INTRODUCTION

Why should we care about stroke? We should care because stroke kills nearly 160,000 Americans each year. About 750,000 people suffer a new or recurrent stroke each year and there are approximately 4.5 million stroke survivors currently living in the United States. Stroke remains the nation’s third leading cause of death and the leading cause of adult disability. With current interventions for ischemic stroke being extremely time dependent, the burden of stroke diagnosis rests squarely on the shoulders of Emergency Medicine physicians. Recent advances in biomarker discovery may provide an invaluable tool in the evaluation of patients with potential stroke.

To date, the diagnosis of many acute neurologic conditions remains largely one of exclusion. This is perhaps most true for acute ischemic stroke (AIS). In AIS, the history and clinical exam may suggest a patient’s physical findings are due to an ischemic stroke, and blood chemistry analyses and CT scans can exclude patients with hypoglycemia, intracranial hemorrhage, or neoplasm. Unless early ischemic changes are found on CT (less than a third of CT scans in AIS have ischemic changes within three hours from symptom onset) or advanced MRI imaging clearly defines decrease cerebral perfusion, the diagnosis of AIS remains based largely on clinical examination. With the development of acute interventions for stroke, especially thrombolytic therapy with its narrow therapeutic time window, the certainty of diagnosis is paramount.

Similar to the development of serum markers in the diagnosis of acute coronary syndromes, several studies in the past decade have investigated direct and indirect biochemical markers of neuronal and glial cell damage in the human central nervous system. Biochemical markers of vascular injury, inflammation, and coagulation activation in stroke also show promise. Recent human studies have investigated potential markers in acute stroke, anoxic brain injury status post cardiac arrest, ischemia and embolic stroke during cardiopulmonary bypass, neonatal hypoxia, spinal cord injury, and traumatic brain injury. The hope is that either individually or as a panel these markers will aid in not only diagnosing

OBJECTIVES:

1. Review the current challenge in stroke diagnosis
2. Discuss the early diagnosis of acute ischemic stroke using biomarkers and review potential candidate proteins
3. Review other conditions which may benefit from the use of protein biomarkers in their diagnosis and management
neurologic conditions and their severity, such as infarct volume, but also will assist in tailoring therapies, measuring therapeutic efficacy, and providing some insight into patient prognosis. While not typically an issue in the Emergency Department, similar markers may also assist in diagnosing early Alzheimer’s disease, multiple sclerosis exacerbations, vascular dementia, and Creutzfeldt-Jakob disease.

Before discussing individual markers and their applications, it is important to understand the differences when comparing the release kinetics of markers of myocardial damage to neurovascular injury. The brain is far more structurally heterogeneous than the heart, with multiple neuronal and glial cell types present and in varying distribution throughout the brain. Each cell line has varying degrees of sensitivity to ischemia and direct injury. Additionally, neurons in different regions of the brain show a wide range of susceptibility to injury. Thus the type of injury, the severity, and the duration will directly affect marker release.

Perhaps the most important difference from the cardiac analogy is the presence of the blood-brain barrier (BBB). When intact, it is very effective at limiting the egress of proteins from the CSF into the serum. Unless significant permeability changes occur, such as in injury, many markers cannot enter the serum freely. It is not uncommon to have over a thousand-fold difference when comparing the CSF to serum marker concentrations. Even when damaged, the BBB may still delay the presence of a marker in the serum, limiting its early diagnostic utility.

Markers can be classified based on the cell of origin or by their general activity. Table 1 is just a partial list of biomarkers that have been studied in acute stroke, traumatic brain injury, subarachnoid hemorrhage, and global ischemia. The markers vary greatly in their sensitivity for injury or activation, and their specificity.

It is unlikely that a single marker under current investigation will have the sensitivity and specificity necessary to be used alone in diagnosing cerebrovascular events. Rather, a combination of markers will be optimized for their sensitivity, specificity, accuracy, and time-to-positivity characteristics. Our long-term goal is to develop a sensitive and specific panel of serum protein markers for use in acute ischemic stroke that may help predict extent of focal brain injury and long-term outcome, identify patients at increased risk of hemorrhage following thrombolytic therapy, and provide early diagnosis of acute cerebral ischemia to assist in treatment decisions.

