The Use of Blood Biomarkers to Predict Poor Outcome After Acute Transient Ischemic Attack or Ischemic Stroke

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Background and Purpose—The prediction of death or disability (“poor outcome”) after stroke by validated clinical models might be improved by the addition of blood biomarker measurements. We investigated whether such measurements improved the classification of patients into 4 categories of predicted risk of poor outcome: very high, intermediate high, intermediate low, and very low.

Methods—We prospectively recruited symptomatic patients within 24 hours of ischemic cerebrovascular events. We measured clinical prognostic variables in each patient. We drew blood soon after admission and measured markers of inflammation, thrombosis, cardiac strain, and cerebral damage. We assessed poor outcome at 3 months with the modified Rankin Scale and recovery of symptoms at 24 hours. We measured the association between blood marker levels and poor outcome after adjustment for stroke severity and age with multivariate logistic regression. Where these associations were statistically significant, we calculated the net reclassification index.

Results—We recruited 270 patients with acute ischemic cerebrovascular events. At 3 months, 112 patients had a poor outcome. After adjustment for stroke severity and age, only interleukin-6 and N-terminal pro-brain natriuretic peptide were significantly associated with poor outcome. The addition of either interleukin-6 or N-terminal pro-brain natriuretic peptide to National Institutes of Health Stroke Scale and age did not improve the prediction of a poor outcome.

Conclusions—Neither interleukin-6 nor N-terminal pro-brain natriuretic peptide had sufficient predictive power to be of clinical use to predict poor outcome after stroke. The search for better markers to improve the classification of patients across clinically relevant boundaries of predicted probabilities of outcome events needs to continue. (Stroke. 2012;43:00-00.)

Key Words: biomarkers ▪ brain infarction ▪ prognosis

After ischemic stroke, the early prediction of death or disability (“poor outcome”) is of great interest. Statistical models, constructed with clinical variables such as age or neurological impairment, make similar predictions of poor outcome to experienced stroke physicians.1 Biomarkers of the processes active in ischemic stroke might add predictive power to these simple statistical models based on bedside clinical examination.

Many studies have examined the association between blood marker levels and poor outcome after stroke.2,3 However, the associations seen in group data, unless very strong, do not often lead to better predictions of outcome in individuals.4 To be useful, a given marker should at least improve on the predictions from validated prognostic variables.

We hypothesized that the addition of biomarker levels to the National Institutes of Health Stroke Scale (NIHSS) and age5 would improve the prediction of poor outcome (or recovery at 24 hours) in patients with ongoing symptoms due to cerebral ischemia who presented to the hospital soon after the onset. We decided to measure improvement in prediction with a novel statistic, the net reclassification index, which gives a clinically meaningful measure of improvement in prediction.6

We examined biomarkers of pathophysiological processes that were plausibly associated with poor outcome after stroke after a systematic review of the available literature.3

Methods

Ethics Statement
The study was approved by the Scotland A Research Ethics Committee (REC Reference No. 06/MRE00/119). All patients or their relatives provided written informed consent.

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Cohort Recruitment

Between March 2007 and February 2009 we included consecutive patients with ischemic stroke or transient ischemic attack who presented in normal working hours with symptoms of <24 hours' duration that were still present at the time of initial assessment. Because of laboratory availability, patients were only eligible if they could be recruited within normal working hours. All patients were assessed at the time of presentation when they still had ongoing symptoms of their acute "brain attack." A study neurologist assessed each patient in the acute stage and recorded prognostic factors for poor outcome after stroke (age, stroke severity, symptoms in the previous week consistent with infection, medical history, atrial fibrillation, NIHSS); medications at admission; electrocardiographic findings; and time when last seen well. We defined prior cognitive impairment as a history of cognitive problems before the onset of symptoms that were sufficient to impair activities of daily living, elicited by the study neurologist.

For each patient, a panel of experts agreed on a final diagnosis of confirmed ischemic stroke, transient ischemic attack, or not a vascular event after considering the presentation, CT or MRI brain imaging, and clinical course blinded to the results of blood marker levels. We defined an ischemic stroke as a clinically definite stroke in a patient whose brain imaging showed either positive evidence of a relevant ischemic lesion or was normal and excluded intracranial hemorrhage and stroke mimics and where the symptoms lasted for >24 hours. We used a similar definition for transient ischemic attack, although symptoms had to last for <24 hours.

