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Biomarkers for acute diagnosis and management of stroke in neurointensive care units

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Abstract:

The effectiveness of current management of critically ill stroke patients depends on rapid assessment of the type of stroke, ischemic or hemorrhagic, and on a patient's general clinical status. Thrombolytic therapy with recombinant tissue plasminogen activator (r-tPA) is the only effective treatment for ischemic stroke approved by the Food and Drug Administration (FDA), whereas no treatment has been shown to be effective for hemorrhagic stroke. Furthermore, a narrow therapeutic window and fear of precipitating intracranial hemorrhage by administering r-tPA cause many clinicians to avoid using this treatment. Thus, rapid and objective assessments of stroke type at admission would increase the number of patients with ischemic stroke receiving r-tPA treatment and thereby, improve outcome for many additional stroke patients. Considerable literature suggests that brain-specific protein biomarkers of glial [i.e. S100 calcium-binding protein B (S100B), glial fibrillary acidic protein (GFAP)] and neuronal cells [e.g., ubiquitin C-terminal hydrolase-L1 (UCH-L1), neuron-specific enolase (NSE), αII-spectrin breakdown products SBDP120, SBDP145, and SBDP150, myelin basic protein (MBP), neurofilament light chain (NF-L), tau protein, visinin-like protein-1 (VLP 1), NR2 peptide] injury that could be detected in the cerebrospinal fluid (CSF) and peripheral blood might provide valuable and timely diagnostic information for stroke necessary to make prompt management and decisions, especially when the time of stroke onset cannot be determined. This information could include injury severity, prognosis of short-term and long-term outcomes, and discrimination of ischemic or hemorrhagic stroke. This chapter reviews the current status of the development of biomarker-based diagnosis of stroke and its potential application to improve stroke care.

Key words:

Biomarker, blood, cerebrospinal fluid (CSF), blood, clinical trial, intracerebral hemorrhage (ICH), ischemic stroke, serum, transient ischemic attacks (TIAs)

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Introduction

In the United States, over 700,000 people are affected by strokes annually, and stroke has remained a major cause of disability for many years.[1-3] Stroke is currently the fifth leading cause of death in the United States and the third leading cause of death worldwide.[4] Although mortality caused by stroke has significantly declined over recent years, cerebrovascular diseases remain a highly significant global health burden.[5,6] The World Health Organization (WHO) defines stroke as "rapidly developing clinical signs of focal disturbance of cerebral function lasting more than 24 hours with no apparent cause other than of vascular origin."[7] There are two main types of strokes: ischemic and hemorrhagic. In addition, transient ischemic attacks (TIAs), which have traditionally been classified as a separate cerebrovascular disease because their duration (by definition) is less than 24 h, are more appropriately classified as ischemic stroke if brain lesion(s) are evident on magnetic resonance imaging (MRI).[8]

Ischemic stroke is the most common type, accounting for over 85% of all strokes. The most common causes of ischemic stroke are arterial occlusion from a thrombus or embolus, hypoperfusion from decreased blood pressure, or oxygen deprivation from systemic hypoxia.[9] Hemorrhagic stroke is caused by the rupture of an artery or vein in the brain and leaking of blood into the brain tissues. Mass effect from a hematoma may injure neurons from the effects of direct pressure. Elevated blood pressure and cerebral aneurysms are common conditions that can cause a hemorrhagic stroke.

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Hemorrhagic stroke includes intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH). ICH occurs when blood accumulates in the brain parenchyma, whereas SAH refers to bleeding within the space between the brain and the arachnoid. ICH is the most common type of hemorrhagic stroke, comprising 10% of all strokes. SAH represents 1-7% of all strokes and affects about 30,000 people in the United States annually.[10] Even though the incidence of hemorrhagic stroke is significantly lower than that of ischemic stroke, the contributions that ICH, SAH, and ischemic stroke make to the total number of years of productive life lost from all strokes are comparable: ICH: 34.2%, SAH: 27.3%, and ischemic stroke: 38.5%.[11,12]

Diagnosis and Management of the Stroke Patients in Neurointensive Care Unit

Management of a patient who has sustained an acute stroke is focused on stabilizing the patient and restoring cerebral blood flow as soon as possible to prevent further brain damage. Optimal management requires rapid assessment and early intervention to facilitate maximal reperfusion of brain tissue.[13] Currently, thrombolytic therapy with recombinant tissue plasminogen activator (r-Tpa) remains the only effective treatment option for ischemic stroke patients that is approved by the Food and Drug Administration (FDA).[14] Administration of r-tPA has a very narrow therapeutic window and requires assessment prior to treatment to rule out a hemorrhagic component of stroke.[15] The use of intravenous r-tPA was approved by the FDA in 1996 based on the National Institute of Neurological Disorders and Stroke r-tPA Stroke Study, in which ischemic stroke patients were treated with r-tPA within 1.5-3 h of symptom onset.[16] According to the guidelines recently proposed by the American Heart Association/American Stroke Association based on several clinical trials, intravenous r-tPA is the standard treatment for eligible ischemic stroke patients within 3 h of symptom onset and in selected patients up to 4.5 h after ictus, and selective cerebral intraarterial r-tPA administration has a 6-h time window.[15] Nevertheless, both intravenous and intraarterial treatments should be applied as soon as feasible to maximize the likelihood of a beneficial outcome.[15]

The workup for a patient with suspected stroke first includes a history (especially the time when neurologic symptoms began), a physical examination [including scoring on the National Institutes of Health Stroke Scale (NIHSS)], and imaging studies (to rule out hemorrhagic components). In addition, several diagnostic studies may be obtained that include prothrombin time and international normalized ratio (PT/INR), glucose, complete blood count, metabolic panel, creatine kinase, electrocardiogram, echocardiogram, lipid panel, carotid Doppler ultrasonography, magnetic resonance angiography (MRA), and computed tomographic angiography (CTA). This assessment should be performed quickly enough to preserve the time-sensitive option of thrombolytic treatment if appropriate. The main goals during the initial assessment of ischemic stroke patients include: 1) exclusion of intracranial hemorrhage, 2) assessment for contraindications to thrombolysis, and 3) characterization of the infarct.[17]

Neurological assessment plays a central role in both therapeutic decision-making and prognostication. Neurological deterioration is a common complication of acute stroke, occurring in as many as 20-40% of all the cases.[18,19] However, this rate might be underestimated due to different definitions of neurological deterioration (for example, increase in NIHSS $\geq$2 points or $\geq$4 points).[20] There exists clinical evidence that early neurological deterioration may be predictive of a poor short-term prognosis.[19] Patients who undergo hemorrhagic transformation of an ischemic stroke are at especially high risk of neurological deterioration, with poor outcomes and increased mortality.[21] A retrospective cohort study in ICH patients suggested that relatively easy and effective risk stratification of early neurological deterioration is possible at admission based on the presence of a "spot sign" (suggestive of active hemorrhage) on the initial brain CTA imaging or extensive degree of intraventricular hemorrhage, whereas risk stratification of late neurological deterioration is possible based on specific clinical parameters assessed at admission such as degree of comorbidity (Charlson index), stroke severity, and/or degree of intraventricular hemorrhage.[22]

Clinical Implementation of Stroke Biomarkers for Acute Diagnosis in Neurointensive Care Unit

Historically, the use of diagnostic serum biomarkers such as creatinine kinase and troponin for evaluation of possible myocardial ischemia significantly improved diagnostics in the emergency room at admission and thereby expedited timely treatment. Similarly, the use of sensitive and specific central nervous system (CNS)-biomarkers of stroke-associated brain injuries could significantly impact the current treatment of stroke and improve overall patient outcomes. Current diagnostic tests for stroke rely on the neurological assessment, which may have poor interexaminer reliability and on neuroimaging, which might not be promptly available at all locations. Thus, inexpensive blood-based biomarkers could potentially provide a much needed objective assessment tool. Biomarkers could improve stroke care by allowing early diagnosis even by clinical providers without extensive neurological training as well as by facilitating serial monitoring of patients and rapid assessment of the severity of brain injury.

The rapid and objective discrimination of stroke type in acute care environments is likely to increase the number of patients with ischemic stroke receiving thrombolytic therapy, thereby improving overall patient outcome and health care for stroke patients. In addition, using a panel of neuronal injury biomarkers would complement the present neuroimaging modalities for the diagnosis of stroke. These biomarkers would be particularly important in patients with nonlocalizing or transient neurological symptoms, those in whom neuroimaging cannot be obtained, or those who are nondiagnostic. The biomarker assessment could be performed during initial triage, avoiding delays in transporting stroke patients to appropriate care centers and allowing expedited treatment of patients at high risk for early stroke recurrence.

