Blood Markers for the Prognosis of Ischemic Stroke

A Systematic Review

William Whiteley, BM BCh, MRCP; Wei Li Chong, BM BCh; Anshuman Sengupta, BM BCh; Peter Sandercock, MD, FRCP

Background and Purpose—The performance of validated prognostic clinical models in acute ischemic stroke might be improved by addition of data on blood biomarkers.

Methods—We searched Medline and EMBASE from 1966 to January 2007 for studies of blood markers in patients with ischemic stroke and an assessment of outcome (death, disability, or handicap). We adopted several strategies to reduce bias.

Results—Studies were generally small (median number of subjects, 85; interquartile range, 49 to 184). Few had evidence of a sample size calculation (7 of 82 [9%]) or reported blinding to whether patients had stroke (21 of 82 [26%]). Of the 66 studies reporting a measure of association, 10 did not adjust for age or stroke severity, 14 adjusted for age, 7 adjusted for severity, and 35 adjusted for both; 30% (20 of 66) used a data-dependent threshold to predict good or bad outcome. There was evidence of within-study reporting bias and publication bias. Cardiac markers showed the most consistent association with poor outcome.

Conclusions—Blood biomarkers might provide useful information to improve the prediction of outcome after acute ischemic stroke. However, this review showed that many studies were subject to bias. Although some markers had some predictive ability, none of the studies was able to demonstrate that the biomarker added predictive power to a validated clinical model. The clinical usefulness of blood biomarkers for predicting prognosis in the setting of ischemic stroke has yet to be established. (Stroke. 2009;40:e380-e389.)

Key Words: biomarkers ■ CRP ■ fibrinogen ■ prognostic variables ■ stroke

The prediction of outcome after ischemic stroke is important for clinicians, patients, and researchers. The best validated clinical prognostic models1 are probably not accurate enough to predict outcome in individual patients with stroke. The performance of clinical models might be improved by blood markers of any of the pathological processes in acute ischemic stroke such as inflammation, hemostasis, neuronal or glial injury, and cardiac dysfunction. Markers of inflammation and hemostasis have been associated with ischemic stroke and heart attack in prospective cohorts of stroke-free people, and it is plausible that markers of neuronal, glial, and cardiac damage could aid prediction of poor outcome after stroke. To examine the relationship between blood markers of ischemic stroke and outcome after acute ischemic stroke, we report a systematic review of the available evidence.

Methods

Study Identification

We searched Medline and EMBASE from 1966 to January 2007 for studies in patients with acute ischemic stroke, which examined venous blood markers and assessed clinical outcome. The search strategy included 13 terms for ischemic stroke, 4 for generic biomarkers, and 780 specific biomarker terms. Prognostic studies were identified using high-sensitivity search terms2 together with common outcome measurements from stroke research (Rankin, National Institutes of Health Stroke Scale, Glasgow Outcome Scale). The electronic search strategy is available as supplemental appendix II.

Study Inclusion

Studies were eligible for inclusion if they: (1) reported results for patients with acute ischemic stroke (not transient ischemic attack); (2) assayed a venous blood marker not routinely measured in patients with ischemic stroke; (3) drew blood within the first week after stroke onset; and (4) measured outcome using death, disability, or handicap scales at a week or later after stroke onset. There was no study quality threshold or language restriction for inclusion. We considered only papers published in full because our resources were limited and abstracts did not contain sufficient detail to permit either methodological quality assessment or meta-analysis. We did not include studies that examined only the risk of subsequent stroke or myocardial infarction in patients with stroke or risk of stroke in asymptomatic study subjects.

Data Extraction

One author (W.W.) selected potentially eligible studies and these were reviewed by 2 of the other authors (A.S., W.L.C.). Data were

Received June 13, 2008; final revision received August 5, 2008; accepted September 8, 2008.

From the Division of Clinical Neurosciences, University of Edinburgh, Edinburgh, UK.

This work has not been published in full elsewhere, although it was presented to the European Stroke Congress 2008 and is available in abstract form Cerebrovasc. Dis. 2008;25(suppl 2):18.

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Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.108.528752

e380
extracted by one of the authors (W.W.) from all relevant studies; 2 other authors (A.S., W.L.C.) each re-extracted data from half of these and any disagreements were resolved by discussion. When we identified duplicate publication, we included the most informative cohort. We assessed study quality using the assay methods and study design sections of the REporting recommendations for tumor MARKer prognostic studies (REMARK) (supplemental Appendix III) reporting recommendations for prognostic tumor markers. To reduce bias in the assessment of studies with multiple blood draws and multiple outcomes, we prespecified which measures of association we would collect when more than one was given. When more than one biomarker was reported in a single study, we recorded data for each biomarker. When more than one outcome had been reported from a single study, we recorded the handicap measure (usually the modified Rankin Scale). If the handicap measure had been reported at more than one time point, we extracted the measure of effect taken closest to 3 months. When a single biomarker had been measured at multiple time points, we recorded the measure of effect for the sample taken soonest after the stroke. To ensure the review was comprehensive, and hence reduce the risk of introducing selection bias, we aimed to include studies irrespective of the method used to measure the association between biomarker and outcome. We noted the measure of association with outcome for each biomarker, which included ORs, hazard ratios (HRs), relative risk ratios, differences in mean marker levels between poor and good outcome, and correlation coefficients between outcome and marker levels. When unadjusted and adjusted measures of effect were reported, we took the most adjusted measure. After discussion with other experts (G.L., M.M.), biomarkers were classified by function and tissue of origin.

Analysis
Excel was used to draw plots of measures of effect (OR/HR/relative risk ratio) and standardized differences in means (difference in means/pooled SD) and their 95% CIs for each biomarker. After review of the data, summary estimation was felt to be inappropriate because of the differences in reported marker thresholds and units used in regression analysis. Vote counting of statistically significant studies, although superficially appealing, was rejected as an analysis method because of the risk of Type 2 error. No one class of marker had a stronger association with poor outcome than others, although the effect of cardiac markers (troponin or natriuretic peptides) on outcome was remarkably consistent. Within each class of marker, no one marker clearly performed better than the rest. Most information was available for the markers fibrinogen and C-reactive protein, although meta-analysis of measures of effect was precluded by differences in reported units and thresholds for both markers; however, both seemed to have a weak and positive association with poor outcome, consistent across OR, HR, differences in means, and correlation coefficients.

Results
The Medline/EMBASE search identified 6033 publications, and a further 61 were identified from reference lists. All abstracts were reviewed, and 232 papers were read in full; 82 studies measuring a total of 70 markers were relevant (Table, supplemental Appendix I). Lists of articles are available on request from the authors. Studies were from: China (2), Denmark (4), Estonia (one), Finland (3), France (one), Germany (10), Greece (5), Israel (2), Italy (9), Japan (one), Norway (2), Poland (2), South Korea (2), Spain (16), Taiwan (2), Turkey (2), the United Kingdom (12), and the United States (5).

Methodological Assessment
Studies were generally small (median sample size, 85; interquartile range, 49 to 184). Few studies reported a sample size calculation (7 of 82 [9%]), reported that the marker was measured blind to stroke status (21 of 82 [26%]), or examined an unselected cohort of patients with stroke (30 of 82 [37%]; Figure 1). Twenty (25%) studies excluded patients with cancer or infection, 9 (11%) patients with cancer, and 7 (8%) patients with infection. The median number of biomarkers measured per study was 2 (range, 1 to 9), markers were sampled at a median of one time point (range, 1 to 10), and the median number of outcomes measured was one (range, 1 to 24). Of the 66 studies that performed a regression analysis, 10 adjusted for neither age nor stroke severity, 14 for age only, 7 for stroke severity only, and 35 made adjustment for both. No study reported the additional predictive value of models containing one or more markers to validated clinical prognostic models or to particular clinical features. Of the 51 studies that developed a logistic regression model and reported the numbers of outcome events and adjustment variables, 24 did not have sufficient outcome events to develop a reliable model (recommended minimum >10 outcomes/variable).

