

Blood Biomarkers of Ischemic Stroke

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Abstract This review provides a summary of the protein and RNA biomarkers that have been studied for the diagnosis and assessment of ischemic stroke. Many of the biomarkers identified relate to the pathophysiology of ischemic stroke, including ischemia of CNS tissue, acute thrombosis and inflammatory response. These biomarkers are summarized by their intended clinical application in ischemic stroke including diagnosis, prediction of stroke severity and outcome, and stratification of patients for stroke therapy. Among the biomarkers discussed are recent whole genome studies using RNA expression profiles to diagnose ischemic stroke and stroke etiology. Though many candidate blood based biomarkers for ischemic stroke have been identified, none are currently used in clinical practice. With further well designed study and careful validation, the development of blood biomarkers to improve the care of patients with ischemic stroke may be achieved.

Keywords Ischemic stroke · Biomarker

Introduction

Ischemic stroke is a leading cause of disability worldwide [1]. Though clinicians are excellent at assessing stroke and its causes, biomarkers to support a clinical diagnosis of

stroke, identify patients at risk of disease, and guide treatment and prognosis would be valuable [2]. A biomarker may be a molecule measured in blood, CSF, or tissue; a recording such as an EKG or electroencephalograph; or an imaging test. In this review we will focus on blood based biomarkers and their application in ischemic stroke. For a peripheral blood biomarker to be of optimal use, it should be rapid, cost effective, specific and sensitive, as is the case for troponin in the assessment of myocardial infarction. Though a biomarker for ischemic stroke is currently not used in clinical practice, efforts to develop such a test are ongoing. We present a summary of this work, focusing primarily on protein and RNA biomarkers in ischemic stroke. Several recent reviews on biomarkers in ischemic stroke should be consulted for additional details [3–5].

In ischemic stroke, the majority of biomarker work has been performed on single proteins selected because of their known relationship to ischemic stroke pathophysiology. Among these include markers of brain tissue damage, inflammation, endothelium, and coagulation/thrombosis. These studies are summarized in Table 1. Though many such proteins are associated with ischemic stroke, the successful translation to a biomarker useful in clinical practice has proven difficult. Part of the challenge arises from the heterogeneity of ischemic stroke. An alternate method to identify blood biomarkers for ischemic stroke is high throughput screening of many molecules, such as RNA or protein. Though there are associated challenges, whole genome or proteome methods offer the potential to identify markers with sufficient sensitivity and specificity to be of clinical use that would otherwise not be readily identified by our current understanding of stroke pathophysiology. In RNA expression studies, all of the known RNAs can be assessed using whole genome platform to identify predictors of disease. This approach is similar to genome wide association studies (GWAS) in which the entire genome is searched for possible DNA single nucleotide polymorphisms associated with a disorder. Similar whole proteome studies

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Table 1 Survey of reported ischemic stroke biomarkers and their clinical application

| | Biomarker | Origin | Description | Application to Ischemic Stroke |
|-------------------------------------|-----------------------------------|---|---|---|
| CNS Tissue Injury Biomarkers | S100B | Astrocytes | Calcium binding protein, involved in cell cycle progression & differentiation | Diagnosis [19, 102] Infarct volume [48, 49, 103] Stroke severity [48, 74, 103] Hemorrhagic transformation [85] |
| | GFAP | Astrocytes Testis, Liver | Intermediate filament protein, role in cell structure, blood brain barrier, communication | Diagnosis [22, 104] |
| | NSE | Neurons Neuroendocrine neoplasms, uremia | Neuronal glycolytic enzyme | Diagnosis [102] Infarct volume [48] Stroke severity [103] |
| | NMDA-R Ab | Glutamate excitotoxic response | Autoantibodies to the NR2A / NR2B subunits of the NMDA-receptor | Diagnosis [13, 14] Stroke severity [13, 14] |
| | MBP | Myelin damage Oligodendrocytes, Schwann cells | Myelination of CNS>PNS | Diagnosis [104] Infarct volume [103] Stroke severity [103] |
| Inflammatory Biomarkers | CRP | Liver synthesis | Acute phase reactant, role in atherothrombosis & ischemic injury [105] | Diagnosis [106] Stroke risk [62, 107–109] |
| | IL-6 | Cytokine from T-cells+ macrophages | Acute phase response, fever | Infarct volume [53–55, 110] Stroke Severity [110] END [53, 54] |
| | TNF- α | Cytokine, inflammatory cells | Inflammation, Acute phase response | Infarct volume [53] Stroke Severity [53] END [72] |
| | VCAM 1 | Immunoglobulin | Leukocyte-endothelial cell interaction | Diagnosis [19] Infarct volume [53] |
| | ICAM 1 | Immunoglobulin | Leukocyte-endothelial cell interaction | Infarct volume [53] Stroke severity [53] END [72] |
| | MMP2, MMP9 | Gelatinase Proteolytic enzymes | Degrades collage causing endothelial damage, plaque matrix disruption | Diagnosis [19, 20] Infarct volume [53] Stroke severity [53] Hemorrhagic transformation [77–80] |
| | Lp-PLA2 | Inflammatory cells | Hydrolyzes oxidized phospholipids in LDL | Stroke risk [59–63, 111] |
| Coagulation / Thrombosis Biomarkers | ApoC-I ApoC-III | Lipoproteins | Lipid metabolism | Diagnosis [23] |
| | Fibrinogen | Glycoprotein cleaved by thrombin to fibrin | Blood coagulation, platelet activation, acute phase reactant | Stroke risk [112–114] |
| | D-Dimer | Breakdown of fibrin blood clot | Acute thrombosis | Diagnosis [115] Cardioembolic stroke [35] |
| | vWF | Glycoprotein | Coagulation, platelet adhesion, binds factor VIII | Diagnosis [19, 20] |
| Other Biomarkers | Plasma DNA | Marker of lyzed ischemic cells | Sheered DNA | Stroke outcome [116] Infarct volume [116] |
| | PARK7 | Elevated in neurodegenerative disease | RNA binding protein regulatory subunit | Diagnosis [117] |
| | NDKA | | Nucleoside phosphate transfers | Diagnosis [117] |
| | B-type neurotrophic growth factor | Growth factor | Growth and differentiation of neurons | Diagnosis [20] |