Biomarker Measurement

We drew blood from each patient before infusion of any thrombolytic therapy into 2.25 mL ethylenediaminetetraacetic acid tubes and an 8-mL tube containing clot activator and gel for serum separation. Samples were transferred on water ice and centrifuged at 3000 revolutions per minute for 10 minutes and the supernatant stored at −80°C.

In serum and plasma blinded to clinical information, clinical and research laboratories measured as markers of inflammation: adiponectin, C-reactive protein, intracellular adhesion molecule 1, interleukin-6 (IL-6), IL-10, matrix metalloproteinase 9, tissue necrosis factor α, von Willebrand Factor, and white cell count; thrombosis: D-dimer, fibrinogen, and tissue-type plasminogen activator; cardiac strain: N-terminal pro B-type natriuretic peptide (NT proBNP) and troponin T; and neural and glial damage: tau, S100B, and creatinine, and glucose (see Supplementary Material for detailed methods; http://stroke.ahajournals.org).

Outcome Measurement

At 24 hours, we determined whether the presenting symptoms had resolved completely (to differentiate transient ischemic attack from ischemic stroke). We ascertained vital status by contacting the patient’s general practitioner at 3 months after onset and sent surviving patients a short questionnaire based on the modified Rankin Scale (mRS) by mail. If a patient failed to return an interpretable questionnaire by mail, we interviewed them in a structured way by telephone to measure the mRS. We dichotomized a patient’s outcome into “poor” if he or she was dependent on others for activities of daily living (mRS scores 3, 4, and 5) or dead and “good” if he or she was independent in activities of daily living 3 months after stroke onset (mRS 0, 1, and 2). We determined the cause of death by inspection of the hospital or general practitioner records.

Statistical Analysis

We examined the association between biomarker levels and delay to admission with a series of correlation analyses. We made adjustment for NIHSS at the time of presentation with multiple linear regression analysis.

We examined the association between clinical variables and blood markers with poor outcome at 3 months with a series of univariate logistic regression analyses. We constructed 2 logistic regression models to adjust for potential confounders. First, in a simple model, we adjusted the association between blood markers with poor outcome for the baseline NIHSS and age. Then in a second series of more complex models, we adjusted not only for NIHSS, age, and premorbid disability, but also for the potentially confounding effects of infection and statins on the associations with inflammatory markers and for the potentially confounding effects of current or previous atrial fibrillation, cardiac failure, or previous cardiac vascular disease on the associations with cardiac markers. We measured the crude association of blood markers with recovery at 24 hours and adjusted these associations for NIHSS and age.

The Additional Predictive Value of Blood Markers Over NIHSS and Age

We assessed if the addition of biomarkers significantly associated with poor outcome made better predictions of poor outcome than a model built based on NIHSS and age alone. We made the most conservative estimate of the improvement in prediction after the addition of blood markers by allowing the association of each clinical covariate with poor outcome to vary. We assessed changes in goodness of fit (Bayes information criterion), calibration ( Hosmer Lemeshow χ²), and discrimination (area under receiver operator characteristic curves) after the addition of biomarkers to the baseline clinical model. We calculated the net reclassification index across the clinically relevant thresholds of predicted risk: 0.1 ("very low risk"), 0.5 ("intermediate"), and 0.9 ("very high risk"). We prespecified thresholds of <10% and >90% for predicted probability of poor outcome because we believe that one would need to be very certain of a good or poor outcome before avoiding treatments such as thrombolysis or selecting patients for palliative care only. The net reclassification index examines whether the addition of a biomarker moves those with poor outcome to higher risk categories more often than lower risk categories and those with a good outcome to lower risk categories more often than higher risk categories. Because Bonferroni adjustments for over 5 comparisons are likely to be too conservative, we considered a 2-tailed P<0.01 to be statistically significant. We analyzed the data with Stata 11.

Results

We recruited 270 patients who presented with ongoing symptoms due to cerebral ischemia. In 40 patients, the symptoms resolved completely within 24 hours, and in 230, they were persistent (Figure 1). At 3 months, a mRS score was available for 268 (99%) patients and vital status for all patients. At 3 months, 34 (11%) patients had died and 112 (42%) had a poor outcome. The cause of death was the effects of the original stroke (21), cancer (3), extracranial hemorrhage (2), cardiac arrhythmia (1), myocardial infarction (1), ischemic colitis (1), heart failure (1), and unavailable in 1. Blood marker data were missing for some patients, although each patient had blood drawn. Data appeared to be missing at random. The clinical characteristics of the study participants are summarized in Table 1 and the levels of blood markers are shown in an online Data Supplement.

