Transl. Stroke Res. (2011) 2:455–462
DOI 10.1007/s12975-011-0137-6

REVIEW ARTICLE

Protein Biomarkers for Traumatic and Ischemic Brain Injury: From Bench to Bedside

Zhiqun Zhang · Stefania Mondello · Firas Kobeissy · Richard Rubenstein · Jackson Streeter · Ronald L. Hayes · Kevin K. W. Wang

Received: 6 October 2011 / Revised: 7 November 2011 / Accepted: 8 November 2011 / Published online: 7 December 2011

Abstract Stroke is the second leading cause of death worldwide and the third leading cause of death in the USA. A clinically useful biomarker for the diagnosis of stroke does not currently exist. Biomarkers could improve stroke care by allowing early diagnosis by non-expert clinical providers, serial monitoring of patients, and rapid assessment of severity of brain injury. With the introduction of highly advanced multidimensional separation techniques coupled with high throughput genomics/proteomics platforms, several components of the pathophysiological and biochemical pathways have been elucidated in the areas of brain trauma. A major outcome of these approaches is the discovery of biomarkers that would have important applications in diagnosis, prognosis, and even development of experimental neuroprotective drugs that have been used in different paradigms of brain injury. In this paper, we reviewed the recent advances of current and novel brain injury protein biomarkers and their utilities in different models of brain injury with an emphasis on stroke, an area that has been understudied. This will include the utility of neuroproteomics/neurosystems biology analysis as a novel discipline leading to the identification of novel biomarkers that can reach the pipeline of bench side. Additionally, an outline of biomarker-based management of traumatic brain injury and stroke patient assessments of therapeutic interventions has been included. Finally, comparison of current biomarker occurrence between preclinical models and biomarker data from human clinical studies for stroke has been summarized.

Keywords Biomarkers · Brain injury · Cell death proteolysis

The Need for Biomarkers for Brain Trauma

There have been significant advances in our understanding of the pathobiology and biochemical pathways relevant to the areas of traumatic brain injury (TBI). At the same period, numerous experimental drugs have been tested and shown to be neuroprotective in experimental animal model of brain injury; however, these efforts failed to translate successfully into TBI clinical trials [1]. These unsuccessful outcomes have led researchers and pharmaceutical companies to modify their approach for TBI clinical trials by switching into utilizing drug intervention-tracking biomarkers' changes [2].

Z. Zhang · S. Mondello · F. Kobeissy · R. Rubenstein · J. Streeter · R. L. Hayes · K. K. W. Wang (✉)
Center of Innovative Research, Banyan Biomarkers Inc,
12085 Research Drive,
Alachua, FL 32615, USA
e-mail: kawangwang17@gmail.com

F. Kobeissy · K. K. W. Wang
Department of Psychiatry, Department of Neuroscience,
McKnight Brain Institute,
University of Florida,
Gainesville, FL 32610, USA

R. L. Hayes
Department of Anesthesiology, University of Florida,
Gainesville, FL 32610, USA

R. Rubenstein
Department of Neurology, Laboratory of Neurodegenerative Diseases and CNS Biomarkers,
SUNY Downstate Medical Center,
Brooklyn, NY 11203, USA

K. K. W. Wang
Taipei Medical University,
Taipei, Taiwan

For this purpose, the areas of proteomic/genomic methods have been the key instruments in the discovery of novel brain injury biomarker candidates [3–6]. As discussed below, several brain injury biomarkers have the potential to revolutionize medical practice and biomedical research in the area of TBI [7]. However, we and others strongly believe that there is an unmet medical need for designing a simple biofluid-based rapid diagnostic test for both the management of severe and moderate TBI patients in the intense care unit as well as for triaging mild TBI patients arriving in the emergency room.

Diagnostic tests based on protein biomarkers have already demonstrated proven clinical diagnostic utility in acute care environments. For example, in the area of cardiac injury, cardiac troponin proteins (T and I) and various forms of brain natriuretic peptide (BNP), often in combination with other biomarkers, are routinely used to facilitate accurate diagnosis of congestive heart failure and myocardial infarction in patients presenting with chest pain [8–10]. With the growing recognition of the importance of biomarkers, a *Biomarkers Consortium* was launched in the USA in October 2006 as a public-pharmaceutical industry partnership including the National Institutes of Health (NIH), the Food and Drug Administration (FDA) as a part of the FDA's critical path initiative, the Centers for Medicare and Medicaid Services, as well as pharmaceutical industry representatives and nonprofit organizations and advocacy groups. An NIH workshop on improving diagnosis of TBI for targeting therapies highlighted the need for biomarker identification [11].

Current and Novel Brain Injury Protein Biomarkers

Several brain injury biomarkers have been cited in literature. Table 1 describes a number of studied traumatic and ischemic brain injury biomarkers documented in literature. Among these, neuron-specific enolase (NSE) [12], glial protein S-100β [13], glial fibrillary acidic protein (GFAP), and myelin basic protein (MBP) have been shown to have great utility in TBI specifically [5, 7]. Different studies illustrated the diagnostic potential of these brain injury biomarkers; however, other studies have provided conflicting results. NSE, for example, initially held great potential as a specific brain injury biomarker since it was originally believed to be strictly neuronal. Of interest, assays of serum NSE together with S100β proteins have been valuable in predicting TBI outcomes [13, 14]. However, additional research found that NSE was also present in red blood cells and platelets, decreasing its diagnostic utility as a marker due to possible cross contamination from blood [15]. After multiple traumas, increase in NSE levels has been observed; however, this increase was not specific to the occurrence of TBI, limiting its ability to be a discriminator of brain injury magnitude [12, 16, 17].