are on the horizon and will likely become more common as the technology develops. Such whole genome, whole proteome approaches are advantageous in that many potential biomarker candidates can be screened rapidly, with subsequent focused investigation of target molecules found to be of greatest biomarker promise.

Protein Biomarkers in Ischemic Stroke

Proteomics is the study of the entire complement of proteins, including protein modifications. Common techniques to study protein biomarkers include Western blot, immunohistochemical staining, enzyme linked immunosor-

bent assay (ELISA), 2D gel electrophoresis, and mass spectrometry (matrix assisted laser desorption/ionization analysis). Using these techniques a number of proteins and panels of proteins have been identified as biomarkers of ischemic stroke (Table 1).

Protein biomarkers can be classified by their pathophysiological role in stroke. Markers of ischemic brain injury include S100 calcium binding protein B (S-100B), neuron-specific enolase (NSE), myelin basic protein (MBP), and glial fibrillary acidic protein (GFAP). Brain injury biomarkers are limited by several factors as markers for ischemic stroke. They are not specific to ischemic stroke, as many disease processes can damage brain tissue. The blood brain barrier (BBB) restricts release of these biomarkers into systemic circulation. As a result, biomarker levels may not correlate with infarct volume or stroke severity given that the BBB breakdown is variable between ischemic strokes and the anatomic location of stroke has different clinical impacts.

Several proteins involved in inflammation and immune response have also been identified as biomarkers of ischemic stroke, including C-reactive protein (CRP), interleukin-6 (IL-6), tissue necrosis factor- α (TNF- α), vascular cell adhesion protein 1 (VCAM 1), inter-cellular adhesion molecule 1 (ICAM 1), N-methyl-D-aspartate (NMDA) receptor antibodies and matrix metalloproteinases (MMPs) (Table 1). Similarly, molecules involved in acute thrombosis have also been associated with ischemic stroke, including fibrinogen, D-Dimer and von-Willebrand factor (vWF). Finally, there are a number of proteins that have been associated with ischemic stroke, which as yet do not have a clear pathophysiological role in the disease, including PARK7, nucleotide diphosphate kinase A (NDKA), and B-type neurotrophic growth factor.

Ribonucleic Acid (RNA) Biomarkers in Ischemic Stroke

An approach for identifying RNA biomarkers of disease emerged from gene chip technologies. Gene expression analysis allows quantitative assessment of all genes expressed as RNA in a cell, tissue, or blood. Similar to protein profiles that have been associated with ischemic stroke, profiles of genes expressed in ischemic stroke have also been identified as markers. Genes can be grouped into expression clusters, and up regulated and down regulated clusters can be used to characterize functional pathways associated with stroke.

In animals, a specific gene expression profile has been shown in both brain tissue and blood following ischemic stroke [6–9]. In humans, brain tissue is rarely available. Thus, peripheral blood is used as a source of RNA. The rationale for using peripheral blood is based on the inflammatory response to brain injury that occurs after ischemic stroke. Changes in RNA expression patterns are

observed in the inflammatory cells involved in this response, including polymorphonuclear leukocytes, monocytes, and lymphocytes [10, 11].