Clinical Features and Outcome

The risk of poor outcome doubled per decade of patient age (OR, 2.03; 95% CI, 1.58–2.62) and per 3-U increase in the NIHSS (OR, 1.98; 95% CI, 1.63–2.39; Table 1). A patient with poor outcome was significantly (P<0.01) more likely to have atrial fibrillation, prior cognitive impairment, or have been admitted more quickly to the hospital. Treatment with statins before stroke was associated (P=0.02) with poor outcome, but not symptoms of infection at admission.
The relationship between blood marker levels and poor outcome was approximately log linear for all markers except loge IL-6 (75th:25th centile OR, 2.05; 95% CI, 1.23–3.93) and NT pro-BNP (OR, 1.26; 95% CI, 1.24–4.12) reached statistical significance. Further adjustment of the association (Supplemental Table II) between IL-6 with poor outcome for the prescription of statin medication before admission and symptoms of infection before stroke attenuated this relationship by a small amount (OR, 1.86; 95% CI, 1.10–3.18). Adjustment of the relationship between loge NT pro-BNP with poor outcome for a prior diagnosis of cardiac failure, atrial fibrillation at the time of admission and prior cardiac vascular disease, also weakened the association to a small degree (OR, 2.09; 95% CI, 1.11–3.93). For other markers, further adjustment made little material difference to the direction, strength, or statistical significance of the associations.

There were no important differences in the magnitude or statistical significance of the associations between any of the blood markers and poor outcome when the analyses were repeated in the subgroup of 190 patients with positive imaging findings.

There was an association of higher levels of NT-pro BNP with continuing symptoms at 24 hours, which remained after adjustment for NIHSS and age (OR, 2.72; 95% CI, 1.25–5.96). The levels of other markers were not significantly associated with clinical outcome at 24 hours (Supplemental Figure I).

The Addition of Blood Markers to NIHSS and Age
We tested the addition of those markers associated significantly with poor outcome to a baseline model constructed with the covariates NIHSS and age (Table 2). The baseline model was well calibrated and showed good discrimination in this cohort.

The addition of NT pro-BNP or IL-6 to the baseline model significantly improved the goodness of fit but made little additional difference to either the discrimination (measured by area under the receiver operating characteristic curve) or the calibration of the models. Although a few patients moved across clinically relevant boundaries of predicted probability of poor outcome, these numbers were small and not statistically or clinically significant. The full models are summarized in Supplemental Table III.

Discussion
Higher levels of NT pro-BNP and IL-6 were strongly associated with poor outcome 3 months after stroke or transient ischemic attack after adjustment for age and neurological impairment. Despite this strong association, the addition of either NT pro-BNP or IL-6 to NIHSS and age did not improve the classification of patients to an important degree into “very high risk” or “very low risk” of poor outcome 3 months after symptom onset. These findings were not affected by delay to admission, statin prescription, or the presence of symptoms of prior infection, which was a concern in our previous study of IL-6 and poor outcome. It is therefore unlikely that doctors will find predictive instruments that use the measurement of IL-6 or NT pro-BNP clinically more useful than age and NIHSS to predict short-term functional outcome in patients with acute stroke. In addition, only NT pro-BNP was associated with failure to recover by 24 hours after symptom onset, although there are wide limits of uncertainty about the estimates of other markers, because the number of patients with complete recovery at 24 hours was small.
Our finding of the association between NT pro-BNP and poor outcome after cerebrovascular disease is consistent with several published studies.9–11 Plausible explanations for the association are: (1) NT-pro BNP is a measure of early cardiac dysfunction after stroke, which is associated with more severe stroke and worse outcome; (2) NT-pro BNP is associated with cardioembolic strokes, which tend to have a worse outcome than other causes of ischemic stroke; or (3) NT pro-BNP is released from the brain after cerebral ischemia. However, despite the association of BNP with many potential causes of poor outcome after stroke, it does not seem to have a role in clinical prediction of poor outcome.

The significant association of higher levels of adiponectin with poor outcome after adjustment for potentially confounding variables is also of interest, although this did not reach our chosen threshold of statistical significance for this study (P=0.02). In previous studies of the association of adiponectin with death after stroke, lower levels of adiponectin were associated with worse outcome12; our finding suggested that this may not be the case.