There was marked asymmetry in a funnel plot (OR or HR against the standard error of log OR/HR), suggesting small study bias. This may represent differences in the methodology of small studies (which may have poorer methodology or patients with more severe stroke) or publication bias (ie, small studies showing little association between markers and outcome are less likely to be published).

Biomarkers as Prognostic Factors
Many markers showed an association with poor outcome whether by difference in means, regression coefficients, or relative measures of effect (Figures 2 and 3). Most correlations were weak (of 66 reported OR/HR/relative risk ratio, 37 <3) and so could be potentially explained by bias. Larger studies tended to have more modest measures of effect, and studies that calculated a threshold (34 of 64) had larger measures of effect. Thresholds were frequently data-derived. No one class of marker had a stronger association with poor outcome than others, although the effect of cardiac markers (troponin or natriuretic peptides) on outcome was remarkably consistent. Within each class of marker, no one marker clearly performed better than the rest. Most information was available for the markers fibrinogen and C-reactive protein, although meta-analysis of measures of effect was precluded by differences in reported units and thresholds for both markers; however, both seemed to have a weak and positive association with poor outcome, consistent across OR, HR, differences in means, and correlation coefficients.

Many studies that did not report a significant finding did not report the association of marker with outcome numerically; this could lead to bias in the assessment of those markers in which the majority of studies did not report significant findings. It is only for the following markers that the majority of the studies show a significant association between marker levels and outcome: adiponectin, brain natriuretic peptide, C-reactive protein, glial fibrillary acidic protein, glutamate, homocysteine, insulin-like growth factor, intercellular adhesion molecule, matrix metalloproteinase 9, platelet activator inhibitor, prothrombin fragments, soluble tumor necrosis factor receptors 1, tau, troponin i, troponin t, and thrombomodulin.

Discussion
Many of the blood markers in this review are associated with poor outcome after ischemic stroke. However, many publications have not established whether these markers add information to established clinical variables such as age or stroke severity let alone whether when added to a validated clinical prognostic scale that predictive power increases. Therefore, most markers are of uncertain clinical significance.
Table. Markers Reported in the Systematic Review

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Hypothesized Role in Ischemic Stroke</th>
<th>Hypothesized Origin in Ischemic Stroke</th>
<th>No. of Studies</th>
<th>Mean Study Size (Smallest, Largest)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated protein C resistance</td>
<td>Hemostasis</td>
<td>Adipocytes</td>
<td>1</td>
<td>219</td>
</tr>
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<td>Adiponectin</td>
<td>Anti-inflammatory</td>
<td>Adipocytes</td>
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<td>164</td>
</tr>
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<td>α-2 antiplasmin</td>
<td>Antifibrinolysis</td>
<td>Liver</td>
<td>1</td>
<td>63</td>
</tr>
<tr>
<td>Anticardiolipin antibodies</td>
<td>Hemostasis</td>
<td>B lymphocytes</td>
<td>1</td>
<td>300</td>
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<td>Antithrombin III</td>
<td>Anticoagulant</td>
<td>Endothelium</td>
<td>1</td>
<td>55</td>
</tr>
<tr>
<td>Atrial natriuretic peptide</td>
<td>Cardiac</td>
<td>Atrial myocardium</td>
<td>2</td>
<td>44 (37, 51)</td>
</tr>
<tr>
<td>β-thromboglobulin</td>
<td>Platelet</td>
<td>Platelets</td>
<td>2</td>
<td>71 (70, 72)</td>
</tr>
<tr>
<td>Brain-derived neurotrophic factor</td>
<td>Neurotrophic</td>
<td>Neurones, renal, retinal</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Brain natriuretic peptide</td>
<td>Cardiac</td>
<td>Atrial and ventricular myocardium</td>
<td>4</td>
<td>107 (51, 175)</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Anti-inflammatory/stress/neuronal survival</td>
<td>Adrenal cortex</td>
<td>4</td>
<td>90 (34, 184)</td>
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<tr>
<td>C-reactive protein</td>
<td>Inflammation</td>
<td>Liver</td>
<td>14</td>
<td>125 (11,467)</td>
</tr>
<tr>
<td>D-dimer</td>
<td>Hemostasis</td>
<td>Clot breakdown</td>
<td>8</td>
<td>118 (46, 231)</td>
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<td>Endothelin-1</td>
<td>Vasoconstrictor</td>
<td>Endothelium</td>
<td>2</td>
<td>69 (37, 101)</td>
</tr>
<tr>
<td>Factor VIIc</td>
<td>Hemostasis</td>
<td>Liver</td>
<td>2</td>
<td>141 (63, 219)</td>
</tr>
<tr>
<td>Factor VIIIc</td>
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<td>Liver</td>
<td>2</td>
<td>121 (70, 171)</td>
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<td>Hemostasis</td>
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<tr>
<td>Factor XIII</td>
<td>Hemostasis</td>
<td>Liver</td>
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<td>Ferritin</td>
<td>Inflammation</td>
<td>Astrocytes/glia/liver</td>
<td>4</td>
<td>95 (51, 162)</td>
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<tr>
<td>Fibrinogen</td>
<td>Hemostasis</td>
<td>Liver</td>
<td>14</td>
<td>156 (22, 469)</td>
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<tr>
<td>Fibrinopeptide A</td>
<td>Hemostasis</td>
<td>Cleaved from fibrinogen I</td>
<td>2</td>
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<td>GABA</td>
<td>Neurotransmitter</td>
<td>Neurones</td>
<td>2</td>
<td>113</td>
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<tr>
<td>GFAP</td>
<td>Glial protein</td>
<td>Glia</td>
<td>1</td>
<td>53</td>
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<tr>
<td>Glutamate</td>
<td>Neurotransmitter</td>
<td>Neurones</td>
<td>5</td>
<td>100 (46, 128)</td>
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<tr>
<td>Glycine</td>
<td>Neurotransmitter</td>
<td>Neurones</td>
<td>2</td>
<td>121 (113, 128)</td>
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<tr>
<td>Homocysteine</td>
<td>Endothelial apoptosis</td>
<td>?Macrophages</td>
<td>1</td>
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<tr>
<td>Insulin-like growth factor</td>
<td>Neuroprotective</td>
<td>Most tissues</td>
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<td>85</td>
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<td>Intercellular adhesion molecule</td>
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<td>81.5 (50, 113)</td>
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<td>Interleukin 1-β</td>
<td>Inflammation</td>
<td>Endothelium</td>
<td>4</td>
<td>68 (18, 162)</td>
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<tr>
<td>Interleukin 1 receptor antagonist</td>
<td>Anti-inflammatory</td>
<td>Lymphocytes/macrophages</td>
<td>3</td>
<td>102 (41, 162)</td>
</tr>
<tr>
<td>Interleukin 4</td>
<td>Inflammation</td>
<td>CD4 T cells/macrophages</td>
<td>1</td>
<td>231</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>Inflammation</td>
<td>CD4 T cells/macrophages</td>
<td>10</td>
<td>101 (11, 231)</td>
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<td>Interleukin 8</td>
<td>Inflammation</td>
<td>Endothelium</td>
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<td>50</td>
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<tr>
<td>Interleukin 10</td>
<td>Anti-inflammatory</td>
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<td>Iron</td>
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<td>Matrix Metalloproteinase 2</td>
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<td>Matrix Metalloproteinase 9</td>
<td>Inflammation</td>
<td>Endothelium</td>
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<tr>
<td>Matrix Metalloproteinase 1</td>
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<td>Endothelium</td>
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<td>50</td>
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<td>Monocyte chemoattractant protein</td>
<td>Inflammation</td>
<td>Endothelium</td>
<td>1</td>
<td>50</td>
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</table>

(Continued)
The association of marker levels with poor outcome after ischemic stroke are in general higher than the association of the same markers with other outcomes, for example with the recurrence of vascular disease in patients with prior vascular disease. This stronger association could be because marker levels in patients soon after ischemic stroke predict (1) an increased risk of myocardial infarction or stroke over and above people with stable vascular disease; (2) markedly reduced brain recovery; (3) increased risk of other complications of stroke; or (4) biased studies.

