Published in final edited form as:
Biomark Med. 2009 August 1; 3(4): 363–383. doi:10.2217/bmm.09.30.

Molecular biomarkers in stroke diagnosis and prognosis

Matthew B Maas[1,2] and **Karen L Furie**[2]

[1]175 Cambridge Street, Suite 300, Boston, MA 02114, USA, Tel.: +1 617 643 2713; Fax: +1 617 643 3939; mmaas@partners.org

[2]Department of Neurology, Massachusetts General Hospital, Harvard Medical School, MA, USA

Abstract

Serum biomarkers related to the cascade of inflammatory, hemostatic, glial and neuronal perturbations have been identifed to diagnose and characterize intracerebral hemorrhage and cerebral ischemia. Interpretation of most markers is confounded by their latent rise, blood–brain barrier effects, the heterogeneity of etiologies and the wide range of normal values, limiting their application for early diagnosis, lesion size estimation and long-term outcome prediction. Certain hemostatic and inflammatory constituents have been found to predict response to thrombolysis and worsening due to infarct progression and secondary hemorrhage, offering a potential role for improved treatment selection and individualization of therapy. Biomarkers will become increasingly relevant for developing targets for neuroprotective therapies, monitoring response to treatment and as surrogate end points for treatment trials.

Keywords

biomarkers; cytokines; diagnosis; inflammation; prognosis; severity; stroke; worsening

Stroke is a sudden loss of neurologic function resulting from focal disturbance of cerebral blood flow due to ischemia or hemorrhage. Transient ischemic attacks (TIAs) are due to the same physiological process but without acute infarction. Stroke is the third leading cause of death in the USA, and 87% of strokes are ischemic [1]. The expanding use of biomarkers in the field of stroke has made a substantial impact in our understanding of the pathophysiology of stroke and the approach to treatment.

Types of biomarkers

A biomarker is any measurable physiological characteristic or substance that marks the risk for or manifestation of a stroke-related process (Box 1). Several categories of biomarkers are studied in stroke – physical markers, imaging markers, electrophysiological markers, histological markers, genetic markers, systemic (serum) markers and neuronal markers. A physical marker associated with stroke may be as simple as the discovery of hypertension. Neuroimaging is widely used to aid in the diagnosis of stroke, to assist in determining a likely etiology for the event, to estimate severity, and to predict functional outcome and risk of

Correspondence to: Matthew B Maas.

Financial & competing interests disclosure

Mathew B Maas and Karen L Furie are supported by NIH (grant P50NS051343). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

recurrence. Cardiac imaging is used to investigate risk factors and to search for a cardioembolic mechanism. Histological markers can be especially useful in evaluating uncommon causes, such as vasculitis and collagen vascular diseases. Genetic markers can identify heritable cerebrovascular conditions that are associated with stroke and may be useful in individualizing treatments. Serum markers are widely used to test for metabolic risk factors, such as diabetes and dyslipidemia. New research is expanding our understanding of the systemic response to stroke (e.g., inflammation) and demonstrates the potential for applying new serum biomarkers to routine clinical use.

Various markers in nearly all of these listed categories have been evaluated for their association with prospective risk for stroke, for diagnostic purposes and for their value in predicting long-term outcome. Markers of risk factors and prevention strategies have been extensively reviewed in treatment guidelines. The American Stroke Association guidelines for stroke prevention contain a thorough review of the markers of prospective stroke risk for primary and secondary prevention [2–4]. The use of biomarkers in diagnosing stroke and assessing prognosis is an emerging and rapidly evolving field. In this article, we will focus on molecular markers in the cerebrospinal fluid (CSF) and serum, and their use in diagnosing stroke, characterizing severity, optimizing treatment and predicting outcome. This article will provide a brief overview of key cellular mechanisms in cerebral ischemia and hemorrhage, will summarize the important recognized molecular markers of those processes and will discuss the application of these markers to assess a patient's clinical status, and to guide therapy.

Relatively little biomarker research has been conducted with a focus on hemorrhagic stroke compared with the extensive data on ischemic stroke markers. Relevant data are noted when available, but insufficient data exist to draw broad conclusions regarding how to best translate hemorrhagic stroke biomarkers into routine clinical use.

Box 1. Biomarker categories for stroke

Physical

- Neuroimaging
- Cardiac imaging
- Electrophysiological
- Histological

Genetic

- Serum/plasma/cerebrospinal fluid
  - Systemic
  - Neuronal

Applying molecular biomarkers

The CNS cellular response to stroke results in characteristic upregulation and release of particular neuronal markers into the CSF and blood-stream. Increased levels of neuronal isoenzymes and other molecules with CNS-specific expression signal damage to the brain parenchyma. The primary focus of molecular biomarkers of stroke has been their application to diagnosis. The model for a diagnostic marker is the cardiac isoenzyme test. Development of high-sensitivity techniques to detect the cardiac isoenzymes of troponin I and troponin T – products of myocardial degradation – led to the eventual revision of the clinical definition of myocardial infarction to elevate the role of biomarkers in diagnosis [5]. The key property of

the troponin test that led to its widespread incorporation into clinical care was a nearly absolute specificity for myocardial tissue (unlike creatine kinase-MB), as well as high sensitivity. 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 (Box 2).

Biomarkers in stroke provide insight into the pathophysiologic mechanisms of brain injury. Studies of cytokine markers, for example, have enhanced our understanding of the role of inflammation in the infarct core and ischemic penumbra. The observation that higher levels of certain infammatory cytokines have been correlated to progression of infarct size and clinical deterioration emphasizes this relationship. Certain biomarkers may be useful in differentiating between stroke mechanisms, such as cardioembolism or atherothrombosis. Many of the biomarkers reviewed in this paper correlate with the size and clinical severity of the stroke. The only approved pharmacological therapy for acute stroke, thrombolysis, acts on the hemostatic axis. The activity of the hemostatic system has been shown to predict response in terms of likelihood of recanalization and probability for secondary hemorrhage. Finally, selected serum markers have been shown to correlate with long-term outcome measures.

Box 2. Applications for serum biomarkers of stroke

- Clarify pathological mechanisms
- Facilitate diagnosis
- Identify stroke mechanism
- Characterize size and clinical severity
- Identify significant penumbra and estimate risk of progression/worsening
- Select appropriate treatment option and predict likelihood of response
- Monitor treatment efficacy
- Estimate long-term prognosis

Heterogeneity of stroke

In stroke, brain injury, ultimately due to necrosis, is a result of the deprivation of metabolic substrates and apoptosis owing to the combined effects of inflammatory and oxidative damage. In cerebral infarction, the inciting event in the ischemic process is obstruction of arterial flow. This obstruction may occur as a result of a myriad of pathological processes. In addition, a variety of pathological processes contribute to the occurrence of intracerebral hemorrhage (ICH). In hemorrhagic stroke, the products of hemorrhage are inherently inflammatory and the swelling is due to inflammation, and bulk interposition of blood into the neuropil raises local pressure, inhibiting effective circulation and leading to secondary ischemia. Given the vast array of pathological conditions that can lead to stroke, an exhaustive list of all markers for each of these etiologies would be unhelpful. Although the triggering events are quite different in ischemic and hemorrhagic stroke, the ultimate mechanisms of neuronal injury and death are similar. Familiarity with the pathways of endothelial, glial and neuronal injury that occur in stroke is crucial for understanding the relevance of identified biomarkers. Comprehensive reviews of the cellular mechanisms of stroke injury are available elsewhere, but a brief orientation will provide the context for discussing the relevance of individual biomarkers. The following paragraph provides an abbreviated and simplified overview of the most studied pathways involved in ischemic cellular damage.

Neurons are heavily dependent on aerobic metabolism and have a disproportionately high energy demand compared with other cell types. In circumstances of ischemia, oxygen and glucose are rapidly depleted, oxidative phosphorylation in the mitochondria ceases within minutes and cellular function becomes deranged. The onset of energy failure triggers a cascade of events that exacerbate and perpetuate damage. Neuronal membranes depolarize owing to failure of the Na^+/K^+-ATPase pumps, leading to influx of calcium and sodium, and dysfunctional release of neurotransmitters. Excitatory neurotransmitters, such as glutamate, which are released from depolarized cells overstimulate nearby cells, depolarizing them and, thus, inciting an excitotoxic cycle. The cessation of mitochondrial activity increases oxidative and nitrative stress, promoting apoptosis. An inflammatory response begins to develop in the affected area [6–9]. Certain inflammatory cytokines, most importantly TNF, interact with cellular messaging systems and promote apoptosis [10]. Cell membrane dysfunction leads to a net passive movement of water into cells – cytotoxic edema. In response to cytokine signals and oxidative stress, degradative enzymes, such as matrix metalloproteinases (MMPs), are activated and begin to erode the integrity of the blood–brain barrier (BBB) and vascular structures [11]. Within 24 h, the resulting increased permeability leads to vasogenic edema. Ischemia causes autoregulatory mechanisms to fail and prompts upregulation and release of prothrombotic molecules that lead to intravascular thrombosis [12,13]. The increasing local pressure due to cytotoxic and vasogenic edema, and intravascular thrombosis impair the regional microcirculation, and the decrease in cerebral blood flow leads to further ischemia in regions at risk on the periphery of the infarct core. Damaged cells lose membrane integrity and release their contents into the neuropil.

Summary of serum biomarker properties

Table 1 summarizes important properties of molecular markers derived from constituents of damaged neurons and glia, elements of hemostasis, cytokines, inflammatory acute-phase reactants, markers of tissue disruption and apoptosis, measurable alterations in oxidative stress and other miscellaneous molecular signs associated with stroke.

Cellular constituents of neurons & glia as biomarkers

Glial biomarkers

S100-β—S100-β (S100B) is a calcium-binding peptide secreted by astrocytes in the context of brain injury, neurodegenerative processes and psychiatric disorders. The interaction of S100B with the cerebral immune system can lead to enhanced survival of developing neurons at low concentrations, however, at higher concentrations S100B has been shown to stimulate proinflamamtory cytokine production and apoptosis *in vitro*. Animal studies have demonstrated a potential neuroprotective effect of S100B by activating expression of cellular processes in neurons that block NMDA-induced excitotoxicity [14]. Increased serum levels of S100B are found in association with ischemic stroke, but are also present in many other acute and chronic conditions, such as traumatic brain injury (TBI), Alzheimer's disease and schizophrenia [15].

In ischemic stroke, the serum concentration of S100B reaches a maximum concentration between day 2 and 4, and the peak concentration correlates with higher NIH Stroke Scale (NIHSS) scores, infarct size and the clinical course in both infarcts and subarachnoid hemorrhage [16–18]. Possibly owing to its correlation to infarct size, a higher S100B concentration is also associated with higher risk for hemorrhagic transformation after treatment [19]. S100B levels have also been found to be increased in primary hemorrhagic stroke, associated with initial hematoma volume and predicting a worse clinical course with higher morbidity [20]. S100B levels do not necessarily rise quickly. In one study, for example, serum S100B elevation was only 32% sensitive for acute ischemic stroke on admission [21].

The interpretation of serum S100B levels has been complicated by the fact that S100B was thought to be detected in cell types other than astrocytes and Schwann cells, and high levels were found in the context of a wide variety of other conditions [22,23]. Early attempts to clarify the source by analyzing dimers was unfruitful [24]. Further investigation into the potential extracerebral sources of S100B led to a more rigorous assessment of the immunoassay that was being used, identifying extracerebral sources as false-positive contaminants [23]. Although S100B has now been clearly established as CNS specific, erroneous references to results obtained by the tainted assay continue to appear in the literature [25]. There is evidence from several studies that serum S100B levels are affected by BBB integrity owing to acute disruption or the effect of chronic microvascular disease [26,27].

Glial fibrillary-associated protein—Glial fibrillary-associated protein (GFAP) is a monomeric intermediate filament protein present in astrocytes and, to a lesser degree, in ependymal cells of the brain, where it functions as a part of the cytoskeleton. GFAP is widely used in neuropathology as an immunohistochemical marker of glial cell lineage, and increased serum GFAP is specific for neurological injury [28].

In CSF, both S100B and GFAP levels increase early in proportion to size for medium–large-sized strokes, peaking at day 1–2 and returning to baseline by 3 weeks [29]. The serum concentration of GFAP correlates well with that of S100B, as well as to the volume of infarcted brain and the neurological status at discharge from hospital [29,30]. Serum GFAP was found to be more sensitive than S100B as a marker of brain injury in small lesions and minor strokes, but the delayed rise in concentration limits its application as a diagnostic tool [30,31].