To be useful for predicting outcome in clinical practice, markers must either outperform established clinical models when measured alone or improve predictions when added to these models. The net reclassification index is a measure of the number of patients who are better classified across clinically meaningful thresholds after the addition of a new biomarker. Because there is no widely agreed definition of clinically important boundaries for the prediction of poor outcome after stroke, in this study, we chose boundaries after discussion with experienced stroke physicians. However, further work is still needed to determine the boundaries that are most important to patients and less experienced physicians.

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**Limitations**

This study did not aim to elucidate the causal role of particular molecules or the underlying physiological pathways. There are many plausible hypotheses about how higher levels of each marker might cause a poor outcome after cerebral ischemia. However, even in a perfect observational study, free of the influence of selection or information bias, causality is not the only factor that might explain the observed associations. Other explanations include reverse causality, the choice of confounders used for statistical adjustment, and imperfect biomarkers.

We recruited patients with a wide range of severity of baseline neurological impairments and time points of study entry up to 24 hours after symptom onset. A study of a large group of patients with a uniform baseline stroke severity or at a sooner time would have more power to detect important

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**Table 1. The Association Between Baseline Clinical Features and Poor Outcome (Dead or Dependent on Other for Activities of Daily Living) 3 Months After Presentation With Acute Transient Ischemic Attack or Ischemic Stroke**

<table>
<thead>
<tr>
<th>Baseline Characteristic</th>
<th>All (n=268)</th>
<th>Good Outcome (n=156)</th>
<th>Poor Outcome (n=112)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, no. (%)</td>
<td>112 (41.8)</td>
<td>84 (53.9)</td>
<td>46 (41.1)</td>
<td>0.60 (0.37–0.98)</td>
<td>0.04</td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>74.4 (12.5)</td>
<td>70.6 (12.7)</td>
<td>79.6 (10.0)</td>
<td>2.03 (1.58–2.62)*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Clinical Variables**

<table>
<thead>
<tr>
<th>No. (%)</th>
<th>No. (%)</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent infection</td>
<td>25 (9.3)</td>
<td>11 (7.1)</td>
</tr>
<tr>
<td>Prior cardiac vascular disease</td>
<td>65 (24.3)</td>
<td>35 (22.4)</td>
</tr>
<tr>
<td>Prior peripheral vascular disease</td>
<td>15 (5.6)</td>
<td>9 (5.8)</td>
</tr>
<tr>
<td>Prior TIA or stroke</td>
<td>73 (27.2)</td>
<td>34 (21.8)</td>
</tr>
<tr>
<td>Prior heart failure</td>
<td>22 (8.3)</td>
<td>8 (5.2)</td>
</tr>
<tr>
<td>AF (before onset or in ED)</td>
<td>73 (27.3)</td>
<td>26 (16.8)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>34 (12.7)</td>
<td>19 (12.1)</td>
</tr>
</tbody>
</table>

**Imaging lesion**

<table>
<thead>
<tr>
<th>No. (%)</th>
<th>No. (%)</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical infarction</td>
<td>123 (45.9)</td>
<td>57 (36.5)</td>
</tr>
<tr>
<td>Lacunar infarction</td>
<td>40 (14.9)</td>
<td>29 (18.5)</td>
</tr>
<tr>
<td>Posterior circulation infarction</td>
<td>21 (7.8)</td>
<td>14 (9.0)</td>
</tr>
<tr>
<td>&gt;1 territory infarction</td>
<td>4 (1.5)</td>
<td>0</td>
</tr>
<tr>
<td>No visible lesion</td>
<td>74 (27.6)</td>
<td>54 (34.6)</td>
</tr>
<tr>
<td>No scan</td>
<td>4 (1.5)</td>
<td>2 (1.3)‡</td>
</tr>
</tbody>
</table>

**Continuous Variables**

<table>
<thead>
<tr>
<th>Median (IQR)</th>
<th>Median (IQR)</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well to admission, hr†</td>
<td>4.0 (1.7–8.5)</td>
<td>4.8 (2.0–11.7)</td>
</tr>
<tr>
<td>NIHSS</td>
<td>3 (2–9)</td>
<td>3 (1–4)</td>
</tr>
</tbody>
</table>

Prior cardiac vascular diseases are: a history of angina, myocardial infarction, or cardiac revascularization.

TIA indicates transient ischemic attack; AF, atrial fibrillation; ED, emergency department; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; CI, confidence interval.

*Per decade of age.
†Time in hours from when the patient was last seen well to their arrival in the emergency department.
‡Per hour increase.
§Per unit increase.