Our initial studies of blood in animals showed that the expression of large numbers of genes changed at 24 hours after ischemic strokes, hemorrhagic strokes, status epilepticus, hypoxia and hypoglycemia [7]. There were several important observations that have remained consistent in subsequent human studies. First, there were a large number of genes that differed from each type of injury and controls. Second, no single gene distinguished each type of injury from the others. Finally, a gene expression profile (group or set of genes) was required to distinguish each injury from controls and to distinguish each type of injury from each other. These studies were the first to demonstrate proof of principle that gene expression could differentiate ischemic stroke from a variety of other types of brain injury, including intracerebral hemorrhage [7].

Clinical Application of Biomarkers in Ischemic Stroke

Biomarkers can be classified by their intended clinical application [12]. In ischemic stroke, studies have evaluated biomarkers to distinguish ischemic stroke from stroke mimics, determine stroke etiology, predict stroke severity and outcomes including early neurological deterioration and hemorrhagic complications, and identify patients who may benefit from specific therapies including decompressive hemicraniectomy and arterial recanalization.

Biomarkers for the Diagnosis of Ischemic Stroke

Presently the diagnosis of ischemic stroke relies on clinical assessment in combination with neuroimaging. Physicians are very good at diagnosing stroke, therefore the use of a blood test to diagnose stroke is generally limited to specific scenarios where time and/or imaging resources are limited. In a pre-hospital setting or facilities where acute neuroimaging is not available, a blood test could guide the triage and evaluation of acute ischemic stroke. Though a CT scan will likely be required prior to initiation of thrombolysis, a blood test that rapidly identifies ischemic stroke could speed patient transfer to centers and physicians able to perform evaluation for thrombolysis. Additionally, in a minority of stroke patients the diagnosis of ischemic stroke remains unclear in spite of clinical evaluation and imaging. In such a situation, a blood test could add confidence to a physician's diagnosis of stroke.

Numerous attempts have been made to develop a blood test to diagnose stroke using one or several proteins. In total, over 58 proteins and 7 panels of proteins have been studied as possible biomarkers for the diagnosis of ischemic stroke – which are the subject of three recent reviews [3–5].

In spite of this effort, a blood based biomarker to diagnose stroke remains to be established. A biomarker that showed initial promise was antibodies to the NR2A / NR2B subunits of the glutamate NMDA-receptor (NMDA-R). Elevated levels of this antibody could distinguish ischemic stroke from controls at three hours with 97% sensitivity and 98% specificity [13, 14]. However, NMDA-R antibodies have also been associated with hypertension, atherosclerosis, prior stroke, epilepsy, systemic lupus erythematosus and encephalitis. Thus, the specificity of these antibodies to patients with acute ischemic stroke remains uncertain [15–18].

Panels of proteins also show promise as biomarkers in ischemic stroke. A panel of four markers (S100B, vWF, MMP9 and VCAM) were able to distinguish ischemic stroke from controls with a 90% sensitivity and specificity [19]. When a similar panel of 5 markers (S100B, vWF, MMP9, BDNF and MCP-1) was used, ischemic stroke could distinguish from healthy controls with 92% sensitivity and 93% specificity [20]. However, the clinical problem is not whether an ischemic stroke can be distinguished from a healthy control, but whether an ischemic stroke can be distinguished from disease that mimics stroke such as hemorrhagic stroke, seizure, migraine, syncope or hypoglycemia [21]. Making this distinction tends to be more challenging as many of the diseases that mimic stroke can also influence markers studied. Of significant importance is distinguishing ischemic stroke from hemorrhagic stroke, due to the implications in acute thrombolytic therapy. In 135 patients, GFAP was able to distinguish ischemic from hemorrhagic stroke with 79% sensitivity and 98% specificity

within 6 hours of symptom onset [22]. In a study of 31 patients, apolipoprotein CI and apolipoprotein CIII were shown to distinguish hemorrhagic from ischemic stroke with 94% sensitivity and 87% specificity [23]. In a larger study of 1146 patients, a 4 panel marker (S100B, MMP9, D-dimer and brain natriuretic factor) was able to distinguish ischemic stroke from stroke mimics including hemorrhagic stroke with 85% sensitivity; however, specificity was only 34% [24]. Thus, though ischemic stroke can be identified reasonably well, many non-ischemic stroke patients are also incorrectly predicted to be ischemic stroke. The challenge of developing a biomarker of sufficient sensitivity and specificity for clinical applications is further demonstrated in an 8 panel biomarker study. Though the panel could identify patients with ischemic stroke, the diagnostic performance did not add to that of an emergency department physician [25].