Myelin basic protein—Myelin basic protein (MBP) is a hydrophilic protein important for the correct structure of myelin sheaths. Elevated levels of MBP are found in a variety of neurological conditions. For example, MBP levels in CSF has been used as an index of disease activity in multiple sclerosis [32]. MBP has also been found to be elevated in the CSF of patients after stroke [33]. A more detailed study of CSF MBP in the context of acute ischemic stroke found that concentrations were not significantly elevated until 1 week after the ictus, and returned to low levels after 3 weeks [29]. One study sampling the serum levels in patients with ischemic stroke on admission found that MBP was elevated in 39% of patients, and higher peak levels correlated with higher admission NIHSS scores [21]. In a study using serum from subjects who had enrolled in the National Institutes of Neurological Disease and Stroke (NINDS) Recombinant Tissue Plasminogen Activator (tPA) Stroke Study, peak MBP concentration was significantly associated with higher baseline NIHSS scores and larger lesion volumes [18]. The latent increase in this marker is a limiting factor.

Fatty acid-binding proteins—Fatty acid-binding proteins (FABPs) are a class of intracellular molecules involved in buffering and transporting long-chain fatty acids. There are nine types of FABPs that are variably expressed in different tissues. These proteins are rapidly released from damaged cells into circulation. Four FABPs are associated with the nervous system, but only two are found in the adult CNS: brain-type (B-FABP) in glia and heart-type (H-FABP) in neurons, with H-FABPs present at more than tenfold higher concentration [34–36]. H-FABP is present in multiple tissue types and has found application as a biomarker for acute myocardial infarction [37]. B-FABP is thought to be present only in central nervous tissue and is undetectable in the serum of healthy individuals [36,38].

Heart-type and brain-type FABPs peak within 2–3 h of stroke onset. The rapid and early peak is attributable to the small molecular size that allows quick passage through the BBB and the short serum half-life of 20 min, owing to renal clearance. B-FABP was found to be most sensitive to small subcortical and lacunar infarcts, but did not correlate to the degree of neurological injury. By contrast, elevation in H-FABP levels was proportionate to infarct size

and severity of neurological deficits [35]. A pilot study using H-FABP along with troponin I to exclude a cardiac source found no false positives and 68% sensitivity when sampled on admission, which was a better performance than neuron-specific enolase (NSE) and S100B [39]. Elevated B-FABP concentrations in serum is not specific for stroke, having been detected in a variety of brain injury conditions, including mild TBI and electroconvulsive therapy [36].

Neuronal biomarkers

Neuron-specific enolase—Neuron-specific enolase is one of three recognized forms of enolase, an enzyme in the glycolysis pathway. Although relatively specific for neurons, NSE is also found in neuroendocrine cell lines and their associated cancers. Similar to S100B, NSE concentration rises in the CSF following ischemic stroke as well as other brain tissue injuries, such as subarachnoid hemorrhage, ICH and head injury. Peak concentration after ischemic stroke correlates with baseline NIHSS scores, but not the volume of infarcted tissue [18]. NSE first becomes detectable between 4 and 8 h after the onset of stroke. Although CSF levels of NSE increase in proportion to brain injury with high sensitivity, serum NSE levels are less sensitive and a greater extent of injury is required to produce a consistently measurable change [40–42]. Serum and CSF levels of NSE vary widely across the normal population and no clear relationship has been shown between serum and CSF levels [43]. Although significant trends are detected in various studies, values rise slowly and rarely exceed the reference range of normals (13% within first 24 h, as reported by one study) [40,42,44,45].

Tau protein—Tau protein (TP) is a structural microtubule-associated protein that is well known for its association with a variety of neurodegenerative disorders, including Alzheimer's disease. The six isoforms of tau are abundant in the brain, facilitating the formation of microtubules by interaction with tubulin [46]. Aside from its pathological aggregation in neurodegenerative disorders, TP has been explored as a biomarker in acute ischemic stroke. Although TP levels sampled at late time points correlate with infarct size and clinical severity, levels rise slowly and only 27% of TP concentrations were elevated over the normal range within the first 24 h [44]. TP levels in CSF have also been studied in the context of acute stroke, showing the same poststroke increase that also correlated to infarct size [47,48]. Further exploration of neurodegenerative-associated biomarkers applied to stroke has been less fruitful. CSF levels of β-amyloid, Apo E and clusterin were not impacted by stroke [47,49].

Neurofilament light protein—Neurofilament is a triplet protein that forms part of the structural scaffold of neurons. Neurofilament light protein (NFL) is the subunit that forms the core of the filament. Increased levels of NFL have been observed in a variety of neurodegenerative diseases. Increased CSF concentration of NFL has also been shown in a small series of patients following ischemic stroke [50].

N*-acetylaspartate—N*-acetylaspartate (NAA) is a free amino acid representing approximately 1% of the dry weight of the brain. It is synthesized in neurons and functions as a molecular water pump, drawing water out of neurons and through the overlying myelinating oligodendrocytes where NAA is recycled. Owing to the neuron's high metabolic rate compared with other tissue types, metabolic water must be removed at approximately 12-times the rate of that removed in the rest of the body. Therefore, brain NAA is heavily utilized, completely replenishing every 16.7 h [51]. Perturbations in NAA can be identified by magnetic resonance spectroscopy and are useful in characterizing brain lesions. A study investigating NAA in acute ischemic stroke found that serum concentrations were significantly higher in all patients on admission and all points sampled until 48 h. NAA concentration was maximal at time 0 and declined after-ward [45]. This is consistent with prior findings that NAA levels decrease rapidly after damage because the rate of efflux from brain exceeds that of metabolism [52–54].

Combining information about NAA levels with traditional MRI sequences can predict the outcome in patients, where those with lower NAA levels in ischemic tissue do more poorly [55]. Magnetic resonance spectroscopy shows that NAA levels in ischemic injury continues to fall gradually for more than 1 week postinfarction, although any NAA detected beyond 24 h may simply be trapped in cellular debris [54,56,57]. Early reperfusion has been shown to cause a quick, transient increase in serum NAA concentration [58]. This may be due to resumed synthesis from restored metabolic function during a time of persistent BBB dysfunction, or simply from efficient clearance of NAA from the interstitium by restored regional blood flow.

NMDA autoantibodies—The NMDA glutamine receptor is implicated in mediating the excitotoxic response in cerebral ischemia. Autoantibodies (aAbs) to the NR2 subtype of the NMDA receptor have been demonstrated to be associated with neurotoxicity. A pilot study to examine serum levels of the neurotoxic biomarkers, glutamate, homocysteine and NMDA NR2 aAbs, found that subjects presenting with TIA had significantly elevated levels of NMDA NR2 aAbs compared with controls, and those with ischemic stroke had even higher concentrations [59]. Interestingly, in the pilot study and a follow-up larger trial, patients with ICH demonstrated no significant elevation of NMDA NR2 aAbs whereas patients with both TIA and ischemic stroke demonstrated markedly elevated levels that correlated with the NIHSS score and diffusion-weighted imaging (DWI) lesion volume [60]. In addition to showing high sensitivity, the NMDA NR2 aAb assay may be one of the only biomarkers of stroke that can reliably differentiate between hemorrhagic and ischemic stroke, along with DNA and Apo CI and CIII levels [25,61].

Visinin-like protein-1—Visinin-like protein (VLP)-1 is the human homolog of a protein that was identified by screening mice with gene-array analyses for biomarkers that are preferentially and abundantly produced in brain. VLP-1 was then detected in CSF using a rat model of ischemic stroke, and in the serum of humans after stroke. VLP-1 is a neuronal Ca^{2+} sensor protein expressed primarily in brain but also found in other tissues [62]. VLP-1 was detected in 8% of controls with no stroke and in 44% of subjects with stroke in samples taken within the first 24 h after onset of stroke [63]. Although the approach to screening potential brain biomarkers is novel, these early results are not compelling.

Nonspecific markers

Hemostatic biomarkers

Thrombomodulin—Thrombomodulin (TM) is an endothelial membrane protein that plays an important role in hemostasis by binding thrombin and activating protein C, thereby exerting an antithrombotic mechanism. TM expression in the brain is controlled through astrocyte regulation of TM mRNA expression [64]. TM is present in serum and urine in a soluble form. Various TM gene polymorphisms have been associated with coronary events and stroke, although serum TM levels are not correlated [65]. A study investigating 492 stroke patients and matched controls found that serum TM did not correlate to stroke exposure, however, among case subjects, high TM levels were associated with a higher death rate [66].

A small pilot study found that TM was elevated on admission in 43% of patients, and peak levels correlated with discharge modified Rankin scale scores but was contradicted by a larger study that found no significant difference between subjects with stroke and controls on admission or at 1 month [21,67]. Both this study and another by the same group found elevated serum levels of TM only in the subacute phase [67,68]. Likewise, serum TM concentration from NINDS recombinant tPA Stroke Study subjects did not correlate to NIHSS score or stroke lesion volume at any time point or functional outcome [18]. Soluble TM in serum does not appear to be a product of endothelial degradation, but rather a regulated component in the

body's complex hemostasis axis. At present, the significance of serum TM concentration is not clear.

Nonspecifc markers of coagulation—Abnormalities in the hemostasis axis are an uncommon cause of ischemic stroke [69–71], nevertheless, perturbations are seen following stroke onset that may aid in diagnosis, be informative about the severity of the event and predict response to therapies. Some coagulation markers have been used extensively for diagnostic purposes for other conditions. The D-dimer test in cases of suspected pulmonary embolism is a classic example [72]. The limitation to the use of coagulation markers is that they are nonspecifc in nature.

An increase in fibrinogen levels is seen after ischemic stroke and is associated with infarct size and outcome [73–75]. High levels of D-dimer, a small protein fragment produced by fibrin degradation, predicts progression in ischemic stroke and poor outcome in ICH [76–79]. The case is similar for other bio-markers of hemostasis: higher concentrations of fibrinopeptide A, b-thromboglobulin, prothrombin fragments 1 and 2, thrombin–anti-thrombin complexes, platelet factor 4 and von Willebrand factor have all been associated with a worse clinical course in ischemic stroke and increased mortality [76,80,81]. Fibrinopeptide A, thrombin–antithrombin complexes, D-dimer and, possibly, β-thromboglobulin and platelet factor 4, are elevated in patients presenting with TIA compared with matched controls, suggesting a dysfunction in coagulation and platelet activation [82–84]. Several studies that have looked concurrently at multiple hemostatic biomarkers have concluded that the D-dimer level is very sensitive to stroke, it may help differentiate between stroke etiologies and is the strongest prognostic indicator for worsening or recurrence within the subacute period [79,85,86].

Constituents of the hemostasis axis may provide information beyond identifying stroke occurrence and severity. The activity of many agents in the coagulation and fibrinolysis cascade increase in the context of stroke. Measuring the activity of various components may be useful in predicting response to therapies or for individualizing treatments. Patients presenting with ischemic stroke whose international normalized ratio from warfarin treatment is therapeutic have smaller infarcts and perfusion deficits, lower NIHSS score on admission and lower modified Rankin score at discharge [87]. Plasma levels of thrombin-activable fibrinolysis inhibitor (TAFI) are elevated in the acute phase of ischemic stroke, and plasminogen activator inhibitor (PAI)-1 is elevated in patients that show progression of intracranial atherosclerosis, along with other proinflamamtory markers [88,89]. Patients with high levels of PAI-1 are less likely to achieve recanalization after receiving tPA and experience poorer outcomes [90]. Baseline levels of TAFI and PAI-1 also predict symptomatic hemorrhage after tPA treatment [91]. Similarly, polymorphisms of both the *TAFI* and *PAI-1* genes influence the probability of recanalization after tPA treatment [92]. A point-of-care assay to measure the activity of these fibrinolysis inhibitors could allow for adjustments to thrombolytic dosing, which may increase efficacy in patients with high inhibitor levels and reduce the likelihood of hemorrhagic transformation in patients with a concerning profile.

Inflammatory markers

Proinflammatory & anti-inflammatory cytokines—Cytokines are extracellular proteins and glyco-proteins that function as short-range molecular messages, exerting a paracrine or autocrine effect on local tissue. Triggered by a variety of stimuli, cytokines are critical mediators of the tissue inflammatory and immune responses [10]. Inflammatory reactions are intricately mediated by proinflamamtory and anti-inflammatory pathways that often operate in tandem to regulate the extent and intensity of the response. There has been significant interest in delineating the role of proinflamamtory cytokines in the perpetuation and propagation of damage postinfarction. Since inflammation generates the formation of edema and can promote

apoptosis, inflammatory biomarkers are potentially useful for prognosis and as prospective targets for neuroprotective therapies. Although none of the cytokines that activate in the brain following stroke are CNS specific, careful investigation has confirmed that the elevation in plasma levels of these signaling molecules is owing to their production in the CNS and not from peripheral blood cells [93].