Along the same lines, our group and many others have characterized αII-spectrin protein and its breakdown products SBDPs. αII-spectrin generates a number of BDPs; SBDP150 and SBDP145 are produced by calpain activation in acute necrosis phase, while SBDP120 is produced by caspase-3 activation during delayed apoptosis phase. These specific SBDPs serve as potential biomarkers for excitotoxic, traumatic, and ischemic brain injury in rat as well as in human brain trauma [5, 7, 18]. Others have proposed that the cleaved tau protein (c-tau) and a fragmented form of the glutamate-*N*-methyl-D-aspartate (NMDA) receptor (NR2A/2B subtype) [19] might have similar utility as potential biomarkers in brain trauma. In addition, studies by Petzold and Shaw have identified neurofilament-H as a promising axonal injury biomarker for various forms of acute brain damage [20, 21].

Using differential neuroproteomic methods, a systematic assessment was made to identify additional unidentified protein biomarkers for TBI, ischemic and penetrating brain injury with relevant animal models [22–26]. As a follow-up, proteomics-based approach was applied to select top-down candidate markers that represent distinct pathways and hot spots [4]. One such candidate biomarker identified was ubiquitin C-terminal hydrolase-L1 (UCH-L1). It has recently published by us that UCH-L1 is released into both the CSF and blood following both experimental TBI (controlled cortical impact) and ischemic stroke (transient middle cerebral artery occlusion); [18, 27, 28] and in a relevant model of blast overpressure wave-induced brain injury in rats [29]. Similarly, Papa et al. and Brophy et al. have also reported that UCH-L1 was released into the CSF and blood almost immediately following a severe TBI incident [18, 27]. Siman and his colleagues described that UCH-L1 (in addition to phosphorylated neurofilament-H, αII-spectrin breakdown product, and 14-3-3 proteins) is elevated in human CSF following surgically induced circulation arrest [30, 31], as well as in a small severe TBI cohort [30–32]. Independently, UCH-L1 was found to be released into the CSF following aneurysmal subarachnoid hemorrhage in humans [28]. Table 1 summarizes the major identified biomarker candidates in TBI.

In the area of stroke research, Allard and colleagues (2005) have identified PARK7 (DJ-1) protein and nucleotide diphosphate kinase A protein as potential ischemic stroke markers, but their brain specificity and distribution have not been well characterized [33]. Endothelial monocyte-activating polypeptide II precursor (EMAP-II) is another potential microglia biomarker identified by differential neuroproteomics [24, 25], which is unregulated in the CSF and plasma after experimental TBI (Table 1). S100β, UCH-L1, and GFAP proteins are also useful markers for stroke therapeutic development [32]. GFAP

Table 1 Current and novel biomarkers for ischemic and traumatic brain injury

Putative biomarker	Key characteristics	Animal model evidence	Human evidence	Key references
S100β	Glial and blood brain barrier marker	CSF, serum	CSF, serum	Missler et al. 2003 [49]; Raabe et al. 1999 [50]; Romner et al. 2002 [51]; Marchi et al. 2003 [52]; Blyth et al. 2009 [53]; Ehrenreich et al. 2011 [32]
MBP	Demyelination marker	–	Serum	Yamazaki et al. 1995 [54]; Wang et al. 2005 [5]
NSE	Neural damage marker	–	Serum	Missler et al. 2003 [49]; Ross et al. 1996 [55]; Yamazaki et al. 1995 [54]
GFAP and its BDP	Gliosis	–	Serum	Pelinka et al. 2004a [56], 2004b [57]; Vos et al. 2004 [58]; Lumpkins et al. 2008 [59]; Dvorak et al. 2009 [60]; Foerch et al. 2007 [34]; Mondello et al. 2011 [62]; Papa et al. 2011 [61]
αII-spectrin BDPs (SBDP150, SBDP145, SBDP120)	Neural necrosis/apoptosis (calpain/caspase), axonal injury	CSF	CSF, serum	Pike et al. 2001 [63]; Ringger et al. 2004 [64]; Siman et al. 2004 [66], 2005 [65]; 2009 [31] Pineda et al. 2007 [67]; Mondello et al. 2010 [68]
C-tau	Axonal injury marker	CSF, serum	CSF	Zemlan et al. 2002 [69]; Shaw et al. 2002 [70]
IL-6, 8, TNF-α, MMP9	Neuroinflammation	–	CSF, serum	Maier et al. 2005 [71]; Chiaretti et al. 2008 [72]; Folkersma et al. 2008 [73]; Reynolds et al. 2003 [36]
NMDAR fragment	Postsynatic receptor marker	CSF, serum (?)	Serum	Dambinova et al. 2003 [19]
FABPs (brain, heart types)	Neural protein	–	Serum	Pelsers et al. 2005 [74], 2004 [75]
Neurofilament proteins (NF-H, -M, -L)	Axonal injury markers	CSF, serum	CSF	Petzold et al. 2005 [76]; Petzold and Shaw 2007 [21]; Norgren et al. 2004 [77]
UCH-L1	Neural cell body marker	CSF, serum	CSF, serum	Papa et al. 2010 [18]; Liu et al. 2010 [78]; Siman et al. 2005 [65], 2009 [31]; Ehrenreich et al. 2011 [32]
EMAP-II	Microglia activation	CSF, serum	–	Yao et. al 2008, 2009 [24, 25]

might have the ability to distinguish hemorrhagic from ischemic stroke [34], allowing for the use of TPA, which is contraindicated for hemorrhagic stroke patients. These biomarkers could represent different pathways that can be at play at various time points after the initial injury.

