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# The Promise and Potential Pitfalls of Serum Biomarkers for Ischemic Stroke and Transient Ischemic Attack

**Matt B Jensen, MD**, **Marcus R Chacon, MD**, **Justin A Sattin, MD**, **Aitziber Aleu, MD**, and **Patrick Lyden, MD**

## Abstract

Ischemic stroke and transient ischemic attack can be difficult to diagnose clinically, and both acute and preventive therapies carry some risk. Serum biomarkers could increase diagnostic certainty by helping to distinguish cerebral ischemia from common mimics such as focal seizure, complicated migraine, and psychogenic spells. Biomarkers could also identify patients at high risk for future vascular events, which would aid in management decisions. There are many potential obstacles to finding these biomarkers including the blood brain barrier, confounding by other conditions, and imperfect gold standards for use in validation. Diagnostic biomarkers are likely to be molecules found predominantly in brain tissue with rapid entry into the blood, while prognostic biomarkers may be related to the concept of an active atherosclerotic plaque. Many promising serum molecules have been examined in small series of patients with cerebrovascular disease. Large series examining many candidate molecules will be needed to find valid biomarkers, and this should be followed by use in future intervention trials to prove their utility.

## Background

The spectrum of ischemic stroke (IS) and transient ischemic attack (TIA) starts with cerebral arterial occlusion and ischemia of brain tissue, with the resulting volume of infarction ranging from none to an entire hemisphere, depending on the amount of recanalization, collateral blood flow, and secondary neurotoxicity in the ischemic penumbra. All of these are active areas of research for interventions to decrease the eventual stroke severity. Recanalization via intravenous tissue plasminogen activator is currently the only approved treatment for IS, but several other thrombolytic agents, with or without the acute use of other antithrombotic agents, appear promising in achieving or maintaining recanalization and restoration of perfusion. Catheter-based drug delivery and mechanical interventions at the site of the occlusion are also under study. Improving collateral blood flow and minimizing secondary neurotoxicity in the penumbra also have promising candidates under investigation, and may be forthcoming as approved therapies soon. Besides these potential acute therapies, there is also a proliferation of preventive treatments ranging from new antithrombotic agents to surgical and catheter-based interventions for cardiac and arterial lesions that may lower the risk of future events.

While the prospect of multiple acute and preventative therapeutic options on the near horizon is good news for future stroke patients, for clinicians it increases the complexity of management of an already complex disease, where there can be a myriad of clinical presentations due to the variety of functions served by the different brain areas. Confirming the true probability of IS or TIA versus mimic conditions - such as complicated migraine, focal seizure, or psychogenic

Matt Jensen, MD UCSD Stroke Center 200 W Arbor Dr #8466 San Diego, CA 92103 619-543-7760 fax 619-543-3771 E-mail: m1jensen@ucsd.edu.

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spells – challenges even clinicians with extensive experience in the diagnosis and management of stroke. Diagnostic uncertainty increases the difficulty in advising patients about treatment with serious potential risks such as thrombolysis, surgery, or warfarin. Smaller medical centers may not have neurologists available in person or via telemedicine, and the treating clinician may be uncomfortable offering therapies with known risks. A great aid to both the clinician and the patient would be a test or set of tests with both high sensitivity and specificity for cerebral ischemia, to increase the diagnostic certainty or give an idea about future risk. Imaging the brain and cerebral vasculature with magnetic resonance imaging (MRI) has already demonstrated the ability to increase the diagnostic certainty in acute IS, but MRI is not available in many smaller hospitals, and is often available only during daytime hours even in larger centers.

Serum biomarkers might provide a solution to many of these issues; biomarkers could be available in small centers, would not need outside interpretation by a neurology or radiology consultant, and might help both the treating clinician and patient feel assured that the diagnosis is correct, thus allowing the choice of potentially risky therapies with some confidence. The analogy to acute coronary syndromes is obvious; we need a correlate to troponin for diagnosis, and a correlate to high sensitivity C reactive protein (hsCRP) for prognostication. First we will review some promising potential biomarkers for diagnosis of IS and TIA, then focus on some that may help in predicting risk of future stroke.

## Diagnostic Biomarkers

An ideal biomarker to distinguish IS and TIA from mimics should appear or elevate in the blood quickly in the former but not the latter, and not elevate in any other disease or patient population. A good candidate would be a molecule found predominantly in brain rather than other body tissues, such as S-100b[1-4], neuron-specific enolase[2, 4], myelin basic protein[2], and glial fibrillary acidic protein[2]; all of which have shown some promise in small studies. The rational selection of a biomarker is complicated by the fact that unlike the relatively homogeneous myocardium, the brain consists of multiple cell types in varying proportions among different brain areas. Another problem is that the blood brain barrier (BBB) prevents molecules from leaving the infarcted brain tissue and entering the blood stream. Infarction does disrupt the BBB, but there may be little or no perfusion through the disrupted area, so while molecules may leak into the blood, there may be a substantial delay to elevate serum levels, which could mean the marker becomes elevated long after management decisions need to be made. There is also the possibility that two common mimics of stroke, complicated migraine and focal seizure, could potentially cause both molecular leakage from brain cells and disruption of the BBB. Another approach would be to look at markers associated with acute thrombosis, such as D-dimer[5], fibrinogen[6-8], fibrinopeptide A[5], and von Willebrand factor[1, 3], all of which have been shown to elevate after IS in small studies.

