Clinical Usefulness of a Biomarker-Based Diagnostic Test for Acute Stroke

The Biomarker Rapid Assessment in Ischemic Injury (BRAIN) Study

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Background and Purpose—One of the significant limitations in the evaluation and management of patients with suspected acute cerebral ischemia is the absence of a widely available, rapid, and sensitive diagnostic test. The objective of the current study was to assess whether a test using a panel of biomarkers might provide useful diagnostic information in the early evaluation of stroke by differentiating patients with cerebral ischemia from other causes of acute neurological deficit.

Methods—A total of 1146 patients presenting with neurological symptoms consistent with possible stroke were prospectively enrolled at 17 different sites. Timed blood samples were assayed for matrix metalloproteinase 9, brain natriuretic factor, D-dimer, and protein S100β. A separate cohort of 343 patients was independently enrolled to validate the multiple biomarker model approach.

Results—A diagnostic tool incorporating the values of matrix metalloproteinase 9, brain natriuretic factor, D-dimer, and S-100β into a composite score was sensitive for acute cerebral ischemia. The multivariate model demonstrated modest discriminative capabilities with an area under the receiver operating characteristic curve of 0.76 for hemorrhagic stroke and 0.69 for all stroke (likelihood test \( P < 0.001 \)). When the threshold for the logistic model was set at the first quartile, this resulted in a sensitivity of 86% for detecting all stroke and a sensitivity of 94% for detecting hemorrhagic stroke. Moreover, results were reproducible in a separate cohort tested on a point-of-care platform.

Conclusions—These results suggest that a biomarker panel may add valuable and time-sensitive diagnostic information in the early evaluation of stroke. Such an approach is feasible on a point-of-care platform. The rapid identification of patients with suspected stroke would expand the availability of time-limited treatment strategies. Although the diagnostic accuracy of the current panel is clearly imperfect, this study demonstrates the feasibility of incorporating a biomarker based point-of-care algorithm with readily available clinical data to aid in the early evaluation and management of patients at high risk for cerebral ischemia. (Stroke. 2009;40:77-85.)

Key Words: brain natriuretic peptide D-dimer diagnosis hemorrhagic stroke ischemic stroke matrix metalloproteinase 9 S100

Despite a decade of intense public education and medical advancement, stroke continues to represent a leading cause of long-term disability and death, affecting over 700,000 people annually in the United States. At present, tissue plasminogen activator represents the only pharmacological intervention approved by the Food and Drug Administration for the acute treatment of ischemic stroke and must be administered within 3 hours from symptom onset. However, intravenous fibrinolysis remains underused, and only approximately 2% to 6% of patients with stroke receive treatment with intravenous tissue plasminogen activator. Although a variety of obstacles may limit tissue plasminogen activator use, there is evidence associating diagnostic uncertainty with underuse of fibrinolytic treatment. Even when presenting outside the 3-hour window for fibrinolysis, early management decisions regarding glycemic, blood pressure, and temperature control may influence clinical outcomes. Furthermore, patients who present late may still be eligible for acute treatment trials if the diagnosis of stroke can be established within the study window. Thus, it is critical for patients to be rapidly evaluated, diagnosed, and considered for acute therapy.