Table 1.
Biomarkers for neurovascular injury

<table>
<thead>
<tr>
<th>Direct neuronal and glial markers</th>
<th>Vascular markers</th>
<th>Apoptosis / Miscellaneous</th>
<th>Markers of coagulation / fibrinolysis</th>
<th>Inflammatory mediators:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine kinase isoenzyme (BB)</td>
<td>Endothelin 1 (ET 1)</td>
<td>Brain natriuretic peptide</td>
<td>C reactive protein</td>
<td>MMP-2,9</td>
</tr>
<tr>
<td>Glial fibrillary acid protein (GFAP)</td>
<td>Selectin</td>
<td>Caspase 3</td>
<td>D-dimer</td>
<td>Transforming growth factor β (TGF)</td>
</tr>
<tr>
<td>Myelin basic protein (MBP)</td>
<td>Thrombomodulin</td>
<td>Calbindin-D</td>
<td>Heat-shock proteins (HSP 70, HSP 130)</td>
<td>Tumor necrosis factor (TNF)</td>
</tr>
<tr>
<td>Neuron specific enolase</td>
<td></td>
<td>Heat shock protein 60</td>
<td>Monocyte chemoattractant protein-1</td>
<td>Ionized calcium and magnesium</td>
</tr>
<tr>
<td>Phosphoglycerate Mutase-B</td>
<td></td>
<td>Cytochrome C</td>
<td>Plasmin-α2 antiplasmin complex</td>
<td>Neuronal cell adhesion molecule (NCAM)</td>
</tr>
<tr>
<td>S100β</td>
<td></td>
<td></td>
<td>Protein C and S</td>
<td>VCAM</td>
</tr>
<tr>
<td>Synapsin</td>
<td></td>
<td></td>
<td>Interleukins (IL-1, IL-6, IL-8)</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>Tau protein family (tau, MAP-2)</td>
<td></td>
<td></td>
<td>Thrombin-antithrombin III complex.</td>
<td>Excitatory amino-acids (glutamate, etc.)</td>
</tr>
</tbody>
</table>
Protein Markers of Neurovascular Injury in Stroke

Many biochemical markers have been studied in-vitro and in animal models, but relatively few have been evaluated in human in-vivo applications. The development and understanding of the biokinetics of neurovascular markers parallels that of the cardiac markers. Structural proteins, such as tau, require complete cell death and degradation before they are found in the CSF. Biochemical markers located in the cytoplasm are released not only in cell death, but also in reversible ischemic conditions. Cytoplasmic markers are typically released earlier in the development of injury, but are often less specific for neuronal tissue (i.e. NSE). Structural CNS proteins are detected later in the CSF and serum but tend to be more specific for neuronal and glial injury. From the cardiac marker experience and animal research it is clear that no one single marker will have the qualities of being the “perfect test”.

Markers under investigation indicate neuronal injury, glial cell injury, and vascular injury, as well as coagulation and platelet activation. In addition to stroke, several markers are also being studied in-vivo in neurovascular and traumatic brain injury. When a panel approach is developed and introduced to clinical practice it will undoubtedly contain at least one direct marker of neuronal or glial injury. The following is a brief introduction to several potential direct markers of cellular injury.

Myelin Basic Protein (MBP)

MBP has been extensively studied in demyelinating diseases. MBP is a membrane proteolipid produced by oligodendrocytes and has a molecular weight of 18.5 kD. Elevated levels of MBP have been found in the CSF and serum of patients with ischemic stroke, intracerebral hemorrhage, multiple sclerosis, and birth anoxia. It may be best suited for detecting deep strokes and intracerebral hemorrhage (ICH).

Neuron Specific Enolase (NSE)

NSE is the dimeric (γγ) isomer of the glycolytic protein enolase, and is mainly located in the cytoplasm of neurons and cells of neuroendocrine origin. NSE (γγ) is also found in smaller concentrations in erythrocytes and platelets. NSE has a molecular weight of 78kD. Its release into the CSF has been founds in patients with acute head injury, stroke, seizures, encephalitis, and transient ischemic attacks. It is also being used as a surrogate marker for the effectiveness of neuroprotective agents in focal cerebral ischemia in animal models.

S100β

S100β is a 21 kD dimeric (αβ) calcium-binding protein found throughout astroglial and Schwann cells in a homodimer form (ββ). Elevated CSF and serum levels of S-100 (ββ) have been found in patients with stroke, head injury, anoxic brain injury, and ICH. Ongoing studies are also investigating the use of S-100 in detecting cerebral injury in patients undergoing cardiopulmonary bypass or cardiothoracic surgical procedures.

