Blood Biomarkers in the Diagnosis of Ischemic Stroke
A Systematic Review

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Background and Purpose—The diagnosis of ischemic stroke can be difficult. CT may be normal in the early stages of ischemic stroke or in patients with minor symptoms and MR is not always possible. Many blood markers have been proposed for the diagnosis of stroke in the acute setting.

Methods and Results—We have systematically reviewed the diagnostic literature and found 21 studies testing 58 single biomarkers and 7 panels of several biomarkers. Although all show either a high sensitivity or specificity, there are limitations in the design and reporting of all the studies that mean no biomarker can be recommended for use in clinical practice.

Conclusions—We make recommendations for the design and reporting of studies of diagnostic blood biomarkers in stroke.

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Key Words: biomarker | stroke | hemorrhagic stroke | ischemic stroke | diagnosis

A rapid blood test to confirm a clinical and imaging diagnosis of ischemic stroke (or to aid risk stratification in confirmed cases), based on a simple and low-cost near-patient technology, would be extremely useful. At the moment, the diagnosis of ischemic stroke is based on an experienced stroke clinician’s examination of the patient, supplemented by the results of brain imaging. However, in people who suddenly become unwell with a suspected stroke, the clinical assessment within the first hours is not always straightforward. Many patients with acute stroke are not assessed by a stroke specialist; the initial evaluation is often performed by a family practitioner, paramedic, or triage nurse. For those assessed in hospital, interpretation of brain imaging appearances can be difficult, as computerized tomography (CT) is often normal after the onset of ischemia and may remain normal in patients with mild ischemic strokes. MRI, though undoubtedly more sensitive in detecting ischemia than CT, especially in the diagnosis of mild stroke, is still not 100% sensitive or specific. MRI may not be feasible in acutely ill patients because they are restless, have a contraindication to MRI, or MRI may not be immediately available.

Achieving an accurate diagnosis quickly in patients with suspected acute stroke is extremely important. Patients with ischemic stroke, even with relatively mild symptoms, may be eligible for intravenous thrombolysis or other means of brain reperfusion if treatment can be started within a few hours of symptom onset. Patients who are not suitable for such acute treatments are at risk of early recurrent stroke; 8% of high risk patients have a recurrent stroke within the first 2 days. Prompt initiation of secondary preventative treatment can substantially reduce the risk of further stroke.

The development of blood biomarkers for ischemic stroke faces difficulties. The blood-brain barrier slows the release of brain tissue proteins into blood after stroke, delaying the release of glial and neuronal proteins. Many potential blood markers of cerebral ischemia and inflammation are found in other conditions that may mimic stroke, such as severe myocardial infarction and brain infection. Also, the volume of damaged tissue may not correlate with disability; small volumes of tissue damaged by ischemia in an “eloquent” area of the brain may lead to a more disabling deficit than a large volume of brain damaged by stroke in another part of the brain.

There has been a substantial investment in translational medical research programs to discover new diagnostic markers. We therefore felt that a systematic review of published reports to assess the accuracy of blood markers for the diagnosis of ischemic stroke was both relevant and timely. We aimed to describe the methodological quality of the studies, to compare the accuracy of diagnostic markers, and assess the extent to which methodological weaknesses might have biased diagnostic test accuracy.

Methods

Study Identification
We searched Medline and EMBASE from 1966 to March 15, 2007 for all studies of the use of diagnostic blood biomarkers in stroke. We maximized retrieval by searching using both generic biomarker terms and individual biomarkers (and their synonyms) obtained from...
a previous search of the literature.5–6 The search strategy included 13 terms for ischemic stroke, 4 for generic biomarkers, and 780 specific biomarker terms. Diagnostic studies were identified by searching for the words “sensitivity,” “specificity,” “likelihood ratio,” or “diagnosis” in the title or abstract and keywords. The full search strategy is listed in the supplemental materials (available online at http://stroke.ahajournals.org). The search was not restricted by language. We searched the reference lists of relevant papers, conference abstract books and our personal files. We also searched the internet for patents (using www.freepatentsonline.com and www.google.com) and papers citing each relevant paper with the Google Scholar tool (http://scholar.google.com/).