### Table. Continued

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<th>No. of Studies</th>
<th>Mean Study Size (Smallest, Largest)</th>
</tr>
</thead>
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<tr>
<td>Myelin basic protein</td>
<td>Glial damage</td>
<td>Glia</td>
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<td>Neurone specific enolase</td>
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<td>Neurone</td>
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<td>Adrenal medulla</td>
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<td>75</td>
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<td>Nucleosomes</td>
<td>Cell damage</td>
<td>All cells</td>
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<td>Inflammation</td>
<td>Platelets</td>
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<td>73.5 (45, 102)</td>
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<td>Endothelial/liver</td>
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<td>70 (44, 102)</td>
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<td>Protein S</td>
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<td>S100-β</td>
<td>Glial damage</td>
<td>Glia</td>
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<td>100 (26, 359)</td>
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<td>Selectin</td>
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<td>Endothelium</td>
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<td>238</td>
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<td>Serum amyloid A</td>
<td>Inflammation</td>
<td>Liver</td>
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<td>203</td>
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<td>Soluble intracellular adhesion</td>
<td>Inflammation</td>
<td>Endothelium</td>
<td>1</td>
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<tr>
<td>molecule 1</td>
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<td>Soluble TNF-α receptor 1</td>
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<td>Modulate NMDA</td>
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<td>53</td>
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<td>Hemostasis</td>
<td>Coagulation</td>
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<td>Endothelium</td>
<td>3</td>
<td>445 (359, 510)</td>
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<td>Fibrinolysis</td>
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<td>342 (219, 465)</td>
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<td>Cardiac myocyte</td>
<td>Cardiac myocyte</td>
<td>4</td>
<td>230 (175, 330)</td>
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<tr>
<td>Troponin T</td>
<td>Cardiac myocyte</td>
<td>Cardiac myocyte</td>
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<td>209 (172, 279)</td>
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<td>Tumor necrosis factor-α</td>
<td>Inflammation</td>
<td>CD4 T cells</td>
<td>8</td>
<td>124 (18, 231)</td>
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<td>Uric acid</td>
<td>Purine catabolism</td>
<td>?All cells</td>
<td>2</td>
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<td>von Willebrand Factor</td>
<td>Hemostasis</td>
<td>Endothelium</td>
<td>4</td>
<td>127 (46, 219)</td>
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</table>

The association of marker levels with poor outcome after ischemic stroke are in general higher than the association of the same markers with other outcomes, for example with the recurrence of vascular disease in patients with prior vascular disease. This stronger association could be because marker levels in patients soon after ischemic stroke predict (1) an increased risk of myocardial infarction or stroke over and above people with stable vascular disease; (2) markedly reduced brain recovery; (3) increased risk of other complications of stroke; or (4) biased studies.

### Recurrence of Myocardial Infarction or Stroke

In patients with minor stroke or transient ischemic attack, the risk of stroke recurrence is highest in the first few weeks after stroke. However, in patients with more severe stroke, it is difficult to identify stroke recurrence. The association between blood markers and poor outcome after stroke might arise because of an association with an increased risk of stroke recurrence or myocardial infarction.

Most blood markers have a modest association with the development of coronary heart disease in population-based prospective studies of blood biomarkers. The ORs from meta-analysis include adiponectin (OR, 0.84; 95% CI, 0.7 to 1.01) top third to bottom third; d dimer (OR, 1.7; 95% CI, 1.3 to 2.2) top third to bottom third; ferritin (OR, 1; 95% CI, 0.8 to 1.3) cutoff 200; intercellular adhesion molecule (OR, 1.21; 95% CI, 0.95 to 1.55) top third to bottom third; C-reactive protein (OR, 1.7; 95% CI, 1.6 to 2.0) top third to bottom third; fibrinogen (OR, 1.8; 95% CI, 1.6 to 2.0) top third to bottom third; and brain natriuretic peptide (OR, between 1.3 and 5.7 for survival).

In patients with vascular disease, the association between marker levels and incident stroke was modest with CIs overlapping with those from the prospective cohorts of those asymptomatic at baseline: fibrinogen (OR, 1.34; 95% CI,
1.13 to 1.60) comparing the group above to the group below the median, and C-reactive protein (HR, 2.16; 95% CI, 1.32 to 3.53) comparing highest with lowest tertiles.

In stroke-free patients with unstable angina, inflammatory and hemostatic markers also have a modest association with recurrence of coronary events: C-reactive protein (relative risk ratio per SD, 1.45; 1.15 to 1.83 per SD) and serum amyloid A (relative risk ratio per SD, 1.14; 0.99 to 1.44 per SD).

The associations between D-dimer, fibrinogen, C-reactive protein, ferritin, interleukin-6, and serum amyloid A and a poor outcome after stroke were also modest in studies in which no threshold was calculated. However, many other markers have much larger measures of effect; this could be due to a much stronger association of these markers with myocardial infarction and stroke recurrence than previously recognized, or there is another mechanism responsible for their association with poor outcome.

Stroke Recovery
An association between blood markers and poor outcome could arise because markers predict poor brain healing or the development of other stroke complications. For example, inflammatory markers after stroke are associated with increased lymphocyte infiltration and poorer recovery of brain tissue in experimental stroke, the release of excitatory neurotransmitters such as glutamate as part of the ischemic cascade can increase apoptosis and neuronal and glial death, and higher levels of anti-inflammatory markers might indicate strengthened intrinsic antiatherosclerotic mechanisms. Increases in neurotrophic or neuroprotective markers may be associated with improved neuronal recovery. Raised inflammatory markers are also associated with other conditions responsible for poor outcome such as patients who already have either cancer or deep venous thrombosis.

Cardiac markers (natriuretic peptides and troponins) show a consistent association with poor outcome. Because cardio-embolic stroke seems to have a poorer outcome than other stroke subtypes, a possible explanation could be an association of cardiac markers with this stroke subtype. However, only brain natriuretic peptide (and not troponin I) has been associated with cardioembolic rather than other stroke subtypes. Cardiac dysfunction simultaneously or shortly before the stroke or pre-existing cardiac disease could also account for the association, although an association between marker levels and electrocardiographic changes was seen in only some studies.

Another potential role of blood biomarkers is to distinguish groups of patients most likely to benefit from, or to be harmed by, a particular therapy. In the context of acute ischemic stroke, thrombolytic therapy is the most relevant. Only one randomized, controlled trial has reported on this, although it did not report on the presence of a treatment effect biomarker level interaction for the markers measured (myelin basic protein, neurone specific enolase, and S100). Several studies, based on groups of patients, all of whom had received thrombolytic therapy, reported on markers that might predict posttreatment cerebral hemorrhage, but the outcomes reported in these papers were largely radiological and no study reported results from a nontreated comparison group for comparison.

There are several strong clinical predictors for poor outcome after stroke, for example, stroke severity, premorbid disability, and age, which may themselves be strongly associated with marker levels. Many studies of stroke prognosis, although by no means all, adjust for these potential confounders. However, adjusting for stroke severity is imperfect, and therefore residual confounding for stroke severity is likely to account for at least some of the association between markers and poor outcome.