Finally, neuroinflammatory-linked cytokines [interleukin (IL)-6, IL-8, tumor necrosis factor-α (TNF-α), and MMP9] have been also studied in the area of stroke biomarkers. It is also appropriate to think of TBI biomarkers as a continuum of biomarkers that might be released at different time points following the initial brain injury event (Fig. 1) [35].

Neurosystems Biology Analysis in Brain Trauma

In the area of stroke, one major hurdle arises from the fact that the diagnosis and management of stroke patients are limited by the lack of rapid diagnostic assays for use in emergency settings as will be discussed later. Thus, the hunt for specific and sensitive stroke biomarkers has been recently initiated since these signature proteins would aid in discriminating among different stroke phenotypes and help in designing rapid diagnostic assays [36]. In our work in the area of neurotrauma, we have utilized different "Omics" approaches coupled with systems biology (SB) analysis to identify novel key brain injury markers that can be used as a clinical end point in neurotrauma [37, 38].

Coupled to current genomics and proteomics analysis utilized in biomarker identification, SB represents a mathematical model capable of predicting the altered processes or functions of a complex system under normal and perturbed conditions. It combines experimental, basic

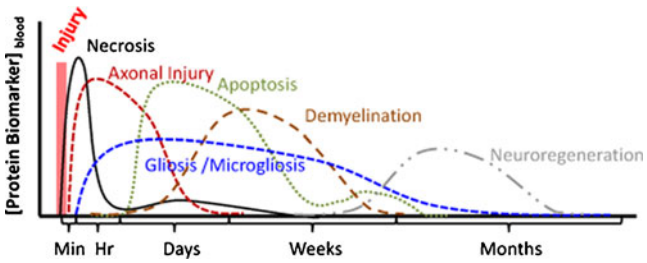

Fig. 1 Biomarkers for monitoring various temporally and biochemically distinct events following brain injury

science data sets, proteomic and genetic data sets, literature, and text mining. When constructed properly, SB databases can provide a context or framework for understanding biological responses within physiological networks at the organism level, rather than in isolation [39].

In the area of brain trauma, neurosystems biology platform harnesses data sets that, by themselves, would be overwhelming, into an organized, interlinked database that can be queried to identify non-redundant brain injury pathways or convert hot spots. These can be exploited to determine their utilities as diagnostic biomarkers and/or therapeutic targets. The ultimate goals of system biology are: first by exploring the systems component (gene, protein, small molecule, metabolite, etc.), help biologists, pharmaceutical companies, and doctors to better understand the mechanisms underlying the disease components [40]. In the field of neurotrauma, identifying and analyzing brain injury-related networks play important and practical clues relating to biological pathways relevant to disease processes. However, the more important underlying goal is to provide important cues that may suggest radically new approaches to therapeutics. In the brain injury for example, it has been shown that calpain and caspase proteases are major components in cell death pathways taking part in two destructive proteolytic pathways that not only contribute to key forms of cell death (necrosis and apoptosis) but also in the destruction of important structural components of the axons [alpha II-spectrin breakdown products (SBDPs) and tau], dendrites (MAP2), and myelin (MBP; Fig. 2). Interestingly, two different forms of SBDPs reflect either neuronal necrosis (SBDP150 and SBDP145 cleaved by calpain) or neuronal apoptosis (SBDP120 cleaved by caspase-3) [5]. These SBDPs and other similar neural protein breakdown products can serve as target pathway specific biomarkers as illustrated in Fig. 2.

For this purpose, rather than focusing on individual molecular components, systems biology seeks to understand the system dynamics that govern protein networks, the functional set of proteins that regulate cellular decisions related to TBI. From the perspectives of drug discovery and