Serum sampling of patients presenting with ischemic stroke has shown elevation in many cytokine markers, including IL-1, IL-6, IL-8, IL-10, IL-13, TGF-β, ICAM-1, VCAM-1, E-selectin, L-selectin, P-selectin, TNF-α, chitotriosidase and monocyte chemoattractant protein-1 [73,89,94–101]. Levels of soluble selectin proteins are increased following stroke, indicating the activation of leukocyte trafficking [68]. Elevated levels of proinflamamtory cytokines and low levels of anti-inflammatory markers are associated with severity, infarct volume, clinical worsening and long-term outcome [102–104]. Elevated levels anti-inflammatory mediators, such as IL-6, IL-10 and IL-1-receptor antagonist, concurrent with elevated proinflamamtory markers suggest a complex interaction of pro-and anti-inflammatory mechanisms at work in the hours and days following ischemic and hemorrhagic stroke [73, 96,100]. The appearance of TNF-α and IL-6 has also been correlated to the volume of perfusion-impaired tissue, suggesting the role of inflammation in the ischemic penumbra [97]. Although systemic anti-inflammatory medications, such as corticosteroids, have not proven to be effective treatments for stroke, inflammatory biomarkers have been used as surrogate markers of efficacy for neuroprotective medications [98,105].

C-reactive protein & other acute-phase reactants—C-reactive protein (CRP) is an acute-phase protein associated with inflammation. Produced in the liver in response to IL-6, CRP binds and aggregates a variety of soluble ligands and activates the classical complement pathway [106]. More recent research has also advanced a hypothesis that CRP may directly cause disruption of the BBB [107]. Owing to the inflammatory nature of atherosclerosis, modest elevations in CRP levels have been correlated to increased risk for stroke in large population studies, independent of other traditional cardiovascular risk factors [108]. CRP and fibrinogen levels rapidly increase as a result of stroke, and CRP on admission has been shown to predict mortality in tPA-treated patients [73,99,109]. As in the case of many other inflammatory biomarkers, levels of CRP are correlated with infarct volume, stroke severity and long-term outcome [104]. Levels of CRP and other acute-phase markers, such as the erythrocyte sedimentation rate, remain elevated up to 3 months following stroke [99]. Higher CRP levels measured within the first day of TIA or stroke onset and 3 months later are both associated with an increased risk for recurrent stroke following the initial event [110,111].

Markers of tissue destruction

Matrix metalloproteinases—Astrocytes and microglia that are involved in an inflammatory response are stimulated to release extracellular matrix degrading proteases. The most well recognized of these proteolytic enzymes are serine proteases and MMPs. MMPs are a family of 14 enzymes, a subset of which have been found to be active in brain tissue. The various MMPs function as stromelysines, gelatinases, colagenases, a membrane-type proteinase and a matrilysin. Serine proteases include elastase and plasminogen activators. Owing to their toxic potential, these enzymes are produced in latent form and require activation. Once active, MMPs are implicated in opening the BBB, damaging capillaries, perpetuating inflammation and, thereby, promoting secondary progression of ischemia and hemorrhagic transformation [11]. Inflammation and associated alteration of the vascular wall structures due to MMP activity have been implicated in the chronic, progressive damage of intracranial atherosclerosis, as well as acute-phase injury [89]. Various cytokines act on astrocytes and microglia in order to promote the production of MMPs and serine protease. The activity of

these matrix-degrading systems are regulated by the opposing effects of tissue inhibitors of metalloproteinases (TIMPs).

Matrix metalloproteinase levels are elevated after both ischemic and hemorrhagic stroke, and serial measurements of MMP-2 and MMP-9 levels demonstrate a correlation with proinflamamtory cytokine expression [95,112,113]. In ischemic stroke, serum concentration of MMP-9 correlates to NIHSS scores and initial and final DWI infarct volumes [97,100, 114], whilst peak concentration is associated with late hemorrhagic transformation and post-tPA hemorrhage [115–118]. MMP-9 and MMP-13 are correlated with DWI expansion [119]. In hemorrhagic stroke, higher MMP-9 is associated with perihematomal edema, this is probably owing to its role in opening the BBB. The MMP inhibitor TIMP-1 is negatively correlated to the extent of edema. MMP-2, MMP-3 and TIMP-2 all show characteristic rises after ICH [120].

Apoptosis pathway markers—TNF is an inflammatory cytokine with a myriad of effects. It can be expressed by endothelial cells, microglia and astrocytes in response to injury, where it exerts a local proinflamamtory and procoagulant effect. There is also animal-based experimental evidence implicating TNF in the pathophysiologic processes that trigger stroke. Once a stroke has occurred, TNF activates MMP and, thereby, increases vascular permeability, promoting edema formation and entrance of proinflamamtory plasma constituents through the impaired BBB. Importantly, TNF stimulates the production of reactive oxygen species (ROS), potentiates glutamate-mediated excitotoxicity and directly signals cells to induce apoptosis [10,100]. The combined effects on the microvasculature impairing local microcirculation, promoting excitotoxicity, facilitating edema formation, inflammation and apoptosis stimulation all contribute to expand the zone of ischemic tissue from the infarct core out into the penumbra.

Neuronal apoptosis is the pathway by which damage extends in a delayed fashion beyond the infarct core that undergoes necrosis. The importance of apoptosis pathways in stroke has been demonstrated in multiple studies, including neuroprotective trials in which blockade of important components of the apoptosis cascade substantially reduced infarct volumes in animal models [121]. Caspases are a family of cysteine proteases that constitute the main molecular cascade in the apoptosis pathway. Initiator caspases activate precursor forms of effector caspases, and those effector caspases, in turn, degrade specific molecular targets within the cell. Caspase-3 is an effector that functions as the final step in the chain of reactions that results in DNA fragmentation, and has been explored as a potential biomarker that would estimate the degree of active apoptosis [122–124]. The basis for interest in caspase-3 is due in part to animal studies demonstrating a potential therapeutic role in decreasing the activity of this enzyme. In a study investigating human stroke patients, plasma levels of caspase-3 were elevated in acute stroke, and correlated to infarct growth and short- and long-term neurological outcome [125]. The Fas system is a parallel pathway that induces apoptosis through a receptor in the TNF-receptor family [124]. Decreased levels of soluble Fas-variant molecules in the serum that act as apoptosis inhibitors, along with increased stimulatory Fas ligands in the perihematomal region measured in deceased patients with ICH shows a correlation between perihematomal edema growth and these markers of active apoptosis [126].

DNA—Circulating levels of DNA measured in plasma increase in response to a variety of injurious conditions, including stroke. Plasma concentration of DNA is elevated within 3 h of ischemic and hemorrhagic stroke onset, and patients who experience a poor outcome or die as a result of their stroke have higher levels than those who do not. The level of DNA also correlates to the volume of hematoma in ICH [127]. DNA concentration is significantly higher among patients with hemorrhagic versus ischemic stroke, and the combination of DNA and

S100B levels has been proposed as a way to differentiate those conditions using molecular biomarkers [25].

Indicators of oxidative stress & other nonspecific markers

Indicators of oxidative stress—Metabolic perturbations caused by ischemia lead to an increased production of ROS, such as free radicals, superoxide radical anions and hydrogen peroxide. At the same time, mitochondrial function fails and the brain's limited stores of antioxidant substances are rapidly exhausted. Reperfusion may actually exacerbate the degree of oxidative stress by repleting oxygen in ischemic tissue. ROS activate MMPs, whose function in broadening the zone of ischemic damage was described previously. In addition to the secondary ischemia that occurs in hemorrhagic strokes, iron released from hemoglobin degradation also catalyzes hydrogen peroxide formation. As many ROS are inherently unstable, biomarkers are necessary as a surrogate method of measuring oxidative stress load. Candidate molecules are those whose chemical structure is altered by oxidation into a new stable entity in a predictable pattern. Oxidative damage occurs via lipid peroxidation, DNA oxidation and protein oxidation. Each of these processes produce markers that can be measured in the serum, along with circulating levels of antioxidants [8,128–130].

Measuring the plasma concentration of various antioxidants is confounded by a few factors. First, an increase in oxidative stress can upregulate the production of antioxidant enzymes, such as members of the superoxide dysmutase family. Second, delivery of peripheral antioxidant molecules is limited owing to impaired circulation and, therefore, the depletion of body stores is unpredictable. Finally, there is wide individual variation in baseline levels of antioxidant activity that may obscure the effects of oxidative stress in an individual case. Stroke patients have been found to have lower levels of retinol, ascorbic acid, uric acid, a-tocopherol and carotenoids, but many studies have demonstrated inconsistent or negative results. Markers of lipid peroxidation include malondialdehyde, thiobarbituric acid-reactive substances and F2-isoprostanes. Of these three groups, F2-isoprostanes have the most favorable chemical characteristics for use as a reliable marker, but clinical studies thus far have not produced definitive results [131]. For DNA oxidation, the measurement of 8-hydroxy-2-deoxy-guanosine demonstrates promise [132]. There is limited literature on biomarkers of protein oxidation, but measuring the carbonyl content of IgG has been proposed as feasible [128].

Ischemia-modified albumin—Albumin is the most abundant protein found in plasma, functioning as a nonspecific carrier molecule and maintaining oncotic pressure. In the context of ischemia, structural changes occur at the N-terminus of the protein, possibly as a result of exposure to ROS. This change in the albumin protein is detected by a cobalt-binding test. Over the last decade, increased ischemia-modified albumin concentration has been linked to acute myocardial ischemia, limb ischemia, mesenteric ischemia and deep venous thrombosis [133–136]. Serum levels of ischemia-modified albumin are increased in subjects with both ischemic and hemorrhagic stroke compared with controls at baseline [137].

Nucleoside diphosphate kinase A & *PARK7*—Nucleoside diphosphate kinase A (NDKA) and *PARK7* (also called *DJ-1*) were selected as potential biomarkers of stroke by screening samples of postmortem CSF for markers that are not found in the CSF of healthy controls. *PARK7* is a redox-sensitive molecular chaperone activated in the context of oxidative stress. NDKA is a kinase that catalyzes the transfer of the terminal phosphate from ATP to nucleotides. Using plasma samples from several cohorts, one team of investigators demonstrated that concentrations of NDKA and *PARK7 were* both increased within 3 h of stroke, with higher sensitivities and specificities than those typically observed using other individual stroke biomarkers [138]. These findings await validation. Furthermore, neither of

these markers are specific to the CNS and both have been implicated in the pathogenesis of neurodegenerative disorders, which may complicate their application.

Polyamine metabolites—Polyamines complex with RNA and facilitate translation. The polyamine spermine has been evaluated in acute stroke, together with spermine oxidase (SMO) and acetylpolyamine oxidase (AcPAO), enzymes that catalyze its degradation, and protein-conjugated acrolein, one of its metabolites. Following acute stroke, plasma levels of spermine decrease in tandem with an increase in SMO, AcPAO and acrolein. Researchers have hypothesized that SMO and AcPAO are released from CNS tissue following stroke and catalyze the degradation of spermine into acrolein in the periphery. Unfortunately, the sensitivity and specificity of all these markers is poor compared with controls, and levels of these markers are altered by other conditions, such as renal dysfunction [139].

Ubiquitin fusion degradation protein & glutathione S-transferase P—Ubiquitin fusion degradation protein (UFDP) is an enzyme in the ubiquitin degradation pathway that is expressed in many body tissue types. UFDP has been tested as a stroke biomarker in several cohorts along with glutathione S-transferase P, a molecule involved in detoxifying ROS. The sensitivity of UFDP ranged from 62 to 95%, but only when the cutoff levels were adjusted by a factor of 8 to optimize the outcome [140,141]. Tested in a single cohort, glutathione S-transferase P1 was 72% sensitive [141].

Limitations & applications

The introduction of a new biomarker has the potential to profoundly revolutionize the field of stroke if it targets an appropriate need. The use of the neuroimaging biomarkers applied in TIA is a case in point. The purpose of defining TIA was to identify cases of cerebral ischemia that do not involve tissue infarction. The early definition of TIA included cases whose deficits persisted up to 24 h [142]. Over the last several decades, work using imaging biomarkers identified DWI evidence suggestive of infarction in up to half of patients who were clinically diagnosed with TIA, mostly those with deficits lasting from 1 to 24 h [143,144]. These biomarker data led to a widespread reconsideration of the definition of TIA with a move to employ neuro-imaging evidence for infarction as indicative of stroke, regardless of the resolution of clinical symptoms [145].