In cases where stroke onset is uncertain, for example, in so-called "wake-up" strokes occurring during sleep, which account for up to 25% of all stroke cases,[23] the presence of signature biomarkers (those with either acute or delayed

release) or the levels of certain markers that could identify the timing of stroke onset (i.e., recent if acute marker levels are high, or relatively remote if they are low) could help ascertain the time of stroke onset.

Certain biomarker signatures could help the selection of appropriate treatment plans for patients with acute stroke. The information obtained from biomarker measures could be used in conjunction with acute neuroimaging patterns to determine if salvageable tissue is present and potentially to lead to more appropriate therapy.[24] It is conceivable that early determination of certain biomarkers such as matrix metalloproteinase-9 (MMP-9) or fibronectin could potentially identify patients at risk of secondary complications of stroke, particularly hemorrhagic transformation leading to ICH and edema.[24] In patients with high levels of such biomarkers, caution may be warranted while low levels of biomarkers may identify patients at lower risk of bleeding who would benefit from more aggressive revascularization measures or thrombolytic treatment. In addition, certain biomarkers of endothelial damage may identify patients at risk for developing malignant edema, for which presently no reliable clinical or imaging predictors exist.[25]

Monitoring biomarker levels during the first few days of hospitalization may provide further insight into stroke progression and predict or evaluate the possible causes of worsening including infection, fever, metabolic derangement, edema, hemorrhagic transformation in ischemic stroke, or vasospasm in SAH. Serial monitoring of biomarker activity could potentially identify patients with continuing or delayed ischemia who may benefit from more aggressive stroke management.

Although to date no clinically approved biomarker for stroke diagnostics is available, several blood biomarkers associated with different pathophysiological pathways of stroke have been identified as potentially useful in clinical management, possibly contributing additional information to current diagnostics, interventions, risk stratification, and monitoring of efficacy of therapy. Well-designed, large-scale clinical studies addressing relevant clinical questions are needed.[26]

Selection of Central Nervous System-Specific Protein Biomarkers for Acute Stroke Diagnosis

A wealth of clinical and experimental data indicate that different CNS injuries including ischemic and hemorrhagic strokes and traumatic brain injury (TBI) promote upregulation of specific characteristic proteins and their subsequent release in cerebrospinal fluid (CSF). On the other hand, these CNS injuries may promote upregulation of specific proteolytic enzymes catalyzing the degradation of cell-specific structural and membrane proteins that result in build-up of characteristic proteolysis products. It is widely recognized that the serum levels of many of these proteins or protein fragments may reflect the severity of brain injury, and that their cellular origin might be indicative of the mechanisms or cellular type affected by injury. These specific protein markers accumulate within brain parenchyma and CSF and then leak into the peripheral circulation at levels proportional to those observed in the brain tissue or CSF. Thus, the primary focus of many stroke biomarker studies is to assess brain pathologies and elucidate pathophysiological processes underlying these pathologies to facilitate evidence-based clinical decisions about timely intervention and management in stroke patients. Although a troponin-like diagnostic biomarker for stroke would be of interest, several other biomarker applications are possible and more likely to be of clinical importance.[27] Considerable literature suggests that biomarkers specific for glial and neuronal injuries might provide valuable and timely diagnostic information for stroke such as time of stroke onset when the exact time could not be determined, severity, discrimination of ischemic or hemorrhagic stroke, and short-term and long-term outcomes and prognosis. Because discrimination between ischemic and hemorrhagic strokes is critical to provide clinical decision for appropriate patient management, one of the main focuses of stroke biomarker research is to identify specific biomarkers that reflect ischemic and hemorrhagic insults. Of interest are studies of CNS-specific biomarker dynamics performed in TBI, which share several common features with strokes including brain ischemia, parenchymal brain hemorrhage, and SAH often coexisting at varied degrees; comparisons of the biomarker profiles between these two acute insults would provide valuable information regarding pathophysiological aspects of a specific brain injury phenotype.[28-30] A number of clinical trials have been performed to establish the utility of several biomarker candidates for stroke diagnosis. The clinical trials with the most promising biomarkers of glial and neuronal brain injuries for diagnosis of acute ischemic stroke and ICH are summarized in Table 1.

Glial-specific biomarkers of acute brain injury

S100 calcium-binding protein B (S100B)

S100B is a Ca^{2+}-binding protein, which belongs to the S100 EF-hand type calcium-binding protein family and is a commonly used astrocytic marker.[60] In the CNS, S100B is found primarily in mature astrocytes and NG2 cells.[61] NG2 cells have an astrocytic appearance but are commonly considered as oligodendrocyte precursor cells based on their NG2 proteoglycan expression, which is characteristic for oligodendrocyte progenitor cells. They do not express glial fibrillary acidic protein (GFAP) or other antigens characteristic of other types of mature glial cell or markers for neurons.[62,63] Under normal physiological conditions, S100B is located predominantly intracellularly and plays important roles in Ca^{2+} homeostasis, astrocytic glutamate uptake, and neurite outgrowth stimulation. However, following brain injuries associated with glutamate excitotoxicity, S100B can be released from astrocytes where it plays complex and differential roles in astrocytic proliferation and differentiation, neuronal survival, and/or apoptotic neuronal and astrocytic cell death.[64] S100B has been identified as a prospective marker to predict early neurological outcomes and potentially discriminate ischemic stroke from TIA and other acute brain injuries including TBI.[31,32] Thus, S100B levels may have the potential to diagnose and predict human neurological disorders associated with glutamate excitotoxicity and astrogliosis including TBI,[65,66] traumatic and nontraumatic ICH, and ischemic stroke.[67]

CSF and serum S100 protein and NSE levels have long been considered as markers of glial and neuronal cell damages in the CNS, respectively, in many neurological disorders and brain injuries.[31,68] Serum S100B levels correlate with neurological

Table 1: Clinical trials of serum protein biomarkers of neuronal and glial injury for diagnostic acute ischemic stroke and ICH

Biomarker (other markers in the study)	Study design	Sample Time point (s)	Patient Groups	Size	Biomarker Levels	Major findings	Clinical implication
Glial-specific biomarkers of acute brain injury							
S100B[31]	Prospective cohort study	Serial 1-10 d	Ischemic stroke	26	>0.2 μg/L 0.25±0.15 μg/L (day 0) 1.80±3.30 μg/L (day 3) 0.40±0.33 μg/L (day 10)	S100B was elevated in ischemic stroke compared to control S100B correlated with neurological deficits, infarction, and edema at admission but was not significantly correlated with the functional prognosis	A prospective marker for diagnostic applications in ischemic stroke
			Control	26	<0.2 μg/L		
S100B[32]	Prospective cohort study	0-24 h	Ischemic stroke	21	0.08-6.73 ng/mL	Positive correlation of serum S100B with neurological deficit in Ischemic stroke and TBI patient	A prospective marker to predict early neurological outcomes and discriminate ischemic stroke from TIA and other acute brain injuries (i.e., TBI)
			TIA	18	0.01-0.73 ng/mL		
			TBI	10	0.1-3.48 ng/mL	Significant difference in temporal profiles of S100B levels between ischemic stroke and TIA or TBI patients	
			Control	28	0.01-0.34 ng/mL		
S100B (NSE)[33]	Prospective cohort study	6-120 h	Ischemic stroke	32		Positive correlation with the neurological deficit and the final infarct volume S100B concentrations at 6 h were associated with the functional outcome S100B above 0.2 μg/L at 48 h indicated a poor functional outcome at 3 months	A prospective marker for diagnostic and prognostic application in ischemic stroke
S100B (MBP, NSE, sTM)[34]	Retrospective (samples from NINDS r-tPA trial)	0-24 h	Ischemic stroke (r-tPA)	359	Baseline: 0.21 ng/mL (0.0-0.309) 2 h — 0.22 ng/mL (0.0-0.255) 24 h-0.34 ng/mL (0.0-0.762)	Higher peak concentrations of S100B was associated with larger CT lesion volumes (r=0.263, P<0.0001) Patients with favorable outcomes had smaller changes in S100B (P<0.05) concentrations in the first 24 h No difference between r-tPA and placebo groups	A prospective marker to predict CT brain lesion and early functional outcome in ischemic stroke Not sensitive for assessment of r-tPA treatment efficacy
			Ischemic stroke (placebo)				
S100B (GFAP, NSE, APC-PCI)[35]	Prospective multicenter cohort study	24 h	Ischemic stroke	83	Median 11.2 μg/L (6.0-40.4)	No significant differences in S100B between ischemic stroke and ICH	Not a sensitive mark to discriminate ischemic stroke and ICH in a mixed stroke population
			ICH	14	Median 11.7 μg/L (5.9-18.7)		
S100B (UCH-L1, GFAP)[36]	Randomized controlled trial German Multicenter EPO Stroke Trial	Serial 1-7 d	Ischemic stroke (EPO-treated)	76	~1-1100 pg/mL	AUC for S100B alone corrected for day 1 NIHSS before drug treatment showed a tendency but was not significantly lower in EPO vs placebo groups Composite AUCs of all three markers were different between treatment groups	A prospective marker in biomarker panel (i.e., UCH-L1, GFAP, S100) to assess drug treatment efficacy in ischemic stroke The panel may not provide additional information obtained with UCH-L1 alone
			Ischemic stroke (placebo)	87			