Studies were eligible for inclusion if they examined the ability of a single or several venous blood (not CSF) markers to discriminate between patients with ischemic stroke and a group without stroke (either controls without disease, stroke mimics, or other neurological diseases), or between ischemic and hemorrhagic stroke, where a cutoff value for the test had been calculated (or arbitrarily set) and there was sufficient information to fill a 2×2 contingency table. There was no minimum sample size for study inclusion. Both conference abstracts and published articles were included.

Data Extraction
Two authors (W.W. and M.T.) reviewed the list of titles and abstracts of potentially relevant articles independently; we then obtained full copies of articles meeting our eligibility criteria, and 2 authors independently extracted data from the eligible papers. Any disagreements were resolved by discussion. We assessed the quality of the study reports with a modified QUADAS instrument7 (web supplement). Where more than one cohort was examined within a study, the results for each cohort were extracted separately.

Statistical Analysis
We calculated 95% confidence intervals for the estimates of sensitivity and specificity in each cohort.8 We made no attempt to assess for publication bias, although this probably exists, as there are currently no well established methods to assess the scale and direction of this form of bias in studies of diagnostic test accuracy.9

Results
The MEDLINE/Embase search identified 3093 studies. A further 8 were found through reading conference reports and the reference lists of relevant articles. All the abstracts were read, and 70 publications were read in full. Twenty-one publications were relevant to the review—6 conference abstracts10–15 and 15 articles16–30 (supplemental materials).

Methodological Assessment
We used a modified QUADAS instrument to assess the quality of the reports of diagnostic biomarkers. The performance of blood biomarkers was examined in patients with suspected stroke—the clinical scenario for any stroke test—in 4/21 studies.14,25,27,31 The remaining studies compared cohorts of patients with a diagnosis of stroke with a control group. The sensitivity and specificity of a biomarker with a prespecified threshold for a positive test was examined in 6/21 studies.14,18,21,23,24,29 The remainder derived a diagnostic threshold cut-off value from the cohort examined. Of the 15 studies that used a data-dependent cutoff, none validated the sensitivity estimates in a separate cohort. Only 2/21 studies20,22 reported that the assessment of biomarker diagnostic accuracy was performed blinded to stroke status. All diagnoses of stroke appeared to be blinded to biomarker status.

The clinical comparisons in each study were different. Some studies classified TIA as an acute ischemic stroke,10,15,17–19,30 though others did not.16,25,27 However the number of patients symptomatic at the time blood was drawn was not defined, and there were no explicit means of measuring recovery at 24 hours. Most studies classified a patient as having a definite ischemic stroke only if they had both appropriate symptoms and a visible appropriate lesion on imaging, even though it is well recognized that many patients with definite stroke can have initially normal neuroimaging. Six studies classified subarachnoid hemorrhage (which generally has a very different clinical presentation to acute stroke) as a hemorrhagic stroke15,17–19,28 though most did not. Only one study14 examined a cohort of suspected stroke patients, and compared the performance of a panel of biomarkers to another assessment method (in this case a triage nurse)—both performed with a similar sensitivity and specificity.

Nine studies16,18–20,23,25–27,30 reported the delay between symptom onset and blood taking for biomarkers—the range was between 30 minutes and 5 days after stroke. Four16,23,25,26 only examined diagnostic performance within the first 24 hours of symptom onset. One study27 reported the sensitivity of a biomarker panel for a diagnosis of stroke at different time points, though there was no clear relationship between the delay to blood taking and sensitivity.

