Blood markers

for the diagnosis and prognosis of stroke

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Table of contents

Table of tables

Table of figures

Abstract

Many blood markers have been associated with stroke. I set out to determine whether blood markers can be applied to: (i) improve the accuracy of the clinical diagnosis of stroke or TIA, and/or (ii) improve the prediction of poor outcome in patients who are still symptomatic at the time of admission with stroke or TIA.

I systematically reviewed the existing literature on the diagnostic performance of a range of blood markers measured soon after stroke onset, to inform the choice of markers for my subsequent prospective studies in this thesis. Many studies had deficiencies in their design, which may have explained the apparently – and perhaps spuriously - impressive diagnostic performance of several markers. In the light of these data I was able to improve the design of my own studies and suggest how future studies of diagnostic markers could be improved.

In order to define an appropriate comparator test for assessing the diagnostic accuracy of blood markers, I first examined the performance of emergency room nurses and doctors. I assessed the accuracy of their diagnosis of TIA or stroke ('acute cerebrovascular disease') in patients presenting with symptoms of suspected stroke, and compared them with a number of stroke diagnostic scales. In the 405 patients recruited to the study, the sensitivity of emergency department staff was 77% and specificity 58%. Each stroke diagnostic scale had a slightly better sensitivity, though worse specificity, than an emergency department clinician. I decided to use the diagnosis by an emergency department clinician of 'probable or definite acute cerebrovascular disease' as the best clinical performance reference standard.

In blood taken from the same cohort of 405 patients, accredited research laboratories measured markers of inflammation, thrombosis, thrombolysis, cardiac strain and cerebral damage. Tissue plasminogen activator and $\log_e$ N-terminal pro brain natriuretic peptide were associated positively with a diagnosis of acute

cerebrovascular disease, though each marker did not add diagnostic value to the diagnosis of an emergency department doctor or nurse.

I systematically reviewed the literature examining the association between the levels of blood markers with poor outcome (i.e. death or dependency) after stroke. I found that although almost all markers studied had a positive association with poor outcome, there were methodological problems with many studies, chiefly small sample size, publication bias or within study reporting biases, and lack of adjustment for important confounders such as age or stroke severity.

With data from the Edinburgh Stroke Study, I examined the association between circulating markers of the inflammatory response (white cell count, interleukin-6, C-reactive protein and fibrinogen) and poor outcome after stroke. After adjustment for age, whether the patient lived alone, was independent of activities of daily living, was orientated, able to lift both arms and able to walk, I found that higher levels of interleukin-6, white cell count and glucose were associated with poor outcome. The relevant test of a biological marker is not its predictive ability alone, but whether, when added to a validated predictive model based on clinical variables, it improves the prediction of outcome. No individual marker improved the prediction of poor outcome when added to a validated prognostic model based on clinical variables alone.

From my cohort of 405 patients with suspected stroke 285 patients had a confirmed diagnosis. Follow up of these 285 patients with confirmed acute cerebrovascular disease showed that, after adjustment for neurological impairment and age, only interleukin-6 and N-terminal pro brain natriuretic peptide were significantly associated with death or disability at 3 months. Neither marker improved the predictions of a model to predict poor outcome based on clinical variables alone.

To examine the relationship between circulating markers of the inflammatory response and recurrent stroke, myocardial infarction, and vascular death ('recurrent vascular events'), again I used data from the Edinburgh Stroke Study. After

adjustment for clinical predictors (age, prior MI, stroke, or TIA and AF) I found that higher levels of interleukin-6, C-reactive protein and fibrinogen remained significantly associated with an increased risk of recurrent vascular events. However, the relationship with deaths from all causes was somewhat stronger for each marker, perhaps suggesting that higher marker levels were associated with debility rather than vascular events per se.

In conclusion, I found no marker measured could improve on the diagnostic accuracy of an emergency department clinician for acute cerebrovascular disease, nor improve the prediction of poor outcome by a prognostic model based upon clinical variables. The work of this thesis does not support the routine use of blood markers as an aid to the diagnosis of, or the prediction of outcome of, acute stroke.

Declaration

I confirm I composed this thesis and that it is my own original work. I have not submitted any part of the thesis for any other degree or professional qualification.

William Nichol Whiteley

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Publications and awards relating to the work of this thesis

Awards

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Papers in peer-reviewed journals

1. Whiteley, W., Chong, W. L., Sengupta, A., & Sandercock, P. 2009a, "Blood Markers for the Prognosis of Ischemic Stroke: A Systematic Review", Stroke, vol. 40, no. 5, p. e380-e389.
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1. Whiteley, W. N., Wardlaw, J. M., Dennis, M. S, Welsh, P., Lowe, G. D. O, Rumley, A., Satter, N., Green, A., and Sandercock, P. A. G. Blood Markers and the diagnosis of stroke or TIA in the emergency department: a prospective cohort study. Cerebrovascular Diseases 29 [Supplement 2], 17. 2010.
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outcome after acute cerebrovascular disease. Cerebrovascular Diseases 29[Supplement 2], 260. 2010.

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1. Whiteley W, Jackson C, Lewis S, Lowe GD, Rumley A, Sandercock P, Wardlaw JM, Dennis M, Sudlow C. The association of circulating inflammatory markers with recurrent vascular events after stroke: a prospective cohort study. *In press* Stroke
2. Whiteley W, Wardlaw J, Dennis M, Welsh P, Green A, Sattar N, Rumley A, Lowe G, Sandercock P. Blood markers and the diagnosis of stroke or TIA in the emergency department: a prospective cohort study *Submitted* to: Cerebrovascular Diseases
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Abbreviations

ACvD	acute cerebrovascular disease
AF	atrial fibrillation
ARU	acute receiving unit, Western General Hospital
AUROC	area under a receiver operator curve
BBISS	blood biomarkers in suspected stroke study
BNP	brain natriuretic peptide
CI	confidence interval
CPSS	Cincinatti prehospital stroke scale
CRP	C-reactive protein
CT	computerised tomography
DWI	diffusion weighted imaging
EDTA	ethylene-diamine-tetra-acetic acid
ECG	electrocardiogram
ED	emergency department
ELISA	enzyme-linked immunosorbent assays
ESS	Edinburgh stroke study
FAST	face arm speech test
FLAIR	fluid-attenuated inversion recovery
GP	general practitioner
GFR	glomerular filtration rate
GSTP	glutathione S transferase P
HR	hazard ratio

ICH	intracerebral haemorrhage
IDI	integrated discrimination improvement
ICAM-1	intercellular adhesion molecule-1
IL-6	interleukin-6
IL-10	interleukin-10
IMA	ischaemia modified albumin
LAPSS	Los Angeles prehospital stroke scale
LR+	positive likelihood ratio
LR-	negative likelihood ratio
MASS	Melbourne ambulance stroke screen
MBP	myelin basic protein
MI	myocardial infarction
MMP-9	matrix metalloproteinase 9
MRI	magnetic resonance imaging
NDKA	nucleoside diphosphate kinase A
NIHSS	National Institutes of Health stroke scale
NPV	negative predictive value
NRI	net reclassification index
NSE	neurone specific enolase
OHS	Oxford handicap scale
OCSP	Oxfordshire community stroke project
OR	odds ratio
PE	pulmonary embolism

PPV	positive predictive value
QUADAS	quality assessment of diagnostic accuracy studies
ROC	receiver operator curve
ROSIER	recognition of stroke in the emergency room scale
SAH	subarachnoid haemorrhage
TIA	transient ischaemic attack
tPA	tissue plasminogen activator
TNF	tumour necrosis factor
UFD-1	ubiquitin fusion degradation protein-1
VLP	visin like protein
WCC	white cell count
WTCRF	Wellcome Trust clinical research facility

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Systematic review

I designed the studies, the bibliographic search strategies, searched the literature, extracted data from papers, and wrote the published papers. Brenda Thomas helped me to design the comprehensive search strategies for both systematic reviews. Dr Wei Li Chong and Dr. Anshuman Sengupta, both house officers in neurology at the Western General Hospital, and Professor Mei-Chiun Tseng, from the National Sun-Yat Sen University, Taiwan, also extracted data from papers, and discussed disagreements with my own data extraction with me.

BBISS cohort recruitment

I applied for funding, ethical approval, NHS research and development approval and approval from the Caldicott guardian. I designed the baseline data collection and follow-up forms, electronic databases, specimen labelling, and barcode systems. I recruited the great majority of the patients, took blood and oversaw specimen handling, transfer to other units and quality control, entered data and performed all follow up. The design and performance of the statistical analysis was my own work.

The doctors and nurses of the acute receiving unit of the Western General Hospital referred patients with suspected stroke and kindly recorded their opinion on the likely diagnosis in each case. Dr. Rayessa Rayessa, Dr Ralph Thomas, Dr Enda Kerr, Dr Evan Mamaloukas and Dr Bartosz Karaszewski, all stroke fellows or registrars, collected baseline data and blood samples during periods when I could not personally recruit patients. The radiographers of the SFC Brain Imaging Research Centre performed MR brain scans at short notice on the patients with uncertain

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Professor Gordon Lowe, Professor Naveed Sattar, Dr. Ann Rumley, Dr. Paul Welsh of the Division of Cardiovascular and Medical Sciences, Royal Infirmary, University of Glasgow and Dr. Alison Green and Mrs. Mary Andrews, National CJD Surveillance Unit, University of Edinburgh measured the blood markers in plasma and serum for this project.

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Chapter 1. Introduction

The importance of diagnosis and prognosis

Many important decisions rest upon the diagnosis and prognosis of stroke. For example, whether to admit a patient to a stroke unit or a medical ward, to start aspirin to reduce the risk of future stroke or heart attack, or to consider nursing home care or a period of rehabilitation. Usually, clinicians determine the probability of a stroke diagnosis and its likely prognosis with an informal assessment of the clinical features of the presenting syndrome, past history and brain imaging. However the clinical diagnosis of stroke is often difficult to make, and prediction of outcome is hard.

It is possible to monitor physiological processes in patients with acute stroke by measuring circulating markers in the blood. The acute inflammatory response may be measured by C-reactive protein (CRP), turnover of thrombosis by D-dimer and axonal damage by tau protein. These 'biomarkers' could add value to the bedside clinical evaluation and to radiological investigations performed in routine clinical practice, to improve the diagnosis of patients with suspected stroke and the prediction of outcome in patients with confirmed stroke.

Aims of the thesis

The main aims of this thesis are therefore to:

(1) Examine whether blood markers of inflammation, thrombosis, thrombolysis, cardiac strain, neuronal and glial damage improve the accuracy of the clinical diagnosis of stroke.

(2) Determine if blood markers of inflammation, thrombosis, thrombolysis, cardiac strain, neuronal and glial damage predict stroke outcome, and if so, whether they add prognostic utility to existing clinical models.

Stroke and population health

In each age group, fewer Western Europeans will die from stroke this year than at any time over the past 50 years (Levi et al. 2009). The dramatic 80% fall in age specific stroke death rates over this period is most likely due to falls in the incidence of stroke as populations change to healthier lifestyles. This conclusion is supported by a falling trend of age specific stroke incidence in well conducted epidemiological studies (Anderson et al. 2005, Rothwell et al. 2004a). Alternative explanations may be that improvements in the care of people after stroke have led to reductions in case fatality, or there has been an improvement in the attribution of deaths to stroke over time with better diagnostic techniques, particularly brain imaging.

Despite these falls in mortality, stroke remains a global health priority. Stroke kills 5.5 million people a year and leads to the loss of 38 million years of healthy life worldwide (Mackay J & Mensah G.A. 2004). It is the third leading cause of death after coronary heart disease and all cancers combined. As stroke incidence rises with age, and the proportion of elderly people in the population rises, the demands that stroke places on health services are likely to remain steady or even rise (Mathers & Loncar 2006).

The definition of stroke

Strokes are a group of diseases of the brain vasculature that share a clinical syndrome: the rapid onset of a focal cerebral disturbance. The World Health Organisation definition of the clinical stroke syndrome is the most widely used;

> A stroke [is] defined as rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 h[ours] or leading to death with no apparent cause other than that of vascular origin
>
> (Hatano 1976)

In the standard definition, focal symptoms that last for less than 24 hours are classified as transient ischaemic attacks (TIA). However, this threshold presents difficulties when assessing patients within 24 hours of symptom onset. Researchers

need to follow up patients assessed within hours of focal neurological symptoms for full recovery – itself difficult to define - until the end of the 24 hours period, to determine short term prognosis and to differentiate transient ischaemic attacks from strokes.

Implicit in the differentiation of transient ischaemic attack from stroke is a difference in prognosis, aetiology or patho-physiology between patients with clinical syndromes classified as 'stroke' and clinical syndromes classified as 'TIA'. Some advocate that these important differences are captured by measuring the duration of symptoms, and others (Saver 2008) believe that the most important distinction is the presence or absence of objective evidence of cerebral tissue damage as evidenced by changes on diffusion weighted MR imaging.

Stroke subtypes

The two major mechanisms of stroke are damage to brain tissue due to a failure of arterial blood supply (i.e. cerebral ischaemia), and intracranial haemorrhage. The most common causes of occlusion of cerebral arteries are: (i) emboli from the heart, (ii) emboli from the arch of the aorta, carotid artery or larger intracerebral vessels, (iii) thrombosis forming in the intracerebral arteries, and (iv) occlusion in the small penetrating arteries of the deep white matter, which may be due to in-situ thrombosis or oedema. Attributing a mechanism to a particular stroke patient is difficult, as several potentially causative lesions may co-exist in the same patient, and the investigations necessary to attribute a cause may be difficult to obtain.

The aetiology of intracerebral haemorrhage is less clear. The risk of haemorrhage is higher in patients with risk factors for atherosclerotic vascular disease – such as smoking and hypertension – though whether most haemorrhages are due to rupture of an atherosclerotic vessel or other mechanisms is not certain. A small proportion of intracerebral haemorrhages are due to structural lesions, such as intracranial vascular abnormalities, though the proportion that can be attributed to these causes

is limited by the difficulty and risks of performing invasive vascular imaging in acutely unwell people.

The clinical diagnosis of stroke

Much depends upon a correct diagnosis of stroke. In the early stages, a correct diagnosis leads to rapid assessment by the right clinical team, referral for timely brain imaging and stroke unit care. The positive diagnosis that a stroke is due to ischaemia, rather than intracerebral haemorrhage, means a number of interventions may be used appropriately: intravenous thrombolysis, antiplatelet agents to prevent recurrent vascular events, anticoagulants to prevent recurrent cardiac embolism in patients with atrial fibrillation (AF), and carotid artery imaging to identify stenosis.

A stroke specialist is rarely the first health professional the patient encounters after the onset of stroke symptoms. Instead, it is usually either a general practitioner (GP), ambulance crew member or one of the emergency department (ED) staff – so called 'first responders' – who refer patients to stroke services when they suspect a stroke. The diagnostic performance of first responders is therefore of great importance. If their diagnostic approach is not sufficiently specific, large numbers of patients without stroke could overwhelm stroke services: if not sufficiently sensitive, patients with stroke may not receive important treatments. The benefits of improving tools for the diagnosis of stroke are therefore potentially large. The main methods to diagnose stroke are clinical examination and brain imaging; the best method used to improve the clinical diagnosis of stroke will depend on the health care setting.

Pre-hospital clinical diagnostic scales

Ambulance staff have a variety of diagnostic stroke scales to choose between to assess patients with suspected stroke: the face arm speech test (FAST), the Los Angeles pre-hospital stroke scale (LAPSS), the Cincinatti prehospital stroke scale (CPSS) and the Melbourne ambulance stroke screen (MASS) (Bray et al. 2005, Harbison et al. 2003b, Kidwell et al. 2000, Kothari et al. 1999). The diagnostic

performance of these scales as assessed in their original (development) cohorts is summarised in Table 1.1. The important comparison between an ambulance crew member's diagnosis of stroke with and without the use of one of these scales was not made in any of these studies.

Table 1.1 The sensitivity and positive predictive value of diagnostic stroke scales for a diagnosis of stroke, when performed by ambulance crews.

	Study setting	Items	Reference standard diagnosis	Completed forms/ ambulance transfers	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)
FAST	Acute stroke unit referrals by ambulance crews	Facial weakness, arm weakness, speech disturbance	Review of medical notes by medical assessors, unblinded	487/?	~79*	NC	78 (72 to 84)
CPSS	Sample of patients from emergency department	Facial weakness, arm weakness, speech disturbance	Review of medical notes for diagnoses of stroke made by a stroke service	n/a	59 (51 to 67)[†]	89 (86 to 91)	NC
LAPSS	All ambulance transfers in 7 months	Facial weakness, grip weakness, arm weakness, blood sugar, no seizure, not wheelchair user, >45 yrs, symptoms <24 hours	Diagnosis by study neurologist soon after paramedic assessment	206/1298	91 (76 to 98)	97 (93 to 99)	86 (70 to 95)
MASS	All ambulance transfers in 12 months	Facial weakness, grip weakness, arm weakness, blood sugar, speech disturbance, >45 yrs, not wheelchair user	Medical notes reviewed for discharge diagnosis	100/3,327	90 (81 to 96)	74 (53 to 88)	90 (81 to 96)

FAST: face arm speech test; CPSS: Cincinnati pre-hospital stroke scale; LAPSS: Los Angeles pre-hospital stroke scale; MASS: Melbourne ambulance stroke screen; NC: not calculated; n/a not available. * non-referrals to stroke unit not reviewed, so approximate [†]sensitivity and specificity calculated by completion of 860 scales in 171 patients by 24 ambulance crew members

These stroke scales were often evaluated as part of multi-faceted interventions to improve the speed of transfer of patients with acute stroke to hospital. These studies showed that, with such complex interventions, the identification of patients with stroke by paramedics could either remain the same or improve (Frendl et al. 2009, Wojner-Alexandrov et al. 2005). The additional effect of introducing a rating scale to the other components of the new intervention (such as extra training or stroke teams) was not clear. With the use of formal clinical scales, ambulance crews appear to have an acceptable performance in the diagnosis of patients with stroke, though additional effect of scales on paramedics' clinical skills is uncertain.

Hospital based stroke scales

A scale to improve the diagnostic ability for stroke of emergency department staff (recognition of stroke in the emergency room scale, ROSIER) was developed in Newcastle (Nor et al. 2005g). After developing a logistic regression model in 343 patients presenting to the emergency department, the authors derived a 7 item scoring system (the items: asymmetrical facial, arm, or leg weakness, speech disturbance, visual field defect, evidence of syncope, seizure) which they validated in a separate cohort of 160 patients from the same institution. A positive ROSIER – i.e. one or more item positive - had a sensitivity of 93% and specificity of 83% for a stroke diagnosis, and in their population, a positive predictive value of 90%. Although ROSIER performed better than the CPSS, LAPSS and FAST scores in this cohort, it did so at the expense of additional complexity.

In a group of patients presenting to a medical admissions unit with suspected stroke from Edinburgh, Hand derived predictors for the diagnosis of stroke (Hand et al. 2006c). A model with eight variables correctly classified 80% of patients into stroke/not stroke categories, with an area under a receiver operator curve (AUROC) of 0.87. The eight variables were: cognitive impairment, time of symptom onset, focal neurological symptoms, abnormal vascular findings, abnormal findings in other organ systems, laterisable symptoms, Oxford community stroke project (OCSP) classification possible, and neurological impairment measured by the

National Institutes of Health stroke scale (NIHSS). Hand also developed simpler models for use by paramedics and emergency department nursing staff, with fewer variables that might be easier to apply.

The effect of applying a scale may be limited, as although doctors can disagree about individual aspects of a patient's history and examination (Hand et al. 2006b, Lindley et al. 1993), they tend to agree with one another – albeit imperfectly - about the diagnosis of stroke within hours of symptom onset (κ =0.77) (Hand et al. 2006a).

After training, there seems to be good inter-observer agreement for a measure of neurological impairment, the NIHSS scale, between doctors (Goldstein, Bertels, & Davis 1989) and doctors with nurses, though the NIHSS alone was not designed as a diagnostic tool (Goldstein & Samsa 1997a, Hand et al. 2006h).

Clinical scoring systems designed to differentiate ischaemic from haemorrhagic stroke have not proved sufficiently discriminatory to be useful for patients presenting with suspected stroke (Allen 1983, Besson et al. 1995, Poungvarin, Viriyavejakul, & Komontri 1991). As they are unable reliably to exclude haemorrhage, they are not useful when making decisions about treatments that could potentially exacerbate intracranial haemorrhage – such as thrombolysis, heparin and aspirin.

Therefore, though clinical scales may help to identify patients with a high probability of stroke amongst the many presenting with suspected stroke, it is not clear whether they add discrimination to a doctor's routine diagnostic abilities.

Difficulties of acute imaging

Brain imaging is essential for the accurate diagnosis of stroke and its subtypes. Imaging seeks to identify non-vascular causes of a clinical stroke syndrome, such as brain tumors, and amongst patients with stroke positively to identify brain ischaemia or haemorrhage.

Brain computerised tomography

In the acute stages almost all intracranial haemorrhages are potentially visible on brain imaging that is of good quality and performed within hours of symptom onset. There are several case reports of patients who have had an intracerebral haemorrhage whilst in a CT scanner that confirm haemorrhage is visible immediately after onset of symptoms (Franke, Ramos, & van 1990, Masson et al. 1984). However, less experienced observers may overlook haemorrhages or mistake non-pathological calcification for blood (Schriger et al. 1998).

In the early stages of stroke, cerebral ischaemia may be difficult to identify positively on CT. About 60% of patients with acute stroke have early CT signs of infarction (Wardlaw & Mielke 2005), depending upon case mix. Neurologists detected early signs of infarction 14% less frequently than neuroradiologists (Wardlaw et al. 2007a). More experienced readers and the use of a scoring system improved the detection of early ischaemic change (Coutts et al. 2004, Wardlaw et al. 2007b). Estimates of the sensitivity of CT for changes of cerebral ischaemia range from 11 to 75% (median 45%), but specificity is in general very high – 100% (Brazzelli et al. 2008).

In summary, signs of intracranial haemorrhage are almost always evident on a CT soon after stroke onset, and most observers are able to identify it reliably. Positive evidence of cerebral ischaemia is seen less frequently, and may be harder for observers to detect, particularly if they have not had special training in radiology or stroke medicine.

Brain magnetic resonance imaging

MRI is currently the main alternative to CT for brain imaging in the early stages of stroke. Some authors state that intracerebral haemorrhage can easily be detected on MRI in the acute stages of stroke by observers of different levels of imaging experience though considerable uncertainty exists (Brazzelli et al 2008, Fiebach et al. 2004, Kidwell et al. 2004). Diffusion weighted imaging (DWI) is the most sensitive

sequence to detect early signs of ischaemic stroke. In patients with minor stroke, DW MRI is sensitive to early ischaemic signs, and may be more sensitive than CT. Estimates of sensitivity – which may be overly optimistic - range from 73% to 100% (median 100%) and specificity from 86 to 100% (median 100%) (Brazzelli et al 2008, Chalela et al. 2007). However, DW MRI does not always show changes in patients with ischaemic stroke (Ay et al. 1999, Doubal, Dennis, & Wardlaw). MRI is more expensive than CT, and is less rapidly available for patients with acute stroke services, in the UK and elsewhere (Kane et al. 2008, Leys et al. 2007). There are also practical barriers to performing MRI in unwell patients with acute stroke (Hand et al. 2005b). MR scanning may not be possible in patients who are too medically unstable to enter the isolated environment of the scanning room, too confused to lie still for the investigation, or who have medical implants (such as pacemakers) or loose metallic foreign bodies.

Potential advantages of blood markers for stroke diagnosis

Blood markers for diagnosis of diseases other than stroke

Blood markers are used routinely in the management of several diseases. In patients presenting to hospital with chest pain, a raised cardiac troponin is indicative of myocardial infarction, rather than angina or other causes of chest pain (Brott et al. 1989c): the measurement of troponin is recommended in the early stages of assessment of all patients with suspected acute coronary syndromes (NICE 2009). In patients presenting to hospital with suspected pulmonary embolus (PE), a raised serum D-dimer measured with a quantitative ELISA has a sensitivity of 96% and specificity of 44% for a diagnosis of PE in the subsequent months; measurement of D-dimer is therefore recommended as part of a clinical pathway to rule out the diagnosis of PE, and helps to reduce inappropriate use of more resource intensive diagnostic methods with a higher hazard from radiation (e.g. CT pulmonary angiography) (British Thoracic Society 2003).

Near patient testing

Blood markers could have a number of advantages compared with current techniques in the diagnosis of stroke. If simple, rapid assays were available for the measurement of blood proteins in patients with stroke (as they are for patients with myocardial infarction (McDonnell et al. 2009)), blood markers would have the advantages of:

(i) *Speed:* Near patient devices for the measurement of troponin and D-dimer give results within minutes. The small immunometric chips could be adapted to other blood proteins, were they of value in stroke.

(ii) *Ease of use*: Almost all point of care devices are designed to be used by people with little training, and without extensive laboratory preparation (Warsinke 2009).

(iii) *Reduced costs*: Implementation of point of care testing devices in patients with myocardial infarction led to estimates of substantial cost savings, by shortening hospital stay and reducing the demand for further diagnostic resources (Apple et al. 2006).

However, there are a number of concerns about point of care testing. The machines may not be as reliable as those designed for laboratory use; the tests may be inappropriately over- or under-used; and the direct costs of the test and machines fall on the ED budget, though savings accrue elsewhere in the health service (predominantly in specialist services).

Role of new tests

Often a new diagnostic test does not overturn previous diagnostic methods. Brain imaging has been an exception. Since it became widely available, several uncomfortable and potentially harmful tests have been abandoned - such as air encephalograms and intra-arterial angiography by direct carotid puncture. Blood markers are unlikely to revolutionise diagnosis to the same extent; it is more likely that if they prove useful they will form part of a management or diagnostic pathway.

Predicting outcome after stroke

Tools for the reliable prediction of outcome after stroke could be very useful. Patients, clinicians and health care planners might use predictions to guide decisions about medical treatment, select an appropriate level and type of care, or for planning services. Auditors could compare observed with predicted outcome to assess the performance of stroke services. There are three main methods – each with their own advocates – to predict outcome after the onset of illness: usual bedside clinical judgement, single predictive measures and multivariable statistical models.

Usual bedside clinical judgment

The most widely used method to predict outcome in clinical practice is the judgment of the assessing doctor. However, such predictions can often be improved upon. For example, only 24% of physicians gave an accurate prediction of recurrent cardiovascular events in patients with cardiac vascular disease, most often making an overestimate (Pignone et al. 2003). When predicting operative mortality, cardiac surgeons overestimated the chance of death and the need for intensive care (Ivanov et al. 2000b). In stroke, clinicians were over-optimistic about the chance of recovery: only 65% of those predicted to recover had gained independence by one year (Counsell, Dennis, & McDowall 2004e).

Single predictive measures

A single easily remembered measurement – for example stroke severity – might be predictive for outcome after stroke. However, the relationship between outcome and a single variable may demonstrate both ceiling and floor effects, and the relationship between a measurement scale (e.g. the NIHSS) and the outcome of interest may not be linear. As relationships between a scale and outcome are not always simple, they cannot always be interpreted without computation.

Multivariable models

Multivariable models may make better predictions of outcome than clinicians. For example, in patients with coronary vascular disease the predictions of survival from a validated Cox regression model were better (and more consistent) than a cardiologist's clinical judgment; furthermore in this case, more senior clinicians did not perform better than the model (Kong et al. 1989). By contrast, in stroke, the predictions from a simple model were as good as those of an experienced clinician (Counsell, Dennis, & McDowall 2004d).

The best prediction of outcome after stroke will very likely come from multivariable models, rather than single predictor variables. Many models have been developed for predicting death and disability in patients soon after acute stroke, though only two – the six simple variable score (Counsell et al. 2002g) and a score containing the NIHSS and age (Konig et al. 2008b) – have been extensively validated. Both these scores contain a measure of stroke severity (the NIHSS score or 3 dichotomous variables with ability to walk, lift arms and orientation to time, place and person) and age; in addition the six simple variable model contains a measure of premorbid function (whether the patient was independent prior to stroke) and a factor that might influence the chance of returning home (whether the patient lived alone before the stroke).

Role of prediction in clinical practice

Although the results of clinical decision rules based upon prognostic models may be reliable, they may not alter clinical decisions (Lee et al. 1995, Reilly et al. 2002, Selker et al. 1998). For example when cardiothoracic surgeons were given access to the results of a reliable clinical prognostic model they did not revise their own personal judgement of a poor outcome (Ivanov et al. 2000a). The key characteristics of a rule that is robust enough to change a clinician's behaviour are not yet defined.

Blood markers in improving prediction of stroke outcome

In stroke, the performance of validated prognostic models to predict stroke outcome could be improved. The major components of the two valid prognostic models do not include measurements of a number of key patho-physiological processes that might help to refine predictions of a poor outcome, such as cardiac dysfunction, the extent of neuronal or glial damage and activation of inflammatory pathways.

In other fields of medicine, blood markers may improve the prediction of outcome, over and above existing clinical risk scores. For example the addition of CRP seems to add prognostic information to the Framingham risk score for the prediction of vascular events (Ridker et al. 2002), and the addition of brain natriuretic peptide to the GRACIE score improves the prediction of outcome after myocardial infarction (Lorgis et al. 2009), though further validation of both of these tests is needed before they are introduced into routine clinical practice.

Blood markers might therefore be useful in addition to validated prognostic models in predicting outcome after stroke.

Chapter 2. Blood markers in the diagnosis of ischaemic stroke: a systematic review

Introduction

A rapid blood test to confirm a clinical and imaging diagnosis of ischaemic stroke (or to aid risk-stratification in confirmed cases), based on a simple and low-cost near-patient technology, would be extremely useful. At the moment, the diagnosis of ischaemic stroke is based on an experienced stroke clinician's examination of the patient, supplemented by the results of brain imaging. However, in people who suddenly become unwell with a suspected stroke, the clinical assessment within the first few hours is not always straightforward. Many patients with acute stroke are not assessed by a stroke specialist; the initial evaluation is often by a GP, paramedic or triage nurse. For those assessed in hospital, interpretation of brain imaging appearances can be difficult, as computerised tomography (CT) is often normal after the onset of ischaemia and may remain normal in patients with mild ischaemic strokes. Magnetic resonance imaging (MRI), though undoubtedly more sensitive in detecting ischaemia than CT, especially in the diagnosis of mild stroke, is still not 100% sensitive or specific. MRI may not be feasible in acutely ill patients because they are restless, have a contraindication to MRI or MRI may not be immediately available (Chalela et al 2007).

Achieving an accurate diagnosis quickly in patients with suspected acute stroke is extremely important. Patients with ischaemic stroke, even with relatively mild symptoms, may be eligible for intravenous thrombolysis or other means of brain reperfusion if treatment can be started within a few hours of symptom onset. Patients who are not suitable for such acute treatments are at risk of early recurrent stroke: 8% of high risk patients have a recurrent stroke within the first 2 days (Johnston et al. 2007). Prompt initiation of secondary preventative treatment can substantially reduce the risk of further stroke.

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

There has been a substantial investment in translational medical research programmes to discover new diagnostic markers. I therefore wished to undertake a systematic review of published reports to assess the accuracy of blood markers for the diagnosis of ischaemic stroke as a precursor to my own study of the subject. I aimed to describe the methodological quality of the studies, to compare the accuracy of diagnostic markers and assess the extent to which methodological weaknesses might have biased diagnostic test accuracy.

Methods

Study identification

I searched Medline and EMBASE from 1966 to 15th March 2007 for all studies of the use of diagnostic blood biomarkers in stroke. I maximised retrieval by searching using both generic biomarker terms and individual biomarkers (and their synonyms) obtained from a previous search of the literature (Anderson 2005, Marcovina et al. 2007, Ridker et al. 2004, Vasan 2006b). The search strategy included 13 terms for ischaemic stroke, 4 for generic biomarkers and 780 specific biomarker terms. Diagnostic studies were identified by searching for the words 'sensitivity', 'specificity', 'likelihood ratio' or 'diagnosis' in the title or abstract and keywords.

The full search strategy is listed in the appendix. The search was not restricted by language.

I searched the reference lists of relevant papers, conference abstract books and my personal files. I also searched the internet for patents (using www.freepatentsonline.com and www.google.com) and papers citing each relevant paper with the 'Google Scholar' tool (http://scholar.google.com/).

Studies were eligible for inclusion if they examined the ability of a single or a set of several venous blood (not CSF) markers to discriminate between patients with ischaemic stroke and a group without stroke (either controls without disease, with stroke mimics or with other neurological diseases), or between ischaemic and haemorrhagic stroke, where a cutoff value for the test had been calculated (or arbitrarily set) and there was sufficient information to fill a 2x2 contingency table. There was no minimum sample size for study inclusion. Both conference abstracts and published papers were included.

Data extraction

I and a colleague, Dr. Mei-Chiun Tseng, reviewed the list of titles and abstracts of potentially relevant papers independently; I then obtained full copies of papers meeting my eligibility criteria and we independently extracted data from the eligible papers. Any disagreements were resolved by discussion. We assessed the quality of the study reports with a modified QUADAS instrument (Whiting et al. 2003) (see appendix). Where a study examined more than one cohort within a study, the results for each cohort were extracted separately.

Statistical analysis

I calculated 95% confidence intervals for the estimates of sensitivity and specificity in each cohort (Agresti & Coull 1998). I made no attempt to assess for publication bias, although this probably exists, as there are currently no well-established methods to assess the scale and direction of this form of bias in studies of diagnostic test accuracy (Begg 2005).

Results

The MEDLINE/Embase search identified 3093 studies. A further 8 were found through reading conference reports and the reference lists of relevant papers. All the abstracts were read, and 70 publications were read in full. 21 publications were relevant to the review - 6 conference abstracts and 15 papers (Abboud et al. 2007, Allard et al. 2007, Allard et al. 2005i, Allard et al. 2005h, Allard et al. 2004a, Dambinova et al. 2003b, Dambinova et al. 2003a, Delgado et al. 2005, Fassbender et al. 1997d, Foerch et al. 2006d, Hill et al. 2000f, Laterza et al. 2006d, Lynch et al. 2004h, Montaner et al. 2005, Rainer et al. 2007, Reynolds et al. 2003h, Rouanet et al. 2006, Takahashi et al. 1999a, Tomitori et al. 2005d, Turck et al. 2006, Zimmermann-Ivol et al. 2004a) (Table 2.1).

Methodological assessment

I used a modified QUADAS instrument to assess the quality of the reports of diagnostic biomarkers (Table 2.2). The performance of blood biomarkers was examined in patients with suspected stroke – the clinical scenario for any stroke test - in 4/21 studies (Laskowitz et al. 2005a, Lynch et al. 2004g, Reynolds et al. 2003g, Rouanet et al 2006). The remaining studies compared cohorts of patients with a diagnosis of stroke with a control group. The sensitivity and sensitivity of a biomarker with a prespecified threshold for a positive test was examined in 6/21 studies (Abboud et al 2007, Fassbender et al. 1997c, Hill et al. 2000e, Laterza et al. 2006c, Rouanet et al 2006, Tomitori et al. 2005c). The remainder derived a diagnostic threshold cut-off value from the cohort examined. Of the 15 studies that employed a data-dependent cutoff, none validated the sensitivity estimates in a separate cohort. Only 2/21 studies reported that the assessment of biomarker diagnostic accuracy was performed blinded to stroke status (Dambinova et al 2003b, Foerch et al. 2006c). All diagnoses of stroke appeared to be blinded to biomarker status.

The clinical comparisons in each study were different. Some studies classified TIA as an acute ischaemic stroke though others did not (Abboud et al 2007, Allard et al 2007, Allard et al 2005i, Allard et al. 2005g, Allard et al. 2004b, Lynch et al. 2004f,

Reynolds et al. 2003f, Turck et al 2006, Zimmermann-Ivol et al. 2004b). However the number of patients symptomatic at the time blood was drawn was not defined and there were no explicit means of measuring recovery at 24 hours. Most studies classified a patient as having a definite ischaemic stroke only if they had both appropriate symptoms and a visible appropriate lesion on imaging, even though it is well recognised that many patients with definite stroke can have initially normal neuroimaging. Six studies classified subarachnoid haemorrhage (which generally has a very different clinical presentation to acute stroke) as a haemorrhagic stroke though most did not (Allard et al 2007, Allard et al. 2005f, Allard et al. 2004c, Takahashi et al. 1999b, Turck et al 2006). Only one study examined a cohort of suspected stroke patients, and compared the performance of a panel of biomarkers to another assessment method (in this case a triage nurse): both performed with a similar sensitivity and specificity (Rouanet et al 2006).

Nine studies reported the delay between symptom onset and blood taking for biomarkers – the range was between 30 minutes and 5 days after stroke (Abboud et al 2007, Allard et al 2007, Allard et al. 2005e, Dambinova et al 2003b, Hill et al. 2000d, Lynch et al. 2004e, Rainer et al 2007, Reynolds et al. 2003e, Zimmermann-Ivol et al. 2004c). Four only examined diagnostic performance within the first 24 hours of symptom onset (Abboud et al 2007, Hill et al. 2000c, Lynch et al. 2004d, Rainer et al 2007). One study reported the sensitivity of a biomarker panel for a diagnosis of stroke at different time points, though there was no clear relationship between the delay to blood taking and sensitivity (Reynolds et al. 2003d).

Markers measured

The 21 studies tested 58 single biomarkers and 7 panels of made up of several markers. The exact number of cohorts, and therefore the total number of patients involved, was difficult to calculate as some studies examine part cohorts from other studies included in the review (Table 2.1). The estimated upper limit was 2928 stroke patients and 1569 controls in 24 cohorts.There was sufficient information to extract 2x2 tables on 21 markers for the diagnosis of ischaemic stroke vs not stroke

or control (Figure 1). Of these markers, 5 had reported sensitivities over 90% (NDKA, PARK7, UFD-1, NMDA receptor (NR) 2 fragment, NR2A/B antibodies) and 14 had a specificity over 90% (PARK 7/RNA-BP , UFDP, NDKA, GSTP, ischaemia modified albumin (IMA), visin like protein (VLP-1), beta globin DNA, NR2 fragments, S100 B, FABP, neurone specific enolase (NSE), NR2A/2B Ab, myelin basic protein (MBP) and thrombomodulin). For 5 biomarkers (S100 beta, MBP, thrombomodulin, NSE and beta globin DNA) the specificity was defined by a 95% or 98% reference interval in subjects without disease. Five markers were tested in more than one cohort of patients, though only neurone specific enolase (NSE) and S100 beta were tested by different research groups. Only S100 B was tested in different cohorts of patients using the same cutoff for a postive result (0.02μg/L). Information about 4 panels of markers was extracted (Figure 2); in no case was the regression equation given for the marker panel (i.e. the formula which permits a calculation of the probability of stroke if the results of the individual component biomarker tests are known) in the original publication, though one became available subsequently. No panel of markers was validated in an independent cohort of patients.

Chiefly as a result of the very substantial clinical heterogeneity of the populations studied, and heterogeneity of results, I did not perform a meta-analysis to derive an overall summary receiver operator curve for any of the individual markers or any of the panels of markers.

In 5 studies, 6 individual markers and 2 panels of markers were assessed to determine the ability of blood biomarkers to distinguish between ischaemic and haemorrhagic stroke. There was sufficient information to extract 2x2 tables on 6 markers and 4 panels (Figure 3). Most studies used the diagnosis of haemorrhagic stroke as the diagnosis of interest in a population of haemorrhagic and ischaemic stroke patients. Two (Allard et al. 2004d, Dambinova et al 2003a) reported a positive test as a diagnosis of ischaemic stroke in a mixed population, both with a high

sensitivity and specificity. No single marker or panel of markers was reported in more than one cohort of patients.

Discussion

I set out to assess the utility of blood biomarker tests for improving the diagnosis of ischaemic stroke in the acute phase. Most of the studies in this review reported biomarkers with high sensitivity and specificity which, if confirmed in validation studies, could be useful in clinical practice. However, all the blood biomarker studies had weaknesses in their methodology. Thus the apparently very high specificities and sensitivities reported may be substantially due to bias and not reflect the true clinical utility of the test. The main problems identified in this review were: small sample size; poor choice of reference standard (lesions required on imaging rather than clinical diagnosis supported by imaging); poor choice of controls (rarely reflecting the clinical setting in which the test would be used); data-dependent thresholds and lack of validation.

Implications of this review for subsequent studies

The important diagnostic questions in the management of acute ischaemic stroke can be summarised as follows:

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

Methodological aspects of the diagnosis of stroke in the emergency department before scanning or expert assessment

If a test is designed to be used for the diagnosis of ischaemic stroke in unselected patients by clinicians in an emergency setting, then cohorts of suspected stroke patients for biomarker development or validation should be recruited by clinicians in the emergency department. In this systematic review, only one (Rouanet et al 2006) attempted this explicitly; three other studies examined suspected stroke patients though it was unclear from the reports whether non-expert clinicans recruited patients, and they also used healthy controls to enlarge the control group (Lynch et al. 2004c, Montaner et al 2005, Reynolds et al. 2003c). Studies should also evaluate whether biomarker tests perform better than the clinical judgement of non-expert clinicians or prehospital screening tools such as the FAS test (Harbison et al. 2003a).

Two useful diagnostic tests employing a biomarker in venous blood for conditions other than stroke, that are used in a similar setting, are BNP for exclusion of diagnosis of congestive cardiac failure and D-dimer for the exclusion of a diagnosis of PE. Both are very sensitive. BNP has a sensitivity range between 68 to 98% (Wang et al. 2005) and D-dimer ELISA has a sensitivity for PE of 96% (Stein et al. 2004). D-dimer is used as part of a diagnostic algorithm that includes an initial assessment of the clinical probability of PE by means of a validated scale. Similarly blood biomarkers of stroke might be most useful when combined either with clinical judgement alone or with clinical judgement plus brain imaging.

Differentiating haemorrhagic from ischaemic stroke before brain imaging

In the developed world a blood test that aims to differentiate between patients with haemorrhagic and ischaemic stroke would be of greatest utility before brain imaging (e.g. during ambulance transfer to hospital). In the developing world, where brain imaging is not available, a low cost blood test to supplement the bedside diagnosis would be extremely useful. In studies to evaluate such a test, the

patients should be recruited at the earliest possible opportunity and certainly before brain imaging is ordered. CT is a rapid test, and very good at identifying acute intracranial haemorrhage, so a biomarker test is likely to be redundant after imaging has been performed. However, in the studies in this review, which compared diagnostic test accuracy for distinguishing ischaemic from haemorrhagic strokes, there were no patients in whom stroke was suspected, who turned out to have an alternative diagnosis on imaging. Hence the cohorts were too highly selected to be useful to assess the diagnostic utility of biomarkers in this particular setting.

Supporting a diagnosis of ischaemic stroke in patients with normal CT brain

In many countries, most patients with suspected stroke have a brain CT as their first investigation. When CT is normal, clinicians are often uncertain whether the diagnosis of stroke is secure enough to justify thrombolysis or the use of aggressive stroke preventative treatments. In patients with clinical symptoms of stroke, but a normal CT brain scan, a blood biomarker could be useful, as ischaemic stroke may be the condition most likely to lead to a rise in specific proteins. Studies evaluating a blood biomarker in this setting should recruit patients in whom the clinicians are uncertain about the appearances of an imaging test, with blood drawn immediately after the CT. If advanced CT or MR scanning become more widely available in the emergency departments, the diagnostic performance of blood biomarkers would need to be compared with these techniques.

Short term prognosis after non-disabling stroke or TIA

In patients with non-disabling stroke or TIA, short term prognosis has a major influence on patient management: it informs decisions about admission to hospital, the intensity and speed of investigation, and the likelihood of successful early discharge. In this context, studies of diagnostic test accuracy should identify whether patients were still symptomatic when they were first assessed and how their recovery was measured. In patients with very short-duration symptoms, imaging tests are much less likely to give positive confirmation, so the place of

imaging as part of the reference standard diagnosis of stroke or transient ischaemic attack (TIA) is altered, though imaging remains of value to exclude stroke mimics. In this context, expert clinicial assessment, and detailed clinical follow up to detect recurrent clinical events are the key methodological determinants.

Defining the reference standard

It is very difficult to define a test which can act as a reference standard test for the diagnosis of stroke; it is recognised that CT, MR and even autopsy may be 'negative', even in patients considered to have a clinically definite acute stroke by all other criteria. Therefore the reference standard for a diagnosis of ischaemic stroke remains a diagnosis by an expert clinician, based on the initial clinical features, supported by appropriate imaging and the patient's subsequent clinical course suuported where necessary by repeated imaging on follow-up.

Validation studies

In this review, I found validation studes were limited. Only a few studies examined the same diagnostic threshold for the same marker in more than one cohort. In one set of papers, different diagnostic thresholds have been calculated in different cohorts for the same biomarker to optimise sensitivity and specificity by AUROC analysis, though the same diagnostic threshold was not examined in more than one cohort (Allard et al 2007, Allard et al. 2005d, Turck et al 2006).

Choice of patient cohorts and controls

It has been proposed that biomarker development should take a linear path; identification of blood biomarker candidates either in animal or human models, before testing them in cohorts of stroke patients versus normal controls before testing them in cohorts of suspected stroke patients (Pepe et al. 2001, Vasan 2006a). However, when considering genomic, proteomic or metabonomic approaches to discovery of biomarker candidates, there are compelling reasons to use cohorts of patients with suspected disease (after all this is the context that the test will be used in) for the discovery phase.

The biomarkers identified in this review are expressed in diverse cell types and part of many different cellular processes (Table 2.3). Some proteins are found mainly in the nervous system: B-type neurotrophic growth factor, S100B, myelin basic protein, neurone specific enolase and visin like protein; others indicate endothelial processes: matrix metalloproteinase-9, thrombomodulin, vascular cell adhesion molecule and von Willebrand factor. Some are not clearly linked to stroke pathogenesis, such as acrolein, nucleoside disphosphate kinase, antibodies to NR2A/2B and glutathione S transferase. Two studies have examined messenger RNA expression in peripheral blood leucocytes soon after stroke (Moore et al. 2005, Tang et al. 2006). The results of prediction analysis for microarrays (PAM) algorithm were different in the two studies; of a PAM gene list of 22 in one study and 29 in the other, there was overlap only of N-acetyl neuraminate pyruvate lyase.

This review has a number of shortcomings. The searching for studies was hampered by the lack of a suitably sensitive yet specific electronic bibliographic search strategy to identify reports of studies which focus on diagnostic test accuracy. This is in distinct contrast to other research designs, especially randomised controlled trials, for which highly sensitive yet specific search strategies have been developed. Searching the 'grey' literature was very difficult to perform comprehensively, so it is likely that there are unpublished reports of biomarker sensitivity and specificity that have not been identified. In this study, the assessment of report quality was necessarily limited, as many of the studies were conference abstracts, with limited space to report the details of the methods of their studies. It is likely that the timing of sampling after stroke onset will affect the performance of a blood biomarker test: in these studies, it was not possible to analyse this because of a paucity of data.

Implications for research

- There are a number of blood biomarkers that perform impressively well in their development cohorts

- To estimate the sensitivity and specificity of markers in clinical practice, they need be examined in unselected cohorts of patients with suspected stroke .
- The design of and reporting of studies of blood biomarkers for the diagnosis of ischaemic stroke could be improved (Table 2.4).

Implications for practice

- No marker can be recommended yet for use in routine clinical practice.

Tables

Table 2.1 Description of diagnostic marker studies

Study	All stroke (n)	Ischaemic stroke (n)	Definition of ischaemic stroke	Haemorrhagic stroke (n)	Control patients (n)	Control patients characteristics	Analysis	Markers measured	Marker model
(Abboud et al 2007)	102	84	Symptoms + MR lesion	18	16	TIA (symptoms+ no MR lesion)/epileptic seizures	IS+ICH vs TIA + seizures	Ischemia modified Albumin	No
(Allard et al 2005i) (3 cohorts)	35	27 (IS) + 6 (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	3	35	Various non-stroke diagnoses	IS+TIA+ICH vs Various non-stroke diagnoses	UFD-1, RNA-BP, NDKA	No
	53	24 (IS) + 23 (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	6	30	Unclear	IS+TIA+ICH + SAH vs control	UFD-1, RNA-BP, NDKA	No
	533	183 (IS) + 124 (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	226	100	Unclear	IS+TIA+ICH + SAH vs control	RNA-BP, NDKA	No
(Allard et al. 2005c) (3 cohorts)	36	27 (IS) + 6 (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	3	35	Various non-stroke diagnoses	IS+TIA+ICH vs Various non-stroke diagnoses	PARK 7, NDKA	No
	53	24 (IS) +23 (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	6	30	Unclear	IS+TIA+ICH + SAH vs control	PARK 7, NDKA	No
	533	183 (IS) + 124 (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	226	100	Unclear	IS+TIA+ICH + SAH vs control	PARK 7, NDKA	No

Study	All stroke (n)	Ischaemic stroke (n)	Definition of ischaemic stroke	Haemorrhagic stroke (n)	Control patients (n)	Control patients characteristics	Analysis	Markers measured	Marker model
(Allard et al. 2004e)	45	26	Symptoms + visible imaging lesion (CT/MR) + TIA	19	21	Orthopaedic diagnoses	IS+TIA vs orthopaedic disorders	ApoC-I, ApoCIII, Serum Amyloid A, Antithrombin 3 fragment	No
(Allard et al 2007) (1 unique of 3 cohorts)	31	22 (IS) + 6 (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	3	31	Various non-stroke diagnoses	IS+TIA+ICH vs control	UFD-1, PARK 7 NDKA	No
	49	29 (IS) + 5 (TIA)	Unclear	10	29	Unclear	IS+TIA+ICH vs control	UFD-1, PARK 7 NDKA	No
	53	24 (IS) + 23 (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	6	30	Unclear	IS+TIA+ICH + SAH vs control	UFD-1, PARK 7 NDKA	No
(Dambinova et al 2003b)	49	31	Symptoms with consistent imaging	18	230	Blood donor/ hypertensives	IS vs control	NR2A/2B antibodies	No
(Dambinova et al 2003a)	48	48	Unclear	-	28	Normal, ICH	IS vs control +ICH	NR2 fragment	No
(Fassbender et al. 1997b)	24	24	Symptoms with lesion on CT	-	24	Unclear	IS vs control	NSE	No
(Foerch et al. 2006b)	135	93	Symptoms with lesion on imaging	42	-	-	IS vs ICH	GFAP	No
(Hill et al. 2000b)	28	28	Unclear	-	-	-	IS vs control	NSE, MBP, S100b, Thrombomodulin	Any positive
(Laskowitz et al. 2005b)	130	130	Unclear	23	-	-	IS vs control	BNP, CRP, D-dimer, MMP-9, S100b	Multiple logistic regression with BNP, CRP, D-dimer, MMP-9, S100b
(Laterza et al. 2006b)	18	18	Unclear		39	Healthy controls	IS vs control	VLP-1	No

Study	All stroke (n)	Ischaemic stroke (n)	Definition of ischaemic stroke	Haemorrhagic stroke (n)	Control patients (n)	Control patients characteristics	Analysis	Markers measured	Marker model
(Lynch et al. 2004b)	44	44	Symptoms >24 hrs and imaging appearance	-	21 + 157	TIA, syncope, misc + no vascular disease	IS vs TIA + SS + control	S100b, GFAP, MMP-9, VCAM, IL-6, ICAM, TNF, NCAM, IL-1rA, IL-1b, IL-8, MCP-1, VEGF, vWF, TAT3, DD, CPK, TF, MBP, PLP, Mal, BNP, Caspase 3, Calbindin D, HSP, Cytochrome C	Multiple logistic regression for MMP9,vWF,VCAM and S100beta,VCAM,vWF
(Montaner et al 2005)	1,100	776(IS) + 185 (TIA)	Unclear	139	90 + 99	Seizures, migraine, tumours, hypoglycaemia + healthy controls	Stroke vs SS	CRP, DD, RAGE, MMP-9, S100b, BNP, NT3, casopase-3, chimerin, secretagogin	All above/below normal range: caspase -3, DD, RAGE, Chimerin, Secretagogin, MMP-9
(Rainer et al 2007)	197	118 (imaging lesion) + 44 (no imaging lesion)	Symptoms >24 hrs	35	Unclear	Healthy volunteers	IS vs control	Plasma DNA, S100b	Both above cutoff:Plasma DNA, S100b
(Reynolds et al. 2003b)	185	82	Symptoms >24 hrs and imaging appearance	103	214 + 38 + 51	Healthy volunteers + closed head injury + TIA	IS + ICH vs control	S100b, BNGF, vWF, MMP-9, MCP-1	3 or more above cutoff: S100b, BNGF, vWF, MMP-9, MCP-1, multiple logistic regression: S100b, BNGF, vWF, MMP-9, MCP-1
(Rouanet et al 2006)	131	85 (IS) + 33 (TIA)	Senior neurologist	13	65	Referred suspected stroke, neurologist not stroke	IS + TIA + ICH vs control	BNP, DD, MMP-9, S100b ('Triage stroke panel')	Multiple logistic regression for: BNP, D-dimer, MMP-9, S100b
(Takahashi et al. 1999c)	32	26 (IS) + 2 (TIA)	unclear	3+1	103	Healthy subjects	IS+TIA+ICH + SAH vs control	S100b	

Study	All stroke (n)	Ischaemic stroke (n)	Definition of ischaemic stroke	Haemorrhagic stroke (n)	Control patients (n)	Control patients characteristics	Analysis	Markers measured	Marker model
(Tomitori et al. 2005b)	62	62	Focal imaging abnormality	0	35	Unclear	IS vs control	acetylpolyamine oxidase, spermine oxidase, total polyamine oxidase	No
(Turck et al 2006)	28	22 (IS) + 6 (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	-	29 + 13	Unclear + conditions mimicking stroke (MS, nerve palsy)	IS+TIA+ICH vs SS + control	UFD-1	No
	34	29 (IS) 5 (TIA)		-	29	Unclear	IS+TIA+ICH vs SS + control	GSTP-1, UFD-1	No
	47	24 (IS) 23 + (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	-	29	Unclear	IS+TIA+ICH + SAH vs control	UFD-1	No
(Zimmermann-Ivol et al. 2004d)	22	IS (11) + 5 (TIA)	Symptoms + visible imaging lesion (CT/MR) + TIA	6	22+20	No disease + AMI	IS+TIA+ICH vs control	H-FABP, CK-MB, TnI, NSE, S100b	No

Abbreviations: AMI: acute myocardial infarction, TIA: transient ischaemic attack, IS: ischaemic stroke, SAH: subarachnoid haemorrhage, ICH: intracerebral haemorrhage; ApoC-I: apolipoprotein CI, ApoCIII: apolipoprotein CI, BNP: brain natriuretic peptide, BNGF: B-type neurotrophic growth factor, CK-MB: creatinine kinase MB, CPK: creatinine phosphokinase, CRP: C-reactive protein, DD: D-dimer, GFAP: glial fibrillary acidic protein, GSTP-1 glutathione S-transferase P, H-FABP: heart fatty acid binding protein, HSP: heat shock protein, IL6: interleukin 6, IL-1RA : interleukin 1 receptor antagonist, IL-1b : interleukin 1 beta, IL-8 : interleukin 8, ICAM: intracellular adhesion molecule, MBP: myelin basic protein, MCP- 1 : monocyte chemoattractant protein, MMP-9 matrix metalloproteinase 9, NDKA: nucleoside diphosphate kinase A, NCAM: neuronal cell adhesion molecule, NSE: neurone specific nolase, NT3: neurotrophin 3, PARK 7: DJ-1 protein, PLP: proteolipid protein, RAGE: receptor of advanced glycosylation end products, RNA-BP : RNA binding protein, TAT3: thrombin –antithrombin 3, TF: tissue factor, TNF: tumour necrosis factor, TnI: troponin I, UFD-1: ubiquitin fusion degradation protein, VLP 1:Visin –like protein 1, VCAM: vascular cell adhesion molecule, VEGF : vascular endothelial growth factor, vWF: von Willebrand factor

Table 2.2 Modified QUADAS instrument to assess quality of reporting of results

	All had same reference test	All reference standard whether BM + or BM-	Marker not part of stroke diagnosis	Marker measurement blind to diagnosis	Clinician blind to biomarker	All have results reported	Threshold established prior to study	No Commercial interests
(Abboud et al 2007)	✓	✓	✓	?	✓	✓	✓	✓
(Allard et al 2005i)	?	?	✓	?	?	?	×	×
(Allard et al. 2005b)	✓	✓	✓	×	✓	?	×	×
(Allard et al. 2004f)	✓	✓	✓	?	✓	×	×	×
(Allard et al 2007)	?	✓	✓	?	✓	✓	×	×
(Dambinova et al 2003b)	✓	✓	✓	✓	✓	✓	×	×
(Dambinova et al 2003a)	?	?	✓	?	?	?	?	×
(Fassbender et al. 1997a)	✓	✓	✓	?	✓	✓	✓	✓
(Foerch et al. 2006a)	✓	✓	✓	✓	✓	✓	×	×
(Hill et al. 2000a)	✓	✓	✓	?	✓	✓	✓	×
(Laskowitz et al. 2005c)	?	?	✓	?	?	?	×	×
(Laterza et al. 2006a)	?	✓	✓	?	✓	✓	✓	×
(Lynch et al. 2004a)	✓	✓	✓	?	✓	×	×	×
(Montaner et al 2005)	?	?	✓	?	✓	✓	×	✓
(Rainer et al 2007)	✓	✓	✓	?	✓	✓	×	×

	All had same reference test	All reference standard whether BM + or BM-	Marker not part of stroke diagnosis	Marker measurement blind to diagnosis	Clinician blind to biomarker	All have results reported	Threshold established prior to study	No Commercial interests
(Reynolds et al. 2003a)	✓	✓	✓		✓	?	✗	✗
(Rouanet et al 2006)	✓	✓	?	?	?	✓	✓	✗
(Takahashi et al. 1999d)		✓	✓	?	✓	✓	✓	✗
(Tomitori et al. 2005a)	✓	✓	✓	?	✓	✗	✗	✗
(Turck et al 2006)	?	?	?	?	?	?	?	✗
(Zimmermann-Ivol et al.	✓	✓	✓	?	✓	?	✗	✗

✗ : No, ✓ : Yes, ?: insufficient information

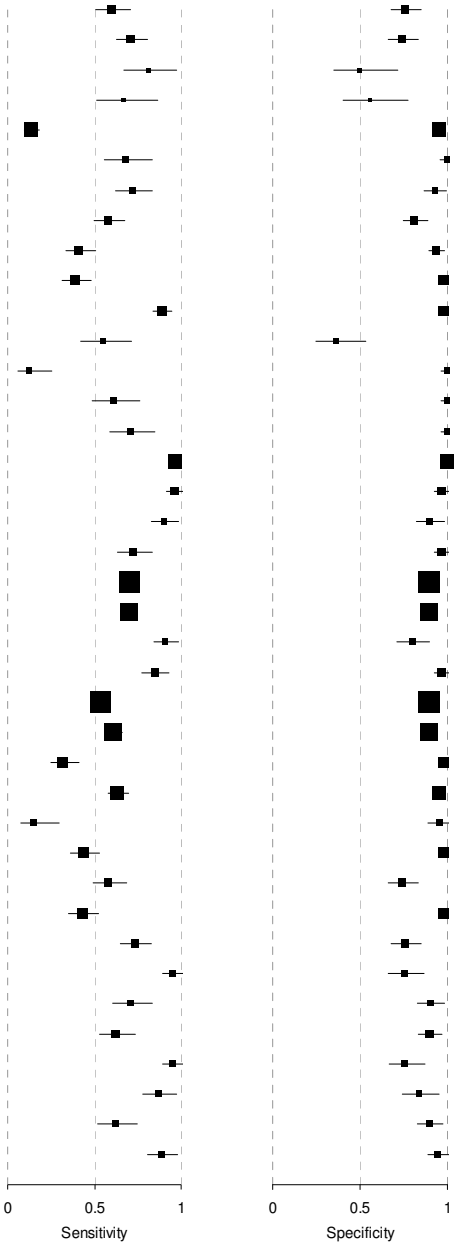
Table 2.3 Putative biological role of markers in the diagnostic systematic review

Biomarker	Description
Acetyl polyamine oxidase	Polyamine catabolism (the function of polyamines is not clear)
Acrolein	Polyamine catabolism
Apolipoprotein C1	Plasma protein found in LDL and VLDL
Apolipoprotein C3	Component of VLDL, HDL and LDL, produced in liver
Beta globin DNA	DNA released after cell damage
Brain natriuretic peptide	Hormone secreted from the ventricular myocardium during periods of increased ventricular stretch and wall-tension
B-type neurotrophic growth factor	Supporting neuronal growth and differentiation
C-reactive protein	Acute phase protein
D-dimer	Breakdown product of fibrin after factor XIII stabilisation; indicative of thrombus formation
Fatty acid binding protein	Proteins involved in intracellular transport, oxidation of fatty acids and membrane lipid trafficking
Glial fibrillary acidic protein	Intermediate filament protein, found mainly in astrocytes
Glutathione S transferase P	One of many glutathione transferases, role in cellular detoxification
Ischaemic modified albumin	Altered cobalt binding on the N-terminus of albumin
Matrix metalloproteinase 9	Collagenase, associated with destruction of plaque matrix and endothelial damage
Myelin basic protein	Constituent of the CNS myelin synthesized by oligodendrocytes and Schwann cells
Neurone specific enolase	Dimeric neuronal glycolytic enzyme
NR2A/2B antibodies	Antibodies to NMDA receptor fragments
Nucleoside diphosphate kinase A	An enzyme catalysing the transfer of phosphate groups between nucleoside triphosphates and nucleoside diphosphates (eg. ATP to GDP)
PARK 7	RNA binding protein regulatory subunit
S100 beta	Acidic calcium binding protein found in glia and Schwann cells
Spermine oxidase	Polyamine catabolism
Thrombomodulin	Endothelial cell thrombin receptor that converts thrombin from a procoagulant to an anticoagulant enzyme
Total polyamine oxidase	Polyamine catabolism
Ubiquitin fusion degradation protein	Enzyme in the pathway for degrading ubiquitin-protein conjugates
Vascular cell adhesion molecule	Part of the immunoglobulin superfamily important in inflammation, immune responses and in intracellular signalling events
Visin like protein	Intracellular neuronal calcium sensor
von Willebrand factor	Binds to factor VIII form a stable complex.