Because of the time sensitivity for effective reperfusion, a major obstacle to the appropriate treatment of patients with suspected stroke is rapid entry into a care pathway that expedites the initial clinical examination and diagnostic
testing requisite for initiating early management strategies. This initial evaluation includes a focused history and physical, brain imaging, coagulation testing, and determination of glucose and electrolytes. Even with a strong clinical suspicion for ischemic stroke, most diagnostic studies are performed to rule out other metabolic or structural causes for neurological symptoms such as hypoglycemia or infection. A noncontrast head CT can be performed rapidly at most institutions; although insensitive for ischemic stroke, this may eliminate the possibility of intracranial hemorrhage, subdural hematoma, or mass lesion. Multimodal imaging techniques such as CT angiography and CT perfusion techniques may also add diagnostic information regarding vascular occlusion but are not widely available on a timely basis at many institutions. MRI-based techniques have shown greatly enhanced sensitivity in the early diagnosis of stroke as well as the identification of intracerebral hemorrhage. In theory, a full MRI study, including diffusion-weighted imaging, perfusion-weighted imaging, MR angiography, and standard T1- and T2-weighted images could be performed within 30 minutes. However, as a practical issue, most hospitals do not have these specialized MRI services available in the acute setting, and these studies require a more advanced interpretation. Thus, although multimodal imaging techniques may provide valuable information, these studies may be difficult to perform in a timely fashion, and should not delay the treatment of a patient who is otherwise eligible for treatment with intravenous thrombolysis. Coupled with limited neurological training, the absence of confirmatory testing or on-site availability of neurological consultation, and the potential for hemorrhagic complications after fibrinolytic therapy, many emergency physicians are reluctant to affirmatively make the diagnosis of acute ischemic stroke. Although most diagnostic approaches for the evaluation of acute stroke rely on neuroimaging techniques, an alternative strategy would be the evaluation of bloodborne biochemical markers of tissue injury. This approach has precedent in the triage and early management of other urgent medical conditions. For example, biomarkers such as troponin, CK-MB, d-dimer, and B-type natriuretic peptide play an important role in the evaluation of myocardial ischemia, pulmonary embolism, and congestive heart failure. Although there is an extensive literature evaluating the role of biomarkers in cerebral ischemia, the brain poses several unique challenges. These include the heterogeneity of different cell populations and their ischemia tolerance and distributions within the central nervous system, the complexity of the ischemic cascade, and presence of the blood–brain barrier. Thus, although statistical associations with stroke have been demonstrated with individual markers of inflammation, gli activation, and neuronal injury, no single biomarker has ever been demonstrated to be clinically useful as a standalone diagnostic test. One way to address this difficulty is by simultaneously evaluating multiple biomarkers that contribute complementary information. For example, by simultaneously targeting different components of the ischemic cascade, a panel of biomarkers can distinguish patients with acute stroke from age and gender-matched control subjects with a sensitivity and specificity of 90%. When integrated with other clinical and radiographic information, such an approach could provide meaningful clinical information and facilitate early management decisions. In the correct clinical context, such a rapid noninvasive test would help to identify a population of patients at risk for cerebral ischemia that need rapid evaluation and triage as well as provide adjunctive diagnostic information in patients for which acute intervention is being contemplated.

In the current study, we explore the feasibility of using a biomarker-based approach to provide adjunctive diagnostic information in patients with suspected stroke. We incorporated 4 biomarkers previously associated with cerebral ischemia (matrix metalloproteinase 9 [MMP-9], d-dimer, S100β, and B-type natriuretic peptide [BNP]) with readily obtainable clinical variables such as age, gender, and presence of atrial fibrillation. To demonstrate the feasibility of this approach as a point-of-care test, on completion of the primary study, a separate validation cohort of 343 patients was enrolled and blood samples measured on Triage Stroke Panel devices using the Triage Meter point-of-care platform.

**Methods**

**Participants and Study Design**

Approval from each Institutional Review Board was obtained before study initiation. Patients were enrolled from July 2002 through June 2005 and were eligible for study participation if they were older than 18 years of age, had no recent history of trauma, and presented with new neurological symptoms consistent with stroke within 24 hours of the enrollment blood draw. Written informed consent was obtained from the study participant or legal designate. Demographic, clinical, laboratory, and radiographic data were collected per a standardized protocol. All radiographic studies performed as standard of care that were pertinent to the diagnosis were included in the case report form and were reviewed during the adjudication process. The validation cohort of 343 patients was prospectively enrolled under the identical protocol.