Whether high throughput screen techniques will identify blood biomarkers to improve the sensitivity and specificity of current biomarkers remains to be determined. Preliminary studies of RNA expression in blood have identified panels of genes able to distinguish ischemic stroke from controls. RNA isolated from blood mononuclear cells identified 190 genes differentially expressed in ischemic stroke compared to controls. A 22 gene panel of the 190 genes was able to distinguish ischemic stroke from controls with 78% sensitivity and 80% specificity [26]. A subsequent study of whole blood RNA identified 1335 genes differentially expressed in acute ischemic stroke compared to controls [27]. The majority of these genes

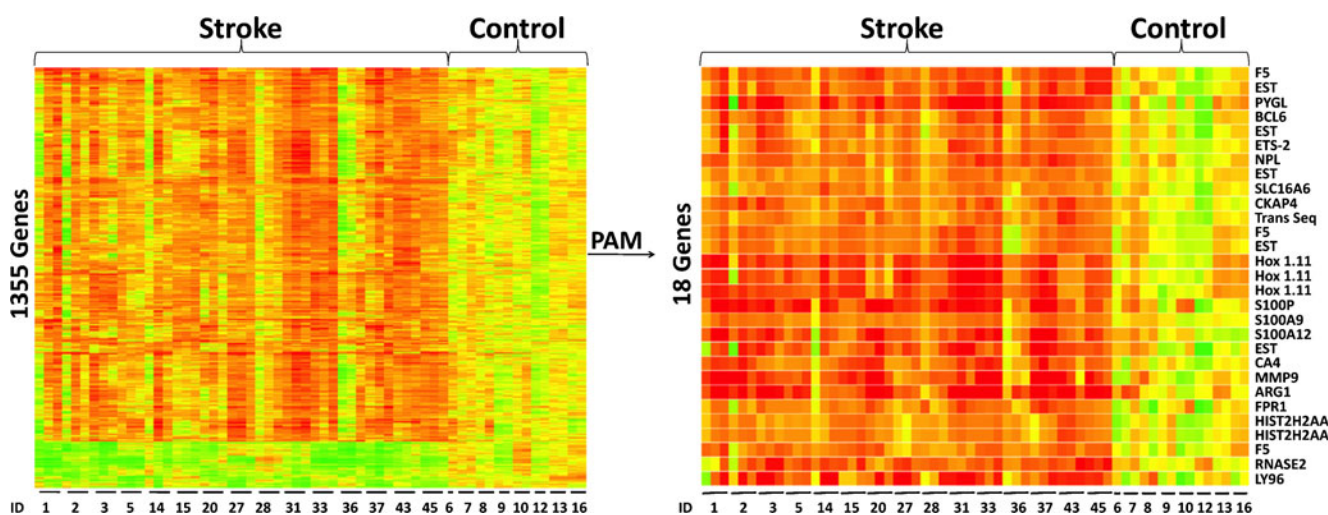


Fig. 1 Gene expression profiles to distinguish ischemic stroke from controls at <3, 5 and 24 hours after stroke onset. Hierarchical cluster plot of the 1355 genes found to be differentially expressed between ischemic stroke and controls (FDR0.05, fold change >|1.2|). Genes are shown on the Y-axis, and patients are shown on the X-axis. Genes that have a high level of expression are shown in red, and genes with a low level of expression are shown in green. From the larger list of 1355 genes, a list

of 18 genes (29 probesets) were identified that optimally discriminate between ischemic stroke and controls. This 18 gene panel could distinguish ischemic stroke from control with 89% sensitivity and 100% specificity. This 18 gene profile has subsequently been studied in a larger set of stroke patients and demonstrated similar sensitivity and specificity (Table 2). This figure is adapted from Tang Y et al. *Annals of Neurology*, 2006, pages 1089–1102 [27]

were expressed in neutrophils and monocytes. An 18 gene panel of the 1335 genes was able to distinguish ischemic stroke from controls subjects in all patients at 24 hours (Fig. 1). More recent RNA expression studies report similar findings to these first two studies. A study by Barr et al. described a panel of 9 genes to distinguish stroke from controls [28]. Despite use of a different microarray platform (Illumina versus Affymetrix arrays), 5 of the 9 genes had been previously identified [27, 28]. Thus, these results represent the first validation of a gene expression study in ischemic stroke. Further validation has been obtained in for the 18 gene panel previously reported by Tang et al. [29]. This 18 gene panel predicted a new set of ischemic strokes (n=70, 199 samples) with 93.5% sensitivity and 89.5% specificity. A summary of these gene panels is shown in Table 2. As with the studies of protein biomarkers for stroke diagnosis, the comparison of greater clinical interest is to distinguish acute ischemic stroke from disease that mimics ischemic stroke rather than healthy controls. Though this study has yet to be performed, a 97 gene profile has been identified to distinguish ischemic stroke from 75% of patients with myocardial infarction and 96% of patients with vascular risk factors. A multigene approach shows promise as a method to identify acute ischemic stroke, though further study comparing to a broad range of stroke mimics is required. Additionally, the use of a profile of RNA as a biomarker of disease is a recent concept that is developing. The technology to measure RNA is evolving, as is the understanding of the many types of RNA that exist. Indeed, most studies to date have focused on