Markers Measured
The 21 studies tested 58 single biomarkers and 7 panels made up of several markers. The exact number of cohorts, and therefore the total number of patients involved, is difficult to calculate as some studies examine part cohorts from other studies included in the review (supplemental materials). The estimated upper limit is 2928 stroke patients and 1569 controls in 24 cohorts. There was sufficient information to extract 2×2 tables on 21 markers for the diagnosis of ischemic stroke versus not stroke or control (Figure 1). Of these markers, 5 had reported sensitivities over 90% (NDKA, PARK7, UFD-1, NMDA receptor [NR] 2 fragment, NR2A/B antibodies), and 14 had a specificity more than 90% (PARK 7/ RNA-BP, UFDP, NDKA, GSTP, ischaemia modified albumin [IMA], visin like protein [VLP-1], beta globin DNA, NR2 fragments, S100 beta, FABP, neurone specific enolase [NSE], NR2A/2B Ab, myelin basic protein [MBP], and thrombomodulin). For 5 biomarkers (S100 beta, MBP, thrombomodulin, NSE, and beta globin DNA) the specificity was defined by a 95% or 98% reference interval in subjects without disease. Five markers were tested in more than one cohort of patients, though only NSE and S100 beta were tested by different research groups. Only S100 beta was tested in different cohorts of patients using the same cutoff for a postive result (0.02 μg/L). Information about 5 panels of markers was extracted (Figure 2); in no case was the regression equation given for the marker panel (ie, the formula which permits a calculation of the probability of stroke if the results of the individual component biomarker tests are known). No panel of markers was validated in an independent cohort of patients.

Chiefly as a result of the very substantial clinical heterogeneity of the populations studied and heterogeneity of results, we did not perform a meta-analysis to derive an
Figure 1. Sensitivity and specificity of individual blood biomarkers, for the diagnosis of stroke (ischemic or any stroke) with 95% confidence intervals.
overall summary receiver operator curve for any of the individual markers or any of the panels of markers. Plots of sensitivity and specificity against study quality and types of control showed no clear relationship (data not shown).

Six individual markers and 2 panels of markers were assessed in 5 studies to determine the ability of blood biomarkers to distinguish between ischemic and hemorrhagic stroke. There was sufficient information to extract 2 tables on 6 markers and 4 panels (Figure 3). Most studies used the diagnosis of hemorrhagic stroke as the diagnosis of interest in a population of hemorrhagic and ischemic stroke patients. Two\textsuperscript{11,17} reported a positive test as a diagnosis of ischemic stroke in a mixed population, both with a high sensitivity and specificity. No single marker or panel of markers was reported in more than one cohort of patients.

**Discussion**

We set out to assess the utility of blood biomarker tests for improving the diagnosis of ischemic stroke in the acute phase. Most of the studies in this review reported biomarkers with high sensitivity and specificity which, if confirmed in validation studies, could be useful in clinical practice. However, all the blood biomarker studies had weaknesses in their methodology, which may explain the very high specificity and sensitivities reported. The main problems identified in this review were: small sample size; poor choice of reference standard (lesions required on imaging rather than clinical diagnosis supported by imaging); poor choice of controls (rarely reflecting the clinical setting in which the test would be used); data-dependent thresholds; and lack of validation.

**Recommendations for Design of Diagnostic Blood Biomarkers Studies**

The important diagnostic questions in the management of acute ischemic stroke can be summarized as follows:

- Does this patient have a stroke—especially if brain imaging is normal?
- Does this patient have an ischemic or a hemorrhagic stroke?
- What is the short term prognosis of patients with these acute symptoms? Is the prognosis sufficiently grave to merit more intensive diagnostic investigation requiring ionising radiation or administration of contrast (eg, CT angiography or CT perfusion?) or potentially risky treatments such as intravenous or intra-arterial thrombolysis?