Limitations

First, nearly all of the serum biomarkers research that has been reported thus far in the field of stroke is exploratory in nature. As previously noted, some markers have been explored because of their association with known mechanisms of stroke, and others have been selected empirically by screening postmortem CSF or performing comparative proteomic analysis [61,140,146]. A consortium is in place (the Human Brain Proteome Project) to facilitate the identification of potential brain markers with proteomic techniques [63]. At present, serum biomarkers have performed poorly when tested prospectively for use in acute diagnosis [21]. There are several reasons. Although a difference in mean concentration of these markers can be demonstrated between a group of stroke patients versus controls, most demonstrate considerable overlap in their distribution. Significant heterogeneity exists in the reference cutoffs used and between the populations that these markers are tested in, even between different cohorts evaluated by the same research group [41,140,141]. In some studies, a cutoff was selected to set specificity at 100% based on a small set of controls since the normal distribution of the marker had not yet been established [39]. When a marker is used as a diagnostic test, a diagnosis is made only when the level is less than or greater than the range that encompasses 95% of normal individuals. Therefore, a clear statistical difference does not translate into a high sensitivity test when the mean levels of the exposed and unexposed

populations both lie within the normal range [147]. Many biomarkers of stroke rise slowly after the event. Owing to the delayed change, they are not useful in detecting a stroke in an emergency department setting, and furthermore, only subacute levels correlate well with other outcome measures [18,21].

The heterogeneity of the causes of stroke further complicates interpretation. As with the diagnosis of stroke, other correlations between biomarker levels and severity of outcomes are generated by demonstrating a difference in mean levels between groups with differing clinical status. What is true for a population may not be accurate on an individual level. A patient with infectious endocarditis may have very high elevations in inflammatory and cytokine markers despite having a small infarction, for instance, which would confound the normal correlation with infarct size. Further confounding evidence exists owing to the many other comorbid processes that can alter CNS biomarker levels, including migraine, seizures, neurometabolic disorders, TBI, chronic microvascular white matter disease and, possibly, dementia – a list that encompasses many of the important stroke mimics to be excluded by a diagnostic test [27, 148–150].

The biomarker characteristics of the molecules reviewed here cannot be interpreted without recognizing the complex role they play in their highly regulated systems. The biology of IL-6 is a good example. Elevated levels of IL-6 have been correlated with both an increased and decreased risk for worse outcome in various studies [91,92,94–96]. This is because the signaling outcome of IL-6 depends on the milieu of other cytokines and the cellular state, exerting either a proinflamamtory or anti-inflammatory effect. Changes that take place in the acute to subacute period can also affect biomarker levels but otherwise may not be apparent, making it difficiult to impossible to control for those effects. NAA levels, for example, can quickly spike owing to reperfusion, but it is frequently not possible to know whether, or at what point, reperfusion has occurred with respect to when a sample was drawn. Perhaps the most critical confounder of biomarker concentrations is the effect on the BBB.

The blood–brain barrier effect

Biomarker concentration shifts are generally more pronounced in the CSF, which receives cellular degradation products directly, than the serum. CSF sampling avoids the confounding effect of the BBB, but is not practical for routine use when reliable clinical and imaging markers exist for diagnosis and prognosis. Even in the CSF, many of the described biomarkers demonstrate age dependency for the range of normal reference values [150,151]. The BBB plays an important role in mediating the release of neuronal and glial cellular constituents to the systemic circulation. In tissues outside the CNS, products of cellular degradation are either swept directly into circulation or are spilt into the interstitium and pass into circulation via lymphatic channels. By contrast, the unique interaction of astrocytic foot processes (or glia limitans) and endothelial cells seal the CNS from systemic circulation exposure by a series of tight junctions. This prevents the passive movement of cellular constituents from the CNS into circulation. A similar barrier exists between the CSF and circulation in the choroid plexus [152]. Alterations in the integrity of the BBB can alter the ability of CNS molecules to enter circulation. In instances such as stroke, the same pathological processes that damage neurons and glia disrupt the BBB, causing release of cellular constituents and their passage into systemic circulation. A recent study of BBB function after stroke demonstrated that lacunar stroke patients had widespread BBB disruption, indicating that the relationship between BBB and infarct size is not proportional [153]. The BBB dysfunction may be due to the stroke or may be baseline dysfunction, as patients with white matter disease (leukoaraiosis) are known to have disrupted BBB function [27]. Since the extent of BBB integrity and extent of brain tissue damage are not strictly correlated, serum concentrations of CNS biomarkers may not correlate well to the rate of biomarker release within the CSF or the absolute CSF concentration [43].

The dependency of serum biomarker levels on BBB function was directly demonstrated in a study of patients who underwent BBB disruption with intra-arterial mannitol [154]. A mathematical model to account for potential BBB effects has been proposed for S100B, but the model was based on one sample set of patients and is not applicable to other markers [26]. Biomarkers can pass into circulation even when the BBB is fully intact. All neuronal biomarkers except B-FABP have been detected in the serum of normal individuals. The ability of various molecules to pass through an uncompromised BBB may be mediated, in part, by molecular weight and solubility, and the movement of some markers into serum appears to be unrelated to BBB function [155,156]. Interpreting serum biomarker concentrations is challenging as perturbations may represent BBB dysfunction alone, concurrent BBB and tissue damage or substantial tissue damage with good preservation of BBB integrity.

Attempts at using molecular markers to estimate the integrity of the BBB have typically required the sampling of the CSF, although the serum level of the monomeric form of transthyretin, a protein produced by cells in the choroid plexus, may be useful [155,156]. The benefit of transthyretin is that it is not expressed in neurons or astrocytes and, therefore, may be independent of parenchymal brain damage.

Application of biomarkers for diagnosis & prognosis

To consider the potential applications of biomarkers to stroke, refer to the various potential biomarker applications that are summarized in Box 2. Several well-studied biomarkers are nonspecifc CNS constituents and others, such as NDKA and *PARK7*, have been selected without regard to pathways of ischemic damage. By contrast, some of the markers that have been studied were selected from knowledge of their cellular function. Research that has clearly demonstrated associations between these molecules and the occurrence of stroke has clarified and validated hypotheses regarding the pathways of cellular damage in stroke. Monitoring the activity of molecules known or suspected to be involved in cellular injury has been shown to be a valid method of exploring pathological mechanisms. The utility of biomarkers in delineating and confirming pathological molecular pathways will ensure they continue to be a resource in stroke research.

Several potential clinical applications have been considered – facilitating diagnosis, identifying stroke mechanisms, characterizing size and clinical severity, identifying an ischemic penumbra and estimating risk of progression or worsening. Many molecular markers have been associated with brain ischemia, but at present they offer little additional information on an individual basis beyond that gained from standard clinical evaluation and neuroimaging. From a diagnostic perspective, a major current challenge is to identify a way in which these markers can add value to existing routinely obtained data. Some neuroimaging markers in stroke face similar obstacles in gaining widespread application. Many correlations have been drawn between imaging parameters (e.g., diffusion- and perfusion-weighted imaging volumes, degree of leukoaraiosis and location) and outcomes (e.g., length of hospital stay, long-term disability, odds of infarct progression and clinical severity) [157–162]. Despite these many detailed associations, the clinical evaluation of the patient is still more informative [163–165]. There have been several attempts to address these limitations by developing more complex models that measure up to 50 markers simultaneously. Sets of four to five of the most predictive markers from these multimarker studies have achieved sensitivity and specificity up to just over 90% [166–168]. The addition of a few basic demographic data (age, sex and presence of atrial fibrillation) or initial computed tomography results slightly improves performance [168]. The strategy in these studies was to combine sensitive, but nontissue-specific, markers with at least one marker specific to the CNS. The use of nonspecific biomarkers may be necessary to achieve useful sensitivity. Furthermore, biomarker testing in one of the studies was performed on a point-of-care platform that is feasible in an emergency department setting [168].

It is unlikely that a single biomarker or panel of markers will supplant the current method of diagnosing stroke by a clinical and radiographic approach. Established imaging techniques also continue to be superior at characterizing infarct and penumbra size. MRI equipment is increasingly available at community hospitals. The most promising application for stroke biomarkers is in realms where current clinical capabilities are limited – in accurately determining stroke etiology, in predicting response to treatment, in individualizing treatment and in monitoring treatment effects. One study utilizing a combination of inflammatory, hemostatic, neural and apoptotic biomarkers (CRP, D-dimer, soluble receptor for advanced glycation end products, MMP-9, S100B, brain natriuretic peptide, neurotrophin-3, caspase-3, chimerin and secretagogin) found that it was useful in differentiating cardioembolic from other types of strokes [169]. Given the correlation between the serum levels and genetic polymorphisms of hemostatic modulators, such as TAFI and PAI-1, and tPA recanalization, including the association between several biomarkers (NSE, TAFI, PAI-1, D-dimer and MMP-9) and the risk for secondary hemorrhagic transformation, better selection of thrombolysis candidates and individualization of thrombolytic doses may be possible [170, 171].

Finally, if the correlations between serum biomarkers of stroke and patient outcome become more firmly established, those markers should be used as surrogate outcome measures. Serum stroke biomarkers have already been used to monitor efficacy in acute several stroke drug trials. Two trials studying the effects of simvastatin in acute ischemic stroke and vasospasm in aneurysmal subarachnoid hemorrhage used panels of biomarkers as surrogate markers of ischemic injury, and a trial of arundic acid, a modulator of astrocyte activation, used the astrocyte-associated marker S100B as a surrogate marker of neurological deficit [98,172, 173]. New US FDA rules allow for drug approval based on one large Phase III trial with supportive evidence from a Phase IIB trial demonstrating an effect on a relevant biomarker [174]. As a result of the enormous expense and complexity of conducting a clinical trial in acute stroke, the use of well-established biomarkers could have a significant impact in drug development and approval. Aside from the need for more firmly established correlations between biomarkers and outcomes, the crucial impediment to using biomarkers in clinical trials is adjusting for the effects of the confounders, as discussed in the limitations section previously – systemic inflammation, natural variability in levels and comorbid processes that activate glia or damage the CNS. Furthermore, biomarker levels generally correlate to the volume of CNS damage, whereas stroke-related disability is a function of many factors – volume, localization, age, comorbidities and psychosocial determinants. To date, since most biomarker research has been exploratory in nature, the lack of rigor in study designs has impeded the development of biomarkers as outcome measures.

Conclusion

In a recent commentary on the use of biomarkers in stroke, Hill posed two “key clinical questions in the acute phase:

- Is this a stroke or not?
- If this is a stroke, is this ischemia or hemorrhage?” [146].

Perhaps more important is the question, what value does this test add beyond the clinical examination and neuroimaging? Serum biomarkers related to the cascade of inflammatory, hemostatic, glial and neuronal perturbations have been identified to diagnose and characterize ICH and cerebral ischemia. Interpretation of most markers is confounded by their latent rise, BBB effects, the heterogeneity of conditions that cause stroke and the wide range of normal values. In the realm of stroke diagnosis, no serum biomarker or panel of markers has been proven to add to the data obtained through the history, physical examination and imaging.

Associations have been identified between biomarker concentrations and lesion size, clinical severity, worsening due to infarct progression and secondary hemorrhage and long-term outcomes. Animal-based research and human trials that have used biomarkers of stroke to identify therapeutic targets and monitor response to treatment demonstrate promising potential applications.

Future perspective

Given the widespread availability of advanced neuroimaging technology and the many factors that confound interpretation of serum biomarker levels, stroke diagnosis may not be the most relevant application for molecular markers at present. Intriguing correlations between hemostatic and inflammatory markers and clinical outcomes, such as response to thrombolysis, infarct progression and secondary hemorrhage, offer an opportunity to use biomarkers in the acute setting to individualize treatment selection and dosing. Biomarkers of inflammation and tissue disruption have become useful in developing a more complete understanding of the pathophysiology of stroke. New therapies have already been tested in animals to modulate the tissue response to injury. Relevant markers will continue to play an important role in developing targets for neuroprotective therapies. Finally, biomarkers may play an important role in monitoring response to treatment and as surrogate end points for treatment trials. A transition from the current exploratory phase to achieve further integration of biomarkers into clinical care will require large-scale, well-designed, prospective research to yield well-controlled outcome associations.

