of moderate to severe hypoxic-ischemic encephalopathy [73]. Elevated GFAP, IL-1, IL-6, IL-8, tumor necrosis factor, interferon γ, and vascular endothelial growth factor in the serum at 6-24 h were also associated with abnormal neurological outcomes. The rationale for the use of molecular biomarkers for the estimation of hypoxic-ischemic injury at birth is strengthened by the fact that neuronal biomarkers in cord serum correlated with the severity of HIE and outcomes [74]. However, systemic hypothermia therapy can decrease serum UCH-L1 levels and increase serum GFAP levels in neonates with HIE which handicapped them from monitoring the brain injury development during the therapy. Based on their diagnostic value of brain injury, GFAP and UCH-L1 are still promising to be novel early biomarkers of brain injury after hypoxic-ischemic insult at birth [75]. Protein S100B in neonates suffering from hypoxic-ischemic insults showed elevated in umbilical cord blood at birth as well. The S100B concentrations were positively associated with the severity of the disease and the risk of suffering from neurodevelopmental sequelae and even death [76].

In contrast, another study refused the correlation of HIE severity with the levels of biomarkers in cord sera of newborns. The cord sera of 15 neonates with hypoxic-ischemic encephalopathy were analyzed for UCH-L1 and GFAP. No differences in cord blood UCH-L1 and GFAP concentrations were found between HIE neonates and controls, and no associations were found between the biomarker concentrations and the severity of disease, or whether the condition developed into a permanent or fatal injury [77]. This controversy has to be solved by performing new well controlled clinical studies before the decision on the reliability of the molecular biomarkers in evaluation of the hypoxic ischemic injury in newborns can be made.

Conclusions

Although many efforts in prevention have been made to minimize the incidence of brain injury due to various causes, it still represents a significant health and socio-economic burden. Early diagnosis and estimation of the extent of the injury are crucial in deciding on the treatment. Although neuroimaging methods such as CT or MRI provide reasonable results, in some cases and situations they burden several crucial disadvantages. Thus, in the past decades' dramatic progress in our understanding of molecular processes ongoing after brain injury led to the design of various candidate molecules which were suggested to serve as biomarkers of the condition. In this review, we provided an overview of current knowledge on serum biomarkers of brain injury which has been already tested in both animal models and human brain pathologies. We covered specificities, time profiles and available experimental and clinical data from the literature for brain injury-specific serum biomarkers namely NSE, UCH-L1, S100B, GFAP, and HMGB1. Although it has not been possible to identify a molecule that can serve as a universal serum biomarker of brain injury till now, it seems, however, that a combination of two markers of different mechanisms provides reasonable results. Indeed, the FDA in 2018 approved an easy diagnostic test evaluating brain injury based on a combination of rapid detection of serum levels of UCH-L1 and GFAP. It has been shown reliable for adult patients where it can help in the triage of patients needing CT. However, in pediatric and especially newborn patients this method has not been confirmed, and thus seeking relevant biomarkers still remains. Since unstable newborns could not be transferred to the radiological department for MRI or CT imaging, reliable serum biomarkers thus could dramatically improve early diagnostics, treatment, and finally the outcome of injured neonates.

Conflict of Interest

There is no conflict of interest.

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