Table 1: Continued

Biomarker (other markers in the study)	Study design	Sample Time point (s)	Patient Groups	Size	Biomarker Levels	Major findings	Clinical implication
S100B (Tau)[37]	Prospectively enrolled into the	1-10 d	Ischemic stroke	56	Day 1: 51.0 pg/mL (30.1-77.2) Day 3: 57.9 pg/mL (27.5-109.1) Day 5: 52.9 pg/mL (30.8-92.5) Day 10: 36.0 pg/mL (25.6-59.2)	S100B was detected in all patients on days from 1 to 5 and decreased on day 10. S100B was also detected in the control group (55.2%)	Limited diagnostic value in ischemic stroke
			Control (crural varices)	38			
S100B (NSE)[38]	Prospective cohort study	Serial 12-48 h	Ischemic stroke	61	127,0 nmol/L (57,0-639,5)	S100B was elevated in stroke vs control S100B correlated with depression symptoms at 60 days	A prospective marker for stroke diagnosis and to predict poststroke depression in ischemic stroke and ICH
			ICH	79	183,5 nmol/L (83,0-3102,5)		
			Control	79	84,6 nmol/L (13,6-284,2)		
S100B (RAGE)[39]	Prospective cohort study	24 h	Ischemic stroke	776	58.70 pg/mL	S100B levels were significantly increased in ischemic stroke vs ICH (P<0.001)	A prospective marker to discriminate ischemic stroke and ICH Combination of biomarkers S100B/RAGE pathway may provide useful information for diagnosis of ischemic stroke vs ICH in the first hours from symptoms onset
			ICH	139	107.58 pg/mL		
S100B (GFAP, MMP-9, sVCAM-1)[40]	Prospective single-center pilot study	24 h	Ischemic stroke	31	0.069 ng/mL	Serum levels of S100B were significantly higher in stroke patients than in nonvascular vertigo patients Detect stroke in vertigo group (sensitivity 94.4%, specificity 31.8%)	Serum S100B levels is a prospective marker to distinguish stroke and vertigo of nonvascular causes
			ICH	12			
			Vertigo (nonvascular)	22	0.047 ng/mL		
			Control	15			
S100B[41]	Prospective observational study	48 h	Ischemic stroke (66.19%)	142	1.12±1.58 ng/mL	S100B was increased in both ischemic stroke and ICH but not in TIA, compared to the control	A prospective marker to distinguish ischemic stroke and ICH from TIA S100B protein might be a copredictor of outcome in ischemic stroke and ICH
			ICH (24.64%)		0.6317±0.782 ng/mL		
			TIA (9.15%).		TIA (0.22±0.25 ng/mL)		
			Control	40	0.1782±0.1622 ng/mL		
S100B (NSE, HSP70)[42]	Prospective cohort study	24 h	ICH	35	0.13±0.03 (day 0) 0.13±0.04 (day 5)	S100B on days 0 and 5 was significantly increased in ICH compared to the control group (P<0.001)	A prospective marker for diagnosis of ICH
			Control	32	0.08±0.03	Positive correlation with NIHSS and bleeding volume, negative correlation with GCS	

Continued

Table 1: Continued

Biomarker (other markers in the study)	Study design	Sample Time point (s)	Patient Groups	Size	Biomarker Levels	Major findings	Clinical implication
GFAP[43]	Prospective cohort study	6 h	Ischemic stroke	93	14 ng/L	GFAP with a cutoff point of 2.9 ng/L detected ICH in acute stroke (sensitivity 0.79, specificity 0.98, positive predictive value 0.94, negative predictive value 0.91; *P*<0.001)	A prospective marker to discriminate ischemic stroke and ICH
			ICH	42	11 ng/L (0-3096)		
GFAP[44]	Prospective cohort study	Serial 1-48 h	Ischemic stroke	45		GFAP was not detected in ischemic stroke within 24 h	A prospective marker to discriminate ischemic stroke and ICH
			ICH	18		GFAP at 2 h was significantly correlated with ICH volume (*r*=0.755, *P*=0.007). Differentiate between ICH and ischemic stroke within 2-6 h (diagnostic accuracy was>0.80)	
GFAP (S100B, NSE, APC-PCI)[35]	Prospective multicenter cohort study	Serial 24 h	Ischemic stroke	83	Median <30 ng/L (<30-1280)	Levels of GFAP were significantly higher in ICH patients (*P*=0.0057)	A prospective marker in combination with APC-PCI to discriminate ischemic stroke and ICH prior to neuroimaging in a mixed stroke population
			ICH	14	Median 55 ng/L (<30-1850)	Combination of GFAP and APC-PCI in patients with NIHSS score >3 sensitivity and negative predictive value of 100% for ICH (*P*=0.0052)	
GFAP (UCH-L1, S100B)[36]	Randomized controlled trial German Multicenter EPO Stroke Trial	Serial 1-7 d	Ischemic stroke (EPO)	76	~0.1-12 ng/mL	AUC for GFAP alone corrected for day 1 NIHSS before drug treatment showed a tendency but was not significantly lower in EPO vs placebo groups.	A prospective marker in the biomarker panel (i.e., UCH-L1, GFAP, S100) to assess drug treatment efficacy in ischemic stroke
			Ischemic stroke (placebo)	87		Composite AUCs of all three markers were different between the treatment groups	The panel may not provide additional information obtained with UCH-L1 alone
GFAP[45]	Prospective multicenter cohort study	4.5 h	Ischemic stroke	163	Median 1.91 μg/L (0.02-236.27)	GFAP cutoff of 0.29 μg/L differentiated ICH from ischemic stroke and stroke mimic (sensitivity 84.2%, specificity 96.3%)	A prospective marker to discriminate ICH from ischemic stroke and stroke mimic
			ICH	39	median 0.08 g/L (0.00-0.97)		
			Control (stroke mimic)	3	Median 0.19 μg/L (0.16-0.21)		
GFAP (S100B, MMP-9, sVCAM-1)[40]	Prospective single-center pilot study	24 h	ischemic stroke	31	0.069 ng/ml (0.051-0.135)	No significant differences among groups were found for GFAP levels	Serum GFAP levels is not a sensitive marker to distinguish stroke and vertigo of nonvascular causes
			ICH	12			
			Vertigo (nonvascular)	22	0.047 ng/mL (0.034-0.06)		
			Control	15			
GFAP (NR2 antibodies)[46]	Prospective cohort study	12-72 h 1 and 2 w	Ischemic stroke	49		GAFAP and NR2 antibodies when used in combination discriminated ischemic stroke and ICH at 12 h after onset (sensitivity 94%, specificity 91%)	A prospective marker in combination with NR2 antibodies to discriminate ischemic stroke and ICH at 12 h after onset
			ICH	23			
			Control	52			
GFAP[47]	Prospective cohort study	2-6 h	Ischemic stroke	65	0.6±0.4 ng/mL	GFAP in ICH was significantly higher than in ischemic stroke group (*P*<0.001)	A prospective marker to discriminate ischemic stroke and ICH
			ICH	43	1.6±0.8 ng/mL	GFAP at the cut point of 0.7 ng/mL differentiate ICH from ischemic stroke (sensitivity 86.0%, specificity 76.9%)	