**Diagnosis of Stroke in the Emergency Department Before Scanning or Expert Assessment**

If a test is designed for the diagnosis of ischemic stroke in unselected patients by clinicians in an emergency setting, then cohorts of suspected stroke patients for biomarker...
development or validation should be recruited by clinicians in the emergency department. In this systematic review, only 1\textsuperscript{14} attempted this explicitly; 3 other studies examined suspected stroke patients\textsuperscript{13,25,27} though it is unclear from the reports whether nonexpert clinicians recruited patients, and they also used healthy controls. Studies should evaluate whether biomarker tests perform better than the clinical judgment of nonexpert clinicians or prehospital screening tools such as the FAS test.\textsuperscript{32} Two useful tests for conditions other than stroke that are used in a similar setting are BNP for exclusion of diagnosis of congestive cardiac failure and D-dimer for the exclusion of a diagnosis of PE. Both are very sensitive. BNP has a sensitivity range between 68% to 98%\textsuperscript{33} and D-dimer ELISA has a sensitivity of 96%.\textsuperscript{34} D-dimer is used as part of a diagnostic algorithm that includes an initial assessment of the clinical probability of PE by means of a validated scale; biomarkers of stroke could be most useful when combined either with clinical judgment alone or with clinical judgment plus brain imaging.

**Differentiating Hemorrhagic From Ischemic Stroke Before Brain Imaging**

A blood test that aims to differentiate between patients with hemorrhagic and ischemic stroke would be of greatest utility before brain imaging (eg, during ambulance transfer to hospital). In studies to evaluate such a test, patients should be recruited at the earliest possible opportunity and before brain imaging is ordered. CT is a rapid test and is very good at identifying acute intracranial hemorrhage, so a biomarker test is likely to be redundant after imaging has been performed. However, in the studies in this review, which compared diagnostic test accuracy for distinguishing ischemic from hemorrhagic strokes, there were no patients in whom stroke was suspected, who turned out to have an alternative diagnosis on imaging. Hence the cohorts were too highly selected to be useful to assess the diagnostic utility of biomarkers in this particular setting.

**Supporting a Diagnosis of Ischemic Stroke in Patients With Normal CT Brain**

Most suspected stroke patients have a brain CT as their first investigation. When CT is normal, clinicians are often uncertain whether the diagnosis of stroke is secure enough to justify thrombolysis or the use of aggressive stroke preventative treatments. In patients with clinical symptoms of stroke, but a normal CT brain scan, a blood biomarker could perform well, as ischemic stroke may be the condition most likely to lead to a rise in specific proteins. Studies evaluating a blood biomarker in this setting should recruit patients in whom the clinicians are uncertain about the appearance of an imaging test, with blood drawn immediately after the CT. If more advanced CT and MR methods become easier in the emergency department, the diagnostic performance of blood biomarkers should be compared with these techniques.
Table 1. Recommendations for Good Quality Studies of Blood Biomarkers for Acute Stroke Diagnosis

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>Description</th>
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<tr>
<td><strong>Patients</strong></td>
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<td>Prospectively collected, consecutive patients with suspected stroke from an</td>
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<td>emergency setting</td>
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<td>Recruit patients in whom nonexpert clinicians suspect stroke</td>
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<tr>
<td>Recruit patients at a clear point in the diagnostic pathway for example,</td>
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<td>pre-hospital, emergency department, pre- or post-CT brain scan</td>
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<td>Record pre test probability of stroke, using either clinician judgment or a</td>
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<td>recognised clinical rating scale</td>
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<td>Define the delay between stroke symptom onset and initial assessment &amp; blood</td>
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<tr>
<td>sampling</td>
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<td>Define whether stroke symptoms still present at time of blood sampling</td>
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<tr>
<td><strong>Reference standard diagnosis</strong></td>
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<td>Expert clinical opinion, with appropriate brain imaging supplemented by data</td>
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<td>from other test results and the patient’s subsequent clinical course</td>
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<tr>
<td>Reference standard diagnosis made blind to biomarker status</td>
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<tr>
<td>Define stroke type—haemorrhagic or ischaemic</td>
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<tr>
<td><strong>Biomarker measurement</strong></td>
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<tr>
<td>Fully describe laboratory technique for marker measurement</td>
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<tr>
<td>Describe intra- and inter-assay reliability of tests</td>
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<tr>
<td>Fully describe logistic regression models of biomarkers</td>
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<td>Measurement blind to clinical status</td>
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<tr>
<td>Validate biomarker and diagnostic threshold in an independent cohort</td>
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<td>Give numerical value of threshold for a positive test</td>
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<td><strong>Reporting</strong></td>
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<td>Show raw data wherever possible</td>
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<td>Use the STARD guideline when preparing study reports</td>
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**Short-Term Prognosis After Nondisabling Stroke or TIA**