The criterion standard of this study was a final diagnosis of stroke as rendered by the treating site clinician who was blinded to biomarker results and by independent stroke experts after review of all clinical, imaging, and conventional laboratory information gathered during the admission. Stroke was defined as persistent neurological deficit lasting >24 hours felt to be of vascular etiology and associated with compatible neuroimaging studies. Four board-certified neurologists with subspecialty training in cerebrovascular disease (D.T.L., S.E.K., J.S., K.S.R.) served as adjudicators and reviewed primary medical records and case report forms on all patients in blinded fashion. Both site clinicians and adjudicators were asked to assess whether patients had ischemic stroke: intracerebral hemorrhage based on CT (excluding subarachnoid hemorrhage), transient ischemic attack (TIA; defined as transient focal neurological deficits believed to be of ischemic vascular etiology but with clinical symptoms lasting less than 24 hours), or stroke mimic (defined by historical, radiographic, or laboratory evidence of an underlying nonvascular medical condition resulting in a neurological deficit). All cases were assigned a diagnosis by the site investigator and by one independent adjudicator. Adjudicators were blinded to site diagnosis and results of the biomarker test. If there was a discrepancy between the site diagnosis and primary adjudication, all records were reviewed by a second adjudicator who arrived at an independent diagnosis, declared the case unadjudicable, or recommended the case be presented for evaluation by the full adjudication committee. The adjudication committee was convened by telephone with all adjudicators present; each adjudicator was assigned as the primary reviewer for one case for presentation to the committee, and the consensus of the adjudication committee served as the final diagnosis.
Pathophysiology of brain ischemia. Four biomarkers (MMP-9, D-dimer, S100, BNP) with a high independent correlation with existing heparin locks using sterile technique. Samples were collected in EDTA-containing tubes and promptly centrifuged at 10,000 g; the resulting supernatant was immediately frozen at −70°C until analysis. Blood samples were sent to Biosite, Inc., where biomarker analysis was performed on pilot Stroke Panel devices. On completion of the primary study, a separate validation cohort of 343 patients was enrolled and blood samples measured on manufactured and validated Triage Stroke Panel devices comprised of the final 4 biomarkers using the Triage Meter point-of-care platform. The Triage Stroke Panel is a rapid, point-of-care fluorescence immunoassay to be used with the Triage Meters for the rapid, quantitative measurement of BNP, D-dimer, MMP-9, and S100β in EDTA-anticoagulated whole blood or plasma specimens. The analytic range for MMP-9 was 25 to 1300 ng/mL; D-dimer was 150 to 5000 ng/mL; S100β 100 to 8000 pg/mL; and BNP 10 to 5000 pg/mL.

### Statistical Analysis
Statistical analysis was performed using SAS version 9.1 (SAS, Cary, NC). Descriptive statistics, including median and interquartile ranges, were obtained for demographic data. Wilcoxon test was used to compare continuous variables, and χ² test was used to compare categorical variables between groups. We have previously performed a systematic analysis of multiple predefined biomarkers related to the pathophysiology of brain ischemia. Four biomarkers (MMP-9, D-dimer, S100β, BNP) with a high independent correlation with stroke and a low degree of colinearity were targeted for further study. In addition to the 4 biomarkers of stroke, 3 clinical variables (age, gender, and presence of atrial fibrillation) were chosen based on their ready availability even in the absence of prior medical documentation or the patient’s ability to give a complete history. The study sought to derive algorithms for using the serum biomarker panel and clinical variables to yield a probability value that the patient was harboring a stroke. These probabilities were used to classify patients into 4 quartiles of stroke likelihood. A separate analysis was performed to assess the discriminative capacity of the 4 biomarkers in the absence of any clinical data.

To preserve independence in the analysis, only the first sample from each patient was used in the analysis. Natural logarithm transformation of each of the preidentified biomarkers was performed and a logistic model developed to evaluate the relationship between biomarker and adjudicated diagnosis. A final multivariate logistic regression model combined the clinical information and biomarker data in predicting final adjudicated diagnosis of stroke versus mimic. Maximum likelihood ratio tests were used to test the lack of fit of the multivariate model. Multivariate logistic regression analysis was also used within hemorraghic stroke subgroup.

The predicated probabilities of the selected multivariate logistic models were categorized to quartiles, and the OR and 95% CI were calculated using the lowest quartile as the reference. The first quartile cutoff was used as the threshold for a positive test result. The interval likelihood ratio and clinical sensitivity and specificity were also calculated using the quartile cutoffs on the predicted probabilities.

The final multivariate logistic model was applied to a validation data set (n=343) in which biomarker measurement was performed on a multiple biomarker platform (the Triage Stroke Panel). To measure and compare the predictive accuracy of the model derived here and its performance on the validation data set, receiver operator characteristic curves, in which sensitivity is plotted as a function of 1-specificity, were generated.