messenger RNA, whereas more recently described RNA, such as microRNA and snoRNA, still require further evaluation as potential biomarkers in stroke [30].

Preliminary whole proteome studies in ischemic stroke are beginning to be performed. Progress has been slow, in part due to the developing nature of the technology required to measure many proteins. Mass spectroscopy is often used, which, as previously reported, has limitations in biomarker discovery. This includes sensitivity to method of sample collection and processing in addition to challenges of data analysis [31, 32]. A study of brain microdialysates from six patients with ischemic stroke identified 53 proteins associated with cerebral infarction [33]. Among these were glutathione S-transferase P1, peroxiredoxin-1, and S100B. Another study compared protein expression in brain tissue from six ischemic strokes to three controls [34]. A total of 132 differential expressed protein spots were identified, 39 of which were characterized by mass spectrometry. As the technology to perform whole proteome analysis improves, similar larger proteomic discovery of biomarkers for ischemic stroke will likely become possible.

Biomarkers of Ischemic Stroke Etiology

Ischemic stroke is often classified by etiology dividing patients into cardioembolic, large vessel, small vessel, and cryptogenic causes. This classification system is unable to determine an etiology of ischemic stroke in as many as 30% of patients. A biomarker could serve to improve identification of ischemic stroke etiology in cryptogenic stroke

Table 2 Microarray studies that have identified panels of genes to distinguish ischemic stroke from controls. Gene panels represent the genes that were found to optimally discriminate stroke from controls

| | Origin of RNA | Microarray Platform | No of Samples | Genes (gene symbols) | Sensitivity / Specificity |
|--------------------|------------------------------------|----------------------------------|---------------|---|---------------------------|
| 22 Gene Panel [26] | Peripheral blood mononuclear cells | Affymetrix U133A | 40 | ADM, APLP2, BST1, C1QR1, CD14, CD163, CD36, CSPG2, CYBB, DUSP1, ENTPD1, ETS2, FCGR1A, FLJ22662, FOS, IL13RA1, KIAA0146, LTA4H, NPL, PILRA, TLR2 | 78% / 80% |
| 18 Gene Panel [27] | Whole Blood | Affymetrix U133 Plus 2.0 | 60 | ARG1, BCL6, CA4, CKAP4, ETS-2, HIST2H2AA, HOX1.11, F5, FPR1, LY96, MMP9, NPL, PYGL, RNASE2, S100A9, S100A12, S100P, SLC16A6 | 89% / 100% |
| 9 Gene Panel [28] | Whole Blood | Illumina HumanRef-8v2 bead chips | 64 | ARG1, CA4, CCR7, CSPG2, IQGAP1, LY96, MMP9, ORM1, S100A12 | — |
| 18 Gene Panel [29] | Whole Blood | Affymetrix U133 Plus 2.0 | 237 | ARG1, BCL6, CA4, CKAP4, ETS-2, HIST2H2AA, HOX1.11, F5, FPR1, LY96, MMP9, NPL, PYGL, RNASE2, S100A9, S100A12, S100P, SLC16A6 | 93% / 95% |

in the cohort analyzed. They were derived from larger lists of genes found to be differentially expressed between ischemic stroke and controls

patients, and thus allow initiation of preventative therapy targeted to the underlying cause.

A number of biomarkers have been found to distinguish cardioembolic from non-cardioembolic ischemic stroke. In 707 ischemic stroke patients, cardioembolic stroke was predicted by levels of BNP >76 pg/ml with 72% sensitivity and 69% specificity [35]. In the same study, D-dimer >0.96 g/mL predicted cardioembolic etiology with a 56% sensitivity and 64% specificity. When BNP and D-dimer were combined, the positive predictive value of cardioembolic stroke was 70%. D-Dimer has been associated with cardioembolic stroke in other studies, with Ageno et al reporting a D-Dimer level >2.0 ug/mL to be predictive of cardioembolic stroke with specificity of 93.2% and sensitivity of 59.3% [36, 37]. A more recent study confirms these findings [38]. BNP has also been associated with embolism in patients with atrial fibrillation [39, 40].