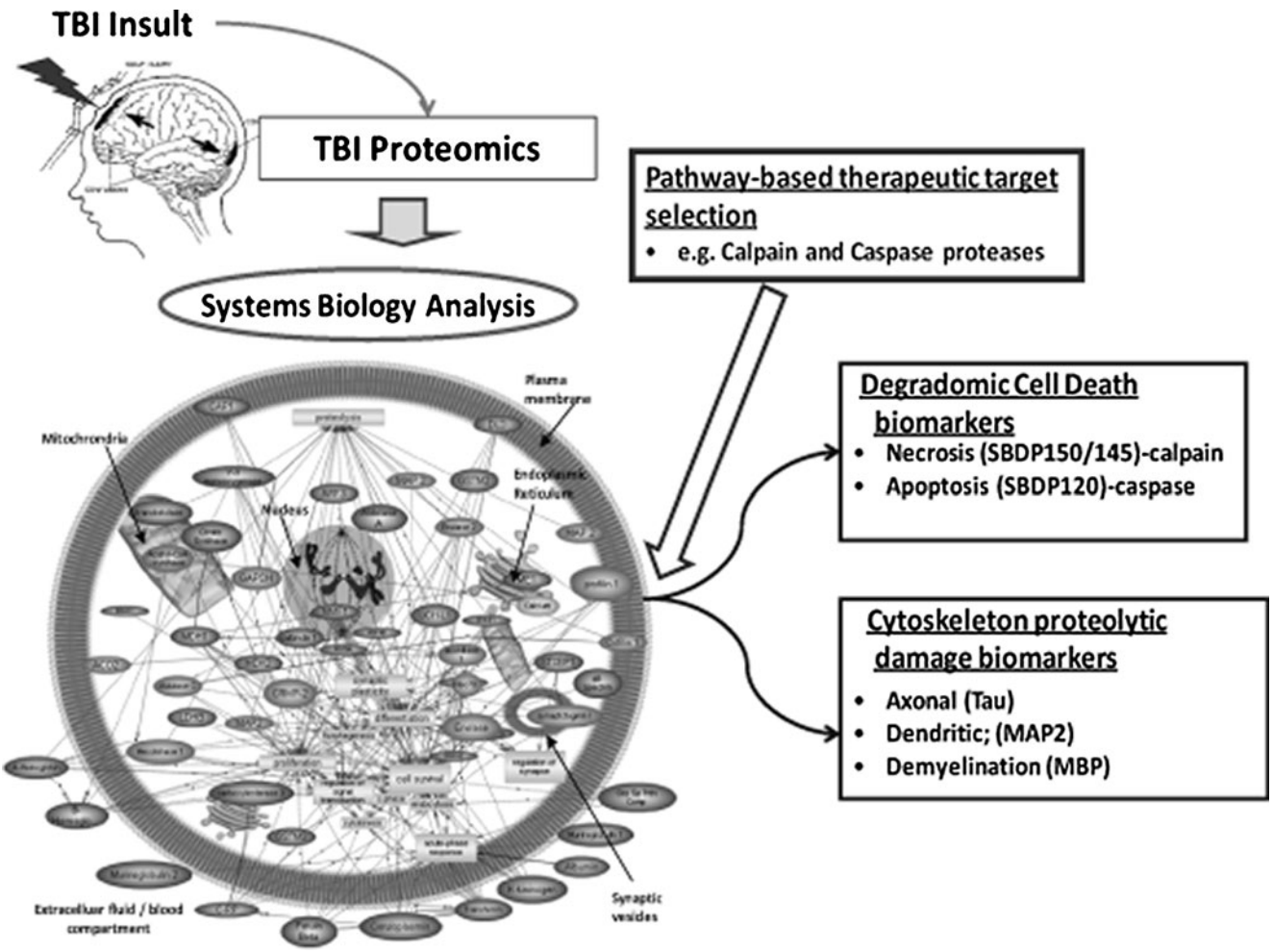

Fig. 2 System biology based on TBI biomarker study

diagnostics, systems biology gives important and practical clues concerning the pathways relevant to TBI and the effects that drugs might have on them. Therefore, it enhances the entire biomarker and therapeutic drug discovery, development, and commercialization process.

Biomarker-Based Management of TBI and Stroke Patients Assessing Therapeutic Intervention

There have been more than 200 unsuccessful clinical trials assessing potential therapies for traumatic brain injury (TBI), and currently, there are no FDA-approved therapies. As early as 2002, an NIH workshop recognized the need for development of more refined surrogate measures to improve design and execution of clinical trials in the field of head injury [41]. There are a number of areas in which incorporation of biomarkers could significantly improve clinical trial design and execution. Injury magnitude is an important criterion for determining patient eligibility for TBI clinical trials. Obviously, it is important to have patients with similar magnitudes of injury in different treatment groups. At present, the Glasgow Coma Scale (GCS) is the primary, if not the exclusive, entry criterion assessment tool for injury magnitude. Given the difficulties associated with accurate GCS assessment outlined above, biomarkers could provide an objective, quantitative assessment of injury magnitudes [41].

Secondary brain insults worsen neurologic outcome after TBI. In an effort to prevent occurrences of these insults, physiologic vital signs (e.g., intracranial pressure, mean arterial blood pressure, and tissue oxygenation) are routinely assessed in intensive care environments, although usually recorded only intermittently in the medical record. Conventional manual recording of vital signs can underestimate the total number of secondary insults. Undetected occurrence of secondary insults and increased neurologic damage in different treatment groups can significantly enhance variability in clinical trials. Detection of elevated biomarker levels, in conjunction with physiological assessment and management, could provide critical information to reduce the number of undetected secondary insults and allow for stratification of patients by occurrence of these insults [32].

As discussed previously, management of severe TBI patients can importantly influence clinical outcome, potentially by altering the number of secondary insults. In spite of various educational programs, the American Association of Neurological Surgeon Guidelines for Management of Severe TBI Patients is not uniformly followed. Moreover, even when efforts are made, failure to rigorously standardize clinical management in different centers could contribute to outcome variability in severe TBI clinical trials. For example, in a recent trial assessing the effects of moderate hypothermia on severe TBI, treatment effects for the five largest centers varied between 14% positive and 20% negative. Although there were significant differences in cerebral perfusion pressure management among the centers, investigators did not detect a correlation with treatment effect and attributed center differences to other baseline variables. In any case, a systematic assessment of biomarkers on admission and during the course of management could provide critical insights into potential differences between patient cohorts among centers [41].

Accurate prediction of outcome is a critical component of the design for the clinical TBI trials. In severe TBI, the Glasgow Outcome Score (GOS) or an extended version of this scale (GOS-E) is typically used. These scales provide broad distinctions and include categories such as “good outcome,” “severely disabled,” “vegetative,” or “dead.” New more powerful clinical trial designs for phase III trials in TBI and stroke employ statistical techniques based on outcome predictions as better as or worse than expected, taking into account each individual patient’s baseline prognosis. Thus, the use of biomarkers to enhance the accuracy of early predictions of outcome could importantly improve the power of phase III trial designs.