### Results
A total of 1146 patients were enrolled at 17 different sites from July 2002 through June 2005 (Supplemental Table I, available online at http://stroke.ahajournals.org). The demographics for the study population were as follows: mean age 60±16.7 years (range, 19 to 102 years); 611 (53%) men and 535 (47%) women; and 690 (60%) white, 407 (36%) black, and 49 (4%) other race. In this cohort, the median time from last known well to serum biomarker blood draw was 9.3 hours (4.5, 18.2 hours interquartile range). Brain imaging was performed in 1092 patients (95%) of the cohort and included CT alone in 48%, MR alone in 11%, and both CT and MR in 41%.

On initial adjudication, site and adjudicator diagnoses of stroke were concordant in 83% of patients, discordant in 14%, and 3% were deemed unadjudicable due to insufficient medical information. Among the 162 diagnoses that were
discordant, final adjudication was reached by secondary review in all but 16 cases that were considered unadjudicable. After excluding unadjudicable patients, protocol violations (patients who were enrolled >24 hours after symptom onset), and patients with missing data fields, 946 patients were included in the analysis.

The final adjudicated diagnoses included 293 (31%) ischemic stroke, 95 (10%) intracranial hemorrhage, 197 (21%) TIA, and 361 (38%) stroke mimics. The mean initial National Institutes of Health Stroke Scale (NIHSS) score for patients presenting with ischemic stroke was $8.4 \pm 7.6$; among patients with hemorrhagic stroke, the mean NIHSS score was $13.5 \pm 0.1$.

**Table 3. Multivariate Logistic Model Incorporating BNP, D-dimer, MMP-9, and S100B**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beta</th>
<th>SE</th>
<th>Z Statistic</th>
<th>P Value</th>
<th>OR Estimate</th>
<th>Lower 95% CI</th>
<th>Higher 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-3.51</td>
<td>1.19</td>
<td>-2.95</td>
<td>0.0031</td>
<td>1.37</td>
<td>1.24</td>
<td>1.53</td>
</tr>
<tr>
<td>BNP</td>
<td>0.32</td>
<td>0.05</td>
<td>5.97</td>
<td>0.0000</td>
<td>1.37</td>
<td>1.24</td>
<td>1.53</td>
</tr>
<tr>
<td>D-dimer</td>
<td>0.13</td>
<td>0.07</td>
<td>1.84</td>
<td>0.0655</td>
<td>1.14</td>
<td>0.99</td>
<td>1.32</td>
</tr>
<tr>
<td>MMP-9</td>
<td>0.30</td>
<td>0.08</td>
<td>3.65</td>
<td>0.0003</td>
<td>1.35</td>
<td>1.15</td>
<td>1.58</td>
</tr>
<tr>
<td>S100B</td>
<td>0.05</td>
<td>0.21</td>
<td>0.22</td>
<td>0.8285</td>
<td>1.05</td>
<td>0.69</td>
<td>1.59</td>
</tr>
</tbody>
</table>

*Marker concentrations were natural log-transformed before fitting the logistic regression model.

**Figure 1.** A, Receiver operator characteristic curve demonstrating sensitivity as a function of 1-specificity for discriminating stroke from mimic based on the logistic model incorporating all 4 biomarkers and the relative contribution of each biomarker alone (initial cohort). This logistic model had an area under the receiver operator characteristic curve of 0.69. B, Receiver operator characteristic curve demonstrating sensitivity as a function of 1-specificity for discriminating intracranial hemorrhage from mimic based on the logistic model incorporating all 4 biomarkers and the relative contribution of each biomarker alone. This logistic model had an area under the receiver operator characteristic curve of 0.76.
Patients presented primarily with language, sensorimotor, visual, or level of consciousness changes (Table 1). Comorbidities associated with final diagnosis of stroke included atrial fibrillation, diabetes mellitus, hypertension, male gender, and age > 55 (Table 2). The most common mimics included headache/migraine (n=61), seizure/postictal state (n=39), infectious/systemic disease (n=35), cardiovascular (including syncope; n=35), no organic substrate (functional or conversion disorder; n=29), neuromuscular (n=24), subdural hematoma or tumor (n=22), intoxication/metabolic (n=20), vertigo (n=17), change in mental status unrelated to ischemia (n=14), Bell’s palsy (n=7), and decompensation of prior neurological deficit (n=5).