Table 1: Continued

Biomarker (other markers in the study)	Study design	Sample Time point (s)	Patient Groups	Size	Biomarker Levels	Major findings	Clinical implication
GFAP (RPB4)[48]	Retrospective cohort study	6 h	Ischemic stroke	36	Median 0.075 ng/mL (0.04-0.68)	Plasma GFAP was significantly higher in ICH vs ischemic stroke (P<0.0001)	A prospective marker to discriminate ischemic stroke and ICH
			ICH	10	Median 0.04 ng/mL (0.04-0.04)	GFAP and RBP4 showed a specificity 100% for both stroke subtypes	Combination with RPB4 may improve diagnosis within the first hours after stroke
Neuron-specific biomarkers of acute brain injury							
UCH-L1 (S100B, GFAP)[36]	Randomized controlled trial German Multicenter EPO Stroke Trial	Serial 1-7 d	Ischemic stroke (EPO)	76	~0.035-0.36 ng/mL	AUC for UCH-L1 alone corrected for day 1 NIHSS before drug treatment was significantly lower in EPO vs placebo groups	A prospective marker alone or in biomarker panel (i.e., UCH-L1, GFAP, S100) to assess drug treatment efficacy in ischemic stroke
			Ischemic stroke (placebo)	87		Composite AUCs of all three markers were different between treatment groups	The panel may not provide additional information obtained with UCH-L1 alone
NSE (S100B)[33]	Prospective cohort study	6-48 h	Ischemic stroke	32		Positive correlation with the neurological deficit and the final infarct volume	A prospective marker for diagnostic and prognostic application in ischemic stroke
NSE (MBP, S100B, sTM)[34]	Retrospective (samples from NINDS r-tPA trial)	0-24 h	ischemic stroke (r-tPA)	359	Baseline: 16,2 ng/mL (2.9-118.7) 2 h-17.3 ng/mL (0.0-172.7) 24 h-15.6 ng/mL (0.0-189.2)	Higher peak concentrations of MBP was associated with larger CT lesion volumes (r=0.117, P<0.0001) No differences between r-tPA and placebo groups	A prospective marker to predict CT brain lesion and early functional outcome in ischemic stroke Not sensitive for assessment of r-tPA treatment efficacy
NSE (Tau)[49]	Prospective cohort study	3-120 h	Ischemic stroke	66	12.5 μg/L	NSE was associated with the neurovascular status on admission. NSE was significantly correlated with the functional outcome at 3 months (P<0.001)	A prospective marker for diagnostic and prognostic application in ischemic stroke
NSE (S100B, GFAP, APC-PCI)[35]	Prospective multicenter cohort study	24 h	Ischemic stroke	83	median 0.11 μg/L (0.02-1.66)	No significant differences in NSE between ischemic stroke and ICH	Not a sensitive mark to discriminate ischemic stroke and ICH in a mixed stroke population
			ICH	14	Median 0.14 μg/L (0.6-0.96)		
NSE[50]	Prospective cohort study	72 h	Ischemic stroke	150	>25 ng/mL (>35 ng/mL, n=13).	Positive correlation NSE with degree of disability and neurological worsening	A prospective marker to predict stroke severity and early functional outcome in ischemic stroke
			Control	101	≤25 ng/mL	Positive correlation NSE with severity of stroke at the time of admission (r=0.919; P<0.001)	
						Positive correlation between serum NSE and degree of disability (i.e., mild, moderate, and severe NIHSS score) (χ^2=94.905, P<0.001)	
NSE (S100B)[38]	Prospective cohort study	12-48 h	ischemic stroke	61	11.2 nmol/L (3.3-54.5)	NSE was elevated in stroke vs control	A prospective marker for stroke diagnosis and to predict functional outcome in ischemic stroke and ICH
			ICH	79	19.3 nmol/L (9.7-26.9)	NSE was not associated with stroke severity on admission	
			Control (high risk)	79	9.5 nmol/L (2.2-23.0)	NSE was associated with functional neurological outcome at 60 days and to the degree of recovery	

Continued

Table 1: Continued

Biomarker (other markers in the study)	Study design	Sample Time point (s)	Patient Groups	Size	Biomarker Levels	Major findings	Clinical implication
NSE[51]	Prospective cohort study	24 h	Ischemic stroke	75	15.68-198.42 ng/l	Positive correlation with infarct volume (CT) (r=0.955, P<0.001) Negative correlation with GCS (r=0.806, P<0.001) Positive correlation with functional neurological outcome (mRS) at day 30 (r=0.744, P<0.001)	A prospective marker to predict stroke severity and early functional outcome in ischemic stroke
NSE (IL-10)[52]	Prospective cohort study	72 h	Ischemic stroke Control	100	17.95±4.54 ng/mL 7.48±1.51 ng/mL	Significantly increased in stroke patients compared to control Positive correlation with degree of neurological deficit (NIHSS score) (r=0.8, $P\leq$0.001)	A prospective marker to predict stroke severity and early functional outcome in ischemic stroke
NSE[53]	Prospective cohort study	24 h	Ischemic stroke	83	9.9±1.7 (unchanged) 9.7±3.4 (increased) 10.6±4.5 (decreased) 16.7±6.4 (1 peak) 18.7±6.6 (2 peaks)	NSE levels were stationary [unchanged (26.5% of the patients), increased (10.8%), and decreased (21.7%)] and NSE increase showed 1 peak (20.5%) and 2 peaks (20.5%) NSE increase with 2 peaks correlated with the incidence of atrial fibrillation and hemorrhagic transformation (P=0.02)	A prospective marker for monitoring hemorrhagic transformation and the status of blood-brain barrier disruption in ischemic stroke
NSE (CRP)[54]	Prospective cohort study	72 h	Ischemic stroke ICH Control	88 32 50	22.6±7.7 ng/mL 7.48±1.51 ng/mL	Increased in acute stroke cases compared to the controls (P<0.05) Not statistically different between ischemic stroke and ICH	A prospective marker to predict stroke severity and early functional outcome in ischemic stroke
NSE (S100B, HSP70)[42]	Prospective cohort study	24 h	ICH control	35 32	Day 0 — 31.66±13.43 Day 5 — 26.56±12.77 21.97±11.13	NSE on day 0 was significantly increased in ICH compared to the control group (P<0.01) No significant correlation with NIHSS score, bleeding volume or GCS	A prospective marker for diagnostic of ICH
NSE[55]	Prospective study	24 h	Ischemic stroke (r-tPA)	67	15.60 ng/mL (8.480-30.69)	Positive correlation with NIHSS score at 24 h after r-tPA (R=0.342, P=0.005) rather than baseline NIHSS score Serum NSE at 24 h showed an increase patients with atrial fibrillation compared to those without. (18.37±4.83 ng/mL vs 14.64±4.14 ng/mL; P=0.003)	NSE levels at 24 h may help predict long-term outcome of ischemic stroke patients with intravenous rtPA treatment
MBP (NSE, S100B, sTM)[34]	Retrospective (samples from NINDS r-tPA trial)	0-24 h	Ischemic stroke (r-tPA) Ischemic stroke (placebo)	178 181	Baseline — .036 ng/mL (0.0-3.606) 2 h — 0.035 ng/mL (0.0-2.149) 24 h — 0.131 ng/mL (0.0-11.835)	Higher peak concentrations of MBP was associated with larger CT lesion volumes (r=0.209, P<0.0001) Patients with favorable outcomes had smaller changes in MBP (P<0.05) concentrations in the first 24 h No difference between r-tPA and the placebo groups	A prospective marker to predict CT brain lesion and early functional outcome in ischemic stroke Not sensitive for assessment of r-tPA treatment efficacy
MBP (IMA)[56]	Prospective study	12 h	Ischemic stroke Control	34 34	82.4±63.9 pg/mL 73±47.5 pg/mL	No statistically significant difference between the stroke and control groups Statistically significant correlations with and NIHSS score (P=0.002, r=0.43) and IMA (P=0.015, r=0.344) levels	MBP levels do not increase in early period of stroke cases