Fagnart et al. performed a survey of S100β concentrations in 50 healthy individuals, 325 patients with neurological disorders, and 20 patients with malignant melanoma. No healthy control, dementia, or meningitis patient had elevations, while less that 10% of patients with meningoradiculitis, peripheral neuropathy, encephalitis, Gillian-Barre syndrome, and AIDS had detectable levels. Nearly 90% of all patients with acute cerebrovascular disorders had detectable S100β concentrations. Of note, this study was conducted in 1988 and new forms of the S100β protein have since been identified. Current assays can measure S100 levels well below the 0.3 microgram/L sensitivity in this study. The S100 family of proteins is in the process of being reclassified but S100β will likely be one of the markers that will be useful.
**Tau Protein (TP)**

Tau protein is one of a class of proteins that are microtubule-associated proteins (MAP). Tau is a major intracellular structural cytoskeletal protein located solely in neurons, and as such has no detectable levels in healthy patients. It is synthesized in 1 of 6 isoforms in the proximal axon and then transported down the axon to stabilize microtubules. The isoforms have molecular weights between 48kD and 68kD. After neuronal injury, tau is proteolytically cleaved and released into the CSF. Initial studies showed that CSF tau had high sensitivity for Alzheimer’s disease, but a low specificity due to elevations also in vascular dementia. More recently it was found to be elevated in stroke patients and in TBI, but its sensitivity remains an issue. Because of its size (30-50kDa cleaved) it is assumed that the blood-brain barrier must be compromised for detectable levels of TP to be found in the serum.

**Other Recent Marker Advances:**

Several studies and surveys have identified the largest obstacle to widespread use of thrombolytics for stroke is the risk of causing an intracerebral hemorrhage. To this end, several recent studies have reported efforts to identify patients at increased risk. In studies by Montaner, matrix metalloproteinases (MMP-9) levels at baseline predicted parenchymal hemorrhages following both cardioembolic strokes and following tPA therapy in acute ischemic stroke. Similarly, Trouillas, Derex and colleagues published data suggesting early fibrinogen degradation coagulopathy is predictive of parenchymal hematomas in cerebral rt-PA thrombolysis. Further work will need to validate and refine these findings.

Some of the first investigators to approach the stroke biomarker problem from a panel approach have been investigators at Duke University and Biosite, Inc. Reynolds, Lynch, and Laskowitz have published their results from several studies, including a large plasma protein screening project that resulted in marker panel comprised of 5 unique proteins associated with stroke ([Figure 1](#)). Utilizing plasma levels of S-100beta, B-type neurotropic growth factor, von Willebrand factor, matrix metalloproteinases-9, and monocyte chemotactic factor-1, the derived panel algorithm provided a sensitivity of 92% at 93% specificity for ischemic stroke in patients within 6 hours from symptom onset. These studies represent the beginning of the second

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**Figure 1.** Box and whisker plots of biomarker concentrations in patient samples (12 h from symptom onset) TIA patients with a discharge diagnosis of TIA; Isch., acute ischemic stroke; Hemor., intracerebral hemorrhage; Controls, samples from healthy controls.
generation of research into biomarkers for brain injury, one with the promise of making an impact in the practice of emergency physicians.

**Extension of Biomarkers for Stroke**

Since the brain responds to stress and injury in a fairly constant manner, irrespective of the precipitating event, researchers have expanded the search for biomarkers into other acute neurologic conditions. Serum markers are also being investigated in both subarachnoid (SAH) and intracerebral hemorrhages (ICH). In SAH, CSF concentrations of S100β have been shown to be reflective of the degree of immediate neurologic injury but also the later injury due to vasospasm. Persson’s study of 43 patients with SAH found a threshold for CSF S100β concentrations that correlated with favorable outcomes.

**Status post cardiac arrest**

Several recent studies have used S100β and NSE as markers for the duration and extent of circulatory arrest. These markers have a high correlation between marker levels and duration of arrest, as well as being able to predict persistent coma.

**Traumatic Brain Injury**

Recently, traumatic brain injury (TBI) has become a focal point of public health. Similar to AIS and TIA, the diagnosis is largely by clinical exam and history, since common neuroimaging modalities are not able to detect subtle injuries. While several markers have been shown to be powerful predictors of outcome in severe TBI, it is hoped that serum markers will be able to identify minor TBI patients who are at increased risk for short and long term impaired neuropsychological performance.

**The Future is Now**

Based on the preliminary studies and several ongoing pilot studies, it is reasonable to expect commercially available products to enter use within the coming years. Equally exciting is the use of these markers in traumatic brain injury and acute spinal cord injury. As they are refined, they will have the potential to assist in the diagnosis and management of an even broader scope of neurologic disorders.

**REFERENCES**