Table 2.4 Recommendations for good quality studies of blood biomarkers for acute stroke diagnosis

Patients
Prospectively collected, consecutive patients with suspected stroke from an emergency setting
Recruit patients in whom non-expert clinicians suspect stroke
Recruit patients at a clear point in the diagnostic pathway for example, pre-hospital, emergency department, pre- or post CT brain scan
Record pre test probability of stroke, using either clinician judgement or a recognised clinical rating scale
Define the delay between stroke symptom onset and initial assessment & blood sampling
Define whether stroke symptoms still present at time of blood sampling
Reference standard diagnosis
Expert clinical opinion, with appropriate brain imaging supplemented by data from other test results and the patient's subsequent clinical course
Reference standard diagnosis made blind to biomarker status
Define stroke type – haemorrhagic or ischaemic
Biomarker measurement
Fully describe laboratory technique for marker measurement
Describe intra- and inter- assay reliability of tests
Fully describe logistic regression models of biomarkers
Measurement blind to clinical status
Validate biomarker and diagnostic threshold in an independent cohort
Give numerical value of threshold for a positive test
Reporting
Show raw data wherever possible
Use the STARD (Bossuyt et al. 2003b) guideline when preparing study reports

Figures



		Diagnostic threshold
APO	Tomitori 2005	1 nmol/ml↑SPD
Acrolein	Tomitori 2005	18 FDP lysine nmol/ml
Apoprotein C 1	Allard 2004	80 RFU
Apoprotein C3	Allard 2004	39 RFU
Beta globin DNA	Rainer 2007	1486 kilogenome-equivalents/l
FABP	Zimmerman 2004	0.531 ODU
GST	Turck 2006	18.3 ng/ml
IMA	Abboud 2007	80 U/ml
IMA	Abboud 2007	85 U/ml
MBP	Hill 2000	
NSE	Hill 2000	
NSE	Zimmerman 2004	10 ng/ml
NSE	Fassbender 1997	0.06ng/ml 4 hrs post stroke
NSE	Fassbender 1997	0.06ng/ml 24 hrs post stroke
NSE	Fassbender 1997	0.06ng/ml ever post stroke
NR2A/2B Ab	Dambinova 2003	2 µg/L
NR2 fragment	Dambinova 2003	
NDKA	Allard 2005	2 µg/L
NDKA	Allard 2005	2.55 µg/L
NDKA	Allard 2005	22 µg/L
NDKA	Allard 2005	
PARK7	Allard 2005	9.33 µg/L
PARK7	Allard 2005	1.55 µg/L
PARK7	Allard 2005	14.1 µg/L
RNA BRS	Allard 2005	
S100beta	Hill 2000	0.02 µg/l
S100beta	Rainer 2007	0.104 µg/l
S100beta	Zimmerman 2004	0.02 ng/ml
S100beta	Takahashi 1999	0.021 µg/L
Spermine Oxidase	Tomitori 2005	4 nmol/ml↑SPD
Thrombomodulin	Hill 2000	
TPO	Tomitori 2005	5 nmol/ml↑in SPD
UFDP	Allard 2007	2.26 µg/L
UFDP	Allard 2007	3.76 µg/L
UFDP	Allard 2007	0.45 µg/L
UFDP	Turck 2006	2.2 ng/ml
UFDP	Turck 2006	2.47ng/ml
UFDP	Turck 2006	0.445ng/ml
VLP 1	Latzera 2006	0.04 µg/L

Figure 1 Sensitivity and specificity of individual blood biomarkers for the diagnosis of stroke (ischaemic or any stroke)

The size of markers is proportional to study size, and 95% confidence intervals**.**

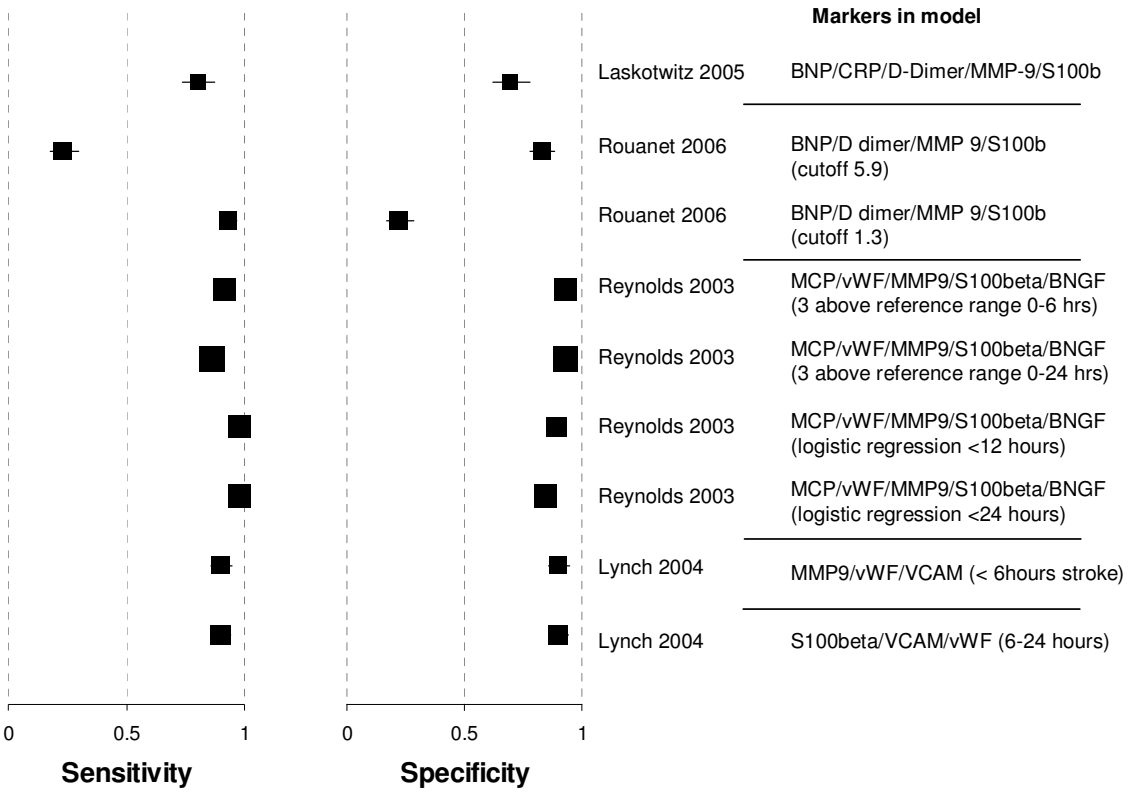
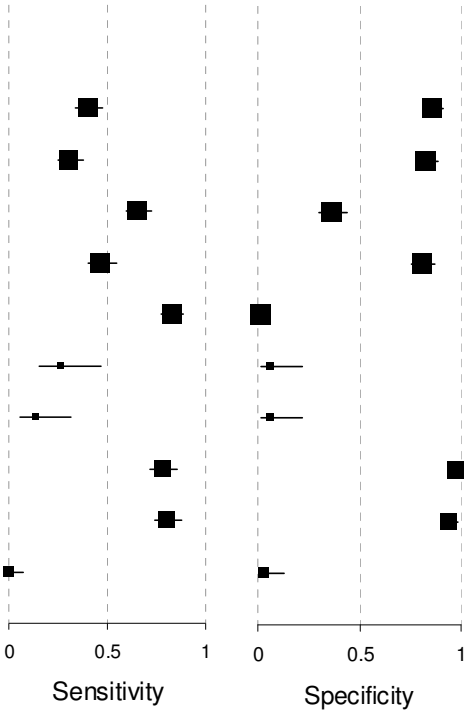


Figure 2 Sensitivity and specificity for blood biomarker models for the diagnosis of stroke

The size of the marker is proportional to the study size and lines show 95% confidence intervals. The markers in each model are shown. BNP: brain natriuretic pepetide; CRP: C-reactive protein; MMP-9: matrix metalloproteinase-9; BNGF: B-type neurotrophic growth factor; vWF: von Willebrand Factor; VCAM: vascular cell adhesion molecule



		Diagnostic threshold
β globin DNA	Rainer 2007	1486 kilogenome equivalents/L
β globin DNA	Rainer 2007	2500 kgenome equ/L
S100β	Rainer 2007	0.104 µg/L
S100β	Rainer 2007	0.25 µg/L
S100β/β gl DNA	Rainer 2007	DNA>2500 kgenome equ/L and S100 > 0.25µg/L
Apoprotein C 1	Allard 2004	59 RFU
Apoprotein C3	Allard 2004	39 RFU
GFAP	Foerch 2006	2.9 ng/L
GFAP	Foerch 2006	1.8 ng/L
NR2A/2B Abs	Dambinova 2003	<2µg/L

Figure 3 Sensitivity and specificity of biomarkers for the diagnosis of haemorrhagic stroke in studies of patients with haemorrhagic and ischaemic stroke.

The size of them markers is proportional to the size of the study and the lines show with 95% confidence intervals. GFAP: glial fibrillary acidic protein; RFU: relative fluorescence units; NR2A/2B Abs: antibodies to the NR2A/2B subunits of the NMDA receptor.

Chapter 3. Validation of clinical scores for the diagnosis of stroke and transient ischaemic attack: BBISS, a prospective cohort study

Introduction

The gold standard for the diagnosis of acute stroke and transient ischaemic attack (from here on I will refer to them together as 'acute cerebrovascular diseases') includes an assessment by a senior stroke physician, careful review of timely brain imaging by a neuro-radiologist and follow up of a patient's clinical course. However, very soon after symptom onset, a gold standard diagnosis is difficult to achieve. In particular, the first person to assess patients with suspected stroke is often an emergency department nurse or doctor, who may not have particular expertise in the diagnosis of acute cerebrovascular diseases or rapid access to brain imaging.

Formalised assessment tools based upon easily collected clinical variables may improve the sensitivity or specificity of emergency staff in their diagnosis of acute cerebrovascular disease (Hand 2002, Nor et al. 2004a, Nor et al. 2005f). Accurately identifying the patients with acute cerebrovascular disease in the emergency department with a more sensitive clinical test could lead to faster referral of patients for thrombolysis, stroke unit care or secondary preventative agents. However, an instrument with a high sensitivity but poorer specificity that identifies most patients with acute cerebrovascular disease will also misclassify patients with stroke mimics. These misclassified patients without acute stroke could overwhelm the future capacity of a stroke service, if the predictive value of a positive test were too low.

The sensitivity and specificity of a diagnostic instrument may vary with the study cohort (Feinstein 2002). External validation of diagnostic scales in different clinical cohorts is therefore important to ensure they perform well in different clinical settings. In a small emergency department validation cohort (n=160), the face arm speech test (FAST) had a sensitivity of about 82% and specificity of 83%, and the recognition of stroke in the emergency room (ROSIER) instrument a sensitivity of

93% and specificity of 83% (Nor et al. 2005e). If these scales had a similar performance in different emergency departments, we would be more confident to introduce them into routine emergency practice.

A new diagnostic tool should improve upon existing diagnostic methods. A new instrument for identifying patients with acute cerebrovascular disease should have better sensitivity than the informal opinion of an emergency department clinician, identifying more patients with stroke, but have no worse specificity, avoiding over-referral of patients without stroke.

In this chapter, I will:

- describe the recruitment of the Blood Biomarkers in Suspected Stroke (BBISS) cohort of patients with suspected stroke in an emergency department in Edinburgh
- summarise the clinical features of the patients with and without acute cerebrovascular disease
- compare the sensitivity and specificity of the FAST (Nor et al. 2004b) and the ROSIER scoring systems (Nor et al. 2005d) and two previously developed multivariate logistic regression models for the diagnosis of stroke (Hand 2002) with the clinical opinion of emergency department staff and stroke specialists.

Methods

BBISS cohort recruitment

I prospectively recruited consecutive patients with suspected stroke who presented to the Acute Receiving Unit (ARU), an emergency department (ED) in the Western General Hospital, Edinburgh. Patients were referred to the emergency department from: (i) general practitioners (GPs) in the North of Edinburgh and East Lothian, (ii) paramedical ambulance staff, and (iii) a walk in clinic in the hospital grounds.

I made strenuous efforts to ascertain all cases, as soon after arrival in the emergency department as possible (a 'very hot' pursuit model) by: (i) holding a pager as first point of contact for all patients brought into the unit with suspected stroke, (ii) visiting the emergency department every 2 hours during the working day and screening admission logs in real time, (iii) assessing patients with suspected stroke admitted to the medical admissions unit and stroke unit within 24 hours of symptom onset, (iv) periodically advertising the study to any new staff in the ED, and (v) providing teaching sessions about acute stroke for nursing and medical staff in the department.

During active recruitment to the study, NHS Scotland had set a target for all emergency departments to assess and discharge each patient within four hours (either home or to another unit). This target was an additional incentive to refer patients to the study as, in general, I assessed and discharged patients more rapidly than more junior emergency medical staff, freeing doctors for other work in the department. When I was unavailable, other stroke fellows recruited patients (Dr. Ralph Thomas, Dr. Evan Mamaloukas, Dr. Bartosz Karaszewski, Dr. Rayessa Rayessa, and Dr. Enda Kerr). I took a history from and examined each patient, and for those who consented to take part in the study, recorded my assessment with a structured proforma (see appendix). Emergency department staff recorded their clinical impression and assessment on the same form.

BBISS inclusion criteria

I recruited patients with *suspected stroke*. I defined suspected stroke patients as those: (i) whose symptoms began less than 24 hours before admission, (ii) who were still symptomatic at the time of assessment and, (iii) suspected to have stroke by a GP, a paramedic or a member of the emergency department staff.

Emergency department nurse or doctor assessment

I asked nurses and emergency department doctors to record whether they felt that acute cerebrovascular disease (TIA or stroke) was: (i) *definite*, where they were sure the diagnosis was TIA or stroke, (ii) *probable* where they thought that TIA or stroke

was the most likely of a number of other diagnosis, and (iii) *possible*, where they thought that TIA or stroke was possible, though other diagnoses were more likely. They then recorded their own assessment with the Face Arm Speech Test (FAST) and the Recognition of Stroke in the Emergency Room (ROSIER) instrument (Nor et al. 2004c, Nor et al. 2005c). I considered patients to be 'FAST positive' when an emergency clinician found they had one of facial weakness, arm weakness or speech disturbance. I calculated a ROSIER score from clinical variables determined by a member of the emergency staff (Equation 1), and the stroke fellow who measured the variables 'syncope' and 'seizure'. I considered patients to be 'ROSIER positive' when their score was 1 or more.

Equation 1 ROSIER Score

ROSIER score = -1 x (syncope) -1 x (seizure) +1 x (face weak) +1 x (arm weak) + 1 x (leg weak) +1 x (speech disturbance) +1 x (visual field defect), if blood sugar >3.5mmol/l

(Nor et al. 2005b)

Variables take the value 1 if present and 0 if absent.

Fellow assessment and variable definition

I recorded demographic details (date of birth, GP and contact information); the time of stroke onset, or where that was unknown, the last time the patient was seen well; and the time of assessment in the emergency department. I defined variables collected during my assessment as follows:

The presenting event

- *Focal symptoms*: symptoms that could arise from focal cerebral disturbance such as hemiparesis, aphasia, hemisensory loss, hemianopia, unilateral ataxia, facial or hand weakness. I did not define as focal any events that could be attributed to dysfunction of a single cranial nerve (such as isolated diplopia) or where the neurological symptoms were poorly localized, for example isolated dysarthria or vertigo. In this, I have followed prior

convention (Warlow C.P et al. 2008), although I acknowledge that sometimes these symptoms can be due to cerebral damage from ischaemia.

- *Headache at onset*: a headache at any time between the onset of symptoms and the assessment of the patient in the emergency department reported by the patient.
- *History of infection*: symptoms best explained by infectious aetiology, for example productive cough, fever, dysuria, diarrhoea, or definite diagnosis of infection by a GP or hospital doctor, within the 2 weeks preceding assessment. I did not routinely classify the type or grade the severity of infection, as I expected too few events for subgroup analysis.

Previous diagnoses

- *Cardiac vascular disease*: prior myocardial infarction, angina or coronary artery procedure (bypass grafting or angioplasty) recorded in GP or hospital notes, or by the patient where neither where available. The accuracy of self report of myocardial infarction in self administered questionnaires in population based epidemiological studies is high (Okura et al. 2004a), though probably is less so in the emergency department where the effects of acute illness may affect the patient's accuracy of recall.
- *Cardiac failure*: a diagnosis of cardiac failure made prior to admission, either on clinical grounds, or with the support of echocardiography, or where a patient was prescribed drugs commonly used for the treatment of heart failure (e.g. spironolactone).
- *Prior stroke or TIA*: a stroke or TIA prior recorded in GP or hospital notes. Patient reports were confirmed by inspection of the GP or hospital records where they were available at the initial assessment. A patient's recall of TIA or stroke is less good than MI (Okura et al. 2004b).

- *Migraine*: a report of intermittent severe headache and nausea and photophobia with complete recovery, with or without focal features to suggest a migraine aura, or record of migraine diagnosis in GP or hospital records.
- *Epilepsy*: a prior diagnosis of epilepsy, recorded in GP or hospital records.
- *Atrial fibrillation* (AF): persistent or paroxysmal atrial fibrillation, in the past (recorded in GP or hospital records) or at admission, observed on an electrocardiogram (ECG).
- *Cognitive impairment*: report by a carer of cognitive impairment sufficient to interfere with activities of daily living prior to the onset of suspected stroke symptoms.

Medications

- *Drugs*: each patient's medications were obtained either from the GP summary record (by inspection or by telephone), a repeat prescription, or dosette box.

Prior handicap

- *Independence of activities of daily living*: patients who were able to wash, dress, toilet and feed themselves without any assistance were 'independent'. I attempted to quantify the extent of pre-admission impairment by judging the Oxfordshire Handicap Scale prior to admission to hospital after discussion with patients and their relatives or carers (Bamford et al. 1989b).

Neurological impairment

- *National Institute of Health Stroke Scale* (NIHSS): I, or another stroke fellow, measured the NIHSS in all patients with suspected stroke after appropriate training (American Heart Association 2009, Brott et al. 1989b). I used the NIHSS rather than developing a new impairment scale for the project, as although there is no generally agreed measure of neurological impairment

for undifferentiated patients with neurological disease, the NIHSS measures many important domains of the neurological examination, is familiar to stroke physicians and has good inter-rater reliability (intraclass correlation coefficient >0.9 (Goldstein & Samsa 1997b)).

- The severity of impairment was also measured with dichotomous variables: whether the patient was able to speak in sentences, whether the patient was orientated (could give the correct time of day (morning or afternoon), was able to describe where they were, and able to give their date of birth), whether the patient was able to take steps without help, and whether the patient was able to lift both arms from the bed (Counsell et al. 2002f).

Other examination findings

- *Temperature, blood pressure and pulse*: were measured on admission by nursing staff using routine clinical equipment. The first measurement of each was recorded.
- *Pedal pulse*: I palpated the dorsalis pedis and posterior tibial pulses bilaterally.

After recording these variables, I noted my own clinical impression: whether I felt the symptoms were *definitely*, *probably* or *possibly* due to acute cerebrovascular disease, with same definitions as 0. If I diagnosed a mimic of acute cerebrovascular disease, I also specified an alternative diagnosis. At the time of clinical assessment, I also assigned the stroke syndrome according to the Oxford Community Stroke Project classification (Bamford et al. 1991c).

Imaging

Patients had CT or MR brain imaging for the following reasons: (i) where it was part of their routine clinical care, (ii) as part of another research project, or (iii) where the diagnosis was substantially uncertain, and MR imaging could improve diagnostic certainty. In almost all cases, brain imaging was performed after the stroke fellow had made a provisional diagnosis. MR brain imaging in the SFC Brain Imaging

Research Centre included at least the following sequences: T1 weighted spin echo, diffusion weighted (DWI), fluid-attenuated inversion-recovery (FLAIR), T2 weighted fast spin echo, and T2 gradient recall echo. Other research studies performed further imaging sequences in a few patients.

Gold standard diagnosis

A panel of stroke experts met weekly and reviewed each patient's clinical assessment, imaging, and clinical progress. The panel determined whether the diagnosis of stroke or transient ischaemic attack was definite, probable or possible and clarified non-stroke diagnoses as far as practicable. Over the course of the two years of recruitment, the members of the panel varied somewhat, but always included at least one experienced consultant in stroke medicine, a stroke neuroradiologist, a stroke fellow, and often a neurologist. Most meetings included more than one member of each category.

The fellow who had assessed the patient (usually myself), first presented the history and the findings on examination. The panel reviewed the relevant imaging and clinical progress, and then reached a consensus diagnosis through discussion. Where additional clinical or radiological information became available at a later date, a fellow presented the case again and altered the final diagnosis if appropriate.

Definitions of diagnostic entities

- *Stroke*. I diagnosed stroke in those patients with a focal neurological deficit that lasted for more than 24 hours, and the panel judged the cause to be an ischaemic stroke or intracranial haemorrhage.
- *Ischaemic stroke*: I diagnosed ischaemic stroke where the patient presented with focal neurological symptoms, brain imaging either showed positive evidence of cerebral infarction in a relevant location (an appearance consistent with the time after stroke onset), or was normal, and the panel judged brain ischaemia to be the cause of the symptoms.

- *Intracranial haemorrhage*: I diagnosed intracranial haemorrhage where the patient presented with focal neurological symptoms, and brain imaging showed positive evidence of acute intracranial haemorrhage, either into the brain parenchyma, ventricles or subarachnoid space relevant to the patient's clinical features.
- *Transient ischaemic attack (TIA)*: I diagnosed TIA in those patients with a focal neurological deficit that lasted for less than 24 hours, where the panel judged the cause to be brain ischaemia or haemorrhage (though all proved to be ischaemic). I did not include cases of amaurosis fugax.
- *Acute cerebrovascular disease (ACvD)*: I defined acute cerebrovascular diseases as those patients who were symptomatic at the point of admission and had a final diagnosis either of stroke or transient ischaemic attack.
- *Mimic*: Those patients with suspected stroke who had neither stroke nor TIA.
- *Definite, probable and possible*: these terms were used to describe different levels of certainly. Whilst these do not map onto numerical prior probabilities for use in a Bayesian analysis, they do have thresholds easily understood by diagnosticians. A *definite* diagnosis was one where no other diagnosis could be countenanced to explain the symptoms. A *probable* diagnosis was one where other diagnostic entities were considered, though felt to be less likely. A *possible* diagnosis was one where other diagnostic entities were felt to be more likely. I analysed definite and probable cases of stroke and TIA together as acute cerebrovascular disease, and possible cases of stroke and TIA with stroke mimics.

Data checking

I employed a number of methods to ensure data consistency. I piloted the data collection form (see appendix) with several patients and made modifications to ensure ease of use before starting recruitment. I used categorical answers to force yes/no decisions for each variable where possible. The data collection form was very

similar in design to the screen appearance of the Access database used for data entry and management to reduce transcription errors. I personally entered the data into the database immediately after collecting it, to allow a rapid check of missing fields at the time when the medical records were still easily available.

The data entry system of the database permitted only very few fields to have missing data and had a number of range checks to ensure continuous data were within reasonable bounds. At the end of the project, all the clinical diagnoses were checked against the discharge diagnoses available through TRAK (the hospital electronic record system) in NHS Lothian, to ensure no other illness had developed that could have explained the presenting symptoms (for example, a changed diagnosis to a malignant brain tumour or to multiple sclerosis). I checked the whole dataset for missing or incomplete records of blood pressure and checked these against the clinical notes. I performed a number of consistency checks between the database record and clinical notes prior to analysis: whether the name matched the sex; if time to imaging was more than 1 day; if the pre-morbid modified Rankin scale was greater than 2 and the patient was recorded 'independent'.

Data management

I designed a Microsoft Access 97 database for data entry, storage and coordinating follow up. Access, a relational database, gives flexibility in data collection, particularly where there are an unpredictable and large number of events. However, the problem of translation of data from a relational database to a flat field spreadsheet for statistical analysis was not trivial and needed considerable programming input.

I added data into each patient's record at three time points: once after their initial assessment, once after discussion of their diagnosis and relevant imaging and with the results of three months follow up.

The database was housed on the central server in the Division of Clinical Neurosciences. This had three advantages (i) regular backup of data to tapes held

with the Division and to the University's central server, (ii) secure access on the local area network (LAN) to locations outside DCN (for example the WTCRF), and (iii) data security. I permitted only named users on the University network (itself password protected) who also knew the current password to access the database. Each user was only able to access areas of the database that were relevant to their role.

To determine the number of stroke patients admitted to the Western General Hospital over the period of recruitment, Mike McDowall queried the Scottish Stroke Care Audit Database on 14th October 2009.

Statistical analysis

Baseline analyses

I made comparisons between patients with and without acute cerebrovascular disease with Student's *t* test for normally distributed continuous variables with the Stata command `ttest`, Wilcoxon rank-sum test for positively skewed variables with `ranksum` and χ^2 tests for dichotomous variables with the `chi` command. To measure the association between variables and ACvD I fitted a series of univariate logistic regression models with the `logistic` command, and report odds ratios, their 95% confidence in intervals as a measure of uncertainty of estimates and Wald tests to test the null hypothesis that OR=1.

The performance of ROSIER, FAS and clinical opinion

I assessed the diagnostic stroke scales FAST and ROSIER at their published thresholds, and the clinical impression of an emergency department clinician and a stroke fellow at two thresholds: (a) 'definite cerebrovascular events' (b) 'definite or probable cerebrovascular events'. I calculated the sensitivity, specificity, positive and negative predictive values and likelihood ratios at these thresholds and their 95% confidence intervals, using the gold standard diagnosis of acute cerebrovascular disease or stroke made by the panel. As each patient had more than one test performed, and each is correlated with another, I used McNemar's test for

paired proportions (Equation 2) to compare the results of the clinical scoring systems to the baseline assessment by a member of the emergency staff (for example see Table 3.1 for the comparison of test sensitivities), and report the McNemar's significance probability, and the exact test where the number of discordant pairs was less than 20 (Leisenring, Alonzo, & Pepe 2000, McNemar 1947).

Equation 2 McNemar's χ^2

$$\text{McNemar's } X^2 = \frac{|b-c|-1}{\sqrt{b+c}}$$

I also tested the performance of the clinical scoring systems for patients in whom the eventual diagnosis was stroke rather than acute cerebrovascular disease, for patients seen less than six hours after the onset of their symptoms, and those for whom the first recorded assessment was by a nurse rather than a doctor.

Logistic regression models for the diagnosis of stroke

I examined the calibration and discrimination of a previously developed logistic regression models for the diagnosis of stroke, and examined their sensitivity and specificity at the suggested thresholds (Hand 2002, Hand et al. 2006g).The first model was designed for use by a registrar in stroke medicine (:

Equation 3):

Equation 3 Model to predict stroke diagnosis, stroke/neurology registrar

$\log_e$(odds of stroke)=
-3.324
- [1.118 x (known cognitive impairment)]
- [0.824 x (abnormal findings in any other system)]
+ [0.952 x (exact time of onset determined)]
+ [1.975 x (definite history of focal neurological symptoms)]
+ [0.934 x (any abnormal vascular findings)]
+ [0.651 x (NIHSS 1 to 4)]
+ [1.145 x (NIHSS 5 to 10)]
+ [1.979 x (NIHSS >10)]

+ [0.707 x (signs lateralisable to right or left)]
+ [1.627 x (OCSP classification possible)]

Variables take the value 1 if present and 0 if absent.

(Hand 2002)

The second model (Equation 4) was designed for use by emergency department nurses:

Equation 4 Model to predict a stroke diagnosis, emergency department nurse

$\log_e$(odds of stroke)=
-3.080
+ [1.047 x exact time of onset determined)]
+ [2.483 (definite history of focal neurological symptoms)]
+ [0.595 x (abnormal verbal output)]
+ [1.637 x (arm weakness)]

(Hand 2002)

In my cohort, I defined the variables as follows:

- Onset time known: time found = time last seen well
- Definite history of focal neurological symptoms: collected by stroke fellow or registrar.
- Abnormal vascular findings: systolic blood pressure>150 or AF or heart murmur or absent pulses
- Abnormal findings in other systems: suspected infection
- Abnormal verbal output and arm weakness: where measured by the emergency department clinician in the FAS test.

I used :

Equation 3 and Equation 4 to calculate $\log_e$(odds of stroke) and subsequently Equation 5 to calculate the predicted probability of stroke or acute cerebrovascular disease.

Equation 5 To calculate predicted probability of stroke from log (odds stroke)

$$\Pr(Stroke) = \frac{e^{\log_e(\text{odds of stroke})}}{1 + e^{\log_e(\text{odds of stroke})}}$$

I assessed the performance of the logistic regression models as follows. First, I assessed *calibration*, or how well predicted probabilities of an acute cerebrovascular disease diagnosis compared with observed probabilities of stroke, by plotting predicted against observed probabilities, and calculation of the Hosmer-Lemeshow χ^2 goodness of fit test with the `hl` command in Stata (Lemeshow & Hosmer, Jr. 1982). This statistic compares the estimated to observed likelihood of acute cerebrovascular disease in deciles of predicted probability. The smaller the χ^2 the closer the predicted is to the observed probability of acute cerebrovascular disease.

Second, I assessed *discrimination* by measuring the area under a receiver operator curve (AUROC) and its exact binomial confidence intervals with the `roctab` command in Stata. This can be interpreted as the chance a randomly chosen patient with acute cerebrovascular disease has a higher predicted probability of stroke than a randomly chosen patient without stroke. An AUROC of 0.5 indicates a model with no better discrimination than chance and an AUROC of 1 a model with perfect discrimination.

Thirdly, I assessed the sensitivity and specificity of the logistic regression models at the thresholds suggested in their development cohorts. The registrar model aimed to be specific, with a threshold of predicted probability of stroke of Pr=0.9. The nurse model aimed to be sensitive at a predicted probability of stroke of Pr=0.3.

I investigated the role of the clinical assessment scales in series by calculating the sensitivity and specificity of a strategy combining the clinical opinion of a member of the emergency department staff with the negative variables in the ROSIER scale.

Stata 10 was used for all statistical analysis. Because of the number of comparisons, I considered a $P<0.01$ to be statistically significant. All P values are two sided.

Ethical considerations

I explained the study to each participant or their welfare guardian and gave them an information sheet to read. Each participant then gave consent or their welfare guardian gave assent to taking part in the study (see appendix for forms). The Multi-centre Research Ethics Committee for Scotland (A) gave ethical oversight to the study (Reference No. 06/MRE00/119). This committee has responsibility for studies of adults with incapacity. Approval was also received from the Lothian Local Research Ethics Committee (Reference No. 06/S11ADMIN/161) and the NHS Lothian Research and Development Office (Reference No. 2006/W/NEU/09).

Sample size

I based the sample size of the study on the reports of the sensitivity and specificity of a biomarker tests for the diagnosis of stroke (Laskowitz et al. 2005d).

Feasibility: About 380 patients present to the Western General Hospital each year with definite stroke (Scottish Stroke Care Audit 2006) of whom about 300 present within 24 hours of their symptom onset. As about two thirds of patients with suspected stroke have a stroke in settings similar to ours (Hand et al. 2006f), I expected personally to be able to recruit 330 patients with symptoms of acute stroke during the working week per year. Over 2.5 years of recruitment, about 800 patients might therefore be eligible to enter the study.

Precision of estimates Laskowitz found a panel of four biomarkers had a sensitivity of 80% and specificity of 70%. This sample size would give reasonably narrow confidence intervals should the panel of markers have a similar performance in my cohort (95% confidence intervals for sensitivity ± 3% and specificity ± 5%).

Revised feasibility: After a few months of recruitment, it was clear that the recruitment of 800 patients within 24 hours of their symptom onset would not be feasible. This may have been due to: (a) a reconfiguration of emergency services in Lothian just before the start of the project, leading to fewer ambulance service referrals to the Western General Hospital, (b) though patients were admitted within

24 hours of symptoms, stroke was not suspected until later in the admission, or (c) suspected strokes were missed in the emergency department. In an attempt to increase the rate of recruitment I enlisted the help of my clinical colleagues at the Royal Infirmary of Edinburgh: this led to the recruitment of an additional 5 patients. I redoubled my efforts to recruit patients from the ARU, but found this did not increase the rate of recruitment (~0.9 patients/working day). I estimated that the study would recruit 400 patients over two years if it continued to recruit at the initial rate (Figure 4).

I recalculated the confidence intervals we would expect around a biomarker panel test if recruitment continued at the observed rate and the biomarker test had a similar performance. The 95 % confidence intervals around sensitivity were ± 5% and specificity ± 7%. These estimates were sufficiently narrow to justify continuation of the study. I used Confidence Interval Analysis v2.1.2 to calculate these estimates. This 'sample size samba' is common (Schulz & Grimes 2005), and I communicated the revised sample size to both the ethics committee and the funder of the project, the Chief Scientist's Office.

This chapter was prepared with reference to the Standards for Reporting of Diagnostic accuracy (STARD) checklist (Bossuyt et al. 2003a).

Results

Recruitment

Between 21st March 2007 and 27th February 2009 during working hours I recruited 405 patients with suspected stroke to the study, of whom 285 (70%) had symptoms due to probable or definite acute cerebrovascular diseases, and 120 (30%) due to other illness. During the recruitment period, a total of 823 stroke and 89 TIA patients were admitted to the Western General Hospital. Of the stroke patients, 545 were admitted on the first day of their symptoms (46 haemorrhagic and 499 ischaemic) (Table 3.2). No data were available on the timing of admission, relative to their symptom onset, of patients with TIA. Of these potentially eligible patients, I

saw 230/499 (47%) of the patients admitted with ischaemic stroke in the first day after symptoms, 15/46 (32%) of the patients admitted with haemorrhagic stroke and 40/89 (45%) of the patients with TIA. The age and routinely collected measures of neurological impairment of the study patients and all those patients seen less than 24 hours in the Western General Hospital were similar (Table 3.3).

Baseline characteristics

Just over half the patients were women (207/405, 51%). Patients with acute cerebrovascular disease were: older (74 versus 68 years, t=-5, P<0.0001); had more severe symptoms measured by the median NIHSS (4 versus 1, Z=-5.7, P<0.0001); more often had arm weakness and were able to talk, though there was no appreciable difference in the proportion of patients with confusion between the two groups at the time of baseline assessment (Table 3.3, Table 3.4). There was no significant difference between the two groups in the number of patients with cardiac, or peripheral vascular disease or prior stroke/TIA. Those who had symptoms due to mimics were more likely to have lost consciousness (14% versus 5%, χ^2=7.6, P=0.006), to have had a seizure (14% versus 5%, χ^2=3.0, P<0.0001), a prior diagnosis of epilepsy (9% versus 2%, χ^2=10.3, P=0.001), or a headache (30% versus 15%, χ^2=10.9, P=0.001) at the onset of symptoms. When I restricted the analysis to those who were able to talk normally, although headache was more common in patients with mimic, this difference was not statistically significant (31% versus 20 % χ^2 =2.1, P=0.15). Patients with acute cerebrovascular disease had higher systolic and diastolic blood pressures (systolic 157 versus 143, t=-4.16, p<0.0001, diastolic 86 versus 80 t=-2.8, P=0.006) and were more likely to have a missing peripheral pulse. There was no evidence that patients with acute cerebrovascular disease (ACvD) were seen faster those with other diagnoses (last seen well to admission: ACvD 6 hr 20 minutes versus other diagnoses 6 hrs).

Brain imaging

Most patients had brain imaging (Table 3.5). Almost all patients with acute cerebrovascular disease had either a CT or MR brain scan (98%), and the majority of

patients with mimics (81%). About a third of patients with stroke, TIA and mimics had an MR brain at some point during their admission to hospital. Most patients with acute cerebrovascular disease had a relevant ischaemic lesion on brain imaging, more in patients with stroke (72%) than TIA (34%).

Differential diagnosis

Table 3.6 summarises the diagnoses in patients with suspected stroke. Ischaemic stroke was the most common diagnosis, making up just over a half of all patients seen, and about four fifths of those with acute cerebrovascular disease. The remaining patients with acute cerebrovascular disease had a cerebral TIA (14%), intracerebral haemorrhage (6%) or subarachnoid haemorrhage (1%).

The most frequent non-cerebrovascular diagnoses were primary headache disorders (14% of patients with mimics), seizures (12%), non-neurological sepsis (11%) and functional symptoms (10%). It is worth noting that, in most of the patients with non-cerebrovascular disorders, the diagnosis was not made positively with neuroimaging. Imaging only contributed to the positive diagnosis of non-stroke in brain tumours, (4%), subdural haematomas (2%), and one case of brainstem compression due to an intracranial aneurysm.

Emergency clinicians, ROSIER and FAS scores and the diagnosis of acute cerebrovascular disease

Where a member of the emergency department staff thought that a patient probably or definitely had acute cerebrovascular disease, their assessment had a sensitivity of 77 % (95% CI: 72 to 82%) and specificity of 58% (49 to 67%) versus the gold standard diagnosis of acute cerebrovascular disease (Table 3.7 and Table 3.9). The clinical impression of an emergency department clinician was more specific than the FAS test (58% versus 37%) and had a similar sensitivity (77% versus 82%). The clinical impression of an emergency department clinician was a little less sensitive (77 versus 82%) though more specific than the ROSIER scale (58% versus 42%).

A diagnosis of definite or probable cerebrovascular disease by a stroke fellow was more sensitive (92% versus 77%) and specific (84% versus 58%) than an emergency department clinician's diagnosis.

I found no qualitative or important quantitative difference in these results where I compared emergency department opinion, FAS and ROSIER against a diagnosis of stroke rather than acute cerebrovascular disease (Table 3.8, Table 3.9). In patients seen less than six hours after the onset of their symptoms (Table 3.10), the sensitivity of an emergency department clinician's impression was similar to the FAS and ROSIER scales though poorer than the stroke fellow's assessment. An emergency department clinician's clinical impression had a better sensitivity than the FAS score though was worse than the stroke fellow's clinical assessment (Table 3.10). Where a patient's first recorded assessment was by a nurse, rather than a doctor, each of the stroke scales had a better sensitivity than the clinical impression, though each scale had a worse specificity (Table 3.11).

Validation of multivariate diagnostic models from (Hand 2002)

Stroke or neurology registrar model: The predicted probability of acute cerebrovascular disease was on average higher in patients with a final diagnosis of acute cerebrovascular disease than in those without (mean 0.9 versus 0.5 t=13, $P<0.0001$) (Figure 5). A calibration plot (Figure 6) showed the model was reasonably calibrated though the difference from perfect calibration is statistically significant (Hosmer Lemeshow $\chi^2=29.4$, $P=0.0003$). The discrimination of the model was good (Figure 7) with an area under a receiver operator curve of 0.80 (95% CI: 0.74 to 0.85). At the suggested threshold (Pr=0.90) the model had a sensitivity of 43% (38 to 49%) and specificity of 86% (79 to 91%) for a diagnosis of acute cerebrovascular disease. The sensitivity was worse than the performance of a stroke registrar though specificity was not (Table 3.7).

Emergency nurse model: The calibration of this model in this dataset was poor (Hosmer Lemeshow $\chi^2= 95.4$, $P<0.0001$) (Figure 8) though discrimination, measured by the area under the receiver operator curve was reasonable (0.70, 95% CI: 0.65 to

0.75). At the suggested probability threshold (Pr=0.3) the model had a sensitivity of 94% (90 to 96%) and specificity of 43.9 (35 to 53). The sensitivity was better than the performance of an emergency department clinician though the specificity was probably worse (Table 3.7).

The addition of stroke scales to clinical opinion.

Where an emergency department clinician thought that a patient probably or definitely had a stroke, and that patient had none of the positive variables in the ROSIER scale or had one of the two negative features, the serial combination of these two tests had a sensitivity of 72% (95% CI: 67 to 78%) and specificity of 64% (54 to 72%).

Discussion

Key results

Each stroke scale had a better sensitivity, though worse specificity, than a member of the emergency team diagnosing a patient's ongoing symptoms as definitely or probably due to acute cerebrovascular disease. The specificity of the FAS, ROSIER and Hand nurse model were similar to one another (~40%), though the Hand nurse model had the best sensitivity (94%). Therefore no scale or model had better specificity and better specificity than an emergency department clinician. This finding was robust to patients seen very early (<6 hours) after their stroke, and to whether they were seen only by a nurse, rather than doctor.

When an emergency department clinician's clinical opinion was combined in series with a specific test (i.e. none of the positive features of the ROSIER scale, or one of the negative features), the specificity of the two tests in combination was better than either in isolation, though at the expense of worse sensitivity.

A stroke fellow or registrar had better sensitivity and specificity for the diagnosis of acute cerebrovascular disease than an emergency department clinician or the ROSIER, FAS or Hand models though the Hand nurse model that had a very similar sensitivity.

Limitations

Incorporation bias: The gold standard diagnosis of stroke and TIA relies heavily upon the presenting symptoms. The components of a clinical stroke scale, if measured with sufficient accuracy, form part of the gold standard diagnosis of stroke and TIA: incorporation of these elements into the final clinical diagnosis is inevitable. This need not preclude the use of a diagnostic study design to evaluate these scales, but does need acknowledgement that the gold standard is being compared with some of its components (albeit imperfectly measured).

Selection bias: Over the period of the study, I was unable to recruit all potentially eligible patients, because: (a) I was only able to recruit during working hours and on weekdays, and (b) some patients were not identified in the ARU, despite intensive efforts. Furthermore, the origin of referral to the Western General Hospital may have may have skewed the spectrum of patients towards milder symptom severity. Despite this the proportion of mimics in my cohort is similar to previous studies (Hand et al. 2006e), and the age and neurological impairment of the recruited patients were similar to all stroke patients admitted to the Western General Hospital.

Completion of some of the scales and missing data: I was unable to collect a clinical opinion, ROSIER and FAS score for each patient. Emergency staff completed the more complex scale (ROSIER) in only 85% of patients. As I have analysed only cases with complete information, rather than imputed missing data, it is possible that I have over-estimated the sensitivity and specificity of the ROSIER score as a result of this bias.

Measurement of variables: The emergency department clinician only measured the variables that were components of the FAS and ROSIER scales. The stroke fellow, rather than a member of the emergency department staff measured two of the variables in the Hand model designed for use by the nursing staff. As I probably

measured these variables with greater accuracy and consistency than the emergency department staff, the reported sensitivity and specificity of this model are likely to be overestimates. It is also possible that the training of the nurses included the FAS test, and they already incorporated the results of the test into their informal assessment of clinical probability.

Accuracy of stroke and acute cerebrovascular diagnosis: The definition of the gold standard diagnosis of stroke adds a tension to the design and interpretation of a study. The simpler the gold standard, the more easily it can be applied to all patients, which avoids bias from the exclusion of patients unable to undergo more complex investigations and the uncertainly of measurement of associations because of small sample sizes. A more complex gold standard, perhaps with compulsory MR imaging, is less likely to misclassify patients, avoiding bias from the random misclassification of patients, and so attenuation of important associations that maybe found in studies with a simpler gold standard definition. This study follows a middle path, though is not immune to random misclassification of patients.

Study strengths

It is worth considering the strengths of the study. I have recruited consecutive patients where stroke was suspected by a member of the emergency team, rather than, for example, examining the performance of stroke scales in patients referred to a stroke service. The study therefore has immediate relevance to the use of these scales by staff in emergency departments. The gold standard diagnosis was consistently and prospectively made after a uniform delay soon after admission, when both relevant clinical information and short term follow up were easily available, rather than retrospectively obtained from patient discharge records. Most of the variables that were relevant to the scores FAS and ROSIER were measured by members of the emergency staff rather than a stroke physician, and so importantly have the measurement errors one would expect from their routine use in the emergency department.

Interpretation

It therefore seems unlikely that the ROSIER, FAS or various logistic regression models simultaneously improve on the sensitivity of the assessment of a member of the emergency department staff and the specificity of their assessment. Using a scale in combination with a clinical assessment improves the overall specificity though at the expense of lower sensitivity.

It is extremely difficult, and perhaps impossible, to measure separately the diagnostic performance of a clinical diagnostic scale and clinical judgment. However, the impacts of two strategies; clinical judgment versus clinical judgment + a clinical scale can be compared. In this study, a strategy based upon clinical judgment alone, where clinical judgment deemed acute cerebrovascular disease probable or definite would miss about a fifth of patients with stroke or TIA, and of those patients positively identified, four fifths would have acute cerebrovascular disease. A strategy of using the FAS or ROSIER scale in patients where clinical judgment deemed acute cerebrovascular disease possible would miss about a fifth of patients with acute cerebrovascular disease and of those patents positively identified about three quarters would have acute cerebrovascular disease. A strategy of identifying patients where clinical judgment deemed acute cerebrovascular disease probable or definite, and patients had none of the positive features of the ROSIER or one of the negative features would identify about three quarters of patients with acute cerebrovascular disease, and of those patient positively identified about four fifths would have acute cerebrovascular disease.

Note that the sensitivity of each scale increases as the clinical suspicion of the emergency department member for stroke increases; for example the sensitivity of the FAS score for acute cerebrovascular disease in the whole sample is 82%, though in those where the emergency department staff was definite or probable about the diagnosis of acute cerebrovascular disease this increased to 94%, and in those where the emergency department staff was definite about the diagnosis of acute cerebrovascular disease this increased to 98%.

The ROSIER scale was first developed and validated in Newcastle in patients referred to a stroke service and was evaluated by emergency department doctors (Nor et al. 2005a). The association of individual variables of the ROSIER scale with a diagnosis of stroke was much stronger in the original dataset than mine – for example arm weakness (OR 5.3 versus 1.7), leg weakness (4.1 versus 1.3) and facial weakness (4.8 versus 2.4), though seizures (0.1 versus 0.1) and LOC (0.1 versus 0.3) were similar. Nor et al found, in a smaller validation cohort, that the sensitivity and specificity of the ROSIER was 93% and 83% respectively. In a small Irish series of 50 patients, the ROSIER had a sensitivity of 98% and specificity of 25%, though the authors used an earlier version of the ROSIER scale which included hand weakness (Jackson et al. 2008a).

Generalisability

The results of this study are probably generalisable to emergency departments where staff have a similar level of training to those in the UK and the case mix was similar to patients in my study.

Implications for research

- We are unable to exclude a training effect from the use of these scales in the apparent performance of the clinical opinion of emergency department staff.
- It is reasonable to compare the performance of new diagnostic methods with the informal clinical opinion of a member of an emergency staff member.

Implications for practice

- About two thirds of patients with suspected stroke prove to have a stroke or TIA
- The results of clinical scales alone do not perform a great deal better for the diagnosis of stroke than the informal opinion of a trained member of the emergency department staff.

Tables

Table 3.1 Comparing the sensitivity of two tests for the diagnosis of acute cerebrovascular disease

Subjects with acute cerebrovascular disease		**Test 1**	
		Positive	**Negative**
Test 2	**Positive**	**a**	**b**
	Negative	**c**	**d**

To compare specificities, a similar table could be drawn for subject without acute cerebrovascular disease. McNemar's $X^2 = \frac{|b-c|-1}{\sqrt{b+c}}$

Table 3.2 All patients admitted to the Western General Hospital with a discharge diagnosis of stroke from 21st March 2007 to 27th February 2009.

Data from the Scottish Stroke Care Audit System. There were no records of delay to admission of patients with TIA. ICD-10 codes I61, I63, I64X were used to define stroke and its subtypes.

Stroke onset to admission (days)	Stroke type			**Total (n, %)**
	Haemorrhagic (n, %)	Ischaemic (n, %)	Uncertain (n, %)	
0	46 (72)	499 (67)	8 (8)	553 (67)
1	6 (9)	107 (14)	1 (1)	114 (14)
2	6 (9)	41 (5)	1 (1)	48 (6)
3	2 (3)	23 (3)	-	25 (3)
4	-	23 (3)	-	23 (3)
5	-	5 (1)	-	5 (1)
>5	4 (6)	51 (7)	-	55 (7)
Total	64 (100)	749 (100)	10 (100)	823 (100)

Table 3.3 Comparison of routinely collected data of stroke severity and age with study cohort

	SSCA data 21st March 2007 to 27th February 2009		Current study data
	All patients	**Patients admitted <24 hours**	**Stroke patients**
Number	873	579	245
Age (mean, SD)	74.5 (13.1)	75.4 (13.1)	74.9 (12.3)
Living alone (n, %)	355 (40.7)	232 (40.1)	80 (32.7)
Able to talk (n, %)	694 (79.5)	436 (75.3)	178 (72.7)
Orientated to time, place and person (n, %)	553 (63.3)	338 (58.3)	172 (70.2)
Able to lift arms (n, %)	591 (67.7)	353 (61.0)	146 (59.6)
Able to walk without help (n, %)	346 (39.6)	200 (34.5)	92 (37.6)
NIHSS score (median, IQR)	4 (2 to 9)*	5 (2 to 12)†	4 (2 to 11)

* recorded in 580 patients † recorded in 392 patients. SSCA: Scottish Stroke Care Audit, a record of all stroke admissions at the Western General Hospital.

Table 3.4 Baseline clinical characteristics of patients with suspected stroke

		Diagnosis		Odds ratio (95% CI)	
	All (n=405)	**ACvD* (n=285)**	**Mimic† (n=120)**	**ACvD vs mimic**	***P* value**
Male sex (n, %)	198 (46.7)	136 (47.7)	53 (44.2)	1.15 (0.75 to 1.77)	0.513
Age (years) (mean, SD)	72.4 (13.9)	74.4 (12.4)	67.5 (15.9)	1.43 (1.22 to 1.67)‡	<0.001
Fellow collected variables	n, %	n, %	n, %		
Head trauma	11 (2.7)	8 (2.8)	2 (2.5)	1.12 (0.29 to 4.33)	0.860
Loss of consciousness	31 (7.8)	15 (5.4)	16 (13.5)	0.37 (0.17 to 0.76)	0.008
Seizure at onset	20 (5.0)	3 (1.1)	17 (14.3)	0.07 (0.02 to 0.23)	<0.001
Headache at onset	80 (20.1)	44 (15.8)	36 (30.3)	0.43 (0.26 to 0.71)	0.001
Infective symptoms	47 (11.6)	27 (9.5)	20 (16.7)	0.52 (0.28 to 0.98)	0.041
Prior cardiac vascular disease	90 (22.3)	67 (23.5)	23 (19.3)	1.28 (0.75 to 2.18)	0.358
Prior peripheral vascular disease	20 (5.0)	17 (6.0)	3 (2.5)	2.47 (0.71 to 8.60)	0.155
Prior TIA or stroke	121 (30.0)	76 (26.8)	45 (37.8)	0.60 (0.37 to 0.94)	0.026
Prior heart failure	29 (7.3)	22 (7.8)	7 (5.9)	1.36 (0.56 to 3.27)	0.494
AF (prior or during ED)	93 (23.0)	74 (26.0)	19 (15.8)	1.92 (1.13 to 3.26)	0.015
Prior epilepsy	17 (4.3)	6 (2.1)	11 (9.2)	0.21 (0.08 to 0.59)	0.003
Diabetes mellitus	46 (11.4)	37 (13.0)	9 (7.5)	1.84 (0.86 to 3.94)	0.117
Prior cognitive impairment	57 (14.2)	39 (13.7)	18 (15.3)	0.88 (0.48 to 1.62)	0.691
Migraine	44 (11.1)	26 (9.3)	18 (15.3)	0.57 (0.30 to 1.08)	0.086
Independent prior to admission	341 (84.2)	247 (86.7)	94 (78.3)	1.80 (1.03 to 3.12)	0.037

Table 3.3 continued		**Diagnosis**		**Odds ratio (95% CI)**	
	All (n=405)	**ACvD* (n=285)**	**Mimic† (n=120)**	**ACvD vs mimic**	***P* value**
Living alone	136 (33.6)	96 (33.7)	40 (33.3)	1.02 (0.64 to 1.59)	0.946
Able to talk	321 (79.3)	216 (75.8)	105 (87.5)	0.45 (0.24 to 0.81)	0.009
Orientated to time place & person	297 (73)	210 (73.7)	87 (72.5)	1.06 (0.66 to 1.71)	0.806
Able to lift arms	279 (68.9)	181 (63.5)	98 (81.7)	0.39 (0.23 to 0.66)	<0.001
Able to walk without help	195 (48.2)	127 (44.6)	68 (56.7)	0.61 (0.40 to 0.94)	0.027
Medications	n, %	n, %	n, %		
Any antiplatelet agent	172 (42.5)	121 (42.5)	51 (42.5)	1.00 (0.65 to 1.53)	0.993
Warfarin	24 (6.0)	13 (4.6)	11 (9.2)	0.48 (0.21 to 1.10)	0.081
Antihypertensive	213 (52.6)	155 (54.4)	58 (48.3)	1.27 (0.83 to 1.95)	0.266
Statin	146 (36.5)	99 (35.2)	47 (39.5)	0.83 (0.54 to 1.30)	0.418
Current smoker	94 (23.4)	65 (23.1)	29 (24.2)	0.94 (0.57 to 1.55)	0.809
Examination findings	n, %	n, %	n, %		
Any focal neurological deficit	323 (81.0)	261 (92.9)	62 (52.5)	11.78 (6.5 to 21.1)	<0.001
Normal pedal pulses	208 (51.7)	133 (47.0)	75 (63.0)	0.52 (0.34 to 0.81)	0.004
Heart Murmur	35 (8.8)	25 (8.9)	10 (8.4)	1.06 (0.49 to 2.29)	0.873
Carotid Bruit	15 (3.7)	13 (4.6)	2 (1.7)	2.83 (0.63 to 12.73)	0.175
Continuous variables (mean,SD)	n, %	n, %	n, %		
Systolic BP (mmHg)	153 (29)	157 (30)	143 (25)	1.18 (1.09 to 1.28)¶	<0.001
Diastolic BP (mmHg)	84 (18)	86 (18)	80 (18)	1.19 (1.05 to 1.36) ¶	0.006
Temperature (^{o}C)	36.4 (0.7)	36.4 (0.6)	36.4 9 (0.8)	0.95 (0.69 to 1.30)	0.727

Table 3.3 continued		**Diagnosis**		**Odds ratio (95% CI)**	
	All (n=405)	**ACvD* (n=285)**	**Mimic† (n=120)**	**ACvD vs mimic**	***P* value**
Continuous variables	median, IQR	median, IQR	median, IQR		
Last seen well to admission (hrs)	6.2 (12.4)	6.3 (13.4)	6.0 (9.9)	1.00 (0.98 to 1.02)§	0.800
Found unwell to admission (hrs)	4.2 (6.2)	3.8 (6.5)	4.6 (5.3)	1.00 (0.97 to 1.01) §	0.424
Admission to stroke fellow (hrs)	0.9 (1.4)	0.9 (1.4)	0.9 (1.4)	1.02 (0.98 to 1.07)	0.294
NIHSS (per unit)	3 (7)	4 (8)	1 (5)	1.11 (1.06 to 1.17)‖	<0.001
ED collected variables (n, %)	n, %	n, %	n, %		
Arm weakness	205 (55.1)	152 (58.9)	53 (46.5)	1.65 (1.06 to 2.57)	0.027
Facial weakness	149 (39.8)	119 (45.8)	30 (26.3)	2.36 (1.46 to 3.83)	<0.001
Leg weakness	144 (38.8)	105 (40.9)	39 (34.2)	1.32 (0.83 to 2.1)	0.226
Speech disturbance	166 (44.5)	134 (51.7)	32 (28.1)	2.75 (1.71 to 4.42)	<0.001
Visual disturbance	52 (14.5)	47 (18.9)	5 (4.6)	4.83 (1.86 to 12.53)	0.001

Percentages are given as a proportion subjects with complete data. Odds ratios >1 indicate a variable is positively associated with a diagnosis of ACvD. I used Wald tests to calculate *P* values.

*ACvD: acute cerebrovascular disease (probable or definite cerebral ischaemia or intracerebral haemorrhage responsible for symptoms at time of clinical assessment) †ACvD definitely not, or only possibly responsible for symptoms at time of clinical assessment ‡ per 10 years, § per hour ‖ per unit increase NIHSS ¶ per 10mmHg increase

Table 3.5 Brain imaging findings in patients with acute cerebrovascular disease or mimic.

		ACvD			Mimic
	All (n=405)	**All (n=285)**	**Stroke (n=243)**	**TIA (n= 40)**	**(n=120)**
First imaging modality (n, %)					
CT	301 (74)	228 (80)	193 (79)	33 (83)	73 (61)
MR	75 (19)	51 (18)	46 (19)	5 (13)	24 (20)
No imaging	29 (7)	6 (2)	4 (2)	2 (5)	23 (19)
Ever MR imaging	126 (31)	93 (33)	83 (34)	10 (25)	33 (28)
Imaging findings (n, %)	n=376	n=279	n=239	n=38	n=97
Relevant ischaemic lesion (first scan) ‡	162 (40)	162 (58)	151 (63)	11 (28)	0
Cortical§	109 (67)	109 (67)	103 (68)	6 (55)	0
Lacunar§	33 (20)	33 (20)	29 (19)	4 (36)	0
Brainstem§	16 (10)	16 (10)	15 (6)	1 (9)	0
>1 lesion	4 (2)	4 (2)	4 (2)	0	0
Relevant ischaemic lesion (any scan) ‡	186 (49)	186 (66)	173 (72)	13 (34)	0
Relevant haemorrhagic lesion‡	17 (4.5)	15 (5.4)	15 (6.1)	0	2 (2)¶
Relevant other lesion‡	6 (1.6)	0	0	0	6 (5)
Non-relevant infarction‡	125 (33)	88 (31)	47 (34)	115 (34)	37 (38)

Relevant lesions are those considered to be responsible for the presenting symptoms.* definite or probable acute cerebrovascular disease ‡as a percentage of those with a scan § as a percentage of those with a relevant ischaemic lesion ¶2 patients with subdural haematoma.

Table 3.6 Diagnoses of patients with suspected stroke seen in the emergency department of the Western General Hospital, Edinburgh.

	N	**% ACvD**	**% total (n=405)**
Acute cerebrovascular disease (ACvD)	**285**		**70.4**
Ischaemic stroke	230	80.7	56.8
Definite	*205*	*71.9*	*50.6*
Probable	*25*	*8.8*	*6.2*
Transient ischaemic attack	40	14.0	9.9
Definite	*31*	*10.9*	*7.7*
Probable	*9*	*3.2*	*2.2*
Intracerebral haemorrhage	13	5.6	3.2
Subarachnoid haemorrhage	2	0.7	0.5
	N	**% mimics**	**% total**
Mimics	**120**	**100**	**29.6**
Primary headache disorders	17	14.2	4.2
Seizures	14	11.7	3.5
Sepsis	13	10.8	3.2
Functional disorders	12	10	2.5
Peripheral nerve disorders	10	8.3	2.5
Syncope	8	6.7	2.0
Vestibulopathy	8	6.7	2.0
Metabolic*	6	5.0	1.5
Brain tumours†	4	3.3	1.0
Dementia fluctuation	4	3.3	1.0
Musculoskeletal disorders	3	2.5	0.7
Other cancers	2	1.7	0.5
Subdural haematoma	2	1.7	0.5
Transient global amnesia	2	1.7	0.5
Other diagnoses‡	15	12.5	3.7

*alcohol (3), hypoglycaemia (1) hypothermia (1), symptoms due to nitrazepam (1); †glioma(2), meningioma (1), metastatic (1); ‡transient symptoms after invasive procedures (3), aneurysmal brainstem compression(1), conjunctivitis (1), uncertain (3)

Table 3.7 Diagnostic performance of different diagnostic approaches for a diagnosis of acute cerebrovascular disease among patients with stroke suspected by emergency staff, compared with 'gold standard'

		Sensitivity		Specificity	
	N	**% (95% CI)**	***P****	**% (95% CI)**	***P****
ED staff informal diagnosis of definite or probable ACvD	389	77 (72 to 82)	Reference	58 (49 to 67)	Reference
Stroke fellow informal diagnosis of definite or probable ACvD	405	92 (90 to 96)	<0.001	84 (77 to 90)	0.009
FAS, measured by ED staff	374	82 (76 to 86)	0.07	37 (29 to 46)	<0.001
ROSIER, variables measured by ED staff/stroke fellow	350	82 (77 to 87)	0.007	42 (33 to 52)	<0.001
Logistic regression model designed for nurse use (Hand 2002) ‡	369	94 (90 to 96)	<0.001	44 (35 to 53)	0.017
Logistic regression model designed for stroke fellow (Hand 2002) §	398	43 (38 to 49)	<0.001†	86 (79 to 91)	0.37†

* *P* obtained by comparing the sensitivity or specificity of an emergency department clinician's assessment with the other diagnostic scales in a series of paired comparisons, using a McNemar's test. Where the number of discordant pairs is <20, I have reported the Exact McNemar's *P* †compared to stroke fellow.‡predicted probability threshold=0.3 §predicted probability threshold=0.9

ED: emergency department; FAS: face, arm speech tests; ROSIER: recognition of stroke in the ED.

A *P*<0.01 indicates a statistically significant difference over ED staff informal diagnosis of acute cerebrovascular disease. ROSIER & FAS (375 assessments) were assessed using data collected by the first qualified ED assessor (104 doctors, 27.7%, 270 nurses, 72%, 1 paramedic, 0.27%)

Table 3.8 Diagnostic performance of different diagnostic approaches for a diagnosis of stroke among patients with stroke suspected by emergency staff, compared with 'gold standard'

	n	Sensitivity (95% CI)	Specificity (95% CI)
ED staff informal diagnosis of definite stroke	389	35 (29 to 41)	93 (88 to 96)
ED staff informal diagnosis of definite or probable stroke	389	80 (75 to 85)	58 (0 to 61)
FAS, measured by ED staff	374	85 (80 to 89)	37 (30 to 45)
ROSIER, variables measured by ED staff/stroke fellow	350	86 (81 to 90)	41 (33 to 49)
Stroke fellow informal diagnosis of definite stroke	405	68 (62 to 74)	83 (76 to 88)
Stroke fellow informal diagnosis of definite or probable stroke	405	93 (89 to 95)	66 (58 to 73)

ED: Emergency department, FAS: face, arm speech tests, ROSIER: recognition of stroke in the ED.

ROSIER & FAS (375 assessments) were assessed using data collected by the first qualified ED assessor (104 doctors, 27.7%, 270 nurses, 72%, 1 paramedic, 0.27%)

Table 3.9 Positive and negative predictive values and likelihood ratios of stroke scales for a diagnosis of acute cerebrovascular disease or stroke.