Table 1: Continued

Biomarker (other markers in the study)	Study design	Sample Time point (s)	Patient Groups	Size	Biomarker Levels	Major findings	Clinical implication
Tau (NSE)[49]	Prospective cohort study	3-120 h	Ischemic stroke	66	60 pg/mL	Tau was correlated with severity of neurological deficits and infarct volume (P=0.001) but was not associated with the neurovascular status on admission	A prospective marker for diagnostic and prognostic application in ischemic stroke
Tau (S100B)[37]	Prospective study	1-10 d	Ischemic stroke	56	Day 1 — 89.1 pg/mL (77.6-104.2) day 3 — 87.6 pg/mL (67.1-140.9) day 5 — 94.2 pg/mL (72.1-197.0) day 10 — 99.6 pg/mL (77.6-246.3)	Tau protein concentrations measured within the early phase did not correlate with degrees of neurological deficit and disability in the early phase and after 3 months A significant correlation between pau protein level and Barthel Index measured at the same time point was observed only on day 10 (r=0.43; P=0.03	Detection of tau protein in the serum of patients with ischemic stroke but not its concentration can be considered as a bad prognostic factor for the clinical outcome in early and late phases of ischemic stroke
Tau[57]	Prospective cohort study	6 h	ICH	176	168.2 pg/mL	Tau levels with cutoff 91.4 pg/mL predicted 3-month poor outcome (sensitivity 83.6%, specificity 75.8%; AUC, 0.826; 95% CI, 0.762-0.879)	A prospective marker to predict mortality and poor outcomes at 3 months in ICH
VLP-1[58]	Prospective cohort study		Ischemic stroke	16	1.78 μg/L	VILIP-1 was elevated in ischemic stroke vs control (sensitivity 100%, specificity 100% at 0.093 mcg/L VILIP-1)	A prospective marker for stroke diagnostic in ischemic stroke
			Control	17	0.03 μg/L		
NR2 peptide[59]	Prospective blinded study	72 h	Ischemic stroke	192	Median 5.44 μg/L (0.1-62.71)	Increased in ischemic stroke Positive correlation with lesion volume (r_s=0.73) At cutoff 1 μg/L, NR2 levels detected ischemic stroke (sensitivity 92%, specificity 96%)	A prospective marker for diagnostic and prognostic application in ischemic stroke

deficit resulting from both stroke and TBI,[32] and levels have been shown to correlate with the severity of brain injury.[66] S100B has also been reported as a biomarker of blood-brain barrier disruption in various conditions.[69,70] A small study has shown that following aneurysmal SAH, the CSF level of S100B as well as of some other biomarkers mentioned below including UCHL-1, NSE, and spectrin breakdown products (SBDPs) increased up to 100-fold and that a panel of selected neurodegeneration markers might be valuable as surrogate endpoints for assessment in SAH patients.[71] In addition, changes in S100B levels correlated positively with acute changes in depressive symptoms' severity following coronary artery bypass grafting.[72] Several studies have demonstrated that serum concentrations of S100B are significantly increased following stroke between days 2 and 4 after symptom onset and peaking after 48 h.[31,32] There may be a role for serum S100B measured before specific treatment as a copredictor of outcome in patients with acute stroke admitted to a hospital emergency department.[41,73] Significant correlations between S100B in CSF and volume of infarction have also been demonstrated in early-phase severe TBI.[74]

Studies performed in TBI patients have shown elevated S100B levels at the acute stage of head trauma. These levels were related to alternations in intracranial pressure and cranial computed tomography (CT) findings, suggesting that S100B may serve as a reliable marker for the evaluation of injury severity[75,76] and as a potential outcome predictor.[77] Serum S100B concentrations have been reported to increase after closed head injury in children.[78] However, temporal profiles of the serum S100B protein levels following stroke and TBI were different, peaking on day 3 or 4 after stroke and on day 1 or 2 after TBI.[32] S100B serum levels are associated with different types of traumatic intracranial lesions, notably epidural and subdural hematomas, SAH, and brain edema.[79] The concentration of S100B increases in the plasma of patients with ischemic stroke, and its elevated levels are associated with larger infarct size, poor neurovascular status on admission, and worse outcomes.[33] A recent study including 35 patients with nontraumatic ICH and 32 healthy controls has shown significant increases in S100B levels in the serum of ICH patients at admission and at day 5, and the S100B levels were negatively correlated with Glasgow Coma Scale scores and

positively correlated with NIHSS, bleeding volume, and in-hospital mortality.[42] S100B protein levels were significantly higher in stroke patients compared to the controls, and these levels were significantly higher in the ischemic stroke group as compared to the ICH or TIA groups, with the highest levels in patients who did not survive.[41] A related study including a total of 80 patients including vertigo patients with ischemic stroke in the posterior circulation (n = 31), ICH (n = 12), and nonvascular cause (n = 22), and matched controls (n = 15) has shown that serum levels of S100B at 24 h of symptom onset were significantly higher in stroke patients than in patients with vertigo from nonvascular causes or controls, whereas the levels of other biomarkers used in the study [i.e., GFAP, soluble vascular cellular adhesion molecule-1 (sVCAM-1), and MMP-9] were not significant in the groups.[40]

The diagnostic and prognostic values of S100B as single biomarker are limited but it shows great potential as part of a biomarker panel for stroke.[34,39] S100B levels in combination with levels of other biomarkers such as NSE and GFAP have been reported to predict TBI outcome.[80-84] In ischemic stroke and ICH patients, the levels of S100B at admission were significantly higher than in high-risk controls but not different between the two types of stroke, and the S100B levels correlated with depression symptoms but not with functional recovery at 2 months following stroke onset.[38] Importantly, it has been shown that using a panel comprising S100B and its scavenger receptor, a soluble receptor for advanced glycation end products (sRAGE), allowed differentiation between ischemic stroke and ICH in a sample of 915 stroke patients [area under the curve (AUC) 0.76].[39] In patients with first-ever acute lacunar stroke,[85] higher serum levels of S100B and lower levels of sRAGE measured within 7 days of symptom onset were independently associated with the presence and number of cerebral microbleeds assessed by using susceptibility-weighted magnetic resonance imaging (SWI).[86]

Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is a brain-specific type III intermediate filament protein found primarily in mature astrocytes and to a lesser extent in some other glial cells.[87] Various types of brain injuries and neurological disorders are associated with gliosis and subsequent GFAP upregulation in the brain. Numerous studies documenting different spatiotemporal profiles of GFAP upregulation in different brain disorders suggest that GFAP could be an attractive marker for screening for the presence of astroglial damage. Several studies have found that changes in GFAP levels have potential clinical utility in acute brain injuries such as TBI and stroke and could provide unique information for determining injury severity and even differential diagnosis of different types of stroke.[88,89] Recent studies have documented elevated GFAP or its breakdown products (GFAP-BDP) levels in both CSF and serum after mild, moderate, or severe TBI in adult patients, and these elevated GFAP levels correlate with TBI magnitude and outcomes[66,81,83,90-95] in children as well.[96-98] These findings are consistent with preclinical models suggesting an association of CSF and serum GFAP levels with TBI outcomes.[99] Importantly, the aforementioned preclinical study demonstrated significant correlation of GFAP levels in serum and CSF following experimental TBI, suggesting the validity of serum analyses to assess changes of this biomarker in the brain.[99] Another TBI study has shown that although the levels of GFAP were increased in TBI, the levels GFAP have poor prognostic value.[100] In addition, clinical studies suggest a possible role for GFAP as a brain-specific marker for malignant gliomas.[89]

GFAP has long been also suggested as a marker of glial injury in stoke. Even in an early study, GFAP was identified as a biomarker indicative of ICH within 6 h of symptom onset, and a cutoff point of 2.9 ng/L was found to provide a sensitivity of 79% and a specificity of 98% for the differentiation of ICH from ischemic stroke.[43] It has been suggested that after ICH, GFAP can be found in the serum of patients due to necrosis of brain cells and subsequent blood-brain barrier disruption. A subsequent multicenter study of S100B, NSE, GFAP, and activated protein C-protein C inhibitor complex (APC-PCI) demonstrated a significant ability of GFAP to distinguish ICH from ischemic stroke.[35] In a recent multicenter cohort study including 205 patients diagnosed with ischemic stroke, ICH, or stroke mimics, plasma GFAP on admission had a sensitivity of 84% and specificity of 96% for differentiating ICH from ischemic stroke and stroke mimics (AUC 0.915, 95% CI 0.847-0.982).[45] GFAP was increased in ischemic stroke and demonstrated its potential application in stroke therapy development studies when used along with other biomarkers.[36]