The recent Workshop on Classification of TBI emphasized the need for surrogate markers based on pathophysiological mechanisms of TBI in humans in clinical trials assessing targeted TBI therapies [1]. For example, recent work by us has confirmed the potential utility of biochemical markers to assess pathophysiological mechanisms of necrosis and apoptosis, and specific forms of cell death occurring after severe TBI. The biochemical markers identify the activity of specific destructive proteases (e.g., calpain and caspases) related to these pathological processes. Appropriate drug therapies targeting proteolytic by-products could use these biochemical markers as indices of therapeutic efficacy. This therapeutic marker would be available to investigators during the acute phase of injury in advance of 6 months’ outcome assessments typically used in such studies. Obviously, these biochemical markers are more closely linked to therapeutic effects on brain tissue compared to the neurological outcome measures such as the GOS or GOS-E.

Acute ischemic stroke is a significant international health concern, representing a potentially catastrophic debilitating medical emergency with poor prognosis for long-term disability. Stroke is the second leading cause of death worldwide [1] and the third leading cause of death in the USA [42]. Stroke affects more than 700,000 Americans annually [43]; every 45 s, someone in America has a stroke, and every 3 min, someone dies of a stroke [42]. In the USA, this type of injury is the primary cause of serious, long-term disability and the main reason for nursing home

admissions [42, 43]. In 2007, the estimated direct and indirect cost of stroke is $62.7 billion [42]. This number will continue to escalate as the aging US population grows.

Precise diagnosis of stroke patients is typically made by trained clinicians, supported by neuroimaging, usually a brain computed tomogram (CT), in some cases supplemented by diffusion- and perfusion-weighted magnetic resonance imaging (MRI). This allows ready differentiation of ischemic stroke from hemorrhagic ones. However, CT is often normal in the acute phase of stroke and negative in the presence of small ischemic lesions or in certain brain locations (posterior fossae). As a result, a diagnosis of stroke often requires clinical interpretation by highly trained personnel. Emergency Room personnel, likely the first providers to see a patient with potential stroke, are less confident in making a diagnosis in the absence of objective laboratory confirmation. Stroke diagnosis is further complicated by the diversity of presenting symptoms. In the presence of focal deficits (i.e., weakness), stroke is relatively obvious, but with nonlocalizing symptoms such as delirium, seizures, dizziness/vertigo, or transient symptoms, the diagnosis can be more challenging. The presence of aphasia (left hemisphere) or profound neglect (right hemisphere) is often interpreted as confused delirium and is frequently misdiagnosed by nursing or primary care physicians even in hospitalized patients [8]. Stroke mimics include postictal states, hemiplegic migraines, brain tumors, epilepsy, encephalopathies, and at times metabolic derangements (e.g., hypoglycemia), all of which make the early diagnosis of stroke difficult. The diagnosis of transient symptoms, such as a transient ischemic attack (TIA), essentially viewed as a stroke equivalent, is difficult for trained clinicians, with substantial disagreement even between neurologists themselves [10, 13]. On the other hand, MRI can be helpful if ordered, but MRI is not readily available in all facilities and requires cooperation of frequently agitated patients, and may be contraindicated in some patients. In addition, the need to identify patients with acute stroke or TIA is highlighted by the high incidence of early stroke recurrence. Approximately 11% of TIA patients will experience a stroke at 90 days and half of those will occur within 48 h. Identifying such patients early would allow secondary stroke prevention treatment to be implemented more rapidly. The difficulties of stroke diagnosis are greatly increased in the acute settings. In the treatment of acute stroke, intravenous tissue plasminogen activator (i.v. tPA) has to be administered within 3 h of symptoms onset (although this window may be closer to 4.5 h). Pressed for time, a careful clinical diagnosis or complete neuroimaging evaluation may not be possible. Only 4–5% of all stroke patients receive i.v. tPA, in part due to the reluctance of non-neurologically trained emergency room personnel to administer a potentially dangerous treatment to patients with unclear diagnosis [44].

In addition, the presence of intracranial hemorrhage, in which case these agents are contraindicated, must first be ruled out. Therefore, blood biomarker-based diagnostic test in the differential diagnosis of acute stroke will be highly valued in stroke clinical onset. Neuron-specific enolase (NSE), S100β, ischemia modified albumin, matrix metalloproteinase-9 (MMP9), D-dimer, glial fibrillary acidic protein (GFAP), BNP, urea, and creatinine have been reported as biomarkers in the diagnosis of acute stroke. In one recent stroke biomarker study which include 100 stroke patients, it was reported that MMP-9 and D-dimer were found to be effective separately at differential diagnosis of ischemic–hemorrhagic stroke; there was no significance for S100β [45]. However, S100β and BNP have no place in the differentiation of hemorrhagic from ischemic stroke when used individually. However, when combined with BNP, D-dimer, MMP9, and S100β, it has more significance. Thus, it would be better to use a panel of biomarker tests rather than being used individually to differentiate hemorrhagic from ischemic stroke [46].

Another candidate stroke marker is glial fibrillary acidic protein (GFAP) which is highly specific to the brain and is located in glia cell cytoskeleton. GFAP is released when astrocytes become activated (gliosis) after injury. GFAP has been shown to be a sensitive serum biomarker of brain damage in patients with smaller lacunar lesions or minor stroke [47]. In a recent publication, GFAP showed a late upregulation at 24-h post-ischemia/reperfusion injury, indicating the presence of reactive gliosis in the middle cerebral artery territory [48]. Along the same line, our group assessed the glial markers S100β and GFAP and UCH-L1 by swELISA in patient serum 1, 2, 3, 4, and 7 days after stroke onset. Of interest, all biomarkers increased post-stroke indicative to be a good measure to evaluate brain damage [47].