Bias in Studies
Many studies calculated a threshold level of the marker for the prediction of poor outcome, although this approach has flaws. When there is an association between marker level and outcome, this is in most cases continuous rather than dichot-
Figure 2. Measures of association of venous blood biomarkers and poor outcome with 95% CIs. +, adjustment for age or stroke severity; ***, adjustment for age and stroke severity; ****, adjustment for age, stroke severity, and other factors. Data points are proportional to study size.
Figure 2. Continued.
omous. Calculating thresholds in a data-dependent fashion (for instance by receiver operating characteristic curve analysis) to optimize the prognostic performance of a blood biomarker can lead to implausibly large effect sizes. Although thresholds or reference intervals can be useful in clinical practice, they must be validated by applying the calculated marker threshold in a new separate cohort before being adopted into clinical practice. Unfortunately, data-derived thresholds are rarely repeated, and when they are repeated, they often are not confirmed by other groups of researchers.31

The lack of sample size calculations in most studies suggests that the studies were performed opportunistically rather than with a careful, prespecified study design. Sample

<table>
<thead>
<tr>
<th>Inflammatory</th>
<th></th>
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<tbody>
<tr>
<td>C-reactive protein</td>
<td>Kocer 2005</td>
<td>mg/L</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Davalos 1994</td>
<td>µg/L</td>
</tr>
<tr>
<td></td>
<td>Ergooglou 2002</td>
<td>µg/L</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>Viola 2000</td>
<td>pg/mL</td>
</tr>
<tr>
<td>Matrix Metalloproteinase - 2</td>
<td>Kim 2006</td>
<td>units</td>
</tr>
<tr>
<td>Matrix Metalloproteinase - 9</td>
<td>Kim 2006</td>
<td>units</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Cha 2002</td>
<td></td>
</tr>
<tr>
<td>Intracellular Adhesion Molecule 1</td>
<td>Wang 2006</td>
<td>ng/mL</td>
</tr>
<tr>
<td>Tumour necrosis factor alpha</td>
<td>Viola 2000</td>
<td>pg/mL</td>
</tr>
<tr>
<td>Vascular cell adhesion molecule</td>
<td>Wang 2006</td>
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<td>D-dimer</td>
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<td>mg/L</td>
</tr>
<tr>
<td></td>
<td>Szajzko 2006</td>
<td>µg/L</td>
</tr>
<tr>
<td>Factor VIIIC</td>
<td>Landi 1987</td>
<td>% activity</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Viola 2003</td>
<td>g/L</td>
</tr>
<tr>
<td></td>
<td>Viola 2000</td>
<td>g/L</td>
</tr>
<tr>
<td></td>
<td>Lund 1987</td>
<td>mg/dL</td>
</tr>
<tr>
<td></td>
<td>Zuliani 2006</td>
<td>mg/dL</td>
</tr>
<tr>
<td></td>
<td>Audiebert 2004</td>
<td>mg/dL</td>
</tr>
<tr>
<td></td>
<td>Wang 2006</td>
<td>mg/dL</td>
</tr>
<tr>
<td></td>
<td>Davalos 1997</td>
<td>mg/dL</td>
</tr>
<tr>
<td></td>
<td>Davalos 2006</td>
<td>mg/dL</td>
</tr>
<tr>
<td>von Willebrand Factor</td>
<td>Audiebert 2004</td>
<td>% activity</td>
</tr>
<tr>
<td></td>
<td>Landi 1987</td>
<td>% activity</td>
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<tr>
<th>Excitatory neurotransmitters</th>
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<tbody>
<tr>
<td>Glutamate</td>
<td>Audiebert 2004</td>
<td>µmol/L</td>
</tr>
<tr>
<td></td>
<td>Serena 2001</td>
<td>µmol/L</td>
</tr>
<tr>
<td></td>
<td>Castillo 1997</td>
<td>µmol/L</td>
</tr>
<tr>
<td>Glycine</td>
<td>Serena 2001</td>
<td>µmol/L</td>
</tr>
<tr>
<td></td>
<td>Castillo 1997</td>
<td>µmol/L</td>
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<tr>
<td>Homocysteine</td>
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<td>pmol/L</td>
</tr>
<tr>
<td>Neurone Specific Enolase</td>
<td>Kim 2006</td>
<td>ng/dL</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor</td>
<td>Lip 2002</td>
<td>IU/mL</td>
</tr>
<tr>
<td>Selectin</td>
<td>Wang 2006</td>
<td>ng/mL</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>Chamorro 2002</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

Figure 3. Standardized differences in means: (mean level in poor outcome—mean level in good outcome)/pooled SD, and 95% CIs. Data points are proportional to study size.
sizes need to be large to allow the detection of the moderate effect sizes that can be realistically expected and to overcome the problems of multiple comparisons in univariate analysis. Very often this problem is compounded by the measurement of biomarkers at multiple time points and the measurement of multiple clinical outcomes. When a logistic regression model is used to analyze study results, as a rule of thumb, sample size calculations should aim for at least 10 outcomes per variable to be entered in the final model. Known prognostic variables for poor outcome such as age, stroke severity, and premorbid disability should be forced into logistic regression models, because their association with poor outcome after stroke is robust.

Biomarker measurement is subject to both inter- and intrapatient random variation. Different batches of the same measurement kit and of different kits can have different performance for the same marker. Very few studies have attempted to compare the performance of different kits to predict outcome.

Publication bias probably exists, because the funnel plot showed marked asymmetry, although other reasons for larger effect sizes in smaller studies such as less methodological rigor or increased stroke severity and stronger association with outcome in smaller studies are also possible. We have attempted to minimize within-study reporting bias by reporting both studies in which a relative measure of effect (Figure 2) and a difference in means was reported (Figure 3).

Limitations of Systematic Reviews of Prognostic Variables
Assessing the quality of prognostic studies is difficult. There is no generally accepted scale to assess the quality of reports comparable to the consolidated standards of reporting trials (CONSORT) guidelines for randomized, controlled trials and the standards for the reporting of diagnostic accuracy studies (STARD) guidelines for studies of diagnostic tests. There is a paucity of evidence to support many of the suggested measures of quality of prognostic studies such as well-defined inception cohorts.

There is no widely accepted way of correcting for publication bias, and furthermore within-study reporting bias becomes a problem when many markers and outcomes have been measured. Frequently, markers are reported to be “nonsignificant” without an estimate of the measure of effect with CIs. When thresholds have been chosen, they usually differ between studies. The interval for analysis in multiple regression analyses may be for different variables in different studies.

Conclusions
Blood biomarkers may be useful in acute ischemic stroke either by suggesting possible mechanisms for the etiology of poor outcome or as part of a clinically useful prognostic scale. The reported associations between particular markers and outcome may arise because markers predict recurrent stroke or myocardial infarction, stroke complications, or new diseases such as cancer. There is a sufficient risk of bias in the studies we assessed that really reliable conclusions cannot be drawn from the current literature.

Until we have further evidence on the ideal components of a prognostic study, it would seem reasonable that the following are attempted to minimize bias: a well-defined cohort of patients are assembled at an early and uniform stage in the disease, define subsequent treatment (eg, thrombolysis, stroke unit care), and multiple logistic regression should include known clinical prognostic variables (like age and stroke severity) whether or not they reach statistical significance in univariate analysis. To be clinically useful, markers should add predictive power to a validated clinical model and should be tested in a separate cohort. Although the REMARK guidelines were initially reported for prognostic markers of cancer, the recommendations stand for all other fields of measurement of prognostic markers, including stroke, and we urge authors to read them before designing and reporting their studies.

Individual patient data meta-analysis of the best quality studies from this review could help to improve the precision of the measures of association between blood markers and poor outcome. However, for many markers, larger, better designed studies are needed before this can be attempted.

Acknowledgments
We are grateful for expert advice from Dr Malcolm MacLeod, Prof Gordon Lowe, Dr Cathie Sadlow, Dr Ann Rumley, and Dr Steff Lewis.

Disclosures
W.W. holds a Clinical Academic Training Fellowship from the Chief Scientist’s Office, Scottish Executive. The funder had no involvement in the design, data collection, analysis, interpretation of data, or writing of the final report.

References


Blood Markers for the Prognosis of Ischemic Stroke: A Systematic Review
William Whiteley, Wei Li Chong, Anshuman Sengupta and Peter Sandercock

Stroke. 2009;40:e380-e389; originally published online March 12, 2009;
doi: 10.1161/STROKEAHA.108.528752

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Data Supplement (unedited) at:
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WEB APPENDIX 1: Studies included in the systematic review


(24) Efstathiou SP, Tsioulos DI, Tsiakou AG, Gratsias YE, Pefanis AV, Mountokalakis TD. Plasma adiponectin levels and five-year survival after first-ever ischemic stroke.[see comment]. *Stroke* 2005 September;36(9):1915-9.


Rallidis LS, Vikelis M, Panagiotakos DB, Liakos GK, Krania E, Kremastinos DT. Usefulness of inflammatory and haemostatic markers to predict short-term risk for death in middle-aged ischaemic stroke patients. Acta Neurologica Scandinavica 2000;0(0):???