Executive summary

Stroke etiologies & the range of biomarkers

- Stroke is a heterogeneous condition, the end result of many diverse pathological conditions that can lead to intracerebral hemorrhage or obstruction of arterial blood flow.
- Many forms of biomarkers have been established for stroke: physical findings, neuroimaging, cardiac imaging, electrophysiological, histological, genetic and neuronal and systemic molecules found in serum or cerebrospinal fluid.
- Potential applications of stroke biomarkers include clarifying pathological mechanisms, facilitating diagnosis, identifying stroke mechanisms, characterizing size and clinical severity, identifying significant penumbra, estimating risk of progression/worsening, selecting appropriate treatment options, predicting the likelihood of response, monitoring treatment efficacy and estimating long-term prognosis.

Specific biomarkers & their correlations

- Cellular constituents from neurons and glia enter the systemic circulation after brain injury. Many markers have been correlated to infarct size, clinical severity and long-term outcomes.
- The hemostatic axis is altered in the context of stroke. Levels of particular constituents predict likelihood of recanalization after thrombolysis.
- Inflammatory cytokines are implicated in propagating damage and causing apoptosis in cells on the periphery of ischemic and hemorrhagic lesions. High levels of proinflamamtory cytokines and low levels of anti-inflammatory cytokines are associated with infarct progression and clinical worsening.

- Enzymes involved in tissue destruction after stroke are measurable in serum, along with their metabolites. Elevated levels are linked to greater risk for clinical deterioration and secondary hemorrhage.
- Multiple nonspecific and acute-phase reactant molecules have been identified as markers of acute stroke. Inclusion of these nonspecific, yet sensitive, markers in a panel of serum tests can increase the sensitivity of the test when biomarkers are used for the diagnosis of stroke.

Limitations

- Virtually all biomarkers of stroke, whether CNS-specific or nonspecific, are present in the serum of normal individuals over a wide range of concentrations. Although mean levels among individuals with stroke are different than in normals, there is considerable overlap in the distribution of values.
- The blood–brain barrier plays a role in modulating serum levels of stroke biomarkers in an unpredictable way.
- Significant latency exists in between the onset of stroke and changes in serum biomarkers.

Applications & future perspective

- In the realms of early diagnosis, lesion-size estimation and long-term outcome prediction, no serum biomarker or panel of markers has been proven to add to the data obtained through history, physical examination and imaging.
- Correlations between hemostatic and inflammatory biomarkers and response to thrombolysis, infarct progression and secondary hemorrhage demonstrate a role for biomarkers in treatment selection and individualization of therapy.
- Animal studies and clinical trials have begun using biomarkers. Biomarkers will become increasingly relevant for developing targets for neuroprotective therapies, monitoring response to treatment and as surrogate end points for treatment trials.
- Further well-designed trials are required to delineate the associations between these markers and clinical outcomes.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics — 2008 update: a report from the american heart association statistics committee and stroke statistics subcommittee. Circulation 2008;117(4):e25–e146. [PubMed: 18086926]
2. Goldstein LB, Adams R, Alberts MJ, et al. Primary prevention of ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council: cosponsored by the Atherosclerotic Peripheral Vascular Disease Interdisciplinary Working Group; Cardiovascular Nursing Council; Clinical Cardiology Council; Nutrition, Physical Activity, and Metabolism Council; and the Quality of Care and Outcomes Research Interdisciplinary Working Group: the American Academy of Neurology affrms the value of this guideline. Stroke 2006;37(6):1583–1633. [PubMed: 16675728]
3. Sacco RL, Adams R, Albers G, et al. Guidelines for prevention of stroke in patients with ischemic stroke or transient ischemic attack: a statement for healthcare professionals from the American Heart

Association/American Stroke Association Council on Stroke: co-sponsored by the Council on Cardiovascular Radiology and Intervention: the American Academy of Neurology affrms the value of this guideline. Stroke 2006;37(2):577–617. [PubMed: 16432246]

4. Adams RJ, Albers G, Alberts MJ, et al. Update to the AHA/ASA recommendations for the prevention of stroke in patients with stroke and transient ischemic attack. Stroke 2008;39(5):1647–1652. [PubMed: 18322260]
5. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined — a consensus document of the joint european society of Cardiology/ American college of cardiology committee for the redefinition of myocardial infarction. J. Am. Coll. Cardiol 2000;36(3):959–969. [PubMed: 10987628]
6. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 1999;22(9):391–397. [PubMed: 10441299]
7. Lee JM, Zipfel GJ, Choi DW. The changing landscape of ischaemic brain injury mechanisms. Nature 1999;399(6738):A7–A14. [PubMed: 10392575]
8. Schaller B, Graf R. Cerebral ischemia and reperfusion: the pathophysiologic concept as a basis for clinical therapy. J. Cereb. Blood Flow Metab 2004;24(4):351–371. [PubMed: 15087705]
9. Doyle KP, Simon RP, Stenzel-Poore MP. Mechanisms of ischemic brain damage. Neuropharmacology 2008;55(3):310–318. [PubMed: 18308346]
10. Hallenbeck JM. The many faces of tumor necrosis factor in stroke. Nat. Med 2002;8(12):1363–1368. [PubMed: 12457181] Review of cytokine-mediated injury in stroke, with a focus on the role of TNF
11. Mun-Bryce S, Rosenberg GA. Matrix metalloproteinases in cerebrovascular disease. J. Cereb. Blood Flow Metab 1998;18(11):1163–1172. [PubMed: 9809504] Comprehensive discussion of tissue damage mechanisms due to hemorrhagic and ischemic stroke, with a focus on the role of metalloproteinases
12. Rose JC, Mayer SA. Optimizing blood pressure in neurological emergencies. Neurocrit. Care 2004;1 (3):287–299. [PubMed: 16174926]
13. Adhami F, Liao G, Morozov YM, et al. Cerebral ischemia-hypoxia induces intravascular coagulation and autophagy. Am. J. Pathol 2006;169(2):566–583. [PubMed: 16877357]
14. Kögel D, Peters M, König HG, et al. S100B potently activates p65/c-rel transcriptional complexes in hippocampal neurons: clinical implications for the role of S100B in excitotoxic brain injury. Neuroscience 2004;127(4):913–920. [PubMed: 15312903]
15. Rothermundt M, Peters M, Prehn JH, Arolt V. S100B in brain damage and neurodegeneration. Microsc. Res. Tech 2003;60(6):614–632. [PubMed: 12645009]
16. Foerch C, Otto B, Singer OC, et al. Serum S100B predicts a malignant course of infarction in patients with acute middle cerebral artery occlusion. Stroke 2004;35(9):2160–2164. [PubMed: 15297628]
17. Persson L, Hardemark H, Edner G, Ronne E, Mendel-Hartvig I, Pahlman S. S-100 protein in cerebrospinal fluid of patients with subarachnoid haemorrhage: a potential marker of brain damage. Acta Neurochir. (Wien) 1988;93(3–4):116–122. [PubMed: 3177026]
18. Jauch EC, Lindsell C, Broderick J, et al. Association of serial biochemical markers with acute ischemic stroke: the National Institute of Neurological Disorders and Stroke recombinant tissue plasminogen activator stroke study. Stroke 2006;37(10):2508–2513. [PubMed: 16960091]
19. Foerch C, Wunderlich MT, Dvorak F, et al. Elevated serum S100B levels indicate a higher risk of hemorrhagic transformation after thrombolytic therapy in acute stroke. Stroke 2007;38(9):2491–2495. [PubMed: 17673718]
20. Delgado P, Alvarez-Sabín J, Santamarina E, et al. Plasma S100B level after acute spontaneous intracerebral hemorrhage. Stroke 2006;37(11):2837–2839. [PubMed: 17008613]
21. Hill MD, Jackowski G, Bayer N, Lawrence M, Jaeschke R. Biochemical markers in acute ischemic stroke. CMAJ 2000;162(8):1139–1140. [PubMed: 10789628]
22. Anderson RE, Hansson LO, Nilsson O, Dijlai-Merzoug R, Settergren G. High serum S100B levels for trauma patients without head injuries. Neurosurgery 2001;48(6):1255–1258. discussion 1258–1260. [PubMed: 11383727]
23. Fazio V, Bhudia SK, Marchi N, Aumayr B, Janigro D. Peripheral detection of S100β during cardiothoracic surgery: what are we really measuring? Ann. Thorac. Surg 2004;78(1):46–43. [PubMed: 15223400]

24. Anderson RE, Hansson LO, Nilsson O, Liska J, Settergren G, Vaage J. Increase in serum S100A1-B and S100BB during cardiac surgery arises from extracerebral sources. Ann. Thorac. Surg 2001;71 (5):1512–1517. [PubMed: 11383792]
25. Rainer TH, Wong KS, Lam W, Lam NY, Graham CA, Lo YM. Comparison of plasma β-globin DNA and S-100 protein concentrations in acute stroke. Clin. Chim. Acta 2007;376(1–2):190–196. [PubMed: 17027951]
26. Marchi N, Cavaglia M, Fazio V, Bhudia S, Hallene K, Janigro D. Peripheral markers of blood-brain barrier damage. Clin. Chim. Acta 2004;342(1–2):1–12. [PubMed: 15026262] Detailed discussion of the role of the blood–brain barrier and other confounding dynamics affecting measurements of serum biomarkers of brain injury
27. Vogelbaum MA, Masaryk T, Mazzone P, et al. S100β as a predictor of brain metastases: brain versus cerebrovascular damage. Cancer 2005;104(4):817–824. [PubMed: 15971200]
28. Pelinka LE, Kroepfl A, Schmidhammer R, et al. Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma. J. Trauma 2004;57(5):1006–1012. [PubMed: 15580024]
29. Aurell A, Rosengren LE, Karlsson B, Olsson JE, Zbornikova V, Haglid KG. Determination of S-100 and glial fibrillary acidic protein concentrations in cerebrospinal fluid after brain infarction. Stroke 1991;22(10):1254–1258. [PubMed: 1926235]
30. Herrmann M, Vos P, Wunderlich MT, de Bruijn C, Lamers KJ. 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):2670–2677. [PubMed: 11062293]
31. Foerch C, Curdt I, Yan B, et al. Serum glial fibrillary acidic protein as a biomarker for intracerebral haemorrhage in patients with acute stroke. J. Neurol. Neurosurg. Psychiatr 2006;77(2):181–184. [PubMed: 16174653]
32. Cohen SR, Brooks BR, Herndon RM, McKhann GM. A diagnostic index of active demyelination: myelin basic protein in cerebrospinal fluid. Ann. Neurol 1980;8(1):25–31. [PubMed: 6157350]
33. Matias-Guiu J, Martinez-Vazquez J, Ruibal A, Colomer R, Boada M, Codina A. Myelin basic protein and creatine kinase BB isoenzyme as CSF markers of intracranial tumors and stroke. Acta Neurol. Scand 1986;73(5):461–465. [PubMed: 2425541]
34. Veerkamp JH, Zimmerman AW. Fatty acid-binding proteins of nervous tissue. J. Mol. Neurosci 2001;16(2–3):133–137. [PubMed: 11478368]
35. Wunderlich MT, Hanhoff T, Goertler M, et al. Release of brain-type and heart-type fatty acid-binding proteins in serum after acute ischaemic stroke. J. Neurol 2005;252(6):718–724. [PubMed: 15834650]
36. Pelsers MM, Hanhoff T, Van dV, et al. Brain- and heart-type fatty acid-binding proteins in the brain: tissue distribution and clinical utility. Clin. Chem 2004;50(9):1568–1575. [PubMed: 15217991]
37. Liao J, Chan CP, Cheung YC, et al. Human heart-type fatty acid-binding protein for on-site diagnosis of early acute myocardial infarction. Int. J. Cardiol 2009;133(3):420–423. [PubMed: 18571749]
38. Zimmerman AW, van Moerkerk H, Veerkamp JH. Ligand specificity and conformational stability of human fatty acid-binding proteins. Int. J. Biochem. Cell Biol 2001;33(9):865–876. [PubMed: 11461829]
39. Zimmermann-Ivol CG, Burkhard PR, Le Floch-RohrJ, Allard L, Hochstrasser DF, Sanchez JC. Fatty acid binding protein as a serum marker for the early diagnosis of stroke: a pilot study. Mol. Cell. Proteomics 2004;3(1):66–72. [PubMed: 14581522]
40. Persson L, Hardemark HG, Gustafsson J, et al. S-100 protein and neuron-specific enolase in cerebrospinal fluid and serum: markers of cell damage in human central nervous system. Stroke 1987;18(5):911–918. [PubMed: 3629651]
41. Anand N, Stead LG. Neuron-specific enolase as a marker for acute ischemic stroke: a systematic review. Cerebrovasc. Dis 2005;20(4):213–219. [PubMed: 16123539] Review of neuron-specific enolase in stroke, including a good portrayal of the difficulty in trying to develop a coherent sense of a marker's usefulness, based on many small exploratory studies with disparate methods and findings
42. Fassbender K, Schmidt R, Schreiner A, et al. Leakage of brain-originated proteins in peripheral blood: temporal profile and diagnostic value in early ischemic stroke. J. Neurol. Sci 1997;148(1):101–105. [PubMed: 9125396]