	N	PPV (95%	NPV(95% CI)	LR+(95% CI)	LR-(95% CI)
For a diagnosis of acute cerebrovascular diseases amongst patients with suspected stroke					
ED staff informal diagnosis of definite ACvD	389	93 (86 to 97)	38 (33 to 44)	6.3 (2.8 to 14)	0.7 (0.7 to 0.8)
ED staff informal diagnosis of definite or probable ACvD	389	81 (75 to 85)	53 (45 to 62)	1.9 (1.5 to 2.3)	0.4 (0.3 to 0.5)
FAS, measured by ED staff	374	75 (69 to 79)	47 (37 to 58)	1.3 (1.1 to 1.5)	0.5 (0.4 to 0.7)
ROSIER, variables measured by ED staff/stroke fellow	350	77 (71 to 82)	51 (40 to 61)	1.4 (1.2 to 1.7)	0.4 (0.3 to 0.6)
Stroke fellow informal diagnosis of definite ACvD	405	97 (94 to 99)	54 (48 to 61)	13 (6.1 to 9.1)	0.4 (0.3 to 42)
Stroke fellow informal diagnosis of definite or probable ACvD	405	94 (90 to 96)	82 (74 to 88)	5.8 (3.9 to 8.8)	0.1 (0.1 to 0.1)
For a diagnosis of stroke amongst patients with suspected stroke					
ED staff informal diagnosis of definite stroke	389	88 (80 to 93)	49 (44 to 55)	5.0 (2.7 to 9.0)	0.7 (0.6 to 0.8)
ED staff informal diagnosis of definite or probable stroke	389	72 (66 to 77)	65 (56 to 73)	1.7 (1.5 to 21)	0.4 (0.3 to 0.5)
FAS, measured by ED staff	374	67 (61 to 72)	63 (53 to 72)	1.4 (1.2 to 1.6)	0.4 (0.3 to 0.6)
ROSIER, variables measured by ED staff/stroke fellow	350	68 (62 to 73)	67 (56 to 76)	1.5 (1.3 to 1.7)	0.3 (0.2 to 0.5)
Stroke fellow informal diagnosis of definite stroke	405	86 (81 to 90)	63 (56 to 69)	3.9 (2.8 to 5.5)	0.4 (0.3 to 0.5)
Stroke fellow informal diagnosis of definite or probable stroke	405	81 (76 to 85)	85 (78 to 91)	2.7 (2.2 to 3.3)	0.1 (0.1 to 0.2)

ACvD: acute cerebrovascular disease; PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio; ED: Emergency department, FAS: face, arm speech tests, ROSIER: recognition of stroke in the ED.

ROSIER & FAS (375 assessments) were assessed using data collected by the first qualified ED assessor (104 doctors, 27.7%, 270 nurses, 72%, 1 paramedic, 0.27%)

Table 3.10 Diagnostic performance of different diagnostic approaches for a diagnosis of acute cerebrovascular disease among patients seen less than 6 hours after symptom onset with stroke suspected by emergency staff, compared with 'gold standard'

		Sensitivity			Specificity		
	N	**%**	**95% CI**	***P****	**%**	**95% CI**	***P***
ED staff informal diagnosis of definite or probable ACvD	185	83	75 to 88	Ref	59	47 to 71	Reference
FAS, measured by ED staff	178	85	78 to 91	0.42	41	29 to 54	0.03
ROSIER, variables measured by ED staff/stroke fellow	170	86	78 to 91	0.21	51	38 to 64	0.21
Logistic regression model designed for nurse use (Hand 2002)	175	94	88 to 97	<0.01	44	31 to 57	0.06
Stroke fellow informal diagnosis of definite or probable ACvD	185	93	88 to 97	<0.01	86	76 to 93	<0.01

* *P* obtained by comparing the sensitivity or specificity of an emergency department clinician's assessment with the other diagnostic scales in a series of paired comparisons, using a McNemar's test. Where the number of discordant pairs is <20, I have reported the Exact McNemar's *P* † compared to stroke fellow. A *P*<0.01 indicates a statistically significant improvement over ED staff informal diagnosis of ACvD

ACvD: acute cerebrovascular disease; ED: Emergency department; FAS: face, arm speech tests; ROSIER: recognition of stroke in the ED.

Table 3.11 Diagnostic performance of different diagnostic approaches for a diagnosis of acute cerebrovascular disease among patients seen by a nurse with suspected stroke, compared with 'gold standard'

		Sensitivity			Specificity		
	N	**%**	**95% CI**	***P****	**%**	**95% CI**	***P***
ED staff informal diagnosis of definite or probable ACvD	267	73	66 to 79	Reference	56	45 to 66	Reference
FAS, measured by ED staff	255	81	75 to 86	0.02	36	27 to 48	<0.01
ROSIER, variables measured by ED staff/stroke fellow	236	81	75 to 87	<0.01	42	31 to 54	0.02
Logistic regression model designed for nurse use (Hand 2002)	251	96	91 to 98	<0.01	44	33 to 55	0.01

P obtained by comparing the sensitivity or specificity of an emergency department clinician's assessment with the other diagnostic scales in a series of paired comparisons, using a McNemar's test. Where the number of discordant pairs is <20, I have reported the Exact McNemar's *P*;

ACvD: acute cerebrovascular disease; ED: Emergency department; FAS: face, arm speech tests; ROSIER: recognition of stroke in the ED.

Table 3.12 Missing data

Variable	All	Acute cerebrovascular	Mimic
Fellow collected variables			
Systolic BP	0	0	0
Diastolic BP	0	0	0
Temperature	16 (3.9)	15 (5.3)	1 (0.8)
Well to admission	5 (1.2)	5 (1.2)	0
Found to admission	5 (1.2)	5 (1.2)	0
Admission to stroke fellow	6 (1.5)	5 (1.2)	1 (0.3)
NIHSS	0	0	0
Sex	0	0	0
Head trauma	4 (1.0)	3 (1.1)	1 (0.8)
LOC	7 (1.7)	6 (2.1)	1 (0.8)
Seizure	7 (1.7)	6 (2.1)	1 (0.8)
Headache	7 (1.7)	6 (2.1)	1 (0.8)
Infective symptom	0	0	0
Cardiac vascular disease	1 (0.2)	0	1 (0.8)
Peripheral vascular disease	3 (0.7)	2 (0.7)	1 (0.8)
TIA or stroke	1 (0.2)	0	1 (0.8)
Heart failure	5 (1.2)	4 (1.4)	1 (0.8)
AF (prior, during)	0	0	0
Epilepsy	6 (1.5)	5 (1.8)	1 (0.8)
Diabetes	0	0	0
Dementia	3 (0.7)	1 (0.4)	2 (1.7)
Migraine	7 (1.7)	5 (1.8)	2 (1.6)
Independent of ADL	0	0	0
Living alone	0	0	0
Able to talk	0	0	0
Orientated to time place & person	0	0	0
Able to lift arms	0	0	0
Able to walk without help	0	0	0

Variable	All	Acute cerebrovascular	Mimic
Antiplatelet	0	0	0
Warfarin	5 (1.2)	4 (1.4)	1 (0.8)
Antihypertensive	0	0	0
Statin	5 (1.2)	4 (1.4)	1 (0.8)
Current smoker	3 (0.7)	3 (0.1)	0
Any focal neurological deficit	6 (1.5)	4 (1.4)	2 (1.7)
Normal pedal pulses	3 (0.7)	2 (0.7)	1 (0.8)
Heart murmur	5 (1.7)	4 (1.4)	1 (0.8)
Carotid bruit	1 (0.2)	1 (0.3)	0
ED collected variables			
Arm weakness	33 (8.1)	27 (9.5)	6 (5.0)
Facial weakness	31 (7.6)	25 (8.7)	6 (5.0)
Leg weakness	34 (8.4)	28 (9.8)	6 (5.0)
Speech disturbance	32 (7.9)	26 (9.1)	6 (5.0)
Visual disturbance	47 (11.6)	36 (12.6)	11 (9.1)

Figures

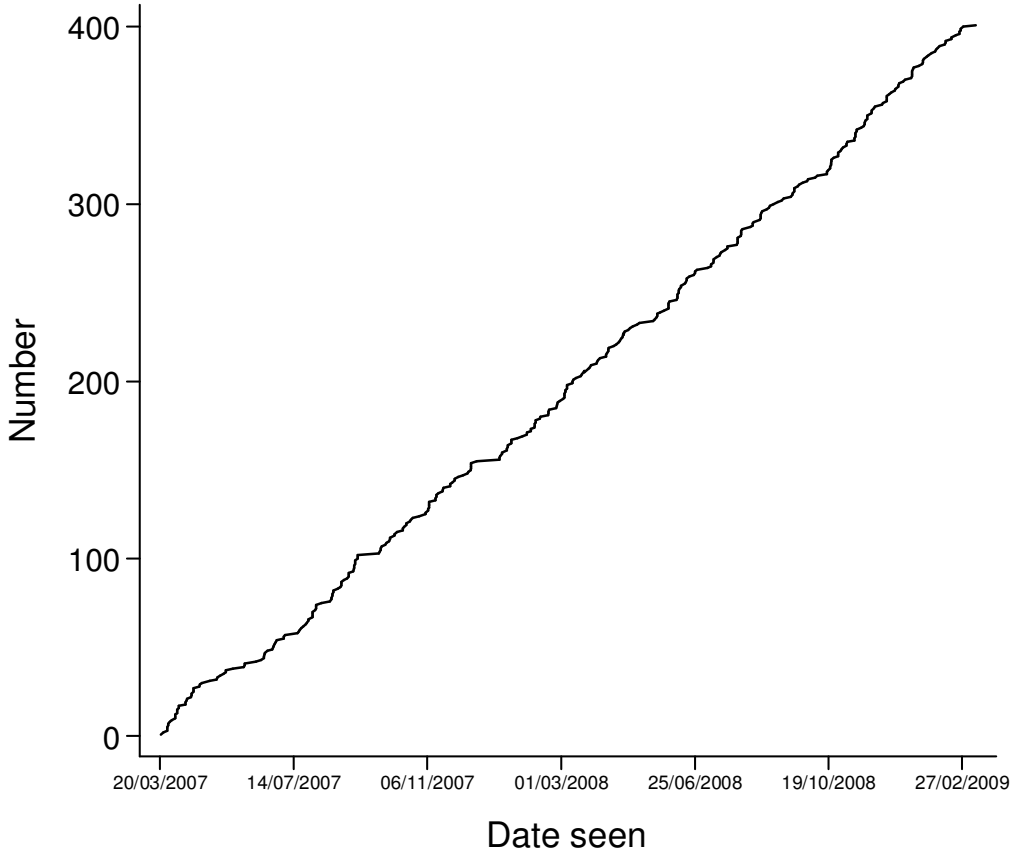


Figure 4 Recruitment to the Blood Biomarkers In Suspected Stroke study.

Note the constant rate of recruitment throughout the study period (~1 patient/day).

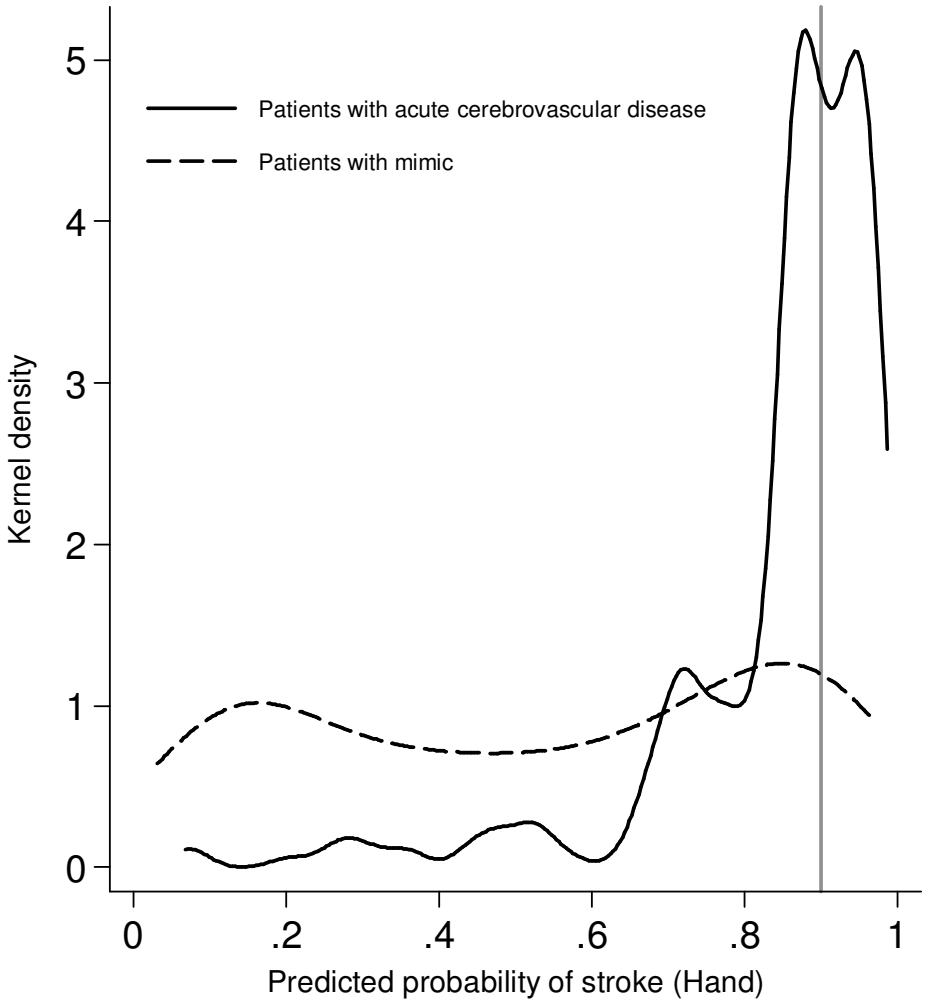


Figure 5 Predicted probabilities of a diagnosis of stroke, derived from a logistic regression model for stroke registrars.

The vertical grey line shows the suggested threshold for the use of the model (P=0.90) for a high positive predictive value (95%). (Hand 2002). At this threshold, in this cohort, the model's sensitivity for acute cerebrovascular disease is 43% and specificity 86%.

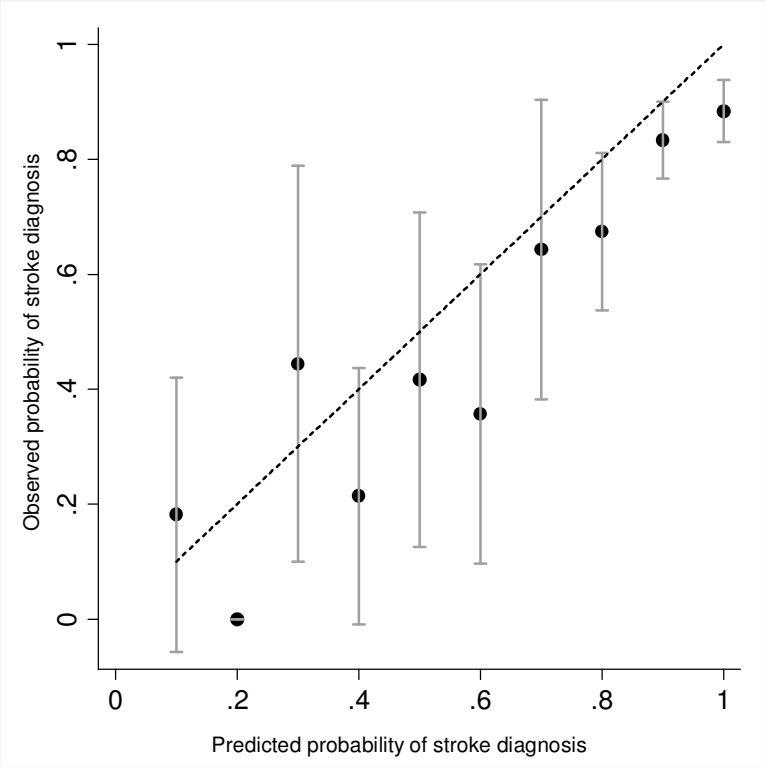


Figure 6 Predicted versus observed probability of stroke from a logistic regression model designed for stroke registrars in patients with suspected stroke.

Predictions derived from a logistic regression model for stroke for neurology registrars (Hand 2002). Bars indicate 95% confidence intervals and the dotted line represents perfect calibration.

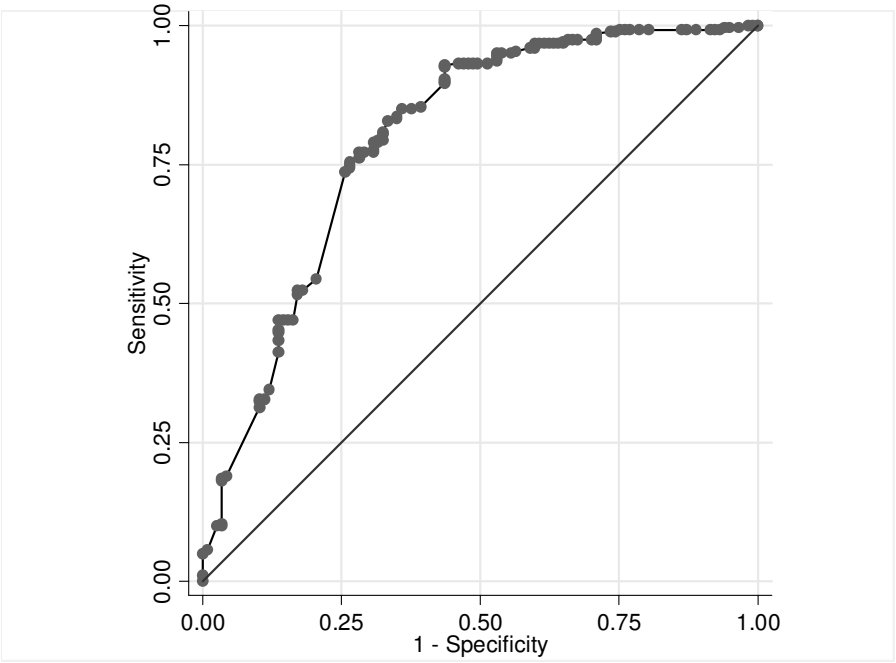


Figure 7 Receiver operator curve for logistic regression model for use by stroke registrars (Hand 2002) applied to BBISS dataset.

AUROC=0.80 (95% CI: 0.74 to 0.85). The AUROC is a measure of the discrimination of a model; 1 indicates perfect discrimination and 0.5 no better discrimination than chance (the diagonal line). N=398

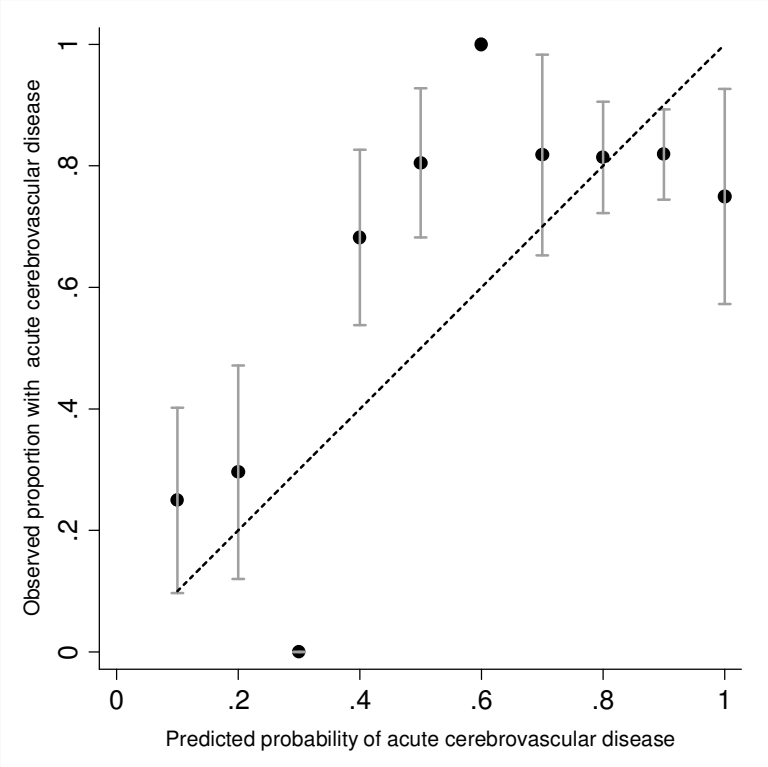


Figure 8 Predicted versus observed probability of stroke for a logistic regression model designed for use by nurses.

Predicted probabilities derived from a clinical prediction model designed for use by emergency department nurses. The dotted line shows perfect calibration

Chapter 4. Blood markers of inflammation, thrombosis, thrombolysis, cardiac strain, neural and glial damage and the diagnosis of acute cerebrovascular diseases in an emergency department: BBISS, a prospective cohort study

Introduction

A clinical diagnosis of stroke or TIA (which I will call 'acute cerebrovascular diseases', ACvD) relies on a few key features: sudden onset of symptoms, a focal neurological deficit, and the absence of positive symptoms of stroke mimics such as a seizure or loss of consciousness. However, the accuracy of clinical diagnosis made by doctors or nurses may depend on their training and expertise in stroke medicine. Further tests can improve upon the accuracy of the clinical diagnosis of stroke. Brain imaging serves positively to identify brain ischaemia or haemorrhage, and exclude the presence of conditions that may mimic stroke in the emergency department such as brain tumours or extradural haemorrhage.

However, brain imaging may take some time to perform, and MR brain imaging – probably the most sensitive technique – is not available to many patients as they may be too sick or their stroke service may not have rapid access to such a scanner (Hand et al. 2005a, Kane et al 2008). Blood markers of various aspects of stroke patho-physiology have the potential to improve the clinical diagnosis.

Some patho-physiological processes may be more common in patients with acute cerebrovascular disease than patients with stroke mimics. For example, markers of glial and neuronal damage, inflammation and cardiac strain are higher in patients with stroke than controls (Dassan, Keir, & Brown 2009, Whiteley, Tseng, & Sandercock 2008a). Ideally, the measurement of a single marker of any one of these processes would be diagnostic of acute cerebrovascular disease. Alternatively, a panel of several markers, assessing markers of several patho-physiological processes might be helpful. Panels of markers have been developed for the

diagnosis of conditions as diverse as lung cancer and myocardial infarction (Apple et al. 2007, Patz, Jr. et al. 2007). A previously developed panel that predicted a diagnosis of stroke included markers of cardiac strain (brain natriuretic peptide, BNP), inflammation/endothelial activation (matrix metalloproteinase-9, MMP-9), glial damage (S100 B) and thrombosis (D-dimer)(Laskowitz et al. 2009f).

Blood markers therefore have the potential to help to confirm or strengthen a clinical diagnosis of acute cerebrovascular disease in patients presenting to the emergency department with suspected stroke.

In this chapter I will seek to:

- describe the univariate association between acute cerebrovascular disease and the plasma or serum concentration of markers of inflammation, thrombosis, thrombolysis, cardiac strain, cerebral damage, renal function, and glucose,
- describe the correlation of the plasma or serum concentration of each marker with potentially confounding variables such as neurological impairment, age, blood pressure, and delay to blood draw,
- describe the association of the plasma or serum concentration of markers with acute cerebrovascular disease after adjustment for those variables,
- validate a previously published logistic regression model for the diagnosis of stroke with four blood markers (Laskowitz et al. 2009e),
- develop a multiple variable logistic regression model based upon plasma or serum levels of blood markers to predict a diagnosis of acute cerebrovascular disease and,
- test whether adding data from any marker positively associated with a diagnosis of acute cerebrovascular diseases improves diagnostic performance of the clinical opinion of a member of the emergency department staff to a clinically useful degree.

Methods

BBISS cohort recruitment

I have described the process of recruiting the BBISS cohort of patients in detail in the preceding chapter. In brief, I recruited all patients presenting to the Western General Hospital where an emergency department doctor or nurse suspected TIA or stroke, and the patient had been symptomatic for less than 24 hours. Each patient had brain imaging, where clinically indicated, or where there was substantial clinical uncertainly. A gold standard diagnosis of stroke or TIA was made by a panel of experts, including stroke physicians and neuroradiologists with access to the clinical findings, relevant imaging, and subsequent clinical course, blinded to the serum or plasma marker levels.

Blood draw

I drew blood from each patient as soon as possible after assessment into two 2.5 ml EDTA tubes, and an 8 ml tube containing clot activator and gel for serum separation. I took the samples on water ice to the Wellcome Trust Clinical Research Facility (WTRCF) at the Western General Hospital. A technician centrifuged the blood at 3000 revolutions per minute for 10 minutes, pipetteted the supernatant from each blood tube into two screw-topped tubes, labelled each sample with a 'Cryotag' bar code and transferred them to a -80 ^{0}C fridge.

Nurses from the WTCRF took blood samples from patients at 24 hours after symptom onset, when this fell within normal working hours. I personally transferred samples in batches to the University of Glasgow for the analysis of markers of cardiac strain, inflammation, thrombosis and thrombolysis, and to the National Creutzfeld-Jacob Disease Surveillance Unit in Edinburgh for the analysis of markers of neuronal and glial damage.

Sample management

Bar codes labels marked each sample with a unique identifying number. An Access database linked each sample number to the patient identification number, recorded

the time and date the sample was drawn and where each sample was to be analysed.

Measurement of blood markers

Experienced biochemists in academic laboratories measured markers according to their standard practice. Dr Ann Rumley and Dr Paul Welsh (Division of Cardiovascular and Medical Sciences, Royal Infirmary, University of Glasgow) measured markers of inflammation, cardiac strain and thrombosis and Dr Alison Green and Mrs. Mary Andrews (National CJD Surveillance Unit, University of Edinburgh) measured markers of neuronal and glial damage. Blood markers were measured in plasma or serum that had been stored at -80◦C for a maximum of 2 years. In general, the plasma or serum from the first sample (<24 hours) was analysed. Where that sample was unavailable, or there was insufficient plasma or serum, the subsequent sample taken at 24 hours after symptom onset was used. All markers were measured blind to acute cerebrovascular disease status.

The coefficient of variation

One measure of the analytic random variation or imprecision of a test, is the *coefficient of variation*. It is calculated by dividing the standard deviation of repeat measures by the mean of all measurements, and it is usually reported as a percentage. The advantage of this measure is that, in general, as the mean of samples increases so does the standard deviation; the coefficient of variation allows comparison of the variability regardless of the mean of samples. Simulation has demonstrated that the chance of finding a more than 1.5 fold difference in two measurements of the same sample where the coefficient of variation is <10% has a probability of <0.001 (Reed, Lynn, & Meade 2002).

I 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.

- *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.

Clotting

- *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 imunonephelometry (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.

Thrombolysis

- *Tissue plasminogen activator (tPA) (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 479pg/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%.

Other markers

- *Creatinine (μmol/):* I used the measurement of serum creatinine made by the clinical laboratories at the Western General Hospital. An Ortho Clinical Diagnostics Fusion 5.1 measured creatinine with an enzymatic method on dry slides. The coefficient of variations at 85 and 480 μmol/L was <1.4%.

- *Glucose (mmol/l)*: I used the measurement of glucose in fluoridated serum made by the clinical laboratories at the Western General Hospital. An Ortho Clinical Diagnostics Fusion 5.1 measured glucose with a glucose oxidase/peroxidase on dry slides. The coefficient of variation at 4.3 and 16 mmol/L was <1.4%.

Statistical analysis

Univariate associations between plasma or serum concentration of blood markers and acute cerebrovascular disease

I calculated the association between plasma or serum concentration of blood markers and the diagnosis of stroke using a series of univariate logistic regression analyses, with a diagnosis of acute cerebrovascular disease as the dependent variable, and blood marker marker level the independent variable. For each marker I assessed the association as a linear, continuous variable, to maintain power in the analysis (Altman & Royston 2006), and presented the results as the ratio of odds of acute cerebrovascular disease in the 75th to 25th centile of marker (i.e. (OR per unit increase)$^{(75th\ -25th\ centile)}$) to allow comparison between marker distributions.

Testing the assumption of a linear relationship between serum blood marker concentration and log odds of acute cerebrovascular disease

A logistic regression model assumes a linear association between a continuous variable and the log odds of the outcome of interest. However, the relationship may not be log-linear, and a transformation of the variable may give a better model fit.. There a number of ways of modeling a non-linear relationship. The easiest is with a simple transformation of the variable of interest (for example Ln(X), X^2, etc.).

However, simple transformations are limited in the shapes they can take. More complex transformations may model relationships better (though with an increased risk of overfitting); for example fractional polynomials and restricted cubic splines (Steyerberg 2009a). Both complex transformations have their advocates, and it is not yet clear which should be preferred for modeling non-linear relationships. As

restricted cubic splines can be easily implemented in Stata 10 using the `mkspline` command, I investigated linearity using this transformation, at the default setting of three knots (at quantiles 10, 50 and 90%), recommended for smaller datasets (Harrell F.E 2001).

I compared linear and nested restricted cubic splines by calculating the likelihood ratio statistic (Equation 6) and its associated probability (from a χ^2 distribution with 4 degrees of freedom) with the `lrtest` command, which was possible as a linear model is nested within the restricted cubic spline model (Dupont 2009).

Equation 6 Likelihood ratio statistic, 1 degree of freedom. *L*=likelihood

$$\chi^2 = -2(L_0 - L_1)$$

I investigated the effect of outliers by repeating this process, truncating the distribution of the plasma or serum levels of markers at their 5th and 95th centiles. Where there was evidence of significant non-linearity I investigated this further by plotting the relationships.

Adjusting for age, neurological impairment, cardiac disease and infection

The most important confounders of the relationship between plasma or serum concentration of blood markers and a diagnosis of stroke are degree of neurological impairment and age. I measured the association between plasma or serum blood marker levels with NIHSS, age (in years), delay to blood draw (in hours) and systolic blood pressure (mmHg) with Spearman's rank correlation coefficients (and associated *P*-values) using the `spearman` command in Stata. I did not use a parametric test as I expected markers to have a skewed distribution.

I used the NIHS score as a measure of neurological impairment for this analysis, as it had an approximately linear association with the log odds of a diagnosis of acute cerebrovascular disease. For the association with cardiac markers (NT pro-BNP and troponin T) I made additional adjustments for AF, cardiac failure and previous

cardiac vascular disease, and for the association with inflammatory markers I made adjustment for 'symptoms due to infection'. I performed this analysis with multivariate logistic regression using the `logistic` command in Stata, and present the odds ratios, their associated 95% confidence intervals and *P*-values derived from Wald tests.

Validating a published blood marker model

I assessed the performance of a published predictive model for the diagnosis of stroke (Laskowitz et al. 2009d) constructed with blood marker variables. As the model was developed to predict an outcome of stroke rather than acute cerebrovascular disease, I validated the model with the outcome of 'all stroke' as well as 'acute cerebrovascular disease'. The authors constructed a simple logistic regression model with the natural logarithm of four markers: S100 B, D-dimer, MMP-9 and BNP. I applied the coefficients from the published model to my cohort (Equation 7), substituting NT pro-BNP for BNP (the relationship in plasma is 1:1).

Equation 7 Logit (Stroke) from Laskowitz et al 2009

Logit (Stroke)=
-3.51
+ [0.32 x Ln(BNP)]
+ [0.13 x Ln(D-dimer)]
+ [0.3 x Ln(MMP-9)]
+ [0.05 x Ln(S100 B)]

I calculated: (a) the predicted probability of stroke, (b) the area under a receiver operator curve (AUROC) to predict the diagnosis of both stroke and acute cerebrovascular disease and stroke in my cohort (`roctab`), and (c) applied the thresholds reported in the paper for the diagnosis of stroke (Pr<0.39 and Pr>0.64) to this cohort to calculate sensitivity and specificity.

Generation of predictive models

This section of the analysis concentrates upon the generation and validation of predictive biomarker models for the diagnosis of stroke. I constructed statistical models using a number of methods, and compared their performance to one another. The methods of model development ranged from those with a high degree of flexibility, which potentially had a better fit to the data but at a greater risk of poor external validity, to those where the choice of variables was limited by external information (from systematic review), where external validity might be higher.

I generated multivariable logistic regression models with only blood marker variables to predict a diagnosis of acute cerebrovascular disease.

Rather than generating a model with all 19 potential candidate markers, I attempted to reduce the number of markers in the final model, because: (a) as a rule of thumb there should be more than 10 outcome events per variable, the lower bound for reasonable selection of a pre-specified variables; this number may need to be even higher (up to 50) when variables are not pre-specified (Harrell F.E, Lee K.L., & Mark D.B 1996, Steyerberg 2009b), (b) because a model with all 19 variables would very likely over-fit the data and replication in a validation dataset would be unlikely to be successful (and so be of little practical use), and (c) a model with all variables would be difficult to implement in clinical practice, because of the expense and technical difficulty of measuring a large number of proteins simultaneously.

To restrict the number of variables, I first investigated the number of missing values and their distribution for each of the blood markers. Stata deletes any cases missing a variable in logistic regression, leading to a complete case analysis. Complete case analysis may not only lead to substantial loss of power, and but also selection bias, if the number of cases falls substantially in multivariate analysis. I excluded any marker with substantial missing data.

Second, I considered collinearity. I calculated correlation coefficients between each marker pair using the Stata command `spearman,matrix`, keeping only one from a

pair when the Spearman's rank correlation coefficient was >0.8 (Katz 2008b, Steyerberg 2009b).

Third, I constructed a model with those markers that, from previous systematic review, were associated with a diagnosis of stroke (Whiteley, Tseng, & Sandercock 2008b).

Fourth, I considered only those markers that had associations with acute cerebrovascular disease at $P<0.1$ in univariate analysis, and only those with $P>0.1$ in subsequent multivariate models. Despite the popularity and simplicity of this approach, it risked over-fitting data.

Fifth, I investigated both forward and backward automated variable selection. Forward selection procedures select variables and enter them in order of strength of association with acute cerebrovascular disease into the model, continuing to enter variables until the fit of the model is no longer improved, at a threshold of significance (here, $P=0.10$). Backward selection procedures begin with a full model, and remove the variable with the weakest association with outcome, at a threshold of significance (here, $P=0.05$). I used the Stata command `stepwise` to implement this procedure. However, stepwise selection procedures have a number of disadvantages: the selection of variables may be unstable; they may artificially inflate estimates of coefficients and *P*-values, and may give worse predictions than a full model (Steyerberg 2009a).

Sixth, I attempted a more modern approach. Proponents of boosted logistic regression (Schonlau 2005) with shrinkage and bagging claim that it generates models with improved predictive accuracy, and better generalisability. I implemented the `boost` command in Stata with the following settings: a randomly chosen half of the dataset for training and half for validation; all possible 3-way interactions between markers; shrinkage set at 0.01, and half the dataset used for bagging.

Seventh, I examined the standardised Pearson residuals and Pregibon leverage statistic from each logistic regression model, and plotted each statistic against the predicted probability. I retested model excluding patients who showed a high Pearson residual (>2 standard deviation) or undue leverage (>2*(number of independent variables /sample size)) (Katz 2008a).

Eighth, for each model I considered all two way interactions between variables within the model generated with the `fitint` command. There is a risk of chance findings when investigating all two way interactions, so when a two-way interaction had $P<0.05$ in an individual model, I attempted to replicate that interaction by creating a logistic regression model with just two variables in the whole dataset. Where these remain significant ($P<0.01$) I report them.

I then compared the performance of a model developed from variables from systematic review, a model developed by univariate selection and a model developed by stepwise variable selection. I considered (a) model discrimination using the AUROC and its 95% confidence interval. The AUROC ranges from 0.5 to 1, where 0.5 implies no better discriminative ability that chance and 1, perfect discrimination. It can be interpreted as the probability that a randomly chosen patient with acute cerebrovascular disease has a higher predicted probability of acute cerebrovascular disease than a randomly chosen patients without acute cerebrovascular disease, and (b) as a measure of calibration, I report the Hosmer-Lemeshow statistic, which compares the predicted to the observed probability of a diagnosis of acute cerebrovascular disease, by dividing the sample into deciles on the basis of predicted probability and performing a χ^2 test (Stata command `estat gof`). A non-significant result supports a well calibrated model. I also report the Cragg-Uhler (Nagelkerke) pseudo R^2, which is a measure of explained variance in risk (in a similar, though non-equivalent, way to R^2 in linear regression models) (Stata command `fitstat`). Whilst pseudo R^2 measures should not be used to compare models using different estimating equations, they may be useful in comparing models using the same data and the same equations (in this case the

logistic regression model). Of the number of measures of pseudo R^2, Cragg-Uhler's has been recommended for assessment of prognostic models (Steyerberg 2009a).

Markers in addition to clinical opinion

I examined whether any single marker positively associated with a diagnosis of stroke, after adjustment for other markers in previous models, added useful diagnostic power to the clinical opinion of a member of the emergency department staff or the stroke fellow. As some other confounders are easily measured, for example age and AF, I forced these into models. I considered the markers that were positively, rather than negatively, associated with a diagnosis of acute cerebrovascular disease as I felt that 'acute cerebrovascular disease' was a more stable and less heterogeneous construct than 'stroke mimic'. First I assessed whether the addition of a blood marker significantly improved the log likelihood of a model containing only clinical opinion and age (and in addition, for models with NT pro-BNP, I added AF) with a likelihood ratio test. Second, I added all markers to the model. Thirdly, I examined for interactions with time last seen well to admission and abnormal brain imaging.

I used Stata 10 for all statistical analysis. I measured 19 markers. Therefore the probability of finding at least one significant result at the $p<0.05$ level by chance in any table of all markers is Pr=0.6. Because of this, I considered a $P<0.01$ to be statistically significant. Even with this more stringent criterion, the probability of at least one 'statistically significant' result by chance in each table is Pr=0.17; at $P=0.001$, this probability is Pr=0.02. My interpretation of results has, therefore, been extremely cautious. All *P* values are two sided.

Ethical considerations

The Multi-centre Ethics Committee for Scotland A gave ethical oversight to the study. This committee has responsibility for studies of adults with incapacity in Scotland. Approval was also received from the Local research Ethics Committee. A grant from ReMIND supported the measurement of serum tau and S100 B.

Results

The median delay to blood draw from symptom onset was 7 hours (interquartile range 3 to 19 hours, 5th to 95th centiles 1 to 28 hours). All markers had a positively skewed distribution. I have presented their median and interquartile ranges in Table 4.1 and Table 4.2, though I have not compared statistically these measures of central tendency and spread to avoid repeating similar analyses in the following paragraphs.

Linearity of univariate associations of plasma or serum blood marker levels and a diagnosis of acute cerebrovascular disease

In this dataset non-linear, restricted cubic spline models fitted data better than linear models for fibrinogen, NT pro-BNP, and S100 B (Table 4.4). After truncation (removing the top and bottom 5% of observations), there was no evidence of any change in the likelihood ratio statistics between linear and non-linear models, indicating that extreme outliers did not explain non-linearity (Table 4.4).

I plotted predicted against observed probability for NT pro-BNP and S100 B (Figure 9, Figure 10, Figure 11, Figure 12) and found that a natural logarithm transformation of the plasma or serum marker concentration appeared to fit the data as well as a 3-knot restricted cubic spline at default values. As the logarithmic transform has fewer degrees of freedom than a restricted cubic spline model, and appears to explain the data well, I used it in subsequent analyses. Linear models of the association between marker levels and probability of acute cerebrovascular disease underestimated the probability of acute cerebrovascular disease at lower marker levels. The relationship between levels of fibrinogen and probability of acute cerebrovascular disease was not clear (Figure 13).

Univariate associations of untransformed blood markers with acute cerebrovascular disease diagnosis

Only tissue plasminogen activator was associated positively with a diagnosis of acute cerebrovascular disease (OR 1.6, 75th to 25th centile) (Table 4.3). The positive and negative associations between acute cerebrovascular disease and

untransformed markers of inflammation, thrombosis, cardiac strain, cerebral damage, renal dysfunction or glucose could be explained by chance (P>0.01), though there are plausible physiological explanations for the positive association of acute cerebrovascular disease with D-dimer and NT pro-BNP.

There was no difference in the direction, magnitude or significance of these results when considering a diagnosis of all stroke, or ischaemic stroke, versus all other diagnoses (including TIA).

Adjustment of the association between blood markers and potential confounders

Table 4.5 summarises the relationship between blood markers, age, NIHSS, delay to blood draw and blood pressure. All of the markers, except ICAM-1, TNF-α, MMP-9, tau and creatinine were positively correlated with severity of neurological impairment measured by the NIHSS. All markers, except ICAM, interleukin-10, MMP-9, white cell count, tau and glucose, were positively correlated with age. No marker was correlated strongly and significantly with the delay to blood draw. Most markers were correlated negatively with blood pressure, though this was only significant for CRP, IL-6, MMP-9, D-dimer and troponin T. After adjustment, the association between adiponectin and mimic strengthened. No marker of any physiological process had a positive, strong and statistically significant association with a diagnosis of acute cerebrovascular disease after adjustment for neurological impairment and age.

Validation of a multi-marker model for the diagnosis of stroke

In 355 patients where there was sufficient blood marker data to validate a previously published model to predict stroke diagnosis (Laskowitz et al. 2009c), the median predicted probability of stroke from the model in patients with stroke was Pr=0.83 and in patients without stroke Pr=0.75 (Figure 14). The area under a receiver operator curve (AUROC) for discriminating acute cerebrovascular disease from mimics was 0.63 (0.57 to 0.69) and stroke from not stroke 0.68 (0.63 to 0.74) (Figure

15). This was similar to its performance in the Laskowitz cohort where AUROC=0.69.

I was unable to examine the lower published threshold for the model (P<0.39), as the lowest predicted probability from the model in my series was Pr=0.42; this threshold therefore had 100% sensitivity, though 0% specificity. The upper threshold (Pr>0.64) had a sensitivity of 92% (95% CI: 89 to 95%) and specificity of 18% (95% CI: 13 to 24%) in my cohort. In the published development cohort, the higher threshold had a sensitivity of 27% and specificity of 89%.

The model was therefore unable reliably to classify patients into those with and without stroke, and therefore had poor external validity in my cohort.

Development of a model for the diagnosis of acute cerebrovascular disease using only markers: can a valid model be developed that is better than an emergency department nurse or doctor?

Reducing candidate predictors

(i) Those with missing values (Table 4.8**)**

Values are missing in this dataset because of errors in sample transport, and different volumes in each sample available for analysis. These are likely to be random errors, and so probably not associated with a significant selection bias. For example, though more values were missing for NT pro-BNP for patients with acute cerebrovascular disease (7%) than without (3%), there was no significant association between missingness of NT pro-BNP and acute cerebrovascular disease (OR=1.75, 95% CI: 0.16 to 1.46). Therefore I did not reject any marker on the grounds of the amount of missing data. I performed subsequent analyses on all available data.

(ii) Collinearity

I constructed a Spearman correlation matrix between all 19 variables. No correlation coefficient was >0.8. Correlations over 0.5 were: between CRP and fibrinogen, 0.65; between IL-6 and D-dimer, 0.61; between IL-6 and vWF 0.55; between IL-6 and CRP

0.61 and; between total white cell count and MMP-9, 0.60. Therefore there was no justification to reject a marker on the basis of collinearity.

(iii) Selection by previous described associations

Of the 19 markers measured in the BBISS cohort, the following have been previously found to be positively associated with a diagnosis of ischaemic or haemorrhagic stroke or TIA: TNF-α, IL-6, S100 B, NT pro-BNP, vWF, CRP, MMP-9, vWF and D-dimer (Whiteley, Tseng, & Sandercock 2008c). In a simple logistic regression model with plasma or serum concentration of each marker as a linear term, save S100 B and NT pro-BNP which were log transformed, only higher levels of NT pro-BNP were positively and significantly associated with a diagnosis of acute cerebrovascular disease and higher levels of CRP with a diagnosis of mimic after adjusting for the levels of all other markers. I could not be certain whether TNF-α, MMP-9, IL-6, D-dimer, Ln S100 B and vWF were associated more with a diagnosis of acute cerebrovascular disease or mimic in this model.

(iv) Selection by strength of univariate association

I considered markers with associations with a $P<0.1$ in univariate analysis: IL-10, vWF, D-dimer, tPA and NT pro-BNP (as its log transform) and S100 B (as its log transform). After excluding non-significant variables ($P>0.1$) in the resulting model (D-dimer and vWF), I arrived at the model in Table 4.7. After adjustment for other markers, associations between each marker and acute cerebrovascular disease remained the same or differed very slightly from those found in unadjusted analysis.

(v) Stepwise selection

I constructed models using both forward and backwards selection to examine the stability of variable selection (Table 4.7). Both forward and backwards selection procedures gave the same model. Higher levels of the adiponectin, TNF-α, CRP, IL-10 and the proportion of neutrophils in the total white cell count predicted mimic

rather than ACvD. Higher levels of tPA, Ln NT pro-BNP and Ln S100 B predicted acute cerebrovascular disease. After adjustment each association with ACvD strengthened, in comparison to unadjusted univariate associations.

(vi) Boosted logistic regression

Whilst a boosted model explained variance very well in the training dataset (n=166, R^2=0.97), it had very little explanatory power in the test dataset (n=166, R^2=0.07). The following variables explained over 50% of the log likelihood; tPA (19.6%), Ln NT pro-BNP (15.6%), fibrinogen (11.2%), IL-10 (8.3%) in the development model. Because of its poor external validity even in this dataset, I took analysis of this modeling strategy no further.

(vii) Comparing model performance

Each model was well calibrated in this dataset (Hosmer Lemeshow χ^2 *P*>0.15). The AUROC and Cragg-Uhler R^2 for variables derived from my systematic review and from univariate selection from the data were similar. Unsurprisingly, a model derived from stepwise selection (which was very likely overfitted) had a higher AUROC (0.77) and higher Cragg-Uhler R^2.

Note: a model created with only those variables from the Biosite model (Ln NT pro-BNP, Ln MMP-9, Ln D-dimer and LnS100 B), where the coefficients were allowed to vary had an AUROC of 0.67 (95% CI:0.61 to 0.73), Hosmer Lemeshow χ^2 357 *P*=0.38 and R^2 of 0.10.

(vii) Residuals and patients with undue leverage

Model developed with variables from my systematic review: After excluding 71 patients with a Pearson residual >2, or a leverage > 0.04, Ln NT pro-BNP was the only significant variable (*P*<0.001) left in the model, though the point estimate of the OR for each marker strengthened.

Model developed with variables from strength of univariate association: After excluding 30 patients with a Pearson residual >2, or a leverage > 0.04, each variable remained

statistically significant, and the point estimate of the OR for each marker strengthened.

Model developed by stepwise selection: After excluding 81 patients with a Pearson residual >2, or a leverage > 0.04, only CRP, Ln NT pro-BNP and tPA remained significantly associated with a diagnosis of acute cerebrovascular disease.

(viii) Interaction

I fitted all two-way interactions in each model. Because of the number of possible interactions in each model, I investigated each significant interaction in the whole dataset. Of 12 two-way interactions with $P<0.05$ in individual models, 3 remained significant when tested in the whole dataset: creatinine and tPA ($P=0.01$), TNF-α and IL-10 ($P=0.0001$) and vWF and D-dimer ($P=0.0058$). These two-way interactions were not pre-specified, subject to non-independence error, and should therefore be interpreted with caution. I have not added these interactions to the models.

(ix) Missing data

Replacement of missing data for Ln NT pro-BNP with values for the highest quartile of Ln NT pro-BNP (7.22) when the diagnosis was acute cerebrovascular disease and the lowest quartile (4.72) when the diagnosis was mimic made only a very small difference to the performance of the models or the magnitude of associations.

Does addition of any single blood marker improve upon clinical opinion?

NT pro-BNP, tPA and Ln S100 B were associated positively and significantly with a diagnosis of acute cerebrovascular disease in more than one model. Ln NT pro-BNP did not improve the fit of a model containing a member of the emergency department staff's clinical opinion (definite or probable ACvD), age and AF (n=366, LR χ^2=2.2. P=0.14), nor did Ln S100 B (n=372, LR χ^2=3.4. P=0.06), nor did tPA (n=375, LR χ^2=2.5. P=0.11). Addition of all of these markers to a model containing the clinical opinion of an emergency department clinician, age and AF did not significantly improve the basic model (n=340, LR χ^2=7.1. P=0.07).

I examined whether there was evidence of interaction in these models with either 'time from when the patient was last seen well', or whether brain imaging was normal. Multiplicative interaction terms with blood levels of blood marker and delay to admission or relevant imaging findings did not improve the log likelihood of models containing Ln NT pro-BNP, Ln S100 B or tPA (all $P>0.15$).

Discussion

Summary of main findings

In unadjusted analyses, only tPA and NT pro-BNP (as its natural logarithm transform) were associated positively and significantly with a diagnosis of acute cerebrovascular disease. After adjustment for severity of neurological impairment and age, neither marker was associated with a diagnosis of acute cerebrovascular disease. Two markers, IL-10 (an anti-inflammatory cytokine) and adiponectin (a hormone with anti-inflammatory and insulin sensitising effects) were associated significantly with a diagnosis of a mimic of acute cerebrovascular disease, an association that strengthened after taking account of the severity of neurological impairment and age.

A logistic regression model for the diagnosis of acute cerebrovascular disease with 9 markers, developed using a data-dependent stepwise technique, had a better sensitivity and specificity than an emergency department clinician's diagnosis of probable or definite stroke. However, simpler models (4 variables) and models with markers pre-specified by systematic review (8 markers) did not. Neither BNP, nor tPA nor S100 B (each associated positively with a diagnosis of acute cerebrovascular disease, after adjustment for other markers), improved the model fit of logistic regression models which included age, AF and an emergency department clinician's diagnosis of probable or definite acute cerebrovascular disease. It is unlikely any of these blood markers would help to improve the accuracy of an emergency department clinician's diagnosis of acute cerebrovascular disease, either measured individually or in combination.

Study limitations

Missing data: I did not have a complete data set of all 19 markers for every patient. This reduced the power of multivariate analyses, and meant some samples taken >24 hours after symptom onset were used in the analysis. The cause of missing data was either a problem with transfer of samples to other laboratories for analysis or insufficient sample volume. Although of some concern, the analysis do not suggest that missing data have led to a material bias in any of the main analysis.

Measurement variability: The coefficient of variation for the measurement of most markers was low, particularly for the measurements made in a clinical laboratory. Although the coefficient of variation was small, it will have led to random errors in marker measurement and a tendency of the OR to approach 1, though, only to a small degree.

Markers do not track one patho-physiological process: The concept 'biomarker' implies that the levels of a particular blood marker are strongly correlated with a particular patho-physiological process. However, most hormones, cytokines, damage markers and other proteins have associations with many biological processes; for example the 'inflammatory' biomarker, IL-6, not only has effects on white cells and other inflammatory cytokines, but also haematopoiesis, liver and neuronal regeneration. Which of the many functions of an individual molecule is important in a particular clinical situation is often unclear. One solution is to investigate the relationship between markers with multivariate linear regression or structural equation modelling. However, as the biological meaning of the relationships between different markers in physiology is often unclear, these complex models may well to lead to confusion rather than real insight.

Misdiagnosis: A panel of experts, blinded to the results of the marker assessments, made the diagnoses after careful consideration of the patient's clinical course, presentation and imaging findings. Despite this, it is possible that they have misclassified some patients; for example patients in whom the stroke mimic

diagnosis was of 'functional disorder' (usually thought to be a condition without any major alteration of underlying physiology) in fact had higher levels of tau and D-dimer than patients in whom the non-stroke diagnosis was migraine.

Tau: Serum tau is a marker of axonal damage but it did not rise as expected in patients with stroke, nor did it show an association with severity of neurological impairment, nor an association with the delay to blood draw since the onset of symptoms. This is contrary to a previous, smaller report (Bitsch et al. 2002). Although tau is found in neuronal axons, and is believed to be a CNS specific protein, its levels were not higher in patients with dementia or minor head injury (Ingelson et al. 1999, Kavalci et al. 2007).

Study strengths

The study strengths deserve consideration. I recruited a large series of patients and made their diagnoses in a uniform way based on all available clinical and radiological data. I carefully considered diagnostic categories that would be meaningful to clinicians seeing patients very soon after the diagnosis of suspected stroke, and specified the main analysis comparisons prior to analysis. The pre-analysis handling of samples was consisted and performed to a high standard. All markers were measured in high throughput research laboratories with extensive experience and CPA (UK) Ltd accreditation, or in clinical laboratories with ongoing auditing and inspection procedures. Bias was minimised by blinding of the marker measurement to clinical diagnosis, and near consecutive recruitment of patients.

Interpretation

The markers of inflammation, thrombosis, thrombolysis, cardiac strain, neuronal and glial damage measured in this cohort are very unlikely to be useful additions to the clinical diagnosis of stroke, either when measured individually or as a panel. As most marker levels were strongly correlated with clinical features such as age or neurological impairment, which were also associated strongly with a diagnosis of

acute cerebrovascular diseases, they are unlikely to be helpful in addition to a clinical diagnosis.

A major challenge in the diagnosis of stroke is both the variety of conditions that mimic stroke, and the heterogeneity of stroke itself. It is very difficult to imagine a pathophysiological process that is unique to stroke or one of its subtypes and not found in a stroke mimic. This is quite unlike the situation for myocardial infarction, where there are very few conditions, other than cardiac ischaemia, that cause severe acute chest pain and lead to a rise in markers of myocardial necrosis.

Generalisability

The results of this study are applicable to situations where the differential diagnosis of suspected stroke is similar to this study, and the severity of neurological impairment encountered is broad. Where patients are more severely affected, or where the range of mimics of acute cerebrovascular disease is narrower, the diagnostic performance of blood markers may be better; however the performance of clinical assessment is also likely to improve, perhaps by a similar amount.

Implications for research

- There are positive associations between markers of thrombolysis and of cardiac strain with a diagnosis of acute cerebrovascular disease.
- Marker levels add very little to clinical features for the diagnosis of acute cerebrovascular disease.

Implications for practice

- I cannot recommend any marker of inflammation, thrombosis, thrombolysis, cardiac strain, neuronal or glial damage measured in this study for the diagnosis of acute cerebrovascular disease.
- I cannot recommend a combination of markers (including one previously marketed as the 'Stroke Triage Panel') for the diagnosis of acute cerebrovascular disease.

Table 4.1 Baseline blood marker levels (median, IQR) by diagnostic category

			ACvD (all)				***Most frequent mimics***			
Blood marker	**All**	**ACvD (all)**	***IS***	***ICH***	***TIA***	**Mimic (all)**	***Seizure***	***Sepsis***	***Functional***	***Migraine***
	N=405	**N=285**	***N=230***	***N=15***	***N=40***	**N=120**	***N=14***	***N=13***	***N=12***	***N=17***
Markers of inflammation										
Adiponectin (µg/ml)	11.65 (11.4)	11.45 (11.2)	11.38 (11.5)	13.95 (11.17)	11.74 (9.73)	11.71 (11.80)	19.27 (15.8)	11.65 (7.30)	8.54 (9.67)	5.61 (6.34)
CRP (mg/l)	4.02 (7.4)	4.08 (6.68)	4.69 (7.24)	2.22 (11.18)	2.40 (3.39)	3.95 (13.98)	6.17 (12.54)	12.22(27.93)	3.89 (3.99)	2.15 (4.04)
ICAM (ng/ml)	163 (73)	165 (83)	165 (88)	184 (66)	157 (68)	159 (77)	179 (55)	142 (76)	165 (111)	119.5 (68.5)
Interleukin-6 (pg/ml)	4.32 (6.71)	4.71 (6.75)	4.83 (6.85)	9.75 (4.42)	2.88 (3.65)	3.74 (7.19)	4.94 (5.10)	8.26 (5.01)	2.73 (1.76)	1.39 (1.2)
TNF-α (pg/ml)	1.36 (0.53)	1.39 (0.53)	1.39 (0.55)	1.31 (0.42)	1.40 (0.53)	1.32 (0.57)	1.36 (0.81)	1.59 (0.83)	1.28 (0.59)	1.23 (0.33)
Interleukin-10 (pg/ml)	4.5 (4.4)	4.4 (3.4)	4.4 (3.3)	5.2 (2.2)	4.1 (4.5)	4.6 (6.1)	6.35 (5.3)	10.85 (22.1)	3.65 (4.8)	4.3 (3.55)
MMP-9 (ng/ml)	916 (811)	900 (807)	854 (781)	1135 (1145)	909 (838)	985 (797)	1114 (815)	1666 (1435)	758 (705)	731.5 (709)
vWF (IU/dl)	156 (101)	161 (94)	162 (91)	166 (139)	133 (115)	148 (110)	173.5 (88)	229 (140)	102.5 (83)	93 (28)
White cell count (x10^9 cell/l)	8.4 (4)	8.7 (3.9)	8.7 (3.9)	9.8 (4.1)	7.4 (4.1)	8.1 (4.1)	6.6 (4.8)	9.6 (7.4)	7.9 (1.25)	7.4(2.9)
Neutrophil / WCC (mean, SD)	0.72 (0.12)	0.71 (0.17)	0.71 (0.11)	0.86 (0.17)	0.66 (0.21)	0.72 (0.19)	0.71 (0.20)	0.80 (0.11)	0.63 (0.11)	0.64 (0.11)
Markers of thrombosis										
D-dimer (ng/ml)	198 (324)	229 (355)	251 (381)	161 (416)	148 (168)	144 (249)	270 (256)	321 (414)	134 (257)	61 (85.5)
Fibrinogen (g/l)	4.78 (1.7)	4.82 (1.62)	4.89 (1.68)	4.95 (1.47)	4.45 (1.2)	4.42 (1.77)	3.86 (1.67)	5.18 (3.06)	4.02 (0.78)	4.28 (0.98)
Markers of thrombolysis										
tPA (ng/ml)	10.48 (7.0)	11.26 (6.4)	11.29 (3.5)	12.38 (10.1)	10.0 (3.5)	8.7 (6.0)	10.1 (4.2)	10.5 (10.5)	10.0 (9.5)	8.0 (4.5)

Table 4.1 continued			*ACvD (all)*				*Most frequent mimics*			
Blood marker	All	ACvD (all)	*IS*	*ICH*	*TIA*	Mimic (all)	*Seizure*	*Sepsis*	*Functional*	*Migraine*
	N=405	N=285	*N=230*	*N=15*	*N=40*	N=120	*N=14*	*N=13*	*N=12*	*N=17*
Markers of cardiac strain										
NT pro-BNP (pg/ml)	381 (1252)	515.5 (1635)	701 (1824)	499 (990)	128 (310)	194 (598)	799 (1059)	621.5 (1045)	86 (84)	49 (72)
Troponin-T>0.01 (n,%)*	52 (14)	39 (15.4)	33 (16.2)	3 (33)	2 (5.3)	13 (11.3)	3 (21.4)	5 (41.7)	0	0
Markers of cerebral damage										
Tau (pg/ml)	21 (39)	21 (35)	21 (13)	13 (17)	24 (78)	22 (47)	22.5 (44)	15 (83)	50 (314)	18.5 (18)
S100 B (pg/ml)	60 (59)	63 (72)	59 (72)	109 (119)	63 (56)	55 (43)	55 (73)	77 (35)	50 (69.5)	64 (51)
Other physiological markers										
Creatinine (µmol/)	83 (36)	84 (36)	86 (36)	66.5 (24)	82 (37)	78 (33)	79 (35)	94 (8)	75 (14)	70 (13)
Glucose (mmol/l)	5.8 (1.7)	5.8 (1.7)	5.8 (1.6)	6 (5.1)	5.4 (1.6)	5.6 (1.6)	6.1 (1.9)	7.6 (2.8)	5.2 (0.9)	5.1 (1.0)

* as a proportion of samples with available troponin results. ACvD: acute cerebrovascular disease (probable or definite cerebral ischaemia or intracerebral haemorrhage responsible for symptoms at time of clinical assessment) IS: ischaemic stroke; TIA: transient ischaemic attack; ICH: intracerebral haemorrhage. Data for the 2 sub-arachnoid haemorrhage patients is included in ICH; Mimic: definitely not, or only possibly responsible for symptoms at time of clinical assessment; data are shown for a subset of the most frequent mimics of ACvD

Table 4.2 Quarters and medians of the distributions of blood markers of inflammation, thrombosis, cardiac strain and neuronal and glial damage

Marker	Lower quarter	50th centile (Median)	Upper quarter
Inflammation			
Adiponectin (μg/ml)	1.5 to 6.8	11.6	18.2.5 to 51.2
CRP (mg/l)	0.163 to 1.79	4.02	9.2 to 289
ICAM (ng/ml)	72 to 129	163	208 to 445
Interleukin-6 (pg/ml)	0.12 to 2.04	4.32	8.76 to 15.13
TNF-α (pg/ml)	0.56 to 1.18	1.36	1.72 to 14.55
Interleukin-10 (pg/ml)	0.9 to 3.3	4.5	7.7 to 403.5
MMP-9 (ng/ml)	69 to 580	916	1391 to 3941
vWF (IU/dl)	27 to 116	156	217 to 479
White cells ($x10^9$ cell/l)	2 to 6.6	8.4	10.6 to 21.6
Neutrophil / WCC	0.25 to 0.62	0.71	0.80 to 0.97
Thrombosis			
D-dimer (ng/ml)	11 to 97	198	421 to 2800
Fibrinogen (g/l)	2.5 to 4.08	4.78	5.78 to 9.95
Thrombolysis			
tPA (ng/ml)	1.11 to 7.48	10.48	14.49 to 57.4
Cardiac strain			
BNP (pg/ml)	5 to 112	381	1364 to 28690
Troponin T (ng/ml)	0.1 to 0.01	0.01	0.01 to 0.699
Cerebral damage			
Tau (pg/ml)	0 to 12	21	51 to 3000
S100 B (pg/ml)	0 to 38	60	97 to 2744
Other markers			
Creatinine (μmol/l)	33 to 67	83	103 to 472
Glucose (mmol/l)	1.5 to 5.2	5.8	6.9 to 19.9

Table 4.3 Univariate associations between marker levels and a diagnosis of acute cerebrovascular disease.

Marker (units)	OR, 75th / 25th centile (95% CI)	*P*- value
Inflammation		
Adiponectin (µg/ml)	0.89 (0.67 to 1.19)	0.447
CRP (mg/l)	0.97 (0.92 to 1.02)	0.225
ICAM (ng/ml)	1.15 (0.87 to 1.52)	0.326
Interleukin-6 (pg/ml)	1.26 (0.88 to 1.82)	0.204
TNF-α (pg/ml)	0.92 (0.81 to 1.04)	0.172
Interleukin-10 (pg/ml)	0.97 (0.94 to 0.99)	0.018
MMP-9 (ng/ml)	0.86 (0.68 to 1.10)	0.234
vWF (IU/dl)	1.36 (0.96 to 1.93)	0.088
White cells (x10^9 cell/l)	1.03 (0.78 to 1.35)	0.828
Neutrophil / WCC	0.94 (0.68 to 1.29)	0.688
Thrombosis		
D-dimer (ng/ml)	1.20 (1.02 to 1.42)	0.032
Fibrinogen (g/l)	1.13 (0.87 to 1.46)	0.374
Thrombolysis		
tPA (ng/ml)	1.63 (1.20 to 2.21)	0.002
Cardiac strain		
NT pro-BNP (pg/ml)	1.14 (1.00 to 1.30)	0.045
NT pro-BNP (Ln Unit)	2.15 (1.52 to 3.04)	<0.001
Troponin T (ng/ml)	1.01 (0.99 to 1.03)	0.492
Cerebral damage		
Tau (pg/ml)	0.99 (0.97 to 1.01)	0.385
S100 B (pg/ml)	1.00 (0.97 to 1.03)	0.957
S100 B (Ln Unit)	1.24 (1.03 to 1.51)	0.027
Other markers		
Creatinine (µmol/)	1.00 (0.82 to 1.22)	0.973
Glucose (mmol/l)	1.06 (0.90 to 1.25)	0.504

The OR is the ratio of odds of acute cerebrovascular disease in the 75th to the 25th centile of plasma or serum marker levels assuming a linear relationship between marker level. OR>1 indicates increasing odds of a diagnosis of ACvD with increasing marker levels. *P*-values are derived from Wald tests and determine if the reported OR is significantly different from 1.

