

Biomarkers of Ischemic Stroke

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Abstract

The diagnosis and management of patients with ischemic stroke is primarily based on clinical assessment in conjunction with imaging tests. Development of molecular biomarkers as additional tools to support a clinical diagnosis, identify patients at risk of disease, and help guide patient treatment and prognosis would be of great value. Currently, no such biomarkers are used in the management of patients with ischemic stroke; however, several promising proteomic and genomic markers have been identified, as presented in this review.

Keywords

Biomarker, ischemic stroke, cerebrovascular disease

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Ischemic stroke remains the leading cause of adult disability in developed countries.¹ Although clinical assessment is the key feature of patient management, additional tools such as biomarkers can be used to support a clinical diagnosis, identify patients at risk of disease, and help guide patient treatment and prognosis. In cerebrovascular disease, computed tomography (CT) and magnetic resonance imaging (MRI) are frequently used to confirm clinically suspected disease. However, there are several limitations to neuroimaging in acute ischemic stroke, with the CT brain scan often having subtle findings and MRI not always readily accessible. The limitations of CT and MRI make the development of other supportive biomarkers valuable in the care of patients with acute stroke. Peripheral blood biomarkers are very useful in clinical practice because they are readily available and low-cost, as shown by the use of troponin for myocardial infarction. Currently, no blood test for ischemic stroke is used in clinical practice, although substantial efforts are ongoing to develop such a test. This article presents a summary of this work, focusing primarily on the use of proteomics and genomics to develop a blood-based biomarker of ischemic stroke.

Biomarkers and Pathophysiology

The identification of biomarkers for ischemic stroke stems from a growing understanding of stroke pathophysiology. Initial studies identified biomarkers specific to brain tissue, representing molecules that are released into the systemic circulation following ischemic injury to the brain. Biomarkers relating to the coagulation cascade have been associated with ischemic stroke, representing acute thrombus in cerebral circulation. Inflammation also plays an important role in ischemic stroke. A number of biomarkers relating to atherosclerosis and the inflammatory

response to ischemic brain tissue have been identified. Finally, a number of biomarkers have been associated with ischemic stroke, although their links with its pathophysiology remain unknown.

Classification of Biomarkers Proteomics

Proteomics is the study of the entire complement of proteins, including the modifications made to proteins. Common techniques to identify protein biomarkers include western blot, immunohistochemical staining, enzyme-linked immunosorbent assay, and mass spectrometry (matrix-assisted laser desorption/ionisation analysis). Using these techniques, a number of proteins have been identified as biomarkers of ischemic stroke (see *Table 1*). Protein biomarkers of ischemic brain injury include S100 calcium-binding protein B (S100B), neuron-specific enolase (NSE), myelin basic protein, and glial fibrillary acidic protein (GFAP).²⁻⁴ Brain tissue injury biomarkers are limited by several factors in their use as biomarkers for ischemic stroke. They are not specific to it, as many disease processes can damage brain tissue and cause their release into the systemic circulation. Levels of such biomarkers also depend on the extent of blood-brain barrier breakdown, which is variable between ischemic strokes. Several proteins involved in inflammation have been identified as biomarkers of ischemic stroke. These include C-reactive protein,⁵ interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), vascular cell adhesion molecule 1 (VCAM-1), intercellular cell adhesion molecule 1 (ICAM-1), and matrix metalloproteinases (MMPs).^{6,7} Molecules involved in acute thrombosis have also been associated with ischemic stroke, including fibrinogen,⁸ D-dimer,^{9,10} and von Willebrand factor (vWF).^{11,12} Finally, a number of proteins have been associated with ischemic stroke that as yet do not

have a clear pathophysiological role, including PARK7,¹³ nucleotide diphosphate kinase A,¹³ and B-type neurotrophic growth factor.¹²

Genomics

A new approach for identifying biomarkers of disease has emerged from gene chip technologies. Genomic expression analysis allows quantitative assessment of all genes expressed in a cell, tissue, or blood sample. Similar to protein profiles that have been associated with ischemic stroke, profiles of genes expressed following such an event have been identified as markers of ischemic stroke.^{14–16} Genes can be grouped into expression clusters and upregulated or downregulated clusters in a disease state, allowing the disease to be identified. The changes in blood RNA expression patterns following ischemic stroke primarily reflect the inflammatory response to ischemic brain tissue, including involvement of polymorphonuclear leukocytes, monocytes, and lymphocytes.^{17,18}

Clinical Application of Biomarkers Biomarkers for Diagnosis of Ischemic Stroke

Currently, the diagnosis of ischemic stroke relies on clinical assessment in combination with neuroimaging. A biomarker to aid in the diagnosis of ischemic stroke would be very valuable, particularly to aid rapid triage and referral of patients with acute ischemic stroke to hospitals where acute treatments are available. Although a number of individual molecules have been associated with ischemic stroke, it is likely that a panel of molecules will provide a more reliable test for diagnosis.