WEB APPENDIX 2: ELECTRONIC SEARCH STRATEGY

MEDLINE

1. cerebrovascular disorders/ or basal ganglia cerebrovascular disease/ or exp brain ischemia/ or carotid artery diseases/ or carotid artery thrombosis/ or carotid stenosis/ or cerebrovascular accident/ or exp brain infarction/ or exp hypoxia-ischemia, brain/ or exp intracranial arterial diseases/ or exp “intracranial embolism and thrombosis”/

2 ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemisph$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.

3 (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.

4 1 or 2 or 3

5 exp biological markers/

6 biomarker$.tw.

7 (((biochemical or clinical or immun$ or laboratory or biologic$ or serum or surrogate or viral) adj6 marker$).tw.

8 (((blood or plasma) adj6 marker$).tw.

9 Fructose-Bisphosphate Aldolase/ or activins/ or inhibin-beta subunits/ or Inhibins/ or Adiponectin/ or Antiplasmin/ or alpha-Macroglobulins/ or alpha 1-antichymotrypsin/ or alpha 1-antitrypsin/ or Orosomucoid/ or Peptidyl-Dipeptidase A/ or Fibroblast Growth Factor 2/ or angiotensins/ or angiotensin i/ or angiotensin ii/ or angiotensin iii/ or Antithrombin III/ or apolipoproteins/ or apolipoprotein a/ or apolipoprotein a-i/ or apolipoprotein a-ii/ or apolipoproteins b/ or apolipoprotein b-48/ or apolipoprotein b-100/ or apolipoproteins c/ or apolipoprotein c-i/ or apolipoprotein c-ii/ or apolipoproteins d/ or apolipoproteins e/ or apolipoprotein e2/ or apolipoprotein e3/ or apolipoprotein e4/ or beta 2-Glycoprotein I/ or Natriuretic Peptide, Brain/ or Brain-Derived Neurotrophic Factor/ or caspases, effector/ or caspase 3/ or caspase 6/ or caspase 7/ or caspase 14/ or Cathepsin B/ or antigens, cd40/ or cd40 ligand/ or Ceruloplasmin/ or Chitinase/ or Cholesterol Ester Transfer Proteins/ or Chromogranin A/ or Clusterin/ or Fibronecctins/ or Chimerin Proteins/ or Chimerin 1/ or complement system proteins/ or anaphylatoxins/ or complement activating enzymes/ or complement c1/ or complement c2/ or complement c3/ or complement c4/ or complement c5/ or complement c6/ or complement c7/ or complement c8/ or complement c9/ or complement factor b/ or complement inactivator proteins/ or complement membrane attack complex/ or properdin/ or C-Reactive Protein/ or Fibrin Fibrinogen Degradation Products/ or phosphopyruvate hydratase/ or tau-crystallins/ or cell adhesion molecules/ or antigens, cd22/ or antigens, cd24/ or antigens, cd31/ or antigens, cd146/ or antigens, cd164/ or cadherins/ or carcinoembryonic antigen/ or cd4 immunoadhesins/ or cell adhesion molecules, neuronal/ or integrin alpha beta2/ or intercellular adhesion molecule-1/ or receptors, lymphocyte homing/ or selectins/ or vascular cell adhesion molecule-1/ or endothelins/ or endothelin-1/ or endothelin-2/ or endothelin-3/ or...
Erythropoietin/ or E-Selectin/ or Factor XI/ or Factor IX/ or Factor XII/ or Factor V/ or Factor VII/ or Factor VIII/ or Factor XIIa/ or exp Interleukins/ or exp Fibrinogen/ or Antigens, CD95/ or exp Ferritins/ or fibrinopeptide a/ or fibrinopeptide b/ or exp Fibronectins/ or exp Follistatin-Related Proteins/ or exp Follistatin/ or exp Fatty Acids, Nonesterified/ or exp Glial Fibrillary Acidic Protein/ or exp Glutathione Transferase/ or Granulocyte-Macrophage Colony-Stimulating Factor/ or exp Selectins/ or Platelet Glycoprotein GPIIb-IIIa Complex/ or growth hormone/ or human growth hormone/ or exp Haptoglobins/ or Hemopexin/ or Heparin Cofactor II/ or exp Intercellular Adhesion Molecule-1/ or exp Immunoglobulin G/ or Laminin/ or Leptin/ or Macrophage Colony-Stimulating Factor/ or Malondialdehyde/ or exp matrix metalloproteinases, secreted/ or exp Monocyte Chemoattractant Proteins/ or Myelin Basic Proteins/ or Peroxidase/ or exp S100 Proteins/ or Neurotrophin 3/ or 9 Nicotin Oxide/ or Nucleoside-Diphosphate Kinase/ or Aryldialkylphosphatase/ or Phosphoglcerate Mutase/ or Pregnancy-Associated Plasma Protein-A/ or Plasminogen Activator Inhibitor 1/ or Plasminogen/ or Plasminogen Activator Inhibitor 2/ or Platelet Activating Factor/ or Antigens, CD31/ or Platelet-Derived Growth Factor/ or Platelet Factor 4/ or Protein C/ or Protein S/ or Prothrombin/ or Resistin/ or Plasminogen Inactivators/ or Platelet Activation/ or tau Proteins/ or Thrombin/ or Thrombomodulin/ or Thromboplastin/ or TUMOR NECROSIS FACTOR-ALPHA/ or Transforming Growth Factor beta/ or Vascular Endothelial Growth Factor A/ or Vitronectin/ or von Willebrand Factor/ 10 (Aldolase A or aldolase b or aldolase c or fructose bisphosphonate aldolase or activin$ or inhibin$ or adiponectin or adipocyte specific secretory protein or gelatine binding protein or adipocyte complement related protein or alpha antiplasmin or Alpha-2-antiplasmin precursor or Alpha-2-AP or Alpha-2-PI or Alpha-2-plasmin inhibitor or pigment epithelium derived factor or plasmin inhibitor alpha 2 or alpha-macglobulin$ or alpha 2M or antichymotrypsin or alpha 1-antichymotrypsin or alpha 1-antitrypsin or Seromucoid or serum sialomucin or alpha 1-acid glycoprotein or alpha 1-acid seromucoid or a 1-acid seromucoid or acid alpha 1-glycoprotein or alpha 1-acid glycoprotein or alpha 1-acid glycoprotein acute phase or alpha 1-glycoprotein acid or angiotensin converting enzyme or cd143 or cd143 or kininase ii or angiotensin i-converting enzyme or carboxycathepsin or dipeptidyl peptidase a or kininase a or ACE or kininase 2 or Dipeptidyl carboxypeptidase I or basic fibroblast growth factor or fibroblast growth factor, basic or hbfg-2 or cartilage-derived growth factor or class ii heparin-binding growth factor or fgf-2 or fbg2 or fibroblast growth factor-2 or heparin-binding growth factor class ii or prostate epithelial cell growth factor or prostatropin or Fibroblast Growth Factor 2 or heparin-binding growth factor 2 or angiotensin$ or antithrombin ii or heparin cofactor i or at iii or antithrombin iii, human plasma or antithrombin iii-alpha or atenativ or baxter brand of antithrombin or bayer brand of antithrombin or factor xa inhibitor or grifols brand of antithrombin or heparin co-factor i or pharmacia brand of antithrombin or thrombate iii or antithrombin 3 or antithrombin-3 or antithrombin iii or apolipoprotein$ or beta 2 glycoprotein$ or beta 2-Glycoprotein I or brain natriuretic peptide or nesiritide or b-type natriuretic peptide or bnp gene product or bnp-32 or brain natriuretic peptide-32 or natrecor or natriuretic factor-32 or natriuretic peptide type-b or type-b natriuretic peptide or ventricular natriuretic peptide, b-type or Brain-Derived Neurotrophic Factor or casp3 or apopain or caspase-3 or pro-caspase-3 or procaspase-3 or caspase 3 or cathepsin b-like activity or cathepsin b-like proteinase or cathepsin b1 or cathepsin b or amyloid precursor protein secretase or endoglin$ or CD105 or cd40 or Bp50 or caeruloplasmin or caeruloplasmin ferroxidase or ceruloplasmin ferroxidase or ceruloplasmin oxidase or ferroxidase i or alpha 2-ceruloplasmin or endochitinase or chitinase$ or chitotriosidase or cholesterol ester transport protein or cetyl or cholesteryl ester exchange protein or cholesteryl ester transfer protein or parathyroid secretory protein or secretory protein i, parathyroid gland or Chromogranin A or pancreastatin or parastatin or Pituitary secretory protein i or vasostatin or apoj protein or apolipoprotein j or complement lysis inhibitor or complement-associated protein sp-40,40 or ionizing radiation-induced protein-8 or mac393 antigen or sgp-2 protein or sp 40,40 protein or sulfated glycoprotein 2 or sulfated glycoprotein-2 or trpm-2 protein or testosterone-repressed prostate message-2 protein or x-ray-inducible protein 8 or xip8 protein or aging-