Gene expression profiles in blood have also been shown to distinguish cardioembolic from large-vessel ischemic stroke. RNA was isolated from blood and run on Affymetrix U133 Plus2.0 microarrays. In 33 samples from 11 patients, a 23 gene panel distinguished cardioembolic from large vessel ischemic stroke with >95% sensitivity and specificity [41]. In a larger study of 194 samples from 76 acute ischemic strokes, a 40 gene panel was able to distinguish cardioembolic from large vessel ischemic stroke with >95% sensitivity and specificity at each of 3 hours, 5 hours, and 24 hours after stroke onset [42]. A separate 37-gene profile was identified to differentiate cardioembolic stroke due to atrial fibrillation from non-atrial fibrillation causes with >90% sensitivity and specificity. The identified genes elucidate differences in inflammation between stroke subtypes. When these panels were applied to patients with cryptogenic stroke, 17% were predicted to be large-vessel and 41% to be cardioembolic stroke [42]. Of the cryptogenic strokes predicted to be cardioembolic, 27% were predicted to have atrial fibrillation. Further validation of these profiles in larger cohorts is required. However, gene expression signatures show potential to add valuable diagnostic information in the management of patients with stroke of unknown etiology.

Less is known regarding biomarkers of small vessel ischemic strokes. One study of 116 patients found CRP to be significantly higher in patients with large vessel compared to small vessel ischemic strokes [43]. Lacunar stroke has also been shown to have higher levels of thrombomodulin, ICAM-1, tissue factor, and homocysteine compared to controls, though it is unclear whether this is different compared to other stroke subtypes [44, 45]. Progression of white matter hyperintensities on MRI which may reflect small vessel disease has been associated with a higher plasma level of intercellular adhesion molecule (ICAM) [46]. A profile of differentially expressed genes

has also been reported for WMH in patients with and without Alzheimer's disease [47]. Among 38 subjects with minimal or several WMH, 50 differentially expressed genes ($p < 0.005$, fold change > 1.5) could separate patients by WMH severity. Identified genes were associated with oxidative stress and inflammation, suggesting novel targets for further evaluation in microvascular disease.

Biomarkers of Final Infarct Volume and Outcome

Several biomarkers have been associated with infarct volume, including S-100B, MMP, IL-6, TNF-alpha, ICAM-1 and glutamate. These could be useful to predict clinical outcome in patients with ischemic stroke. However, it should be emphasized that infarct size may not correlate with neurologic outcome, as even small infarcts can cause devastating neurological outcomes when they occur in certain anatomical regions such as the brainstem.

One might predict that a larger infarct volume would lead to increased release of CNS tissue biomarkers into systemic circulation. Missier confirmed this prediction, showing elevated levels of S-100B and NSE were associated with final infarct volume [48]. Other studies have associated infarct volume with S-100B [49, 50], NSE [50, 51], Tau [51] and glutamate [52]. A larger infarct volume might also lead to a greater inflammatory response to the ischemic tissue. This is supported by studies showing elevated inflammatory markers are associated with infarct volume, including TNF- α [53], IL-6 [54, 55], ICAM-1 [53], MMP-2 and MMP-9 [53, 56].

Markers to predict outcome would be useful in the management of ischemic stroke patients as potential surrogate measures. They could be used to monitor the course of stroke therapy or direct a specific therapy to a subgroup of patients most likely to benefit. In 250 ischemic strokes, IL6 levels were elevated in the 14 patients that died at one year [57]. In another study, poor stroke outcome was also associated with IL-6 (OR 2.4, 95% CI 1.4-4.2) and Ln NT pro BNP (OR 2.2, 95% CI 1.2-4.0) [58]. However, neither IL-6 nor Ln NT pro-BNP were able to improve upon outcome prediction achieved by the NIHSS score and age alone (c-statistic 0.84).

Biomarkers have shown some success in the prediction of recurrent ischemic vascular following ischemic stroke. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is associated with a 2 fold increase in stroke occurrence and risk of recurrent stroke (adjusted hazard ratio 2.5, 95% CI 1.0-6.4) [59, 60]. Based on several large trials, it has been approved by the FDA as a predictor of coronary heart disease and stroke [61]. In the Rotterdam trial, Atherosclerosis Risk In Communities (ARIC) studied elevated levels of Lp-PLA2 that were associated with an adjusted HR of 2.0-2.1 for stroke [62, 63]. However, in the Women's Health

Initiative, the relative increase in stroke risk with elevated levels of Lp-PLA2 was less, at 1.07 (95% CI 1.01-1.14) [64]. CRP levels have also been shown to be predictive of stroke risk [62]. Despite the increased risk of stroke predicted by Lp-PLA2 and CRP, the benefit in terms of improving stroke outcomes has yet to be demonstrated. Trials of the Lp-PLA2 inhibitor Darapladib are ongoing and are likely to shed light in this regard.