43. Casmiro M, Maitan S, De Pasquale F, et al. Cerebrospinal fluid and serum neuron-specific enolase concentrations in a normal population. Eur. J. Neurol 2005;12(5):369–374. [PubMed: 15804267]
44. Wunderlich MT, Lins H, Skalej M, Wallesch CW, Goertler M. Neuron-specific enolase and tau protein as neurobiochemical markers of neuronal damage are related to early clinical course and long-term outcome in acute ischemic stroke. Clin. Neurol. Neurosurg 2006;108(6):558–563. [PubMed: 16457947]
45. Stevens H, Jakobs C, de Jager A, Cunningham RT, Korf J. Neurone-specific enolase and N-acetyl-aspartate as potential peripheral markers of ischaemic stroke. Eur. J. Clin. Invest 1999;29(1):6–11. [PubMed: 10092982]
46. Williams DR. Tauopathies: classification and clinical update on neurodegenerative diseases associated with microtubule-associated protein tau. Intern. Med. J 2006;36(10):652–660. [PubMed: 16958643]
47. Hesse C, Rosengren L, Vanmechelen E, et al. Cerebrospinal fluid markers for Alzheimer's disease evaluated after acute ischemic stroke. J. Alzheimers Dis 2000;2(3–4):199–206. [PubMed: 12214084]
48. Hesse C, Rosengren L, Andreasen N, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neurosci. Lett 2001;297(3):187–190. [PubMed: 11137759]
49. Lidstrom AM, Hesse C, Rosengren L, Fredman P, Davidsson P, Blennow K. Normal levels of clusterin in cerebrospinal fluid in Alzheimer's disease, and no change after acute ischemic stroke. J. Alzheimers Dis 2001;3(5):435–442. [PubMed: 12214028]
50. Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelsø C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J. Neurochem 1996;67(5):2013–2018. [PubMed: 8863508]
51. Baslow MH. Evidence supporting a role for N-acetyl-l-aspartate as a molecular water pump in myelinated neurons in the central nervous system. An analytical review. Neurochem. Int 2002;40(4): 295–300.
52. Petroff OA, Graham GD, Blamire AM, et al. Spectroscopic imaging of stroke in humans: histopathology correlates of spectral changes. Neurology 1992;42(7):1349–1354. [PubMed: 1620345]
53. Sager TN, Laursen H, Hansen AJ. Changes in N-acetyl-aspartate content during focal and global brain ischemia of the rat. J. Cereb. Blood Flow Metab 1995;15(4):639–646. [PubMed: 7790413]
54. Sager TN, Hansen AJ, Laursen H. Correlation between N-acetylaspartate levels and histopathologic changes in cortical infarcts of mice after middle cerebral artery occlusion. J. Cereb. Blood Flow Metab 2000;20(5):780–788. [PubMed: 10826528]
55. Pereira AC, Saunders DE, Doyle VL, et al. Measurement of initial N-acetyl aspartate concentration by magnetic resonance spectroscopy and initial infarct volume by MRI predicts outcome in patients with middle cerebral artery territory infarction. Stroke 1999;30(8):1577–1582. [PubMed: 10436104]
56. Saunders DE. MR spectroscopy in stroke. Br. Med. Bull 2000;56(2):334–345. [PubMed: 11092084]
57. Walker PM, Ben Salem D, Lalande A, Giroud M, Brunotte F. Time course of NAA T_2 and ADC(w) in ischaemic stroke patients: ^{1}H MRS imaging and diffusion-weighted MRI. J. Neurol. Sci 2004;220 (1–2):23–28. [PubMed: 15140601]
58. Elting JW, Sulter GA, Langedijk M, Luijckx GJ, Teelken AW, De Keyser J. N-acetylaspartate: serum marker of reperfusion in ischemic stroke. J. Stroke Cerebrovasc. Dis 2004;13(6):254–258. [PubMed: 17903983]
59. Dambinova SA, Khounteev GA, Skoromets AA. Multiple panel of biomarkers for TIA/stroke evaluation. Stroke 2002;33(5):1181–1182. [PubMed: 11988587]
60. Dambinova SA, Khounteev GA, Izykenova GA, Zavolokov IG, Ilyukhina AY, Skoromets AA. 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):1752–1762. [PubMed: 14500616]
61. Allard L, Lescuyer P, Burgess J, et al. ApoC-I and ApoC-III as potential plasmatic markers to distinguish between ischemic and hemorrhagic stroke. Proteomics 2004;4(8):2242–2251. [PubMed: 15274118]

62. Dai FF, Zhang Y, Kang Y, et al. The neuronal Ca^{2+} sensor protein visinin-like protein-1 is expressed in pancreatic islets and regulates insulin secretion. J. Biol. Chem 2006;281(31):21942–21953. [PubMed: 16731532]
63. Laterza OF, Modur VR, Crimmins DL, et al. Identification of novel brain biomarkers. Clin. Chem 2006;52(9):1713–1721. [PubMed: 16858073]
64. Tran ND, Wong VL, Schreiber SS, Bready JV, Fisher M. Regulation of brain capillary endothelial thrombomodulin mRNA expression. Stroke 1996;27(12):2304–2301. [PubMed: 8969798]
65. Olivot JM, Labreuche J, De Broucker T, et al. Thrombomodulin gene polymorphisms in brain infarction and mortality after stroke. J. Neurol 2008;255(4):514–519. [PubMed: 18204921]
66. Olivot JM, Labreuche J, Aiach M, Amarenco P, Investigators G. Soluble thrombomodulin and brain infarction: case–control and prospective study. Stroke 2004;35(8):1946–1951. [PubMed: 15192246]
67. Nomura E, Kohriyama T, Kozuka K, Kajikawa H, Nakamura S, Matsumoto M. Significance of serum soluble thrombomodulin level in acute cerebral infarction. Eur. J. Neurol 2004;11(5):329–334. [PubMed: 15142226]
68. Kozuka K, Kohriyama T, Nomura E, Ikeda J, Kajikawa H, Nakamura S. Endothelial markers and adhesion molecules in acute ischemic stroke – sequential change and differences in stroke subtype. Atherosclerosis 2002;161(1):161–168. [PubMed: 11882328]
69. Bushnell C, Siddiqi Z, Morgenlander J, Goldstein L. Use of specialized coagulation testing in the evaluation of patients with acute ischemic stroke. Neurology 2001;56(5):624–627. [PubMed: 11245714]
70. Bushnell C, Siddiqi Z, Goldstein L. Improving patient selection for coagulopathy testing in the setting of acute ischemic stroke. Neurology 2001;57(7):1333–1335. [PubMed: 11591863]
71. Levine SR, Brey RL, Tilley BC, et al. Antiphospholipid antibodies and subsequent thrombo-occlusive events in patients with ischemic stroke. JAMA 2004;291(5):576–584. [PubMed: 14762036]
72. Tapson VF. Acute pulmonary embolism. N. Engl. J. Med 2008;358(10):1037–1052. [PubMed: 18322285]
73. Beamer NB, Coull BM, Clark WM, Hazel JS, Silberger JR. Interleukin-6 and interleukin-1 receptor antagonist in acute stroke. Ann. Neurol 1995;37(6):800–805. [PubMed: 7778854]
74. Di Napoli M, Papa F, Bocola V. Prognostic influence of increased C-reactive protein and fibrinogen levels in ischemic stroke. Stroke 2001;32(1):133–138. [PubMed: 11136928]
75. Di Napoli M, Singh P. Is plasma fibrinogen useful in evaluating ischemic stroke patients?. why, how, and when. Stroke 2009;40(5):1549–1552. [PubMed: 19299637]
76. Barber M, Langhorne P, Rumley A, Lowe GD, Stott DJ. d-dimer predicts early clinical progression. Stroke 2004;35(6):1421–1425. [PubMed: 15073383]
77. Barber M, Langhorne P, Rumley A, Lowe GD, Stott DJ. d-dimer predicts early clinical progression in ischemic stroke: confirmation using routine clinical assays. Stroke 2006;37(4):1113–1115. [PubMed: 16527998]
78. Delgado P, Alvarez-Sabín J, Abilleira S, et al. Plasma d-dimer predicts poor outcome after acute intracerebral hemorrhage. Neurology 2006;67(1):94–98. [PubMed: 16832084]
79. Welsh P, Barber M, Langhorne P, Rumley A, Lowe GD, Stott DJ. Associations of inflammatory and haemostatic biomarkers with poor outcome in acute ischaemic stroke. Cerebrovasc. Dis 2009;27(3): 247–253. [PubMed: 19176958]
80. Feinberg WM, Erickson LP, Bruck D, Kittelson J. Hemostatic markers in acute ischemic stroke. association with stroke type, severity, and outcome. Stroke 1996;27(8):1296–1300.
81. Carter AM, Catto AJ, Mansfield MW, Bamford JM, Grant PJ. Predictive variables for mortality after acute ischemic stroke. Stroke 2007;38(6):1873–1880. [PubMed: 17446429]
82. Fon EA, Mackey A, Côté R, et al. Hemostatic markers in acute transient ischemic attacks. Stroke 1994;25(2):282–286. [PubMed: 8303732]
83. Carrieri P, Orefice G, Indaco A. No effect of acetylsalicylic acid on B-thromboglobulin and platelet factor 4 plasma levels in patients with transient ischaemic attacks. Stroke 1986;17(6):1153–1155. [PubMed: 2949396]
84. Shah AB, Beamer N, Coull BM. Enhanced *in vivo* platelet activation in subtypes of ischemic stroke. Stroke 1985;16(4):643–647. [PubMed: 3161220]