associated protein 4 or Complement cytolysis inhibitor or clusterin or cold-insoluble globulins or
lets proteins or fibronectin or opsonic glycoprotein or opsonic alpha 2 sb glycoprotein or alpha 2-
surface binding glycoprotein or c-fibronectin or c fibronectin or cellular fibronectin or alpha-1
chimerin or alpha-2 chimerin or alpha-chimerin or arghap2 protein or n-chimerin or rhogap2
protein or chimaerin 1 or alpha-1 chimaerin or alpha-2 chimaerin or alpha-chimaerin or alpha1-
chimaerin or n-chimaerin or chimerin or chimerin$ or collagen synthesis byproduct or
complement or c reactive protein or c-reactive protein or CRP or antithrombin vi or fibrin
degradation products or fibrin fibrinogen split products or Fibrin Fibrinogen Degradation Products
or D-dimer or D dimer or enolase or neuron-specific enolase or 2-phospho-d-glycerate hydratase
or cobalt enolase or nervous system-specific enolase or non-neuronal enolase or alpha, alpha-
enolase or beta-enolase or gamma, gamma-enolase or Phosphoppyruvate Hydratase or Neuron
specific enolase or Neurone-specific enolase or Neuron-specific enolase or endothelial protein
c receptor or endothelial cell protein c receptor or protein c receptor or centrocyclin or CD201
antigen or antigens, cd106 or cd106 antigens or vcam-1 or cd106 antigen or incam-110 or
inducible cell adhesion molecule 110 or vascular cell adhesion molecule or big endothelin or big
endothelin-1 or et-1 endothelin-1 or endothelin type 1 or endothelin, big or preproendothelin or
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molecule-1 or lecam-2 or cd62e antigen or endothelial leukocyte adhesion molecule 1 or e-
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plasma thromboplastin component or blood coagulation factor ix or factor ix complex or factor ix
fraction or coagulation factor xi or plasma thromboplastin antecedent or blood coagulation factor
x or coagulation factor xii or hageman factor or blood coagulation factor xii or coagulation factor
v or proaccelerin or ac globulin or blood coagulation factor v or factor pi or factor v or factor ix or
factor xii or factor xi or proconvertin or proconvertin or stable factor or blood coagulation
factor vii or factor vii or antithemophilic factor or coagulation factor vii or factor vii clotting antigen
or factor vii or procoagulant vii or factor vii or procoagulant activity or thromboplastinogen or blood
coagulation factor vii or f viii-c or factor viii-heavy chain or factor viiic or hemofil or hemofil hm or
hemofil m or hemophil or humate-p or hyatt-c or hyatt-c or monoclate or factor viii or
autoprothrombin iii or coagulation factor x or stuart factor or stuart-prower factor or blood
coagulation factor x or stuart prower factor or factor vii activating protease or coagulation factor
xii or factor xii, activated or activated factor xii or blood coagulation factor xii, activated or
hageman-factor fragments or prekallikrein activator or factor xiiia or interleukin or fibrinogen or
coaagulation factor i or factor i or blood coagulation factor i or gamma-fibrinogen or apo-1 antigen
or apoptosis antigen 1 or cd95 antigens or receptors, fas or tumor necrosis factor receptor
superfamily, member 6 or fas antigens or fas receptors or cd95 antigen or tnf receptor or fas antigen
or fas receptor or basic isoferitlin or ferritin or isoferitin or isoferitin, basic or
fibrinopeptide or cold-insoluble globulins or lets proteins or fibronectin or opsonic glycoprotein or
opsonic alpha 2 sb glycoprotein or alpha 2-surface binding glycoprotein or activin-binding protein
or follistatin or fatty acids, free or free fatty acids or nefa or glial fibrillary acidic protein or GFAP
or glial intermediate filament protein or astroprotein or gfa-protein or glial fibrillary acid protein or
glutathione s-alkyltransferase or glutathione s-aryltransferase or glutathione s-
epoxidetransferase or ligandins or s-hydroxyalkyl glutathione lyase or glutathione organic nitrate
ester reductase or glutathione s-transferase or glutathione s-transferase 3 or glutathione s-
transferase a or glutathione s-transferase b or glutathione s-transferase c or glutathione s-
transferase iii or glutathione s-transferase p or glutathione transferase e or glutathione
transferase mu or glutathione transferases or heme transfer protein or ligandin or b-glutathione-
s-transferase or csf-gm or colony-stimulating factor, granulocyte-macrophage or gm-csf or
histamine-producing cell-stimulating factor or csf-2 or tcm-csf or tumor-cell human gm colony-
stimulating factor or granulocyte macrophage colony stimulating factor or antigens, cd62p or
cd62p antigens or gmp-140 or lecam-3 or p selectin or platelet alpha-granule membrane protein
or cd62p antigen or padgem or antigens, cd62r or cd62l antigens or lecam-1 or cd62l antigen or l
selectin or lam-1 or leu-8 antigen or leukocyte adhesion molecule, lam-1 or mel-14 antigen or
Thrombomodulin or antigens, cd142 or cd142 antigens or coagulation factor iii or factor iii or tissue factor or tissue thromboplastin or blood coagulation factor iii or coagulin or glomerular procoagulant activity or prothrombinase or tissue factor procoagulant or urothromboplastin or thromboplastin or tissue factor pathway inhibitor or tissue inhibitor of metalloproteinase or cachectin or tnf-alpha or tumor necrosis factor ligand superfamily member 2 or cachectin-tumor necrosis factor or tnf superfamily, member 2 or tumor necrosis factor or bone-derived transforming growth factor or platelet transforming growth factor or tgf-beta or milk growth factor or tgfbeta or Transforming Growth Factor beta or ubiquitin fusion degradation protein 1 or Vascular Endothelial Growth Factor A or vascular endothelial growth factor or vascular endothelial growth factor-a or gd-vegf or glioma-derived vascular endothelial cell growth factor or vgf or vegf-a or vascular permeability factor or vasculotropin or vrnecit or factor viii-related antigen or f viii-vwf or factor viii-ag or factor viiir-rc or plasma factor viii complex or ristocetin cofactor or ristocetin-willebrand factor or vwf ag or von willebrand factor type iib or von willebrand protein or von Willebrand Factor).