Biomarkers to Predict Early Neurological Deterioration

Early neurological deterioration (END), defined as worsening of neurological status from admission to 48–72 hours after admission, is associated with a number of biomarkers including glutamate, GABA, ferritin, TNF-alpha, ICAM-1, MMP, S100B, MMP and nitric oxide [65, 66]. Identifying patients at risk of END may be useful for initiating therapies to prevent such worsening.

Both plasma and CSF glutamate levels are elevated in patients who experience END [67, 68]. Patients with hemispheric strokes with levels of glutamate in plasma >200 $\mu\text{mol/l}$ and CSF >8.2 $\mu\text{mol/l}$ predicted END with a probability of 92 and 93% respectively [69]. Glutamate may be associated with END because it is a mediator of increased lesion volume, or an measure of cellular lysis. Glutamate levels have been associated with expansion of DWI lesion volumes from admission to 72 hours. Glutamate released from the infarct core may cause spreading depolarization of peri-infarct tissue, thus increasing metabolic demand of an already compromised tissue.

Inflammatory biomarkers also correlate with END. Plasma ferritin levels >275 ng/ml independently predicted END in patients with hemispheric infarcts [69]. IL-6 levels >21.5 pg/ml in plasma and 6.3 pg/ml in CSF predict END [54]. MMP-9 and MMP-13 independently predict lesion volume expansion in ischemic stroke [56]. CSF nitric oxide levels >5 $\mu\text{mol/ml}$ also predict END [70].

For lacunar infarctions, END is predicted by plasma glutamate concentration >200 $\mu\text{mol/l}$ and plasma GABA concentration <240 nmol/l with a positive predictive value of 67% and 84% respectively [71]. A ratio of plasma glutamate to GABA greater than 106 correctly predicted END in 85% of patients. Another study of lacunar infarction found TNF-alpha >14 pg/ml and ICAM-1 >208 pg/ml to correlate with END even after adjustment for glutamate and GABA concentrations [72].

Biomarkers for Decompressive Hemicraniectomy

Decompressive hemicraniectomy performed on selected patients with large cortical ischemic infarcts can improve outcomes when performed early [73]. A biomarker to identify patients at risk for malignant cerebral infarction

and to predict which patients benefit from such surgery would be useful. Indeed, S-100B predicts malignant infarction in patients with MCA occlusions [74]. A plasma level of S-100B >0.35 g/L predicted malignant infarction at 12 hours with a 75% sensitivity and 80% specificity, and at 24 hours with a 94% sensitivity and 83% specificity. A second study found cellular-fibronectin (c-Fn) and MMP-9 levels to predict malignant cerebral infarction [66]. Admission MMP-9 level >140 ng/mL predicted malignant MCA infarction with 64% sensitivity and 88% specificity. Admission c-Fn level >16.6 g/ml was even more predictive of malignant MCA infarction, with a 90% sensitivity and 100% specificity.

Biomarkers of Hemorrhagic Transformation

Hemorrhagic transformation is a significant complication following ischemic stroke. Identification of patients at increased risk of hemorrhage could help reduce the incidence of this complication and potentially allow extension of the time window for t-PA 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 hemorrhage following administration of tPA, including MMP-9, c-FN, PAI-1, TAFI and S100B [75].

The most evidence exists for elevated levels of MMPs predicting hemorrhagic transformation following ischemic stroke. MMPs are involved in destruction of microvascular integrity by degradation of the basal lamina and extracellular matrix [76]. Levels of MMP-9 predict hemorrhagic transformation in ischemic stroke patients who have and have not been treated with t-PA [75–80]. Serum MMP-9 levels ≥ 140 ng/ml predicted hemorrhagic transformation in ischemic stroke patients with a sensitivity of 87% and specificity of 90% [77]. t-PA itself activates MMP-9 and thus may promote hemorrhagic transformation. Hyperglycemia and diabetes have also been associated with elevated MMP-9 and the development of hemorrhagic transformation [81].^{Uemura, 2001 #2927} BBB disruption independently correlates with serum MMP9 levels [82].

Cellular fibronectin (c-Fn) is another factor that has been associated with increased hemorrhagic transformation. Cellular-Fn is synthesized by endothelial cells and is elevated following vascular injury including ischemic stroke. A study showed c-Fn levels >3.6 $\mu\text{g/ml}$ predict the development of hemorrhagic transformation following t-PA use with a sensitivity of 100% and specificity of 96% [83].