Neuron-specific biomarkers of acute brain injury

Ubiquitin C-terminal hydrolase-L1

Ubiquitin C-terminal hydrolase-L1 (UCH-L1), also known as neuronal-specific protein gene product 9.5, is an abundant protein localized in the cell body of neurons in CNS. Thus, UCH-L1 been proposed as a biomarker specific to neuronal injury following ischemic insult in a preclinical model of ischemic stroke induced by middle cerebral artery occlusion (MCAO).[101] A recent study demonstrated elevation of CSF and serum UCH-L1 levels in a preclinical model of ischemic stroke induced by middle cerebral artery occlusion, whereas no change was observed in a rat ICH model induced by collagenase injection into the striatum.[102] A related study in a preclinical piglet model also suggested the utility of serum UCH-L1 to predict neuronal apoptosis induced by deep hypothermic circulatory arrest, which is routinely used in cardiac surgery.[103] Several studies have demonstrated the utility of CSF and serum levels of UCHL-1 as diagnostic and prognostic biomarkers of severe and mild TBIs.[66,100,104-106, 108] Another TBI study has shown that although serum UCH-L1 levels were elevated in military personnel with low-level occupational blast exposure, the correlation of UCH-L1 levels with blast magnitude was weak and inconsistent, and no correlation has been found between UCH-L1 levels and deficits in behavioral measures.[109] Interestingly, a recent TBI study suggests that serum UCH-L1 concentrations might be able to discriminate TBI patients with abnormal and normal CT findings.[94]

In addition, a significant correlation between serum and CSF levels of UCH-L1 and outcome has been demonstrated in a preclinical TBI model, and the increases in both serum and CSF levels reflected brain pathology.[99] CSF levels of UCH-L1 and other neurodegenerative biomarkers such as phosphorylated neurofilament H (pNF-H) and S100B are elevated in patients with aneurysmal SAH, and the levels of these biomarkers are correlated with neurological outcomes and mortality.[71,110] A

study performed using serum samples from patients enrolled in the trial of erythropoietin for stroke[111] has shown that the levels of UCH-L1, S100B, and GFAP were increased in ischemic stroke samples, and the decrease in the UCH-L1 levels was associated with beneficial outcomes of erythropoietin treatment.[36]

A study performed in 125 patients with dizziness, hypertension, type 2 diabetes mellitus, or dyslipidemia found that UCH-L1 levels in serum were significantly different between the controls and patients with white matter lesions (i.e., patients without white matter lesions; $n = 46$, $P < 0.05$), and these increased UCH-L1 levels were correlated with white matter lesion severity, suggesting that UCH-L1 could serve as a biomarker of neuronal damage associated with white matter lesions.[112] Subgroup analysis of patients with different types of lesions has shown that serum UCH-L1 levels were significantly higher in the patients with subcortical white matter lesions but not in the patients with paraventricular lesions compared to the controls.[112] Interestingly, in the same study an attempt was made also to correlate UCH-L1 levels in the urine of patients with white matter lesions with that of controls but the urine UCH-L1 levels were similar between these groups ($P > 0.05$).[112]

Neuron-specific enolase

Neuron-specific enolase (NSE) is a dimeric isoform of the glycolytic enzyme enolase found mainly in neurons. Although NSE is relatively specific for neuronal cells, it is also found in neuroendocrine carcinomas. Several early studies documented elevated NSE concentrations in CSF and blood samples following different brain disorders including stroke [65,113] and related preclinical models.[114-118] Increased serum concentrations of NSE have been reported in TBI patients, and the NSE levels were correlated with neurological outcomes, suggesting that NSE could be a diagnostic and prognostic biomarker of TBI.[65] An earlier study in acute ischemic stroke found that peak levels of NSE in plasma samples were observed on the second day after symptom onset, and some correlation was found between the levels of NSE and infarct volumes ($r = 0.37$, $P < 0.05$) but not with clinical outcome ($r = 0.18$, $P > 0.05$).[119] In contrast, a further study demonstrated that serum NSE and its peak levels correlated with baseline NIHSS scores but not with infarct volume.[34] Elevated NSE levels in serum within the first 24 h after symptom onset have been reported only in a small fraction of stroke patients,[34,49,68,120-122] probably due to the wide range of NSE levels in the normal population.[123] In addition, no clear relationship has been found between serum and CSF levels of NSE in subjects without neurological disease.[123] Further studies have shown that serum NSE levels at 72 h after stroke significantly correlate with worse neurological outcomes, and these levels have high predictive value for determining stroke severity.[50-52,54] It has been shown that serum levels of NSE in the first few days after ischemic stroke can serve as a useful marker to predict stroke severity and early functional outcome.[51] A study performed in 100 ischemic stroke patients and controls without stroke demonstrated that serum concentrations of NSE were significantly increased (17.95 ± 4.54 pg/mL vs 7.48 ± 1.51 pg/mL, $P \leq 0.05$) and concentrations of interleukin-10 (IL-10) significantly decreased (11.79 ± 2.77 pg/mL vs 15.72 ± 2.69 pg/mL, $P \leq 0.05$) in the blood samples from stroke patients taken within the first 72 h after stroke onset as compared to NSE and IL-10 levels in control patients.[52] This study also demonstrated a significant correlation of NSE concentrations with NIHSS scores, with concentrations of both NSE and IL-10 having a high predictive value for early neurobehavioral outcomes.[52] The maximum serum NSE and C-reactive protein levels within 72 h have been shown to be significantly correlated with the severity of neurological disability, and both markers have high predictive value for early neurobehavioral outcome after acute ischemic stroke.[54] Furthermore, a recent study of 83 ischemic stroke patients found that the temporal profile of NSE levels varied widely among different patients: no changes in NSE levels in 26.5% of the patients, increase in 10.8% of the patients, decrease in 21.7% of the patients, and transient changes with a single peak in 20.5% of the patients or two peaks in 20.5% of the patients. The presence of an NSE peak was significantly correlated with the incidence of atrial fibrillation and hemorrhagic transformation.[53] A study including 44 ischemic stroke patients and 17 ICH patients found that in serum samples from patients with ischemic stroke and ICH, the median concentrations of NSE were significantly higher than in samples collected from a high-risk group ($n = 79$), and the median NSE concentration in ICH patients was significantly higher compared to the ischemic stroke group.[38] However, the NSE levels were not associated with stroke severity at admission though the NSE levels were predictive of functional recovery at 60 days following stroke onset.[38] A recent prospective study has shown that serum NSE concentrations in ischemic stroke patients ($n = 67$) assessed within the first 4.5 h of symptom onset were correlated with neurological outcomes assessed using NIHSS at 24 h after r-tPA therapy ($R = 0.342$, $P = 0.005$), and the patients with favorable neurological outcomes after 90 days ($n = 32$) had lower serum NSE levels and NIHSS scores.[55]

In addition, a small trial provided evidence that a panel of biomarkers including NSE may have predictive value for infarct volume, incidence of vasospasm, and overall outcome in patients with SAH.[71] In patients with nontraumatic ICH, the serum levels of NSE were significantly higher at admission but not at day 5 as compared to the controls though no significant correlation has been found between NSE levels and Glasgow Coma Scale scores, NIHSS, bleeding volume, or in-hospital mortality.[42]

αII-spectrin breakdown products SBDP120, SBDP145, and SBDP150

In CNS, αII-spectrin is a major structural component of the neuronal cytoskeleton and is especially prevalent in axons. It plays a critical role in neuronal integrity. After injury, αII-spectrin can become a major substrate for both calpain and caspase-3 proteases, which play an important role in oncotic necrosis and apoptotic cell death, respectively. Although calpain activation is primarily associated with oncotic cell death, it can also contribute in some situations to apoptotic cell death.[124]

Different SBDPs produced by calpain and caspase-3 specific proteolysis have long been studied as biomarkers of axonal damage following brain injuries. Moreover, there exists substantial experimental evidence showing that αII-spectrin is specifically processed to signature cleavage products that

vary with the particular protease that is involved, including 150 kDa (SBDP150) and 145 kDa (SBDP145) fragments produced by calpain proteolysis, and a major cleavage product of 120 kDa (SBDP120) produced by caspase-3.[125] Studies performed in TBI patients have shown that the temporal profiles of the levels of the various SBDPs in CSF correlate with the acute diagnosis of severe TBI.[126,127]