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improvements in prediction with blood markers in patients presenting at early time points or with particularly severe (or mild) strokes. However, then the results would be less generalizable.

We measured the mRS outcome by a postal questionnaire and telephone interview, similar to large trials of stroke treatments, although we note that there are problems with most methods of mRS measurement. It is possible that with this method of outcome measurement, our results were affected by a nondifferential information bias, which would tend to weaken the observed associations.

Strengths
This study met almost all the methodological criteria for a reliable study of prognosis. We recruited 270 patients, of whom 112 had a poor outcome at the end of the study. Because approximately 10 outcome events per covariate has been suggested as a “rule of thumb” to ensure logistic regression models have sufficient power, we could examine associations between different markers. Probability values are derived from Wald tests and determine if the reported OR is significantly different from 1. OR >1 indicates higher marker levels are associated with worse outcome.

Table 2. Performance of Models to Predict Poor Outcome in Patients With Acute Symptomatic Transient Ischemic Attack or Stroke 3 Mo

<table>
<thead>
<tr>
<th>Goodness of Fit</th>
<th>Likelihood Ratio Statistic (P)</th>
<th>Bayesian Information Criterion</th>
<th>Discrimination AUROC (95% CI) (P)</th>
<th>Calibration Hosmer Lemeshow Statistic (P)</th>
<th>Reclassification Net Reclassification Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS + age</td>
<td>Reference</td>
<td>-1118</td>
<td>0.83 (0.78 – 0.88) Reference</td>
<td>0.91</td>
<td>Reference</td>
</tr>
<tr>
<td>NIHSS + age + IL-6</td>
<td>&lt;0.01</td>
<td>-1120</td>
<td>0.84 (0.79 – 0.89) 0.10</td>
<td>0.41</td>
<td>+0% (P=0.99)</td>
</tr>
<tr>
<td>NIHSS + age + log_NT pro-BNP</td>
<td>0.01</td>
<td>-1120</td>
<td>0.84 (0.79 – 0.89) 0.05</td>
<td>0.96</td>
<td>+1% (P=0.64)</td>
</tr>
</tbody>
</table>

Net reclassification improvement: the difference in correct and clinically useful classifications between models with and without biomarkers at thresholds of predicted probability of poor outcome of 0.1, 0.5, and 0.9. Positive values indicate better prediction with the addition of a marker; negative values indicate worse prediction.

AUROC indicates area under the receiver operating characteristic; NIHSS, National Institutes of Health Stroke Scale; IL-6, interleukin-6; NT pro-BNP, N-terminal pro-brain natriuretic peptide; CI, confidence interval.
Conclusions
We have confirmed the associations between blood markers and poor outcome with effects of a similar magnitude to previous studies. Although these blood markers were therefore of potential clinical value, it is sobering to note that none of the very plausible biomarkers measured in this study added a clinically useful degree of prediction of poor outcome after stroke to that provided by the measurement of the simple clinical variables, the NIHSS and age. Future studies of blood markers for predicting outcome after stroke will need to demonstrate that the candidate marker: (1) has a statistically independent association with outcome; (2) adds statistically significant predictive value to a clinical model; and (3) improves the classification of patients across relevant boundaries of predicted probabilities of poor outcome that have clinical use.

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Disclosures
None.

References
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Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2011/10/24/STROKEAHA.111.634089.DC1

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SUPPLEMENTARY MATERIAL

We list the methods to measure each individual marker below:

**Inflammation**

- **Adiponectin (μg/ml):** we measured total plasma adiponectin with a commercial enzyme-linked immunosorbent assays (ELISA) (R&D Systems). The inter-assay coefficients of variation for the assay was < 7%.

- **C-reactive protein (CRP) (mg/l):** we measured plasma CRP with high-sensitivity immunonephelometry (Prospec, Dade Behring Milton Keynes, UK) following the manufacturer's reagents and standards. Intra- and inter-assay coefficients of variation were 4.7% and 8.3%, respectively.

- **Intercellular adhesion molecule-1 (ICAM-1) (ng/ml):** we measured plasma ICAM-1 with a commercially available ELISA (R&D Systems, Abingdon, UK). The inter-assay coefficient of variation was < 7%.

- **Interleukin-6 (IL-6) (pg/ml):** we assayed serum IL-6 with a high sensitivity ELISA (R & D Systems, Abingdon, UK). Intra- and inter-assay coefficients of variation were 7.5% and 8.9%, respectively.