Table 4.4 Likelihood ratio test for nested linear versus restricted cubic spline models for the prediction of an ACvD diagnosis.

	Likelihood ratio test, H_o: $L_{linear}=L_{nonlinear}$			
	Full distribution		Truncated (5^{th} to 95^{th} centile)	
Markers	χ^2	***P***	χ^2	***P***
Inflammation				
Adiponectin	1.16	0.76	1.79	0.62
CRP	4.28	0.23	4.76	0.19
ICAM	5.33	0.15	6.27	0.10
TNF-α	6.21	0.10	0.70	0.87
Interleukin-6	6.52	0.09	6.48	0.09
Interleukin-10	4.66	0.20	6.07	0.11
MMP-9	0.31	0.96	0.03	1.00
vWF	5.58	0.13	5.13	0.16
White cells	6.76	0.08	7.52	0.06
Thrombosis				
D-dimer	6.3	0.10	6.35	0.10
Fibrinogen	18.1	<0.001	15.78	0.001
Thrombolysis				
tPA	4.99	0.17	5.73	0.12
Cardiac strain				
BNP	12.5	0.002	18.4	<0.001
Troponin T*	-	-	-	-
Cerebral damage				
Tau	0.87	0.26	2.92	0.40
S100 B	14.98	0.002	8.03	0.04
Other markers				
Creatinine	5.78	0.12	All within a clinically feasible range	
Glucose	6.08	0.11	All within a clinically feasible range	

Higher χ^2 indicate non-linear models fit data better (degrees of freedom =3). Truncation investigates the effects of outliers on linear relationships. *Distribution too skewed to allow this analysis

Table 4.5 Association of blood markers with potential confounders.

	Spearman's rank correlation coefficient, *r* (*P*- value)			
Marker	**NIHSS**	**Age (yrs)**	**Time to blood draw (hrs)**	**Systolic BP (mmHg)**
Inflammation				
Adiponectin	0.23 (<0.001)	0.32 (<0.001)	0.10 (0.12)	0.03 (0.56)
CRP	0.26 (<0.001)	0.13 (0.01)	0.06 (0.21)	-0.13 (0.01)
ICAM	0.07 (0.18)	0.03 (0.57)	0.04 (0.41)	-0.03 (0.59)
Interleukin-6	0.40 (<0.001)	0.38 (<0.001)	-0.04 (0.48)	-0.19 (<0.001)
TNF-α	0.08 (0.11)	0.20 (<0.001))	0.05 (0.71)	0.01 (0.92)
Interleukin-10	0.16(<0.001)	0.12 (0.02)	0.01 (0.90)	-0.04 (0.40)
MMP-9	0.06 (0.24)	0.01 (0.78)	0.06 (0.23)	-0.14 (0.006)
vWF	0.25 (<0.001)	0.33 (<0.001)	-0.01 (0.82)	-0.11 (0.03)
White cells	0.22 (<0.001)	0.06 (0.24)	0.11 (0.03)	-0.11 (0.02)
Neutrophil / WCC	0.19 (<0.001)	0.22 (<0.001)	0.12 (0.02)	-0.01 (0.85)
Thrombosis				
D-dimer	0.40 (<0.001)	0.38 (<0.001)	-0.04 (0.38)	-0.16 (<0.001)
Fibrinogen	0.22 (<0.001)	0.22 (<0.001)	0.08 (0.10)	-0.10 (0.05)
Thrombolysis				
tPA	0.26 (<0.001)	0.17 (<0.001)	-0.03 (0.43)	-0.01 (0.71)
Cardiac strain				
NT pro-BNP	0.44 (<0.001)	0.57 (<0.001)	0.01 (0.77)	0.03 (0.52)
Troponin T	0.25 (<0.001)	0.23 (<0.001)	0.01 (0.81)	-0.13 (0.01)
Cerebral damage				
Tau	0.05 (0.28)	-0.04 (0.50)	-0.03 (0.58)	-0.06 (0.23)
S100 B	0.16 (0.002)	0.09 (0.07)	-0.05 (0.37)	-0.07 (0.19)
Other markers				
Creatinine	0.06 (0.20)	0.33 (<0.001)	-0.07 (0.19)	-0.04 (0.40)
Glucose	0.18 (<0.001)	0.06 (0.23)	-0.12 (0.02)	0.09 (0.09)

Spearman correlation coefficients between plasma or serum marker levels and neurological impairment (NIHSS), age, time from last seen well to blood draw and systolic blood pressure. Positive correlation coefficients indicate higher marker levels are associated with higher confounder levels; negative correlation coefficients that higher levels are associated with lower levels of the confounder.

Table 4.6 Adjusted associations between marker levels and acute cerebrovascular disease

Marker (units)	OR, Adjusted for NIHSS & age (95% CI)	*P*- value	OR, with further adjustment (95% CI)	*P*- value
Inflammation				
Adiponectin (µg/ml)	0.57 (0.41 to 0.81)	0.001	0.54 (0.38 to 0.75)	<0.001
CRP (mg/l)	0.92 (0.86 to 0.98)	0.008	0.93 (0.87 to 0.96)	0.04
ICAM (ng/ml)	1.11 (0.83 to 1.48)	0.48	1.08 (0.81 to 1.44)	0.60
Interleukin-6 (pg/ml)	0.65 (0.42 to 0.99)	0.05	0.73 (0.47 to 1.14)	0.17
TNF-α (pg/ml)	0.77 (0.61 to 0.98)	0.04	0.80 (0.63 to 1.03)	0.08
Interleukin-10 (pg/ml)	0.94 (0.90 to 0.98)	0.004	0.94 (0.90 to 0.99)	0.01
MMP-9 (ng/ml)	0.80 (0.62 to 1.04)	0.09	0.84 (0.65 to 1.09)	0.18
vWF (IU/dl)	0.88 (0.61 to 1.26)	0.48	0.97 (0.67 to 1.40)	0.86
White cells (x10^9 cell/l)	0.83 (0.61 to 1.12)	0.23	0.89 (0.65 to 1.22)	0.48
Neutrophil / WCC	0.69 (0.48 to 0.98)	0.04	0.74 (0.52 to 1.08)	0.11
Thrombosis				
D-dimer (ng/ml)	1.02 (0.85 to 1.19)	0.98		
Fibrinogen (g/l)	0.91 (0.69 to 1.19)	0.49		
Thrombolysis				
tPA (ng/ml)	1.29 (0.94 to 1.76)	0.11		
Cardiac strain				
Ln BNP (log_e unit)	1.26 (0.81 to 1.95)	0.31	1.23 (0.76 to 1.97)	0.40
Troponin T (ng/ml)	n/a	n/a		
Cerebral damage				
Tau (pg/ml)	0.99 (0.96 to 1.01)	0.31		
Ln S100 B (log_e unit)	1.17 (0.95 to 1.43)	0.14		
Other markers				
Creatinine (µmol/)	0.90 (0.73 to 1.11)	0.31		
Glucose (mmol/l)	1.00 (0.84 to 1.18)	1.00		

Adjustment made for NIHSS and age. Additional adjustment made for prior infection for markers of inflammation, and cardiac failure, AF and prior cardiac vascular disease for cardiac strain markers. The OR is the ratio of odds of acute cerebrovascular disease in the 75th to the 25th centile of plasma or serum marker. OR>1 indicates that higher levels are associated with a diagnosis of ACvD rather than mimic. *P*-values are derived from Wald tests and determine if the reported OR is significantly different from 1.

Table 4.7 Multivariate models using simple logistic regression models to predict a diagnosis of acute cerebrovascular disease, and measures of model performance.

	Stepwise selection* (n=344)		**Univariate selection† (n= 355)**		**Systematic review‡ (n=350)**	
↑ marker associated with mimic	Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)	*P*
Adiponectin	0.56 (0.38 to 0.83)	0.004				
Neutrophil/total WCC	0.65 (0.42 to 1.00)	0.048				
Creatinine	0.76 (0.59 to 0.97)	0.025				
TNF-α	0.78 (0.62 to 0.98)	0.035			0.86 (0.72 to 1.02)	0.086
CRP	0.91 (0.86 to 0.98)	0.009			0.90 (0.85 to 1.00)	0.003
Interleukin 10	0.95 (0.91 to 0.99)	0.019	0.94 (0.90 to 0.97)	0.001		
MMP-9					0.84 (0.63 to 1.11)	0.214
IL-6					0.82 (0.63 to 1.11)	0.469
↑ marker associated with ACvD						
Ln NT pro-BNP	4.30 (2.49 to 7.43)	<0.001	1.80 (1.23 to 2.64)	0.003	2.69 (1.69 to 4.28)	<0.001
tissue Plasminogen Activator	2.14 (1.40 to 3.28)	<0.001	1.83 (1.25 to 2.66)	0.002		
Ln S100 B	1.40 (1.09 to 1.79)	0.008	1.29 (1.03 to 1.62)	0.029	1.26 (1.01 to 1.58)	0.045
D-dimer					1.09 (0.89 to 1.34)	0.379
von Willebrand factor					1.29 (0.82 to 2.01)	0.267

	Stepwise selection* (n=344)	Univariate selection† (n= 355)	Systematic review‡ (n=350)
Measures of model performance			
AUROC§ (95% CI)	0.77 (0.72 to 0.82)	0.71 (0.65 to 0.77)	0.72 (0.67 to 0.78)
Hosmer Lemeshow χ^2‖ (*P*)	2.3 (0.97)	8.8 (0.36)	3.7 (0.88)
Cragg-Uhler pseudo R^2¶	0.26	0.16	0.19
Comparison with ED clinician**			
Specificity at sensitivity of 77%	70%	53%	50%
Sensitivity at specificity of 58%	82%	73%	70%

75th and 25th centiles are given in Table 4.2. Ln NT pro-BNP 75th centile=7.22, 25th centile = 4.72 ; Ln S100 B 75th centile =4.59, 25th centile=3.69. *P* values are derived from Wald tests.

*Stepwise selection of variables by forward and backward selection of all blood markers, with a threshold of adding a marker to the model of P=0.1 and removal P=0.05

†Univariate selection of variables adding markers associated with ACvD with a P<0.1 in univariate analysis, and removing those with P>0.05 in multivariate analysis

‡Variables selected by systematic review of previous literature.

§AUROC: area under receiver operator curve. A value of 1 indicate perfect discrimination and 0.5 no better discrimination than chance.

‖Hosmer Lemeshow χ^2 is a measure of model calibration. Lower χ^2 indicate better calibrated model

¶Cragg-Uhler pseudo R^2 is a measure of the is a measure of explained variance in risk due to the model

** performance of models at the sensitivity and specificity of an emergency department clinician for the diagnosis of probable or definite ACvD

Table 4.8 Missing data

	Missing n, (%)		
Blood markers	**All (n=405)**	**ACVD (n=285)**	**Mimic (n=120)**
Adiponectin	14 (3.5)	9 (3.2)	5 (4.2)
CRP	18 (4.4)	10 (3.5)	8 (6.7)
ICAM	15 (3.7)	10 (3.5)	5 (4.2)
Interleukin-6	12 (3.0)	9 (3.2)	3 (2.5)
TNF-α	13 (2.0)	10 (3.5)	3(2.5)
Interleukin-10	12 (3.0)	9 (3.2)	3 (2.5)
MMP-9	12 (3.0)	9 (3.2)	3 (2.5)
vWF	11 (3.0)	8 (2.8)	3 (2.5)
White cell count	2 (0.5)	2 (0.7)	0
Neurophil count	4 (1.0)	4 (1.4)	0
D-dimer	15 (3.7)	10 (3.5)	5 (4.2)
Fibrinogen	16 (4.0)	9 (3.2)	7 (5.8)
tPA	15 (3.7)	10 (3.5)	5 (4.2)
NT pro-BNP	23 (5.7)	19 (6.6)	4 (3.3)
Troponin T	37 (9.1)	32 (11.2)	5 (4.1)
Tau	2 (0.5)	1 (0.4)	1 (0.8)
S100 B	3 (0.7)	1 (0.4)	1 (0.8)
Creatinine	4 (1.0)	4 (1.4)	0
Glucose	15 (2.5)	11 (3.9)	4 (3.3)

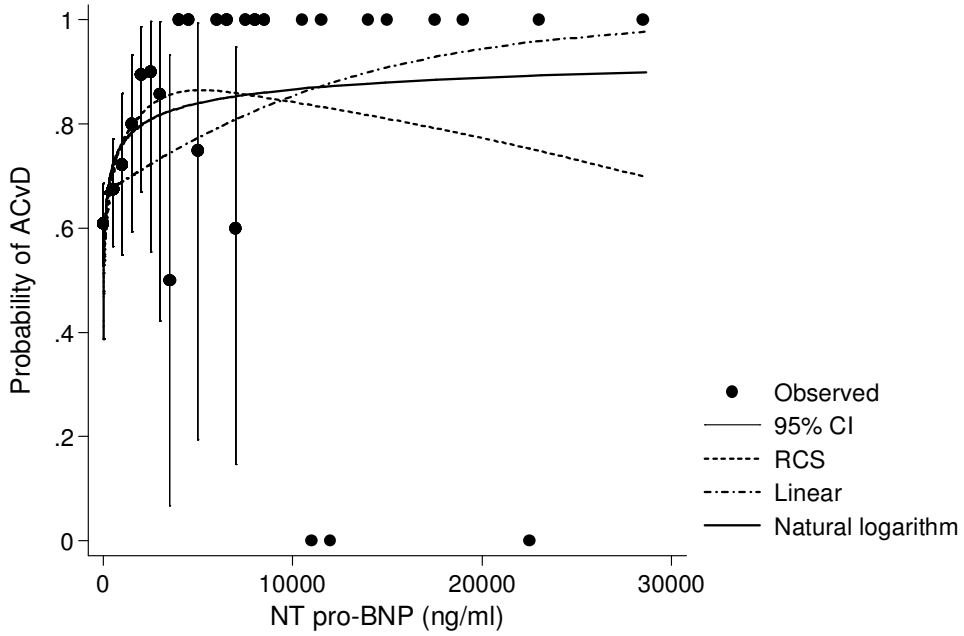


Figure 9 Estimated probability of acute cerebrovascular diseases (ACvD) as a function of serum NT pro-BNP, modelled as a linear relationship, a natural logarithm transform and a 3-knot restricted cubic spline (RCS).

The exact binomial 95% confidence intervals for the observed probability of ACvD are shown.

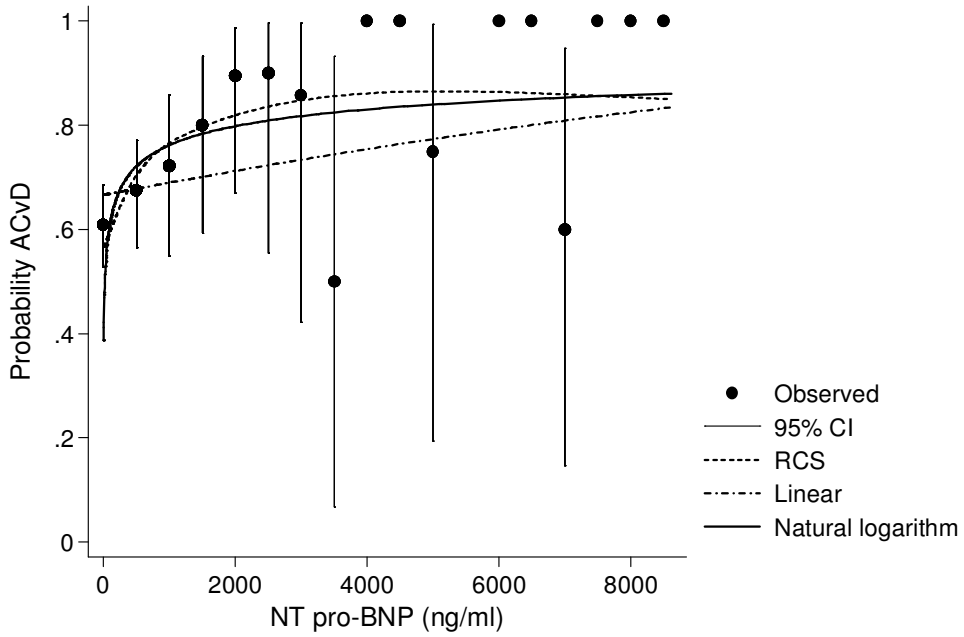


Figure 10 As Figure 9, concentrating on range 0 to 10,000 ng/ml

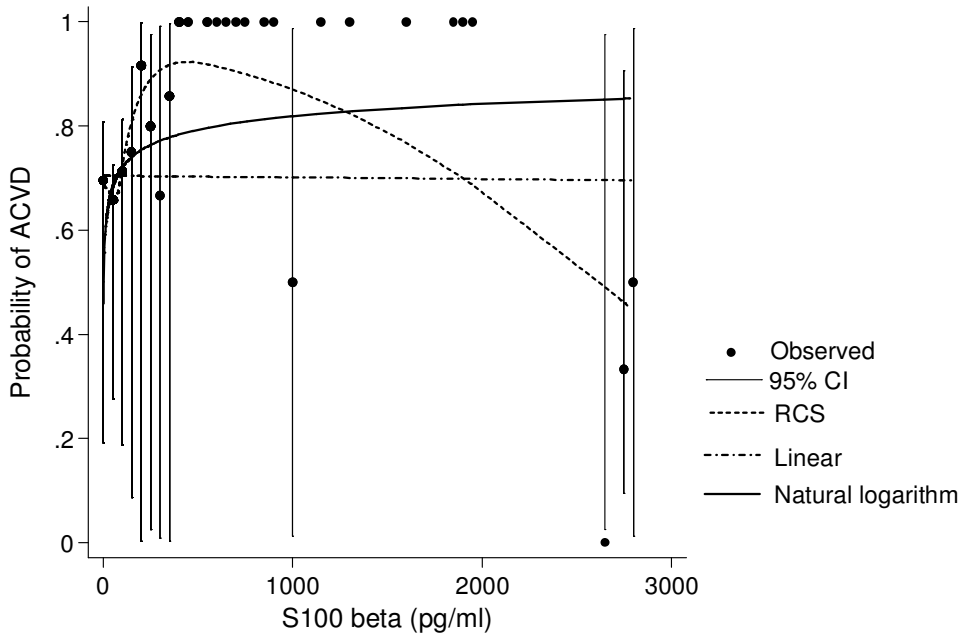


Figure 11 Estimated probabilities of acute cerebrovascular diseases (ACvD) as a function of serum S100 B, modelled as a linear relationship, a natural logarithm transform and a 3-knot restricted cubic spline (RCS).

The 95% exact binomial confidence intervals for the observed probability of ACvD are shown

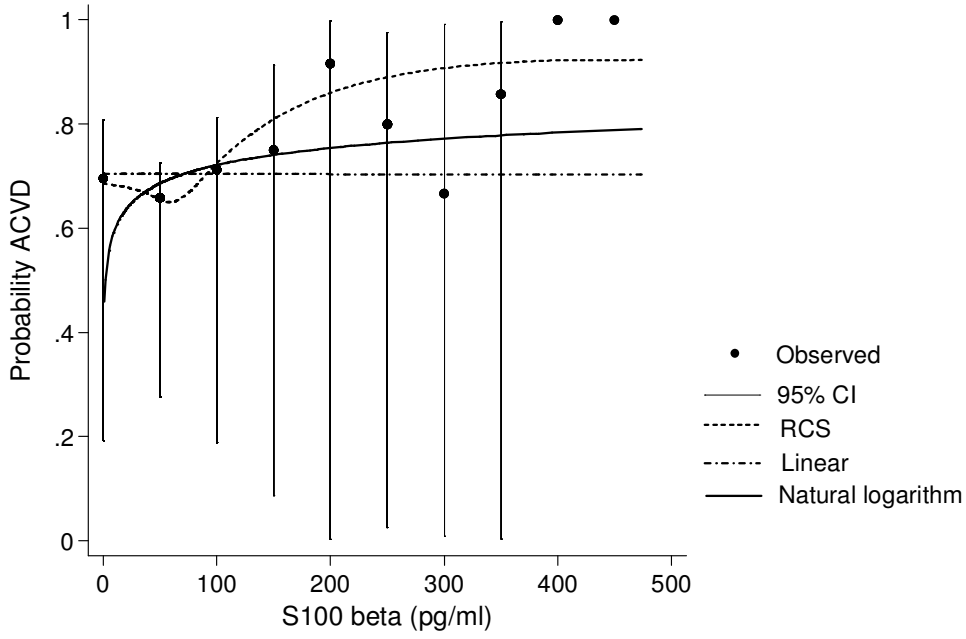


Figure 12 As Figure 11, concentrating on range 0 to 500ng/ml

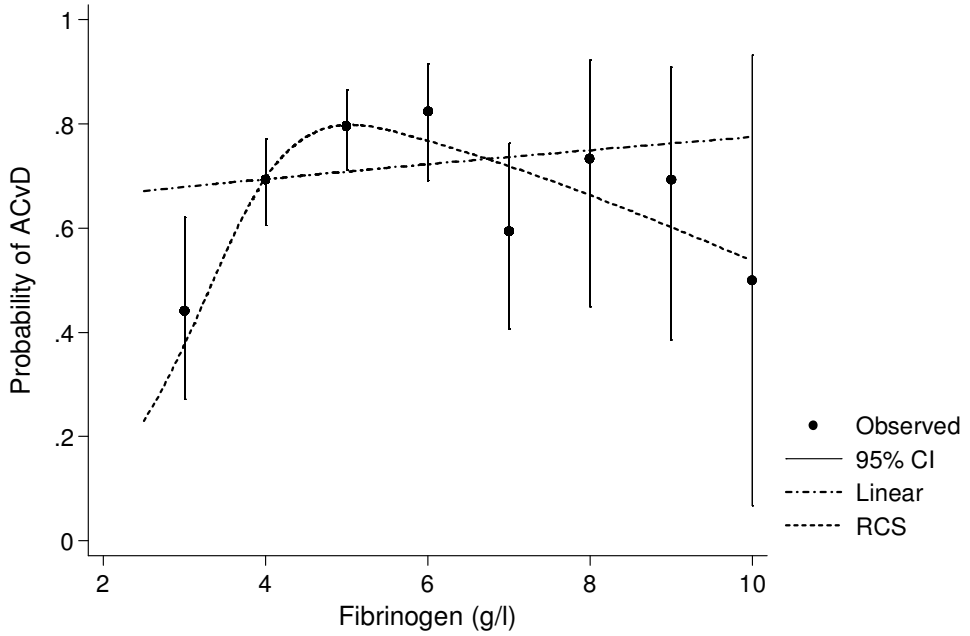


Figure 13 Estimated probabilities of acute cerebrovascular diseases (ACvD) as a function of plasma fibrinogen, modelled as a linear relationship and a 3-knot restricted cubic spline.

The 95% exact binomial confidence intervals for the observed probability of ACvD are shown

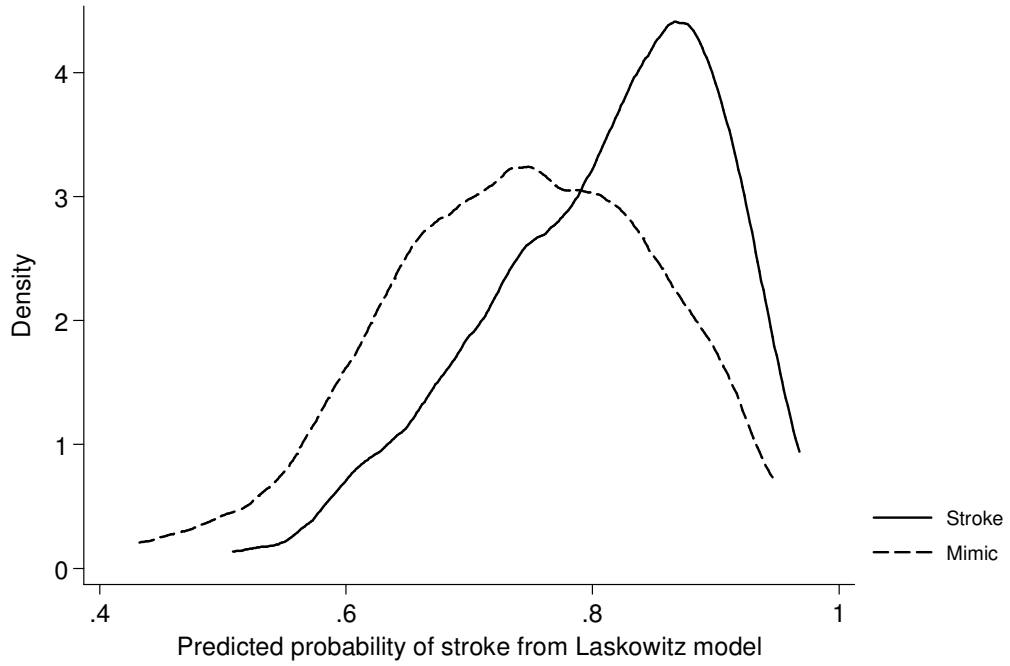


Figure 14 Predicted probability of stroke from published blood marker model (Laskowitz et al. 2009b) in patients with and without a final diagnosis of stroke.

Plots made using kernel density function (Epanechnikov).

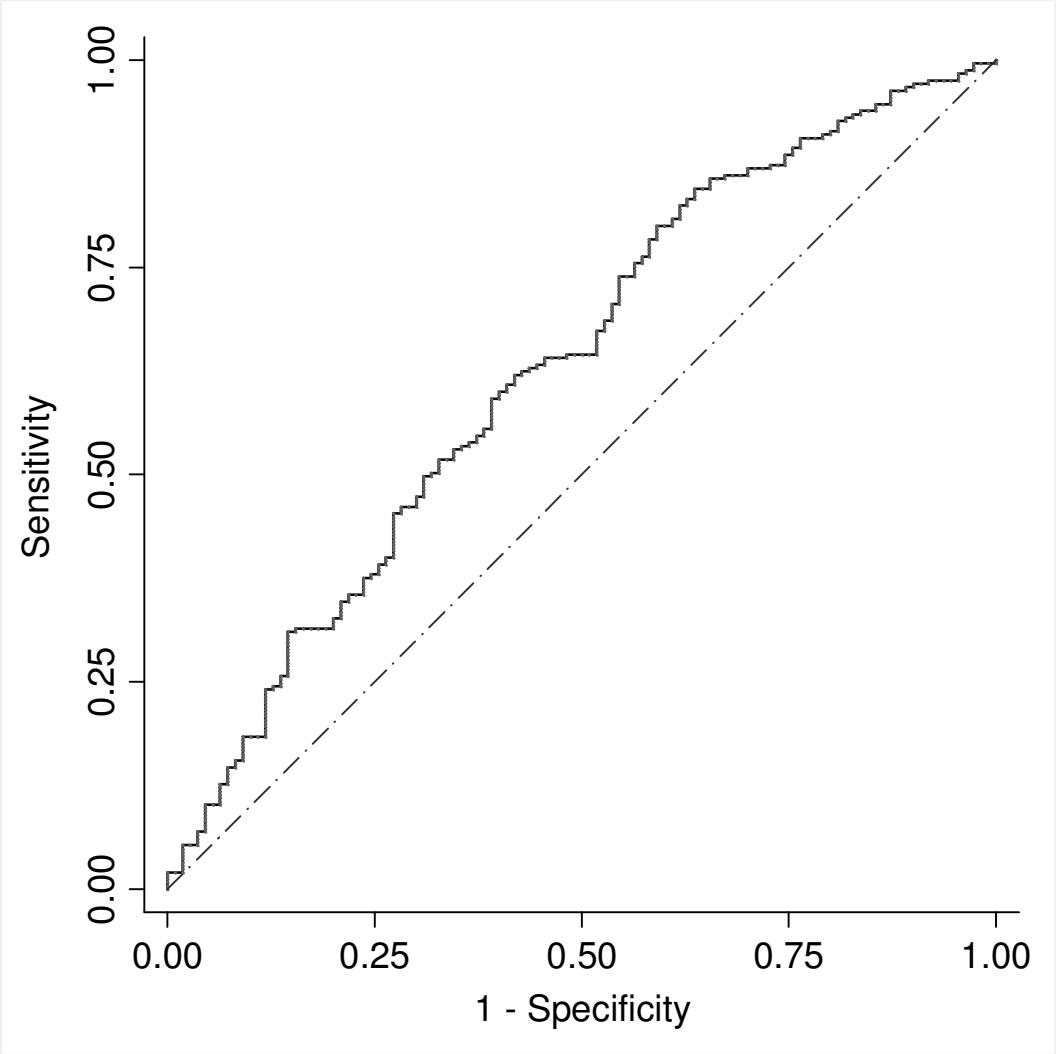


Figure 15 Receiver operator curve for a blood marker model (Laskowitz et al. 2009a) to predict the diagnosis of acute cerebrovascular disease

Area under the curve=0.63, 95% CI: 0.57 to 0.69

Chapter 5. Blood markers for the prognosis of ischaemic stroke: a systematic review

Introduction

The prediction of outcome after ischaemic stroke is important for clinicians, patients and researchers. The performance of models based on clinical variables might be improved by blood markers of any of the pathological processes in acute ischaemic stroke, such as inflammation, haemostasis, neuronal or glial injury and cardiac dysfunction. Markers of inflammation and haemostasis have been associated with ischaemic 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 ischaemic stroke and outcome after acute ischaemic stroke, I undertook a systematic review of the available evidence.

Methods

Study identification

I searched Medline and EMBASE from 1966 to January 2007 for studies in patients with acute ischaemic stroke which examined venous blood markers and assessed clinical outcome. The search strategy included 13 terms for ischaemic stroke, 4 for generic biomarkers and 780 specific biomarker terms. Prognostic studies were identified using high sensitivity search terms (Altman 2001a), together with common outcome measurements from stroke research (Rankin, NIHSS, Glasgow outcome scale). The electronic search strategy is available as an appendix.

Study inclusion:

Studies were eligible for inclusion if they: (a) reported results for patients with acute ischaemic stroke (not transient ischaemic attack), (b) assayed a venous blood marker not routinely measured in ischaemic stroke patients, (c) drew blood within the first week after stroke onset, (d) 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. I considered only papers published in full since our resources were limited and abstracts did not contain sufficient detail to permit either methodological quality assessment or meta-analysis. I did not include studies that examined only the risk of subsequent stroke or MI in patients with stroke or risk of stroke in asymptomatic study subjects.

Data extraction:

I selected potentially eligible studies and these were reviewed by two colleagues, Dr. Anshuman Sengupta and Dr. Wei Li Chong. I extracted data from all relevant studies; Dr. Anshuman Sengupta and Dr. Wei Li Chong each re-extracted data from half of these and we resolved any disagreements by discussion. Where I identified duplicate publication, I included the most informative cohort. I assessed study quality using the assay methods and study design sections of the REMARK reporting recommendations for prognostic tumour markers (Kyzas, Denaxa-Kyza, & Ioannidis 2007, McShane et al. 2005c) (see appendix). To reduce bias in the assessment of studies with multiple blood draws and multiple outcomes, I prespecified which measures of association we would collect where more than one was given. Where more than one biomarker was reported in a single study, we recorded data for each biomarker. Where more than one outcome had been reported from a single study, I recorded the handicap measure (usually the modified Rankin scale). If the handicap measure had been reported at more than one time point, I extracted the measure of effect taken closest to 3 months. Where a single biomarker had been measured at multiple time points, I 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 I aimed to include studies irrespective of the method used to measure the association between biomarker and outcome. I noted the measure of association with outcome for each biomarker which included: odds ratios (OR), hazard ratios (HR), relative risk ratios (RRR), differences in mean marker levels between poor and good outcome and correlation coefficients

between outcome and marker levels. Where unadjusted and adjusted measures of effect were reported, I took the most adjusted measure. After discussion with other experts (Prof. Gordon Lowe and Dr. Malcolm MacLeod), biomarkers were classified by function and tissue of origin.

Analysis

Excel was used to draw plots of measures of effect (OR/HR/RR) and standardised differences in means (difference in means / pooled standard deviation) and their 95% confidence intervals (C.I.), 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, though superficially appealing, was rejected as an analysis method because of the risk of type 2 error (Hedges LV & Olson 2008).

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 1, web appendix 1). Lists of articles are available on request from the authors. Studies were from: China (2), Denmark (4), Estonia (1), Finland (3), France (1), Germany (10), Greece (5), Israel (2), Italy (9), Malaysia (1), New Zealand (1), Norway (2), Poland (2), South Korea (2), Spain (16), Taiwan (2), Turkey (2), UK (12), US (5).

Methodological assessment

Studies were generally small (median sample size 85, interquartile range 49 to 184). Few studies reported a sample size calculation (7/82, 9%), reported that the marker was measured blind to stroke status (21/82, 26%) or examined an unselected cohort of stroke patients (30/82, 37%) (

Figure **16**). 20 (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(Harrell F.E, Lee K.L., & Mark D.B 1996)).

There was marked asymmetry in a funnel plot (OR or HR against the standard error of log OR/HR), suggesting small study bias (Figure 17). This may represent differences in the methodology of small studies (which may have poorer methodology or more severe stroke patients) or publication bias (i.e. small studies showing little association between markers and outcome are less likely to be published).

Biomarkers as prognostic factors

Many markers show an association with poor outcome, whether by difference in means, regression coefficients or relative measures of effect (Figure 18 and Figure 19). Most associations were weak (of 66 reported OR/HR/RR, 37 are less than 3) and so could be potentially explained by bias. Larger studies tended to have more modest measures of effect, and studies which calculated a threshold (34/64) had larger measures of effect. Thresholds were frequently data derived. No one class of marker had a stronger association with poor outcome than others, though the effect of cardiac markers (troponin or natriuretic pepetides) 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 CRP though 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 where the majority of studies did not report significant findings. For example, 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 (BNP), C-reactive protein (CRP), glial fibrillary acidic protein (GFAP), glutamate, homocysteine, insulin like growth factor, intercellular adhesion molecule (ICAM), matrix metalloproteinase 9 (MMP-9), platelet activator inhibitor (PAI-1), prothrombin fragments, soluble TNF receptors 1, tau, troponin i, troponin t and thrombomodulin.

Discussion

Many of the blood markers in this review were associated with poor outcome after ischaemic 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.

The association of marker levels with poor outcome after ischaemic 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 strong association could be because marker levels in patients soon after ischaemic stroke predict: (a) an increased risk of myocardial infarction or stroke over and above people with stable vascular disease, (b) markedly reduced brain recovery, (c) increased risk of other complications of stroke, or (d) biased studies.

Recurrence of MI or stroke

In patients with minor stroke or TIA, the risk of stroke recurrence is highest in the first few weeks after stroke (Coull, Lovett, & Rothwell 2004, Johnston et al. 2000). 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 MI.

Most blood markers have a modest association with the first development of coronary heart disease in population based prospective studies of blood biomarkers. The odds ratios for the association with heart disease from meta-analysis are, comparing the top third of the distribution to the bottom third: adiponectin OR= 0.84 (95% C.I. 0.7-1.01); D dimer, OR=1.7 (95% C.I. 1.3-2.2); ICAM 1 OR=1.21 (95% C.I. 0.95-1.55); CRP, OR=1.7 (1.8 (95% C.I. 1.6-2.0); fibrinogen, 1.8 (95% C.I. 1.6-2.0); BNP, OR between 1.3-5.7 for survival; and ferritin, OR=1 (95% C.I. 0.8-1.3) cut-off 200 (Danesh et al. 1998a, Danesh et al. 1998b, Danesh et al. 2001, Danesh & Appleby 1999, Doust et al. 2005, Malik et al. 2001, Sattar et al. 2006).

In patients with some form of established vascular disease, the association between marker levels and incident stroke was less striking, with confidence intervals overlapping with those from the prospective cohorts of those asymptomatic at baseline: fibrinogen, OR=1.34 (95% CI, 1.13 to 1.60) comparing groups with levels above and below the median, and CRP, HR=2.16 (95% CI, 1.32 to 3.53) comparing highest to lowest tertiles (Rothwell et al. 2004e, Tanne et al. 2006).

In stroke free patients with unstable angina, inflammatory and haemostatic markers also have a modest association with recurrence of coronary events: CRP, RR=1.45 (1.15-1.83) per SD; SAA, RR 1.14 (0.99-1.44) per SD (Haverkate et al. 1997a, Haverkate et al. 1997b).

The associations between D-dimer, fibrinogen, CRP, ferritin, IL6 and SAA and a poor outcome after stroke were also modest in studies where 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 MI and stroke recurrence than previously recognised, 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. Inflammatory markers after stroke are associated with poorer recovery of brain tissue in experimental stroke (Emsley & Tyrrell 2002); excitatory neurotransmitters can increase apoptosis and neuronal and glial death (Kreisel, Bezner, & Hennerici 2006) and higher levels of anti-inflammatory markers might indicate strengthened intrinsic anti-atherosclerotic mechanisms. Increases in neurotrophic or neuroprotective markers may be associated with improved neuronal recovery (Denti et al. 2004, Sotgiu et al. 2006). 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 (Heikkila, Ebrahim, & Lawlor 2007, Roumen-Klappe et al. 2002).

Cardiac markers (natriuretic peptides and troponins) show a consistent association with poor outcome. As cardioembolic 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 (Grau et al. 2001). However, only brain natriuretic pepetide (and not troponin I) has been associated with cardioembolic rather than other stroke subtypes (Di Angelantonio et al. 2005, Montaner et al. 2008). Cardiac dysfunction simultaneously or shortly before the stroke or pre-existing cardiac disease could also account for the association, though an association between marker levels and ECG changes was seen in only seen in some studies (Fure, Bruun, & Thommessen 2006, Sharma et al. 2006).

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 ischaemic stroke, thrombolytic therapy is the most relevant. Only one randomised controlled trial has reported on this, though did not report on the presence of a treatment effect x biomarker level interaction for the markers measured (MBP, NSE and S100)(Jauch et al. 2006). Several studies, based on groups of patients, all of whom had received thrombolytic therapy, reported on markers that might predict post-treatment cerebral haemorrhage, but the outcomes reported in these papers were largely radiological and none of the studies compared results with a non-treated group.

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 (Counsell et al. 2002e). Many studies of stroke prognosis – though 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. Where there is an association between marker level and outcome, this is in most cases continuous rather than dichotomous. Calculating thresholds in a data dependent fashion (for instance by ROC curve analysis) to optimise the prognostic performance of a blood biomarker can lead to implausibly large effect sizes. Whilst thresholds or reference intervals can be useful in clinical practice, they must be validated by applying the calculated marker threshold in a new cohort before being adopted into clinical practice. Unfortunately, data-derived thresholds are rarely replicated, and where they are replicated, they often are not confirmed (Christensen et al. 2002a).

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 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. Where a logistic regression model is used to analyse study results, sample size calculations should aim for at least 10 outcomes per variable to be entered in the final model (Harrell F.E, Lee K.L., & Mark D.B 1996). Known prognostic variables for poor outcome, such as age, stroke severity and premorbid disability should be forced into logistic regression models, as their association with poor outcome after stroke is robust. Reliably to assess the addition of a single biomarker measured at one time point with one outcome, requires that sufficient patients are recruited to ensure that at least 40 people develop the outcome of interest (10 each for age, stroke severity, premorbid disability and the biomarker). Therefore, in patients with moderate to severe stroke (of whom 40% will have a good outcome) the studies should recruit samples of at least 100 patients, assuming no loss to follow up.

Biomarker measurement is subject to both inter- and intra-patient 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 (Barber et al. 2006) to predict outcome.

Publication bias probably exists, as the funnel plot showed marked asymmetry, though other reasons for larger effect sizes in smaller studies, such as less methodological rigour or increased stroke severity and stronger association with outcome in smaller studies are also possible. We have attempted to minimise within study reporting bias by reporting both studies where a relative measure of effect (Figure 18) and a difference in means was reported (Figure 19).

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 of prognosis comparable to the CONSORT guidelines for randomised controlled trials and the 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. Reports frequently state markers are 'non significant' without stating an estimate of the measure of effect with its confidence intervals. Where thresholds have been chosen, they usually differ between studies. The interval chosen for analysis in multiple logistic regression may be per unit, per log unit or per quartile of biomarker. Adjustment in multiple regression analyses may be for different variables in different studies.

Conclusions

Blood biomarkers may be useful in acute ischaemic stroke, either by suggesting possible mechanisms for the aetiology 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 MI, 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.

Implications for research

Until there is an international consensus on the ideal components of a prognostic study analogous to CONSORT, it would seem reasonable to propose that an ideal study should aim to:

- Recruit a well defined cohort of patients are assembled at an early and uniform stage in the disease.
- Define subsequent treatment (e.g. thrombolysis, stroke unit care).
- Multiple logistic regression should include known clinical prognostic variables (e.g. 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 (McShane et al. 2005b).
- 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.

Implications for clinical practice

- None of the markers measured in this review can be recommended to predict the death or disability after acute stroke.

Tables

Table 5.1 Blood markers reported in the systematic review, their putative physiological role in stroke and the size and number of studies examining markers and poor outcome in stroke patients.

Biomarker	Hypothesised role in ischaemic stroke	Hypothesised origin in ischaemic stroke	Number of studies	Mean study size (smallest, largest)
Activated Protein C Resistance	Haemostasis		1	219
Adiponectin	Anti-inflammatory	Adipocytes	1	164
Alpha 2 antiplasmin	Antifibrinolysis	Liver	1	63
Anticardiolipin antibodies	Haemostasis	B lymphocytes	1	300
Antithrombin II	Anti-clotting	Binds at endothelium	1	55
Atrial natriuretic peptide	Cardiac	Atrial myocardium	2	44 (37,51)
Beta globin DNA	Cell damage	All cells	1	44
Beta thromboglobulin	Platelet	Platelets	2	71 (70,72)
BDNF	Neurotrophic	Neurones, renal, retinal	1	50
Brain natriuretic peptide	Cardiac	Myocardium	4	107 (51, 175)
Cortisol	Anti-inflammatory/ neuronal survival	Adrenal cortex	4	90 (34,184)
C-reactive protein	Inflammation	Liver	14	125 (11,467)
D-dimer	Haemostasis	Clot breakdown	8	118 (46, 231)
Endothelin - 1	Vasoconstrictor	Endothelium	2	69 (37, 101)
Factor VIIc	Haemostasis	Liver	2	141 (63, 219)
Factor VIIIC	Haemostasis	Liver	2	121 (70, 171)
Factor IXc	Haemostasis	Liver	1	219
Factor XIII	Haemostasis	Liver	1	63
Ferritin	Inflammation	Astrocytes/Glia/Liver	4	95 (51,162)
Fibrinogen	Haemostasis	Liver	14	156 (22, 469)
Fibrinopeptide A	Haemostasis	cleaved from fibrinogen	2	71 (70, 72)
GABA	Neurotransmitter	Neurones	2	113
GFAP	Glial protein	Glia	1	53
Glutamate	Neurotransmitter	Neurones	5	100 (46, 128)
Glycine	Neurotransmitter	Neurones	2	121 (113, 128)
Homocysteine	Endothelial apoptosis	?Macrophages	1	75
Insulin-like growth factor	Neuroprotective	Most tissues	1	85
Intercellular adhesion molecule	Inflammation	Endothelium	2	81.5 (50, 113)
Interleukin 1 beta	Inflammation	Endothelium	4	68 (18, 162)
IL-1 receptor antagonist	Anti-inflammatory	Lymphocytes/Macrophages	3	102 (41, 162)

Biomarker	Hypothesised role in ischaemic stroke	Hypothesised origin in ischaemic stroke	Number of studies	Mean study size (smallest, largest)
Interleukin 4	Inflammation	CD4 T cells/Macrophages	1	231
Interleukin 6	Inflammation	CD4 T cells/Macrophages	10	101 (11, 231)
Interleukin 8	Inflammation	Endothelium	1	50
Interleukin 10	Anti-inflammatory	Lymphocytes/Macrophages	6	90 (11, 231)
Iron			1	100
L-arginine	Anti-inflammatory	Endothelium	1	113
LAPa2	Lipid metabolism	Vascular smooth muscle	1	467
Matrix metalloproteinase 2	Inflammation	Endothelium	1	49
Matrix metalloproteinase 9	Inflammation	Endothelium	2	50
Matrix metalloproteinase 1	Inflammation	Endothelium	1	50
MCP	Inflammation	Endothelium	1	50
Myelin basic protein	Glial damage	Glia	1	359
Neurone specific enolase	Neuronal damage	Neurone	7	93 (24,359)
Normetanephrines	Sympathetic	Adrenal medulla	1	75
Nucleosomes	Cell damage	All cells	1	63
P selectin	Inflammation	Platelets	2	73.5 (45,102)
Plasminogen activator inhibitor	Antifibrinolysis	Endothelial/liver	3	70 (44, 102)
Procalcitonin	Inflammation	?	1	30
Protein C	Anticlotting	Liver	1	55
Protein S	Anticlotting	Liver	1	55
Prothrombin fragments	Haemostasis	cleaved from prothrombin	2	130 (40, 219)
Resistin	Inflammation	Monocytes/lymphocytes	1	211
S100 beta	Glial damage	Glia	6	100 (26, 359)
Selectin	Inflammation	Endothelium	1	238
Serum Amyloid A	Inflammation	Liver	1	203
sICAM-1	Inflammation	Endothelium	1	238
Soluble TNF alpha receptor 1	Inflammation	Vascular smooth muscle	1	43
Soluble TNF alpha receptor 2	Inflammation	Vascular smooth muscle	1	162
Spermidine	Modulate NMDA	Neurones	1	16
Tau	Neuronal protein	Neurones	1	53
Thrombin/antiThrombin	Haemostasis	Coagulation	3	241 (40,465)
Thrombomodulin	Anticlotting	Endothelium	3	445 (359, 510)
Tissue plasminogen activator	Fibrinolysis	Endothelium	2	342 (219, 465)
Troponin I	Cardiac myocyte	Cardiac myocyte	4	230 (175, 330)
Troponin T	Cardiac myocyte	Cardiac myocyte	4	209 (172, 279)
Tumour necrosis factor alpha	Inflammation	CD4 T cells	8	124 (18, 231)
Uric Acid	Purine catabolism	?all cells	2	2306 (881, 3731)
VCAM	Inflammation	Endothelium	1	238
von Willebrand Factor	Haemostasis	Endothelium	4	127 (46, 219)

Figures

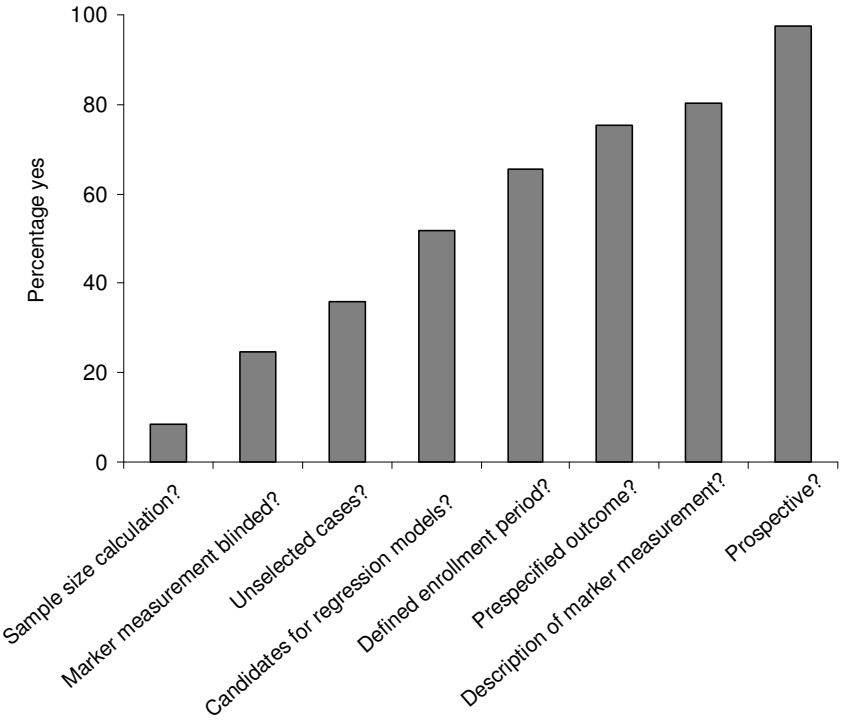


Figure 16 Study quality, using questions modified from the REMARK recommendations.

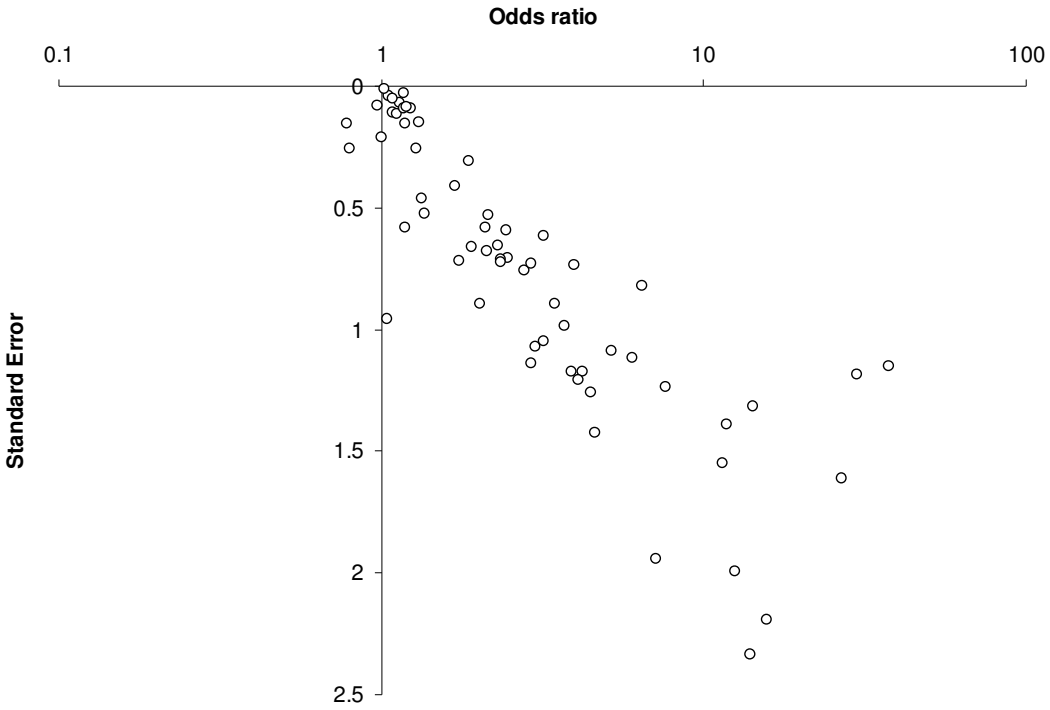


Figure 17 Funnel plot of studies of blood markers and poor outcome after stroke.

Each point represents one estimate of the association between a blood marker and poor outcome in one study. The x-axis represents the odds ratio (OR), a measure of the strength of the association between markers and poor outcome; OR>1 if higher levels markers are positively associated with poor outcome and OR<1 if higher levels of markers are negatively associated with poor outcome. The y-axis represents the standard error, a measure of precision; the lower the standard error, the more precise the estimate of the OR. Note: there are no studies with a low precision that show a negative association between higher levels of blood markers and poor outcome, a phenomenon known as 'small study bias'.

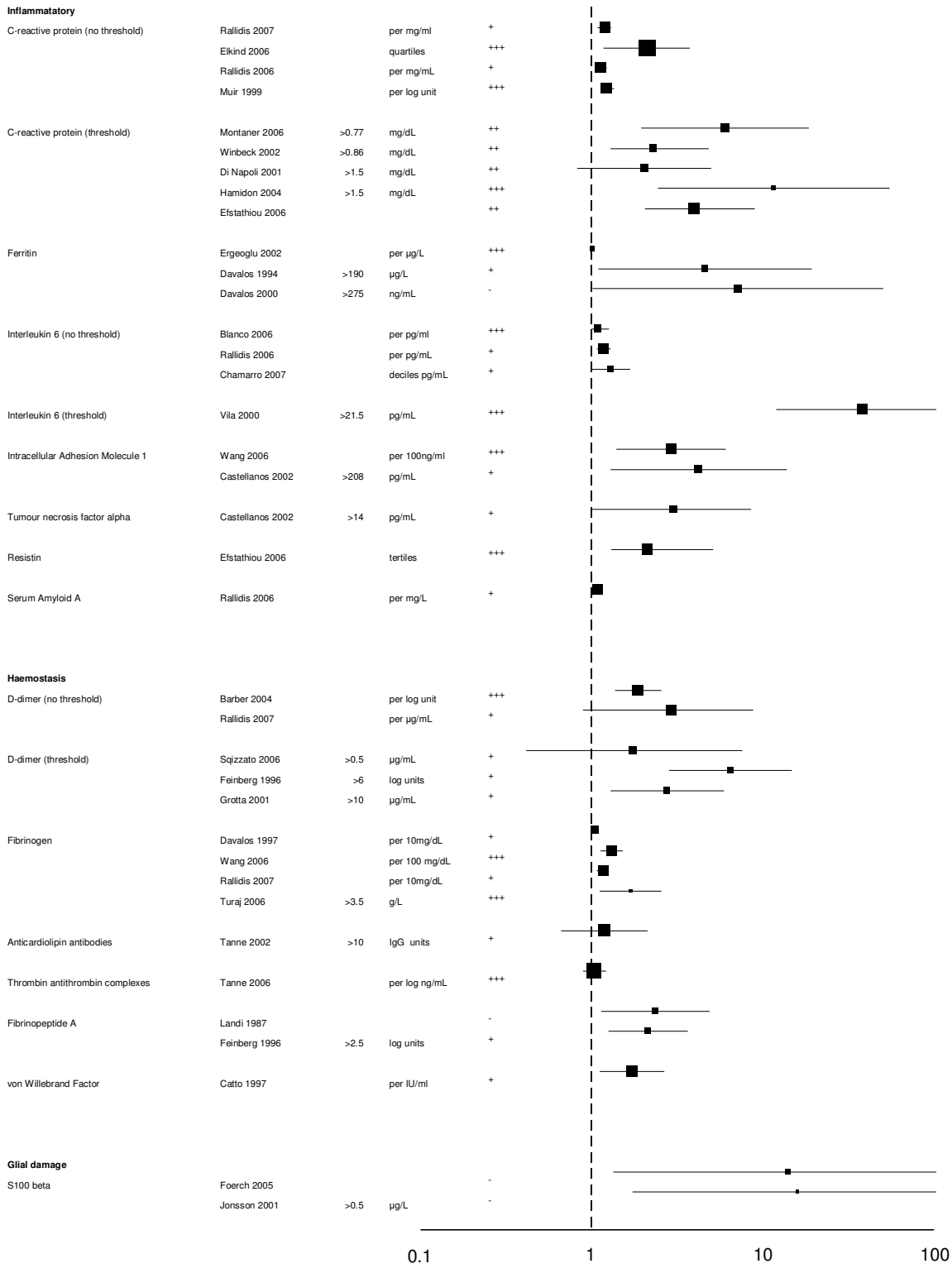


Figure 18 Measures of association of venous blood biomarkers and poor outcome.

95% confidence intervals + = adjustment for age or stroke severity, ++ = adjustment for age and stroke severity, +++ = adjustment for age, stroke severity and other factors.

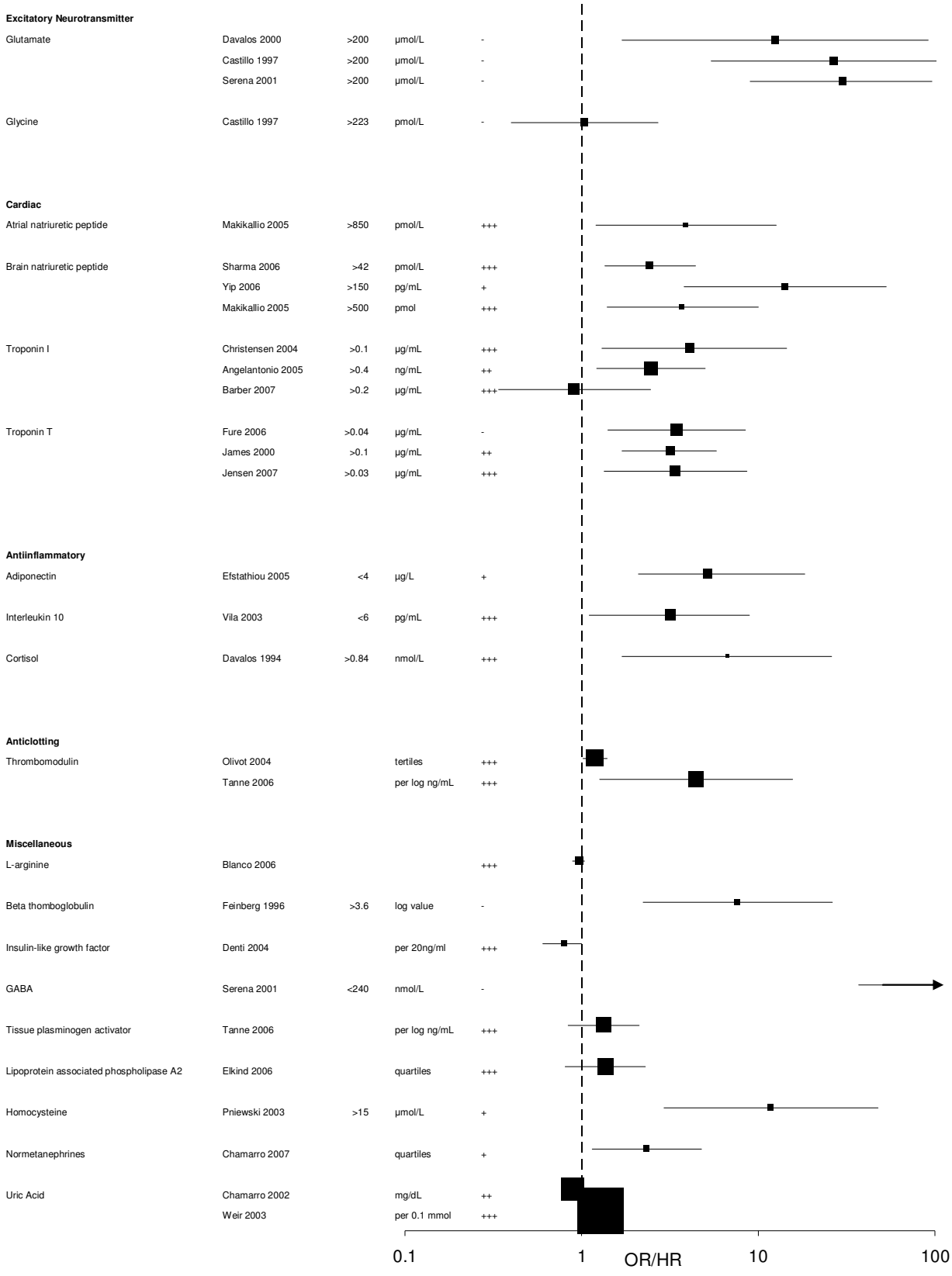


Figure 18 continued

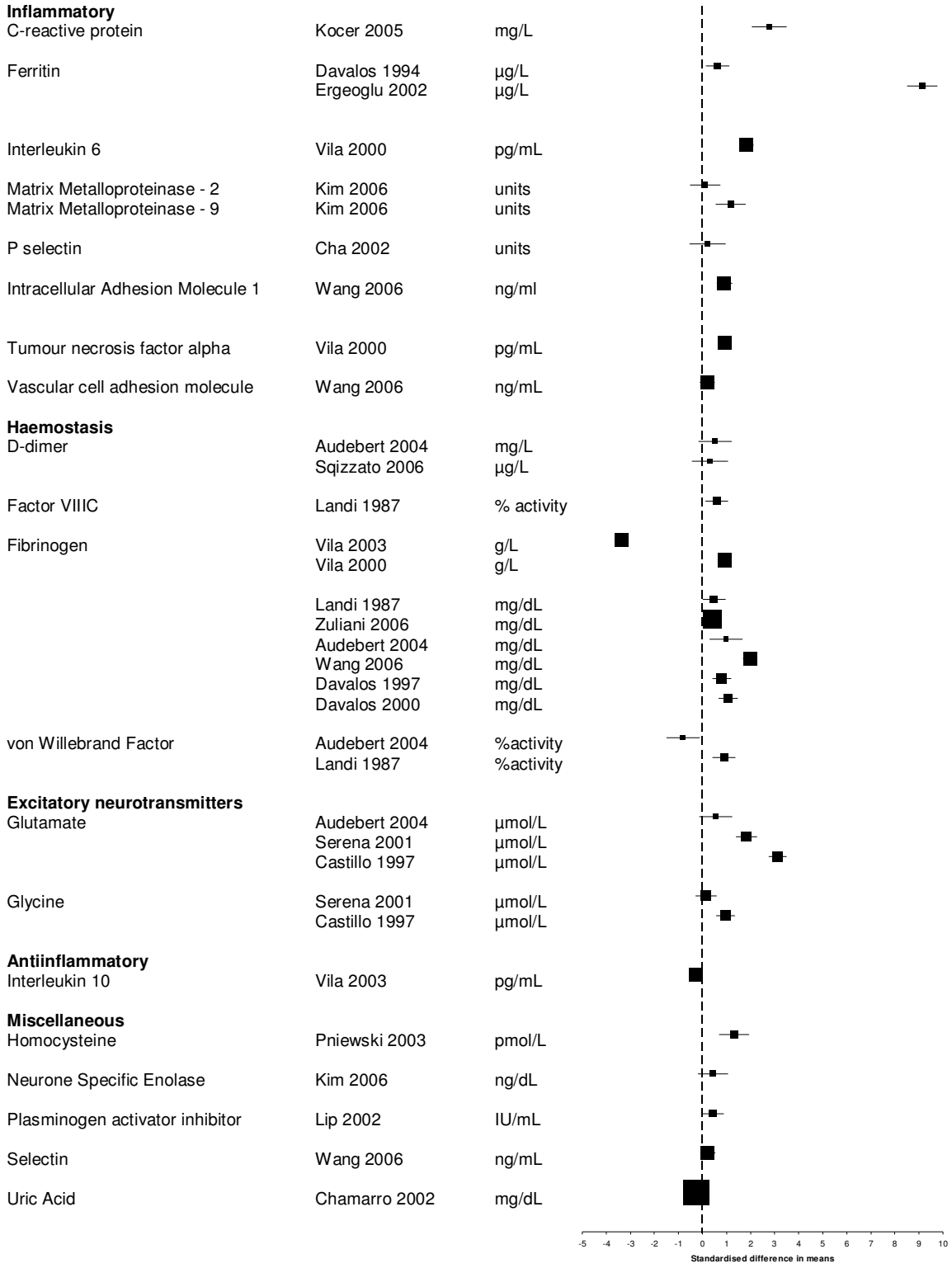


Figure 19 Standardised differences in means : (mean level in poor outcome - mean level in good outcome) /pooled standard deviation, and 95% confidence intervals

Chapter 6. Inflammatory markers and poor outcome after stroke: a prospective cohort study and systematic review of interleukin 6

Introduction

A non-specific systemic inflammatory response occurs after both ischaemic and haemorrhagic stroke, either as part of the process of brain damage or in response to complications such as deep venous thrombosis. Several studies have reported that higher levels of inflammatory markers such as C-reactive protein (CRP) and interleukin 6 (IL-6) are associated with worse outcome after both ischaemic (Whiteley et al. 2009a) and haemorrhagic strokes (Castellanos et al. 2005, Castillo et al. 2002). However, these studies often had methodological weaknesses, chiefly that they were too small, or did not adequately adjust for confounders or assess the clinical utility of the measurements.