These temporal profiles suggest that neuronal death (before 72 h) is mostly due to necrosis, and secondary neuronal death (after 72 h) is primarily due to apoptosis. Furthermore, the severity of brain injury correlates with increased levels of SBDPs.[126-129]

Several SBDPs are detectable in CSF after in patients with aneurysmal SAH.[128] Quantitative analyses of SBDPs in brains from rats that underwent middle cerebral artery occlusion (MCAO) revealed that SBDP150 and SBDP145 were strongly increased 6 h after experimental ischemia. SBDP145 level was elevated after 6 h after ischemic stroke detected by Western blot and enzyme-linked immunosorbent assay (ELISA).[102] Other preclinical studies in animal stroke models demonstrated accumulation of calpain and caspase-3 proteolytic fragments of brain-derived αII-spectrin in CSF.[130,131]

The major advantage of αII-spectrin over other related biomarkers is its ability to reveal both calpain and caspase-3 activation within the acute period after CNS injury. SBDP120 is a sensitive marker of caspase-3 activation exclusively associated with apoptotic cell death following severe TBI.[126] SBDP150 and SBDP145 provide a highly sensitive measure of calpain activation primarily associated with oncotic cell death, and to lesser extent the changes in the levels of these SBDPs might be also be indicative of apoptotic cell death.[124] A significant increase in SBDP150 and SBDP120 levels was observed following SAH.[128] This variability emphasizes the heterogeneous nature of CNS pathology after stroke and points to important implications for individualized treatment of brain-injured patients tailored to specific neurochemical cascades in the injured brain.

Importantly, a recent small study performed in patients with aneurysmal SAH showed that the changes in the CSF levels of SBDPs used in a biomarker panel significantly correlated with brain infarction, cerebral vasospasm, and poor outcomes.[71]

Myelin basic protein and myelin basic protein autoantibody

Myelin basic protein (MBP) is a structural hydrophilic protein that plays a critical role in organization of the structure of myelin sheaths of oligodendrocytes and Schwann cells in the CNS. MBP has long been considered as a marker of active demyelination.[132] It has been shown that the MBP levels in TBI patients with severe intracerebral damage were significantly increased at the time of admission and remained elevated for up to 2 weeks following injury, and higher serum MBP levels were associated with poor outcomes.[133] Recent studies suggested a prospective prognostic value of serum MBP levels in adult and pediatric TBIs.[65] Studies from the 1980s have shown increased levels of MBP in the CSF of patients with ischemic stroke and ICH.[134,135] MBP was found to be elevated in CSF samples from patients with ICH collected at a mean of 1 day after stroke (range: days 0-3). MBP levels increased in samples from both ischemic stroke and ICH patients collected at a mean of day 5 (range: days 4-8) as compared to day 1, whereas no change in MBP levels was found in patients with TIAs in whom the symptoms resolved within 24 h.[135] In this early study, the MBP levels correlated with brain lesions, and higher values were predictive of poor short-term prognosis.[135] These findings were confirmed in a study with 28 ischemic stroke patients, which showed that MBP concentrations in serial CSF specimens were significantly increased at 7 days but not at 48 h or at 18-21 days after stroke onset.[136] Another study found elevated MPB concentrations in 39% (n = 28) of the samples collected from ischemic stroke patients at admission.[137] In a study using retrospective serum samples from subjects enrolled in the original NINDS r-tPA Stroke Study,[16] peak MBP concentrations at 24 h after stroke onset were associated with higher NIHSS baseline scores and larger CT lesion volumes.[34] However, in this study, no significant changes in MBP or other biomarkers (e.g., tau protein, NSE, S100B) were found between the treatment groups despite significantly improved outcomes in the r-tPA-treated group.[34] The results of a recent study of ischemic stroke patients included within 5-10 days of symptom onset demonstrated significantly higher levels of CSF MBP than in the normal population, and the levels of MBP and other markets used in this study (e.g., tau protein, GFAP) correlated with clinical stroke severity.[138] However, in acute ischemic stroke patients, the serum levels of MBP within 12 h of symptom onset were not significantly different compared to the MBP levels in the samples from the healthy control group.[56] Thus, the delay in increase of MBP levels might be a factor limiting its diagnostic application for acute stroke management.

Interestingly, a recent study including subjects with ischemic stroke and controls (n = 112 and n = 40, respectively) demonstrated that although there was a global decrease in MBP autoantibody titers at acute time points after stroke, patients with white matter lesions demonstrated an increased titer of MBP autoantibodies at 1 month after stroke and also demonstrated worse neurological outcomes, suggesting possible pathological consequences involving these autoantibodies.[139] These findings were consistent with a recent preclinical study showing that rats developing Th1 or Th17 responses to MBP had a worse outcome after ischemic stroke.[140] On the other hand, measuring MBP autoantibodies titers in stroke patients could be an alternative strategy for MBP-based diagnosis of white matter injury.

Neurofilament light chain

Neurofilament light chain (NF-L) is a light chain subunit of a structural triplet protein that forms the core of the neurofilament, which is an essential structural element of axons and to lesser extent of the cell body and dendrites. NF-L binds to several structural proteins including spectrin and microtubule-associated protein-2 (MAP-2).[141] Thus, the appearance of NLF in CSF has been postulated to be specific for axonal injury. Elevated NF-L levels in CSF have been reported in different chronic neurodegenerative disorders and following acute brain injuries including ischemic stroke SAH and TBI.[142,143] A recent study demonstrated that patients

with ischemic stroke had significantly higher CSF levels of NF-L and that it correlated with the severity of white matter lesions.[138] A study of patients with cervical artery dissection compared to patients with local symptoms only ($n = 8$), TIAs, ($n = 10$) or ischemic stroke ($n = 31$) found a significant increase in serum NF-L levels in patients with stroke as compared to patients with local symptoms or TIAs, and the NF-L level correlated with clinical stroke severity and poor outcome at 3 months.[144] In addition, the CSF level of NF-L correlates with outcome after aneurysmal SAH.[107,145] Analysis of serial ventricular CSF samples collected from 35 aneurysmal SAH patients for up to 15 days demonstrated elevated NF-L concentrations in all patients, and the NF-L levels were higher in the patients with early cerebral ischemia defined by CT scans within the first 3 days.[107] However the dynamics of NF-L release in neurodegenerative disorders and following CNS injuries are still poorly understood, and further studies are warranted to establish the validity of NF-L as a diagnostic biomarker for acute stroke.

Tau protein

Tau is a highly soluble structural protein that belongs to the neuron-specific type II microtubule-associated protein family. In the CNS, tau protein is predominantly expressed in neurons and to a lesser extent in astrocytes and oligodendrocytes. Tau plays a critical role in the structural stabilization of microtubules by interaction with tubulin and formation of the neuronal cytoskeleton. Under pathological conditions, tau forms insoluble aggregates (pathologies collectively known as tauopathies), which are believed to play role in the pathophysiology of many neurodegenerative disorders.[146] Tau aggregates were first described in Alzheimer's disease, and it was suggested that these aggregates resulted from abnormal modification of tau protein mainly by its hyperphosphorylation.[147,148] Levels of both phosphorylated and total tau proteins in the CSF have long been considered as promising markers to discriminate neurodegenerative disorders from healthy aging.[149,150] Further studies have shown that tau protein also increases in CSF following acute brain injuries including stroke[37,151,152] and TBI.[153,154] Increased levels of tau protein in serum have been demonstrated in patients following stroke, and mild and severe TBI.[155-157] Interestingly, these studies have also shown that CSF and serum concentrations of tau protein in TBI patients measured initially after injury are correlated with short-term and long-term outcomes.[154,157]

After ischemic stroke, tau protein levels in CSF showed a marked transient increase 2-3 days after symptom onset, peak at 1 week, and return to normal levels after 3-5 months.[151,152] A recent study demonstrated that CSF levels of total tau protein in samples collected within 5-10 days of stroke onset as well as levels of other biomarkers including NF-L, MBP, and GFAP were significantly higher in patients with ischemic stroke as compared to controls and that tau protein levels, along with the levels of MBP and GFAP, significantly correlated with clinical stroke severity.[138]