In summary, ideal biomarkers for TBI and stroke that have been identified and validated with preclinical animal models need to be translated and validated in clinical studies as well. They should be tested in terms of their ability to detect injury magnitude as well as drug-based biomarker level reduction. A direct comparison of biomarker occurrence between preclinical models and biomarker data from human clinical studies would allow investigators to gain considerable insight into the validity (or challenges to the validity) of the employed preclinical animal models. Finally, the sensitive and specific cerebral biomarker could impact the delivery of TBI and stroke care in an important manner.

References

1. Saatman KE, et al. *Classification of traumatic brain injury for targeted therapies*. J Neurotrauma. 2008;25(7):719–38.
2. Wang KK, et al. Neuroprotection targets after traumatic brain injury. Curr Opin Neurol. 2006;19(6):514–9.
3. Svetlov SI, et al. Biomarkers of blast-induced neurotrauma: profiling molecular and cellular mechanisms of blast brain injury. J Neurotrauma. 2009;26(6):913–21.
4. Kobeissy FH, et al. Neuroproteomics and systems biology-based discovery of protein biomarkers for traumatic brain injury and clinical validation. Prot Clin Appl. 2008;2(10–11):1467–83.
5. Wang KK, et al. Proteomic identification of biomarkers of traumatic brain injury. Expert Rev Proteomics. 2005;2(4):603–14.
6. Haskins WE, et al. Rapid discovery of putative protein biomarkers of traumatic brain injury by SDS-PAGE-capillary liquid chromatography-tandem mass spectrometry. J Neurotrauma. 2005;22(6):629–44.
7. Papa L, Robinson GMW, Oli MW, Robicsek SA, Gabrielli A, Robertson CS, Wang KKW, Hayes RL. Use of biomarkers for diagnosis and management of traumatic brain injury patients. Expert Opin Med Diagn. 2008;2(8):1–9.
8. Hochholzer W, Morrow DA, Giugliano RP. Novel biomarkers in cardiovascular disease. Am Heart J. 2010;160(4):583–94.
9. Velagaleti RS, et al. Relations of biomarkers of extracellular matrix remodeling to incident cardiovascular events and mortality. Arterioscler Thromb Vasc Biol. 2010;30(11):2283–8.
10. Singh D, et al. Sputum neutrophils as a biomarker in COPD: findings from the ECLIPSE study. Respir Res. 2010;11:77.
11. Denslow N, et al. Application of proteomics technology to the field of neurotrauma. J Neurotrauma. 2003;20(5):401–7.
12. Pelinka LE, et al. Nonspecific increase of systemic neuron-specific enolase after trauma: clinical and experimental findings. Shock. 2005;24(2):119–23.
13. Berger RP, et al. Serum biomarker concentrations and outcome after pediatric traumatic brain injury. J Neurotrauma. 2007;24 (12):1793–801.
14. Berger RP, et al. Identification of inflicted traumatic brain injury in well-appearing infants using serum and cerebrospinal markers: a possible screening tool. Pediatrics. 2006;117(2):325–32.
15. Johnsson P, et al. Neuron-specific enolase increases in plasma during and immediately after extracorporeal circulation. Ann Thorac Surg. 2000;69(3):750–4.
16. Berger RP, et al. Serum neuron-specific enolase, S100B, and myelin basic protein concentrations after inflicted and non-inflicted traumatic brain injury in children. J Neurosurg. 2005;103(1 Suppl):61–8.
17. Ingebrigtsen T, Romner B. Biochemical serum markers for brain damage: a short review with emphasis on clinical utility in mild head injury. Restor Neurol Neurosci. 2003;21(3–4):171–6.
18. Papa L, et al. Ubiquitin C-terminal hydrolase is a novel biomarker in humans for severe traumatic brain injury. Crit Care Med. 2010;38(1):138–44.
19. Dambinova SA, et al. Blood test detecting autoantibodies to *N*-methyl-D-aspartate neuroreceptors for evaluation of patients with transient ischemic attack and stroke. Clin Chem. 2003;49 (10):1752–62.
20. Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci. 2005;233(1–2):183–98.