11 Incidence/ or exp mortality/ or follow up studies/ or mortality/ or prognosis$.tw. or predict$.tw. or course.tw or rankin.tw or Glasgow outcome scale.tw or NIHSS.tw

12 5 or 6 or 7 or 8 or 9 or 10

13 4 and 11 and 12

14 limit 13 to humans

EMBASE

1. cerebral artery disease/ or cerebrovascular accident/ or stroke/ or vertebrobasilar insufficiency/ or wallenberg syndrome/ or exp brain infarction/ or exp brain ischemia/ or exp occlusive cerebrovascular disease/ or cerebrovascular disease/ or exp carotid artery diseases/

2. ((brain or cerebr$ or cerebell$ or vertebrovas$ or cerebell$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.

3. (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.

4. 1 or 2 or 3

5. disease Marker/ or biochemical marker/ or biological marker/ or molecular marker/ or marker/

6. biomarker$.tw.

7. ((biochemical or clinical or immun$ or laboratory or biologic$ or serum or surrogate or viral) adj6 marker$).tw.

8. ((blood or plasma) adj6 marker$).tw.

9. (Aldolase A or aldolase b or aldolase c or fructose bisphosphonate aldolase or activin$ or inhibin$ or adiponectin or adipocyte specific secretory protein or gelatine binding protein or adipocyte complement related protein or alpha 2 antiplasmin or Alpha-2-antiplasmin
adhesion molecule-1 or lecam-2 or cd62e antigen or endothelial leukocyte adhesion molecule 1 or e-selectin or selectin e or autoprothrombin ii or christmas factor or coagulation factor ix or ptc or plasma thromboplastin component or blood coagulation factor ix or factor ix complex or factor ix fraction or coagulation factor xi or plasma thromboplastin antecedent or blood coagulation factor xi or coagulation factor xii or hageman factor or blood coagulation factor xii or coagulation factor v or proaccelerin or ac globulin or blood coagulation factor v or factor pi or factor v or factor ix or factor xii or factor xi or coagulation factor vii or proconvertin or stable factor or blood coagulation factor vii or factor viii or antihemophilic factor or coagulation factor viii or factor viii clotting antigen or factor viii coagulant antigen or factor viii procoagulant activity or thromboplastinogen or blood coagulation factor viii or f viii-c or factor viii-heavy chain or factor viiic or hemophil or hemophil hm or hemophil m or hemophil or humate-p or hyate-c or hyatt-c or monoclate or factor vii or autoprothrombin iii or coagulation factor x or stuart factor or stuart-prower factor or blood coagulation factor x or stuart prower factor or factor vii activating protease or coagulation factor xii or factor xii, activated or activated factor xii or blood coagulation factor xii, activated or hageman-factor fragments or prekallikrein activator or factor xiiia or interleukin or fibrinogen or coagulation factor i or factor i or blood coagulation factor i or gamma-fibrinogen or apo-1 antigen or apoptosis antigen 1 or cd95 antigens or receptors, fas or tumor necrosis factor receptor superfamily, member 6 or fas antigens or fas receptors or cd95 antigen or tnfrsf6 receptor or fas antigen or fas receptor or basic isoform or ferritin or isoform or isoform or basic or fibrinopeptide or cold-insoluble globulins or lets proteins or fibronectin or opsonic glycoprotein or opsonic alpha 2 sb glycoprotein or alpha 2-surface binding glycoprotein or activin-binding protein or follistatin or fatty acids, free or free fatty acids or nef or glial fibrillary acidic protein or GFAP or glial intermediate filament protein or astroprotein or gfa-protein or glial fibrillary acid protein or protein or glutathione s-alkyltransferase or glutathione s-aryltransferase or glutathione s-epoxidetransferase or ligandins or s-hydroxyalkyl glutathione lyase or glutathione organic nitrate ester reductase or glutathione s-transferase or glutathione s-transferase 3 or glutathione s-transferase a or glutathione s-transferase b or glutathione s-transferase c or glutathione s-transferase iii or glutathione s-transferase p or glutathione transferase e or glutathione transferase mu or glutathione transferases or heme transfer protein or ligandins or b-glutathione-s-transferase or csf-gm or colony-stimulating factor, granulocyte-macrophage or gm-csf or histamine-producing cell-stimulating factor or csf-2 or tc-gm-csf or tumor-cell human gm colony-stimulating factor or granulocyte macrophage colony stimulating factor or antigens, cd62p or cd62p antigens or gmp-140 or lecam-3 or p selectin or platelet alpha-granule membrane protein or cd62p antigen or padgem or antigens, cd62l or cd62l antigens or lecam-1 or cd62l antigen or i selectin or lam-1 or leu-8 antigen or leukocyte adhesion molecule, lam-1 or mel-14 antigen or tq1 antigen or antigens, cd62e or cd62e antigens or e selectin or elam-1v or endothelial leukocyte adhesion molecule-1 or lecam-2 or cd62e antigen or endothelial leukocyte adhesion molecule 1 or gp130 or sgp130 or interleukin 11 receptor or gpib-iiia receptors or integrin alphaiaibbalpha or glycoproteins iiib-iiiia or integrin alphaiaaib beta-3 or pituitary growth hormone or somatotropin or growth hormone, pituitary or haptoglobin or haemopexin or hemopexin or heparin co-factor ii or antigens, cd54 or cd54 antigens or icam-1 or cd54 antigen or intercellular adhesion molecule 1 or gamma globulin, 7s or igg or allergobuline or igg t or igg1 or igg2 or igg2a or igg2b or igg3 or igg4 or immunoglobulin gt or polyglobin or immunoglobulin g or insulin or ischaemia modified albumin or merosin or glycoprotein gp-2 or laminin m or laminin m chain or laminin or leptin or ob protein or obese protein or ob gene product or obese gene product or lipoprotein associated phospholipase or lipoprotein lipase or csf-1 or csf-m or colony-stimulating factor 1 or colony-stimulating factor, macrophage or m-csf or macrophage colony stimulating factor or malonaldehyde or propanediol or malonyldialdehyde or malonyldialdehyde or sodium malondialdehyde or Malondialdehyde or interstitial collagenase or mmp-1 metalloproteinase or mmp1
metalloproteinase or matrix metalloproteinase-1 or pro-matrix metalloproteinase-1 or pro-matrix metalloproteinase-1 or prommp-1 or matrix metalloproteinase 1 or gelatinase a or 72-kda gelatinase or 72-kda type iv collagenase or mmp-2 metalloproteinase or mmp2 metalloproteinase or matrix metalloproteinase-2 or matrix metalloproteinase 2 or stromelysin 1 or transin or mmp-3 metalloproteinase or mmp3 metalloproteinase or stromelysin or matrix metalloproteinase 3 or gelatininase b or 92-kda gelatinase or 92-kda type iv collagenase or mmp-9 metalloproteinase or mmp9 metalloproteinase or matrix metalloproteinase-9 or matrix metalloproteinase 9 or metallopeptidase 9 or monocyte chemoattractant protein-1 or myelin basic protein or encephalitogenic basic proteins or basic protein, encephalitogenic or basic proteins, encephalitogenic or encephalitogenic basic protein or neuunitogenic protein or protein, encephalitogenic basic or proteins, encephalitogenic basic or myeloperoxidase or hemi-myeloperoxidase or Peroxidase or antigen s 100 or nerve tissue protein s 100 or s-100 protein or s100 protein family or s 100 or ngf-2 or nerve growth factor 2 or neurotrophin 3 or neutrophil gelatinase associated lipocalin or neutrophil protease 4 or deoxynucleoside diphosphate kinases or gdp kinase or nucleoside diphosphokinases or nucleoside-diphosphate kinases or oxidised ldls or osteoprotogelin or aryl-dialkyl phosphatase or aylalkylphosphatase or homocysteine thiolactone hydrolase or opa anhydrase or oph enzyme or organophosphorus acid anhydrase or organophosphorus acid anhydrase or organophosphorus acid anhydrase or organophosphorus acid anhydrase or organophosphorus acid anhydrase or organophosphorus acid anhydrase or organophosphorus acid anhydrase or organophosphorus acid anhydrase or paraxoaxonase-1 or paraxoaxonase-2 or glycinate 3-2 -phosphomutase or phosphoglyceromutase or phosphoglycerate phosphomutase or phosphoglycerate mutase or papp-a or igfbp-4 metalloproteinase or igfbp-4 protease or igfbp-4-specific proteinase or insulin-like growth factor-dependent igf binding protein-4 protease or insulin-like-growth factor binding protein-4 protease or papp-alpha or pregnancy associated alpha plasma protein or pregnancy-associated alpha-plasma protein or pregnancy associated plasma protein a or prothromboinolysin or glu-plasminogen or glutamic acid 1-plasminogen or glutamyl plasminogen or agep or acetyl glyceryl ether phosphorylcholine or paf-acether or phosphorylcholine, acetyl glyceryl ether or 1-alkyl-2-acetyl-sn-glycerophosphocholine or platelet aggregating factor or platelet aggregation enhancing factor or platelet-activating substance or thrombocyte aggregating activity or platelet activating factor or cd31 antigens or pecam-1 or platelet endothelial cell adhesion molecule-1 or cd31 antigen or platelet derived microvesicles or platelet derived growth factor or antiheparin factor or pf 4 or heparin neutralizing protein or pf4 or gamma-thromboglobulin or prorenin or Protein C or Protein S or Protein Z or coagulation factor ii or factor ii or blood coagulation factor ii or differentiation reversal factor or prothrombin or adipocyte cysteine-rich secreted protein fzz3 or resistin or receptor of AGE or RAGE or receptor of advanced glycation end products or secretagogen or plasminogen activator inhibitors or bae-pai or endothelial plasminogen activator inhibitors or placental plasminogen activator inhibitors or plasminogen activator inhibitors, endothelial or plasminogen activator inhibitors, placental or platelet activation or PARK 7 or SCD40L or Tau or thrombase or thrombomar or thrombostat or alpha-thrombin or beta-gamma-thrombin or beta-thrombin or gamma-thrombin or thrombin or thrombin-antithrombin complex or Thrombomodulin or antigens, cd142 or cd142 antigens or coagulation factor iii or factor iii or tissue factor or tissue thromboplastin or blood coagulation factor iii or coagulin or glomerular procoagulant activity or prothrombinase or tissue factor procoagulant or urothromboplastin or thromboplastin or tissue factor pathway inhibitor or tissue inhibitor of metalloproteinase or cachectin or tnf-alpha or tumor necrosis factor ligand superfamily member 2 or cachectin-tumor necrosis factor or tnf superfamily, member 2 or tumor necrosis factor or bone-derived transforming growth factor or platelet transforming growth factor or tgf-beta or milk growth factor or tgbeta or Transforming Growth Factor beta or ubiquitin fusion degradation protein 1 or Vascular Endothelial Growth Factor A or vascular endothelial growth factor or vascular endothelial growth
factor-a or gd-vegf or glioma-derived vascular endothelial cell growth factor or vegf or vegf-a or vascular permeability factor or vasculotropin or vitronectin or factor viii-related antigen or f viii-vwf or factor viii-ag or factor viii-rco or plasma factor viii complex or ristocetin cofactor or ristocetin-willebrand factor or vwf ag or von willebrand factor type iib or von willebrand protein or von willebrand Factor).tw.