Similarly, serum concentrations of tau proteins in blood samples of patients with ischemic stroke collected at extended time points have shown correlation with stroke outcomes, whereas tau protein concentrations in the majority of the samples collected within 24 h of symptom onset were within normal range.[49] Serum tau protein was detected in 47.8% of ischemic stroke patients, and these patients developed more severe neurological deficits, suggesting that the presence of tau protein in the serum itself, rather that its exact concentration, at early time points after stroke has a predictive value as a prognostic indicator associated with infarction volume, neurological deficits, and functional status measured in the early and late phases of ischemic stroke.[37] In ICH, a study performed with 176 patients demonstrated that serum tau levels measured at admission had a prognostic value for mortality and poor neurological outcomes at 3 months after symptom onset.[57]

Visinin-like protein-1

Visinin-like protein-1 (VLP-1) is a member of the family of neuronal intracellular calcium sensor visinin-like proteins (also known as VILIPs) that are abundantly expressed in the brain and play a role in Ca^{2+}-dependent cell signaling involved in the modulation of cyclic adenosine monophosphate (cAMP) production.[158] Initially, VLP-1 was cloned using cDNA from a rat brain library, which was further demonstrated to have 100% homology with human VLP-1.[159,160] Although VLP-1 is preferentially expressed primarily in CNS neurons, it is also found in pancreatic islets.[161] Initial studies have shown time-dependent increase of VLP-1 levels in a preclinical model of ischemic stroke and in human plasma samples retrospectively collected from stroke patients.[162] In human plasma samples, VLP-1 was not detected in ~92% of unaffected controls (36 from 39 samples tested), whereas VLP-1 was detected in up to 100% of the samples from patients with confirmed stroke after 48 h after stroke onset but VLP-1 was detected only in 44% of the plasma samples from these patients taken within the first 24 h after symptom onset.[162] However, another pilot study performed using serum samples taken after 3 h of stroke onset demonstrated that the VLP-1 level of 0.093 μg/m significantly ($P < 0.01$) differentiated healthy individuals ($n = 17$) from stroke patients ($n = 16$) with 100% sensitivity and 100% specificity.[58]

In addition, recent studies have shown that serum and CSF levels of VLP-1 might be diagnostic of Alzheimer's disease[163,164] and that serum and CSF VLP-1 levels might be diagnostic biomarkers of acute encephalopathy with biphasic seizures and late reduced diffusion (AESD).[163,164]

NR2 peptide and NR2 autoantibody

The N-methyl-D-aspartate (NMDA) receptor is a subtype of ionotropic glutamate receptor that plays a critical role in learning and memory. The NMDA receptor is a tetramer comprising two glycine-binding NR1 and two glutamate-binding NR2 subunits.[165] NMDA receptors are expressed predominantly in CNS neurons though the expression of different NR1 and NR2 subunit receptors has been demonstrated in cerebral epithelial cells,[166] oligodendrocytes,[167] and in neurons of enteric nervous system.[168,169] Alterations in the expression of NMDA receptor subunits have been demonstrated in different neurological disorders. Following cerebral ischemia, the expression of NR2 subunit is upregulated,[170] whereas the expression of the NR1 subunit is downregulated.[171] In addition, there exists evidence that cerebral ischemia and excitotoxicity induce calpain-mediated cleavage of both NR2A and NR2B subunits.[172,173]

A study including 105 stroke patients [i.e., TIAs (n = 56), acute ischemic stroke (n = 31), ICH (n = 18)] and 255 controls has shown that the levels of the autoantibodies to NR2A/2B NMDA receptor subunits in plasma are increased in patients with TIAs and ischemic stroke as compared to controls, and that the levels of NR2A/2B autoantibodies measured within 72 h differentiated ischemic stroke from ICH.[174] Another study from the same group using serum samples collected from patients with suspected TIA and ischemic stroke within 72 h of stroke onset demonstrated that levels of NR2 autoantibodies were elevated in females with acute strokes and in both males and females with multiple recent strokes as compared to patients without stroke or to healthy controls, and the levels of NR2 autoantibodies in stroke patients correlated in a gender-dependent manner with several risk factors for stroke including hypertension, diabetes mellitus, and atrial fibrillation, suggesting that levels of NR2 autoantibodies might be indicative of a history of multiple strokes and be a predictive factor for stroke.[175]

Levels of NR2 peptide (probably a product of calpain-mediated proteolysis of the NR2A/2B subunits[176]) have been shown to be elevated in plasma samples collected at admission from patients with ischemic stroke.[59] This study enrolled 292 subjects with ischemic stroke, TIAs, or vascular stroke factors (ICH patients were excluded from the study) as well as healthy controls. NR2 peptides may have a role as a diagnostic biomarker for acute stroke.

Use of Blood Glial Fibrillary Acidic Protein Levels for Early Discrimination of Ischemic Stroke and Intracerebral Hemorrhage

Rapid differentiation between ICH and ischemic stroke using biomarker testing would facilitate prehospital, cause-specific management of stroke patients. It has been postulated that the more sudden disruption of astrocytes and blood-brain barrier after ICH may be responsible for the rapid appearance of GFAP in serum, in contrast to more delayed astrocytic damage and subsequent release of GFAP in ischemic stroke.[43,89] The predictive value of GFAP for discriminating between ischemic stroke and ICH could be increased when used with an additional biomarker or biomarker panel. An exploratory multicenter study with 97 stroke patients enrolled within 24 h of symptom onset (83 ischemic stroke and 14 ICH) showed that blood levels of GFAP and (APC-PCI) might be useful to rule out ICH in a mixed stroke population prior to neuroimaging, whereas S100B and NSE had no such predictive value.[35]

Elevated serum and CSF levels of GFAP are hallmarks of brain injuries and are associated with overall outcome and prognosis. GFAP may be useful as a surrogate marker for the management of hemorrhagic stroke.[43] Previous studies have suggested that the serum level of GFAP appeared to be a more sensitive marker of brain injury in small lesions and minor strokes as compared to S100B[27] but its application as a diagnostic tool might be limited by its delayed rise in concentration following mild injuries.[43,177] However, a recent study provided strong evidence that the serum levels of GFAP can differentiate ICH and ischemic stroke. Meta-analyses of recent clinical trials suggested that serum GFAP is a sensitive and specific test for differentiating ICH and ischemic stroke in patients within 1-6 h of stroke onset.[178,179] GFAP level is increased after both ischemic stroke and ICH but its concentrations are significantly higher in early ICH patients.[44]

A multicenter cohort study including 205 patients diagnosed with either ischemic stroke, ICH, or stroke mimics suggested that plasma GFAP analysis performed within 4.5 h of symptom onset can differentiate ICH and ischemic stroke with a sensitivity of 84% and specificity of 96%.[45] Similar results were obtained in a study of Chinese patients using blood samples collected within 2-6 h after the onset of symptoms. A blood GFAP level at the cut point of 0.7 ng/mL yielded an AUC of 0.901 (95% CI 0.828-0.950) with high sensitivity (86.0%) and specificity (76.9%) to differentiate ICH from ischemic stroke.[47] In addition, in the ICH group, a significant correlation was observed between GFAP levels and neurological deficits assessed by NIHSS, and between GFAP levels and hemorrhage volume, and the highest GFAP levels were predictive of poor short-term functional outcomes.[47] Furthermore, a recent study suggested that GFAP used in combination with NR2 can differentiate between ischemic stroke and ICH at a time point extended to 12 h after the ictus with a sensitivity of 94% and specificity of 91%.[46] Another study showed that plasma concentrations of retinol binding protein 4 and GFAP at concentrations >61 μg/mL and <0.07 ng/mL, respectively, demonstrated 100% specificity, and these biomarkers might be independent predictors to discriminate stroke subtype, improving discrimination by 29% (P < 0.0001).[48]

Conclusion

Recent clinical trials have identified several prospective brain-specific biomarkers for which their appearance in the peripheral circulation reflects their postinjury dynamics in the brain tissue and CSF. Although many of these biomarkers demonstrate independent diagnostic and prognostic values in ischemic stroke and ICH, the heterogeneity of stroke phenotypes suggests that useful clinical information can be obtained only from a panel of selected biomarkers. Some promising biomarkers are still at an early stage of development, and further clinical trials are warranted to determine their utility in clinical practice. Of interest, especially in the neurointensive care unit (NICU) applications, are early biomarkers capable of estimating the time of stroke onset or discriminating ischemic from hemorrhagic stroke to facilitate clinical decision-making such as whether to administer thrombolytic therapy. Future trials will provide answers to this and many other questions in this rapidly developing area.

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