The logistic model incorporating the 4 biomarkers (BNP, MMP-9, S100β, d-dimer) provided good discriminative capacity to differentiate all stroke from mimic (c=0.69; Table 3; Figure 1A; likelihood ratio test P<0.001). The logistic model performed better at differentiating mimics from patients with intracranial hemorrhage (c=0.76; Figure 1B) than ischemic stroke (c=0.67). The odds ratio of patients with acute stroke having positive test results was highly significant at 6.4 when the threshold for a positive test was set at the highest quartile (Table 4). The model indicated an even stronger relationship with intracranial hemorrhage; in this cohort, a test result in the highest quartile indicated a 15-fold increased probability of intracranial hemorrhage (Table 4). The model performed somewhat less well at predicting ischemic stroke, although a test result in the highest quartile still reflected a 5-fold increased probability of ischemic stroke (Table 4).

A correlation between the logistic values and severity of presenting NIHSS was observed with higher panel scores indicative of a greater degree of initial deficit (Spearman correlation 0.431; P<0.0001). Patients with TIA had predicted probabilities that were intermediate between mimic and stroke populations, and patients with intracranial hemorrhage had the highest predicted probability of stroke in this model. To better define tissue injury in patients with transient symptoms, a secondary radiographic end point was used in patients classified with TIA who received acute diffusion-weighted MRI. Of the subgroup of 116 patients with the final diagnosis of TIA who received acute diffusion-weighted imaging (mean time to imaging 20±26 hours), 13% (15 of 116) had a positive study. When applied to patients with TIA,

### Table 4. Predictive Probability of Logistic Model for Diagnosing Stroke

<table>
<thead>
<tr>
<th>Quartile (range)</th>
<th>Q1 (≤0.39)</th>
<th>Q2 (0.39–0.5)</th>
<th>Q3 (0.5–0.64)</th>
<th>Q4 (&gt;0.64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonstroke</td>
<td>133</td>
<td>109</td>
<td>67</td>
<td>52</td>
</tr>
<tr>
<td>Stroke</td>
<td>54</td>
<td>79</td>
<td>120</td>
<td>135</td>
</tr>
<tr>
<td>OR</td>
<td>1</td>
<td>1.79</td>
<td>4.41</td>
<td>6.39</td>
</tr>
<tr>
<td>95% CI</td>
<td>N/A</td>
<td>1.16–2.74</td>
<td>2.85–6.82</td>
<td>4.08–10.03</td>
</tr>
<tr>
<td>P value</td>
<td>N/A</td>
<td>0.0078</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Interval likelihood ratio</td>
<td>0.38</td>
<td>0.67</td>
<td>1.67</td>
<td>2.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quartile (range)</th>
<th>Q1 (≤0.37)</th>
<th>Q2 (0.37–0.47)</th>
<th>Q3 (0.47–0.61)</th>
<th>Q4 (&gt;0.61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonstroke</td>
<td>108</td>
<td>101</td>
<td>90</td>
<td>62</td>
</tr>
<tr>
<td>Stroke</td>
<td>6</td>
<td>13</td>
<td>24</td>
<td>52</td>
</tr>
<tr>
<td>OR</td>
<td>1</td>
<td>2.32</td>
<td>4.80</td>
<td>15.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>N/A</td>
<td>1.88–12.26</td>
<td>6.13–37.17</td>
<td>6.13–37.17</td>
</tr>
<tr>
<td>P value</td>
<td>N/A</td>
<td>0.0935</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Interval likelihood ratio</td>
<td>0.21</td>
<td>0.49</td>
<td>1.01</td>
<td>3.19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quartile (range)</th>
<th>Q1 (≤0.38)</th>
<th>Q2 (0.38–0.48)</th>
<th>Q3 (0.48–0.62)</th>
<th>Q4 (&gt;0.62)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonstroke</td>
<td>121</td>
<td>106</td>
<td>77</td>
<td>57</td>
</tr>
<tr>
<td>Stroke</td>
<td>43</td>
<td>57</td>
<td>87</td>
<td>106</td>
</tr>
<tr>
<td>OR</td>
<td>1.00</td>
<td>1.51</td>
<td>3.18</td>
<td>5.23</td>
</tr>
<tr>
<td>95% CI</td>
<td>N/A</td>
<td>0.94–2.43</td>
<td>2–5.06</td>
<td>3.26–8.41</td>
</tr>
<tr>
<td>P value</td>
<td>N/A</td>
<td>0.086</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Interval likelihood ratio</td>
<td>0.44</td>
<td>0.66</td>
<td>1.39</td>
<td>2.29</td>
</tr>
</tbody>
</table>

N/A indicates not applicable.
the logistic model had poor discriminative capacity beyond 3 hours from symptom onset (Supplemental Table II).

