

# Identifying blood biomarkers to improve the diagnosis of stroke

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**ABSTRACT** Blood biomarkers are useful for the management of many diseases and could be useful for doctors caring for stroke patients, if they accurately predicted a diagnosis or recurrence of stroke. In a series of studies, we systematically reviewed the blood biomarker literature in stroke, determined the performance of existing blood biomarkers for the diagnosis of stroke and examined the value of markers of inflammation to predict recurrent stroke and myocardial infarction.

**KEYWORDS** Biomarker, diagnosis, prognosis, stroke

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## INTRODUCTION

A rapid and accurate diagnosis of transient ischaemic attack (TIA) or stroke in patients arriving at the emergency department with suspected stroke is important in order to identify patients with ischaemic stroke for intravenous thrombolysis, to avoid unnecessary medication or investigations in those without stroke or TIA and to select patients at a high risk of early recurrent stroke, which might be prevented with antiplatelet, antihypertensive and statin drugs.

A rapid blood test to confirm a clinical or imaging diagnosis of stroke (or to aid risk stratification in confirmed cases), based on simple and low-cost near-patient technology, would be extremely useful. The diagnosis of stroke is currently made by an expert clinician, together with an accurate reading of early brain imaging. However, an expert clinical opinion very soon after symptom onset is not easily available to most stroke patients. Early brain imaging with computed tomography (CT) is often normal at the onset of cerebral ischaemia and more sensitive magnetic resonance (MR) brain imaging can be difficult, either because it is not immediately available<sup>1</sup> or because patients are too restless or have other contra-indications to MR scanning.<sup>2</sup>

Blood markers are used routinely in the management of other diseases. For example in patients presenting to hospital with suspected pulmonary embolus (PE), raised serum D-dimer – a marker of thrombolysis turnover – has a sensitivity of 96% and specificity of 44% for a PE diagnosis. Measurement of D-dimer is therefore

recommended as part of a clinical pathway to rule out the diagnosis of PE and helps to reduce inappropriate use of more resource-intensive diagnostic methods with higher hazard from radiation (e.g. CT pulmonary angiography).<sup>3</sup> If we could find blood markers in patients with stroke that were almost always 'positive', or almost always 'negative', then such markers might be useful in emergency department diagnostic pathways.

In a series of studies, we aimed to measure the diagnostic performance of clinical assessment compared with biomarker tests. We set out to identify potential blood biomarkers of stroke from a systematic literature review of previous studies, to assess the performance of the most promising biomarkers for the diagnosis of acute cerebrovascular disease in a prospective clinical cohort and to measure the performance of markers of inflammation in improving the prediction of recurrent stroke or myocardial infarction (MI) after stroke.

## PERFORMANCE OF EXISTING STROKE SCALES

About one in 20 patients presenting to emergency departments are suspected to have had a stroke or TIA.<sup>4</sup> Between a half and two-thirds of patients with suspected stroke have an eventual diagnosis of stroke or TIA, the proportion depending on the clinical context and experience of the assessors. The most frequent diagnoses in those patients without stroke are focal migraines, focal onset seizures, syncope, sepsis and functional neurological disorders.<sup>5–7</sup> Formalised assessment tools, based on easily measured clinical variables can help to identify stroke patients in the emergency department. Two of the best-known scales are the simple 'face arm

speech test' (FAST) and the more complex 'recognition of stroke in the emergency room' (ROSIER) instrument. The FAST was originally developed for stroke identification by paramedic ambulance crews<sup>8</sup> and the ROSIER for the identification of patients with stroke in emergency departments.<sup>6</sup>

For a blood marker to be useful for the diagnosis of stroke, a single marker should perform better than a simple clinical scale when measured in addition to such a simple clinical scale. As a baseline, I measured the performance of the FAST and the ROSIER in patients presenting to an emergency department with suspected stroke. I found that both scales had similar diagnostic characteristics (FAST vs ROSIER: sensitivity 82% vs 84%  $p=0.23$ , specificity 38% vs 41%  $p=0.42$ ), both performing less well than in their development cohort.<sup>6,7</sup> Each scale appeared to work equally well in patients with different levels of neurological impairment, at different times after symptom onset, and whether performed by a nurse or doctor.

## IDENTIFYING POTENTIAL BIOMARKERS

After stroke, molecules are released into the blood by both brain injury and the body's reaction to stroke. These molecules are released from damaged neurones and glia, by excitatory neurotransmission, systemic inflammation, cardiac strain, thrombosis, as well as many other physiological processes. Higher levels of these molecules have been associated with a diagnosis of ischaemic stroke alone or together with intracranial haemorrhage. In our systematic review of the published literature, there were 21 studies of biomarkers for the diagnosis of stroke, testing 58 single markers and seven panels of more than one marker.<sup>9</sup> Most of these markers were positively associated with a diagnosis of stroke and several of them in their development cohorts had a very high sensitivity and specificity for the diagnosis of stroke which – if confirmed – could be clinically useful.

However, it is likely that the impressive sensitivities and specificities that some of these markers demonstrated were, at least in part, due to the study design. There were a number of specific problems. First, the patients studied were not an unselected group of patients with suspected stroke early in the course of their illness. Instead, in the majority of the studies, the marker levels of patients with stroke were compared with levels in patients with other neurological diseases, or indeed normal subjects. This is likely to have inflated the sensitivity and specificity of the markers for a stroke diagnosis. Secondly, most studies derived their own threshold of marker levels which distinguished patients with and without stroke, although these thresholds were validated in other cohorts for only a few markers.