In patients with nondisabling stroke or TIA, short-term prognosis has a major influence on patient management: it informs decisions about admission to hospital, the intensity and speed of investigation, and the likelihood of successful early discharge. In this context, studies of diagnostic test accuracy should identify whether patients were still symptomatic when they were first assessed and how their recovery was measured. In patients with very short-duration symptoms, imaging tests are much less likely to give positive confirmation, so the place of imaging as part of the reference standard diagnosis of stroke or transient ischaemic attack (TIA) is altered, though imaging remains of value to exclude stroke mimics. In this context, expert clinical assessment and detailed clinical follow-up to detect recurrent clinical events are the key methodological determinants.

It is very difficult to define a test which can act as a reference standard test for the diagnosis of stroke; it is recognized that CT, MR, and even autopsy may be “negative,” even in patients considered to have a clinically definite acute stroke by all other criteria. Therefore the reference standard for a diagnosis of ischemic stroke remains a diagnosis by an expert clinician, based on the initial clinical features, supported by appropriate imaging, and the patient’s subsequent clinical course, with perhaps the need for repeated imaging on follow-up.
In this review, we found validation studies were limited. Only a few studies examined the same diagnostic threshold for a marker in more than one cohort. In one set of papers, different diagnostic thresholds have been calculated in different cohorts for the same biomarker to optimize sensitivity and specificity by ROC analysis, though the same diagnostic threshold was not examined in more than one cohort.\textsuperscript{15,18,19}

It has been proposed that biomarker development should take a linear path; identification of blood biomarker candidates either in animal or human models, before testing them in cohorts of stroke patients versus normal controls, before testing them in cohorts of suspected stroke patients.\textsuperscript{3,35} However, when considering the various “-omic” approaches to discovery of biomarker candidates, there are compelling reasons to use cohorts of patients with suspected disease (after all this is the context that the test will be used in) for the discovery phase.

The biomarkers identified in this review are expressed in diverse cell types and part of many different cellular processes (Table 2). Some proteins are found mainly in the nervous system: B-type neurotrophic growth factor, S100 beta, myelin basic protein, neuropeptide specific enolase, and visin like protein; others indicate endothelial processes: matrix metalloproteinase-9, thrombomodulin, vascular cell adhesion molecule, and von Willebrand factor. Some are not clearly linked to stroke pathogenesis, such as acrolein, nucleoside diphosphate kinase, antibodies to NR2A/2B, and glutathione S transferase. Two studies have examined RNA expression in peripheral blood leukocytes\textsuperscript{36,37} soon after stroke. The results of prediction analysis for microarrays (PAM) algorithm were different in the two studies; of a PAM gene list of 22 in one study, 29 in the other there was overlap only of N-acetyl neuraminic pyruvate lyase.

This review has a number of shortcomings. The absence of a suitable sensitive yet specific search strategy for diagnostic tests hampers the systematic review process. Searching the “gray” literature is very difficult to perform comprehensively, so it is likely that there are unpublished reports of biomarker sensitivity and specificity that have not been identified. In this study, the assessment of report quality was necessarily limited, as many of the studies were conference abstracts, with limited space to report the details of the methods of their studies. It is likely that the timing of sampling after stroke onset will affect the performance of a blood biomarker test; in these studies, it was not possible to analyze this because of a paucity of data.

We have demonstrated that the design of and reporting of studies of blood biomarkers for the diagnosis of ischemic stroke could be improved (Table 1). There are a number of blood biomarkers that perform impressively well in their development cohorts, however all of them need to be examined in unselected cohorts of patients with suspected stroke. None can be recommended yet for use in routine clinical practice.

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Disclosures

None.

References


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