85. Kang DW, Yoo SH, Chun S, et al. Inflammatory and hemostatic biomarkers associated with early recurrent ischemic lesions in acute ischemic stroke. Stroke 2009;40(5):1653–1658. [PubMed: 19265045]
86. Haapaniemi E, Tatlisumak T. Is d-dimer helpful in evaluating stroke patients? A systematic review. Acta Neurol. Scand 2009;119(3):141–150. [PubMed: 18705677]
87. Ay H, Arsava EM, Gungor L, et al. Admission international normalized ratio and acute infarct volume in ischemic stroke. Ann. Neurol 2008;64(5):499–506. [PubMed: 18661516]
88. Montaner J, Ribó M, Monasterio J, Molina CA, Alvarez-Sabín J. Thrombin-activable fibrinolysis inhibitor levels in the acute phase of ischemic stroke. Stroke 2003;34(4):1038–1040. [PubMed: 12649517]
89. Arenillas JF, Alvarez-Sabín J, Molina CA, et al. Progression of symptomatic intracranial large artery atherosclerosis is associated with a proinflammatory state and impaired fibrinolysis. Stroke 2008;39 (5):1456–1463. [PubMed: 18323504]
90. Ribó M, Montaner J, Molina CA, Arenillas JF, Santamarina E, Alvarez-Sabín J. Admission fibrinolytic profile predicts clot lysis resistance in stroke patients treated with tissue plasminogen activator. Thromb. Haemost 2004;91(6):1146–1151. [PubMed: 15175801]
91. Ribó M, Montaner J, Molina CA, et al. Admission fibrinolytic profile is associated with symptomatic hemorrhagic transformation in stroke patients treated with tissue plasminogen activator. Stroke 2004;35(9):2123–2127. [PubMed: 15243150]
92. Fernandez-Cadenas I, Alvarez-Sabín J, Ribo M, et al. Influence of thrombin-activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 gene polymorphisms on tissue-type plasminogen activator-induced recanalization in ischemic stroke patients. J. Thromb. Haemost 2007;5(9):1862–1868. [PubMed: 17723126]
93. Emsley HC, Smith CJ, Gavin CM, et al. Clinical outcome following acute ischaemic stroke relates to both activation and autoregulatory inhibition of cytokine production. BMC Neurol 2007;7:5. [PubMed: 17328808]
94. Kim JS, Yoon SS, Kim YH, Ryu JS. Serial measurement of interleukin-6, transforming growth factor-b, and S-100 protein in patients with acute stroke. Stroke 1996;27(9):1553–1557. [PubMed: 8784129]
95. Montaner J, Alvarez-Sabín J, Barberá G, et al. Correlation between the expression of proinflammatory cytokines and matrix metalloproteinases in the acute phase of an ischemic stroke. Rev. Neurol 2001;33(2):115–118. [PubMed: 11562868]
96. Dziedzic T, Bartus S, Klimkowicz A, Motyl M, Slowik A, Szczudlik A. Intracerebral hemorrhage triggers interleukin-6 and interleukin-10 release in blood. Stroke 2002;33(9):2334–2335. [PubMed: 12215608]
97. Montaner J, Rovira A, Molina CA, et al. Plasmatic level of neuroinflammatory markers predict the extent of diffusion-weighted image lesions in hyperacute stroke. J. Cereb. Blood Flow Metab 2003;23 (12):1403–1407. [PubMed: 14663335]
98. Montaner J, Chacón P, Krupinski J, et al. Simvastatin in the acute phase of ischemic stroke: a safety and efficacy pilot trial. Eur. J. Neurol 2008;15(1):82–90. [PubMed: 18070096]
99. Emsley HC, Smith CJ, Gavin CM, et al. An early and sustained peripheral inflammatory response in acute ischaemic stroke: relationships with infection and atherosclerosis. J. Neuroimmunol 2003;139 (1–2):93–101. [PubMed: 12799026]
100. Sotgiu S, Zanda B, Marchetti B, et al. Inflammatory biomarkers in blood of patients with acute brain ischemia. Eur. J. Neurol 2006;13(5):505–513. [PubMed: 16722977]
101. Sotgiu S, Barone R, Zanda B, et al. Chitotriosidase in patients with acute ischemic stroke. Eur. Neurol 2005;54(3):149–153. [PubMed: 16319488]
102. Vila N, Castillo J, Dávalos A, Chamorro A. Proinflammatory cytokines and early neurological worsening in ischemic stroke. Stroke 2000;31(10):2325–2329. [PubMed: 11022058]
103. Vila N, Castillo J, Dávalos A, Esteve A, Planas AM, Chamorro A. Levels of anti-inflammatory cytokines and neurological worsening in acute ischemic stroke. Stroke 2003;34(3):671–675. [PubMed: 12624290]
104. Smith CJ, Emsley HC, Gavin CM, et al. Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome. BMC Neurol 2004;4:2. [PubMed: 14725719]

105. Qizilbash N, Lewington SL, Lopez-Arrieta JM. Corticosteroids for acute ischaemic stroke. Cochrane Database Syst. Rev 2002;2(2) CD000064.

106. Casas JP, Shah T, Hingorani AD, Danesh J, Pepys MB. C-reactive protein and coronary heart disease: a critical review. J. Intern. Med 2008;264(4):295–314. [PubMed: 18823504]

107. Kuhlmann CR, Librizzi L, Closhen D, et al. Mechanisms of C-reactive protein-induced blood-brain barrier disruption. Stroke 2009;40(4):1458–1466. [PubMed: 19246692]

108. Rost NS, Wolf PA, Kase CS, et al. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. Stroke 2001;32(11):2575–2579. [PubMed: 11692019]

109. Montaner J, Fernandez-Cadenas I, Molina CA, et al. Poststroke C-reactive protein is a powerful prognostic tool among candidates for thrombolysis. Stroke 2006;37(5):1205–1210. [PubMed: 16601211]

110. Arenillas JF, Alvarez-Sabín J, Molina CA, et al. C-reactive protein predicts further ischemic events in first-ever transient ischemic attack or stroke patients with intracranial large-artery occlusive disease. Stroke 2003;34(10):2463–2468. [PubMed: 14500938]

111. Purroy F, Montaner J, Molina CA, et al. C-reactive protein predicts further ischemic events in transient ischemic attack patients. Acta Neurol. Scand 2007;115(1):60–66. [PubMed: 17156267]

112. Rosell A, Ortega-Aznar A, Alvarez-Sabán J, et al. Increased brain expression of matrix metalloproteinase-9 after ischemic and hemorrhagic human stroke. Stroke 2006;37(6):1399–1406. [PubMed: 16690896]

113. Abilleira S, Montaner J, Molina CA, Monasterio J, Castillo J, Alvarez-Sabín J. Matrix metalloproteinase-9 concentration after spontaneous intracerebral hemorrhage. J. Neurosurg 2003;99(1):65–70. [PubMed: 12854746]

114. Montaner J, Alvarez-Sabín J, Molina C, et al. Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke 2001;32(8): 1759–1766. [PubMed: 11486102]

115. Montaner J, Alvarez-Sabín J, Molina CA, et al. Matrix metalloproteinase expression is related to hemorrhagic transformation after cardioembolic stroke. Stroke 2001;32(12):2762–2767. [PubMed: 11739970]

116. Montaner J, Molina CA, Monasterio J, et al. Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. Circulation 2003;107 (4):598–603. [PubMed: 12566373]

117. Ning M, Furie KL, Koroshetz WJ, et al. Association between tPA therapy and raised early matrix metalloproteinase-9 in acute stroke. Neurology 2006;66(10):1550–1555. [PubMed: 16717217]

118. Castellanos M, Sobrino T, Millan M, et al. Serum cellular fibronectin and matrix metalloproteinase-9 as screening biomarkers for the prediction of parenchymal hematoma after thrombolytic therapy in acute ischemic stroke: a multicenter confirmatory study. Stroke 2007;38(6):1855–1859. [PubMed: 17478737]

119. Rosell A, Alvarez-Sabín J, Arenillas JF, et al. A matrix metalloproteinase protein array reveals a strong relation between MMP-9 and MMP-13 with diffusion-weighted image lesion increase in human stroke. Stroke 2005;36(7):1415–1420. [PubMed: 15947272]

120. Alvarez-Sabín J, Delgado P, Abilleira S, et al. Temporal profile of matrix metalloproteinases and their inhibitors after spontaneous intracerebral hemorrhage: relationship to clinical and radiological outcome. Stroke 2004;35(6):1316–1322. [PubMed: 15087562]

121. Rabuffetti M, Sciorati C, Tarozzo G, Clementi E, Manfredi AA, Beltramo M. Inhibition of caspase-1-like activity by Ac-Tyr-Val-Ala-Asp-chloromethyl ketone induces long-lasting neuroprotection in cerebral ischemia through apoptosis reduction and decrease of proinflammatory cytokines. J. Neurosci 2000;20(12):4398–4404. [PubMed: 10844008]

122. Cohen GM. Caspases: the executioners of apoptosis. Biochem. J 1997;326(Pt 1):1–16. [PubMed: 9337844]

123. Bratton SB, MacFarlane M, Cain K, Cohen GM. Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. Exp. Cell Res 2000;256(1):27–33. [PubMed: 10739648]

124. Broughton BR, Reutens DC, Sobey CG. Apoptotic mechanisms after cerebral ischemia. Stroke 2009;40(5):e331–e339. [PubMed: 19182083]

125. Rosell A, Cuadrado E, Alvarez-Sabín J, et al. Caspase-3 is related to infarct growth after human ischemic stroke. Neurosci. Lett 2008;430(1):1–6. [PubMed: 18055116]

126. Delgado P, Cuadrado E, Rosell A, et al. Fas system activation in perihematomal areas after spontaneous intracerebral hemorrhage. Stroke 2008;39(6):1730–1734. [PubMed: 18403741]

127. Rainer TH, Wong LK, Lam W, et al. Prognostic use of circulating plasma nucleic acid concentrations in patients with acute stroke. Clin. Chem 2003;49(4):562–569. [PubMed: 12651807]

128. Cherubini A, Ruggiero C, Polidori MC, Mecocci P. Potential markers of oxidative stress in stroke. Free Radic. Biol. Med 2005;39(7):841–852. [PubMed: 16140205] Comprehensive review of oxidative stress biomarkers in stroke

129. Heistad DD, Wakisaka Y, Miller J, Chu Y, Pena-Silva R. Novel aspects of oxidative stress in cardiovascular diseases. Circ. J 2009;73(2):201–207. [PubMed: 19110503]

130. Alexandrova M, Bochev P, Markova V, et al. Dynamics of free radical processes in acute ischemic stroke: influence on neurological status and outcome. J. Clin. Neurosci 2004;11(5):501–506. [PubMed: 15177393]

131. Kelly PJ, Morrow JD, Ning M, et al. Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke: the biomarker evaluation for antioxidant therapies in stroke (BEAT-stroke) study. Stroke 2008;39(1):100–104. [PubMed: 18063832]

132. Liu H, Uno M, Kitazato KT, et al. Peripheral oxidative biomarkers constitute a valuable indicator of the severity of oxidative brain damage in acute cerebral infarction. Brain Res 2004;1025(1–2): 43–50. [PubMed: 15464743]

133. Sinha MK, Gaze DC, Tippins JR, Collinson PO, Kaski JC. Ischemia modified albumin is a sensitive marker of myocardial ischemia after percutaneous coronary intervention. Circulation 2003;107(19): 2403–2405. [PubMed: 12742986]

134. Gunduz A, Mentese A, Turedi S, et al. Serum ischaemia-modified albumin increases in critical lower limb ischaemia. Emerg. Med. J 2008;25(6):351–353. [PubMed: 18499817]

135. Gunduz A, Turedi S, Mentese A, et al. Ischemia-modified albumin in the diagnosis of acute mesenteric ischemia: a preliminary study. Am. J. Emerg. Med 2008;26(2):202–205. [PubMed: 18272103]

136. Mentese A, Mentese U, Turedi S, et al. Effect of deep vein thrombosis on ischaemia-modified albumin levels. Emerg. Med. J 2008;25(12):811–814. [PubMed: 19033496]

137. Abboud H, Labreuche J, Meseguer E, et al. Ischemia-modified albumin in acute stroke. Cerebrovasc Dis 2007;23(2–3):216–220. [PubMed: 17143006]

138. Allard L, Burkhard PR, Lescuyer P, et al. PARK7 and nucleoside diphosphate kinase A as plasma markers for the early diagnosis of stroke. Clin. Chem 2005;51(11):2043–2051. [PubMed: 16141287]

139. Tomitori H, Usui T, Saeki N, et al. Polyamine oxidase and acrolein as novel biochemical markers for diagnosis of cerebral stroke. Stroke 2005;36(12):2609–2613. [PubMed: 16269634]

140. Allard L, Turck N, Burkhard PR, et al. Ubiquitin fusion degradation protein 1 as a blood marker for the early diagnosis of ischemic stroke. Biomark. Insights 2007;2:155–164. [PubMed: 19662200]

141. Turck N, Allard L, Walter N, et al. GSTP1 and UFD1 as early blood biomarkers of acute stroke. Cerebrovasc. Dis 2006;21:125.