To identify such a panel of biomarkers, 44 acute ischemic stroke patients were screened for 26 blood markers.¹¹ Four markers—S100b, vWF, MMP9, and VCAM—were identified that correctly diagnosed ischemic stroke with a sensitivity of 90% and specificity of 90%. This group went on to study >50 protein biomarkers in 82 patients with ischemic stroke.¹² Using a panel of five markers (S100b, vWF, MMP9, B-type neurotrophic growth factor, and MCP-1), ischemic stroke was predicted with a sensitivity of 92% and specificity of 93% when three out of five of the markers were above specified cut-off values. Initial gene expression studies have identified a panel of genes associated with ischemic stroke. In peripheral blood mononuclear cells, 190 genes were found to be regulated in ischemic stroke compared with controls.¹⁹ A panel of 22 genes selected from the 190 enabled diagnosis with a sensitivity of 78% and specificity of 80%. A second study showed that in whole blood samples, 1,335 genes are differentially expressed in ischemic stroke compared with controls.¹⁹ A panel of 18 of the genes from this list was able to correctly diagnose 15 out of 15 ischemic stroke patients at 24 hours from onset.

Despite the differences between these two studies, most of the 190 genes identified in the first study were present in the 1,335 genes identified in the second study. Using a panel of genes that overlapped in the two studies, ischemic stroke was correctly diagnosed in 13 of 15 patients in the Tang et al. study.²⁰ Distinguishing hemorrhagic stroke from a diagnosis of ischemic stroke is critical to the triage and initiation of acute treatment. It is important for the biomarker used for this purpose to be very sensitive and specific, as a common application will guide the use of intravenous recombinant tissue plasminogen activator (rt-PA) therapy. GFAP shows some promise in this regard, with an elevated level able to distinguish hemorrhagic from ischemic stroke six hours after onset with a sensitivity of 79% and specificity of 98%.²¹

Table 1: Summary of Selected Biomarkers Associated with Ischemic Stroke

Biomarker	Description
Ischemic Brain Tissue Biomarkers	
S100B	Astrocyte marker; a calcium-binding protein
GFAP	Astrocyte marker; an intermediate filament protein
NSE	Neuronal marker; a neuronal glycolytic enzyme
MBP	Marker of myelin damage
Inflammatory Biomarkers	
CRP	An acute-phase reactant involved in atherothrombosis and ischemic injury
IL-6	Cytokine involved in acute-phase response and development of fever
TNF- α	Cytokine involved in inflammation and acute-phase response
VCAM-1	Immunoglobulin involved in leukocyte–endothelial cell interaction
ICAM-1	Immunoglobulin involved in leukocyte–endothelial cell interaction
MMP2, MMP9	Proteolytic enzymes that degrade collagen-disrupting endothelium and plaque matrix
Coagulation/Thrombosis Biomarkers	
Fibrinogen	A glycoprotein involved in blood coagulation, platelet activation, and acute-phase reactant
D-dimer	Marker of acute thrombosis; a breakdown product of fibrin blood clot
vWF	A glycoprotein involved in coagulation, platelet adhesion, and factor VIII binding
Other Biomarkers	
Plasma DNA	Marker of lysed ischemic cells
PARK7	An RNA-binding protein; elevated in neurodegenerative disease
NDKA	Transfers phosphate between nucleosides
BNGF	A growth factor involved in neuronal growth and differentiation
Genomics	
Gene expression profiles	Profiles of RNA expressed following ischemic stroke

BNGF = B-type neurotrophic growth factor; CRP = C-reactive protein; MBP = myelin basic protein; NDKA = nucleotide diphosphate kinase A; for all other abbreviations refer to article.

Biomarkers to Determine Ischemic Stroke Etiology

Ischemic stroke can be classified by etiology using TOAST (Trial of ORG 10172 in Acute Stroke Treatment) criteria into cardioembolic, large-vessel, small-vessel, cryptogenic, and other causes. This classification system is unable to determine an etiology of ischemic stroke in as many as 30% of patients. A biomarker could aid in the identification of its etiology and thus allow initiation of preventive therapy targeted to the underlying cause. Elevated levels of brain natriuretic peptide and D-dimer have been associated with cardioembolic stroke, with a positive predictive value of 70%.¹⁰ On its own, D-dimer has also been associated with cardioembolic stroke.^{22,23} Gene expression profiles in blood may also distinguish cardioembolic from large-vessel ischemic stroke. A panel of 23 selected genes was able to differentiate cardioembolic from large-vessel ischemic stroke with >95% sensitivity and specificity.²⁴ Small-vessel ischemic strokes are less frequently studied, although C-reactive protein has been reported to be higher in patients with large-vessel compared with small-vessel ischemic strokes.²⁵