The biggest difficulty to finding useful biomarkers for IS and TIA is the inaccurate standard available for validation; both are clinical diagnoses, and rather than being made with complete certainty most often the diagnosis is thought of in terms of probability, such as a low, medium, or high likelihood of true IS or TIA versus mimic. Acute brain MRI can increase diagnostic certainty and could be used to help validate potential biomarkers, but there are many cases where the clinical diagnosis is certain and the MRI is negative, as well as the converse, so MRI is unlikely to be the ideal gold standard. Few patients come to autopsy immediately after acute stroke; even if an autopsy series could be attempted, there may be many cerebrovascular events between the initial stroke and biomarker assay and the subsequent autopsy, which would cloud interpretation of the findings. The challenge then is validating a new test with imperfect gold standards; in searching for a biomarker for diagnosis of IS and TIA, multiple studies will need

to show very clear results with significance testing showing an extremely low likelihood of the findings being due to chance alone.

## Prognostic Biomarkers

A biomarker or set of biomarkers to help in the diagnosis of IS and TIA would be very helpful, but in very mild strokes and in all TIAs the diagnosis is of little importance compared to the practical question – what is the risk of future stroke? The issues are related in that a test that reliably distinguishes mimics from true IS and TIA would thereby distinguish a low-risk group from a group with a higher risk, but it would not necessarily stratify risk among true IS and TIA patients. The molecules to look for in risk prediction are less likely to be from injured brain tissue, but rather from the pathological substrate underlying the arterial occlusion, which in most cases would be an atherosclerotic plaque, either at the site of thrombus formation or proximal to it in the case of embolism. The concept of an active plaque that is at high risk for rupture and thrombus formation has been a main focus of research in myocardial infarction, and the most promising biomarker in that setting is hsCRP, which in many large studies has been shown to predict the risk of future vascular events independent of other known risk factors. Studies looking at hsCRP in IS and TIA show a strong suggestion of utility [6, 9-13]. Many other molecules related to the active plaque concept have shown promise such as those associated with inflammation including tumor necrosis factor alpha (TNF-α)[14] and interleukin-6 (IL-6) [14, 15]. Markers related to coagulation such as D-dimer[5] and fibrinogen[6-8] may also indicate active plaques, or other more systemic disorders or clotting, that predict higher risk of recurrence. The plaque of greatest importance is in the proximal internal carotid artery, which is the most amenable to intervention with carotid endarterectomy; biomarkers of active plaque potentially could identify patients most likely to benefit from surgery or catheter-based interventions. Validation of biomarkers to predict future stroke risk would be relatively straightforward, by correlating the serum level of the candidate molecule with the likelihood of subsequent stroke. The challenge will remain that even if we can accurately identify patients with a high risk of future strokes, we will need to prove that current and future therapies are safe and effective in lowering that risk in those populations.

## Conclusion

There is great promise in the search for serum biomarkers to aid in the diagnosis and prognosis of cerebrovascular disease, but many theoretical and practical challenges stand in the way. Several large series systematically examining a large number of molecules will be needed, and will likely need to use probability-based clinical diagnosis, MRI findings, and subsequent stroke incidence as validation measures. If clear diagnostic or prognostic biomarkers do emerge, we will need to incorporate them into future intervention trials to prove their usefulness.

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**Table 1**

A selection of molecules examined in serum of IS and TIA patients.

| |
|---|
| High sensitivity C-reactive protein (hsCRP)[6, 9, 13] |
| S-100b[1-4] |
| Neuron-specific enolase[2, 4] |
| Myelin basic protein[2] |
| Glial fibrillary acidic protein[2] |
| D-dimer[5] |
| Fibrinogen[6-8] |
| Fibrinopeptide A[5] |
| von Willebrand factor[1, 3] |
| Tumor necrosis factor alpha (TNF-α)[14] |
| Interleukin-6 (IL-6)[14, 15] |
| B-type neurotrophic growth factor[1] |
| Matrix metalloproteinase-9[1, 3] |
| Vascular cell adhesion molecule[3] |
| Intercellular adhesion molecule-1[14] |
| Monocyte chemotactic protein-1[1] |
| N-amino terminal fragment of the prohormone brain natriuretic peptide (NT-proBNP) [16] |
| Glutamate[17] |
| Homocysteine[17, 18] |
| *N*-methyl-D-aspartate (NMDA) receptor autoantibodies (aAb)[17] |
| ß$_2$-glycoprotein 1 (ß2GP1)-dependent anticardiolipin antibody (aCL)[19] |
| Matrix metalloproteinase-2/9[14] |
| Plasma DNA concentrations[20] |
| Autoantibodies (aAbs) to the subtype NR2A/2B of *N*-methyl-D-aspartate (NMDA) receptors[21] |
| PARK7[22] |
| Nucleoside diphosphate kinase A (NDKA)[22] |
| Heart-fatty acid binding protein (H-FABP)[23] |
| CD40L[24] |
| Chitotriosidase activity[25] |
| Pregnancy-associated protein-A (PAPP-A)[26] |
| Lipoprotein-associated phospholipase $A_2$ (Lp-$PLA_2$)[27] |
| Apolipoprotein B[28] |
| Apo B/apo A1 ratio[28] |
| Serum lipoprotein(a) [Lp(a)][29] |
| Smaller-molecular-weight apo(a) isoforms[29] |
| Titers of IgG, IgA, and IgM antibodies specific for *C pneumoniae*[30, 31] |
| Vascular endothelial growth factor (VEGF)[32] |
| Transforming growth factor-ß (TGF-ß)[15] |

Apolipoprotein E ε4 Allele[33]

Anticardiolipin antibodies (aCLs)[34]

Thrombin- antithrombin III[5]