The addition of markers of inflammation to validated clinical prognostic models might improve the prediction of poor outcome after stroke. There are at least two validated models for predicting clinical outcome after stroke; one is based on six simple clinical variables which can be applied without specific training (Counsell, Dennis, & McDowall 2004c), and the other includes the more complex National Institutes of Health Stroke Scale (NIHSS) and age (Konig et al. 2008c). The NIHSS is complex, assessing 15 items and requires specific training.

I therefore aimed to replicate the finding that several markers of the acute phase response – CRP, IL- 6, white cell count, fibrinogen or glucose- appear to be associated with poor outcome after ischaemic and haemorrhagic stroke. The data were available from a large prospective cohort of stroke patients which aimed to avoid as many as possible of the methodological weaknesses of the other studies. I then wished to assess whether blood biomarkers could improve an existing prognostic models in the same cohort

IL-6 has shown a particularly strong association with poor outcome after stroke; I therefore performed a systematic review of the existing literature to put these results in context.

Methods

Patients

The Edinburgh Stroke Study prospectively recruited all consenting patients with recent stroke from the emergency department, medical, neurology and occasionally other (e.g. surgical) wards, stroke unit and neurovascular clinics of the Western General Hospital, Edinburgh, UK between April 2002 and May 2005 into the Edinburgh Stroke Study (Jackson et al. 2008c). Clinicians recorded data at the time of assessment using a standardised structured proforma and, in patients who consented, drew blood for measurement of inflammatory markers.

The study definition of a clinically definite stroke was new clinical symptoms or signs of a focal disturbance of cerebral function lasting more than 24 hours of a vascular origin. Patients with subarachnoid haemorrhage were excluded. At a weekly meeting, stroke physicians, neurologists and neuroradiologists reviewed the clinical features of each patient, all brain images and clinical progress. Ischaemic stroke was defined as a clinically definite stroke in a patient whose brain imaging showed either positive evidence of a relevant ischaemic lesion or was normal and excluded intracranial haemorrhage and stroke mimics. Stroke was diagnosed as an intracerebral haemorrhage if the patient's clinical features and brain imaging were consistent with acute haemorrhage. The pathological subtype of stroke was defined as probably ischaemic in patients with a clinically definite stroke in whom the radiological results were equivocal or unavailable, and analysed them together with definite ischaemic strokes. A final ischaemic stroke syndrome was assigned according to the Oxford Community Stroke Project (OCSP) classification based on the clinical syndrome at the time of maximum deficit modified , where appropriate, by the site and size of relevant infarcts on brain imaging (Bamford et al. 1991b). The

diagnosis of stroke was made blinded to the measurement of CRP, IL-6 and fibrinogen.

Measurement of clinical variables

A physician with experience in stroke medicine assessed each patient as soon as possible after presentation and recorded risk factors for stroke, current treatment and electrocardiogram findings, measured impairment using the National Institutes of Health Stroke Scale (NIHSS) and collected variables for a previously validated "six simple variables" prognostic model (Brott et al. 1989a, Counsell et al. 2002d) (age, prior dependence, able to lift both arms from the bed, able to walk without assistance, living alone at the time of the event and orientation in time and person). I defined hypertension as a history of treated hypertension; ischaemic heart disease as a history of myocardial infarction, angina, coronary artery bypass grafting or percutaneous coronary intervention; peripheral artery disease as a history of claudication, peripheral artery intervention or definite signs of vascular disease of the legs (e.g. absent pedal pulses); cardiac failure as definite signs of heart failure, or taking at least two medications for its treatment and independence prior to stroke as not requiring assistance for washing, dressing, feeding or toileting.

Measurement of blood markers

Clinicians drew blood on the same day as clinical assessment, or for patients admitted to hospital, as soon after assessment as possible. A clinical laboratory measured total white cell count (Beckman Coulter LH750 analyser) and blood glucose (Vitros Chemistry analyser). Blood samples for IL-6, CRP and fibrinogen, were transported to the laboratory on water ice, centrifuged to obtain serum and EDTA-anticoagulated plasma and stored at -80^{o}C until analysed. CRP and fibrinogen in plasma were measured by immunonephelometry (Prospec, Dade Behring Milton Keynes, UK) using the manufacturer's reagents and standards. IL-6 was assayed by ELISA (R & D Systems, Oxford, UK). Intra- and inter-assay coefficients of variation were 4.7 and 8.3%, 2.6 and 5.3%, and 7.5 and 8.9%, respectively. All assays were blind to stroke outcome.

Assessment of outcome

Each patient was sent a validated self completion questionnaire by post at 6 months from their stroke onset date, which measured disability with the modified Rankin Scale (mRS), a standard tool for examining outcome after stroke. Non-responders were sent a repeat questionnaire. Each patient was 'flagged' at the General Register Office for Scotland who provided information on the date and place of death. The cause of death was confirmed by inspection of the relevant medical records. In primary analyses, I dichotomised a patient's outcome into: 'poor' if they were dependent on others for activities of daily living (mRS scores 3, 4, 5) or dead, and 'good' if they were independent in activities of daily living (mRS 0,1 and 2) 6 months after stroke onset. In subsidiary analyses, I dichotomised patient outcome at 6 months into alive or dead.

Statistical analysis

Association between marker levels and baseline features

In a series of bivariate analyses, I compared normally distributed baseline characteristic with Student's t-tests, proportions with χ^2 tests and positively skewed data with Wilcoxon rank sum tests. For the calculation of Pearson correlation coefficients, I logarithmically transformed positively skewed blood marker data to obtain a normal distribution. I examined the relationship between biomarker level and delay to blood taking using multivariable regression analysis.

Association between marker levels and outcome:

I investigated the unadjusted associations between inflammatory marker level and outcome with χ^2 for trend tests. I built a logistic regression model for the association of each inflammatory biomarker with poor or good outcome, with the terms from the previously validated six simple variable model added sequentially. I also examined logistic regression models for the association between individual biomarkers and outcome, adjusting stepwise for NIHSS, age, vascular risk factors, sex, and prior independence and living alone (domains not part of the NIHSS). For

these analyses, I compared the upper and lower thirds of inflammatory marker levels for the entire sample, and modelled the marker levels as linear variables. I stratified the analyses by NIHSS, OCSP, delay to blood taking and pathological stroke type to look for evidence of effect modification.

Assessing the contribution of biomarkers to clinical prognostic models

I assessed the additional contribution of those inflammatory markers that were significantly associated with poor outcome after adjustment to previously validated six simple variable model (Counsell et al. 2002c).

First, I assessed whether blood markers improved the goodness of fit of existing models using the likelihood ratio statistic. Second, to compare the ability of models to discriminate between good and poor outcome, I calculated areas under receiver operator curves (AUROC). An AUROC of 1 indicates perfect discrimination and 0.5 no discrimination. Third, I assessed calibration (whether the average predicted risk of poor outcome in subgroups matches that observed in the cohort) with the Hosmer Lemeshow χ^2 statistic. Fourth, I assessed the ability of the best performing model including biomarkers to one without by examining risk stratification tables (Janes, Pepe, & Gu 2008). I used the methods of Pencina et al to calculate net reclassification improvement (NRI) (Pencina et al. 2008). NRI is a measure that takes into account the correct movement of individuals between categories of predicted risk (i.e. the numbers of patients moving correctly or incorrectly between categories) to estimate overall improvement. I pre-specified thresholds of <10% and >90% for predicted probability of poor outcome as I 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.

All *P* values reported are 2 sided and I considered $P < 0.05$ statistically significant. I performed statistical analyses with Stata (version 10.1, College Station, TX, USA).

Systematic review of IL-6

I searched Medline and EMBASE from 1966 to December 2008 for studies in patients with acute stroke that measured IL-6 and assessed clinical outcome. The search strategy included 13 terms for ischaemic stroke and 2 for IL-6. Prognostic studies were identified using high sensitivity search terms (Altman 2001b), together with common outcome measurements from stroke research (Rankin, NIHSS, Glasgow outcome scale) (see MOOSE checklist, appendix). I included studies if they (a) reported results for patients with acute stroke (not transient ischaemic attack); (b) assayed a venous IL-6 in stroke patients; (c) measured outcome using death, disability or handicap scales and (d) reported results in a manner that allowed calculation of OR for poor outcome or death per unit increase in marker, to allow comparison of measures of association between studies. I extracted data from logistic regression models reporting the association between interleukin 6 and poor outcome or death after stroke, and the degree of adjustment for age, stroke severity and other potential confounders. I performed fixed effects meta-analysis with Stats Direct Version 2.7.2.

I prepared this chapter with reference to the STROBE guidelines for reports of observational epidemiological studies, the REMARK guidelines for reports of prognostic variables and the MOOSE guidelines for the meta-analysis of observational studies (McShane et al. 2005a, Stroup et al. 2000, von Elm et al. 2007b).

Ethics

This study was conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Lothian Research Ethics Committee. All patients or their guardians provided written informed consent for the collection of samples and subsequent analysis.

Results

Baseline characteristics

Data completeness is summarised in Figure 20. 1408 patients were recruited into the main ESS cohort, of whom 844 (60%) had blood drawn for markers of inflammation. Of these 785 (93%) had a definite ischaemic stroke, 16 (2%) a probable ischaemic stroke and 43 (5%) a haemorrhagic stroke. Those included in this biomarker subset were similar to those who were not, in age, sex and the proportions with hypertension, peripheral or cardiac vascular disease, diabetes or atrial fibrillation. On average, compared to those without biomarker data, patients with biomarker data had milder strokes (median NIHSS 1 vs 2 $p<0.001$, proportion TACS 7.7% vs 14.7% $p=0.001$ respectively), as patients admitted to hospital and those with more severe symptoms were less likely to be recruited because of practical barriers to obtaining and processing research blood samples and obtaining informed consent or assent(Jackson et al. 2008b). Included patients were also less likely to have a diagnosis of cardiac failure (4.3% vs 8.3% $p=0.002$). The median delay from stroke to blood taking was 13 days (IQR 6 to 22 days). Of those patients who had blood drawn for blood markers, 6 month modified Rankin Scale data were available in 750/844 (89%) and vital status at 6 months was available in all patients. At 6 months, of the 844 patients, 59 were dead, and 238 were dead or disabled. Deaths were due to the initial or recurrent stroke (35/59, 59%), vascular disease of the heart, legs or bowel (9/59, 15%), cardiac failure (5/59, 9%), cancer (5/59, 9%) and bowel perforation, chronic obstructive pulmonary disease or pneumonia (5/59, 8%).

For all markers there was a weak, though statistically significant ($p<0.001$) negative relationship between the natural logarithm of marker and time from stroke onset to blood draw. The Pearson correlation coefficients for the relationship between time in days and the natural logarithm of each marker were: glucose, r= -0.07; white cell count, r= -0.12; fibrinogen, r= -0.12; C-reactive protein, r= -0.14 and interleukin 6, r= -0.19. In multivariate regression models with time as the independent variable, after

adjustment for age and stroke severity measured by NIHSS, these relationships were even weaker and not statistically significant.

Table 6.1 summarises the baseline data for all those patients from whom blood was drawn for markers and for those with good and poor outcome at 6 months. Patients who died or had poor outcome were older, had more severe strokes, and had more ischaemic heart disease, previous strokes or transient ischaemic attacks, diabetes, congestive cardiac failure and atrial fibrillation. They were more likely at the time of stroke to: live alone, be dependent on others for activities of daily living, be disoriented, have arm weakness and be unable to walk. They had higher levels of IL-6, CRP, fibrinogen, white cell count and glucose.

Relation of markers to outcome with and without adjustment for other factors

There were strong positive associations between marker levels and the odds of poor outcome (Figure 21). The risk of poor outcome rose by third of IL-6 distribution (χ^2 trend $p<0.001$), CRP (χ^2 trend $p<0.001$), fibrinogen (χ^2 trend $p<0.001$), white cell count (χ^2 trend $p=0.002$) and glucose (χ^2 trend $p=0.001$). The risk of death also rose by third of marker (χ^2 trend $p<0.001$ for each marker) (data not shown), though in general the association between marker thirds and death was stronger than for poor outcome. After adjustment for age, and at the onset of stroke: whether the patient lived alone, was independent of activities of daily living, was orientated, able to lift their arms or walk, the odds ratios were attenuated for the association with poor outcome (IL-6, OR: 3.1, 95% CI 1.9 to 5.0; CRP, OR: 1.9, 95% CI 1.2 to 3.1; fibrinogen OR: 1.5, 95% CI: 1.0 to 2.4, white cell count, OR: 2.1, 95% CI: 1.3 to 3.4 and glucose OR: 1.3 95%, CI: 0.8 to 2.1) and death (data not shown). Adjustment for the association between marker levels and poor outcome for NIHSS, age, vascular risk factors, sex, and prior independence and living alone, led to only minor changes in the magnitude of these odds ratios for the association with poor outcome. After additional adjustment for other markers, only the association between IL-6 and poor outcome remained independently significant (OR: 2.4 95% CI 1.3 to 4.5). Further adjustment of the associations with death was not performed because of the

relatively small number of events. There was no material difference in the magnitude, direction or significance of the association between IL6, CRP and white cell count (data shown for IL6) and outcome after stratifying the analysis by: stroke subtype, stroke severity, clinical stroke syndrome or delay to blood taking after stroke (Figure 22).

The crude increase in the odds of death or disability per unit increase in marker level, was lowest for CRP and highest for fibrinogen, though the range of the fibrinogen (1.2 to 9.6 g/l) was smaller than CRP (0.159 to 263 mg/L). After adjustment for the 6 simple variables, the associations between IL6, CRP and white cell count remained statistically significant (Table 6.2).

Does the addition of marker data improve the predictive accuracy of clinical predictive models?

I added data for the markers that were independently associated with poor outcome (IL-6, CRP and white cell count) as continuous variables to the previously validated six simple variable model (Counsell et al. 2002b) (Table 6.3). Model fit was improved significantly after the addition of IL-6 or white cell count, though not CRP. Model calibration was adequate after the addition of IL-6, white cell count and CRP. However, the AUC improved significantly only after the addition of IL-6 to the six simple variable model, though not after the addition of white cell count or CRP alone. A model with the six simple variables and all of the inflammatory markers was well calibrated, though had a similar AUC to a model with the six simple variables and IL-6 alone (p=0.8). As the 'NIHSS and age' model was poorly calibrated in this cohort (Hosmer-Lemeshow χ^2 p=0.01), it was not examined further.

I compared the proportions of patients with predicted high (>90%) and low (<10%) risks of poor outcome by the six simple variable model with and without the addition of IL-6 (Table 6.4). The addition of IL-6 to the six simple variable model increased the proportion of patients in the lowest risk category from 2.5% to 4.4% and the proportion in the highest risk category from 2.2 % to 3.0 %, i.e. an extra 2.6%

(95% CI: 1.7 to 4.1) were moved from indeterminate (10-90%) to determinate categories (>90% or <10%). The models correctly classified those in the highest risk category as having a poor outcome, in 91% (95% CI: 73 to 98) of patients for the model including IL-6, and 94% (95% CI: 73 to 99) for model without. The models incorrectly classified patients in the lowest risk category in 12% (95% CI: 5 to 27) for the model including IL-6 and 16% (95% CI 6 to 38) for the model with the six simple variables alone. The net reclassification improvement after the addition of IL-6 to the six simple variable model (5%, p=0.014) was small.

Systematic review and meta-analysis

The literature search identified 146 studies. I excluded studies for the following reasons: non-systematic reviews (20), the full paper was unobtainable (3), participants did not have stroke at baseline (75), blood IL-6 was not measured (12), death or disability was not reported (20), reported odds ratios for the association of IL-6 above and below a threshold (Castellanos et al. 2008, Vila et al. 2003) (2), reported correlation coefficients only (Mazzotta et al. 2004, Smith et al. 2004, Sotgiu et al 2006, Waje-Andreassen et al. 2005) (4), reported mean levels in patients with good and bad outcome only (Basic, V et al. 2008, Christensen et al. 2002b, Domac et al. 2007, Nakase et al. 2008, Shenhar-Tsarfaty et al. 2008) (5) or did not report numerical results (Silvestri et al. 2004) (1). I identified 4 relevant studies (Blanco et al. 2006, Chamorro et al. 2007, Rallidis et al. 2006, Welsh et al. 2009) (Table 6.5), which yielded, for the association between IL-6 and poor outcome 1037 patients, and IL-6 and death 1,122 patients. The summary odds ratios are comparable to the results of the current study (Figure 23).

Discussion

Statement of main findings

In this large cohort of stroke patients, I found that higher levels of IL-6, CRP and white cell count were independently and significantly associated with poor outcome and death at six months after stroke. The association was independent of stroke

severity, age and risk factors for recurrent stroke, though only IL-6 was independent of other markers. The addition of IL-6 to a validated prognostic model increased the proportion of patients with predicted probabilities of a poor outcome of >90 or <10% by only 2.8%, and the net classification index by 5%. These findings lend support to the hypothesis that the inflammatory response is associated with poor outcome after stroke. Although the measurement of the inflammatory response assessed with IL-6 improves prediction of poor outcome, in this cohort the degree was so small that the use of these markers in routine practice is unlikely to be helpful to clinicians aiming to predict the outcome of their stroke patients, for example by selecting individuals for aggressive treatment or palliative care.

Study limitations and potential biases

I did not exclude patients with infection even though this is a potential confounding factor (infection after stroke is associated both with higher levels of inflammatory markers and with poor outcome after stroke independently of other factors) as I sought external validity to determine the role of markers in a clinical setting. However, the delay between blood draw and stroke did leave time for the development of infective or inflammatory complications in some of the more severely affected stroke patients, so a rise in inflammatory markers due to infection rather than brain damage from the stroke may have been responsible for at least part of the observed association. The cohort, consisting of a mixture of outpatients and hospital inpatients, contained relatively mild stroke patients, so models generated from the whole cohort may not be applicable to cohorts containing only patients with severe strokes, as my models may have a ceiling effect at higher stroke severities. The study was limited in its ability to recruit more patients with very severe strokes chiefly because of the practical barriers to blood taking for research purposes out of normal working hours and obtaining informed consent. I dichotomised the Oxford Handicap Scale, measured by postal questionnaire, into 'independent' and 'dependent'. Although crude, this measure has both internal and external validity (Lindley et al. 1994). Although analysing each level of the OHS

may have added power to the study, this methods increases the complexity of the analysis and makes explanation of the results more difficult.

Blood samples for inflammatory marker levels were only drawn at the time of assessment. Although serial measurement might have provided more information, even the single measurement available was still strongly associated with outcome. It seems unlikely that the additional effort of obtaining serial samples would be outweighed by additional predictive power.

The use of the area under the curve to choose between predictive models is a subject of some controversy. The area under the curve analysis is based on rank comparison, which may be problematic for populations in whom the risk of an event is very low (for example incident stroke in asymptomatic cohorts)(Cook 2007). However, as the risk of poor outcome after stroke is high in this cohort (32%), the use of the AUC seems reasonable. While in this study IL-6 has an association with poor outcome, an extremely strong and independent association needs to be demonstrated before a marker usefully improves classification accuracy (Pepe et al. 2004b). I assessed the additional predictive utility of IL-6 with risk stratification tables applying cut points for predicted outcome that are relevant for stroke practice, for the treatments that are currently available. Less stringent thresholds of risk could be examined, though it is hard to see how they would be useful in making decisions about individual patients. I have not demonstrated that IL-6 improves prediction in this cohort, using my chosen thresholds. My conclusions would be strengthened by replication of my findings in a validation dataset.

The systematic review is limited in scope, as several other studies relevant to the association between IL-6 and death or poor outcome reported their results either as a comparison of odds of poor outcome above and below optimised cut points or as correlation coefficients hence extraction of data per unit increase in marker level was not possible.

Interpretation

I have demonstrated that blood markers of the acute inflammatory response, in particular IL-6, are associated with death and poor outcome after stroke. The results from this study are broadly comparable to other studies of IL-6 and poor outcome or death after stroke (Figure 4), which supports the generalisability of the findings.

The strengths of the current study in comparison to other studies merit consideration. It is much larger than previous reports, and has used a measure of handicap (the modified Rankin Scale) as well as death to define poor outcome. It has used a validated prognostic model to adjust for confounding by stroke severity, age and prior dependence and has carefully explored the role of these markers in clinical decision making, which though often proposed, has not been examined before.

IL-6 is induced by TNF α and IL-1β, and then leads to the releases of CRP, fibrinogen and cell adhesion molecules, though the cellular origin of interleukin 6 after stroke is not clear. Whether the higher levels of IL-6 are a bystander to, or a cause of, poor outcome after stroke is uncertain.

Evidence in favour of a causal role for inflammation in poor outcome after stroke comes from animal studies. A peripheral challenge with either a bacterial endotoxin (lipo-polysaccharide) or IL-1β seems to increase the measured volume of brain damage after arterial occlusion in a mouse model of stroke. (McColl, Rothwell, & Allan 2007). The blockade of IL-1 (which reduces further inflammatory responses) reduces the volume of brain damage after arterial occlusion in mice; the results of human studies are not yet available. The inhibition of the ingress of neutrophils into the brain with antibodies to adhesion molecules (e.g. ICAM) also seems to reduce the volume of brain damage in a mouse model. (Muir et al. 2007).

Evidence against a causal role of inflammation comes from a variety of sources. Mice deficient in IL-6 showed similar stroke volume and disability at 24 hours as mice with normal IL-6 expression (Clark et al. 2000), suggesting that it may simply

be part of the inflammatory response to stroke and not directly pathogenic. The association of interleukin-6 with poor outcome has been demonstrated in many conditions such as HIV (Kuller et al. 2008), many cancers (Duffy et al. 2008), and the occurrence of first episodes of vascular disease including stroke (Danesh et al. 2008c), making it more plausible that IL-6 is a general marker of disease severity rather than part of numerous disease specific pathways to poor outcome.

Implications for research

- In this large cohort of stroke patients, blood markers of the acute inflammatory response were associated with poor outcome after stroke, though only IL-6 showed independent association after adjustment for confounding factors including levels of other markers.
- In this cohort, the addition of IL-6 to a previously validated prognostic model added to the prediction of outcome, but by an amount that is unlikely to be useful in clinical practice.
- Whether or not inflammatory markers are useful in prediction of recurrent stroke (Welsh et al. 2008a, Woodward et al. 2005f) or other vascular events is a separate question, which requires further study.

Implications for clinical practice

- The measurement of IL-6, fibrinogen, CRP, white cell count or glucose do not give additional predictive power to easily measured clinical variables for the prediction of poor outcome after stroke.

Tables

Table 6.1 Baseline characteristics of biomarker cohort and their influence on death and poor outcome

Characteristic	Biomarker cohort (n=844)	Good outcome 6 months (n=512)	Poor outcome 6 months (n=238)	*P* value
Age – mean (SD)	72 (11)	70 (11)	75 (11)	<0.001*
Male sex no. (%)	445 (53)	275 (54)	115 (48)	0.169†
NIHSS§ – median (IQR)‖	1 (4)	1 (2)	4 (7)	<0.001c
Laboratory measurements	median	median (IQR)	median (IQR)	
Interleukin-6 (pg/ml)	4.0 (4.8)	3.3 (3.2)	6.1 (7.5)	<0.001‡
C-reactive protein (mg/l)	3.4 (8.1)	2.6 (5.7)	7.1 (18.8)	<0.001‡
Fibrinogen (g/l)	4.5 (1.6)	4.3 (1.4)	5.0 (1.9)	<0.001‡
White cell count (x10^9/l)¶	8.0 (3.1)	7.7 (2.9)	8.5 (3.1)	<0.001‡
Glucose(mmol/l)**	5.6 (1.9)	5.5 (1.7)	6.0 (2.1)	0.0002‡
Cholesterol (mmol/l) – mean (SD)	5.2 (1.3)	5.2 (1.2)	5.1 (1.3)	0.189*
Pathological stroke type	No. (%)	No. (%)	No. (%)	
Definite ischaemic stroke	785 (93)	484 (95)	215 (90)	0.006†
Definite haemorrhagic stroke	43 (5)	18 (4)	21 (9)	
Probable ischaemic stroke	16 (2)	10 (2)	2 (1)	
OCSP ischaemic stroke syndrome				
Total anterior circulation infarction	53 (7)	10 (2)	32 (15)	<0.001†
Partial anterior circulation infarction	352 (44)	225 (46)	96 (44)	
Lacunar infarction	221 (28)	143 (29)	53 (24)	
Posterior circulation infarction	124 (16)	80 (16)	28 (13)	
Unclassified	51 (6)	36 (7)	8 (4)	
Six simple variable model ††				
Living alone	324 (38)	327 (36)	105/237 (44)	0.033†
Independent pre-stroke	799 (95)	502 (98)	209 (88)	<0.001†
Normal verbal Glasgow coma scale	754 (90)	492/509 (97)	185/237 (78)	<0.001†
Able to lift both arms	749 (89)	494/511 (97)	180 (76)	<0.001†

Characteristic	Biomarker cohort (n=844)	Good outcome 6 months (n=512)	Poor outcome 6 months (n=238)	*P* value
Able to walk	640 (76)	464/511 (91)	117 (49)	<0.001†
Co-morbidities	No. (%)	No. (%)	No. (%)	
History of hypertension	453 (54)	244 (52)	143 (60)	0.047†
Prior ischaemic heart disease	234 (28)	125 (24)	86 (36)	0.001†
History of diabetes	103 (12)	52 (10)	41 (17)	0.006†
History of peripheral vascular disease	36 (8)	40 (8)	18/235 (8)	0.941†
History of cardiac failure	40 (5)	11/511 (2)	25/237 (11)	<0.001†
Atrial fibrillation (previous or current)	162 (19)	73 (14)	69 (29)	<0.001†
Prior stroke or transient ischaemic	262 (31)	144 (28)	86 (36)	0.027†
Smoker (current or within 1 year)	275/829 (31)	163/508 (32)	73/232 (31)	0.886†

Good outcome: (mRS =0,1,2) ; Poor outcome: (mRS=3,4,5 or dead) * t test, †Chi squared test, ‡ Wilcoxon rank sum test, §National Institute of Health Stroke Scale, ‖482 good outcome and 224 poor outcome strokes, ¶496 good outcome and 233 poor outcome strokes, **471 good outcome and 218 poor outcome strokes ††the sixth variable is age in this model.

Table 6.2 The association between marker levels and poor outcome after stroke

	Odds ratio per unit increase in marker level (95% CI)		
Markers	**Unadjusted estimate**	**Adjusted for 6 simple variable**	**Further adjustment***
IL6 (pg/ml)	1.14 (1.10 to 1.17)	1.07 (1.03 to 1.11)	1.05 (1.01 to 1.09)
CRP (mg/L)	1.02 (1.01-1.03)	1.01 (1.00 to 1.01)	1.01 (1.00 to 1.01)
Fibrinogen (g/l)	1.35 (1.21 to 1.51)	1.12 (0.98 to 1.28)	1.05 (0.90 to 1.21)
White cell count ($x10^9$/l)	1.14 (1.08-1.21)	1.08 (1.01 to 1.16)	1.06 (0.99 to 1.14)
Glucose (mmol/l)	1.06 (1.00-1.12)	1.04 (0.97 to 1.12)	0.96 (0.87 to 1.05)

* adjusted for NIHSS, age, living alone and prior independence previous diabetes, history of cardiovascular disease, history of peripheral vascular disease, history of cardiac failure, history of hypertension, current or history of atrial fibrillation

Table 6.3 Performance of predictive models to predict poor outcome after stroke

Model	Likelihood ratio statistic	P*	Hosmer-Lemeshow χ^2	P†	AUROC (95% CI)	P‡
1. Six simple variables	Reference	Reference	6.2	0.63	0.78 (0.74 to 0.83)	Reference
2. Six simple variables + IL-6	10.9	<0.01	8.0	0.43	0.80 (0.76 to 0.84)	<0.01
3. Six simple variables + CRP	3.4	0.06	6.7	0.57	0.78 (0.75 to 0.82)	0.09
4. Six simple variables + white cell count	5.62	0.02	3.3	0.91	0.78 (0.74 to 0.82)	0.53
5. Six simple variables + white cell count + CRP + IL-6	13.39	<0.01	12.0	0.15	0.80 (0.76 to 0.83)	0.01

Performance of 6 simple variables model (age, living alone, independent of activities of daily living prior to stroke, normal verbal GCS, able to lift arms from bed, able to walk) and addition of interleukin-6, C-reactive protein and white cell count as continuous variables. *The likelihood ratio test compares a goodness of fit between models with and without biomarker data. $p<0.05$ indicates that the model with biomarkers gives a significantly better fit of the data. † The Hosmer Lemeshow test compares the observed number of people with events to that predicted by the model. $p>0.05$ indicates that the model is well calibrated ‡AUROC =1 indicates perfect discrimination of a model between patients with good and bad outcomes. $p<0.05$ indicates that the model containing biomarkers has a significantly higher AUC than one without.

Table 6.4 Risk stratification tables to assess the clinical significance of added predictive value of IL-6 to the six simple variable model

	Predicted risk of poor outcome from 6 simple variable model + IL- 6				Total % reclassified
Predicted risk from 6 simple variable model	**<10%**	**10-50%**	**50-90%**	**>90%**	
<10%					
Patients (n)	14	5	-	-	-
% reclassified	-	26	-	-	26
Observed % poor outcome	14	20	-	-	-
10-50%					
Patients (n)	19	534	4	-	-
% reclassified	3	-	7	-	4
Observed % poor outcome	11	20	75	-	-
50-90%					
Patients (n)	-	4	137	10	-
% reclassified	-	3	-	7	9
Observed % poor outcome	-	50	69	90	-
>90%					
Patients (n)	-	-	4	13	-
% reclassified	-	-	23	-	23
Observed % poor outcome	-	-	100	92	-
Total					
Patients (n)	33	543	145	23	-
Observed % poor outcome	12	20	70	91	-

Table 6.5 Table of studies included in the systematic review

Study	Stroke Diagnosis	Markers Measured	Blinding	Defined Enrollment Period	Adequate description of measurement	Mean Age	Male %	Outcome	N, All(Poor Outcome)	Covariates in model
Blanco 2006	Clinical supported by imaging	IL6, L-arginine, TNF, Glutamate, GABA,Fibrinogen	?	Yes	Yes	70	58	Poor outcome 3 months	113 (36)	HBP,Age,SBP,Temp, Glucose,CSS,Arginine
Welsh 2009	Clinical supported by imaging	IL6, CRP, IL18, TNF alpha, D dimer	?	Yes	Yes	69	53	Poor outcome 1 month	219 (94)	Age OCSP SSS score CRP IL18 TNF
Chamarro 2007	Clinical supported by imaging	IL6, Normetanephrines	?	No	No	74	43	Death 3 months	136 (16)	NIHSS Infection Neutrophils Monocytes Normetanephrines
Rallidis 2006	Positive imaging only	IL6, CRP, Serum Amyloid A	?	Yes	Yes	54	65	Death in hospital	203 (14)	Age Sex BMI HBP Cholesterol DM Smoking CRP Serum Amyloid A
Whiteley 2009	Clinical supported by imaging	IL6, CRP, fibrinogen	Yes	Yes	Yes	72	53	Poor outcome 1 month	844 (238)	Lives alone, independent prior to stroke, age, able to walk, lift arms, talk

IL6=interleukin 6, IL-18-interleukin 18, TNF=tumour necrosis factor alpha, GABA=gamma-amino-butyric acid, SBP=systolic blood pressure, HBP=high blood pressure, CSS=Canadian stroke scale, SSS=Scandinavian stroke scale, NIHSS=National Institutes of Health Stroke Scale, OCSP=Oxfordshire Community Stroke Project classification,

All studies were prospective, inpatient based studies of patients with ischaemic stroke and drew blood soon after stroke. No study examined unselected admissions of patients with stroke

Figures

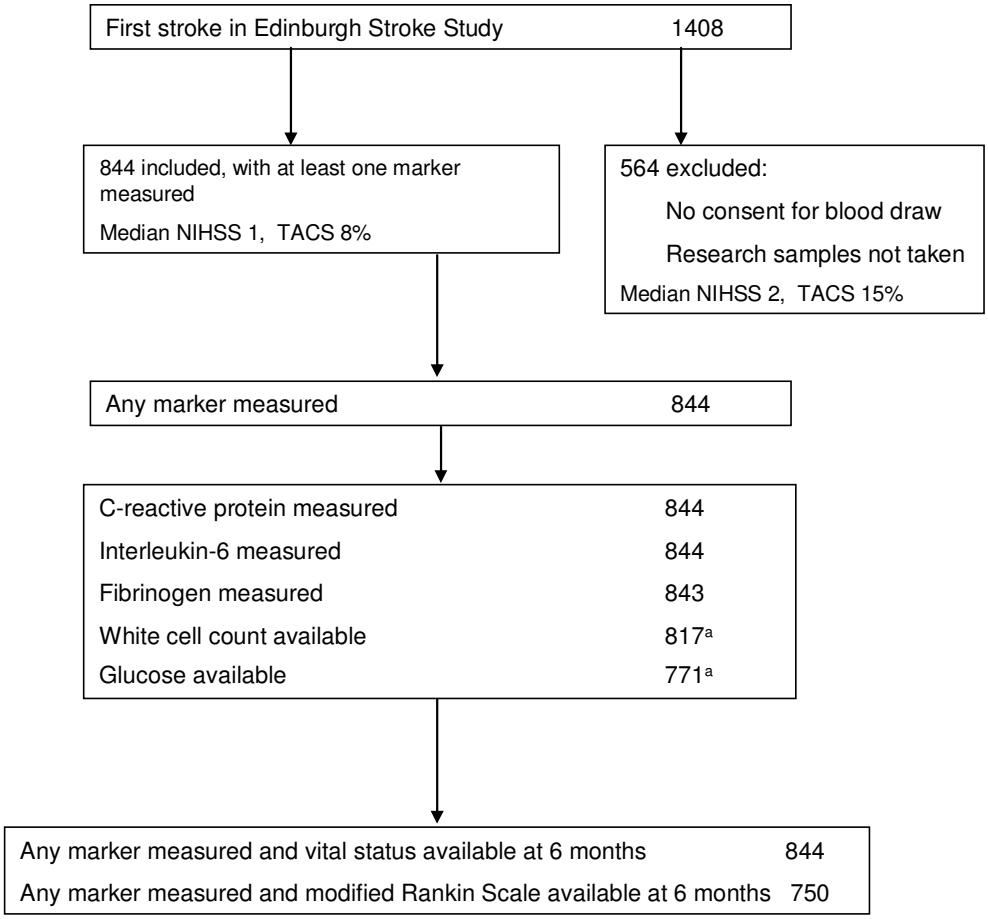


Figure 20 Flowchart of data available in the Edinburgh Stroke Study.

[a] Results are incomplete for glucose and white cell count, as for outpatients these results were sometimes reported to the general practice rather than the central results database.

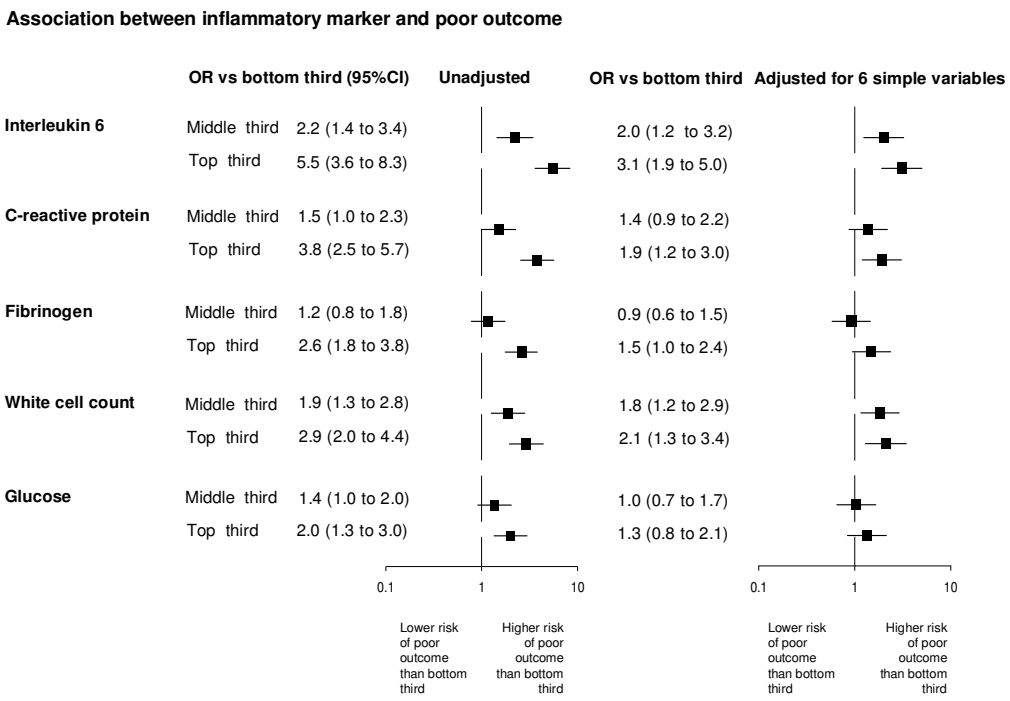


Figure 21 Association between levels of inflammatory marker vs poor outcome (mRS >2 or death) in the Edinburgh Stroke Study.

Expressed as ratio of odds in middle and top thirds of marker distribution, versus the referent lower third. Dotted line indicates OR=1 (i.e. same odds as lower third). OR are reported unadjusted and adjusted for six simple variables (age, living alone, independent of activities of daily living prior to stroke, normal verbal GCS, able to lift arms from bed, able to walk). Tertiles of: IL-6, 2.8 and 5.5 pg/l, CRP: 1.9 and 7.1 mg/l, fibrinogen: 4.1 and 5.1 g/l, white cell count: 7.0 and 9.1 x109 cell/l, and glucose: 5.2 and 6.3 mmol/l.

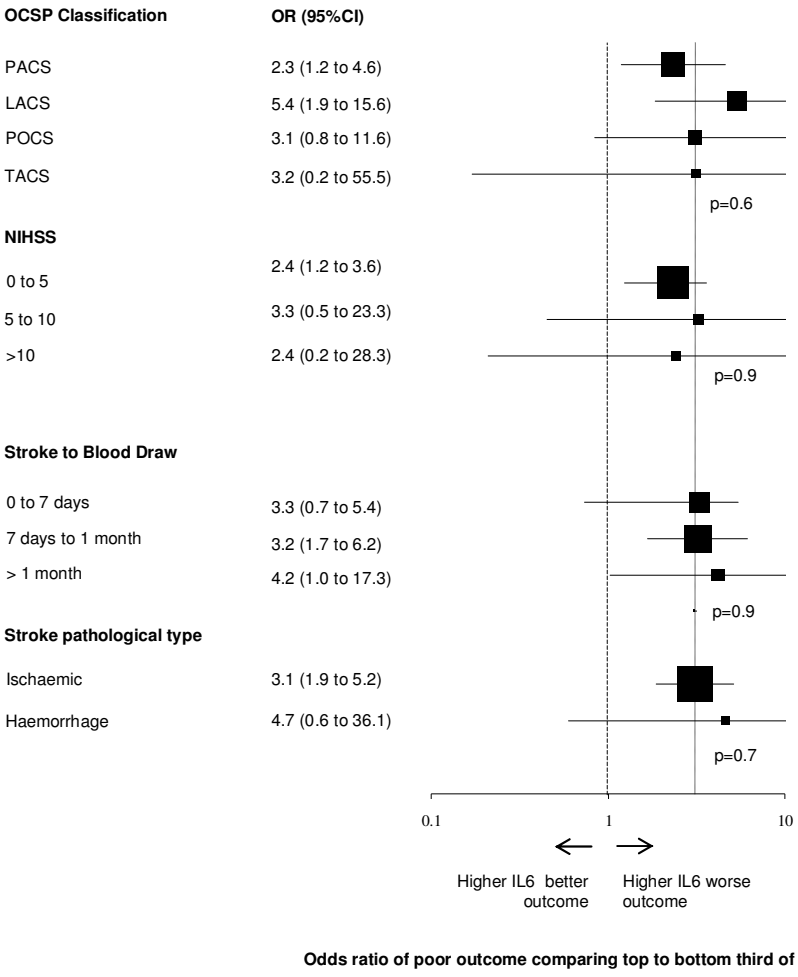


Figure 22 Association between upper third and lower third of interleukin 6 by subgroups in the Edinburgh Stroke Study.

Each OR is adjusted for the six simple variables (age, living alone, independent of activities of daily living prior to stroke, normal verbal GCS, able to lift arms from bed, able to walk), and the estimate for the whole cohort is given by the vertical dashed line. OR of >1 indicates that increased levels of marker are associated with poorer outcome in that category of patient. P values are derived from tests for heterogeneity.

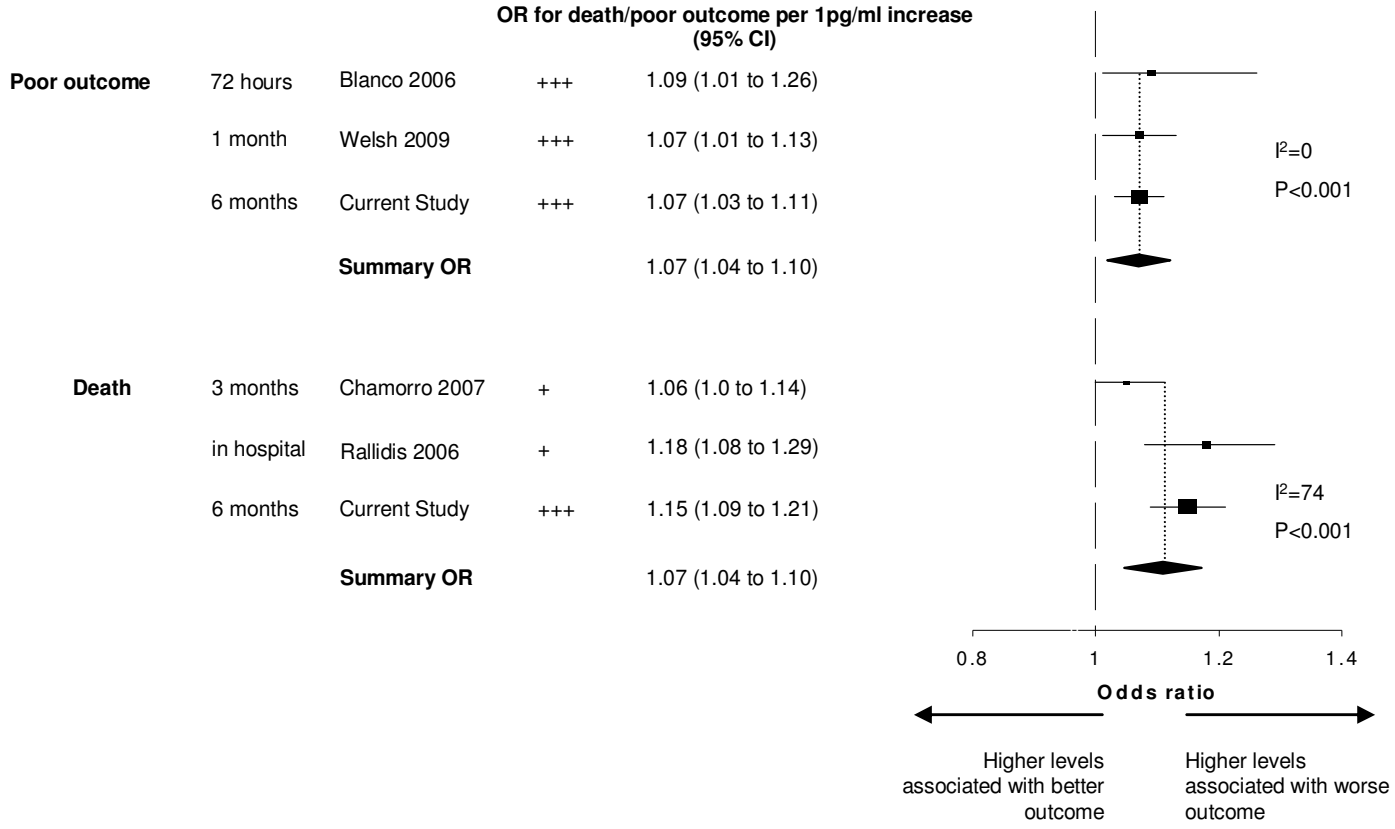


Figure 23 Systematic review and meta-analysis of studies of interleukin 6 (IL-6) with available data OR for death or poor outcome is presented per unit increase in marker levels.

Sizes of squares are proportional to the number of patients in each study. Summary estimates are calculated by fixed effects meta-analysis. P values show statistical significance of summary estimate of effect and I^2 is reported as a measure of heterogeneity between studies used to calculate the summary OR. += adjusted for age or stroke severity, ++= adjusted for age and stroke severity, +++= adjusted for age, stroke severity and other factors

Chapter 7. Plasma and serum markers of inflammation, thrombolysis, thrombosis, cardiac strain, neural and glial damage and poor outcome after acute cerebrovascular disease: BBISS, a prospective cohort study

Introduction

My systematic review of the association between blood biomarkers and the risk of poor outcome after stroke (Chapter 5) showed that many of these studies were either small, did not adjust for neurological impairment or age, or used optimised thresholds which can over-estimate the association between a marker and clinical outcome. Although many of these studies proposed that blood markers might therefore be useful in predicting poor outcome after stroke, none examined whether the addition of a markers to a validated prognostic scores added a useful degree of predictive ability.

Age and clinical measures of neurological impairment, for example inability to walk, talk or lift both arms, are strongly associated with poor outcome after stroke. These clinical variables may also be associated with higher levels of markers of inflammation, thrombolysis, thrombosis, cardiac strain, neural and glial damage, confounding any association between the level of blood markers and outcome. To assess the potential utility of markers as an aid to predicting prognosis in routine clinical practice, appropriate adjustment for these variables is therefore important.

In chapter 6 I have examined the additional value of CRP, IL-6 and white cell count to a validated clinical prognostic model in the ESS cohort, and found that only IL-6 improved the prediction of poor outcome after stroke, by an amount that was unlikely to be clinically important (Whiteley et al. 2009d). However, as blood was drawn at variable time after symptom onset (a median of 13 days) post-stroke, infection may have been responsible for some of the association in that study, and I was unable to make adjustment for symptoms of infection.

In this chapter, I will assess in my own BBISS cohort data whether blood markers of inflammation, thrombosis, thrombolysis, cardiac strain, cerebral damage add to variables in previously validated prognostic models for the prediction of poor outcome after stroke (Counsell et al. 2002a, Konig et al. 2008a).

I will therefore:

- Assess the univariate association of clinical variables at baseline with: (a) disability or death ('poor outcome') by 3 months; (b) death by 3 months; and (c) complete recovery by 24 hours among patients presenting to an emergency department with confirmed acute cerebrovascular disease.
- In this cohort, calculate the univariate association of 19 serum and plasma markers drawn less than 24 hours after stroke or TIA with: (a) poor outcome by 3 months; (b) death by 3 months; and (c) complete recovery by 24 hours.
- Adjustment for stroke severity, age, prior infection and other important covariates, to assess the impact on the association of plasma and serum markers with: (a) poor outcome by 3 months; (b) death by 3 months; and (c) complete recovery by 24 hours.
- Assess the improvement of the prediction of poor outcome at 3 month after stroke or TIA by the addition of serum and plasma markers to prediction models based on established clinical variables alone.

Methods

Cohort recruitment

I have described the recruitment of the cohort in detail in chapter 3 and the measurement of blood markers in chapter 4. In brief, I recruited patients presenting to an emergency department when an emergency department clinician suspected stroke or TIA in a symptomatic patient with symptoms of less than 24 hours duration. At the baseline assessment I collected measures of neurological impairment and co-morbidity. A gold standard diagnosis of confirmed stroke or

TIA ('acute cerebrovascular disease') was made by a panel of experts after considering the presentation, relevant imaging and clinical course of each patient, blinded to the results of blood marker levels. I drew blood as soon as patients were assessed in the emergency department and then at 24 hours after symptom onset, if that fell within normal working hours. Accredited research and clinical laboratories measured adiponectin, CRP, ICAM-1, IL-6, MMP-9, TNF-α, vWF, white cell count, neutrophil fraction, D-dimer, fibrinogen, tPA, NT pro-BNP, troponin T, tau, S100B, creatinine and glucose in serum and plasma blinded to clinical information.

Follow up

I followed up each patient at 24 hours and 3 months after symptom onset. At 24 hours, I spoke to each patient and asked them whether their presenting symptoms had resolved completely (yes or no). At 3 months after symptom onset I posted a questionnaire to each patient, based upon the Oxford Handicap Scale (Bamford et al. 1989a) (Table 7.1). The OHS (a modified Rankin Scale) though intended to measure handicap (participation), actually categorises levels according to a mixture of a description of symptoms and disability. It does, however, yield grades of recovery after stroke that have face validity and are easily understood. Conventionally, grades 0-2 describe people who are independent for activities of daily living, whereas individuals in grades 3-5 require increasing levels of help from other people.

If a patient failed to return their questionnaire by post, or provided an illegible or uninterpretable response, I made a telephone call to them or their carer. I used a structured interview to measure the OHS over the telephone (Wilson et al. 2002). I ascertained vital status by contacting the patient's GP at 3 months after onset (as normally a general practitioner is informed more quickly of death than the General Register Office (Scotland)). For this analysis, I classified patients who were dead or OHS 3-5 as poor outcome and OHS 0-2 as good outcome.

Statistical analysis

Association between clinical variables with poor outcome at 3 months, death at 3 months and recovery within 24 hours

I assessed the association of baseline clinical variables with poor outcome at 3 months, death at 3 months and recovery at 24 hours by calculating odds ratios and their 95% confidence intervals with simple logistic regression analysis (`logistic`).

Linearity of the association between serum and plasma markers with poor outcome at 3 months and recovery at 24 hours

Logistic regression analysis assumes a linear relationship between continuous variables and the log-odds of poor outcome. I tested whether the relationship between blood markers with poor outcome was a log-linear relationship at 3 months and 24 hours. I used likelihood ratio tests to compare models built with a restricted cubic spline transformation of blood marker variables with models built with blood markers as linear variables. Where relationships were significantly nonlinear, I plotted transformations of marker levels against predicted probabilities of poor outcome and chose a variable transformation that fitted the data well on inspection. I further tested these transformations to ensure linearity with model specification link tests (`linktest`). I used linear logistic regression (`logistic`) to estimate the association between blood markers (transformed where necessary) with poor outcome at 3 months and 24 hours after symptom onset.

Univariate associations between marker levels with poor outcome at 3 months, death at 3 months and recovery at 24 hours after symptom onset

I examined the association between poor outcome at 3 months, death at 3 months and recovery at 24 hours with a series of univariate logistic regression analyses, and report odds ratios as a measure of association, 95% confidence intervals as a measure of uncertainly and the Wald test *P* values to test the null hypothesis that OR = 1.

Adjusted associations between marker levels with poor outcome at 3 months, death at 3 months and recovery at 24 hours after symptom onset

I adjusted the association between blood markers and different outcome events (poor outcome, death and recovery at 24 hours) for baseline neurological impairment, measured using the NIHSS score, and age in a series of multivariable logistic regression analysis. I then made further adjustment ofor baseline handicap, and in addition for inflammatory markers I made adjustment for previous symptoms of infection and for cardiac markers current or previous AF, cardiac failure and previous cardiac vascular disease.

The additional prognostic utility of blood markers to clinical prognostic variables, for predicting poor outcome after acute cerebrovascular disease

I first applied the variables, recorded at baseline, that were part of 2 previously described models to my cohort with logistic regression to determine the predicted probability of death or dependency. One model incorporated six baseline variables (age, prior dependence in activities of daily living, ability to lift both arms from the bed, ability to walk without assistance, living alone at the time of the event, and orientation in time and person) (Counsell, Dennis, & McDowall 2004b), and the other incorporated NIHSS and age (Konig et al. 2008d). I then added each of the blood markers that were strongly associated with poor outcome, independent of age or NIHSS, (i.e. IL-6 and NT pro-BNP) to each of the clinical models. I allowed the coefficients of each variable to vary, rather than applying the coefficients from a previous cohort. This gave blood markers and clinical variables a more even chance of success in this cohort. I tested all two way interactions (`fitint`) within each model, then to adjust for different sampling times after stroke onset examined for interactions between marker levels with time to blood draw.

I evaluated the added value of each blood markers to models constructed with clinical variables alone by measuring goodness of fit, discrimination, model calibration and reclassification.

Goodness of fit

(i) I compared the likelihood of nested logistic regression models with and without a biomarker variable with the likelihood ratio statistic (`lrtest`), and report the associated *P* value with reference to the χ^2 distribution.

(ii) I used the `fitstat` command to calculate Akaike's information criterion (AIC) for nested models with and without blood markers.

$$AIC = -2 * \log_e(\text{likelihood}) + 2k$$

Where k= degrees of freedom

AIC measures both fit i.e. -2 Ln (likelihood) and complexity i.e. number of degrees of freedom. Of two models, that with the lower AIC is generally considered better (McGeechan et al. 2008b).

Model discrimination

(iii) I used the predicted probability of poor outcome from each model to calculate the area under receiver operator curves (AUROC) (`roctab`). I compared the AUROC with the `roccomp` command which uses a non-parametric method (DeLong, DeLong, & Clarke-Pearson 1988).

Consider a pair of patients, one randomly selected from patients with a poor outcome, and the other randomly selected from patients with a good outcome. The AUROC can be understood as the probability that the patient with a poor outcome has a higher predicted probability (from one of the logistic regression models) of poor outcome than a patient with a good outcome, with a value of 1 indicating excellent discrimination and a value of 0.5 no better discrimination than chance (McGeechan et al. 2008a). The calculation of the AUROC is based on ranking predicted probabilities of poor outcome, rather than measuring the absolute difference. Therefore the contribution to the AUROC of a pair of patients with predicted risks 1% apart is the same as a pair with

predicted risk 20% apart. It is unlikely that a risk difference of 1% is clinically important, though one of 20% may be.

(iv) The integrated discrimination index (IDI) was developed provide an estimate of the mean improvement in predicted probability when evaluating the additional value of a new marker (Pencina et al 2008). It measures the extent to which a new marker adds a model's ability to improve average sensitivity without sacrificing average specificity. The IDI is calculated as follows:

$$IDI = \left(\overline{p}_{new,poor} - \overline{p}_{new,good}\right) - \left(\overline{p}_{original,poor} - \overline{p}_{original,good}\right)$$

Where *p* is the maximum likelihood estimation of predicted probability, *new* indicates a model with biomarkers, *original* is a model with clinical variables only, *poor* indicated poor outcome and *good* indicates good outcome.

As the magnitude of the IDI is often extremely small, a relative, rather than absolute index may be calculated (Pencina et al 2008):

$$RIDI = \frac{\left(\overline{p}_{new,poor} - \overline{p}_{new,good}\right)}{\left(\overline{p}_{original,poor} - \overline{p}_{original,good}\right)} - 1$$

A simple interpretation of the integrated discrimination improvement is that it measures the average difference in predicted outcome between patients with good and poor outcome, for a model containing biomarkers over a model based upon clinical variables alone.

Model calibration

(v) I used the Hosmer-Lemeshow test as a measure of model calibration (`hl`). This test divides the sample into deciles of predicted probability and performs a χ^2 test, comparing the difference between the observed and expected number of patients with poor outcome in each decile. Where the χ^2 is non-significant, the model may fit the data well and is said to be well calibrated.

I plotted the observed against predicted risks in a series of graphs, to visualize where calibration problems might arise.

Reclassification

(vi) The most important measure of the utility of a new marker is whether it improves the prediction of poor outcome to a clinically important degree. One way of measuring this is to determine the number of people who, as a result of the information from the new marker, are moved correctly between strata of predicted prognosis that are considered 'clinically significantly different'. Before calculating measures of reclassification, I defined the clinically important threshold as a predicted probability of poor outcome of >90%, >50% and >10%, in discussion with senior stroke physicians. The measure I chose for reclassification was the net reclassification index (Pencina et al 2008). The NRI is calculated from four probabilities:

$$p(\text{up} \mid \text{outcome} = \text{poor}) = \frac{N(\text{poor outcome, moving up})}{N(\text{poor outcome})} \quad (1)$$

$$p(\text{down} \mid \text{outcome} = \text{poor}) = \frac{N(\text{poor outcome, moving down})}{N(\text{poor outcome})} \quad (2)$$

$$p(\text{up} \mid \text{outcome} = \text{good}) = \frac{N(\text{good outcome, moving up})}{N(\text{good outcome})} \quad (3)$$

$$p(\text{down} \mid \text{outcome} = \text{good}) = \frac{N(\text{good outcome, moving down})}{N(\text{good outcome})} \quad (4)$$

The NRI is then calculated from the probabilities (1) to (4):

$$\text{NRI} = (p(\text{up} \mid \text{outcome} = \text{poor}) - p(\text{down} \mid \text{outcome} = \text{poor})) - (p(\text{up} \mid \text{outcome} = \text{good}) - p(\text{down} \mid \text{outcome} = \text{good}))$$

The limitation of the NRI are: (a) it depends heavily on the choice of cut-points, (b) gives equal weight to individuals moving up or down across thresholds (c) does not account for patients who move over more than one threshold value.

(vii) Because of the problems with the NRI, I plotted the predicted risk from models with blood markers against the predicted risk of models based on clinical variables alone. I gave some measure of reclassification by highlighting those patients whose predicted probability of poor outcome changed by >10% with the addition of blood markers, and whether the change improved or worsened the categorization.

Statistical significance

I performed all statistical analysis using Stata 10, StataCorp 2009. Each table, with 19 markers therefore tests 19 hypotheses. There is a risk of type I error. The Bonferroni correction for multiple comparisons becomes overly conservative where more that 5 hypotheses are tested, so I considered a *P* value of less than 0.01 (i.e. 0.05/5) to be statistically significant.

Results

I recruited 285 patients with confirmed acute cerebrovascular disease and obtained complete follow up data for all patients for 'recovery at 24 hours' and for death at 3 months. For 283 (99%) patients I had data for handicap at 3 months. I made a baseline assessment of patients at a median of 6.2 hours after the onset of their symptoms. At 3 months after symptom onset, 88 (31%) patients were dependent on others for their activities of daily living and 35 had died (12%). At 24 hours after symptom onset, 40 (14%) of patients with acute cerebrovascular disease reported a complete recovery.

The association of clinical features with poor outcome at 3 month, and complete recovery by 24 hours

Neurological impairment: Patients with more severe neurological deficits at baseline had an increased risk of poor outcome (Table 7.2) or death at 3 months (Table 7.4) and a reduced chance of recovery at 24 hours (Table 7.4) after symptom onset. For each 3 point increase in the NIHSS score, there was a doubling of the odds of poor outcome (OR=2.00, 95% CI: [1.65 to 2.4]), and an approximately 50%

increase in the odds of death (1.58 [1.40 to 1.79]). Figure 24 summarises the non-linear relationship between NIHSS and recovery at 24 hours. A series of dichotomous variables of neurological impairment (orientation to time, place and person, the ability to lift the arms from the bed and the ability to walk) were each strongly associated with increased odds of poor outcome or death; there were negative relationships of a similar strength with recovery by 24 hours.

Age: Increasing age increased the odds of poor outcome (Table 7.2) or death ar 3 months (Table 7.3) in older patients. The odds of poor outcome or death approximately doubled for each decade increase in age. The observed reduction in the odds of recovery at 24 hours with increasing age was not statistically significant (Table 7.4).

Comorbidities: Atrial fibrillation, discovered either before, or at the time of admission to hospital was present in about a quarter of patients, and associated with approximately a tripling of the odds of poor outcome (Table 7.2) or death by 3 months (Table 7.3). Prior cognitive impairment (14% of those with acute cerebrovascular disease) was also very strongly associated with poor outcome. The associations between poor outcome with AF or cognitive impairment were confounded by age and neurological impairment. After adjustment for the NIHSS and age, the association with AF (OR=1.47, 95% CI [0.74 to 2.89]) was attenuated and no longer statistically significant, though cognitive impairment remained a strong predictor (5.40 [1.88 to 15.56]).

I could not exclude a modest positive (or negative) association between death, poor outcome or recovery at 24 hours with history of prior stroke or TIA, MI or angina, heart failure or diabetes; for most of these dichotomous variables, the observed positive association was weak.

Haemorrhagic stroke: The number of patients with intracerebral haemorrhage (n=13) or subarachnoid haemorrhage (n=2) was small; 8/15 of these haemorrhagic strokes had a poor outcome, 6/15 died and none recovered by 24 hours after onset.

Ischaemic stroke syndrome: Almost all patients with the clinical syndrome of total anterior circulation infarction were dead or dependent at 3 months (40/43, 93%) though one 81 year old woman made a remarkable recovery at 24 hours after presenting with left hemianopia, moderate hemiparesis and hemi-inattention. Most patients with clinical syndrome of lacunar infarction had a good recovery (55/75, 73%) and only 1 died.