142. A classification and outline of cerebrovascular diseases. II. Stroke 1975;6:564–616. [PubMed: 1179466]

143. Kidwell CS, Alger JR, Di Salle F, et al. Diffusion MRI in patients with transient ischemic attacks. Stroke 1999;30(6):1174–1180. [PubMed: 10356095]

144. Engelter ST, Provenzale JM, Petrella JR, Alberts MJ. Diffusion MR imaging and transient ischemic attacks. Stroke 1999;30(12):2762–2763. [PubMed: 10583010]

145. Albers GW, Caplan LR, Easton JD, et al. Transient ischemic attack - proposal for a new definition. N. Engl. J. Med 2002;347(21):1713–1716. [PubMed: 12444191]

146. Hill MD. Diagnostic biomarkers for stroke: a stroke neurologist's perspective. Clin. Chem 2005;51 (11):2001–2002. [PubMed: 16244286]

147. Whiteley W, Tseng MC, Sandercock P. Blood biomarkers in the diagnosis of ischemic stroke: a systematic review. Stroke 2008;39(10):2902–2909. [PubMed: 18658039] Review of several important serum biomarkers applied to stroke diagnosis, including studies of multiple concurrent markers, with sensitivity and specificity calculated for many studies

148. Noppe M, Crols R, Andries D, Lowenthal A. Determination in human cerebrospinal fluid of glial fibrillary acidic protein, S-100 and myelin basic protein as indices of non-specific or specific central nervous tissue pathology. Clin. Chim. Acta 1986;155(2):143–150. [PubMed: 2421949]

149. Lamers KJ, van Engelen BG, Gabreels FJ, Hommes OR, Borm GF, Wevers RA. Cerebrospinal neuron-specific enolase, S-100 and myelin basic protein in neurological disorders. Acta Neurol. Scand 1995;92(3):247–251. [PubMed: 7484080]

150. Lamers KJ, Vos P, Verbeek MM, Rosmalen F, van Geel WJ, van Engelen BG. Protein S-100B, neuron-specific enolase (NSE), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP) in cerebrospinal fluid (CSF) and blood of neurological patients. Brain Res. Bull 2003;61 (3):261–264. [PubMed: 12909296]

151. van Engelen BG, Lamers KJ, Gabreels FJ, Wevers RA, van Geel WJ, Borm GF. Age-related changes of neuron-specific enolase, S-100 protein, and myelin basic protein concentrations in cerebrospinal fluid. Clin. Chem 1992;38(6):813–816. [PubMed: 1375875]

152. Segal MB. The choroid plexuses and the barriers between the blood and the cerebrospinal fluid. Cell Mol. Neurobiol 2000;20(2):183–196. [PubMed: 10696509]

153. Wardlaw JM, Doubal F, Armitage P, et al. Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. Ann. Neurol 2009;65(2):194–202. [PubMed: 19260033]

154. Marchi N, Rasmussen P, Kapural M, et al. Peripheral markers of brain damage and blood-brain barrier dysfunction. Restor. Neurol. Neurosci 2003;21(3–4):109–121. [PubMed: 14530574]

155. Anderson RE, Winnerkvist A, Hansson LO, et al. Biochemical markers of cerebrospinal ischemia after repair of aneurysms of the descending and thoracoabdominal aorta. J. Cardiothorac. Vasc. Anesth 2003;17(5):598–603. [PubMed: 14579213]

156. Marchi N, Fazio V, Cucullo L, et al. Serum transthyretin monomer as a possible marker of blood-to-CSF barrier disruption. J. Neurosci 2003;23(5):1949–1955. [PubMed: 12629200]

157. Engelter ST, Provenzale JM, Petrella JR, DeLong DM, Alberts MJ. Infarct volume on apparent diffusion coefficient maps correlates with length of stay and outcome after middle cerebral artery stroke. Cerebrovasc. Dis 2003;15(3):188–191. [PubMed: 12646778]

158. Ay H, Koroshetz WJ, Vangel M, et al. Conversion of ischemic brain tissue into infarction increases with age. Stroke 2005;36(12):2632–2636. [PubMed: 16269639]

159. Ay H, Arsava EM, Rosand J, et al. Severity of leukoaraiosis and susceptibility to infarct growth in acute stroke. Stroke 2008;39(5):1409–1413. [PubMed: 18340093]

160. Wu O, Koroshetz WJ, Ostergaard L, et al. Predicting tissue outcome in acute human cerebral ischemia using combined diffusion-and perfusion-weighted MR imaging. Stroke 2001;32(4):933–942. [PubMed: 11283394]

161. Beaulieu C, de Crespigny A, Tong DC, Moseley ME, Albers GW, Marks MP. Longitudinal magnetic resonance imaging study of perfusion and diffusion in stroke: evolution of lesion volume and correlation with clinical outcome. Ann. Neurol 1999;46(4):568–578. [PubMed: 10514093]

162. Menezes NM, Ay H, Wang Zhu M, et al. The real estate factor: quantifying the impact of infarct location on stroke severity. Stroke 2007;38(1):194–197. [PubMed: 17122428]

163. Thijs VN, Lansberg MG, Beaulieu C, Marks MP, Moseley ME, Albers GW. Is early ischemic lesion volume on diffusion-weighted imaging an independent predictor of stroke outcome? A multivariable analysis. Stroke 2000;31(11):2597–2602. [PubMed: 11062281]

164. Schiemanck SK, Kwakkel G, Post MW, Kappelle LJ, Prevo AJ. Predicting long-term independency in activities of daily living after middle cerebral artery stroke: does information from MRI have added predictive value compared with clinical information? Stroke 2006;37(4):1050–1054. [PubMed: 16497980]

165. Schiemanck SK, Kwakkel G, Post MW, Prevo AJ. Predictive value of ischemic lesion volume assessed with magnetic resonance imaging for neurological deficits and functional outcome poststroke: a critical review of the literature. Neurorehabil. Neural Repair 2006;20(4):492–502. [PubMed: 17082505]

166. Lynch JR, Blessing R, White WD, Grocott HP, Newman MF, Laskowitz DT. Novel diagnostic test for acute stroke. Stroke 2004;35(1):57–63. [PubMed: 14671250]

167. Reynolds MA, Kirchick HJ, Dahlen JR, et al. Early biomarkers of stroke. Clin. Chem 2003;49(10): 1733–1739. [PubMed: 14500614]

168. Laskowitz DT, Kasner SE, Saver J, Remmel KS, Jauch EC. BRAIN Study Group: Clinical usefulness of a biomarker-based diagnostic test for acute stroke: the biomarker rapid assessment in ischemic injury (BRAIN) study. Stroke 2009;40(1):77–85. [PubMed: 18948614] Recent study employing a panel of serum biomarkers to maximize diagnostic sensitivity for ischemic stroke

169. Montaner J, Perea-Gainza M, Delgado P, et al. Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. Stroke 2008;39(8):2280–2287. [PubMed: 18535284]

170. Castellanos M, Serena J. Applicability of biomarkers in ischemic stroke. Cerebrovasc. Dis 2007;24:7–15. [PubMed: 17971634] Descriptive review of the various applications of biomarkers in ischemic stroke, covering a limited subset of exemplifying markers

171. Montaner J. Stroke biomarkers: can they help us to guide stroke thrombolysis? Timely Top. Med. Cardiovasc. Dis 2007;11:E11. [PubMed: 17473898]

172. Lynch JR, Wang H, McGirt MJ, et al. Simvastatin reduces vasospasm after aneurysmal subarachnoid hemorrhage: Results of a pilot randomized clinical trial. Stroke 2005;36(9):2024–2026. [PubMed: 16051891]

173. Pettigrew LC, Kasner SE, Gorman M, et al. Effect of arundic acid on serum S-100β in ischemic stroke. J. Neurol. Sci 2006;251(1–2):57–61. [PubMed: 17092520]

174. Fisher M, Cheung K, Howard G, Warach S. New pathways for evaluating potential acute stroke therapies. Int. J. Stroke 2006;1(2):52–58. [PubMed: 18706045]

Table 1

Summary of evidence for correlations of biomarkers in stroke.

Biomarker type	CNS*	Isch v ICH‡	Early Dx§	Late Dx¶	TIA Dx#	Size**	Severity*‡	Worsening*§	Prognosis*¶
Neuronal									
S100-β	+	−	− (M)	+		+	+	+	+
Neuron-specific enolase	−	−	−	+	+	+/−	+/−		+/−
Glial fibrillary-associated protein	+	+/−	−	+		+	+		+
Heart fatty acid-binding protein	−	−	+/−	+	−	+	+		+
Brain fatty acid-binding protein	+	−	+/−	+		−	−		−
Tau protein	+	−	−	+/−		+	+		+
Hyperphosphorylated tau protein	+	−	−	−		−	−	−	−
Myelin basic protein	+	−	−	+/−		+	+		+
Neurofilament light protein	+	−		+/−					
N-acetyl aspartate	+	−	+	+		−	−		−
N-methyl-D-aspartate NR2 subunit autoantibodies	+	+	+	+	+	+	+		
Visinin-like protein 1	+	−		+/−					
B-type neurotrophic growth factor			(M)	+			+		
Creatine kinase-BB isoenzyme	−								
Hemostatic									
Thrombomodulin	−	−	−	+		−	+/−		+
Thrombomodulin polymorphisms	−	NA	NA	NA		−			−
D-dimer	−	−	+ (M)	+	+	+	+	+	+
Fibrinogen	−	−	+	+		+	+	+	+
Fibrinopeptide A	−	−			+				+
β-thromboglobulin	−	−		+	+/−				+
Prothrombin fragments 1 & 2	−	−						+	
Thrombin–antithrombin complex	−	−			+			+	
von Willibrand factor	−	−	(M)	+				+	+
Platelet factor 4	−	−		+	+/−				
Thrombin-activable fibrinolysis inhibitor	−	−		+				+	

Biomarker type	CNS*	Isch v ICH‡	Early Dx§	Late Dx¶	TIA Dx#	Size**	Severity*‡	Worsening*§	Prognosis*¶
Plasminogen activator inhibitor-1	–	–							
Thrombin-activable fibrinolysis inhibitor polymorphisms	NA	NA	NA	NA					
Plasminogen activator inhibitor-1 polymorphisms	NA	NA	NA	NA					
International normalized ratio	NA					+	+		+
Cytokine									
IL-1	–	–	–	–		–	–	–	–
IL-4	–	–						–	
IL-6	–	–		+		+	+	+	+
IL-8	–	–		–		–			–
IL-10	–	–	–	+/–		–	+/–	+	–
IL-13	–	–							
TGF-β	–	–		+					
ICAM-1	–	–		+		+	+	+	
VCAM-1	–	–	(M)						
E-selectin	–	–		+					
P-selectin	–	–		+					
L-selectin	–	–							
TNF-α	–	–	–	+		+	+	+	+
Chitotriosidase	–	–		+		+	+		
Monocyte chemoattractant protein-1	–	–	(M)	+/–					
IL-1-receptor antagonist	–	–	+	+		+			–
Infammatory phase reactants									
C-reactive protein	–	–	+	+				+	+
Erythrocyte sedimentation rate	–	–	–	+					+
Tissue destruction									
Matrix metalloproteinase-1	–	–		–					
Matrix metalloproteinase-2	–	–		+/–		+/–	+/–	–	
Matrix metalloproteinase-3	–	–				+/–			
Matrix metalloproteinase-9	–	–	+ (M)	+		+	+	+	+

Biomarker type	CNS*	Isch v ICH‡	Early Dx§	Late Dx¶	TIA Dx#	Size**	Severity*‡	Worsening*§	Prognosis*¶
Matrix metalloproteinase-13	–	–		+				+	
Tissue inhibitor of metalloproteinase-1	–	–				+/–			
Tissue inhibitor of metalloproteinase-2	–	–				–			
Caspase-3	–	–	–	–			+	+	+
Soluble Fas	–	–	+	–				+	
DNA	–	+	+	+		+	+		+
Oxidative stress									
Superoxide dysmutases	–	–	–	+/–		+	+	+/–	+
Retinol	–	–	–	+				+	+
Ascorbic acid	–	–	–	+			+	+	
Uric acid	–	–	–	+		+/–		+	+/–
α-tocopherol	–	–	–	–			+	–	
Carotenoids	–	–	–	+				+/–	
Malondialdehyde	–	–		+		+	+		+
Thiobarbituric acid-reactive substances	–	–		+					
F2-isoprostanes	–	–		+/–		–	–		–
8-hydroxy-2-deoxy-guanosine	–	–		+/–				+/–	
Protein carbonyls (including IgG)	–	–		+/–					
3-nitrotyrosine	–	–		+/–					
Miscellaneous									
Ischemia-modifed albumin	–	–	+	+			+		
Nucleoside diphosphate kinase A	–	–	+	+	+				
PARK7 (*DJ-1*)	–	–	+	+	+				
Apo CI	–	+	–	–		–	–		–
Apo CIII	–	+	–	–		–	–		–
B-type natriuretic peptide			(M)						
Spermine	–			+		+			
Spermine oxidase	–			+		+			
Acetylpolyamine oxidase	–			+		+			

Biomarker type	CNS*	Isch v ICH‡	Early Dx§	Late Dx¶	TIA Dx#	Size**	Severity*‡	Worsening*§	Prognosis*¶
Protein-conjugated acrolein	−			+		+			
Ubiquitin fusion degradation protein	−			+					
Glutathione S transferase P	−			+					

*Specific to CNS tissue

‡Differentiate Isch stroke versus ICH;

§Marker potentially useful for early Dx;

¶Marker potentially useful for subacute Dx;

#Marker potentially useful for transient Isch attacks Dx;

**Correlated to stroke volume;

*‡Correlated to clinical severity;

*§Correlated to presence of a significant Isch penumbra or with objective clinical or radiographic worsening

*¶Correlated with long-term outcomes.

+: Marker is well correlated for the application; −: Marker is not well correlated for the application; +/−: Evidence is mixed for a correlation for the application; Dx: Diagnosis; ICH: Intracerebral hemorrhage; Isch: Ischemic; M: Marker is well correlated for the application as part of a multiple marker panel; NA: Not applicable; TIA: Transient ischemic attack.