21. Petzold A, Shaw G. Comparison of two ELISA methods for measuring levels of the phosphorylated neurofilament heavy chain. J Immunol Methods. 2007;319(1–2):34–40.
22. Kobeissy FH, et al. Novel differential neuroproteomics analysis of traumatic brain injury in rats. Mol Cell Prot. 2006;5(10):1887–98.
23. Ottens AK, et al. Neuroproteomics: a biochemical means to discriminate the extent and modality of brain injury. J Neurotrauma. 2010;27(10):1837–52.
24. Yao C, et al. P43/pro-EMAPII: a potential biomarker for discriminating traumatic versus ischemic brain injury. J Neurotrauma. 2009;26(8):1295–305.
25. Yao X, Liu J, McCabe JT. Alterations of cerebral cortex and hippocampal proteasome subunit expression and function in a traumatic brain injury rat model. J Neurochem. 2008;104(2):353–63.
26. Liu MC, et al. Comparing calpain- and caspase-3-mediated degradation patterns in traumatic brain injury by differential proteome analysis. Biochem J. 2006;394(Pt 3):715–25.
27. Brophy GM, et al. alphaII-Spectrin breakdown product cerebrospinal fluid exposure metrics suggest differences in cellular injury mechanisms after severe traumatic brain injury. J Neurotrauma. 2009;26(4):471–9.
28. Lewis SB, et al. Identification and preliminary characterization of ubiquitin C terminal hydrolase 1 (UCHL1) as a biomarker of neuronal loss in aneurysmal subarachnoid hemorrhage. J Neurosci Res. 2010;88(7):1475–84.
29. Svetlov SI, et al. Morphologic and biochemical characterization of brain injury in a model of controlled blast overpressure exposure. J Trauma. 2010;69(4):795–804.
30. Serbest G, et al. Temporal profiles of cytoskeletal protein loss following traumatic axonal injury in mice. Neurochem Res. 2007;32 (12):2006–14.
31. Siman R, et al. A panel of neuron-enriched proteins as markers for traumatic brain injury in humans. J Neurotrauma. 2009;26 (11):1867–77.
32. Ehrenreich H, et al. Circulating damage marker profiles support a neuroprotective effect of erythropoietin in ischemic stroke patients. Mol Med. 2011. doi:10.2119/molmed.2011.00259.
33. Allard L, et al. PARK7 and nucleoside diphosphate kinase A as plasma markers for the early diagnosis of stroke. Clin Chem. 2005;51(11):2043–51.
34. Foerch C, et al. Elevated serum S100B levels indicate a higher risk of hemorrhagic transformation after thrombolytic therapy in acute stroke. Stroke. 2007;38(9):2491–5.
35. Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. J Transl Med. 2009;7:97.
36. Reynolds MA, et al. Early biomarkers of stroke. Clin Chem. 2003;49(10):1733–9.
37. Grant SG. Systems biology in neuroscience: bridging genes to cognition. Curr Opin Neurobiol. 2003;13(5):577–82.
38. Grant SG, Blackstock WP. Proteomics in neuroscience: from protein to network. J Neurosci. 2001;21(21):8315–8.
39. Chen SS, et al. Bioinformatics for traumatic brain injury: proteimic data mining. In: Pardalos PM, Boginski VL, Vazacopoulos A, editors. Data mining in biomedicine. New York: Springer; 2007. p. 1–26.
40. Beltrao P, Kiel C, Serrano L. Structures in systems biology. Curr Opin Struct Biol. 2007;17(3):378–84.
41. Narayan RK, et al. Clinical trials in head injury. J Neurotrauma. 2002;19(5):503–57.
42. Flynn RW, MacWalter RS, Doney AS. The cost of cerebral ischaemia. Neuropharmacology. 2008;55(3):250–6.
43. Zaleska MM, et al. The development of stroke therapeutics: promising mechanisms and translational challenges. Neuropharmacology. 2009;56(2):329–41.
44. Donnan GA, et al. How to make better use of thrombolytic therapy in acute ischemic stroke. Nat Rev Neurol. 2011;7(7):400–9.
45. Whiteley W, Tseng MC, Sandercock P. Blood biomarkers in the diagnosis of ischemic stroke: a systematic review. Stroke. 2008;39 (10):2902–9.
46. Kavalci C, et al. Value of biomarker-based diagnostic test in differential diagnosis of hemorrhagic-ischemic stroke. Bratisl Lek Listy. 2011;112(7):398–401.