Brain imaging: The presence of any visible stroke lesion (haemorrhagic or ischaemic) on brain imaging was not statistically significantly associated with poor outcome or death, though the absence of a lesion was associated with recovery at 24 hours.

Reperfusion therapy: Only 7 patients were treated with rtPA (6) or had a neuron-interventional intra-arterial clot retrieval procedure (1), of whom 5 had a poor outcome.

Physiological variables at baseline: I found no evidence of an association between admission systolic or diastolic blood pressure and poor outcome, death or recovery at 24 hours. A lower admission temperature was associated with poor outcome at 3 months, an association that remained robust to adjustment for age, neurological impairment and delay to admission (OR=0.52 95% CI [0.31 to 0.87], per ^{o}C increase in body temperature).

Linearity of association between serum and plasma markers and outcome at 24 hours and 3 months

The relationship between plasma and serum marker levels and poor outcome at 3 months was approximately linear in univariate analysis for: adiponectin, CRP, ICAM-1, TNF-α, IL-10, vWF, white cell count, fibrinogen, tau, creatinine, glucose, age and NIHSS (data not shown). Models built with restricted cubic splines of serum and plasma markers had a better fit than a linear model for the markers: IL6 (LR test P=0.049), D-dimer (P=0.002), tPA (P=0.004), NT pro-BNP (P=0.002) and S100B (P=0.03). After examining plots of these markers against predicted probabilities from restricted cubic spline models, I decided to keep IL-6 and tPA as a

linear predictors and used the natural logarithms of S100B, NT pro-BNP and D-dimer.

The relationship between plasma and serum marker levels and poor outcome at 24 hours was approximately linear in univariate analysis for: adiponectin, CRP, ICAM-1, IL-6, TNF-α, IL-10, white cell count, D-dimer, fibrinogen, tPA, tau, S100B, creatinine and age. Models built with restricted cubic splines of untransformed serum and plasma markers had a better fit than a linear model for the markers: vWF (LR test P=0.02), NT pro-BNP (P=0.006), glucose (P=0.04) and NIHSS (P<0.0001). After examining plots of these markers against predicted probabilities from restricted cubic spline models, I decided to keep vWF as a linear predictor and took the natural logarithms of NT pro-BNP. I examined a threshold of an NIHSS of 5 to predict recovery by the next day (Figure 24).

Association between markers and poor outcome or death at 3 months and recovery at 24 hours

The pattern seen for most markers, though of differing strength and boundaries of uncertainty, was for positive associations with poor outcome and death and negative associations with recovery at 24 hours, associations that attenuated after adjusting for neurological impairment and age (Figure 25). Because few patients died at 3 months or recovered at 24 hours, there is much uncertainty about the magnitude of the association between markers levels and these outcomes in this cohort. These data are summarized in Table 7.6, Table 7.7 and Table 7.8.

Inflammatory markers: Higher levels of inflammatory markers were, in general, positively associated with increased odds of poor outcome and of death at 3 months. Associations were slightly stronger with death than with poor outcome, though for each individual marker this difference could be explained by chance. Of the markers of inflammation, IL-6 had the strongest association with poor outcome and death at 3 months, and recovery by 24 hours, associations that attenuated after adjusting for neurological impairment and age. Of the other markers, only the association between adiponectin and death remained statistically significant (Wald

test *P*=0.007) after making adjustment for age and severity of neurological impairment.

Markers of thrombosis: Higher plasma levels of log-transformed D-dimer were positively associated with increased odds of both poor outcome and death alone, and reduced odds of recovery at 24 hours. These associations attenuated after adjustment for neurological impairment and age for each outcome, and remained strong (OR=4.07, 95% CI:[1.96 to 8.44]) only for death.

Tissue plasminogen activator: Higher levels of tPA were associated with death, an association that remained after adjustment for neurological impairment and age. After adjustment, no other associations were either strong or statistically significant.

Cardiac strain: Both a higher blood level of log-transformed NT pro-BNP and a detectable troponin T were associated with higher odds of poor outcome or death. Only lower levels of NT pro-BNP were associated with recovery at 24 hours after symptom onset. Note, however, that troponin T was only present in 52/368 patients, and was therefore analysed as a yes/no variable.

Cerebral damage: Neither S100B nor tau were associated with poor outcome or death at 3 months or recovery at 24 hours.

Creatinine and glucose: After adjustment, only the association between glucose and death remained statistically significant.

The addition of blood markers to variables from clinical prognostic models

I tested the addition of markers that were significantly associated with poor outcome to variables in two clinical models to predict poor outcome at 6 months. These markers were interleukin-6 and NT pro-BNP (Table 7.10). The final models are shown in Table 7.9. There were no statistically significant two way interactions (*P*>0.05) between marker levels and time to blood draw (when it was forced into the final model), stroke severity or age.

The six simple variable model and the 'NIHSS and age' model were well calibrated in this dataset, and showed good discrimination measured by the AUROC. The addition of either IL-6 or NT pro-BNP to either of these models based on clinical variables led to an improvement in model fit, when measured either by the likelihood ratio statistic or Akaike's information criterion. All the models were well calibrated; examples of calibration plots are shown for IL-6 in Figure 27 and Figure 26. However, the addition of either IL-6 or pro NT-BNP either to a model with six simple variables or one based on the NIHSS and age improved the AUROC by a very small amount (0.01) which may have been due to chance. There was a small integrated discrimination improvement that was a little more for models with IL-6 than those with pro NT-BNP. A model with interleukin-6 better reclassified 5% of patients across clinically relevant borders when compared to a model with six simple variables alone, though this may have been due to chance; the addition of IL-6 to NIHSS and age, and the addition of NT pro-BNP either set of variables slightly worsened classification of patients by a small amount that was not statistically significant. Figure 28 to Figure 32 confirm the findings from inspection of the summary statistics: few patients were reclassified by an amount greater than 10% of the predicted probability with the addition of IL-6 or NT pro-BNP; there was no clear advantage in accuracy of classification and no patients were reclassified across the clinically relevant bounds of <10% and >90% predicted probability of poor outcome.

Discussion

Principal findings

In patients with acute cerebrovascular disease, markers of inflammation, thrombosis, thrombolysis, and cardiac strain were associated with an increased risk of poor outcome at 3 months. Adjustment for neurological impairment at baseline and age substantially attenuated these associations. After adjustment, and with correction for multiple comparisons, there was robust evidence of an independent and positive association between both NT pro-BNP and IL-6 with poor outcome.

However, the results were also compatible with all of the markers (with the exception of IL-10 and tau), having a similar, though small, positive association with poor outcome.

There was also good evidence that higher levels of D-dimer, adiponectin, tPA, pro NT-BNP, troponin T and glucose were associated with an increased risk of death by 3 months. None of the markers was associated with an increased chance of recovery at 24 hours after symptom onset, after adjusting for neurological impairment and age.

However, despite their strong association with poor outcome, neither IL-6 nor pro NT-BNP improved the prediction of poor outcome beyond that of validated prognostic models based on clinical variables alone.

Strengths and weaknesses of the study

Study power

The estimates of the odds ratios often had bounds of uncertainty that were compatible with a moderately strongly positive or negative association between blood markers and poor outcome. It is likely that a larger sample size would have reduced this uncertainty. However, for those markers that could have been positively or negatively associated with poor outcome, the upper bound of the confidence interval was in general no greater than OR=2.5 and the lower bound no less that OR=0.5. These only moderate association indicate these markers are unlikely to represent clinically useful discriminators (Pepe et al. 2004a). The problem of sample size was compounded by missing values for some blood markers; though these were missing at random, and so unlikely to lead to an important selection bias, they did lead to loss of power in multivariable logistic regression analysis. Despite this, the study was larger than most of the previous studies of the association between serum or plasma markers and poor outcome after stroke (Chapter 5)(Whiteley et al. 2009b).

I measured outcome with the Oxford Handicap Scale, an ordinal scale. To increase power, I could have analysed the data with ordinal logistic regression analysis, rather than dichotomising the OHS into patients who were alive and independent and patients who were dead or dependent. However, ordinal logistic regression assumes that relationships are parallel with each step on the ordered outcome scale; here the relationship between OHS and most markers violated this assumption.

Case Mix

The strokes in this study may have been mild in comparison to other studies, though they are representative of stroke patients presenting to an emergency department. I found no evidence that associations between blood markers and poor outcome were different in patients with different levels of neurological impairment: two way interactions between stroke severity and marker levels did not significantly improve the fit of models to predict poor outcome at 3 months after stroke. However, tests for interaction have a relatively lower power, so larger studies would be needed to be absolutely certain that the associations between blood marker levels and outcome were not different in patients with low and high neurological impairment.

Causality

This study was designed to obtain empirical evidence on whether blood markers have clinical utility as predictive tools, irrespective of the mechanism of the association. This study therefore did not aim to elucidate the causal role of particular molecules or physiological pathways. Although there are many plausible hypotheses for an aetiological role of higher levels of each marker with poor outcome after acute cerebrovascular disease, 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.

First, as I drew blood samples some time after symptom onset, the key processes responsible for poor outcome that start within minutes of symptom onset may

induce a rise in a particular blood marker level, yet have no relationship to the physiological process that the marker purports to measure (e.g. thrombosis and D-dimer). This 'reverse causality' may explain some of the associations between marker level and poor outcome. However, I was unable to demonstrate an interaction between the observed associations and time to blood draw from symptom onset, which might be expected if reverse causality were an important explanation.

Second, the choice of confounding variables used for adjustment can colour the interpretation of the adjusted analyses. Here I adjusted univariate associations for neurological impairment and age, as these are powerful predictors of poor outcome. The adjusted odds ratios are therefore a measure of the effect of markers on poor outcome over and above the effect of neurological impairment and age. However, if a particular physiological process acted solely through greater neurological impairment (hence higher NIHSS) this adjustment would lead to an attenuation of an important association.

Third, many authors assume that there are good markers of particular physiological pathways- i.e. 'biomarkers'. However, as many markers rise with a number of different physiological processes – for example the acute inflammatory response lead to increased levels of putative markers of both inflammation and thrombosis – it is difficult to draw any conclusions about the role of broad categories of markers such as 'inflammation' or 'thrombosis' and poor outcome.

Measurement of outcome

I used the Oxfordshire Handicap Scale to measure impairment in this study. There have been concerns about the reliability of this scale, usually in face to face interview, but also through postal questionnaire. Some patients were followed up by telephone and others by post; as it is likely that postal responders were in better health than telephone responders, potentially there is a difference in the accuracy of measurement of outcome between patients followed up by telephone and those

followed up by post. Also, as measurement of disability was made by the patients themselves, it was not blinded to the severity of the stroke, though was blind to marker levels. However the marker levels and stroke severity are closely related. However, as the direction of all association was in the same direction for death as well as poor outcome, I believe that these associations are robust.

Strengths

This study met almost all the methodological criteria for a reliable study of prognosis (Tugwell P & Sackett D 1981): (i) I assembled an inception cohort at an early and uniform point in the course of the illness and included subjects with a range of disease severities; (ii) I avoided diagnosis access bias, as I made great efforts to assess all patients with suspected stroke; (iii) I achieved almost complete follow up; (iv) I used objective criteria for determining outcome, which were validated and accurate; (iv) I adjusted analyses for other important prognostic variables and confounding factors; and (iv) measured markers blinded to outcome in established research and clinical laboratories with low coefficients of variation.

Unanswered questions and future research

The most remarkable and consistent association in this study was the association between levels of the cardiac markers NT pro-BNP and troponin T, even after adjusting for prior cardiac disease, heart failure and atrial fibrillation. The reason for this association is unclear. Possible explanations are an association between cardiac markers and arrhythmia, or with cardiac dysfunction at the time of acute cerebrovascular disease, or unmeasured cardiac disease.

Implications for research

- The markers NT pro-BNP and IL-6, although they are associated with poor outcome, independent of neurological impairment (measured with the NIHSS) or age, did not help to improve the prediction of poor outcome.
- Further research may identify blood markers which do provide clinically useful predictive power for 'poor outcome'. However, it may be more

fruitful to explore their role in predicting specific events (e.g. recurrent ischaemic stroke or DVT).

Implication for practice

- None of the markers measured in this thesis can be recommended for the prediction of poor outcome at 3 months after presentation with acute cerebrovascular disease.

Tables

Table 7.1 The Oxford Handicap Scale

Handicap level	Description of impact on daily life	Grade
none	no change	0
minor symptoms	no interference	1
minor handicap	some restrictions but able to look after self	2
moderate handicap	significant restriction; unable to lead a totally independent existence (requires some assistance)	3
moderate-to-severe handicap	unable to live independently but does not require constant attention	4
severe handicap	totally dependent; requires constant attention day and night	5

Table 7.2 The association between baseline clinical features and poor outcome (dead or dependent on other for activities of daily living) at 3 months after presentation with acute cerebrovascular disease

Baseline characteristic	All (n=283)	Good outcome (n=160)	Poor outcome (n=123)	Odds ratio (95% CI) Poor vs good outcome	*P* value
Male sex (n, %)	135 (47.7)	86 (53.8)	49 (39.8)	0.66 (0.44 to 0.99)	0.045
Age (years) (mean, SD)	74.4 (12.5)	70.6 (12.6)	79.4 (10.4)	1.96 (1.54 to 2.49)*	<0.001
Fellow collected variables	n (%)	n (%)	n (%)		
Symptoms of infection	27 (9.5)	11 (6.9)	16 (13.0)	2.03 (0.9 to 4.5)	0.090
Prior cardiac vascular disease	67 (23.7)	36 (22.5)	31 (25.5)	1.16 (0.67 to 2.01)	0.601
Prior peripheral vascular disease	16 (5.7)	5 (5.7)	7 (5.7)	1.01 (0.37 to 2.81)	0.978
Prior TIA or stroke	76 (26.9)	34 (21.3)	42 (34.2)	1.92 (1.12 to 3.27)	0.016
Prior heart failure	22 (7.9)	8 (5.1)	14 (11.6)	2.45 (0.99 to 6.05)	0.052
AF (prior or during ED assessment)	74 (26.2)	26 (16.3)	48 (39.0)	3.30 (1.89 to 5.74)	<0.001
Diabetes mellitus	37 (13.0)	20 (12.5)	17 (13.8)	1.12 (0.56 to 2.25)	0.744
Prior cognitive impairment	39 (13.8)	6 (3.8)	33 (26.8)	9.35 (3.8 to 23.2)	<0.001
Recovery by 24 hours	40 (14.1)	34 (21.3)	6 (4.9)	0.19 (0.08 to 0.47)	<0.001
Simple variables					
Independent prior to admission	246 (86.9)	156 (97.5)	90 (73.2)	0.07 (0.02 to 0.20)	<0.001
Living alone	96 (33.9)	53 (33.1)	43 (35.0)	1.09 (0.66 to 1.78)	0.661
Orientated to time place & person	208 (73.5)	144 (90.0)	64 (52.0)	0.12 (0.06 to 0.23)	<0.001
Able to lift both arms	180 (63.6)	131 (81.9)	49 (39.8)	0.15 (0.08 to 0.25)	<0.001
Able to walk without help	126 (44.5)	106 (66.3)	20 (16.3)	0.10 (0.06 to 0.18)	<0.001

Baseline characteristic	**All (n=283)**	**Good outcome (n=160)**	**Poor outcome (n=123)**	**Odds ratio (95% CI) Poor vs good outcome**	***P* value**
Pathological stroke subtype	n (%)	n (%)	n (%)		
Probably ischaemic	34 (12.0)	27 (16.9)	7 (5.7)	-	-
Definitely ischaemic	234 (82.7)	129 (80.6)	105 (85.4)	-	-
Intracerebral or subarachnoid bleed	15 (5.3)	4 (1.9)	11 (8.1)	3.83 (1.19 to 12.34)	0.024
OCSP classification, presentation†					
Total anterior circulation infarction	43 (16.0)	3 (1.9)	40 (35.7)	-	-
Partial anterior circulation infarction	114 (42.5)	72 (46.2)	42 (37.5)	-	-
Lacunar infarction	75 (28.0)	55 (35.3)	20 (17.9)	-	-
Posterior circulation infarction	18 (9.0)	18 (11.5)	6 (5.4)	-	-
Unclassifiable clinical syndrome	12 (4.5)	8 (5.1)	4 (3.6)	-	-
Imaging lesion					
Cortical infarction	123 (43.5)	57 (35.6)	66 (53.6)	-	-
Lacunar infarction	40 (14.1)	29 (18.1)	11 (8.9)	-	-
Posterior circulation infarction	21 (7.4)	14 (8.8)	7 (5.7)	-	-
>1 territory infarction	4 (1.4)	0	4 (3.2)	-	-
Intracerebral or subarachnoid bleed	15 (5.3)	4 (2.5)	11 (8.9)	-	-
No visible lesion	74 (26.1)	54 (33.8)	20 (16.2)	-	-
No scan	6 (2.1)	2 (1.3)	4 (3.3)	-	-
Any visible lesion	191 (67.5)	101 (63.1)	90 (73.2)	1.59 (0.95 to 2.67)	0.075

Table 7.2continued

Baseline characteristic	All (n=283)	Good outcome (n=160)	Poor outcome (n=123)	Odds ratio (95% CI) Poor vs good outcome	*P* value
Medications	n (%)	n (%)	n (%)		
Any antiplatelet agent	121 (42.8)	63 (39.4)	58 (47.2)	1.37 (0.85 to 2.21)	0.190
Warfarin	13 (4.7)	5 (3.1)	8 (6.7)	2.2 (0.70 to 6.90)	0.177
Antihypertensive	153 (54.1)	84 (52.5)	69 (56.1)	1.16 (0.72 to 1.58)	0.547
Statin	99 (35.5)	48 (30.2)	51 (42.5)	1.71 (1.04 to 2.81)	0.034
rtPA (intra-arterial or -venous)	7 (2.5)	1 (0.6)	6 (4.9)	8.15 (0.97 to 68.6)	0.054
Current smoker	65 (23.2)	40 (25.3)	25 (20.5)	0.76 (0.43 to 1.34)	0.344
Examination findings					
Normal pedal pulses	133 (47.3)	93 (58.1)	40 (33.1)	0.36 (0.21 to 0.58)	<0.001
Continuous variables	mean (SD)	mean (SD)	mean (SD)		
Systolic BP (mmHg)	156 (29.9)	159 (29.3)	153 (30.5)	0.93 (0.86 to 1.01)‡	0.080
Diastolic BP (mmHg)	86.0 (18.0)	87.4 (16.5)	84.1 (19.7)	0.90 (0.79 to 1.03)‡	0.126
Temperature (^{0}C)	36.4 (0.6)	36.5 (0.5)	36.3 (0.7)	0.55 (0.36 to 0.84)§	0.005
Continuous variables	median (IQR)	median (IQR)	median (IQR)		
Well to admission (hrs)**	6.3 (13.4)	6.3 (12.2)	6.0 (12.7)	0.98 (0.86 to 1.01)¶	0.192
Found to admission (hrs)**	4.2 (6.2)	5.1 (10.1)	2.7 (4.7)	0.95 (0.92 to 0.99)¶	0.005
Admission to stroke fellow (hrs)	0.9 (1.4)	0.9 (1.6)	0.9 (1.4)	1.01 (0.97 to 1.06)¶	0.507
NIHSS	4 (8)	2 (3)	8 (13)	1.26 (1.18 to 1.34)‖	<0.001

P values are derived from Wald tests, null hypothesis: OR=1. Prior cardiac vascular diseases are a history of angina, MI or cardiac revascularisation*per decade of age; † ischaemic probable and definite acute cerebrovascular disease only; ‡per 10 mmHg; §per ^{0}C rise; ¶per hour increase;‖ per unit increase **from last seen well, or when found

Table 7.3 The univariate association between baseline clinical variables and death at 3 months in patients with acute cerebrovascular disease

Baseline characteristic	All (n=285)	Alive 3 months (n=250)	Dead 3 months (n=35)	Odds ratio (95% CI) Dead vs alive	*P* value
Male sex (n, %)	136 (47.7)	125 (50.0)	11 (34.4)	0.46 (0.22 to 0.98)	0.043
Age (years) (mean, SD)	74.4 (12.4)	73.4 (12.5)	81.8 (1.54)	2.08 (1.40 to 3.09)	<0.001
Fellow collected variables	n (%)	n (%)	n (%)		
Symptoms of infection	27 (9.5)	22 (8.8)	5 (14.3)	1.72 (0.61 to 4.90)	0.086
Prior cardiac vascular disease	67 (23.1)	59 (23.6)	8 (22.9)	0.96 (0.41 to 2022)	0.923
Prior peripheral vascular disease	17 (6.0)	15 (6.0)	2 (5.9)	0.98 (0.21 to 4.46)	0.974
Prior TIA or stroke	76 (26.7)	62 (24.8)	14 (40.0)	2.02 (0.97 to 4.21)	0.060
Prior heart failure	22 (7.8)	17 (6.9)	5 (14.7)	2.33 (0.80 to 6.79)	0.121
AF (prior or during ED assessment)	86 (30.2)	68 (27.2)	18 (51.4)	2.83 (1.38 to 5.82)	0.005
Diabetes mellitus	37 (13.0)	29 (11.6)	8 (22.9)	2.26 (0.94 to 5.44)	0.069
Prior cognitive impairment	39 (13.7)	29 (11.7)	10 (28.6)	3.03 (1.32 to 6.95)	0.009
Recovery at 24 hours	40 (14.1)	39 (15.6)	1 (2.90)	0.16 (0.02 to 1.20)	0.074
Simple variables	n (%)	n (%)	n (%)		
Independent prior to admission	247 (89.7)	225 (90.0)	22 (62.9)	0.19 (0.08 to 0.41)	<0.001
Living alone	96 (33.7)	81 (32.4)	15 (42.9)	1.56 (0.76 to 3.21)	0.223
Orientated to time place & person	210 (73.7)	200 (80.0)	10 (28.6)	0.10 (0.04 to 0.22)	<0.001
Able to lift both arms	181 (63.5)	174 (69.6)	7 (20.0)	0.11 (0.04 to 0.26)	<0.001
Able to walk without help	127 (44.6)	123 (49.2)	4 (11.4)	0.13 (0.05 to 0.39)	<0.001

Baseline characteristic	All (n=285)	Alive 3 months (n=250)	Dead 3 months (n=35)	Odds ratio (95% CI) Dead vs alive	*P* value
Pathological stroke subtype	n (%)	n (%)	n (%)		
Probably ischaemic	34 (11.9)	32 (12.8)	2 (5.7)	-	-
Definitely ischaemic	236 (82.8)	207 (82.8)	29 (82.9)	-	-
Intracerebral or subarachnoid bleed	15 (5.3)	11 (4.4)	6 (17.1)	2.80 (0.84 to 9.34)	0.093
OCSP classification, presentation[†]					
Total anterior circulation infarction	43 (15.9)	27 (11.3)	16 (51.6)	-	-
Partial anterior circulation infarction	115 (42.6)	105 (43.9)	10 (32.6)	-	-
Lacunar infarction	75 (27.8)	73 (30.5)	2 (6.5)	-	-
Posterior circulation infarction	25 (9.3)	24 (10.0)	1 (3.2)	-	-
Unclassifiable clinical syndrome	12 (4.4)	10 (4.2)	2 (6.5)	-	-
Imaging lesion					
Cortical infarction	124 (43.5)	101 (40.4)	23 (65.7)	-	-
Lacunar infarction	40 (14.0)	39 (15.6)	1 (2.9)	-	-
Posterior circulation infarction	21 (7.3)	20 (8)	1 (2.9)	-	-
>1 territory	5 (1.8)	5 (2)	0	-	-
Intracerebral or subarachnoid bleed	15 (5.3)	11 (4.4)	4 (11.4)	-	-
No visible lesion	74 (26.0)	69 (27.6)	5 (12.3)	-	-
No scan	6 (2.1)	5 (2.0)	1 (2.9)	-	-
Any visible lesion	193 (67.2)	167 (66.8)	26 (74.3)	1.43 (0.64 to 3.20)	0.377

Table 7.3 continued

Baseline characteristic	All (n=285)	Alive 3 months (n=250)	Dead 3 months (n=35)	Odds ratio (95% CI) Dead vs alive	*P* value
Medications	n (%)	n (%)	n (%)		
Any antiplatelet agent	121 (42.5)	101 (40.0)	20 (57.1)	1.97 (0.96 to 4.02)	0.064
Warfarin	13 (4.6)	11 (4.5)	2 (5.9)	1.34 (0.28 to 6.33)	0.711
Antihypertensive	155 (54.4)	134 (53.6)	21 (60.0)	1.30 (0.63 to 2.67)	0.477
Statin	99 (35.2)	80 (32.4)	19 (55.9)	2.64 (1.28 to 5.47)	0.009
rtPA (intra-arterial or -venous)	7 (2.5)	4 (1.6)	3 (8.6)	5.77 (1.23 to 26.93)	0.026
Current smoker	65 (23.1)	58 (23.5)	7 (20.0)	0.81 (0.34 to 1.96)	0.648
Examination findings					
Normal pedal pulses	133 (47.0)	123 (49.6)	10 (28.6)	0.41 (0.19 to 0.88)	0.023
Continuous variables	mean, SD	mean, SD	mean, SD		
Systolic BP (mmHg)	157 (30.1)	158.2 (29.5)	146.7 (5.6)	0.87 (0.77 to 0.99) ‡	0.035
Diastolic BP (mmHg)	85.9 (17.9)	86.3 (16.9)	83.4 (24.2)	0.91 (0.74 to 1.12) ‡	0.368
Temperature (^{0}C)	36.4 (0.6)	36.4 (0.56)	36.4 (0.94)	1.06 (0.60 to 1.89) §	0.840
Continuous variables	median, IQR	median, IQR	median, IQR		
Well to admission (hrs)**	6.3 (13.4)	6.85 (14.47)	2.38 (11.18)	0.94 (0.89 to 0.99) ¶	0.023
Found to admission (hrs)**	3.8 (6.5)	4.25 (7.25)	2.12 (1.75)	0.91 (0.83 to 0.98) ¶	0.018
Admission to stroke fellow (hrs)	0.9 (1.4)	1.04 (1.17)	1.01 (2.28)	1.02 (0.96 to 1.07) ¶	0.581
NIHSS	4 (8)	5.6 (5)	15.8 (11)	1.17 (1.11 to 1.22) ‖	<0.001

P values are derived from Wald tests, null hypothesis: OR=1. Prior cardiac vascular diseases are a history of angina, MI or cardiac revascularisation*per decade of age; † ischaemic probable and definite acute cerebrovascular disease only; ‡per 10 mmHg; §per ^{0}C rise; ¶per hour increase;‖per unit increase **from last seen well, or when found

Table 7.4 The univariate association between baseline clinical variables and reported complete recovery of symptoms at 24 hours in patients presenting with acute cerebrovascular disease

Baseline characteristic	All (n=285)	Recovery 24 hours (n=40)	No recovery 24 hours (n=245)	Odds ratio (95% CI) Recovery vs no recovery	*P* value
Male sex (n, %)	136 (47.7)	29 (47.5)	117 (47.8)	0.99 (0.50 to 1.93)	0.976
Age (years) (mean, SD)	74.4 (12.4)	71.3 (12.6)	74.9 (12.3)	0.80 (0.61 to 1.03)*	0.09
Fellow collected variables	n, %	n, %	n, %		
Prior cardiac vascular disease	67 (23.1)	6 (15.0)	61 (24.9)	0.51 (0.28 to 0.94)	0.031
Prior peripheral vascular disease	17 (6.0)	0	17 (7.0)	-	-
Prior TIA or stroke	76 (26.7)	9 (22.5)	67 (27.4)	1.37 (0.85 to 2.19)	0.200
Prior heart failure	22 (7.8)	2 (5.0)	20 (8.3)	0.58 (0.13 to 2.59)	0.477
AF (prior or during ED) assessment)	74 (26.0)	8 (20.0)	66 (26.9)	0.68 (0.29 to 1.54)	0.356
Diabetes mellitus	37 (13.0)	3 (7.5)	34 (13.9)	0.50 (0.14 to 1.72)	0.274
Prior cognitive impairment	39 (13.7)	5 (12.5)	34 (13.9)	0.88 (0.32 to 2.41)	0.807
Simple variables					
Independent prior to admission	247 (89.7)	37 (82.5)	210 (85.7)	2.05 (0.60 to 7.03)	0.251
Living alone	96 (33.7)	16 (40)	80 (32.6)	1.38 (0.69 to 2.73)	0.363
Orientated to time place & person	210 (73.7)	38 (95)	172 (70.2)	8.10 (1.90 to 34.3)	0.005
Able to lift both arms	181 (63.5)	35 (87.5)	146 (60.0)	4.75 (1.80 to 12.5)	0.002
Able to walk without help	127 (44.6)	35 (87.5)	92 (37.6)	11.6 (4.40 to 30.8)	<0.001

Baseline characteristic	All (n=285)	Recovery 24 hours (n=40)	No recovery 24 hours (n=245)	Odds ratio (95% CI) Recovery vs no recovery	*P* value
OCSP classification, presentation†					
Total anterior circulation infarction	43 (15.9)	1 (2.5)	42 (18.3)	-	-
Partial anterior circulation infarction	115 (42.6)	23 (7.5)	92 (40.0)	-	-
Lacunar infarction	75 (27.8)	12 (30.0)	63 (27.4)	-	-
Posterior circulation infarction	25 (9.3)	2 (5.0)	23 (10.0)	-	-
Unclassifiable clinical syndrome	12 (4.4)	2 (5.0)	10 (4.3)	-	-
Imaging lesion					
Cortical infarction	124 (43.5)	7 (17.5)	117 (47.8)	-	-
Lacunar infarction	40 (14.0)	5 (12.5)	35 (14.3)	-	
Posterior circulation infarction	21 (7.3)	1 (2.5)	20 (8.2)	-	-
>1 territory infarction	5 (1.8)	0	5 (2)	-	-
Intracerebral or subarachnoid bleed	15 (5.3)	0	15 (6.1)	-	-
No visible lesion	74 (26.0)	25 (62.5)	49 (20)	-	-
No scan	6 (2.1)	2 (5)	4 (1.6)	-	-
Any visible lesion	205 (50.6)	18 (17.5)	187 (61.9)	0.13 (0.07 to 0.23)	<0.001
Medications					
Any antiplatelet agent	121 (42.5)	14 (35.0)	107 (43.7)	0.69 (0.35 to 1.39)	0.305
Warfarin	13 (4.6)	2 (5.0)	11 (4.6)	1.10 (0.23 to 5.16)	0.903
Antihypertensive	155 (54.4)	17 (42.5)	138 (56.3)	0.57 (0.29 to 1.13)	0.106
Statin	99 (35.2)	8 (20.0)	91 (37.8)	0.41 (0.18 to 0.93)	0.034-

Table 7.4 continued

Baseline characteristic	**All (n=285)**	**Recovery 24 hours (n=40)**	**No recovery 24 hours (n=245)**	**Odds ratio (95% CI) Recovery vs no recovery**	***P* value**
rtPA (intra-arterial or -venous)	7 (2.5)	0	7 (2.9)	n/a	
Current smoker	65 (23.1)	6 (15.0)	59 (24.4)	0.55 (0.22 to 1.37)	0.197
Examination findings	n, %	n, %	n, %		
Normal pedal pulses	133 (47.0)	28 (70.0)	105 (43.2)	3.07 (1.49 to 6.31)	0.002
Continuous variables	mean, SD	mean, SD	mean, SD		
Systolic BP (mmHg)	157 (30.1)	158 (32.7)	157 (29.7)	1.01 (0.91 to 1.13) ‡	0.817
Diastolic BP (mmHg)	85.9 (17.9)	87.7 (2.7)	85.6 (18.1)	1.06 (0.88 to 1.28) ‡	0.499
Temperature (^{0}C)	36.4 (0.6)	36.4 (0.5)	36.4 (0.6)	1.09 (0.62 to 1.87) §	0.769
Continuous variables	median, IQR	median, IQR	median, IQR		
Well to admission (hrs)**	6.3 (13.4)	3.0 (6.1)	7.7 (14.4)	0.94 (0.91 to 0.97) ¶	<0.001
Found to admission (hrs)**	3.8 (6.5)	2.6 (4.1)	4.1 (7.2)	0.97 (0.94 to 1.00) ¶	0.067
Admission to stroke fellow (hrs)	0.9 (1.4)	0.8 (1.05)	0.9 (1.5)	0.95 (0.90 to 1.00) ¶	0.061
NIHSS	4 (8)	1 (2)	4 (9)	0.78 (0.71 to 0.85) ‖	<0.001

P values are derived from Wald tests, null hypothesis: OR=1. Prior cardiac vascular diseases are a history of angina, MI or cardiac revascularisation*per decade of age; † ischaemic probable and definite acute cerebrovascular disease only; ‡per 10 mmHg; §per ^{0}C rise; ¶per hour increase;‖ per unit increase**from last seen well, or when found

Table 7.5 Quarters and medians of the distributions of blood markers of inflammation, thrombosis, cardiac strain and neuronal and glial damage in patients with acute cerebrovascular disease.

Marker	Lower quarter	50th centile (Median)	Upper quarter
Inflammation			
Adiponectin (µg/ml)	1.50 to 6.91	11.45	18.16 to 40.66
CRP (mg/l)	0.16 to 1.89	4.08	8.57 to 289
ICAM (ng/ml)	72 to 132	165	215 to 413
Interleukin-6 (pg/ml)	0.62 to 2.14	4.71	8.89 to 14.89
TNF (pg/ml)	0.78 to 1.18	1.39	1.71 to 6.27
Interleukin-10 (pg/ml)	0.9 to 3.3	4.4	6.7 to 238.3
MMP-9 (ng/ml)	69 to 557.5	900	1364 to 3941
vWF (IU/dl)	27 to 123	161	217 to 479
White cells ($x10^9$ cell/l)	3.7 to 6.8	8.7	10.7 to 21.6
Neutrophil / WCC	0.25 to 0.63	0.71	0.79 to 0.97
Thrombosis			
Ln D-dimer (log_e unit)*	2.40 to 4.71	5.43	6.14 to 7.93
Fibrinogen (g/l)	3.2 to 4.2	4.8	5.8 to 9.8
Thrombolysis			
tPA (ng/ml)	1.11 to 8.34	11.26	14.75 to 57.4
Cardiac strain			
Ln BNP (log_e unit)*	1.61 to 4.95	6.24	7.48 to 10.26
Troponin T (ng/ml)	0.01	0.01	0.01 to 3.11
Cerebral damage			
Tau (pg/ml)	0 to 12.5	21	47 to 3000
Ln S100B (log_e unit)*	1.61 to 3.70	4.17	4.74 to 7.93
Other markers			
Creatinine (µmol/l)	33 to 67	84	103 to 313
Glucose (mmol/l)	3.7 to 5.2	5.8	6.9 to 19.9

* variables log_e transformed to give a log-linear relationship with outcome in logistic regression

Table 7.6 Associations between marker levels and poor outcome at 3 months.

Marker (units)	OR, Unadjusted (95% CI)	*P*-value	OR, Adjusted for NIHSS & age* (95% CI)	*P*-value	OR, with further adjustment† (95% CI)	*P*-value
Inflammation						
Adiponectin (µg/ml)	2.54 (1.74 to 3.69)	<0.001	1.71 (1.08 to 2.69)	0.022	1.80 (1.13 to 2.92)	0.014
CRP (mg/l)	1.29 (1.13 to 1.48)	<0.001	1.17 (1.01 to 1.35)	0.033	1.16 (1.00 to 1.34)	0.045
ICAM (ng/ml)	1.09 (0.79 to 1.18)	0.598	1.16 (0.77 to 1.75)	0.468	1.18 (0.78 to 1.79)	0.425
Interleukin-6 (pg/ml)	5.28 (3.24 to 8.60)	<0.001	2.38 (1.36 to 4.16)	0.002	2.21 (1.24 to 3.95)	0.007
TNF (pg/ml)	1.01 (0.82 to 1.34)	0.673	0.97 (0.72 to 1.31)	0.867	0.90 (0.65 to 1.25)	0.528
Interleukin-10 (pg/ml)	1.06 (1.00 to 1.12)	0.045	1.00 (0.93 to 1.07)	0.981	1.00 (0.92 to 1.08)	0.945
MMP-9 (ng/ml)	1.16 (0.87 to 1.54)	0.305	1.20 (0.84 to 1.70)	0.320	1.23 (0.86 to 1.77)	0.253
vWF (IU/dl)	2.35 (1.63 to 3.40)	<0.001	1.63 (1.02 to 2.61)	0.043	1.57 (0.96 to 2.57)	0.072
White cells (x10^9 cell/l)	1.93 (1.38 to 2.69)	<0.001	1.51 (0.97 to 2.37)	0.070	1.48 (0.93 to 2.35)	0.095
Neutrophil / WCC	2.07 (1.15 to 2.94)	<0.001	1.54 (0.99 to 2.41)	0.057	1.56 (0.98 to 2.49)	0.058
Thrombosis						
D-dimer (log_e unit)	3.32 (2.23 to 1.93)	<0.001	1.55 (0.96 to 2.49)	0.070	1.53 (0.95 to 2.48)	0.080
Fibrinogen (g/l)	1.88 (1.38 to 2.55)	<0.001	1.48 (1.05 to 2.12)	0.024	1.76 (1.00 to 3.10)	0.050
Thrombolysis						
tPA (ng/ml)	1.54 (1.18 to 2.02)	0.002	1.20 (0.88 to 1.63)	0.254	1.24 (0.91 to 1.71)	0.178
Cardiac strain						
NT pro-BNP (log_e unit)	4.98 (3.07 to 8.09)	<0.001	2.22 (1.24 to 3.99)	0.007	2.45 (1.25 to 4.80)	0.009
Troponin T >0.01ng/ml	7.08 (3.10 to 16.2)	<0.001	2.94 (1.09 to 8.00)	0.034	2.48 (0.87 to 7.07)	0.089
Cerebral damage						
Tau (pg/ml)	1.00 (0.98 to 1.03)	0.878	0.99 (0.96 to 1.03)	0.696	0.99 (0.96 to 1.30)	0.597
S100 b (log_e unit)	1.36 (1.06 to 1.73)	0.014	1.24 (0.91 to 1.68)	0.170	1.04 (.91 to 1.71)	0.173
Other markers						
Creatinine (µmol/)	1.24 (1.22 to 1.61)	0.100	0.96 (0.67 to 1.36)	0.813	0.91 (0.63 to 1.31)	0.596
Glucose (mmol/l)	1.22 (1.01 to 1.48)	0.040	1.20 (0.95 to 1.51)	0.125	1.19 (0.93 to 1.51)	0.148

Adjustment made for NIHSS and age* and in addition†, independence of activities of daily living and: for markers of inflammation, prior infection 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.

Table 7.7 Associations between marker levels and death at 3 months.

Marker (units)	OR , Unadjusted (95% CI)	*P*-value	OR, Adjusted for NIHSS & age* (95% CI)	*P*-value	OR, with further adjustment† (95% CI)	*P*-value
Inflammation						
Adiponectin (µg/ml)	3.01 (1.91 to 4.81)	<0.001	2.22 (1.24 to 3.92)	0.007	2.30 (1.28 to 4.10)	0.005
CRP (mg/l)	1.12 (1.03 to 1.48)	0.008	1.10 (1.02 to 1.19)	0.012	1.10 (1.02 to 1.19)	0.130
ICAM (ng/ml)	1.45 (0.93 to 2.27)	0.099	1.85 (1.03 to 3.30)	0.039	1.83 (1.02 to 3.28)	0.043
Interleukin-6 (pg/ml)	5.00 (2.67 to 9.37)	<0.001	2.63 (1.23 to 5.57)	0.013	2.71 (1.24 to 5.90)	0.012
TNF (pg/ml)	1.06 (0.78 to 1.43)	0.719	1.12 (0.76 to 1.64)	0.562	1.08 (0.73 to 1.59)	0.715
Interleukin-10 (pg/ml)	1.05 (1.01 to 1.10)	0.024	0.99 (0.95 to 1.04)	0.786	0.99 (0.94 to 1.04)	0.726
MMP-9 (ng/ml)	1.26 (0.85 to 1.88)	0.243	1.34 (0.84 to 2.19)	0.207	1.37 (0.85 to 2.23)	0.198
vWF (IU/dl)	2.78 (1.73 to 4.48)	<0.001	2.04 (1.40 to 3.64)	0.016	2.07 (1.16 to 3.69)	0.014
White cells (x10^9 cell/l)	2.09 (1.35 to 3.24)	0.001	1.30 (0.79 to 2.14)	0.298	1.26 (0.99 to 2.09)	0.374
Neutrophil / WCC	2.95 (1.64 to 5.31)	<0.001	1.88 (1.02 to 3.50)	0.045	1.90 (1.01 to 3.59)	0.047
Thrombosis						
D-dimer (log_e unit)	6.89 (3.70 to 12.8)	<0.001	3.94 (1.92 to 8.09)	<0.001	4.07 (1.96 to 8.44)	<0.001
Fibrinogen (g/l)	3.54 (0.80 to 2.70)	0.212	0.87 (0.39 to 1.91)	0.721	0.84 (0.37 to 1.87)	0.662
Thrombolysis						
tPA (ng/ml)	1.89 (1.37 to 2.61)	<0.001	1.61 (1.16 to 2.13)	0.004	1.57 (1.16 to 2.13)	0.004
Cardiac strain						
BNP (log_e unit)	8.01 (3.71 to 17.3)	<0.001	4.29 (1.67 to 11.0)	0.002	5.58 (1.93 to 16.2)	0.002
Troponin T >0.01ng/ml	17.72 (7.52 to 1.7)	<0.001	9.09 (3.44 to 24.0)	<0.001	8.48 (3.14 to 22.9)	<0.001
Cerebral damage						
Tau (pg/ml)	0.95 (0.86 to 1.06)	0.371	0.95 (0.85 to 1.07)	0.395	0.95 (0.86 to 1.06)	0.395
S100B (log_e unit)	1.35 (0.98 to 1.86)	0.068	1.14 (0.78 to 1.67)	0.146	1.17 (0.80 to 1.71)	0.422
Other markers						
Creatinine (µmol/)	1.60 (1.16 to 2.20)	0.004	1.24 (0.95 to 1.62)	0.115	1.39 (0.94 to 2.05)	0.103
Glucose (mmol/l)	1.50 (1.21 to 1.87)	<0.001	1.69 (0.98 to 1.58)	0.074	1.50 (1.15 to 1.97)	0.003

Adjustment made for NIHSS and age* and in addition† independence of activities of daily living and: prior infection for markers of inflammation, and cardiac failure, AF and prior cardiac vascular disease for cardiac strain markers. The OR is the ratio of odds of death 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

Table 7.8 Associations between marker levels and recovery by 24 hours.

Marker (units)	OR, Unadjusted (95% CI)	*P*-value	OR, Adjusted for NIHSS & age* (95% CI)	*P*-value	OR, with further adjustment† (95% CI)	*P*-value
Inflammation						
Adiponectin (µg/ml)	0.83 (0.51 to 1.34)	0.439	1.24 (0.71 to 2.17)	0.77	1.23 (0.70 to 2.17)	0.469
CRP (mg/l)	0.65 (0.44 to 0.96)	0.031	0.82 (0.59 to 1.14)	0.235	0.81 (0.57 to 1.13)	0.206
ICAM (ng/ml)	0.81 (0.50 to 1.30)	0.381	0.82 (0.50 to 1.35)	0.435	0.82 (0.50 to 1.35)	0.437
Interleukin-6 (pg/ml)	0.32 (0.15 to 0.67)	0.002	0.77 (0.35 to 1.69)	0.513	0.76 (0.33 to 1.75)	0.523
TNF (pg/ml)	0.81 (0.56 to 1.18)	0.277	0.86 (0.60 to 1.22)	0.387	0.82 (0.58 to 1.16)	0.266
Interleukin-10 (pg/ml)	1.01 (0.96 to 1.06)	0.716	1.12 (1.00 to 1.25)	0.061	1.13 (1.02 to 1.26)	0.023
MMP-9 (ng/ml)	1.08 (0.73 to 1.59)	0.701	1.18 (0.75 to 1.84)	0.476	1.20 (0.76 to 1.90)	0.422
vWF (IU/dl)	0.65 (0.39 to 1.08)	0.094	0.95 (0.56 to 1.61)	0.853	0.97 (0.57 to 1.68)	0.926
White cells ($x10^9$ cell/l)	0.50 (0.29 to 0.84)	0.008	0.67 (0.38 to 1.19)	0.169	0.67 (0.37 to 1.21)	0.185
Neutrophil / WCC	0.60 (0.39 to 0.94)	0.026	0.81 (0.49 to 1.34)	0.418	0.84 (0.50 to 1.41)	0.510
Thrombosis						
D-dimer (ng/ml)	0.31 (0.14 to 0.68)	0.004	0.61 (0.32 to 1.16)	0.128	0.63 (0.34 to 1.18)	0.151
Fibrinogen (g/l)	0.65 (0.41 to 1.04)	0.073	0.90 (0.55 to 1.49)	0.694	0.84 (0.38 to 1.87)	0.668
Thrombolysis						
tPA (ng/ml)	0.73 (0.48 to 1.11)	0.141	1.01 (0.62 to 1.65)	0.960	1.01 (0.93 to 1.65)	0.967
Cardiac strain						
NT pro-BNP (log_e unit)	0.22 (0.12 to 0.42)	<0.001	0.39 (0.18 to 0.84)	0.017	0.24 (0.09 to 0.64)	0.004
Troponin T >0.01ng/ml	0.27 (0.06 to 1.15)	0.078	0.60 (0.11 to 3.20)	0.548	0.52 (0.09 to 2.99)	0.464
Cerebral damage						
Tau (pg/ml)	0.99 (0.95 to 1.04)	0.802	1.00 (0.95 to 1.05)	0.966	1.00 (0.96 to 1.05)	0.962
S100 B (log_e unit)	0.90 (0.64 to 1.27)	0.550	1.02 (0.70 to 1.48)	0.908	1.02 (0.70 to 1.48)	0.914
Other markers						
Creatinine (µmol/)	0.81 (0.53 to 1.24)	0.323	0.92 (0.55 to 1.55)	0.752	0.90 (0.53 to 1.53)	0.707
Glucose (mmol/l)	0.81 (0.58 to 1.15)	0.247	0.93 (0.94 to 1.04)	0.724	0.93 (0.68 to 7.25)	0.657

Adjustment made for NIHSS and age*, and in addition† activities of daily living, and prior infection for markers of inflammation, and cardiac failure, AF and prior cardiac vascular disease for cardiac strain markers. The OR is the ratio of odds of recovery at 24 hours 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

Table 7.9 Predictive logistic regression models to predict poor outcome at 3 months after presenting with acute cerebrovascular disease

Variables	6 simple variables	NT pro-BNP + 6 simple variables	IL-6 + 6 simple variables	NIHSS + age	NT pro-BNP + NIHSS + age	IL-6 + NIHSS + age
Lives alone	0.83 (0.42 to 1.65)	0.78 (0.40 to 1.57)	0.78 (0.39 to 1.57)			
Orientated to time place & person	0.33 (0.15 to 0.75)	0.40 (0.17 to 0.90)	0.36 (0.16 to 0.82)			
Able to lift arms	0.50 (0.24 to 1.03)	0.56 (0.26 to 1.16)	0.61 (0.29 to 1.28)			
Able to walk	0.20 (0.10 to 0.39)	0.23 (0.11 to 0.46)	0.24 (0.11 to 0.49)			
Independent of ADL	0.21 (0.05 to 0.84)	0.21 (0.05 to 0.84)	0.22 (0.05 to 0.92)			
Age*	1.05 (1.01 to 1.08)	1.03 (0.99 to 1.06)	1.04 (1.00 to 1.07)	1.07 (1.03 to 1.10)	1.05 (1.02 to 1.08)	1.06 (1.03 to 1.09)
NT pro-BNP†		2.12 (1.14 to 3.92)			2.22 (1.24 to 3.99)	
IL-6†			2.15 (1.23 to 3.82)			2.38 (1.36 to 4.16)
NIHSS‡				1.24 (1.16 to 1.32)	1.21 (1.13 to 1.30)	1.20 (1.12 to 1.28)
Constant	-0.17	-1.05	-0.81	-6.44	-6.92	-6.45

*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^{β}).

The constant terms come from the underlying logistic regression model of the form: logit (poor outcome)= $\beta_a.a + \beta_b.b$ + constant

Table 7.10 Performance of models to predict poor outcome in patients with acute cerebrovascular disease 3 months.

	Goodness of fit		***Discrimination***			***Calibration***	***Reclassification***
	Likelihood ratio statistic (*P*)*	**Akaike's Information Criterion**	**AUROC (95% CI)**	**Integrated discrimination improvement (95% CI)**	**Relative IDI**	**Hosmer Lemeshow statistic ($P^{\dagger}$)**	**Net reclassification improvement**
6 simple variable model	Reference	260.3	0.86 (0.72 to 0.91)	Reference	Reference	0.90	Reference
6 simple variable model + IL-6	<0.01	255.0	0.87 (0.83 to 0.90)	0.02 (0.01 to 0.04)	6%	0.12	+5% (p=0.32)
6 simple variable model + NT pro-BNP	0.016	256.5	0.87 (0.83 to 0.91)	0.01 (0.00 to 0.02)	2%	0.51	-3% (p=0.56)
NIHSS + age	Reference	261.9	0.84 (0.79 to 0.88)	Reference	Reference	0.76	Reference
NIHSS + age + IL6	<0.01	255.0	0.85 (0.81 to 0.89)	0.02 (0.00 to 0.04)	6%	0.12	-4% (p=0.35)
NIHSS + age + BNP	0.01	256.4	0.85 (0.82 to 0.89)	0.01 (-0.01 to 0.02)	1%	0.99	-4% (p=0.40)

χ^2 tests with: *1 degree of freedom † 8 degrees of freedom

Likelihood ratio statistic compares the goodness of fit between nested models; a significantly better model has a better fit to the data

AUROC =1 indicates perfect discrimination of a model and AUROC=0.5 indicates no better discrimination than chance

IDI: the difference between models with and without markers, of the difference in mean predicted probabilities of poor outcome for those with a good and those with a poor outcome

Hosmer Lemeshow statistic: compares the predicted to observed number of events in deciles of predicted probabilities. A higher *P* value indicate a better calibrated model.

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.

Table 7.11 Missing Data

Variable	All (n=283)	Poor outcome (n=123)	Good outcome (n=160)
Systolic BP	0	0	0
Diastolic BP	0	0	0
Temperature	15 (5.3)	6 (4.9)	9 (5.6)
Well to admission	5 (1.8)	2 (1.6)	3 (1.9)
Found to admission	5 (1.8)	2 (1.6)	3 (1.9)
Admission to stroke fellow	5 (1.8)	2 (1.6)	3 (1.9)
NIHSS	0	0	0
Sex	0	0	0
Headache	6 (2.1)	3 (2.4)	3 (1.9)
Infective symptom	0	0	0
Cardiac vascular disease	0	0	0
Peripheral vascular disease	2 (0.7)	1 (0.8)	1(0.6)
TIA or stroke	0	0	0
Heart failure	4 (1.4)	2 (1.6)	2 (1.3)
AF (prior, during)	0	0	0
Diabetes	0	0	0
Dementia	0	0	0
Migraine	5 (1.8)	3 (2.4)	2 (1.3)
Independent of ADL	0	0	0
Living alone	0	0	0
Able to talk	0	0	0
Orientated time place &	0	0	0
Able to lift arms	0	0	0
Able to walk without help	0	0	0
Antiplatelet	0	0	0
Warfarin	4 (1)	3 (2.4)	1 (0.6)
Antihypertensive	0	0	0

Variable	All (n=283)	Poor outcome (n=123)	Good outcome (n=160)
Statin	4 (1)	3 (2.4)	1 (0.6)
Current smoker	3 (1.1)	1 (0.8)	2 (1.3)
Normal pedal pulses	2 (0.7)	2 (1.6)	0
Blood markers			
Adiponectin	9 (3.1)	4 (3.2)	5 (3.1)
CRP	10 (3.5)	5 (4.1)	5 (3.1)
ICAM	10 (3.5)	4 (3.2)	6 (3.8)
Interleukin-6	9 (3.1)	3 (2.4)	6 (3.8)
TNF	10 (3.5)	4 (3.2)	6 (3.8)
Interleukin-10	4 (1.4)	3 (2.4)	1 (0.6)
MMP-9	4 (1.4)	3 (2.4)	1 (0.6)
vWF	3 (1.1)	2 (1.6)	1 (0.6)
White cells	2 (0.7)	1 (0.8)	1 (0.6)
Neutrophil / WCC	4 (1.4)	2 (1.6)	2 (1.2)
D-dimer	10 (3.5)	4 (3.2)	6 (3.8)
Fibrinogen	9 (3.1)	4 (3.2)	5 (3.1)
tPA	10 (3.5)	4 (3.2)	6 (3.8)
NT pro-BNP	19 (6.7)	6 (4.9)	13 (8.1)
Troponin T	15 (5.3)	0	15 (9.4)
Tau	1 (0.4)	0	1 (0.6)
S100 B	2 (0.7)	0	2 (1.2)
Creatinine	4 (1.4)	2 (1.6)	2 v
Glucose	11 (3.9)	5 (4.1)	6 (3.8)

Figures

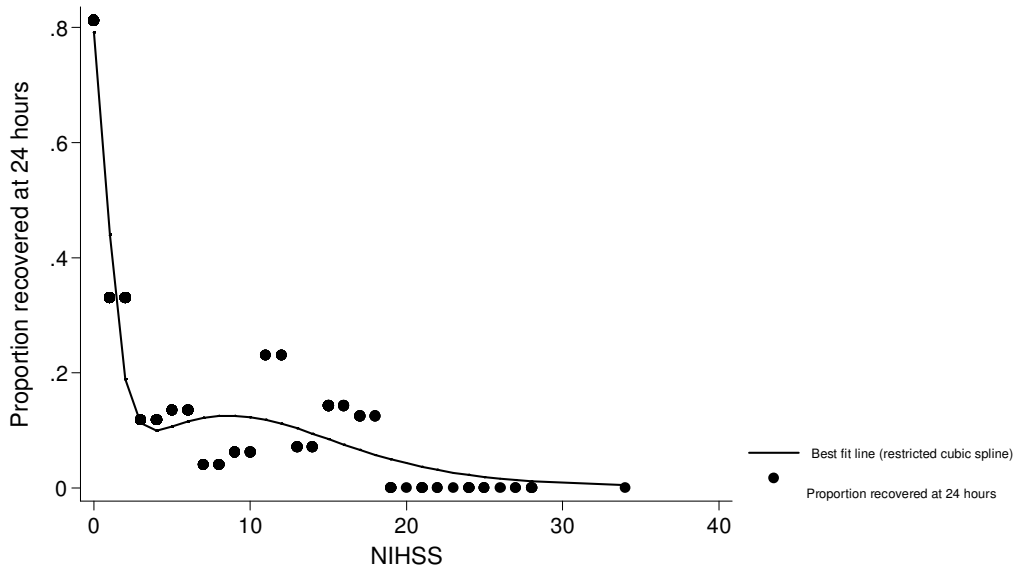


Figure 24 Relationship between NIHSS, a marker of neurological impairment, and the proportion of patients reporting complete recovery by 24 hours.

40/285 patients with acute cerebrovascular disease made a complete recovery. The plot had a similar form for patients seen <6 hours after symptom onset.

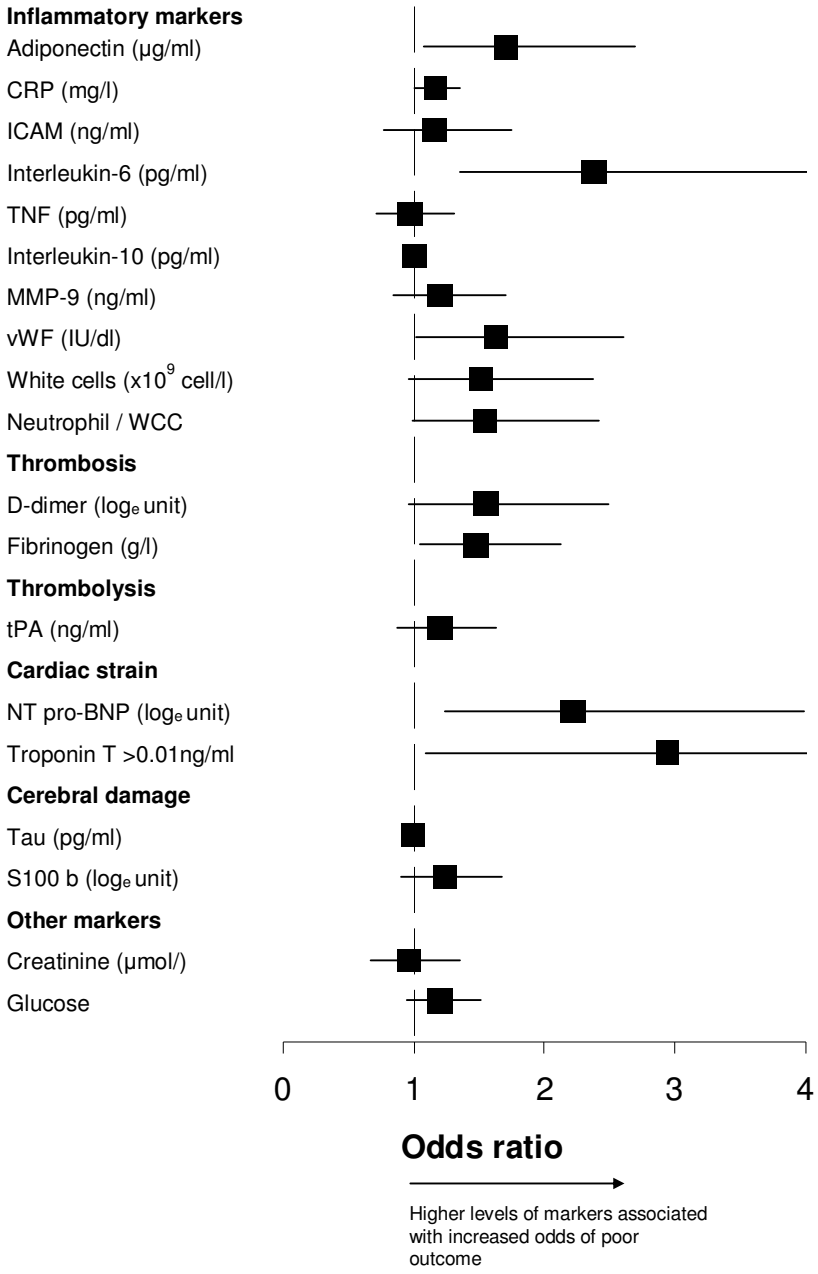


Figure 25 Association between blood markers levels and poor outcome at 3 months after acute cerebrovascular disease.

Adjustment made for NIHSS and age. Ratio of odds between the 75th and 25th centiles of a marker's distribution with 95% confidence intervals. The vertical dotted line indicates OR=1, i.e. no association between marker levels and poor outcome

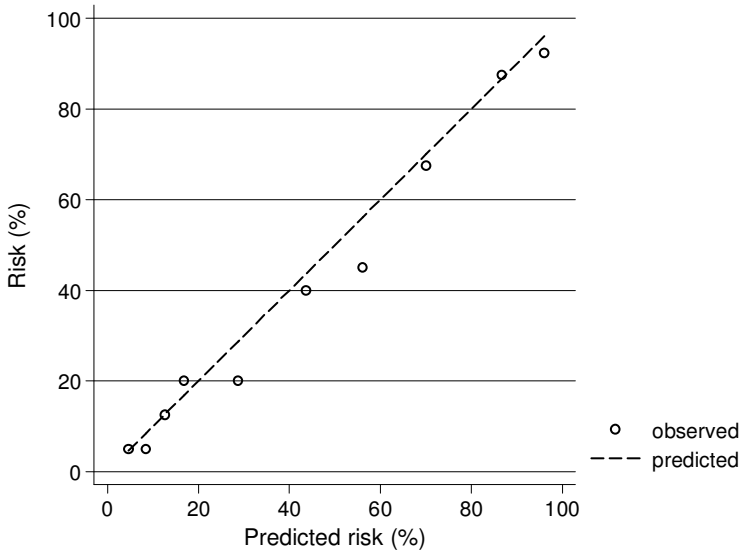


Figure 26 Plot of the observed risk of poor outcome in each decile of predicted risk calculated from a model with only the six simple variables.

The dotted straight line indicates a perfectly calibrated model

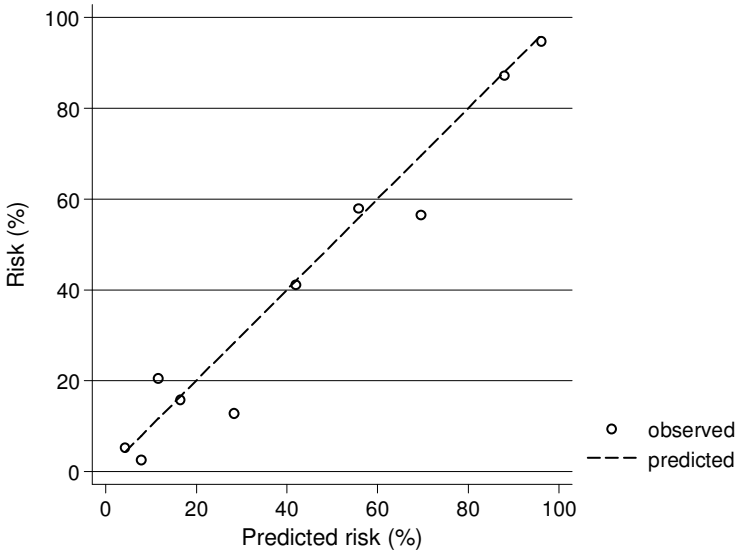


Figure 27 Plot of the observed risk of poor outcome in each decile of predicted risk calculated from a model with interleukin 6 in addition to the six simple variables.