In a separate analysis, we included 3 clinical variables to establish whether this approach might enhance the ability of the biomarker-based test to predict stroke. The clinical variables of gender, age, and presence of atrial fibrillation were chosen for practical reasons, because this information is almost universally obtainable even in the absence of prior medical documentation or ability to give a good medical history. Although the 4 biomarkers contributed the majority of the discriminative power of this test, the addition of the 3 clinical variables did slightly improve test performance for both ischemic stroke and intracranial hemorrhage (Supplemental Figure I, available online at http://stroke.ahajournals.org).

Most interventions for acute stroke are only viable soon after symptom onset, and thus the ability of a stroke diagnostic to detect early cerebral ischemia is critical to its clinical value. The biomarker-based test performed well within in patients with acute symptoms, and in fact displayed a sensitivity of approximately 90% in predicting all stroke (sensitivity 90%, specificity 45%; Table 5), intracranial hemorrhage (sensitivity 88%, specificity 38%; Table 5), and ischemic stroke (sensitivity 91%, specificity 45%; Table 5) within 3 hours of symptom onset. Among the 4 biomarkers included in the model, BNP had the greatest contribution in discriminating mimic versus stroke and S100β had the least (Figure 1). Although S100β has been associated with acute cerebral ischemia in prior studies,16 this biomarker did not significantly contribute to the model in the multivariate analysis. Approximately 8% (92 patients) of this study population had elevated S100β values greater than 150 pg/mL (the analytic sensitivity of the assay); when the analysis is restricted to this subpopulation, the median S100β value for ischemic stroke was 610 pg/mL, nearly twice the level in mimics (316 pg/mL) and statistically significant ($P<0.005$) by Wilcoxon rank sum test.

At present, noncontrast CT constitutes the standard of care in the evaluation of acute stroke at most institutions to rule out intracranial hemorrhage or mass lesion, because the early CT changes associated with ischemia are often absent or quite subtle. The sensitivity of a strategy incorporating the current biomarker test in conjunction with noncontrast CT for the evaluation of early ischemia was significantly greater than CT alone (Figure 2). Additional analysis also demonstrated that the logistic model incorporating all 4 biomarkers was significantly correlated with ischemic lesion on CT (0.61 for CT-positive patients versus 0.495 for CT-negative patients; $P<0.0001$).

Finally, an additional validation cohort of 343 patients was enrolled and samples measured on manufactured and validated Triage Stroke Panel devices with the final 4 biomarkers. Final discharge diagnosis in this cohort included 87 (25%) patients with ischemic stroke, 64 (19%) patients with intracranial hemorrhage, 40 (12%) patients with TIA, and 152 (44%) mimics without acute cerebrovascular disease. When the selected logistic regression model parameters were applied on this independent cohort, our model had virtually identical discriminative characteristics, suggesting that the multiple biomarkers panel model is valid on different study cohorts (Figure 3).