- **Tumour necrosis factor-alpha (TNF-α):** (pg/ml): we assayed serum TNF-α with a high-sensitivity ELISA (R&D Systems, Abingdon, UK). Intra-assay and inter assay coefficients of variation were 8.4% and 12.5%, respectively.

- **Interleukin-10 (IL-10) (pg/ml):** we measured serum IL10 with an ELISA assay (R&D Systems, Abingdon, UK). The inter-assay coefficient of variation was 4.5%. 

Matrix-metalloproteinase-9 (MMP-9) (ng/ml): we measured serum MMP-9 with a commercially available sandwich ELISA (R&D Systems, Abingdon, UK). Intra-assay and inter assay coefficients variation were 4.4% and 10.4%, respectively.

Thombosis

- von Willebrand factor (vWF) (IU/dL): we measured serum vWF antigen with an ELISA using rabbit antihuman polyclonal antibodies obtained from DAKO (High Wycombe, UK). Intra and inter assay coefficient of variation were 3.3% and 4.2%, respectively.

- D-dimer (ng/ml): we measured plasma levels of fibrin D-dimer with a commercially available ELISA from Biopool AB, Umea Sweden. The intra and inter assay coefficients of variation were 4.7 and 5.2%, respectively.

- Fibrinogen (g/l): we measured fibrinogen in plasma by immunonephelometry (Prospec, Dade Behring Milton Keynes, UK) using the manufacturer’s reagents and standards. Intra- and inter-assay coefficients of variation were 7.5 and 8.9%, respectively.

- Tissue plasminogen activator (t-PA) (ng/mL): we measured plasma levels of tissue plasminogen activator (t-PA) antigen with commercially available ELISAs from Biopool AB, Umea Sweden. The intra and inter assay coefficients of variation were 6.6% and 6.5%, respectively.

Cardiac Strain
• **N-terminal-pro-brain-natriuretic-peptide (NT pro-BNP) (pg/ml):** we measured serum levels of NT pro-BNP using the Elecsys 2010 electrochemiluminescence analyser (Roche Diagnostics, Burgess Hill, UK) calibrated using the manufacturer’s reagents. Manufacturer’s controls were used with limits of acceptability defined by the manufacturer. Low control coefficient of variation was 6.7% and high control coefficient of variation was 4.9%.

• **Troponin T (ng/ml):** we determined serum troponin T using the Elecsys 2010 electrochemiluminescence analyser (Roche Diagnostics, Burgess Hill, UK) calibrated using the manufacturer’s reagents. Manufacturer’s control was used with limits of acceptability defined by the manufacturer. The abnormal high (detectable) control coefficient of variation was 2.3%.

**Cerebral damage**

• **Tau (pg/ml):** we measured tau in serum with a sandwich ELISA using the Innotest htau antigen (Innogenetics). The coefficient of variation at 479 pg/ml was 5.8%.

**S100 B (pg/ml):** we measured S100 B in serum. 96-well microtiter plates were coated with 200 µL of 0.05 M carbonate buffer containing monoclonal anti-S100 B (Affiniti Research Products, Exeter, UK). The plates were washed with 0.67 M barbitone buffer containing 5 mM calcium lactate, 0.1% bovine serum albumin, and 0.05% Tween and then were blocked with 2% bovine serum albumin and washed again. Two-hundred microliters of diluted serum (1:1) in 0.67 M barbitone buffer
containing 5 mM calcium lactate was added in duplicate. After incubation and washing, horseradish-peroxidase-conjugated polyclonal anti-S100 B (Dako, Copenhagen, Denmark) was used as a detecting antibody. The o-phenylenediamine color reaction was stopped with 1 M hydrochloric acid, and the absorbances were read at 492 and 405 nm. The antigen concentration was calculated from an internal standard curve ranging from 0 to 250 pg/mL. The coefficient of variation at a concentration of 263pg/ml was 11%.

Supplementary table 1. Distribution of blood markers of inflammation, thrombosis, cardiac strain and neuronal and glial damage in patients with acute cerebrovascular disease.