47. Herrmann M, et al. Release of glial tissue-specific proteins after acute stroke: a comparative analysis of serum concentrations of protein S-100B and glial fibrillary acidic protein. Stroke. 2000;31(11):2670–7.
48. Datta A, Jingru Q, Khor TH, Teo MT, Heese K, Sze SK. Quantitative neuroproteomics of an in vivo rodent model of focal cerebral ischemia reperfusion injury reveals a temporal regulation of novel pathophysiological molecular markers. J Proteome Res. 2011;10(11):5199–213.
49. Missler U, Wiesmann M, Friedrich C, Kaps M. S-100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke. Stroke. 2003;28(10):1956–60.
50. Raabe A, Grolms C, Seifert V. Serum markers of brain damage and outcome prediction in patients after severe head injury. Br J Neurosurg. 1999;13:56–9.
51. Romner B, Ingebrigtsen T, Kongstad P, Borgesen SE. Traumatic brain damage: serum S-100 protein measurements related to neuroradiological findings. J Neurotrauma. 2002;17(8):641–7.
52. Marchi N, Rasmussen P, Kapural M, Fazio V, Kight K, Mayberg MR, et al. Peripheral markers of brain damage and blood–brain barrier dysfunction. Restor Neurol Neurosci. 2003;21(3–4):109–21.
53. Blyth BJ, Farhavar A, Gee C, Hawthorn B, He H, Nayak A, Stöcklein V, Bazarian JJ. Validation of serum markers for blood-brain barrier disruption in traumatic brain injury. J Neurotrauma. 2009;26(9):1497–1507.
54. Yamazaki Y, Yada K, Morii S, Kitahara T, Ohwada T. Diagnostic significance of serum neuron-specific enolase and myelin basic protein assay in patients with acute head injury. Surg Neurol. 1995;43(3):267–70.
55. Ross SA, Cunningham RT, Johnston CF, Rowlands BJ. Neuron-specific enolase as an aid to outcome prediction in head injury. Br J Neurosurg. 1996;10(5):471–6.
56. Pelinka LE, Kroepfl A, Schmidhammer R, Krenn M, Buchinger W, Redl H, Raabe A. Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma. J Trauma. 2004a;57 (5):1006–12.
57. Pelinka LE, Kroepfl A, Leixnering M, Buchinger W, Raabe A, Redl H. GFAP versus S100B in serum after traumatic brain injury: relationship to brain damage and outcome. J Neurotrauma. 2004b;21(11):1553–61.
58. Vos PE, Lamers KJ, Hendriks JC, van Haaren M, Beems T, Zimmerman C, et al. Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. Neurology. 2004;62 (8):1303–10.
59. Lumpkins KM, Bochicchio GV, Keledjian K, Simard JM, McCunn M. Scalea T. Glial fibrillary acidic protein is highly correlated with brain injury. J Trauma. 2008;65(4):778–84.
60. Dvorak F, Haberer I, Sitzer M, Foerch C. Characterisation of the diagnostic window of serum glial fibrillary acidic protein for the differentiation of intracerebral haemorrhage and ischaemic stroke. Cerebrovasc Dis. 2009;27(1):37–41.
61. Papa L, Lewis L, Falk J, Zhang Z, Silvestri S, Giordano P, et al. Glial fibrillary acidic protein breakdown product as a novel serum biomarker for mild and moderate traumatic brain Injury. Ann Emerg Med. (in press)
62. Mondello S, Papa L, Buki A, Bullock MR, Czeiter E, Tortella FC, Wang KK, Hayes RL. Neuronal and glial markers are differently associated with computed tomography findings and outcome in patients with severe traumatic brain injury: a case control study. Crit Care. 2011;15(3):R156.
63. Pike BR, Flint J, Dutta S, Johnson E, Wang KKW, Hayes RL. Accumulation of non-erythroid αII-spectrin and calpain-cleaved αII-spectrin breakdown products in cerebrospinal fluid after TBI in rats. J Neurochem. 2001;78(6):1297–306.
64. Ringger NC, O'Steen BE, Brabham JG, Siler X, Pineda J, Wang KKW, Hayes RL. A novel marker for traumatic brain injury: CSF αII-spectrin breakdown product levels. J Neurotrauma. 2004;21 (10):1443–56.
65. Siman R, Zhang C, Roberts VL, Pitts-Kiefer A, Neumar RW. Novel surrogate markers for acute brain damage: cerebrospinal fluid levels corrrelate with severity of ischemic neurodegeneration in the rat. J Cereb Blood Flow Metab. 2005;25(11):1433–44.
66. Siman R, McIntosh TK, Soltesz KM, Chen Z, Neumar RW, Roberts VL. Proteins released from degenerating neurons are surrogate markers for acute brain damage. Neurobiol Dis. 2004;16 (2):311–20.
67. Pineda JA, Lewis SB, Valadka SB, Papa L, Hannay HJ, Heaton S, et al. Clinical significance of αII-spectrin breakdown products in CSF after severe TBI. J Neurotrauma. 2007;24(2):354–66.
68. Mondello S, Robicsek SA, Gabrielli A, Tepas J, Robinson C, Buki A, et al. αII-spectrin breakdown products (SBDPs): diagnosis and outcome in severe traumatic brain injury patients. J Neurotrauma. 2010;27(7):1203–13.
69. Zemlan FP, Jauch EC, Mulchahey JJ, Gabbita SP, Rosenberg WS, Speciale SG, Zuccarello M. C-tau biomarker of neuronal damage in severe brain injured patients: association with elevated intracranial pressure and clinical outcome. Brain Res. 2002;947 (1):131–9.
70. Shaw GJ, Jauch EC, Zemlan FP. Serum cleaved tau protein levels and clinical outcome in adult patients with closed head injury. Ann Emerg Med. 2002;39(3):254–7.
71. Maier B, Laurer HL, Rose S, Buurman WA, Marzi I. Physiological levels of pro- and anti-inflammatory mediators in cerebrospinal fluid and plasma: a normative study. J Neurotrauma. 2005;22(7):822–35.
72. Chiaretti A, Antonelli A, Mastrangelo A, Pezzotti P, Tortorolo L, Tosi F, Genovese O. Interleukin-6 and nerve growth factor upregulation correlates with improved outcome in children with severe traumatic brain injury. J Neurotrauma. 2008;25 (3):225–34.
73. Folkersma H, Brevé JJ, Tilders FJ, Cherian L, Robertson CS, Vandertop WP. Cerebral microdialysis of interleukin (IL)-1beta and IL-6: extraction efficiency and production in the acute phase after severe traumatic brain injury in rats. Acta Neurochir (Wien). 2008;150(12):1277–84.
74. Pelsers MM, Glatz JF. Detection of brain injury by fatty acid-binding proteins. Clin Chem Lab Med. 2005;43(8):802–9.
75. Pelsers MM, Hanhoff T, Van der Voort D, Arts B, Peters M, Ponds R, et al. Brain- and heart-type fatty acid-binding proteins in the brain: tissue distribution and clinical utility. Clin Chem. 2004;50 (9):1568–75.
76. Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci. 2005;233(1–2):183–98.
77. Norgren N, Sundström P, Svenningsson A, Rosengren L, Stigbrand T, Gunnarsson M. Neurofilament and glial fibrillary acidic protein in multiple sclerosis. Neurology. 2004;63 (9):1586–90.
78. Liu MC, Akinyi L, Larner SF, Oli M, Zheng WR, Kobeissy F, et al. Ubiquitin C-terminal hydrolase-L1 as a novel biomarker for ischemic and traumatic brain injury in rats. Euro J Neurosci. 2010;31(4):722–32.