## ASSESSING PROMISING BLOOD BIOMARKERS

I selected blood markers for testing in a prospective diagnostic cohort study from two systematic reviews of the existing literature.<sup>9,10</sup> This approach has a number of advantages. By reviewing the entirety of the medical literature, it ensures that no potential candidate markers are ignored and that marker selection is unbiased and not dependent on any pre-existing prejudices of the study authors. It also ensures that the chosen markers can be appropriately measured in emergency departments, not relying on the development of new technology or statistical methods. However, this method, which examines a number of studies of different biomarkers in diverse patient populations, does not directly compare the expected comparative strength of association between markers and outcomes of interest. It also will not delineate new blood markers for which other approaches, for example those based on proteomics or biological plausibility, might be more suitable.

The study team measured 19 markers of pathophysiological processes known to be disturbed after stroke that showed promise and could be measured in its laboratories. We measured adiponectin, interleukin-10, intercellular adhesion molecule-1, C-reactive protein, interleukin-6, tumour necrosis factor-alpha, matrix-metalloproteinase-9, von Willebrand factor as markers of inflammation and D-dimer and tissue plasminogen activator as markers of thrombosis and fibrinogen turnover. S100 B and tau were measured as markers of cerebral damage and N-terminal pro-B-type natriuretic peptide and troponin-t as markers of cardiac strain.

To develop a representative cohort of patients with suspected stroke, we prospectively recruited patients seen at the Western General Hospital (WGH) in Edinburgh where an emergency department clinician suspected stroke or TIA as a cause of ongoing symptoms. Each patient was assessed using the FAST, and a member of the study team collected other clinical data. Blood was drawn from each patient as soon as possible after admission and stored at  $-80^{\circ}\text{C}$  until marker measurement.

The blood levels of two markers, tissue plasminogen activator and N-terminal pro-B-type natriuretic peptide, were significantly higher in patients with stroke than in patients with features mimicking stroke. However, neither marker improved the sensitivity or specificity of the FAST test for a diagnosis of stroke to a clinically or statistically significant degree. In two important subgroups, where a blood marker test might be particularly useful – patients with normal brain imaging and those in whom a member of the emergency department staff was substantially uncertain about the diagnosis of stroke – there was no evidence that blood markers significantly improved the diagnostic performance of the FAST. Interestingly, the addition of age to the FAST did make a

statistically significant improvement in the sensitivity and the specificity of the FAST, although this finding awaits prospective validation.

Panels of markers are particularly appealing for the diagnosis of many conditions. One panel has been developed for the diagnosis of stroke in the emergency department<sup>11</sup> that we attempted to replicate. In its development cohort, a panel of four markers (B-type natriuretic peptide, D-dimer, S100 and matrix-metalloproteinase-9) had a sensitivity and specificity of about 80% for the identification of patients with stroke. We found that at the published thresholds we could not replicate this diagnostic performance for the panel.<sup>12</sup>

## BLOOD MARKERS FOR THE PREDICTION OF RECURRENT STROKE AND MI AFTER STROKE

Inflammatory markers, particularly C-reactive protein, have been proposed as predictors for the occurrence of stroke or MI in healthy individuals. We investigated whether they might be useful for the prediction of recurrent stroke or MI in people after stroke. Data from the Edinburgh Stroke Study, which prospectively recruited stroke patients admitted to hospital or seen in outpatient clinics at the WGH, documented markers of inflammation soon after stroke onset (in 817 patients) and followed them for up to four years for fatal or non-fatal recurrent stroke, MI or fatal vascular events and death from any cause. The adjusted incidence of the outcome cluster 'recurrent stroke, MI or vascular death' after stroke was significantly higher if there were higher levels of interleukin-6 (75th to 25th centile: hazard ratio [HR] 1.56, 95% confidence interval [CI] 1.37–1.77), C-reactive protein (75th to 25th centile HR 1.08,

95% CI 1.04–1.11) and fibrinogen (75th to 25th centile HR 1.45, 95% CI 1.24–1.72). However, no inflammatory marker improved the prediction of recurrent vascular events over the variables age, prior TIA, MI/stroke and atrial fibrillation.<sup>13</sup>

## CONCLUSIONS

Although several markers of different physiological processes were associated with a diagnosis of TIA or stroke and with recurrent vascular events after stroke, no marker improved the diagnosis of stroke or prediction of recurrent events over established clinical variables. Major challenges in the diagnosis of stroke are both the variety of conditions that mimic stroke and the heterogeneity of stroke itself: strokes may be due to occlusion of small vessels in the deep white matter, blockage of larger cortical vessels or intracranial haemorrhage. It is very difficult to imagine a pathophysiological process that is unique to stroke or one of its subtypes and not found in any stroke mimic. For example, markers of brain damage rise in people with brain tumours, subdural haematomas and encephalitis, all of which can present as stroke mimics. This is quite unlike the situation for MI, where very few conditions other than cardiac ischaemia cause severe acute chest pain and lead to a rise in markers of myocardial necrosis.

Although there is room for improvement in their performance, the best method of rapid emergency clinical diagnosis of acute cerebrovascular disease remains the simplest: the assessment of a patient using one of the established stroke assessment scales.

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