<table>
<thead>
<tr>
<th>Marker</th>
<th>All Median (IQR)</th>
<th>mRS 0-2 Median (IQR)</th>
<th>mRS 3-6 Median (IQR)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>11.4 (6.8-7.9)</td>
<td>9.2 (5.7-15.5)</td>
<td>14.7 (9.0-19.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>4.2 (1.9-8.3)</td>
<td>3.1 (1.5-6.8)</td>
<td>6.2 (2.5-18.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICAM (ng/ml)</td>
<td>165 (131-215)</td>
<td>163 (131-2.8)</td>
<td>165 (127 to 220)</td>
<td>0.74</td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
<td>4.5 (2.1-8.2)</td>
<td>2.9 (1.7-5.7)</td>
<td>7.4 (3.9-10.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF (pg/ml)</td>
<td>1.4 (1.2-1.7)</td>
<td>1.4 (1.2-1.7)</td>
<td>1.4 (1.3 to 1.8)</td>
<td>0.27</td>
</tr>
<tr>
<td>Marker</td>
<td>All</td>
<td>mRS 0-2</td>
<td>mRS 3-6</td>
<td>(P)</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------</td>
<td>------------</td>
<td>------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>Interleukin-10 (pg/ml)</td>
<td>4.4 (3.3-6.6)</td>
<td>4.3 (3.1-6.0)</td>
<td>4.8 (3.5-7.4)</td>
<td>0.06</td>
</tr>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>872 (555-1337)</td>
<td>859 (538-1322)</td>
<td>872 (619-1364)</td>
<td>0.45</td>
</tr>
<tr>
<td>vWf (IU/dl)</td>
<td>163 (123-218)</td>
<td>147 (113-204)</td>
<td>187 (140-231)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>White cells (x10⁹ cell/l)</td>
<td>8.6 (6.7-10.6)</td>
<td>8 (6.3-10.0)</td>
<td>9.1 (7.3-11.5)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

**Thrombosis**

<table>
<thead>
<tr>
<th>Marker</th>
<th>All</th>
<th>mRS 0-2</th>
<th>mRS 3-6</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>D-dimer (ng/ml)</td>
<td>228 (109-440)</td>
<td>158 (88-302)</td>
<td>347 (199-770)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ln D-dimer (loge unit)*</td>
<td>5.4 (4.7-6.1)</td>
<td>5.1 (4.5-5.7)</td>
<td>5.8 (5.3-6.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>4.8 (4.2-5.8)</td>
<td>4.6 (4.0-5.3)</td>
<td>5.2 (4.5-6.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>11.1 (8.4-14.6)</td>
<td>10.7 (8.4-12.7)</td>
<td>11.6 (8.3-17.2)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Cardiac strain**

<table>
<thead>
<tr>
<th>Marker</th>
<th>All</th>
<th>mRS 0-2</th>
<th>mRS 3-6</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>NT pro-BNP (pg/ml)</td>
<td>518 (140-1800)</td>
<td>281 (100-860)</td>
<td>1342 (406-3862)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ln NT pro-BNP (loge unit)*</td>
<td>6.3 (5.0-7.5)</td>
<td>5.6 (4.6-6.8)</td>
<td>7.2 (6.0-8.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Troponin T (ng/ml)</td>
<td>0.01(0.01-0.01)</td>
<td>0.01(0.01-0.01)</td>
<td>0.01(0.01-0.02)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Cerebral damage**

<table>
<thead>
<tr>
<th>Marker</th>
<th>All</th>
<th>mRS 0-2</th>
<th>mRS 3-6</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>Tau (pg/ml)</td>
<td>21 (13-49)</td>
<td>23 (12-51)</td>
<td>21 (13-50)</td>
<td>0.83</td>
</tr>
<tr>
<td>S100B (pg/ml)</td>
<td>61 (39-109)</td>
<td>59 (37-94)</td>
<td>69 (43-133)</td>
<td>0.08</td>
</tr>
<tr>
<td>Ln S100B (loge unit)*</td>
<td>4.2 (3.7-4.7)</td>
<td>4.1 (3.7-4.6)</td>
<td>4.3 (3.8-5.0)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**Other markers**

<table>
<thead>
<tr>
<th>Marker</th>
<th>All</th>
<th>mRS 0-2</th>
<th>mRS 3-6</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>86 (68 to 104)</td>
<td>83 (68-102)</td>
<td>86 (69-109)</td>
<td>0.19</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.8 (5.2 to 6.8)</td>
<td>5.6 (5.1-6.7)</td>
<td>6.0 (5.4-7.3)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* variables loge transformed to give a log-linear relationship with outcome in logistic regression; †Mann Whitney U test. mRS: modified Rankin scale

Supplementary table 2 Associations between marker levels with death or disability at 3 months.