### Table 5. Sensitivity and Specificity of Model in Discriminating Stroke as a Function of Time From Symptom Onset

<table>
<thead>
<tr>
<th>Latency From Symptom Onset</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Area Under the Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25th Percentile</td>
<td></td>
<td>75th Percentile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All stroke</td>
<td>Nonstroke</td>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3 hours</td>
<td>53</td>
<td>41</td>
<td>0.90</td>
<td>0.45</td>
<td>0.27</td>
</tr>
<tr>
<td>3–6 hours</td>
<td>106</td>
<td>86</td>
<td>0.81</td>
<td>0.39</td>
<td>0.24</td>
</tr>
<tr>
<td>6–12 hours</td>
<td>89</td>
<td>83</td>
<td>0.89</td>
<td>0.31</td>
<td>0.37</td>
</tr>
<tr>
<td>12–24 hours</td>
<td>113</td>
<td>178</td>
<td>0.86</td>
<td>0.35</td>
<td>0.40</td>
</tr>
<tr>
<td>Pooled</td>
<td>361</td>
<td>388</td>
<td>0.86</td>
<td>0.37</td>
<td>0.35</td>
</tr>
<tr>
<td>Hemorrhagic stroke</td>
<td>Nonstroke</td>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3 hours</td>
<td>53</td>
<td>8</td>
<td>0.88</td>
<td>0.38</td>
<td>0.50</td>
</tr>
<tr>
<td>3–6 hours</td>
<td>106</td>
<td>20</td>
<td>0.90</td>
<td>0.29</td>
<td>0.40</td>
</tr>
<tr>
<td>6–12 hours</td>
<td>89</td>
<td>95</td>
<td>0.95</td>
<td>0.28</td>
<td>0.60</td>
</tr>
<tr>
<td>12–24 hours</td>
<td>113</td>
<td>47</td>
<td>0.96</td>
<td>0.28</td>
<td>0.60</td>
</tr>
<tr>
<td>Pooled</td>
<td>361</td>
<td>95</td>
<td>0.94</td>
<td>0.30</td>
<td>0.55</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>Nonstroke</td>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3 hours</td>
<td>53</td>
<td>33</td>
<td>0.91</td>
<td>0.45</td>
<td>0.30</td>
</tr>
<tr>
<td>3–6 hours</td>
<td>106</td>
<td>66</td>
<td>0.83</td>
<td>0.32</td>
<td>0.30</td>
</tr>
<tr>
<td>6–12 hours</td>
<td>89</td>
<td>63</td>
<td>0.87</td>
<td>0.30</td>
<td>0.40</td>
</tr>
<tr>
<td>12–24 hours</td>
<td>113</td>
<td>131</td>
<td>0.84</td>
<td>0.32</td>
<td>0.39</td>
</tr>
<tr>
<td>Pooled</td>
<td>361</td>
<td>293</td>
<td>0.85</td>
<td>0.34</td>
<td>0.36</td>
</tr>
</tbody>
</table>

### Discussion

At present, the absence of a widely available and rapid diagnostic test for acute stroke remains a significant limitation in the evaluation and treatment of this disease. In the
In the current study, we found that a point-of-care device designed to rapidly assess 4 biomarkers, MMP-9, d-dimer, S100β, and BNP, could sensitively identify patients experiencing stroke.

Given the difficulties inherent in making a clinical diagnosis of stroke as well as the compressed timeframe for initiating effective intervention, there is a clear clinical need for adjunctive diagnostic information in the acute setting. The use of a biomarker-based diagnostic approach has proven extremely useful in other medical emergencies such as acute coronary syndromes, and this strategy has been advocated in acute stroke to aid in triage and early management decisions. Such a biomarker-based test might have enhanced usefulness if integrated with clinical data that is universally available for each patient (not dependent on detailed history) and independent from the clinical evaluation.

The negative predictive value of a diagnostic test used in the early hospital or prehospital setting is essential for its clinical usefulness and will vary with the prevalence of true stroke in the tested population. In the current study, approximately 41% of the patients enrolled were ultimately diagnosed with ischemic or hemorrhagic stroke, which would yield a negative predictive value of 88% (corresponding sensitivity of 95% and specificity of 25%). Our results also demonstrate that this biomarker-based approach is extremely sensitive to early ischemia and identified all stroke patients presenting within 3 hours of symptom onset. An additional validation cohort using manufactured and validated devices on a point-of-care platform demonstrated the feasibility of this approach to provide rapid diagnostic information on a point-of-care platform across different medical settings. To ensure that our results were as relevant to current clinical practice as possible, the diagnostic workup at each center was pursued as per the institutional standard of care. Virtually all patients received at least one CT or MRI. Reflective of current practice, acute diffusion/perfusion MRI protocols were only available in select centers.