The dotted straight line indicates a perfectly calibrated model

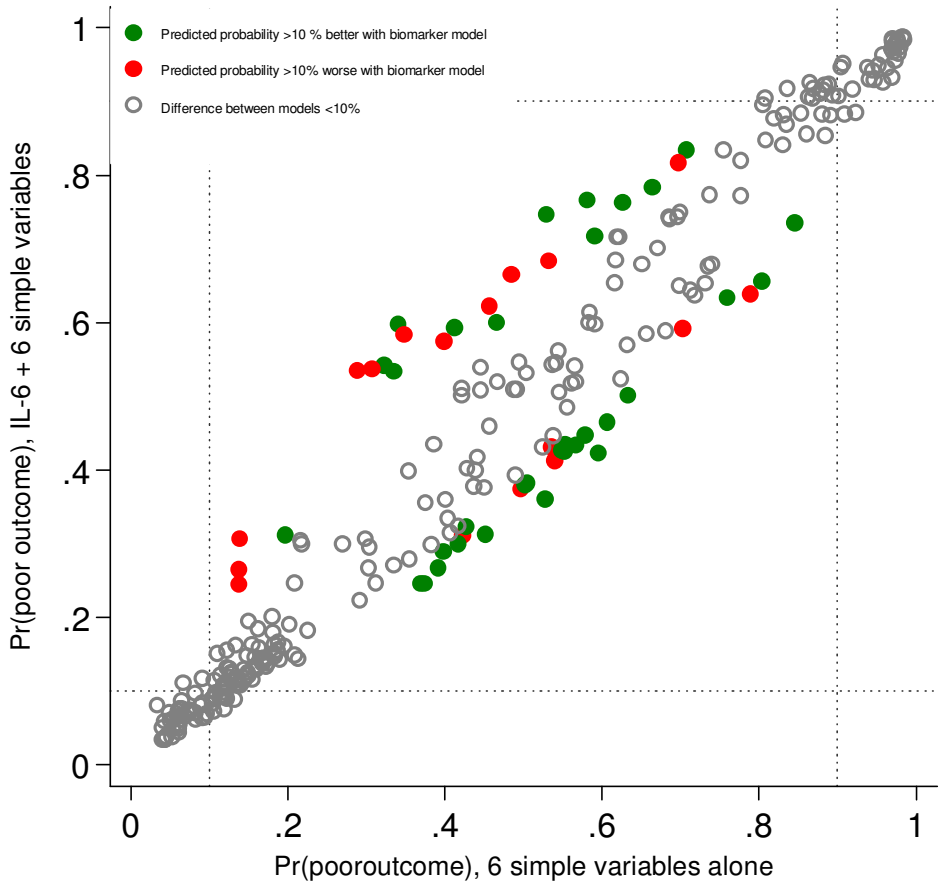


Figure 28 The predicted probability of poor outcome at 3 months after presentation with acute cerebrovascular disease using interleukin-6 in addition to the six simple variable model against the predicted probability of poor outcome with the six simple variable model alone.

Coloured markers indicate those patients in whom the predicted probability of poor outcome from the 2 models differs by 10% or more. Dotted lines indicate predicted probability of poor outcome of 0.1 and 0.9. Markers coloured red indicate the model with interleukin 6 gave a worse prediction of poor outcome compared to the observed outcome than the six simple variable model alone (n(poor outcome)=11, n(good outcome)=6); markers coloured green indicate that the model with interleukin-6 gave a better prediction than the six simple variable model alone (n(poor outcome)=12, n(good outcome)=21). N=274.

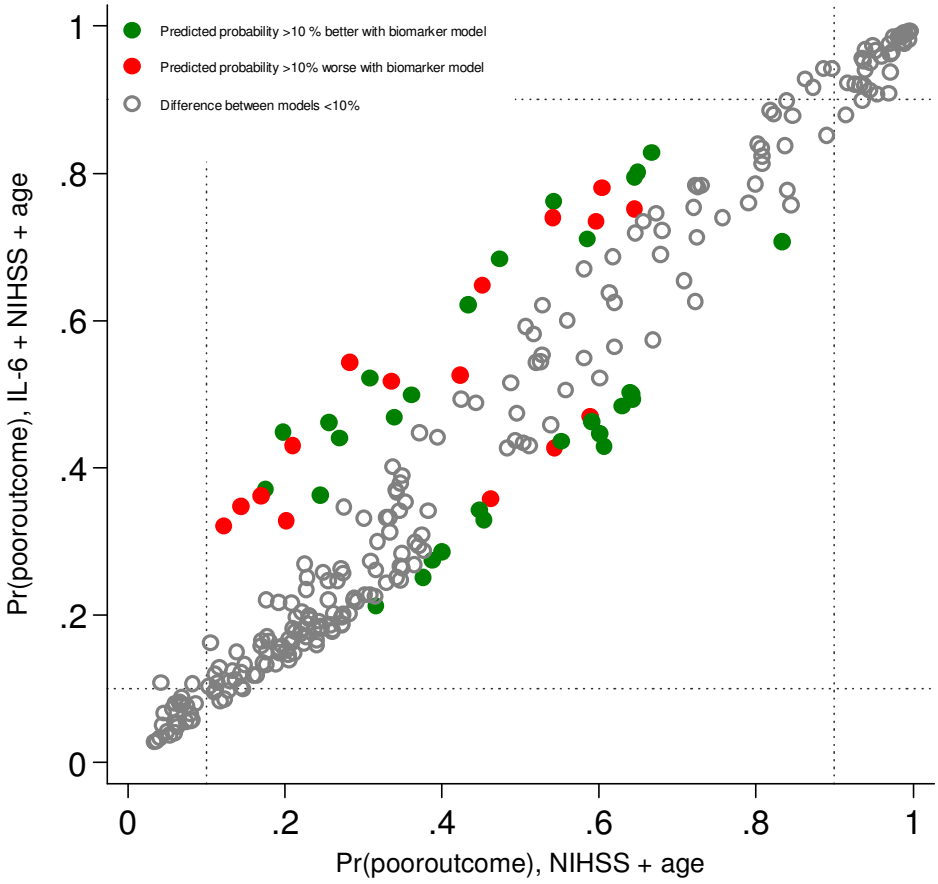


Figure 29 The predicted probability of poor outcome at 3 months after presentation with acute cerebrovascular disease using interleukin-6 in addition to NIHSS + age against the predicted probability of poor outcome with NIHSS + age alone

Coloured markers indicate those patients in whom the predicted probability of poor outcome from the 2 models differs by 10% or more. Dotted lines indicate predicted probability of poor outcome of 0.1 and 0.9. Markers coloured red indicate the model with interleukin 6 gave a worse prediction of poor outcome compared to the observed outcome than NIHSS + age alone (n(poor outcome)=13, n(good outcome)=3); markers coloured green indicate that the model with interleukin-6 gave a better prediction than the six simple variable model alone (n(poor outcome)=15, n(good outcome)=15). N=274.

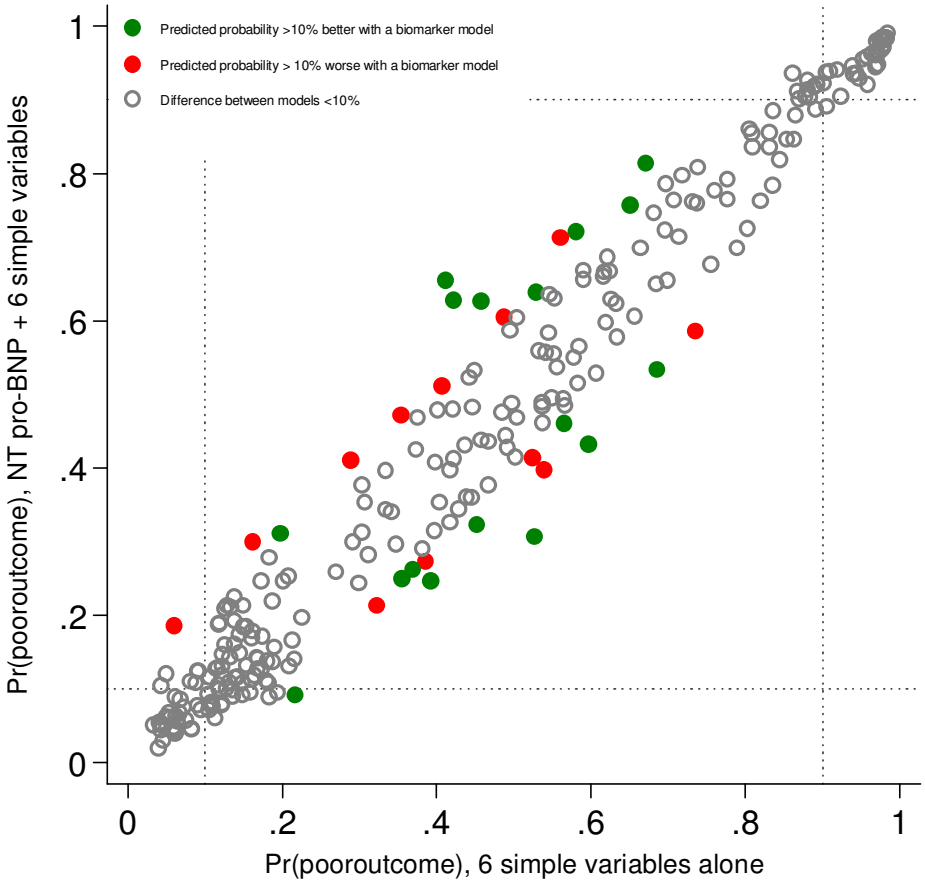


Figure 30 The predicted probability of poor outcome at 3 months after presentation with acute cerebrovascular disease using NT pro-BNP in addition to the six simple variables against the predicted probability of poor outcome with 6 simple variables alone**.**

Coloured markers indicate those patients in whom the predicted probability of poor outcome from the 2 models differs by 10% or more. Dotted lines indicate predicted probability of poor outcome of 0.1 and 0.9. Markers coloured red indicate the model with NT pro-BNP gave a worse prediction of poor outcome compared to the observed outcome than the 6 simple variables alone (n(poor outcome)=5, n(good outcome)=7); markers coloured green indicate that the model with NT pro-BNP gave a better prediction than the six simple variable model alone (n(poor outcome)=8, n(good outcome)=9). N=274.

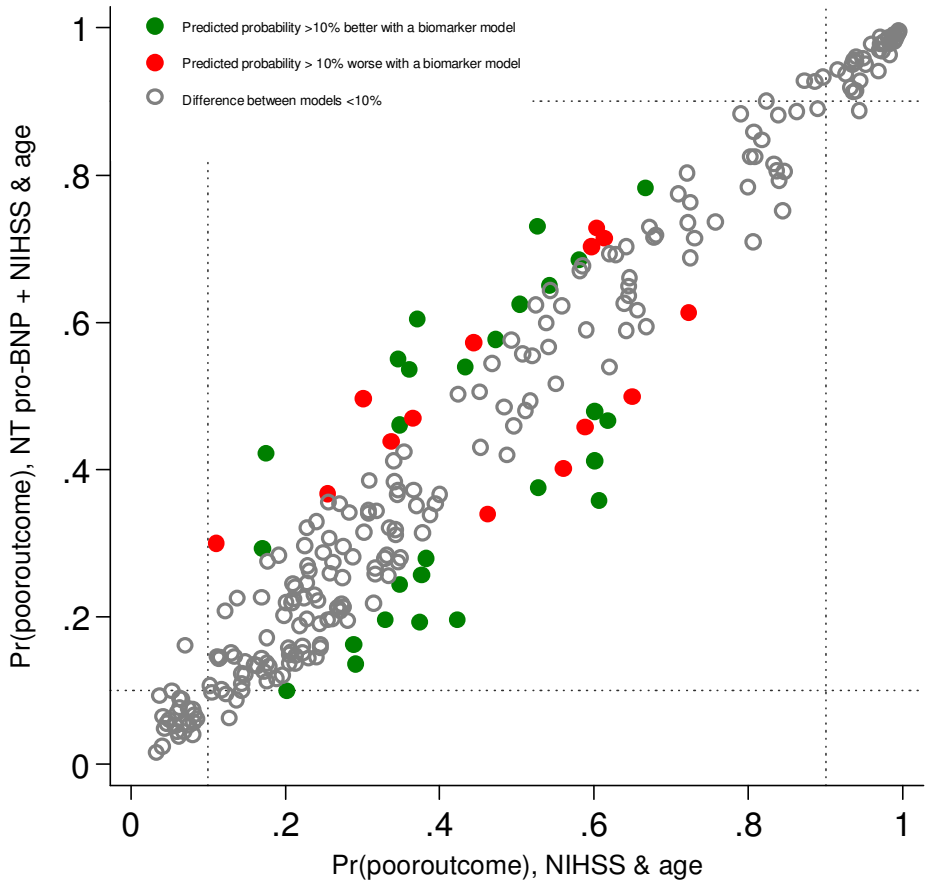


Figure 31 The predicted probability of poor outcome at 3 months after presentation with acute cerebrovascular disease using NT pro-BNP in addition to NIHSS + age against the predicted probability of poor outcome with NIHSS + age alone.

Coloured markers indicate those patients in whom the predicted probability of poor outcome from the 2 models differs by 10% or more. Dotted lines indicate predicted probability of poor outcome of 0.1 and 0.9. Markers coloured red indicate the model with NT pro-BNP gave a worse prediction of poor outcome compared to the observed outcome than the NIHSS + age (n(poor outcome)=5, n(good outcome)=9); markers coloured green indicate that the model with NT pro-BNP gave a better prediction than the six simple variable model alone (n(poor outcome)=13, n(good outcome)=14). N=274.

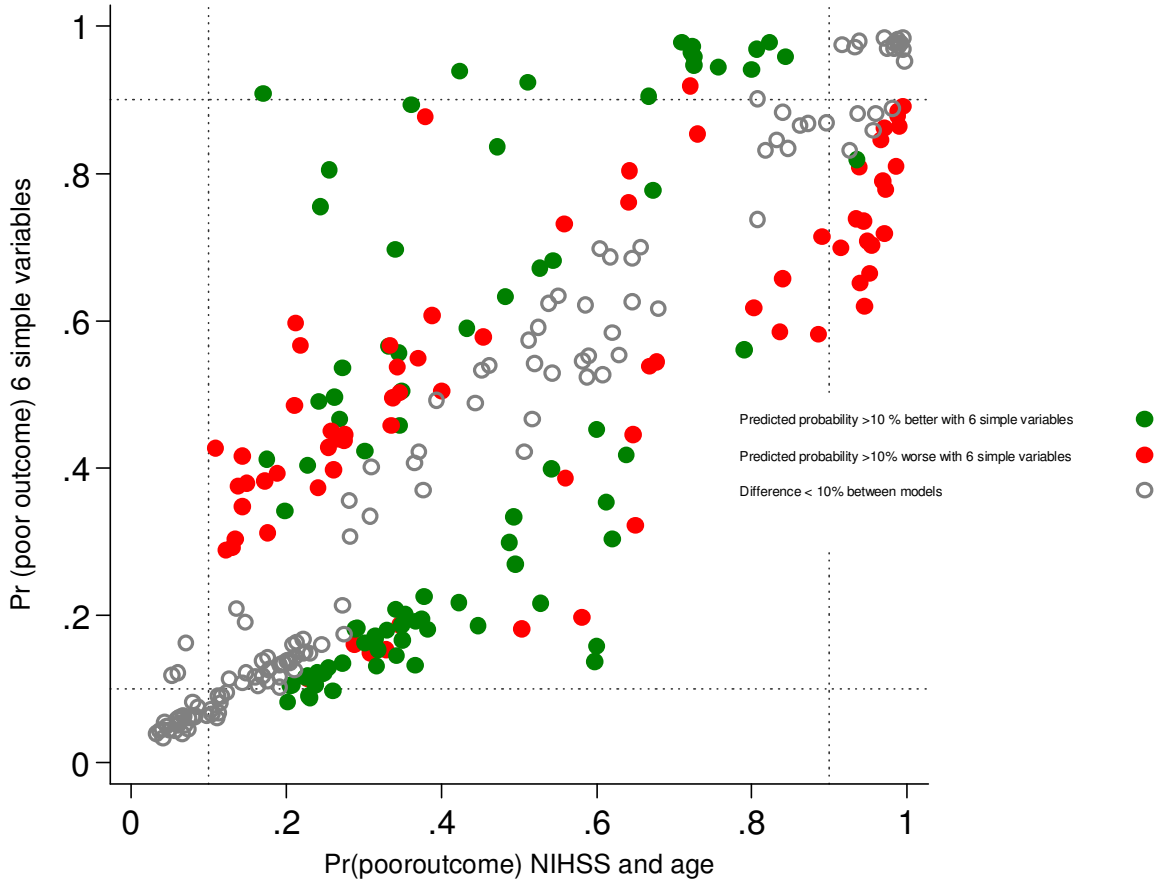


Figure 32 The predicted probability of poor outcome at 3 months after presentation with acute cerebrovascular disease using the 6 simple variables model against the predicted probability of poor outcome with NIHSS + age.

Coloured markers indicate those patients in whom the predicted probability of poor outcome from the 2 models differs by 10% or more. Dotted lines indicate predicted probability of poor outcome of 0.1 and 0.9. Markers coloured red indicate the 6 simple variable model gave a worse prediction of poor outcome compared to the observed outcome than NIHSS + age (n(poor outcome)=36, n(good outcome)=37); markers coloured green indicate that the model with 6 simple variables gave a better prediction than the NIHSS and age model (n(poor outcome)=36, n(good outcome)=47). N=405.

Chapter 8. The association of circulating inflammatory markers with recurrent vascular events after stroke: ESS, a prospective cohort study

Introduction

Circulating levels of inflammatory markers may be elevated soon after stroke. In prospective studies of patients with prior stroke or transient ischaemic attack (TIA), increased levels of markers of acute inflammation – C-reactive protein (CRP) (Di Napoli et al. 2005, Woodward et al. 2005e), interleukin -6 (IL-6) (Welsh et al. 2008b), fibrinogen (Rothwell et al. 2004b, Woodward et al. 2005d) and white cell count (Grau et al. 2004) – were associated with an increased subsequent incidence of recurrent stroke and myocardial infarction (MI). These inflammatory markers are also associated with the risk of death or disability at 3 months after stroke (Whiteley et al. 2009c). IL-6 is a messenger cytokine that is proximal to CRP in the inflammatory process. It stimulates the production of acute phase reactants by the liver, and may have a stronger association than CRP with the risk of recurrent stroke (Welsh et al. 2008c).

If high levels of acute-phase markers in the early stages of stroke appear to contribute to the risk of recurrent vascular events (Danesh & Pepys 2009), I hypothesised that the associations with recurrent events may differ among different stroke subtypes. If inflammatory markers have a causal role in further vascular events after a stroke, I hypothesised the association between inflammatory markers with recurrent vascular events to be stronger than with other, non-vascular outcomes.

In a prospective cohort of patients with recent stroke I aimed to: (1) estimate the association between levels of circulating inflammatory markers and the incidence of 'recurrent vascular events' (recurrent stroke, MI and vascular death), and (2)

compare the strength of the association between the risk of death from vascular and non-vascular causes.

Methods

The Edinburgh Stroke Study was a prospective, hospital based cohort study of patients with stroke followed up for recurrent stroke, MI and death. In brief, consenting stroke patients presenting to the Western General Hospital were recruited in Edinburgh between April 2002 and May 2006. A clinical assessment was made at baseline and blood was drawn at the same time from consenting patients for markers of inflammation (CRP, IL-6, fibrinogen and white cell count and glucose). The Lothian Research Ethics Committee reviewed the project.

Definition of stroke

I defined a clinically definite stroke as new focal disturbance of cerebral function lasting more than 24 hours of a vascular origin. I excluded patients with subarachnoid haemorrhage. I defined an ischaemic stroke as a clinically definite stroke in a patient in whom brain imaging showed either a relevant ischaemic lesion or was normal and excluded both intracranial haemorrhage and stroke mimics. I defined the pathological type of stroke as probably ischaemic in patients with a clinically definite stroke in whom the radiological results were equivocal or unavailable, and analysed them together with definite ischaemic strokes. I assigned a final ischaemic stroke syndrome according to the Oxford Community Stroke Project (OCSP) classification (Bamford et al. 1991a) based on the clinical syndrome at the time of maximum deficit, modified if necessary by the site and size of relevant infarcts on brain imaging (Mead et al. 2000). I used an algorithm based on a modified TOAST classification (Adams, Jr. et al. 1993) to assign aetiological stroke subtypes. The diagnosis of stroke and stroke subtypes was made blind to marker levels.

Assessment of outcome

Patients were followed from their index stroke to the end of the study or to death, whichever occurred first. A recurrent stroke was defined as new focal disturbance of cerebral function lasting more than 24 hours of a vascular origin, occurring after a period of at least 24 hours of neurological stability from the index stroke, and after exclusion of other causes of the symptoms. MI was diagnosed either in patients with at least two of: chest pain, a rise in cardiac enzymes and ECG changes of MI, or in patients who died with autopsy evidence of acute MI or cases of sudden death with no alternative explanation. Where possible, the study team assessed patients with a suspected recurrent stroke. In the remainder, results of brain imaging and medical records were reviewed. I defined 'other vascular death' as deaths due to vascular diseases other than stroke or MI, e.g. ruptured abdominal aortic aneurysm, ischaemic limb, ischaemic bowel or cardiac failure. I classified deaths due to the qualifying stroke (which were often due to pneumonia) or gastrointestinal haemorrhage as non-vascular. I defined the outcome cluster as recurrent vascular events: 'recurrent fatal or non-fatal stroke, subsequent fatal or non-fatal MI or other vascular death'. Infections or other complications between stroke onset and the measurement of vascular outcome or death were not routinely recorded.

Patients were followed for up to four years with multiple overlapping methods. To ascertain new events occurring during follow up, we: wrote to general practitioners; invited clinicians to notify us ; gave patients a study contact card; mailed questionnaires to patients at 6 months and annually afterwards; and flagged patients with the General Register Office for Scotland to notify us of deaths. When a patient died, all medical records were reviewed to verify the cause of death. At the end of the follow up, patients' general practitioners were contacted and an Edinburgh wide stroke audit system was reviewed, which aimed to record all stroke admissions in the city.

Measurement of blood markers

The NHS clinical laboratory measured total white cell count (Beckman Coulter LH750 analyser) and blood glucose (Vitros Chemistry analyser). CRP and fibrinogen

were measured in plasma by immunonephelometry (Prospec, Dade Behring Milton Keynes, UK) using the manufacturer's reagents and standards. IL-6 was assayed by ELISA (R & D Systems, Oxford, UK). Intra- and inter-assay coefficients of variation were for CRP 4.7 and 8.3%, for fibrinogen 2.6 and for IL-6 5.3%, and 7.5 and 8.9%. All assays were performed blind to stroke outcome.

Statistical analysis

I used Stata version 10 (Statcorp 2007) for analysis and prepared the chapter with reference to the STROBE (von Elm et al. 2007a) guidelines for the reporting of observational studies.

I measured time to first recurrent stroke, MI or vascular death and censored patients at the end of follow up or non-vascular death. I compared the baseline characteristics of patients who experienced a stroke, MI or vascular death with those who did not in a number of univariable Cox regression analyses. I examined the relationships between inflammatory markers with correlation coefficients, and used linear regression (after $\log_e$ transformation of markers) to examine the relationship of markers with delay to blood draw. I used Kaplan Meier survival curves to compare event free survival between groups of patients defined by thirds of inflammatory biomarkers and compared curves with log rank trend tests. I used Cox regression analysis to calculate unadjusted hazard ratios (HR) and 95% confidence intervals (CI) per unit increase in marker levels. I built a multivariate Cox regression model to adjust for confounders, adding variables sequentially that were associated with recurrent stroke, MI or vascular death in univariable analysis and had data completeness of over 95%, keeping those variables that significantly improved the fit of the model ($p<0{\cdot}05$). To test the improvement of the model by adding each variable, I used likelihood ratio tests.

When the model was complete, I tested the proportional hazards assumption in the final model by plotting and testing Schoenfield residuals and time dependent covariates. To evaluate goodness of fit, I plotted Nelson-Aalen cumulative hazard

functions against Cox Snell residuals. I looked for first order interactions of inflammatory biomarker levels with other variables in the final model by adding multiplicative terms, performed sensitivity analysis for patients seen as inpatients and as outpatients, and examined the association between inflammatory markers and stroke, other markers, MI or vascular death by stroke subtype with the modified TOAST classification.

I assessed the change in discrimination after the addition of interleukin 6 to a model containing only clinical variables by calculating Harrell's c-statistic for models with and without biomarkers. The c-statistic is analogous to the area under a receiver operator curve for Cox regression models; a vale of 0.5 indicates no better discrimination than chance and a value of 1.0 perfect discrimination.

I replicated the analysis measuring time to death only, censoring at the end of the study. To adjust models examining the risk of death, I used previously validated covariates (Counsell, Dennis, & McDowall 2004a). I also plotted, by thirds of marker levels, the competing risks of vascular deaths, death due to the initial stroke and death due to other causes using the 'stcompet' command (Coviello 2004), which calculates the cumulative incidence of each outcome.

Results

Baseline characteristics

877 of 1408 patients in the Edinburgh Stroke Study (62%), gave consent and had blood drawn for markers of inflammation. Of these 817 (93%) had a definite ischaemic stroke, 17 (2%) a probable ischaemic stroke and 43 (5%) a haemorrhagic stroke. Of those patients who had blood drawn for blood markers, no patient was lost to follow up for the outcomes of death, recurrent stroke or myocardial infarction. Patients were first assessed at a median of 10 days (IQR 3 to 21 days) after onset and blood drawn at a median of 0 days (IQR 0 to 3 days) after assessment. The delay to assessment was longer for patients seen in an out-patient clinic (median 19 days) than in the in-patient stroke unit (median 2 days). During

the 1866 person years of follow up time (mean 2.12 years), 106 recurrent strokes (92 ischaemic, 5 haemorrhagic and 9 of uncertain type) and 34 myocardial infarctions occurred. There was a total of 184 deaths: 113 from vascular causes (63 strokes, 35 from cardiac causes, and death from bowel ischaemia, vascular dementia and presumed vascular renal failure) and 64 from other causes (33 cancers, 13 chest infections, 6 from COPD and the rest pancreatitis, bowel perforation, hip fracture, and extra-pulmonary sepsis).

At the time of the clinical assessment of the index stroke, the median IL-6 was 4·0 (interquartile range [IQR] 2.4 to 7.2) pg/l, median CRP 3.5 (IQR 1.4 to 9.7) mg/l, median fibrinogen 4.5 (IQR 3.8 to 5.4) g/l, median white cell count 8 (IQR 6.6 to 9.7) x109/l and median glucose 5.6 (IQR 5 to 6.8) mmol/l. The correlation coefficients were, between IL-6 and: CRP 0.59, fibrinogen 0.48, glucose 0.06 and white cell count 0.25. Although the blood level of each marker fell with increasing delay to blood draw after stroke, these association between markers attenuated and became statistically insignificant, after adjusting for the level of baseline neurological impairment and age.

Circulating inflammatory markers and recurrent stroke, MI and vascular death

There was a significant increase in the risk of recurrent vascular events for patients who: were older; or had a history of AF, heart failure or previous peripheral vascular disease, coronary heart disease or stroke (Table 8.1).

The log hazard of stroke, MI or vascular death rose with each third of IL-6 and CRP, though not by thirds of glucose, fibrinogen or white cell count. In unadjusted Kaplan-Meier survival analyses, patients survived free of recurrent vascular events for a shorter time in the highest third of IL-6 (log rank trend $\chi 2=13.22$ p=0.0003) (Figure 33) and CRP (log rank trend $\chi^2=13.9$ p=0.0002). This relationship did not reach statistical significance for fibrinogen (log rank trend $\chi^2=2.84$ p=0.0921), glucose (log rank trend $\chi^2=1.07$ p=0.3003) or white cell count (log rank trend $\chi^2=3.09$ p=0.0787).

However, as a linear model fitted the data well for each marker, we modelled each marker as a linear variable.

Table 8.2 shows the association between circulating inflammatory markers and recurrent vascular events. In univariate analyses, all markers except glucose were significantly associated with recurrent vascular events. The relative hazard of recurrent vascular events for an increase of 1 pg/ml of IL-6 was 1.07 (95% CI: 1.04 to 1.10) per pg/ml. The unadjusted associations between IL-6 and recurrent fatal or non-fatal stroke alone (HR 1.04 95% CI 1.00 to 1.08 per pg/ml) were weaker though the HRs for the association with a one unit increase of CRP, fibrinogen, white cell count and glucose were not significantly different from 1. The unadjusted association between IL-6 and fatal or non-fatal MI alone (HR 1.09 95% CI:1.03 to 1.15) was stronger than for recurrent stroke alone.

I adjusted for the following confounders in the final model: age, prior stroke or TIA or ischaemic heart disease, current or prior AF and cardiac failure. Adding markers of stroke severity (i.e. ability to walk or lift arms off bed), blood pressure at assessment, diabetes, carotid stenosis or smoking did not significantly improve models containing a single inflammatory marker. After adjustment, there was still a significant association between recurrent vascular events and increasing levels of IL-6, CRP and fibrinogen (Table 8.2). In this cohort, those patients with highest blood levels (75th centile) of interleukin 6 had a 1.33 fold increase in the incidence of recurrent vascular events compared with those with the lowest levels (25th centile). A similar relative increase in incidence was seen for fibrinogen (HR 1.20), and less for C-reactive protein (HR 1.06).

I added markers sequentially, in order of the strength of their association with recurrent vascular events, to a model containing only clinical variables (age, prior stroke or TIA or heart disease, current or prior AF or cardiac failure). Addition of IL-6 significantly improved the model (likelihood ratio (LR) test χ^2=14.0, p<0.001), though further addition of CRP (LR test χ^2=0.3, p=0.56), fibrinogen (LR test χ^2=0.2,

p=0.68), white cell count (LR test χ^2=0.7, p=0.40), or glucose (LR test χ^2=1.3, p=0.25) did not make further improvement, probably as the markers were correlated. After adjustment for all markers, only the association between IL-6 and recurrent vascular events remained statistically significant. The final model with units of IL-6 fulfilled the proportional hazards assumption and fitted the data well.

A model with only clinical variables (age, prior TIA, MI or stroke and AF) had a Harrell's C statistic of 0.62; when I added interleukin-6 to this model, the Harrell's C statistic increased by a small amount, to 0.64.

First order interactions

There was significant heterogeneity in the association between IL-6 and recurrent vascular events by the subtype of ischaemic stroke at baseline (Figure 34), driven largely by unclassified strokes. Multiplicative interaction terms between stroke subtype (small vessel stroke versus all others (with a modified TOAST algorithm and the OCSP classification)), delay to blood taking after stroke, age, ability to walk and level of interleukin 6 did not make important changes to the association between IL-6 and recurrent vascular events (and none significantly improved the fit of the final Cox proportional hazards model). There were no significant two way interactions between other blood markers and IL-6. The strength of the association between inflammatory markers and recurrent vascular events was consistent between clinic patients and in-patients (Table 8.4).

Circulating inflammatory markers and death

All markers were significantly associated with an increased risk of death (Table 8.3). After adjustment for factors which are known reliably to influence survival after the index stroke (age, being able to walk or talk, independence of daily activities prior to stroke, being able to lift arms from the bed), these associations remained statistically significant though attenuated. IL-6, CRP, fibrinogen and glucose were more strongly associated with death than with recurrent vascular events, though white cell count was less strongly associated. After additional adjustment for all

other markers, only the associations of IL-6 and fibrinogen with death remained statistically significant. The association between higher levels of IL-6, CRP and fibrinogen and an increased incidence of death was consistent for each of the separate causes of death (vascular deaths, deaths due to the initial stroke and deaths due to other causes)(Figure 35, data for CRP and fibrinogen not shown). Where the cause of death was the qualifying stroke, patients in the top third of the IL-6 distribution had the shortest survival time.

Discussion

In this study of inpatients assessed soon after onset and a group of outpatients with milder strokes seen after a short interval, higher levels of IL-6, CRP and fibrinogen were associated with a higher incidence of recurrent stroke, MI or vascular death, independent of atrial fibrillation, prior vascular events and age. In addition, higher levels of each inflammatory marker were associated with a higher incidence of death from all causes, an association that was stronger than for all vascular events. The associations with recurrent stroke alone were weaker: it was weak but statistically significant for IL6, and did not reach statistical significance in this cohort for the other markers.

Somewhat unexpectedly I found no consistent evidence of different strengths of association between higher baseline levels of IL-6 with different ischaemic stroke subtypes, strokes of different severity or different times from stroke onset to blood draw. Stroke patients had qualitatively similar associations between IL-6, CRP and fibrinogen and deaths from vascular and from non vascular causes. However, there was a suggestion that early deaths from the index stroke might be more strongly associated with higher levels of inflammation.

Strengths and limitations

This study had a number of methodological strengths: several overlapping methods were used to ensure all recurrent vascular events were detected; vital status was determined at the end of the follow up period for all of the cohort; data on all

suspected outcome events were checked by the study clinicians, either directly or by review of the medical and imaging records. The majority of patients with recurrent strokes underwent brain imaging (93%), in contrast to previous studies, which had limited access to brain imaging.

Not all patients had blood drawn for inflammatory markers. The most common reasons for not drawing blood were: the patient did not consent and practical constraints in inpatients, chiefly the working hours of research laboratories handling the samples. Patients without blood samples tended to have more severe strokes though were otherwise similar; there was no evidence of an interaction between stroke severity and the association of inflammatory markers and recurrent vascular events or death.

The number of patients with haemorrhagic strokes was too small reliably to explore interactions between haemorrhagic versus ischaemic strokes, or between inflammatory markers and the risk of occlusive vascular outcomes, though no trends were observed (Welsh et al. 2008d, Woodward et al. 2005c). With only a single sample of blood I could not correct my analyses for regression dilution bias (Danesh et al. 2008b).

Blood was drawn as soon as possible after assessment (median 2 days among patient admitted to hospital); hence levels of IL-6 and CRP were higher than in previous studies, which may in some cases have been due to stroke complications such a pneumonia or deep vein thrombosis As I were unable to adjust for these in our analysis, the observed association may have been due to confounding by these complications. However, in the 60% of patients seen as an outpatient, who had milder strokes, and probably fewer infections or other complications the median time to blood draw was 19 days. Despite this, I was unable to demonstrate effect modification by the time to blood draw after stroke on the association between either IL-6 or CRP and recurrent vascular events. It is possible that among patients

in whom the initial assessment was delayed, some early recurrent strokes may have been overlooked.

I did not confirm my hypothesis that large vessel stroke subtype at baseline would have had a stronger association between marker levels and the risk of subsequent vascular events. However, a relatively large number of strokes were unclassified by the TOAST classification, so I cannot exclude the possibility that an association exists.

I used a competing risks survival analysis to examine the association of IL-6, fibrinogen and CRP with of the three main causes of death; vascular, non-vascular and deaths due to the initial stroke. It is possible that there was some misclassification of the cause of death, particularly for deaths occurring soon after stroke when accurate attribution of the cause of death is difficult, even if autopsy is performed.

The epidemiological association between inflammatory markers and recurrent vascular events appears consistent and strong, and similar for IL-6 and CRP, though a somewhat weaker for fibrinogen. However, the clinical utility of adding inflammatory markers to a clinical predictive model is not determined only by independence in multivariate models. The small increase in the c-statistic for a model containing IL-6 makes it unlikely that it will add clinically useful prediction to prediction based on variables that do not require blood draw.

Interpretation

It is unlikely that CRP or IL-6 has a causal role in the generation of recurrent vascular events after stroke, and more likely that the observed association reflects an inflammatory response either to atherosclerosis or to its risk factors, or to an as yet unidentified trigger. In support of this, studies that have examined functional CRP and IL-6 polymorphisms (which produce differences in baseline CRP or IL-6 levels) found no increased risk of stroke (Ladenvall et al. 2006) or other occlusive vascular

events (Elliott et al. 2009, Walston et al. 2007, Zacho et al. 2008) with different polymorphisms.

Generalisability

IL-6 is a key pro-inflammatory cytokine that up-regulates circulating downstream inflammatory markers including CRP, fibrinogen and white cell count. My finding that IL-6 showed the strongest association with recurrent vascular events and with death in this cohort of stroke patients, is consistent with recent reports from population-based prospective studies (Danesh et al. 2008a, Patterson et al.).

Most other studies in patients with acute cerebrovascular diseases found lower levels of inflammatory markers than ours, perhaps because of greater delay between blood draw and stroke (delay to blood draw in these studies was between 12 hours and 30 days). My estimates of the association between: (i) CRP, IL-6, fibrinogen and recurrent stroke (Campbell et al. 2006b, Elkind et al. 2006a, Woodward et al. 2005b), and (ii) CRP, fibrinogen and death (Di Napoli, Papa, & Bocola 2001a, Elkind et al. 2006b) are consistent with previous studies (Table 8.5). Increasing fibrinogen predicted both recurrent ischaemic stroke and myocardial infarction in an analysis of pooled data (Rothwell et al. 2004c) though the association with all death or non-vascular death was not consistent across studies (Di Napoli, Papa, & Bocola 2001b, Rallidis et al. 2008, Rothwell et al. 2004d).

Conclusion

I have demonstrated an association between higher levels of IL-6, CRP and fibrinogen and increased incidence of occlusive vascular events in patients after stroke. The association between IL-6, CRP and fibrinogen and fatal vascular and non-vascular events after stroke seems similar.

Tables

Table 8.1 Baseline characteristics of stroke patients.

	Total	Recurrent Stroke, MI or vascular death	No vascular event	Univariate HR (95% CI)
Number	877	159	718	
Demographic				
Age, years(mean, SD)	71.4 (12.0)	73.6 (10.7)	70.9 (12.2)	1.02 (1.00 – 1.04)†
Male sex, N (%)	463 (52.8)	80 (50.3)	383 (53.3)	0.9 (0.6 to 1.2)
Laboratory results	Median (IQR)	Median (IQR)	Median (IQR)	
Interleukin-6 (pg/ml)	4.0 (2.4-7.2)	4.8 (2.8-9.1)	3.8 (2.3-6.6)	
CRP (mg/L)	3.5 (1.4-9.7)	5.9 (1.9-15.5)	3.3 (1.2-8.8)	
Fibrinogen (g/L)	4.5 (3.8-5.4)	5.7 (3.9-5.7)	4.4 (3.8-5.4)	
White cell count ($x10^9$/l)	8 (6.6-9.7)	8.4 (6.7-9.7)	7.9 (6.6-9.7)	
Glucose (mmol)	5.6 (5-6.8)	5.7 (4.9-7.2)	5.6 (5-6.7)	
Cholesterol (mmol/l)	5.1 (4.4-6.0)	4.9 (4.3-5)	5.1 (4.4-6)	
Pathological type, index stroke	N (%)	N (%)	N (%)	
Definite ischaemic	817 (93.2)	149 (93.7)	668 (93.0)	1.1 (0.6 to 2.1)‡
Definite haemorrhagic	43 (4.9)	9 (5.7)	34 (4.7)	1.2 (0.6 to 2.4) ‡
Subtype unknown	17 (1.9)	1 (0.6)	16 (2.2)	0.3 (0.1 to 1.9) ‡
Clinical stroke syndrome of index stroke (OCSP*)				
TACI	57 (6.8)	10 (6.3)	53 (7.4)	1.2 (0.6 to 2.2) §
PACI	376 (45.1)	81 (50.9)	308 (42.9)	1.1 (0.8 to 1.6) §
LACI	228 (27.3)	38 (23.9)	200 (27.9)	0.8 (0.5 to 1.1) §
POCI	131 (15.7)	22 (13.8)	121 (16.9)	0.8 (0.5 to 1.3) §
Uncertain subtype	42 (5.0)	8 (5.0)	36 (5.0)	0.9 (0.5 to 1.9) §
Severity of index stroke				
Can't walk, can't lift arms	91 (10.4)	12 (7.6)	79 (11.0)	0.9 (0.5 to 1.5)
Can't walk, can lift arms	119 (13.6)	31 (19.5)	88 (12.3)	1.6 (1.1 to 2.4)
Can walk	664 (76.0)	115 (72.9)	549 (76.7)	0.7 (0.5 to 1.0)

Table 8.1 continued	Total	Recurrent Stroke, MI or vascular death	No vascular event	Univariate HR (95% CI)
Cardioembolic	117 (13.3)	28 (17.6)	89 (12.4)	1.2 (0.8 to 2.0) ‖
Large vessel disease	72 (8.2)	12 (7.6)	60 (8.4)	1.0 (0.7 to 1.6) ‖
Mixed aetiology	58 (6.6)	16 (7.6)	42 (5.9)	1.8 (1.2 to 2.7) ‖
Small vessel disease	178 (20.3)	24 (15.1)	154 (21.5)	0.8 (0.6 to 1.2) ‖
Unclassified after complete investigation	355 (40.5)	65 (40.9)	290 (40.4)	0.9 (0.7 to 1.3) ‖
Unclassified after incomplete investigation	40 (15.8)	14 (8.8)	83 (11.6)	0.8 (0.5 to 1.5) ‖
Risk factors				
History of TIA	143 (16.3)	29 (18.2)	114 (15.9)	1.1 (0.8 to 1.7)
History of stroke	166 (18.9)	41 (25.8)	125 (17.4)	1.5 (1.1 to 2.2)
History of ischaemic heart disease	242 (27.6)	62 (39.0)	180 (25.1)	1.9 (1.4 to 2.6)
History of PVD	69 (7.9)	22 (13.9)	47 (6.6)	2.0 (1.3 to 3.2)
Ipsilat. carotid stenosis >70	97 (12.5)	21 (14.9)	76 (12.0)	1.2 (0.8 to 2.0)
Ever AF	168 (20.3)	42 (26.4)	126 (17.6)	1.7 (1.3 to 2.5)
Prior treated hypertension	467 (53.3)	96 (60.4)	371 (51.7)	1.4 (1.0 to 1.9)
Diabetes	110 (12.5)	26 (16.4)	84 (11.7)	1.4 (0.9 to 2.2)
Ever smoker	602 (69.8)	113 (71.1)	489 (69.5)	1.1 (0.8 to 1.5)
Heart failure	40 (4.58)	14 (8.7)	26 (3.6)	2.8 (1.6 to 4.8)
Any antiplatelet at baseline	369 (46.1)	11 (6.9)	33 (4.6)	1.7 (0.9 to 3.1)
Warfarin at baseline	43 (4.9)	32 (4.5)	11 (6.9)	1.5 (0.8 to 2.7)
Systolic BP (Mean, No.observations)	147.2 (874)	147.3 (159)	147.2 (715)	1.00 (0.99 to 1.01) †
Diastolic BP (Mean, No. observations)	80.0 (874)	80.1 (159)	80.0 (715)	1.00 (0.99 to 1.01) †

Footnote. *OCSP=Oxfordshire Community Stroke Project Classification (ischaemic and probable), TACS=total anterior circulation stroke, PACS=partial anterior circulation stroke, LACS=lacunar stroke, POCS=posterior circulation stroke. † per unit increase ‡ versus other pathological types §versus all others in OCSP classification ‖ versus all others in TOAST classification.

Table 8.2 The association between marker level and recurrent stroke, MI or vascular death, assuming a linear association between marker level and log hazards

	Hazard ratio per unit increase in marker (95% CI)			Hazard ratio comparing 75th to 25st centile*
	Unadjusted	Adjusted†	Adjusted for all markers‡	Adjusted†
Interleukin-6 (pg/ml)	1.07 (1.04 to 1.10)	1.06 (1.03 to 1.09)	1.05 (1.01 to 1.09)	1.33 (1.15 to 1.53)
C-reactive protein (mg/l)	1.01 (1.00 to 1.01)	1.01 (1.00 to 1.01)	1.00 (1.00 to 1.03)	1.06 (1.02 to 1.09)
Fibrinogen (g/l)	1.16 (1.05 to 1.28)	1.12 (1.01 to 1.25)	1.02 (0.97 to 1.17)	1.20 (1.01 to 1.43)
White cell count ($x10^9$/l)	1.06 (1.02 to 1.10)	1.05 (1.00 to 1.11)	1.03 (0.97 to 1.09)	1.17 (0.98 to 1.38)
Glucose (mmol/l)	1.04 (0.99 to 1.09)	1.03 (0.98 to 1.08)	1.02 (0.97 to 1.08)	1.06 (0.97 to 1.15)

Footnote * HR per unit increase in marker (99th centile-1st centile level of marker).25th and 75th percentile respectively for: IL6 2.39 and 7.22 pg/ml; CRP 1.39 and 9.65 mg/l; fibrinogen 3.81 and 5.41 g/l; white cell count 6.6 and 9.7 x109/l; glucose 5.0 and 6.8 mmol/l. †Adjusted for confounders: age, cardiac failure, atrial fibrillation (current or past), or prior stroke, TIA, peripheral vascular disease or MI. ‡ adjusted for all confounder in previous column, and other markers.

Table 8.3 The association between marker level and any death, assuming a linear association between marker level and log hazards

	Hazard ratio per unit increase in marker (95% CI)			Hazard ratio comparing 75th to 25th centile*
	Unadjusted	Adjusted†	Adjusted for all markers‡	Adjusted†
Interleukin-6 (pg/ml)	1.13 (1.10 to 1.15)	1.10 (1.07 to 1.12)	1.07 (1.04 to 1.12)	1.56 (1.37 to 1.77)
C-reactive protein (mg/l)	1.01 (1.00 to 1.01)	1.01 (1.00 to 1.01)	1.00 (1.00 to 1.81)	1.08 (1.04 to 1.11)
Fibrinogen (g/l)	1.37 (1.26 to 1.49)	1.26 (1.14 to 1.40)	1.14 (1.01 to 1.28)	1.45 (1.24 to 1.72)
White cell count ($x10^9$/l)	1.07 (1.02 to 1.12)	1.05 (1.00 to 1.11)	1.03 (0.97 to 1.09)	1.17 (1.00 to 1.37)
Glucose (mmol/l)	1.95 (1.02 to 1.10)	1.06 (1.02 to 1.11)	1.04 (0.99 to 1.09)	1.12 (1.03 to 1.21)

Footnote * HR per unit increase in marker (75th centile-25th centile level of marker). 25th and 75th percentile respectively for: IL6 2.39 and 7.22 pg/ml; CRP 1.39 and 9.65 mg/l; fibrinogen 3.81 and 5.41 g/l; white cell count 6.6 and 9.7 $x10^9$/l; glucose 5.0 and 6.8 mmol/l. †Adjusted for confounders: age, ability to walk, living alone, independent prior to stroke, orientated to place time and person, able to lift arms from bed. ‡adjusted for all confounder in previous column, and other markers

Table 8.4 Association between inflammatory markers and recurrent stroke, MI or vascular death, adjusted for age, cardiac failure, AF and previous occlusive vascular disease and reported separately for patients seen as an inpatient and patients seen as an outpatient.

	Inpatient		**Outpatient**	
	Hazard ratio (75^{th} to 25^{th} centile)	***P***	**Hazard ratio (75^{th} to 25^{th} centile)**	***P***
Interleukin-6	1.41 (1.03 to 1.11)	0.001	1.27 (0.96 to 1.71)	0.097
C-reactive protein	1.03 (1.01 to 1.06)	0.018	1.05 (0.98 to 1.23)	0.173
Fibrinogen	1.10 (0.95 to 1.28)	0.191	1.14 (0.95 to 1.37)	0.157
White cell count	1.16 (0.98 to 1.34)	0.079	1.03 (0.86 to 1.24)	0.755
Glucose	1.09 (1.03 to 1.16)	0.004	0.98 (0.90 to 1.07)	0.668

25^{th} and 75^{th} percentile respectively for: IL6 2.39 and 7.22 pg/ml; CRP 1.39 and 9.65 mg/l; fibrinogen 3.81 and 5.41 g/l; white cell count 6.6 and 9.7 $x10^9$/l; glucose 5.0 and 6.8 mmol/l

Table 8.5.Thresholds from other studies of stroke recurrence and inflammatory markers applied to the Edinburgh Stroke Study (ESS), using the same analytical technique.

Study	Marker	Thresholds	Outcome	Reported association	Association when threshold applied to ESS
(Woodward et al. 2005a)	CRP mg/L	1.14, 3.34	Recurrent stroke	OR: 1.26 (0.98 to 1.61)*	1.18 (0.69 to 2.13)‖
	Fibrinogen g/L	3.32, 4.04	Recurrent stroke	OR: 1.24 (0.97 to 1.59)*	0.97 (0.47 to 1.98) ‖
(Welsh et al. 2008e)	IL-6 pg/ml	1.70, 2.94	Recurrent stroke	OR: 1.31 (1.01 to 1.69)*	1.35 (0.61 to 2.97) ‖
(Di Napoli, Papa, & Bocola 2001c)	CRP mg/L	5, 33	Death or any vascular event	HR: 2.89 (1.58 to 5.29)†	1.77 (1.41 to 2.21)**
	Fibrinogen g/L	3.78, 6.17	Death or any vascular event	HR: 2.08 (1.19 to 3.62)†	1.85 (1.41 to 2.42) **
(Rallidis et al 2008)	CRP mg/l	Per 1mg/l	Death by hospital discharge	OR: 1.20 (1.09 to 1.30) ‡	1.01 (1.01 to 1.02) **††
	Fibrinogen	Per 0.1 g/L	Death by hospital discharge	OR: 1.18 (1.08 to 1.30) ‡	1.02 (1.00 to 1.04) **
(Elkind et al. 2006c)	CRP mg/L	4.2, 10.3, 31.1	Recurrent vascular event	HR: 1.86 (1.13 to 3.08)§	1.91 (1.15 to 3.18) ‖
	CRP mg/L	4.2, 10.3, 31.1	Death	HR: 4.50 (2.83 to 7.15)§	3.21 (2.04 to 5.05) **
(Campbell et al. 2006a)	CRP mg/L	0.8, 1.8, 4.4. 92.1	Recurrent ischaemic stroke	OR: 1.00 (0.63 to 1.58) §	1.55 (0.14 to 3.30) ‖

*Top versus bottom third, adjusted; †per third, unadjusted; ‡adjusted; §top to bottom quarter adjusted ‖ adjusted for age, AF, prior TIA, stroke or MI and cardiac failure; ** adjusted for age, ability to walk, living alone, independent prior to stroke, orientated to place time and person, able to lift arms from bed†† death within first year only

Figures

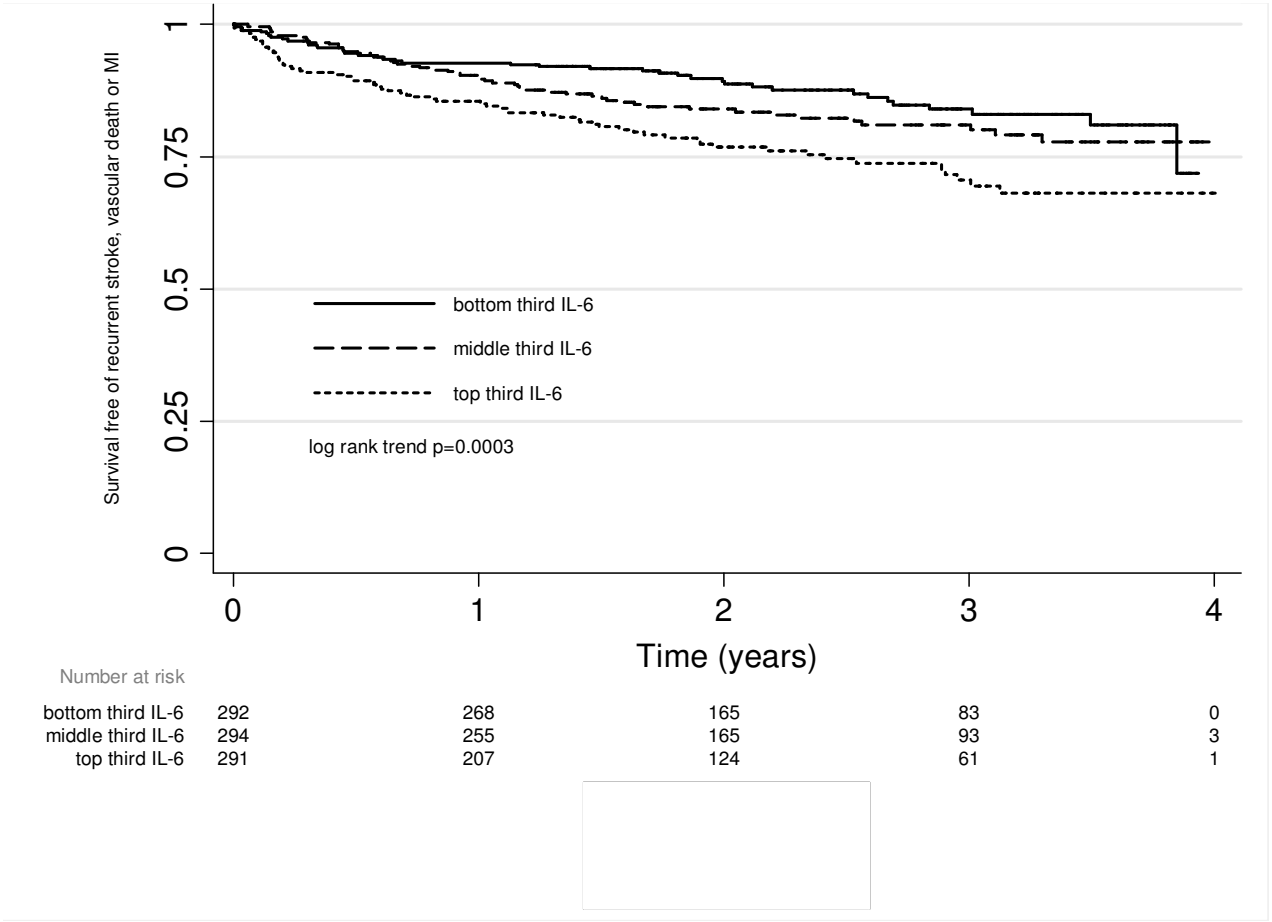


Figure 33 Unadjusted Kaplan Meier survival curve and life table, for survival free from recurrent stroke, myocardial infarction or vascular death by third of interleukin 6

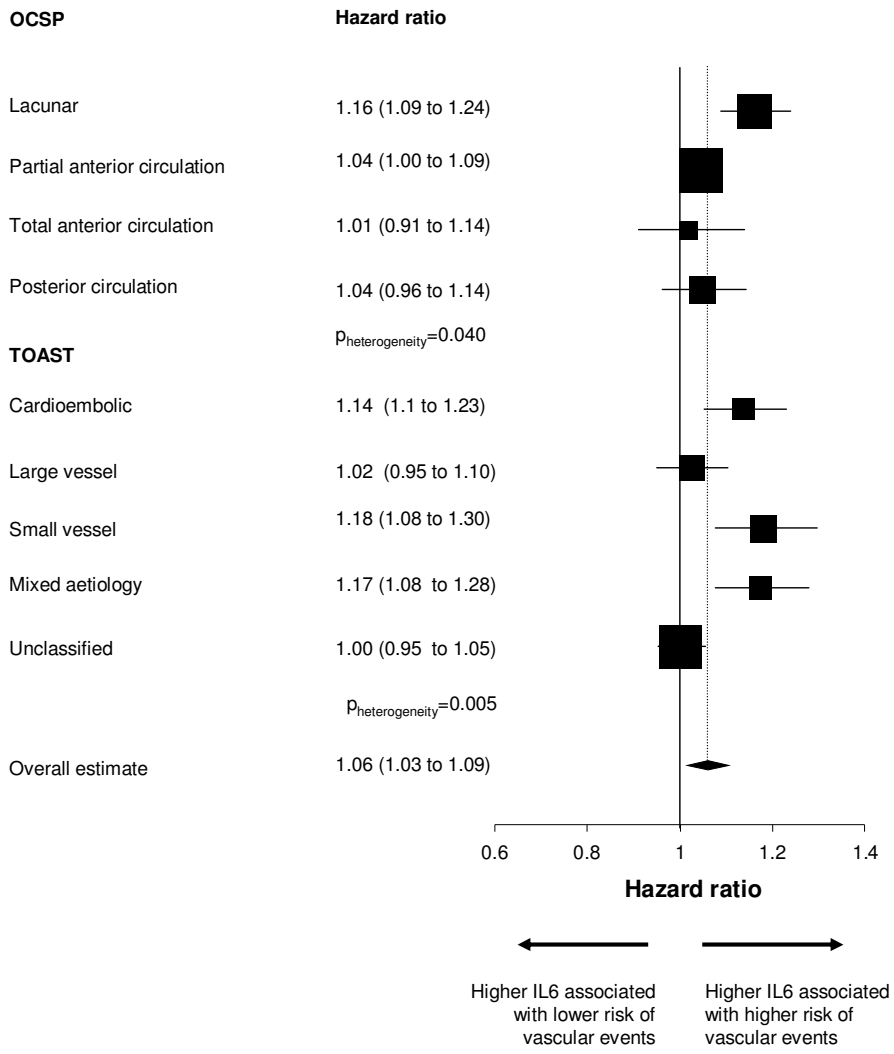


Figure 34 Hazard ratio per pg/ml increase in interleukin-6 for the occurrence of recurrent stroke, myocardial infarction and vascular death, for different baseline subtypes of ischaemic stroke.

Adjusted for age, AF, prior TIA, stroke or MI and cardiac failure. Stroke classified by the Trial of Org 10172 in Acute Stroke Treatment algorithm and the OCSP classification. P value is derived from a χ^2 test of heterogeneity. Each square is placed at the point estimate, and the size of the square is proportional to the number of strokes in that category. Horizontal lines mark 95% confidence intervals

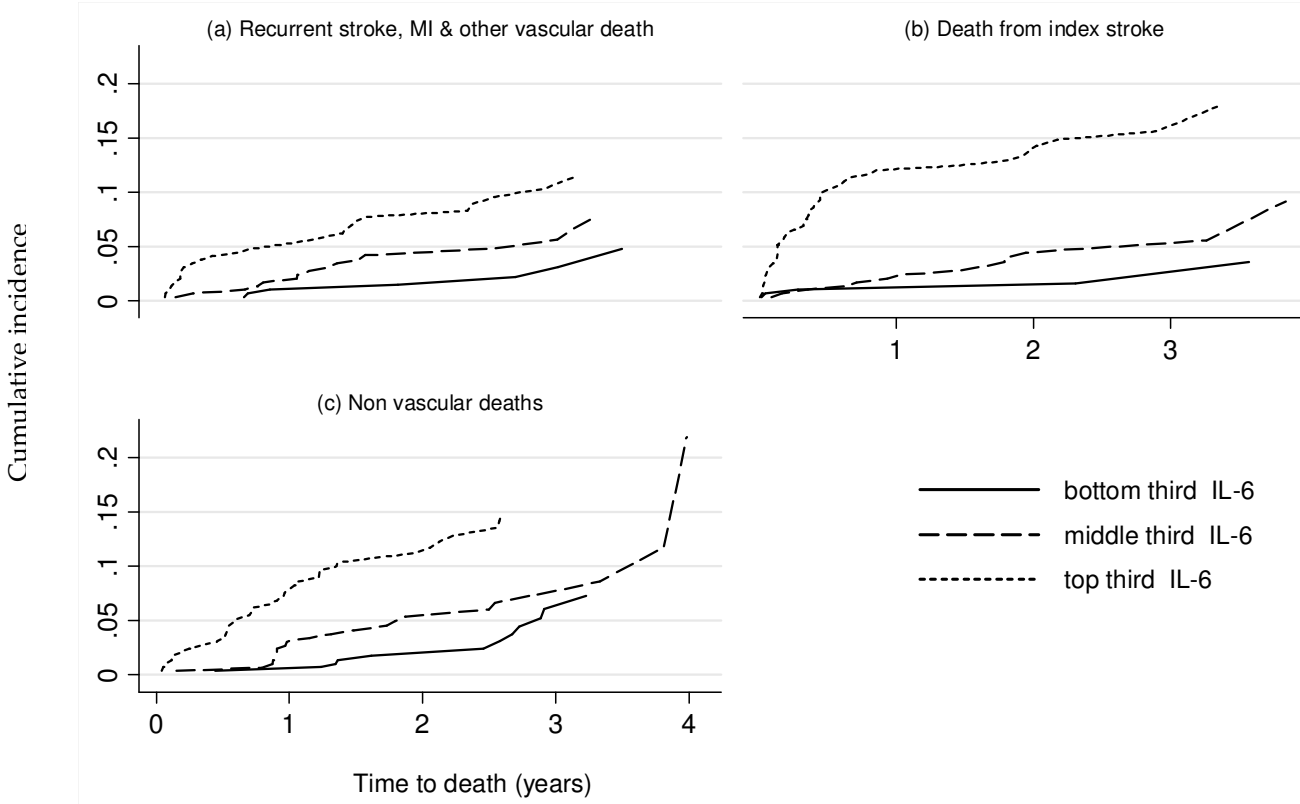


Figure 35 Unadjusted cumulative incidence curves of (a) death from recurrent stroke, MI or other vascular causes, (b) death from the initial stroke or (c) non-vascular death, by thirds of interleukin 6, estimated from a competing risk analysis

Chapter 9. Discussion

Implications for clinical practice

I have studied the clinical utility of blood biomarkers that are easily available to clinical researchers. In chapter 2, I posed a number of clinical questions that could be addressed with the use of blood biomarkers:

Do blood markers identify patients with ischaemic or haemorrhagic stroke ('acute cerebrovascular disease') in a group of patients in whom stroke is suspected by a member of the emergency team?

A member of the emergency department team might use a blood marker that could identify patients with acute cerebrovascular diseases quickly and simply to aid a decision whether or not to admit a patient to a stroke service or prioritise them for urgent brain imaging. This could reduce the delay to time-dependent treatments.

However, I found that - in patients presenting with suspected stroke - none of the markers of inflammation, thrombosis, thrombolysis, cardiac strain or cerebral damage that I measured were likely to improve the clinical diagnosis of acute cerebrovascular disease. I have answered this question satisfactorily in the thesis though subject to the constraints in the recruitment of the cohort (see below). There was no evidence that blood markers could identify patients with acute cerebrovascular disease amongst those patients in whom a member of the emergency department was substantially uncertain about the diagnosis of stroke (i.e. excluding those with definite stroke).

Do blood markers identify patients with ischaemic stroke amongst patients in whom a stroke physician suspects stroke, though the initial imaging is normal?

An emergency department doctor might want positive reassurance that the patient in front of him has had an ischaemic stroke, when the initial CT imaging appears to be normal. Were the diagnosis of positive diagnosis of ischaemic stroke made more easily in patients with normal CT brain scans, then thrombolysis or other important

acute treatments for stroke might be delivered more frequently by non-experts, improving access to treatments for those remote from larger hospitals.

In this study, the use of blood markers did not improve the identification of patients with ischaemic stroke amongst those with a normal scan over and above simple clinical measurements.

Does this patient with a clinical diagnosis of definite stroke have either a haemorrhagic or ischaemic stroke?