10. Fructose Bisphosphate Aldolase/ or ACTIVIN A/ or ACTIVIN/ or INHIBIN A/ or INHIBIN/ or INHIBIN B/ or ADIPONECTIN/ or ANTIPLASMIN/ or ALPHA 2 ANTIPLASMIN/ or Alpha 2 Macroglobulin/ or Chymotrypsin A/ or Alpha 1 Antitrypsin/ or OROSOMUCOID/ or Dipeptidyl Carboxypeptidase/ or Fibroblast Growth Factor 2/ or ANGIOTENSIN I/ or ANGIOTENSIN/ or ANGIOTENSIN BLOOD LEVEL/ or ANGIOTENSIN II/ or Antithrombin III/ or exp Apolipoprotein/ or Beta2 Glycoprotein 1/ or exp Brain Natriuretic Peptide/ or Brain Derived Neurotrophic Factor/ or exp CASPASE/ or Cathepsin B/ or CD40 LIGAND/ or CD40 ANTIGEN/ or exp CERULOPLASMIN BLOOD LEVEL/ or exp CERULOPLASMIN/ or CHITINASE/ or Cholesterol Ester Transfer Protein/ or Chromogranin A/ or exp CLUSTERIN/ or Fibronectin/ or Chimerin/ or exp COMPLEMENT/ or COMPLEMENT BLOOD LEVEL/ or Anaphylatoxin/ or PROPERDIN/ or C Reactive Protein/ or Fibrin Degradation Product/ or Enolase/ or TAU PROTEIN/ or Cell Adhesion Molecule/ or Cd22 Antigen/ or Cd24 Antigen/ or Cd31 Antigen/ or antigens, cd164/ or Cadherin/ or Carcinoembryonic Antigen/ or Cd4 Immunoglobulin/ or Nerve Cell Adhesion Molecule/ or Integrin/ or Intercellular Adhesion Molecule 1/ or Homing Receptor/ or Selectin/ or Vascular Cell Adhesion Molecule 1/ or ENDOTHELIN 2/ or BIG ENDOTHELIN 2/ or ENDOTHELIN 1/ or BIG ENDOTHELIN 1/ or ENDOTHELIN 3/ or ENDOTHELIN/ or ERYTHROPOIETIN/ or P SELECTIN GLYCO PROTEIN LIGAND 1/ or L SELECTIN/ or SELECTIN/ or exp Blood Clotting Factor/ or exp Cytokine/ or exp Fibrinogen/ or Antigens, CD95/ or Ferritin/ or fibrinopeptide a/ or fibrinopeptide b/ or exp Fibronectins/ or exp Follistatin-Related Proteins/ or exp Follistatin/ or exp Fatty Acids, Nonesterified/ or exp Glial Fibrillary Acidic Protein/ or exp Glutathione Transferase/ or Granulocyte-Macrophage Colony-Stimulating Factor/ or exp Selectins/ or Platelet Glycoprotein GPIIb-IIIa Complex/ or growth hormone/ or human growth hormone/ or exp Haptoglobins/ or Hemopexin/ or Heparin Cofactor II/ or exp Intercellular Adhesion Molecule-1/ or exp Immunoglobulin G/ or Laminin/ or Leptin/ or Macrophage Colony-Stimulating Factor/ or Malondialdehyde/ or exp Matrix Metalloproteinase/ or exp Monocyte Chemoattractant Proteins/ or Myelin Basic Proteins/ or Peroxidase/ or exp S100 Proteins/ or Neurotrophin 3/ or 9 Nitric Oxide/ or Nucleoside-Diphosphate Kinase/ or Aryldialkylphosphatase/ or Phosphoglycerate Mutase/ or Pregnancy-Associated Plasma Protein-A/ or Plasminogen Activator Inhibitor 1/ or Plasminogen/ or Plasminogen Activator Inhibitor 2/ or Platelet Activating Factor/ or Antigens, CD31/ or Platelet-Derived Growth Factor/ or Platelet Factor 4/ or Protein C/ or Protein S/ or Prothrombin/ or Resistin/ or Plasminogen Inactivators/ or Platelet Activation/ or tau Proteins/ or Thrombin/ or Thrombomodulin/ or Thromboplastin/ or TUMOR NECROSIS FACTOR-ALPHA/ or Transforming Growth Factor beta/ or Vascular Endothelial Growth Factor A/ or VITRONECTIN/ or von Willebrand Factor/ or Tissue Plasminogen Activator/ec [Endogenous Compound]

11. 5 or 6 or 7 or 8 or 9 or 10

12. Incidence/ or exp mortality/ or follow up studies/ or mortality/ or prognosis.tw. or predict.tw. or course.tw. or rankin.tw. or Glasgow outcome scale.tw. or NIHSS.tw.

13. 4 and 11 and 12

14. limit 13 to human
WEB APPENDIX 3

Quality questionnaire

Was the study prospective?
YES: The study reports that patients and blood samples were collected prior to the development of an outcome
NO: No report or clearly retrospective (e.g. patients with poor prognosis collected prior to biomarker measurement)

Was the evaluation of prognostic marker blinded to patient outcome?
YES: The study reports an attempt to blind the person measuring the level of biomarker to patient outcome
NO: There is no such report.

Was there a defined time period during which patients were enrolled?
YES: Study define time period, end of follow up period and median follow up time
NO: Does not define above criteria

Were there precisely defined clinical outcomes at the beginning of the study?
YES: Study defines which clinical endpoints are to be measured
NO: No such definition

Did the study provide a rationale for study sample size?
YES: Evidence of a sensible sample size calculation (e.g. 10 outcomes/variable in a multiple regression model)
NO: no attempt to define sample size

Did the study provided a list of candidate variables?
YES: A list of variables to be considered in multiple regression analysis is provided at the beginning of the study
NO: evidence that variables were measured and not reported

Were the methods for measuring the prognostic marker adequately described and referenced?
YES
NO

Cases unselected/unbiased?
YES No attempt to select patients with exclusion criteria
NO only a subset of stroke patients enter the study