<table>
<thead>
<tr>
<th>Marker (units)</th>
<th>OR, with further adjustment* (95% CI)</th>
<th>(P)-value</th>
</tr>
</thead>
</table>

**Inflammation**

<table>
<thead>
<tr>
<th>Marker</th>
<th>OR, with further adjustment* (95% CI)</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>1.79 (1.12 to 2.85)</td>
<td>0.02</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>1.11 (0.98 to 1.26)</td>
<td>0.10</td>
</tr>
<tr>
<td>ICAM (ng/ml)</td>
<td>1.06 (0.68 to 1.65)</td>
<td>0.79</td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
<td>1.86 (1.10 to 3.18)</td>
<td>0.02</td>
</tr>
<tr>
<td>TNF (mg/ml)</td>
<td>0.86 (0.62 to 1.18)</td>
<td>0.34</td>
</tr>
<tr>
<td>Marker</td>
<td>Median (IQR)</td>
<td>P-value</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>----------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Interleukin-10 (pg/ml)</td>
<td>1.03 (0.95 to 1.11)</td>
<td>0.52</td>
</tr>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>1.21 (0.85 to 1.73)</td>
<td>0.28</td>
</tr>
<tr>
<td>vWF (IU/dl)</td>
<td>1.45 (0.87 to 2.42)</td>
<td>0.15</td>
</tr>
<tr>
<td>White cells (x10^9 cell/l)</td>
<td>1.59 (0.98 to 2.55)</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Thrombosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-dimer (log_e unit)</td>
<td>1.52 (0.95 to 2.45)</td>
<td>0.08</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>1.33 (0.88 to 2.01)</td>
<td>0.17</td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>1.18 (0.85 to 1.65)</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Cardiac strain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT pro-BNP (log_e unit)</td>
<td>2.09 (1.11 to 3.93)</td>
<td>0.02</td>
</tr>
<tr>
<td>Troponin T &gt;0.01ng/ml</td>
<td>1.84 (0.86 to 3.94)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Cerebral damage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tau (pg/ml)</td>
<td>0.99 (0.95 to 1.03)</td>
<td>0.63</td>
</tr>
<tr>
<td>S100 b (log_e unit)</td>
<td>1.16 (0.84 to 1.61)</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>Other markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>0.94 (0.64 to 1.37)</td>
<td>0.73</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>1.19 (0.94 to 1.50)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Adjustment made for *NIHSS and age, independence of activities of daily living and for markers of inflammation: prior infection and prescription of statins; and for cardiac strain markers: cardiac failure, AF and prior cardiac vascular disease. The OR is the ratio of odds of poor outcome in the 75th to the 25th centile of plasma or serum marker. P-values are derived from Wald tests and determine if the reported OR is significantly different from 1.
Supplementary table 3 Predictive logistic regression models to predict poor outcome at 3 months after presenting with acute symptomatic TIA or stroke

<table>
<thead>
<tr>
<th>Variables</th>
<th>NIHSS + age</th>
<th>Log$_e$ NT pro-BNP + NIHSS + age</th>
<th>IL-6 + NIHSS + age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age*</td>
<td>1.07 (1.03 to 1.10)</td>
<td>1.04 (1.01 to 1.08)</td>
<td>1.06 (1.03 to 1.09)</td>
</tr>
<tr>
<td>NT pro-BNP†</td>
<td>2.26 (1.24 to 4.12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6†</td>
<td></td>
<td>1.99 (1.19 to 3.33)</td>
<td></td>
</tr>
<tr>
<td>NIHSS‡</td>
<td>1.23 (1.15 to 1.32)</td>
<td>1.20 (1.12 to 1.29)</td>
<td>1.20 (1.12 to 1.29)</td>
</tr>
</tbody>
</table>

Constant  | -6.37 | -6.90 | -6.31 |

*per year; †75th to 25 centile; ‡per unit increase;

Odds ratios and 95% confidence intervals for variables in each model. Odds ratios for each variable from each logistic regression model (i.e. $e^{\beta}$).

The constant terms come from the underlying logistic regression model of the form: logit (poor outcome)=$\beta_0 + \beta_1 + \beta_2 + \text{constant}$
### Supplementary figure 1

Associations between blood marker levels and failure to recover by 24 hours after symptom onset after stroke or TIA

*Adjustment made for NIHSS and age. The OR is the ratio of odds of poor outcome in the 75th to the 25th centile of plasma or serum marker to allow comparison of the relative strengths of associations between different markers. *P*-values are derived from Wald tests and determine if the reported OR is significantly different from 1. OR>1 indicate higher marker levels are associated with worse outcome.