If patients with ischaemic stroke could be distinguished from patients with haemorrhagic stroke without the use of a CT scan (for example in an ambulance or an emergency department triage) then important acute treatments, such as intravenous thrombolysis, could be given more rapidly. I have not been able to address this important question in this thesis, as the study recruited too few patients with intracranial haemorrhage, and hence any analysis underpowered. The ideal design for such a study would be to recruit a large number of patients very early after the onset of their symptoms in whom the 'first responder' had made a firm diagnosis of stroke, and then see whether blood markers could identify either those with intracranial haemorrhage, or those with cerebral ischaemia from the remaining patients.

What is the prognosis of the patient with stroke, in the shorter or the longer term?

In my view, no laboratory measurement of a blood marker of inflammation, thrombosis, thrombolysis, cardiac strain or cerebral damage is likely to improve the prediction of poor outcome after acute cerebrovascular disease over and above the simple bedside measurement of neurological impairment and age. The data from this thesis suggests that for patients presenting with acute cerebrovascular disease the currently available markers have limited or no clinical utility.

Therefore, none of the markers measured in this project can be recommended for use in clinical practice for the diagnosis or prediction of poor outcome after stroke.

Limitations of the study

The study had a number of limitations, which may give opportunities for further research.

Selection bias

Despite intensive efforts, I only managed to recruit about one half of patients presenting to hospital with ischaemic stroke within the first day of their symptoms, and fewer patients with transient ischaemic attack. . The routinely collected data from the Scottish Stroke Care Audit (collected on stroke patients only) reveals that the severity and age of stroke patients in the current study are similar to the patients who I failed to recruit. There is no routinely collected data available on patients with TIA, or patients with stroke mimics.

Therefore, I believe that the patients within the study were broadly representative of those presenting to hospital with suspected stroke. These patients have, in general, milder symptoms than those who are studied within acute stroke treatment trials and those who are admitted to stroke units. However, the patients that I recruited were comparable in age and level of neurological impairment to studies recruiting patients in emergency departments with suspected stroke.(Chalela et al 2007, Hand et al. 2006d)

I did not recruit out of hours or at weekends, and during my holidays there was a reduction in the intensity of recruitment as other stroke fellows were recruiting patients to other studies concurrently. The second reason for failure to recruit patients to the study was incapacity on behalf of the patient and absence of a welfare guardian. As I not collect a log of patients not recruited to the trial, I could not study this issue systematically, though clearly it is important source of bias as those patients unable to consent differ from those who do in important, and often unpredictable ways. (Al-Shahi, Vousden, & Warlow 2005)

Selection of blood markers

The selection of the best candidate markers for the diagnosis of prediction of outcome in stroke from amongst the vast array of possible markers is difficult. There are a number of competing approaches.

The first approach could be termed 'biological plausibility'. Here, a marker might be selected because of its theoretical association with stroke pathophysiology, or the presumptive kinetics of marker release after stroke onset, usually based on animal studies. There are many narrative reviews which have taken this approach. (Beaudeux 2009, Foerch et al. 2009). However, the basis of the theoretical associations may not be sound: they are highly dependent on the existing findings in the published literature, which are as prone to publication and other biases as the clinical literature.

Second, proteomic methods might be used to discover new proteins in serum of patients with stroke. In essence these methods compare the protein profile, measured usually as a mass/charge spectrum, of serum or CSF of patients with stroke (or one of its subtypes) to patients without stroke. These studies have identified a number of proteins, though there has yet to be a large scale reproduction of their work.(Allard et al 2007, Allard et al 2005i, Allard et al. 2005a, Allard et al. 2004g). Despite the novelty of proteomic methods, and the exciting potential for the discovery of new proteins, current laboratory and statistical methods limit the usefulness of the technique. First, even after depleting the most abundant proteins from serum (for example albumin and immunoglobulins), the proteins with the lowest concentrations, which might be the most tissue specific, are hard to identify. For example, studies of serum taken soon after myocardial infarction have not identified troponin as potential candidate markers, but instead rather more common, non-specific markers of inflammation and proteins that have subsequently been shown to be storage artifacts.(Marshall et al. 2003) Second, the challenge of identifying an important protein from amongst hundreds (or even

thousands) of others – particularly where differences may be quantitative rather than qualitative – is so difficult as not to be tractable to modern statistical methods.

My approach was to select blood markers for this study by two systematic reviews of the existing literature. This approach has a number of advantages. By reviewing the entirety of the medical literature, it ensures that no potential candidate markers are ignored. It ensures an unbiased selection of markers, not dependent on any pre-existing prejudices of the study authors. It also ensures that those markers chosen can be measured, and does not rely on the development of new technology or statistical methods. However, this method, which identifies a number of different biomarkers studies in different patient populations does not directly compare the expected comparative strength of association between markers and outcomes of interest. This is the reason for the current study.

Of course, the relationship between the levels of blood markers and either the diagnosis of stroke or the prediction of outcome after stroke is complex. My approach in this study has been to examine the role of blood markers in prediction of either outcome after stroke or diagnosis of stroke, and not the causal role of particular biological processes in stroke. The study has therefore measured the effect of adding each marker to a clinical assessment in improving prediction rather than whether a marker has a causal role in stroke pathogenesis. In other settings, causal variables may be weak predictors.

Difficulties of blood marker measurement

For large scale studies examining the causal relationship between marker levels and particular outcomes, it is important to measure the level of blood markers in each individual that most truly reflects the activity of physiological process that the biomarker purports to measure. For example, many important biomarkers are altered by time of day, and timing of blood draw in relation to food or symptom onset. These can lead to important – sometimes several-fold – differences in the concentration of blood marker levels. However, the aim of this study was to try to

determine whether blood markers might be useful in clinical practice. Those markers which change rapidly with minor differences in physiological state or time of day are unlikely to be blood markers which make their way into clinical practice.

However, whereas this biological variability is to be welcomed, it is important that the sample collection and storage is as homogenous as possible. I achieved this in this study by rapid freezing of each sample very soon after each blood sample was taken. All the procedures were done in the same research laboratory by staff experienced in the preparation and measurement of samples for blood markers.

Use of other medications

The levels of CRP and other inflammatory markers are reduced clearly by rosuvastatin. (Ridker et al. 2009). However, in this study the additional confounding due to statin prescription of the relationship between poor outcome after stroke, over and above neurological impairment and age, was small and not statistically significant.

Implications for research

The search for a blood marker for the diagnosis of stroke, or any of its subtypes has many theoretical and practical difficulties. First, it is difficult to imagine a physiological process that is unique to any one subtype of stroke that could not be found in a stroke mimic. Second, as the release into blood of brain proteins is slowed by the blood brain barrier, venous blood levels will not rise early after symptom onset, when their measurement might be most clinically useful.

It is unlikely that other markers of the physiological processes of inflammation, thrombosis, thrombolysis, or cardiac strain will be useful in stroke diagnosis, unless markers are discovered that are unique to stroke. There may be brain proteins or other molecules that are released from damaged brain and are able to pass to pass rapidly through a damaged blood brain barrier (for example very small, or uncharged molecules) though again these may not unique to acute ischaemic stroke, or one of its pathological subtypes.

I have not examined structural proteins that form the tight junctions of the blood brain barrier – for example cadherins, occludins and junctional adhesion molecules – which may be unique to brain, and might be released rapidly after damage to the luminal side of the blood brain barrier after arterial occlusion by thrombus, rather than other pathologies. These proteins are worthy of further study, when they can be measured easily in serum or plasma.

The main challenge facing a blood test for the prediction of poor outcome after stroke is that easily measured clinical variables, particularly neurological impairment and age, are so strongly associated with poor outcome. As most physiological markers that rise after stroke are associated with one or both of these variables, markers will probably add no more than a small improvement in prediction to clinical examination.

One potential use for a blood marker of a physiological process is targeting particular treatment on those patients most likely to benefit from them. The most plausible blood marker candidates are those that reflect the mechanism of action of treatments – for example markers of endogenous thrombolysis (e.g. tPA) or clot burden (D-dimer) in acute ischaemic stroke patients treated. High or low levels of such markers could describe groups of patients who might stand to gain benefit from, or be harmed by, particular interventions. Reliably to identify these patients would need: (i) two groups of patients, one randomly allocated to receive the treatment and the other to avoid treatment, (ii) the measurement of blood marker levels and potentially confounding baseline variables before treatment in all patients, (iii) a sufficiently large sample size to assess the interaction between blood marker level and treatment benefit, and (iv) the validation of thresholds calculated for decision making in other, potentially larger studies. However, blood markers to aid treatment decision making are often suggested after a treatment is already used routinely in clinical practice, when the allocation of patients to a control group would be difficult to justify. As a trial to assess of the role of blood markers in making treatment decisions needs a larger sample size than a trial simply to identify

treatment effects, blood markers are not usually evaluated in the early large-scale studies of a new treatment. The additional complexity of study design and increased sample size mean these studies are very expensive to perform. For pharmaceutical companies, the additional cost of biomarker evaluation might not be economic: even were a trial to show an effective biomarker based approach, a targeted treatment is used (therefore sold) less frequently. The only successful commercial model is probably where the company owns of both the revenue from sale of the blood marker and the drug itself. For example, Roche owns patents for both trastuzumab (herceptin) and the fluorescent in-situ hybridisation test for HER/neu in breast cancer tissue, which identifies the patients most likely to benefit.

Future directions

The prediction of poor outcome and other potentially predictable events (recurrent stroke, MI, DVT, PE, infection) after stroke is of great interest to clinicians and patients. Senior clinicians often use a clinical assessment of prognosis to inform decisions about acute drug treatments or other interventions, the timing of long term placement and suitability for rehabilitation. As a clinician's predictions have a potentially large impact on treatment, improvement and standardisation of prediction might improve patient outcomes.

Clinical predictions are likely based on: experience; a doctor's ability; and the type of outcome they are trying to predict. Outcomes that might be predicted more accurately are those that are frequent, for which a clinician can build experience quickly and have the prediction confirmed by experience, and those which occur soon after stroke, when a clinician would link outcomes with baseline clinical variables.

The work of this thesis has led me to propose a study to improve the prediction of thrombotic and haemorrhagic events after stroke: to build and validate better models; to examine the interaction between predicted outcome and treatment effect of antiplatelets, heparin and thrombolysis; and to implement the prediction from predictive models in clinical practice (see final appendix).

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Appendix 1. Search Strategy

MEDLINE Search Strategy

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 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. 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. 5 or 6 or 7 or 8

10. 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 apolipoproteins 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 Fibronectins/ 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 X/ 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 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/

11. (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 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-macroglobulin$ 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 hbgf-2 or cartilage-derived growth factor or class ii heparin-binding growth factor or fgf-2 or fgf2 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 or 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 cetp 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 arhgap2 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 hydrolase or cobalt enolase or nervous system-specific enolase or non-neuronal enolase or alpha, alpha-enolase or beta-enolase or gamma, gamma-enolase or Phosphopyruvate Hydratase or Neuron specific enolase or Neurone specific enolase or Neurone-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

preproendothelin-1 or proendothelin 1-38 or proendothelin-1 precursor or Erythropoietin or antigens, cd62e or cd62e antigens or e selectin or elam-1 or endothelial leukocyte 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 vii 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 hemofil or hemofil hm or hemofil m or hemophil or humate-p or hyate-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 xiia or factor xii, activated or activated factor xii or blood coagulation factor xii, activated or hageman-factor fragments or prekallikrein activator or factor xiia 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 isoferritin or ferritin or isoferritin or isoferritin, 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 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 l 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 gpiib-iiia receptors or integrin alphaiibbeta3 or glycoproteins iib-iiia or integrin alpha-iib 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 allerglobuline 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 propanedial or malonylaldehyde 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 promatrixmetalloproteinase-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 gelatinase 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 neuritogenic 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 ldl or osteoprotogerin or aryl-dialkyl phosphatase or arylalkylphosphatase or homocysteine thiolactone hydrolase or opa anhydrase or oph enzyme or organophosphorus acid anhydrase or organophosphorus acid anhydrolase or organophosphorus acid hydrolase or organophosphorus hydrolase or paraoxonase or paraoxonase-1 or paraoxonase-2 or glycerate 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 profibrinolysin or glu-

plasminogen or glutamic acid 1-plasminogen or glutamyl plasminogen or agepc 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 fizz3 or resistin or receptor of AGE or RAGE or receptor of advanced glycation end products or secretagogin 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 thrombinar 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 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 vegf or vegf-a or vascular permeability factor or vasculotropin or vitronectin or factor viii-related antigen or f viii-vwf or factor viiir-ag or factor viiir-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.

12. 10 or 11

13. (12 and 4) not (4 and 9)

14. (alzheimer$ or coronary or fibrillation or cardiac or cardio$ or cadasil or diabetes or tumour or tumor or trauma$ or angina or dement$ or child$ or pediatr$ or paediatr$ or newborn$).ti.

15. 14 and stroke.ti.

16. 14 not 15

17. 13 not 16

18. limit 17 to humans

EMBASE Search Strategy

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 cerbrovascular disease/ or exp carotid artery diseases/

2. ((brain or cerebr$ or cerebell$ or vertebrobasil$ 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 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-macroglobulin$ 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 hbgf-2 or cartilage-derived growth factor or class ii heparin-binding growth factor or fgf-2 or fgf2 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 or 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 cetp 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 arhgap2 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 hydrolase or cobalt enolase or nervous system-specific enolase or non-neuronal enolase or alpha, alpha-enolase or beta-enolase or gamma, gamma-enolase or Phosphopyruvate Hydratase or Neuron specific enolase or Neurone specific enolase or Neurone-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 preproendothelin-1 or proendothelin 1-38 or proendothelin-1 precursor or Erythropoietin or antigens, cd62e or cd62e antigens or e selectin or elam-1 or endothelial leukocyte 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 vii 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 hemofil or hemofil hm or hemofil m or hemophil or humate-p or hyate-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 xiia or factor xii, activated or activated factor xii or blood coagulation factor xii, activated or hageman-factor fragments or prekallikrein activator or factor xiia 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 isoferritin or ferritin or isoferritin or isoferritin, 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 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 l 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 gpiib-iiia receptors or integrin alphaiibbeta3 or glycoproteins iib-iiia or integrin alpha-iib 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 allerglobuline 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 propanedial or malonylaldehyde 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 promatrixmetalloproteinase-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 gelatinase 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 neuritogenic 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 ldl or osteoprotogerin or aryl-dialkyl phosphatase or arylalkylphosphatase or homocysteine thiolactone hydrolase or opa anhydrase or oph enzyme or organophosphorus acid anhydrase or organophosphorus acid anhydrolase or organophosphorus acid hydrolase or organophosphorus hydrolase or paraoxonase or paraoxonase-1 or paraoxonase-2 or glycerate 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 profibrinolysin or glu-plasminogen or glutamic acid 1-plasminogen or glutamyl plasminogen or agepc 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 fizz3 or resistin or receptor of AGE or RAGE or receptor of advanced glycation end products or secretagogin 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 thrombinar 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 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 vegf or vegf-a or vascular permeability factor or vasculotropin or vitronectin or factor viii-related antigen or f viii-vwf or factor viiir-ag or factor viiir-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 GLYCOPROTEIN LIGAND 1/ or L SELECTIN/ or SELECTIN/ or exp Blood Clotting Factor/ or exp Cytokine/ or exp Fibrinogen/ or Antigens, CD95/ or exp 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. 4 and 11

13. (alzheimer$ or coronary or fibrillation or cardiac or cardio$ or cadasil or diabetes or tumour ot tumor or trauma$ or angina or dement$ or child$ or pediatr$ or paediatr$ or newborn$).m_titl.

14. stroke.m_titl.

15. 13 not 14

16. 12 not 15

17. limit 16 to human

18. (diagnos$ or sensitivity or specificity or odds ratio or likelihood ratio or LR).tw.

19. 17 and 18

Appendix 2. Modified QUADAS questionnaire: systematic review of blood markers for the diagnosis of stroke

1. Is the reference standard likely to correctly classify the target condition?

Yes: Expert clinical opinion supported by neuroimaging.

No: No imaging, no expert opinion on stroke diagnosis.

2. Did patients receive the same reference standard regardless of the index test result?

Yes: All patients had reference standard. If 'normal controls' recruited, some description of how stroke was excluded in the 'normal' cohort is necessary.

No: Some patients who had the biomarker test did not have a sensible reference standard (i.e. expert clinical opinion + imaging)

3. Was the reference standard independent of the index test?

Yes: The biomarker status was not used to make a diagnosis of stroke

No: The biomarker status could be used to make a diagnosis of stroke

4. Were the index test results interpreted without knowledge of the results of the reference standard?

Yes: The report mention that assessors of biomarker status are blinded to stroke status

No: If the report states that the assessor of biomarker status had knowledge of stroke staus.

5. Were the reference standard results interpreted without knowledge of the results of the index test.

Yes: Either the report mentions the diagnosis was collected before the blood marker was measured, or there is mention of blinding

No: The reporter of the diagnostic study (expert or radiologist) has access to biomarker status

6. Were withdrawals from the study explained?

Yes: all patients who enter the study complete it, or there is explanation of why some patients do not make it to analysis

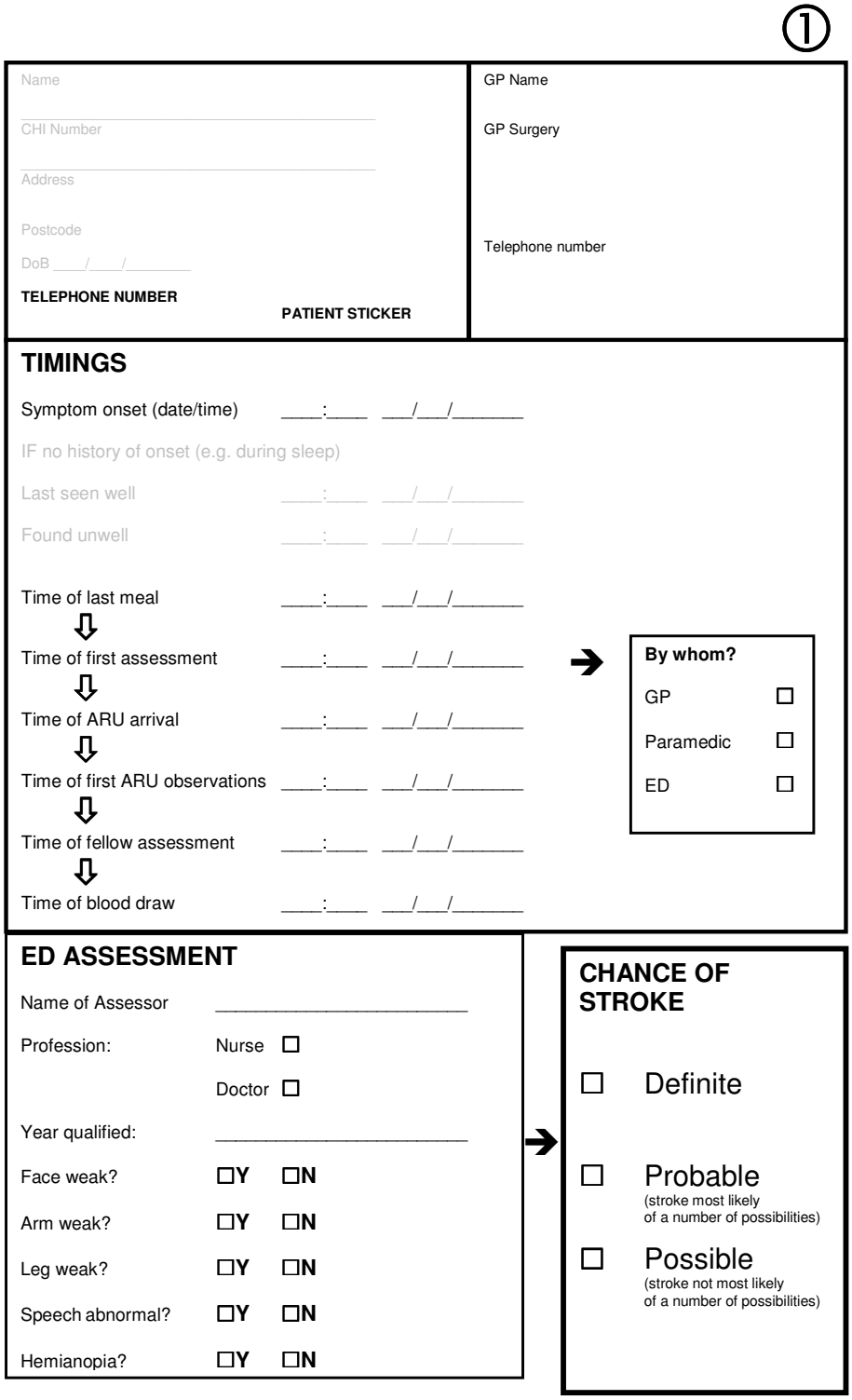
No: Patients missing from final analysis without explanation

7. Was a cut-off established before the study was started?

Yes: A cut off was taken either from the literature, or in a clearly defined pilot study prior to the analysis of the main data.

No: Not true

Appendix 3. Data collection form



①

Name

CHI Number

Address

Postcode

DoB ___/___/_____

TELEPHONE NUMBER

PATIENT STICKER

GP Name

GP Surgery

Telephone number

TIMINGS

Symptom onset (date/time) ____:____ ___/___/_____

IF no history of onset (e.g. during sleep)

Last seen well ____:____ ___/___/_____

Found unwell ____:____ ___/___/_____

Time of last meal ____:____ ___/___/_____

⇩

Time of first assessment ____:____ ___/___/_____ ➔

⇩

Time of ARU arrival ____:____ ___/___/_____

⇩

Time of first ARU observations ____:____ ___/___/_____

⇩

Time of fellow assessment ____:____ ___/___/_____

⇩

Time of blood draw ____:____ ___/___/_____

By whom?

GP ☐

Paramedic ☐

ED ☐

ED ASSESSMENT

Name of Assessor _____

Profession: Nurse ☐

Doctor ☐

Year qualified: _____

Face weak? ☐**Y** ☐**N**

Arm weak? ☐**Y** ☐**N**

Leg weak? ☐**Y** ☐**N**

Speech abnormal? ☐**Y** ☐**N**

Hemianopia? ☐**Y** ☐**N**

➔

CHANCE OF STROKE

☐ Definite

☐ Probable
(stroke most likely of a number of possibilities)

☐ Possible
(stroke not most likely of a number of possibilities)

②

HISTORY OF:

	YES	NO	UNSURE	
Focal brain deficit	☐	☐	☐	➔
Head injury	☐	☐	☐	
Loss of consciousness at onset	☐	☐	☐	
Seizure at onset	☐	☐	☐	focal onset? Y ☐ N ☐ Unsure ☐
History suggestive of infection	☐	☐	☐	which? _____
Headache at onset	☐	☐	☐	describe _____
Movement disorder	☐	☐	☐	describe _____

CIRCLE ONE
hemiparesis, dysphagia, aphasia, hemisensory loss, hemianopia or ataxia, facial or hand weakness
NOT
isolated dysarthria, diplopia or vertigo

PAST HISTORY

	YES	NO	UNSURE	
MI/Angina	☐	☐	☐	last symptomatic? __ /___/____
Heart failure (clinical or echo diagnosis)	☐	☐	☐	
Peripheral vascular (symptoms or operation)	☐	☐	☐	
Renal impairment (previous diagnosis)	☐	☐	☐	
Migraine	☐	☐	☐	with aura? Y ☐ N ☐ Unsure ☐
Epilepsy	☐	☐	☐	focal onset? Y ☐ N ☐ Unsure ☐
AF (permanent or PAF)	☐	☐	☐	
Stroke or TIA	☐	☐	☐	last event? __ /___/____
Diabetes	☐	☐	☐	
Cognitive impairment	☐	☐	☐	

Other

DRUGS

	YES	NO
Aspirin	☐	☐
Clopidigrel	☐	☐
Dipyridamole	☐	☐
Warfarin	☐	☐
ACE inhibitor	☐	☐
Beta blocker	☐	☐
Statin	☐	☐

Other:

_____

_____

_____

Current Smoker?
Y ☐ Never ☐ No < 1 yr ☐
No > 1 yr ☐

SOCIAL HISTORY

	YES	NO	UNSURE
Independent of ADLS	☐	☐	☐
Living alone	☐	☐	☐

OHS – BEFORE EVENT

0. No symptoms at all ☐

1. Minor symptoms ☐
despite symptoms able to carry out all usual duties and activities

2. Minor handicap ☐
unable to carry out previous activities: able to look after own affairs without assistance

3. Moderate handicap ☐
requiring some help but able to walk without assistance

4. Moderately severe handicap ☐
unable to walk without assistance, attends to bodily needs without assistance

5. Severe handicap ☐
bedridden, incontinent requiring constant nursing care and attention day and night

NIH Stroke Scale *(Please circle the most appropriate response for each section. See supplementary notes attached. untestable please state reason. Add the scores for each item to get the total, and do not count untestable items)* ③

1a Level of Consciousness (LOC)	0 1 2 3	Alert – *keenly responsive* Drowsy – *arousable by minor stimulation to obey, answer, or respond* Stuporous – *requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped*) Comatose – *responds only with reflex motor or autonomic effects or totally unresponsive*
1b LOC Questions	0 1 2	Answers both correctly Answers one correctly Incorrect Patient is asked to state the month & his/her age. No credit for partly correct answers.
1c LOC Commands	0 1 2	Obeys both correctly Obeys one correctly Incorrect Patient is asked to close & open eyes, grip & release normal hand
2. Best Gaze	0 1 2	Normal Partial gaze palsy – *gaze is abnormal in one or both eyes, no forced deviation/total gaze paresis* Forced deviation – *or total gaze paresis not overcome by oculocephalic maneouvre*
3. Visual Fields	0 1 2 3	No visual loss Partial hemianopia or visual inattention Complete hemianopia Bilateral hemianopia – *including cortical blindness*
4. Facial Palsy	0 1 2 3	Normal Minor - *flattened nasolabial fold, asymmetry on smiling* Partial – *total or near total paralysis of lower face* Complete - *absent facial movement in upper and lower face on one or both sides*
5. Best Motor RIGHT ARM	0 1 2 3 4 x	No drift – *holds limb at 90 degrees for full 10 seconds* Drift - *drifts down but does not hit bed* Some effort against gravity No effort against gravity No movement *Untestable (only for amputation or shoulder joint fusion – please state which)*
6. Best Motor LEFT ARM	0 1 2 3 4 x	No drift – *holds limb at 90 degrees for full 10 seconds* Drift - *drifts down but does not hit bed* Some effort against gravity No effort against gravity No movement *Untestable (only for amputation or shoulder joint fusion – please state which)*
7. Best Motor RIGHT LEG	0 1 2 3 4 x	No drift – *holds limb at 45 degrees for full 5 seconds* Drift - *drifts down but does not hit bed* Some effort against gravity No effort against gravity No movement *Untestable (only for amputation or hip joint fusion – please state which)*
8. Best Motor LEFT LEG	0 1 2 3 4 x	No drift – *holds limb at 45 degrees for full 5 seconds* Drift - *drifts down but does not hit bed* Some effort against gravity No effort against gravity No movement *Untestable (only for amputation or hip joint fusion – please state which)*
9. Limb Ataxia	0 1 2 x	Absent Present in 1 limb Present in 2 or more limbs ***Untestable*** *(only for amputation or joint fusion – please state which)*
10. Sensory	0 1 2	Normal Partial loss - *patient feels pinprick is less sharp or is dull on affected side* Dense loss - *patient is unaware of being touched on face, arm, leg*
11. Best Language	0 1 2 3	No dysphasia Mild to moderate dysphasia - *obvious loss of fluency or comprehension, without significant limitation in ideas expressed or form of expression. Conversation about provided material difficult or impossible but examiner can identify items from patient's response.* Severe dysphasia - *all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener who carries burden of communication. Examiner cannot identify items provided from patient response.* Mute - *no usable speech or auditory comprehension.*
12. Dysarthria	0 1 2 x	Normal articulation Mild to moderate dysarthria - *patient slurs some words, can be understood with some difficulty.* Unintelligible or worse - *speech is so slurred as to be unintelligible (absence of or out of proportion to dysphasia) or is mute/anarthric* *Untestable (intubation or other physical barrier to producing speech – please state)*
13. Neglect	0 1 2	No neglect Partial neglect - *Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities* Complete neglect - *Profound hemi-inattention (e.g. does not recognise own hand or orients to only one side of space) or hemi-inattention to more than one sensary modality (e.g. visual + tactile).*

④

EXAMINATION

Temperature _____

Blood pressure _____/_____mmHg

Pulse _____

Blood sugar _____mmol

	YES	NO	UNSURE		YES	NO	UNSURE
AF on ECG	☐	☐	☐	Hoover's	☐	☐	☐
Heart murmur	☐	☐	☐	Yawning	☐	☐	☐
≥ 1 absent peripheral pulse	☐	☐	☐				
Movement disorder	☐	☐	☐				
Mirror movements	☐	☐	☐				
Able to talk	☐	☐	☐				
Walks without help	☐	☐	☐				
Lifts arms from bed	☐	☐	☐				
Orientated (time, place, person)	☐	☐	☐				

	L	R	none
Carotid bruit	☐	☐	☐

Other findings:

Immediate management:

IV rtPA ☐ IA rtPA ☐ Clot extraction ☐ IST3 ☐ Not eligible ☐

DIAGNOSIS – STROKE FELLOW

Cerebral ischaemia:

☐ Definite IF ANY: Hemisphere ☐ **L** ☐ **R** ☐ **Unsure**

☐ Probable

☐ Possible

☐ TACS hemiparesis + hemianopia + higher cerebral dysfunction (HCD: aphasia or inattention)

☐ PACS 2 of: motor/sensory, hemianopia or HCD; monoparesis, new HCD alone

☐ POCS CN with contralateral motor/sensory, bilateral motor/sensory, EOM disorder, ataxia + no long tract signs, isolated hemianopia

☐ LACS no visual field or HCD: involves 2 of 3 of face, arm and leg – not hand alone: Pure motor, pure sensory, ataxic hemiparesis,sensorimotor

☐ Unsure

Not Stroke ☐ IF YES: Diagnosis _____

Presenting symptom _____

⑤

BRAIN IMAGING

Scan Time _____:_____ Scan Date ____/_____/_____ MR ☐ CT ☐

Relevant Brain lesion side Left ☐ Right ☐ Both ☐ None visible ☐

Relevant Lesion type Ischaemic ☐ Haemorrhage ☐ None visible ☐

Lesion location *(underline symptomatic lesion)* Lacunar ☐ Cortical ☐ Posterior Fossa ☐

Other diagnosis _____

CAROTID IMAGING

ICA stenosis left _____% Irregularity Yes ☐ No ☐

ICA stenosis right _____% Irregularity Yes ☐ No ☐

ECHO ☐TTE ☐TTE + contrast ☐TOE

LA atrial size _____cm LV function ☐Good

Valve Lesion Aortic ☐ Mitral ☐ ☐Moderate

PFO Yes ☐ No ☐ ☐Poor

OTHER

Test type	**Result**

SHORT TERM OUTCOMES

		YES	**NO**	**UNSURE**
Post thrombolysis:	symptomatic haemorrhage	☐	☐	☐
	non-symptomatic haemorrhage	☐	☐	☐
Resolved by 24 hours		☐	☐	☐

EVENTUAL DIAGNOSIS

	Definite	Probable	Possible
Cerebral ischaemia	☐	☐	☐
Cerebral haemorrhage	☐	☐	☐
Not stroke _____	☐	☐	☐

Cause:

☐ Cardioembolic **Cortical, b'stem or c'bellar, at least one cardiac source identified**, TIA in>1 vascular territory

☐ Large artery **Cortical, b'stem or c'bellar, vessel >50% stenosis**, **no cardiac source**, TIA in same territory, carotid bruit, loss of pulses, CT /MR lesion >1.5cm, intermittent claudication,

☐ Small-vessel **lacunar syndrome**, no **vessel >50% stenosis**, **no cardiac source** NIDDM, HBP, lesion<1.5cm MR/CT, normal carotid and heart

☐ Other aetiology _____

☐ Undetermined

Appendix 4. Patient Consent Form

NHS Lothian

Department of Clinical Neurosciences
Western General Hospital

THE UNIVERSITY OF EDINBURGH

Study of Blood Tests to Improve the Diagnosis of Suspected Stroke

PATIENT CONSENT FORM

	YES	NO
	(please tick)	
The study has been explained to me and I have read the information leaflet about it. I have had time to consider the study and have had all my questions answered.	☐	☐
I give my consent for my usual general practitioner and consultant to be contacted about the study and for follow up, for my medical records to be examined, and for the information collected in this study to be linked to other NHS information on my health care held confidentially by NHS Scotland.	☐	☐
I give my consent for up to 3 samples of my blood to be taken and tested for markers of acute stroke and stored for developing new tests and in future studies.	☐	☐
I give my consent to be contacted in the future about my health and to be invited to attend for follow-up assessments	☐	☐

I understand that I am free to withdraw at any time from any part of the study, without giving a reason, and without it adversely affecting my future medical care.

Signed:……..(patient's signature)

Name:……(patient's name)

Doctor:……

Date:……

Place patient sticker here

Blood Biomarkers in Suspected Stroke v3 22/1/7

Appendix 5 Relative assent form

NHS Lothian

Department of Clinical Neurosciences
Western General Hospital

THE UNIVERSITY OF EDINBURGH

Study of Blood Tests to Improve the Diagnosis of Suspected Stroke

RELATIVE/WELFARE GUARDIAN'S ASSENT FORM

	YES *(please tick)*	**NO** *(please tick)*
The study has been explained to me and I have read the information leaflet about it. I have had time to consider the study and have had all my questions answered.	☐	☐
I give my assent for my usual general practitioner and consultant to be contacted about the study and for follow up, for their medical records to be examined, and for the information collected in this study to be linked to other NHS information on their health care held confidentially by NHS Scotland.	☐	☐
I give my assent for up to 3 samples of blood to be taken and tested for markers of acute stroke and stored for developing new tests and in future studies.	☐	☐
I give my assent to be contacted in the future about their health, and to be invited to attend for follow-up assessments	☐	☐

I understand that my relative is free to withdraw at any time from any part of the study, without giving a reason, and without it adversely affecting their future medical care. If I am not appointed as welfare guardian, I confirm I am the nearest relative and no welfare guardian is available.

Signed:……..(relative's signature)

Name:……(relative's name)

Relationship…… …...(relationship to the patient)

Patient's name……..

Doctor:……

Date:……

Place patient sticker here

Blood Biomarkers in Suspected Stroke v2 4/1/7

Appendix 5. Patient short information leaflet

Department of Clinical Neurosciences
Western General Hospital

PATIENT SUMMARY INFORMATION SHEET

Study of Blood Tests to Improve the Diagnosis of Suspected Stroke

You have come to hospital with a suspected stroke. We are trying to find out if a new blood test helps to make the right diagnosis for people with symptoms like yours. We are therefore asking if you can help with this study.

We will take up to three blood samples over the next 24 hours from a vein in the arm in the same way as the other blood tests you had on arriving in hospital. Your care in the hospital will not be affected by the results of this blood test.

The research team will discuss your diagnosis with the doctors looking after you. We will look at the tests and brain scans done as part of your routine clinical care. We will see how well the diagnosis made by the blood test matches the correct diagnosis.

The blood taken will be stored in the Wellcome Trust Clinical Research Facility at the Western General Hospital. It will be used by researchers in the University of Edinburgh and the University of Glasgow to assess new blood tests for stroke. We will not be able to give you the results of the blood test.

We would like to contact you in 3 months time and between 6 and 18 months to see how you are recovering from your illness. We will usually do this by sending you a questionnaire by post after contacting your GP with a telephone reminder should there be no response.

You do not have to take part in this study. If you do not wish to take part, your care will not be affected in any way. If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

If you like, you can discuss whether to take part in the research with one of the doctors in the department who is not involved in the research. Her name is Dr Sarah Keir. She is a Consultant Stroke Physician at the Western General Hospital. Please ask us if you wish to speak to her, or she can be contacted by telephone on 0131-5371000.

Appendix 6. Patient long information leaflet

NHS Lothian

Department of Clinical Neurosciences
Western General Hospital

PATIENT DETAILED INFORMATION SHEET

Study of Blood Tests to Improve the Diagnosis of Suspected Stroke

We are inviting you to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

We are trying to find a better way to make a correct diagnosis when people arrive at hospital with suspected stroke. We are investigating how well a new blood test makes the diagnosis of stroke. We will also see if blood tests help to predict how well people recover after a stroke.

Why have I been chosen?

You have been chosen to take part in this study as one of your doctors or nurses suspects you may have had a stroke.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

If you choose to take part in this study, we will take up to 3 blood samples from a vein in your arm. The care and tests you get for your symptoms will be unaffected by the results of the blood test. We will contact you in 3 months time to see how well you are recovering from your symptoms. We will contact your GP before contacting you again. We will usually send you a questionnaire by post, and we will make a telephone reminder should there be no response. We may contact you to see how well you are and invite you for further studies one more time over the next 18 months.

What are the possible benefits of taking part?

We cannot promise the study will help you. We will pay very careful attention to the diagnosis of every patient who joins the study. We feel that this should help make sure that your diagnosis will be as accurate as possible. We hope the results of this research will improve the treatment of people with suspected stroke in the future.

What will happen if I don't want to carry on with the study?

If you withdraw from the study, we will destroy all your identifiable samples if you wish, but we will need to use the data collected up to your withdrawal. We will not contact you again.

What happens when the research study stops?

When the study stops, we will keep your information in the Department of Clinical Neurosciences. It will be kept securely and in strict confidence. The blood samples will be stored for a further 10 years before they are destroyed.

Will my taking part in this study be kept confidential?

Any information you give us as well as the results of the scans and blood tests will be treated as confidential and will only be available to the doctors looking after you and the research staff involved in the project. Information that could identify you as an individual (your name, date of birth, address or hospital number) will not leave the University department. Blood collected during the study will be transferred to researchers in Glasgow.

What will happen to any samples I give?

Blood samples taken at your entry into the study will be taken and stored in the Wellcome Trust Clinical Research Facility. The samples will be stored for 10 years and then destroyed. Part of the sample will be sent to the University of Glasgow for analysis. The remaining blood samples will be analysed by researchers in the University of Edinburgh looking at the changes found in blood soon after acute stroke. The samples will be kept anonymous, though linked to information held by the person in charge of the study. The samples may be used in future studies. If a test is developed from the blood sample you have given, you will not benefit financially.

Will information from the study be given to my GP?

We will write a letter to you GP to tell them that you are involved in this study.

How will information from this study be published?

Once the study has been completed and the information analysed, the results of the study will be published in medical journals, so other doctors can make use of the information. None of your personal information will be used in these articles.

Complaints
If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (Dr W. Whiteley 0131532912). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

Who has reviewed the study?

This study has been reviewed by the Multicentre Research Ethics Committee for Scotland A. If you wish to talk to a doctor independent of the study, please contact Dr Sarah Keir, Consultant Stroke Physician at the Western General Hospital. The telephone number is 0131 5371000.

Thank you for reading about this study

Investigation of Blood Biomarkers in Suspected Stroke

Appendix 7. Search Strategy, Systematic review of blood markers for the prognosis of ischemic stroke

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 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 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 apolipoproteins 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 Fibronectins/ 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 X/ 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 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/

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 2 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-macroglobulin$ 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 hbgf-2 or cartilage-derived growth factor or class ii heparin-binding growth factor or fgf-2 or fgf2 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 or 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 cetp 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 arhgap2 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 hydrolase or cobalt enolase or nervous system-specific enolase or non-neuronal enolase or alpha, alpha-enolase or beta-enolase or gamma, gamma-enolase or Phosphopyruvate Hydratase or Neuron specific enolase or Neurone specific enolase or Neurone-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

preproendothelin-1 or proendothelin 1-38 or proendothelin-1 precursor or Erythropoietin or antigens, cd62e or cd62e antigens or e selectin or elam-1 or endothelial leukocyte 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 vii 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 hemofil or hemofil hm or hemofil m or hemophil or humate-p or hyate-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 xiia or factor xii, activated or activated factor xii or blood coagulation factor xii, activated or hageman-factor fragments or prekallikrein activator or factor xiia 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 isoferritin or ferritin or isoferritin or isoferritin, 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 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 l 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 gpiib-iiia receptors or integrin alphaiibbeta3 or glycoproteins iib-iiia or integrin alpha-iib 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 allerglobuline 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 propanedial or malonylaldehyde 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 promatrixmetalloproteinase-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 gelatinase 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 neuritogenic 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 ldl or osteoprotogerin or aryl-dialkyl phosphatase or arylalkylphosphatase or homocysteine thiolactone hydrolase or opa anhydrase or oph enzyme or organophosphorus acid anhydrase or organophosphorus acid anhydrolase or organophosphorus acid hydrolase or organophosphorus hydrolase or paraoxonase or paraoxonase-1 or paraoxonase-2 or glycerate 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 profibrinolysin or glu-

plasminogen or glutamic acid 1-plasminogen or glutamyl plasminogen or agepc 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 fizz3 or resistin or receptor of AGE or RAGE or receptor of advanced glycation end products or secretagogin 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 thrombinar 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 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 vegf or vegf-a or vascular permeability factor or vasculotropin or vitronectin or factor viii-related antigen or f viii-vwf or factor viiir-ag or factor viiir-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.

11 Incidence/ or exp mortality/ or follow up studies/ or mortality/ or prognos$.tw. or predict$.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 cerbrovascular disease/ or exp carotid artery diseases/

2. ((brain or cerebr$ or cerebell$ or vertebrobasil$ 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 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-macroglobulin$ 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 hbgf-2 or cartilage-derived growth factor or class ii heparin-binding growth factor or fgf-2 or fgf2 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 or 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 cetp 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 arhgap2 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 hydrolase or cobalt enolase or nervous system-specific enolase or non-neuronal enolase or alpha, alpha-enolase or beta-enolase or gamma, gamma-enolase or Phosphopyruvate Hydratase or Neuron specific enolase or Neurone specific enolase or Neurone-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 preproendothelin-1 or proendothelin 1-38 or proendothelin-1 precursor or Erythropoietin or antigens, cd62e or cd62e antigens or e selectin or elam-1 or endothelial leukocyte 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 vii 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 hemofil or hemofil hm or hemofil m or hemophil or humate-p or hyate-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 xiia or factor xii, activated or activated factor xii or blood coagulation factor xii, activated or hageman-factor fragments or prekallikrein activator or factor xiia 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 isoferritin or ferritin or isoferritin or isoferritin, 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 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 l 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 gpiib-iiia receptors or integrin alphaiibbeta3 or glycoproteins iib-iiia or integrin alpha-iib 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 allerglobuline 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

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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 GLYCOPROTEIN LIGAND 1/ or L SELECTIN/ or SELECTIN/ or exp Blood Clotting Factor/ or exp Cytokine/ or exp Fibrinogen/ or Antigens, CD95/ or exp 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 prognos$.tw. or predict$.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

Appendix 8. Modified REMARK 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: reporting of the source an ELISA, or a reference to it

NO: no such reference

Cases unselected/unbiased?

YES No attempt to select patients with exclusion criteria

NO only a subset of stroke patients enter the study

Appendix 9. Studies in systematic review of prognostic markers for ischaemic stroke

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Appendix 10. Future plans

TITLE

Targeted treatment for acute stroke: development of prognostic models and decision support tools

BACKGROUND

Each year, 135,000 people in the UK have an acute stroke: their care costs the NHS £2.8 billion. Thrombolytic, antithrombotic and antiplatelet drugs are effective for the treatment of acute stroke by reducing the risk of thrombotic events. However, each drug is associated with an increased risk of serious intracranial and gastrointestinal haemorrhage. Better targeting of these therapies, by stratifying patients according to their risk of these events, could increase their net population health gain by (a) reducing avoidable haemorrhagic and (b) arterial and venous thrombotic events.

The National Institute for Clinical Excellence recently recommended, for the prevention of venous thrombo-embolism, *'offer[ing] prophylactic-dose [heparin] to patients in whom a diagnosis of haemorrhagic stroke has been excluded, [and] the risk of bleeding (haemorrhagic transformation of stroke or bleeding into another site) is assessed to be low.'*[1].

Currently, clinicians do not have the tools available to them to trade the risk of all haemorrhagic against the risk of all thrombotic events after stroke, as there are no reliable, valid models. This study seeks to determine whether new prognostic models based on clinical variables and novel biological markers can usefully provide sufficient gain in predictive power to influence clinical decisions in the use of thrombolytic, antithrombotic and antiplatelet drugs in acute stroke.

The MRC Methodology Hub, based in Edinburgh under the leadership of Professor Gordon Murray (co-sponsor) has extensive experience in predictive model building and meta-analysis of large studies. My own experience of prognostic model development[2] experience within the department[3] and experience amongst my collaborators[4] makes Edinburgh a strong base for this research.

Current treatments have modest net effects: After acute ischaemic stroke, 79 patients need to be treated with aspirin (started <24 hours) to prevent one dying or becoming dependent on others[5] and 10 with rtPA (<3 hours) to prevent one becoming dependent on others[6]. Haemorrhagic events reduce the clinical efficacy of these drugs. When heparin is used in unselected patients with acute stroke, the reductions in arterial and venous occlusive events are offset by similar increases in intra- and extra- cranial haemorrhages. We could improve the net clinical benefit of antiplatelet, antithrombotic and thrombolytic treatments by targeting them to those who are more likely to have a thrombotic than a haemorrhagic event with the prognostic models developed in this project.

Serious thrombotic events after stroke are common: Patients with ischaemic stroke are at risk of recurrent ischaemic stroke (15% in the first year), myocardial infarction (MI) (2% per year), and deep vein thrombosis (DVT) and pulmonary embolus (PE) (18% in the first month[7]).

Haemorrhagic complications after ischaemic stroke are common: In cohorts of acute stroke patients, serious intracranial bleeding occurs in 2-8%[8] and gastro-intestinal (GI)

haemorrhage requiring treatment in 3[9]-8%[10]. These serious events are in part due to the stroke, and part due to treatment with antiplatelet agents, heparin, and recombinant tissue plasminogen activator (rtPA) (Table 1).

Table 1 Relative and absolute risk of major bleeds in patients with ischaemic stroke

	Odds ratio		Events caused per 1000 treated			
	Symptomatic ICH	**Major GI bleed**	**Symptomatic ICH***		**Major GI bleed †**	
			Low risk	***High Risk***	***Low Risk***	***High Risk***
Aspirin	**1.33[5]**	**1.67[5]**	**5**	**23**	**11**	**31**
Heparin	**2.55[11]**	**2.99[11]**	**23**	**10**	**22**	**92**
rtPA	**3.37[6]**	**-**	**43‡**	**58‡**	**-**	**-**

I derived estimates by applying relative risks seen in trials (from systematic reviews) to absolute risks from cohort studies with different levels of baseline risk of bleeding. I have assumed all patients in cohort studies took aspirin.

*symptomatic ICH proportion in untreated patients (at any time) 1.5%[12] in low risk and 7%[12] in high risk population.

†GI bleed proportion in untreated patients (~1st year) 1.6%[9] in low risk, and 4.6%[10] in high risk population

‡Estimates for ICH after rtPA are from trials, which may underestimate their frequency in practice.

***Clinical prediction is imperfect*:** The prediction of outcome in an individual patient – hence choice of treatment - is usually based upon clinical experience informed by current evidence. However, clinicians do not perform as well as prognostic models in predicting recurrent vascular events[13], as analysing many variables simultaneously is difficult without the support of a model. Prognostic models to predict adverse outcomes after stroke could improve treatment decisions particularly by less experienced doctors, nurses and other team members.

***Prognostic models may help decision making*:** The use of prognostic models to predict thrombotic and haemorrhagic complications after stroke could (i) identify patients at a high or low risk of thrombotic or haemorrhagic events, which could influence medical treatment – for example avoiding thrombolytic treatments in ischaemic stroke patients at high risk of haemorrhage, or starting antiplatelet treatment in intracerebral haemorrhage patients at high risk of subsequent thrombotic events, (ii) adjust data for baseline prognostic variables for audit or research purposes which may improve the power of randomised controlled trials[14], (iii) define groups at high risk of particular outcomes, in whom new treatments could be tested and, (iv) inform patients and their families of their future risks.

***The addition of biologically relevant markers to prognostic models*:** Selecting patients for treatment based upon levels of relevant blood or imaging biological markers, rather than clinical variables such as age or stroke severity, could lead to better use of existing treatments. Markers of thrombosis, lipid fractions and others could be useful when selecting patients for treatments to prevent arterial and venous thrombosis after stroke. For example D-dimer is associated with a risk of early recurrent ischaemic lesions (OR=3.2 per log unit)[15].

Evaluating prognostic models: A prognostic model must be carefully developed in appropriate data sets and the predictions validated in separate cohorts. However, the real

test of a model is its impact on outcomes including clinicians' behaviour, quality of care and most importantly patient outcome[16]. Models that achieve this are more likely to be adopted by the NHS.

AIMS

The aim of this project is to determine whether prognostic models can be developed that provide clinically useful guidance and are able to increase net health gains from more effective application of existing treatments for stroke, which could be incorporated into NHS electronic patient record systems.

This aim is timely and relevant. The General Medical Council[17] obliges doctors *'to share with patients'.. 'the information they want or need to know about their condition* [and] *its likely progression'*. Without reliable means of predicting outcome, most clinicians will find this difficult. With reference to the MRC Strategic Plan (2009), this project aims to *translate* work from epidemiological studies into tools for use by clinicians, and *target* particular disease subtypes using biomarkers and other measures of risk (so-called 'stratified medicine'). We will also exploit existing *population data sets* though the SCOPE collaborative (containing data from several MRC funded studies: IST, IST-3, CLOTS) the Kadoorie Study of Chronic Disease in China and the VISTA collaboration. Scotland is an ideal base for this research: electronic clinical records are part of the eHealth strategy (expected in some health boards by 2011) and the NHS Scotland CHD and Stroke Strategy. There is therefore a clear pathway for the implementation of the successful predictive models by embedding them within e-forms for use by frontline clinicians in the NHS.

OBJECTIVES

The objectives of this project are to:

(1) Develop models to predict haemorrhagic and thrombotic events from data in: (i) the Stroke Complications and Outcomes Prediction Engine (SCOPE) collaboration (ii) The Kadoorie Study and (iii) the Virtual Internet Stroke Archive (VISTA) (see below for study details).
(2) Determine whether prediction of haemorrhagic events based on clinical and imaging data has a significant interaction with the treatment benefits of intravenous rtPA, heparin and aspirin with data from the MRC-funded First International Stroke Trial (IST), Third International Stroke Trial (IST-3, data available 2012) and Chinese Acute Stroke Trial (CAST).
(3) Create a decision tool for use in clinical practice, based on the prediction models for haemorrhagic events and arterial and venous thrombosis developed in parts 1-3
(4) Pilot the application of the decision tool in clinical stroke practice in NHS Lothian with a view to establishing a larger scale randomised multi-centre evaluation study.

PLAN OF RESEARCH

(1) Development and validation of clinical prognostic models to predict haemorrhage and thrombosis after stroke

Systematic reviews: With my experience in systematic reviews of prognostic studies[18,19] , I will perform systematic reviews of prognostic models in acute stroke to predict (i) haemorrhagic events and (ii) arterial and venous thrombotic events after stroke, to ensure no potentially useful model is missed. I will develop protocols for this type of review with colleagues from the Cochrane Prognostic Methods Group.

To develop prognositic models for the prediction of haemorrhage and arterial and venous thrombosis after stroke I will develop these models using clinical variables that could be collected at the bedside.

I will use three **data resources**:

(i) Stroke Complications and Outcomes Prediction Engine (SCOPE): Based at the Western General Hospital (PI Professor Martin Dennis, a collaborator on this fellowship) this major international collaboration has collected data from 24 large randomised controlled trials and observational cohort studies of patients with stroke. It includes baseline and outcome data from over 40,000 patients who are representative of the clinical spectrum of acute stroke. Baseline data include measures of age, pre-morbid function, stroke severity, and medical history. Baseline variable and outcome definitions were similar across the studies, reducing methodological heterogeneity.

(ii) The Kadoorie Study[20]*:* This is a bio-banking study of 515,000 people with no major disability between 35-74 years old from 10 regions of China, run from the Clinical Trial Service Unit in Oxford by Professor Zhengming Chen (a collaborator on this project) with the close involvement of the China Center for Disease Control. Sites were chosen to reflect a range of exposures and economic development and participants recruited between 2004 and 2008; to date there has been on average 2.8 years of follow up. Events are collected through established death and disease registers and routinely collected healthcare data. Each 5 years, 10,000 surviving study participants are invited for review, and baseline measures – including blood samples – will be repeated to take account of regression dilution bias.

(iii) Virtual Internet Stroke Archive (VISTA) Based in Glasgow, VISTA contains data from 28 randomized clinical trials in acute stroke. Data are available on 21,822 patients, of whom 18,937 (90.5%) have ischaemic stroke and 1,933 (9.4%) have intracerebral haemorrhage. It includes the German Stroke Registry, the validation dataset for the Essen Stroke Risk Score (which predicted only recurrent stroke, and lost power by dichotomisation of variables, in part leading to its poor predictive performance).

Developing prognostic models: Using data from the SCOPE collaboration, I will build prognostic models using standard statistical methods to predict GI haemorrhage, intracerebral haemorrhage, recurrent stroke, MI and DVT. Informed by results from systematic reviews and inspection of the datasets, I will select variables with: face validity, few missing data, that are easily measured at the time of assessment at a low cost, with a wide range and with low inter-observer variability. I will avoid stepwise selection methods[22] and will not dichotomise continuous variables, as this process risks loss of information and hence power[21]. The source studies have made strenuous efforts to ensure completeness of data. Where, despite this, important variables have missing values, I will use imputation methods (either simple or multiple) to mitigate the selection bias associated with complete case analyses. I will examine the effects of source study, as well as the effect of predicting only fatal events. Pre-specified first order interactions include severity with time of presentation after stroke and baseline stroke subtype.

To validate the statistical models developed*:* Initially, I will develop prognostic models in the SCOPE dataset, and then examine for external validity in the Kadoorie study and the separate VISTA collaboration. I will assess the performance of models using measures of: *calibration* by comparing predictions from the prognostic model with observed outcomes, both in development and validation datasets, and *discrimination* measuring the area under the receiver operating curves. The threshold for a 'good model' is unknown, but should at

least improve upon clinicians' predictions. The Edinburgh Stroke Study (PI Dr. Cathie Sudlow) recorded clinicians' predictions of recurrent vascular events, which I will compare with the predicted probability of vascular events from prognostic models and the observed rate of events. Finally, I will calculate a ratio of predicted probabilities from models predicting haemorrhagic events and models predicting thrombotic events, to see whether individuals can be accurately classified into those more likely to have subsequent haemorrhage and those more likely to have subsequent thrombotic events. I will estimate the weight to place on outcomes of different severity by their effects on the length of stay in hospital and quality of life.

Blood markers: Models with clinical variables alone are likely to be robust, and applicable to clinical practice. However, blood or imaging markers that reflect particular pathological processes after stroke could: (i) improve the performance of these models and (ii) give insight into the pathology of stroke. Within the Kadoorie study, all participants had serum stored at the time of recruitment to the study. Markers of thrombosis (fibrinogen), lipids and other markers of cardiovascular risk (e.g. inflammation, vitamins etc.) will be measured as part of incident stroke studies, and studies of stroke recurrence will 'piggy back' on these analysis. I will determine whether the measured markers improve the prediction of recurrent vascular events after stroke over prognostic models with only clinical variables.

Opportunities for novel observational epidemiology: Whilst the primary aim of this project is to develop predictive models, it is also an opportunity to explore the epidemiology of recurrent stroke. For example, it is uncertain whether the risk factors of subsequent ischaemic events in participants with baseline haemorrhagic strokes and haemorrhagic stroke in participants with baseline ischaemic strokes are different. Even non-analytical studies of recurrence rates alone after haemorrhage are small relative to the Kadoorie study[23]. Collaboration with senior stroke epidemiologists (Dr Cathie Sudlow and Dr. Rustam Al-Shahi-Salman, Edinburgh) will add to the analysis of these data.

Feasibility and statistical power: *(i) Model building* the feasibility of model building to predict individual complications has been shown in preliminary analysis in a subset of SCOPE. Within the datasets of FOOD and IST-1 (168 bleeds), the developed model correctly predicted GI haemorrhage in ~70% of patients. This is sufficiently promising to justify further exploration of models to predict thrombotic and haemorrhagic events post stroke. The sample sizes of the SCOPE and VISTA collaborations are sufficiently large to allow robust model building. A rule of thumb in model development is there should be at least 10 events per outcome per variable[24]; with ~1705 recurrent haemorrhagic and thrombotic events in VISTA and ~3,700 in SCOPE, there is clearly sufficient power to develop a model with a practical (<10) number of variables. With these sample sizes, I will have >80% power to detect a decrease in the c-index by 0.037[22]. *(ii) Observational epidemiology* The Kadoorie study expects 12,000 incident strokes by the time planned for analysis (2013) of which a larger proportion will be due to haemorrhage (about 30%) than in studies based in Europe (6.7% of strokes[25]). Over an average of 2.8 years of follow up, 11% (383/3447) of people with incident stroke have had recurrent stroke. A similar proportion of those with a history of stroke have had stroke recurrence (826/9056 or 9.1%) over 2.5 years of follow up. If events continue to accumulate in a similar fashion, the power of Cox regression analysis to detect variables for predicting recurrence is shown in table 3.

Table 3. Study power to detect a 20%, 50% or 75% effect size at α=0.01 (two sided) in Kadoorie dataset

Baseline stroke type	HR=1.2	HR=1.5	HR=1.75
Ischaemic	**50%**	**99%**	**100%**
ICH	**20%**	**89%**	**100%**

The final output from this period of research will be: A family of validated prognostic models based on clinical variables for the prediction of haemorrhagic and thrombotic events after stroke, which could be used for (a) the development of clinical prediction rules in parts 4 and 5 (b) baseline adjustment in future stroke research and (c) insights into the epidemiology of recurrent stroke.

(2) Prediction of haemorrhagic complications after treatment with heparin, aspirin or rtPA

Thrombolysis*:* The absolute excess of symptomatic intracerebral haemorrhage due to intravenous rtPA in acute stroke is between 2% and 16%[6]. This complication has led many to avoid the use of rtPA for the treatment of acute ischaemic stroke[26]. A model to predict intracerebral haemorrhage after treatment with rtPA could change practice if those with the highest predicted risk of intracerebral haemorrhage had a poorer outcome than those with a lower predicted risk, and so avoid rtPA treatment (Fig 1).

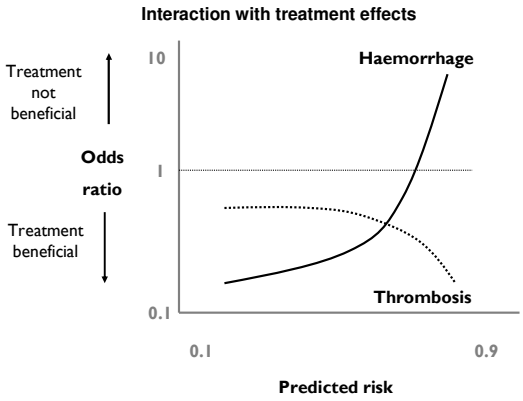

Figure 36 Characteristic of a useful model

Whilst there are a number of clinical and imaging predictors of intracranial haemorrhage after treatment in iv rtPA in stroke[27], whether those predicted to have a haemorrhage would benefit from treatment as much as those who were not is uncertain. By developing a multivariate prognostic model to predict haemorrhage after rtPA within the IST-3 dataset, with the methods in (1) I will create a tool for use by clinicians.

***Aspirin and heparin*:** Using existing data from the CAST and IST studies, I will take a similar approach to study the effect of aspirin and heparin in acute stroke. I will build models to predict intracerebral haemorrhage and test for evidence of an interaction between predicted risk of intracerebral haemorrhage and treatment effect.

Feasibility*:* IST-3 is the largest (3,100 by 2012) trial of thrombolysis in ischaemic stroke, run by Professors Sandercock, Wardlaw (both collaborators) and Lindley. It has complete baseline clinical and imaging data collection, with imaging follow up for intracerebral haemorrhage at 24 hours and reports of clinical deterioration. In the most recent Cochrane review of thrombolysis for ischaemic stroke[5], the range of symptomatic intracranial haemorrhage was 2.4-19.8% in treated and 0.2 to 6.5% in control patients; applying these figures to IST-3 gives a range from 40 to 407 intracranial haemorrhages. I would therefore be able to reliably develop a model with, at the very least, 4 variables. Data are available from

the CAST and IST studies on over 40,000 patients. The sizes of an interaction between predictions of intracerebral haemorrhage with treatment effect that could be reliably detected relative to the overall effect of treatment are shown in table 4.

Table 4 Interaction relative to overall treatment effect (Overall trial power 80%)[28]

	1.1	**1.5**	**2.0**	**3.0**
Chance of a significant interaction test ($p<0.05$)[30]	**30%**	**55%**	**80%**	**95%**

The final output of this period of research will be: (i) A validated model for the prediction of intracerebral haemorrhage after rtPA, heparin and aspirin from clinical and imaging variables (ii) Whether, by predicting intracerebral haemorrhage and avoiding treatment in some, one could improve patient outcome.

(3) To develop an interface for the presentation of the results of the prognostic models.

Validated clinical models would be useful in clinical practice, if there is a threshold above which models predict a higher probability of haemorrhagic than thrombotic events. I aim to develop a prediction tool to present the risks of thrombosis versus those of haemorrhage with recommendations to guide doctors' decision making[29]. This will be web-based, with a link from the electronic record, to allow easy collection of data, in collaboration with the very strong information technology department in the Division of Clinical Neurosciences who have experience developing these interfaces. I will pilot the tool with scenarios with doctors of different levels of experience as subjects, measuring decision performance using a within subject pre- and post- design in collaboration with Dr. Shaun Treweek from the University of Dundee.

(4) A pilot clinical trial of the provision of the result of prognostic models to stroke units. I aim to pilot the interface developed in (3) to (i) test data-collection and randomisation systems (ii) assess problems with the use of the prediction tool in practice and (iii) obtain data to choose an easily measured outcome measure to allow sample size calculations for a larger randomised trial. NHS Lothian is an ideal test bed for this pilot, with (i) a single information technology department and; (ii) electronic patient-records for use in neurovascular clinics and an electronic patient record for acute stroke units soon to be introduced.

The main aim of a large scale multi-centre randomised controlled trial of a clinical prediction model would be to measure improvements in practice. Possible outcome for a trial after stroke include adherence to number of evidence based guidelines, collected through the routine audit system already in place in NHS Lothian e.g. % of ischaemic stroke patients on aspirin, % of ischaemic stroke patients discharged on aspirin, antihypertensive and statin medication. With a sample size of 200 patients, I expect to be able to test the feasibility of, and refine the methodology for a larger study.

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