A study of 77 ischemic stroke patients identified lower levels of plasminogen activator inhibitor-1 (PAI-1) and higher levels of thrombin-activated fibrinolysis inhibitor (TAFI) to be associated with hemorrhagic transformation [84]. In combination, PAI-1 levels >180% and TAFI levels <21.4 ng/ml predicted symptomatic hemorrhagic transformation after t-PA with a sensitivity of 75% and specificity of 97.6%. S100B >0.23 g/L is also associated with an increased risk of hemorrhagic transformation in ischemic stroke with a sensitivity of 46% and specificity of 82% [85]. Identification of hemorrhagic transformation will become more important once there is a treatment to prevent it, or testing could be performed in time to impact hemorrhagic transformation associated with thrombolysis.

Biomarkers of Arterial Recanalization

Recanalization of arterial blood flow is an important predictor of good outcome in acute stroke. A biomarker potentially could be useful in selecting patients most likely to benefit from recanalization therapy. Plasminogen antigen inhibitor 1 (PAI-1) is a marker of fibrinolysis that has been associated with recanalization resistance [86]. Levels of PAI-1 <34 ng/ml predict poor response to thrombolysis. A second study of 63 ischemic stroke found that recanalization with IV-tPA was predicted by lower levels of α 2-antiplasmin and fTAFI (functional thrombin activated fibrinolysis inhibitor) [87]. A level of α 2-antiplasmin >85% predicted recanalization with a sensitivity of 25% and specificity of 85%.

BioMarkers for Stroke Prevention Therapy

Biomarkers might be useful to better understand treatments for stroke [88]. For example, both aspirin and Aggrenox produce fast and sustained recovery of plasma eNOS levels, while Aggrenox but not aspirin was associated with oxLDL in one small trial [89]. This may highlight differences in therapeutic benefit between aspirin and Aggrenox. Another potential application of biomarkers is to identifying patients with aspirin resistance. Despite the proven benefit of aspirin, the concept of aspirin resistance has developed from the observation that some patients do not derive protection from antiplatelet therapy. Identifying patients resistant to aspirin might identify a group of patients that derive greater benefit from alternate stroke prevention therapy. Detecting aspirin resistance is possible by measuring thromboxane A2 production and platelet aggregation [90]. Levels of urine 11-dehydroxythromboxane B2 have been associated with aspirin resistance. However, it tends to overestimate the number of patients with aspirin resistance [91]. Point of care measures of platelet function also have been developed such as the PFA-100 system, mTEG

(modified thromboelastograph) and RPFA (Rapid Platelet Function Analyzer) system. Though these assays can demonstrate differences in a patient's response to aspirin, agreement between assays is variable [92–94]. This may be due in part to factors other than aspirin that influence platelet aggregation, such as altered levels of von Willebrand factor. Additionally, it remains unclear whether changing therapy in a patient with aspirin resistance is associated with improved outcomes. Thus further study is required before clinical decisions are made based on a biomarker for aspirin resistance. Single nucleotide polymorphisms in DNA have been described to guide clopidogrel and warfarin stroke prevention therapy [95, 97]. Whether a protein or RNA marker could be used in a similar manner to guide stroke treatment is certainly an area for further research.

Biomarkers of Ischemic Penumbra

No plasma biomarker of human ischemic penumbra has been reported. Selection of patients for experimental recanalization therapy beyond the 3–4.5 hour thrombolysis window currently is based on clinical and neuroimaging criteria. A biomarker that could identify those patients with salvageable brain tissue in acute ischemic stroke could be of significant clinical utility. In the penumbral area, a number of molecules have been identified that may aid in differentiating tissue with reduced cerebral blood flow from severe ischemic tissue [97, 98]. Glucose tends to be higher and glutamate lower in penumbral tissue [99]. Cells that survive ischemia express a number of stress response proteins including heat shock proteins (HSP-27, HSP-70, HSP-72), α -B-crystallin, heme oxygenase-1, neuregulin-1, cyclo-oxygenase-2 and hypoxia-inducible factor-1 α [100, 101]. Such proteins serve as tissue markers of ischemic penumbra in brain. Whether a corresponding biomarker in blood can be identified requires further study.

Conclusion

A number of blood based biomarkers of ischemic stroke have been identified and show promise to aid in ischemic stroke. Many relate to the underlying pathophysiology of ischemic stroke, including ischemia of CNS tissue, acute thrombosis and inflammatory response. To date, a number of biomarkers have been identified to answer focused clinical questions, however sufficient sensitivity and specificity for use in clinical practice has not been achieved. With further well designed studies that undergo thorough validation, the development of blood biomarkers to improve the care of patients with ischemic stroke may yet be achieved.

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