Interestingly, aside from S100β, which is a marker of astrocyte activation, the biomarkers in this stroke panel are not specific to central nervous system tissues.19 For example, d-dimer is a crosslinked fibrin degradation product. Although previously demonstrated to be elevated after acute stroke,20–23 d-dimer is elevated in any clinical circumstance in which both clot formation and subsequent fibrinolysis are increased, including deep venous thrombosis, pulmonary embolism, disseminated intravascular coagulation, acute myocardial infarction, surgery, and trauma. MMP-9 belongs to a family of zinc-binding proteolytic enzymes, which are capable of degrading all components of the extracellular matrix, including collagen IV, laminin, and fibronectin. Although MMP-9 elevations have been observed in experimental and clinical stroke,24,25 MMP-9 activation is part of an inflammatory response and is implicated in various pathological conditions, including atherosclerosis, multiple sclerosis, tumor growth, and metastasis. BNP was first identified from porcine brain, although it became clear that the primary site of synthesis is in the heart.26 Increased myocardial wall stretch is a stimulus for cardiac natriuretic peptide secretion, and high BNP levels are indicative of heart failure and correlate with disease severity.27 Elevations in BNP have also been reported in the first 24 hours after ischemic stroke, although the exact mechanisms by which this occur remain undefined.16,28,29 Thus, although the majority of the markers used in this panel are not specific for cerebral ischemia, they represent different components of the ischemic cascade and when used in conjunction, they provide complementary information in the diagnosis of stroke.

The initial analysis assessed the ability of this model to discriminate stroke from nonstroke mimics. TIA values were intermediate between stroke and mimic, which is likely reflective of the fact that the clinical syndrome of TIA represents a continuum from fully reversible ischemia to small infarcts not associated with persistent clinical deficit. The question of whether this test can be used to stratify patients with TIA at high risk for subsequent stroke remains an active area of investigation as does the question of whether serial biomarker analysis might provide additional prognostic...
information to guide inpatient management or identify patients at high risk for in-hospital neurological deterioration.

There are significant limitations to this study that should be addressed. One difficulty inherent in the design of a stroke diagnostic study is the lack of a definitive reference standard. In this study, we used the standard clinical definition of stroke (persistence of symptoms >24 hours), although it is likely that in a proportion of patients, transient symptoms may have been associated with tissue injury. This is supported by the fact that in the secondary analysis of radiographic data, we found that 13% of patients classified with TIA had diffusion abnormalities on MRI. Mimic and TIA also had lower presenting NIHSS scores than patients with stroke, and future studies should clarify the incremental benefit of biomarker-based testing above and beyond clinical impression, which will be influenced by severity of deficit at presentation. Another challenge to this type of study is defining the appropriate clinical context in which the test would be used. Clearly, a biomarker-based diagnostic test would not replace the necessity for CT or other early imaging studies, and before contemplating any reperfusion strategy, neuroimaging must be performed to rule out intracranial hemorrhage. The discriminative capacity of current biomarker panel is also not adequate to be useful in isolation and must be taken in context with clinical assessment and judgment before making management decisions. Another potential limitation of this approach is the fact that none of the biomarkers are organ-specific and may be elevated in the setting of medical comorbidities. For example, elevated levels of BNP are associated with congestive heart failure and D-dimer with pulmonary embolism. One advantage to this panel approach is that the effect of any individual biomarker is minimized. However, future studies should examine whether test performance is comparable in these specific patient populations.

It is likely that the diagnostic accuracy of a biomarker-based approach will continue to improve with the identification of new candidate proteins associated with cerebral ischemia. However, even with the current limitations, the approach of using a biomarker-based test that optimizes sensitivity might be useful in the prehospital setting to route at-risk patients to facilities with stroke expertise. To specifically address this, future study design might define the additional value of a biomarker-based test when used in conjunction with a standard prehospital clinical screening tool. Similarly, such a test could be used in the early hospital setting to identify patients at highest risk for stroke and facilitate entry into a fast track care pathway, which would expedite immediate evaluation, laboratory testing, and imaging. Thus, although the diagnostic accuracy of the current panel is clearly imperfect, the current study demonstrates the feasibility of incorporating a biomarker-based point-of-care algorithm with readily available clinical data to aid in the early evaluation and management of patients at high risk for cerebral ischemia. Our results suggest that a biomarker-based assay greatly enhances the sensitivity of early noncontrast CT alone and that this approach is feasible as a point-of-care test in the emergency setting.

Appendix

BRAIN Study Group

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Disclosures

The authors served as site investigators in this study and have served as scientific consultants for Biosite Inc.

References


Clinical Usefulness of a Biomarker-Based Diagnostic Test for Acute Stroke: The Biomarker Rapid Assessment in Ischemic Injury (BRAIN) Study
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