Biomarkers of Final Infarct Volume

A number of biomarkers have been associated with infarct volume, including S100B, MMP, IL-6, TNF- α , ICAM-1, and glutamate. Such markers may be useful in predicting clinical outcome in patients with ischemic stroke and thus aid in selection for recanalisation therapy. However, it should be emphasized that infarct size may not correlate with neurological outcome, since even small infarcts can cause devastating effects when they occur in certain anatomical regions, such as the brainstem. One would predict that a larger infarct volume should lead to increased release of central nervous system tissue biomarkers into the systemic circulation. Missier confirmed this prediction, showing that elevated levels of S100B and NSE were associated with final infarct volume.²⁶ Further studies have identified an association with infarct volume and S100B,^{27,28} NSE,^{28,29} Tau,²⁹ and glutamate.³⁰ A larger infarct volume should also lead to a larger inflammatory response to the ischemic tissue. This is supported by studies showing that increased levels of inflammatory markers are associated with infarct volume, including TNF- α ,⁶ IL-6,^{31,32} ICAM-1,⁶ MMP2, and MMP9.^{6,33}

Biomarkers of Early Neurological Deterioration

Early neurological deterioration (END) following ischemic stroke has been associated with several biomarkers, including glutamate, gamma-aminobutyric acid (GABA), ferritin, TNF- α , ICAM-1, MMP, S100B, and nitric oxide. The identification of patients at risk of END may be of clinical utility by allowing initiation of therapies to prevent such worsening. Elevated glutamate levels are found in the plasma and cerebrospinal fluid of patients who experience END.^{34,35} Glutamate may be associated with END because it is involved in expanding lesion volume and/or an index of cellular lysis. Inflammatory biomarkers have also been associated with END. Elevated levels of plasma ferritin independently predicted END in patients with hemispheric infarcts, although plasma ferritin levels are also associated with glutamate levels.³⁶ Other inflammatory markers associated with END include IL-6,³¹ nitric oxide,³⁷ MMP9, and MMP13.³³

Biomarkers of Malignant Cerebral Infarction

Decompressive hemicraniectomy is an aggressive therapy reserved for patients with large cortical ischemic strokes. Outcomes from this intervention are generally better when hemicraniectomy is performed early.³⁸ A biomarker to identify patients at risk of malignant cerebral infarction may potentially aid in the selection of patients for hemicraniectomy. S100B has been found to predict malignant infarction at both 12 and 24 hours with reasonable sensitivity and specificity.³⁹ Cellular fibronectin and MMP9 levels have been found to predict malignant

cerebral infarction, with cellular fibronectin having a sensitivity of 90% and specificity of 100%.⁴⁰

Biomarkers of Hemorrhagic Transformation

Symptomatic hemorrhagic transformation is a major complication following ischemic stroke. Identification of patients at increased risk of symptomatic hemorrhage could help to reduce the incidence of this complication and allow extension of the time window for rt-PA (Alteplase) administration in selected patients. A number of clinical (age, hypertension, anticoagulant treatment, hyperglycemia) and radiological (diffusion-perfusion mismatch, infarct volume, proximal occlusion, leukoaraiosis) factors have been associated with an increased risk of hemorrhagic transformation. Several biomarkers have also been associated with an increased risk of symptomatic hemorrhage, including MMP9, cellular fibronectin, PAI-1, thrombin-activatable fibrinolysis inhibitor,⁴¹ and S100B.⁴² The most evidence exists for elevated levels of MMPs predicting hemorrhagic transformation following ischemic stroke. MMPs are involved in the destruction of microvascular integrity through degradation of the basal lamina and extracellular matrix.⁴³ Levels of MMP9 have been shown to predict hemorrhagic transformation with variable sensitivity and specificity in ischemic stroke patients who have and have not been treated with rt-PA.⁴⁴⁻⁴⁷ Cellular fibronectin has also been associated with increased hemorrhagic transformation.⁴⁸

Biomarkers of Arterial Recanalization

Recanalization of arterial blood flow is an important predictor of good outcome in acute stroke. A biomarker could potentially be useful in selecting patients for recanalization therapy, such as thrombolysis or mechanical clot disruption. Plasminogen antigen inhibitor 1 is a marker of fibrinolysis and low levels have been associated with resistance to recanalisation with rt-PA.⁴⁹ By contrast, lower levels of α 2-antiplasmin and functional-thrombin activated fibrinolysis inhibitor have been associated with successful recanalization with rt-PA.⁵⁰

Conclusion

A number of blood-based biomarkers of ischemic stroke have been identified and show promise in aiding ischemic stroke diagnosis, staging, and prediction. Many relate to the underlying pathophysiology of ischemic stroke, including ischemic central nervous system tissue, acute thrombosis, and the inflammatory response. With further study and validation, it is likely that several blood biomarkers will serve as useful tools in the care of patients with ischemic stroke. ■

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