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Population-based study of blood biomarkers in prediction of sub-acute recurrent stroke

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Abstract

Background and purpose—Risk of recurrent stroke is high in the first few weeks after TIA or stroke and clinic risk prediction tools have only limited accuracy, particularly after the hyper-acute phase. Previous studies of the predictive value of biomarkers have been small, been done in selected populations and have not concentrated on the acute phase or on intensively treated populations. We aimed to determine the predictive value of a panel of blood biomarkers in intensively treated patients early after TIA and stroke.

Methods—We studied 14 blood biomarkers related to inflammation, thrombosis, atherogenesis and cardiac or neuronal cell damage in early TIA or ischaemic stroke in a population-based study (Oxford Vascular Study). Biomarker levels were related to 90-day risk of recurrent stroke as Hazard Ratio (95% CI) per decile increase, adjusted for age and sex.

Results—Among 1292 eligible patients there were 53 recurrent ischaemic strokes within 90 days. There were moderate correlations ($r > 0.40$; $p < 0.0001$) between the inflammatory biomarkers and between the cell damage and thrombotic subsets. However, associations with risk of early recurrent stroke were weak, with significant associations limited to Interleukin-6 (HR=1.12, 1.01-1.24; $p=0.035$) and C-reactive protein (1.16, 1.02-1.30; $p=0.019$). When stratified by type of presenting event, P-selectin predicted stroke after TIA (1.31, 1.03-1.66; $p=0.028$) and C-reactive protein predicted stroke after stroke (1.16, 1.01-1.34; $p=0.042$). These associations remained after fully adjusting for other vascular risk factors.

Conclusion—In the largest study to date, we found very limited predictive utility for early recurrent stroke for a panel of inflammatory, thrombotic and cell damage biomarkers.

Keywords

Biomarkers; prediction; early recurrent stroke

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Introduction

Prediction of early recurrent stroke after a transient ischaemic attack (TIA) or stroke would be clinically useful. Clinical risk scores, such as the ABCD system, are of some predictive value after TIA,¹ particularly when carotid and brain imaging are incorporated,^{2,3} but better prediction is required and there are no widely used and validated scores to predict early recurrence after stroke. Use of blood biomarkers could improve predictive power of risk scores and help to target secondary prevention. Several biomarkers, including C-reactive protein (CRP), interleukin 6 (IL-6) and D-dimer, have been shown to predict long-term risk of cardiovascular events in primary prevention populations,^{4–6} but those biomarkers studied in the secondary prevention setting after stroke have generally not been found to add to the overall predictive power of clinical risk scores for long-term risk of recurrent events.^{7–10} CRP, IL-6 and fibrinogen have been associated with the long term (greater than 3 years of outcome) risk of recurrent stroke¹¹ and CRP was predictive for stroke and other vascular events,¹² but there have been few small studies of biomarkers in the prediction of early stroke recurrence after TIA and stroke. These studies have used different outcomes and have reported conflicting results about the predictive value of markers such as IL-6 and CRP.^{13–17} There have therefore been calls for larger studies to help reduce publication-bias and type-2 error in determining the role of biomarkers in diagnosis and risk prediction¹³.

We therefore aimed in this large prospective population-based study to establish whether a panel of biomarkers related to inflammation, thrombosis, atherogenesis, and cardiac and/or neuronal function was of prognostic value for the risk of recurrent stroke within 90 days of TIA or stroke. The panel was chosen based on evidence of possible prognostic value from previous studies.^{11–18} The inflammatory biomarkers used were IL-6, CRP, tumour necrosis factor receptor -1 (TNFR-1), and neutrophil gelatinase associated lipocalin (NGAL). Thrombotic biomarkers were thrombomodulin (TM), fibrinogen, P-selectin, D-dimer, von Willebrand Factor antigen (VWF) and Protein Z (PZ). In addition, anti phosphorylcholine (anti PC), an anti atherogenic antibody was included as low levels may be predictive of stroke.¹⁹ Markers of cardiac or neuronal function and/or injury and neurone regeneration used were heart type fatty acid binding protein (hFABP), neurone specific enolase (NSE) and brain derived neurotrophic factor (BDNF).

Methods

Patients and samples

The Oxford Vascular Study (OXVASC) is a large population based study of the incidence of all acute vascular events including TIA, strokes, acute coronary syndromes (ACS) and peripheral vascular events (PVD). The methods are described in detail elsewhere²⁰ and the study was approved by the Oxfordshire Research Ethics Committee. The OXVASC population consists of 92,728 individuals registered with 100 primary-care physicians in Oxfordshire, UK.

Patients with TIA or stroke were identified by multiple methods of ascertainment, which have been shown previously to be near-complete.²¹ Briefly, multiple overlapping methods of “hot” and “cold” pursuit were used to achieve near complete ascertainment of all

individuals presenting to medical attention with TIA or stroke.²¹ Methods of hot pursuit include:

- 1) A 7-day, open-access TIA service to which participating GPs and the local accident and emergency department (A&E) send all individuals with suspected TIA or minor stroke whom they would not normally admit to hospital, with emergency provision at weekends supplementing a weekday clinic.
- 2) Daily searches of admissions to the stroke unit, medical, neurology and other relevant wards.
- 3) Daily searches of the local A&E and eye hospital attendance register.

All patients provided written informed consent and were seen by study physicians as soon as possible after their initial presentation. Relatives provided written assent and clinical details in those unable to provide written consent. A detailed history was obtained from each patient with a standardised questionnaire. This included date of symptom onset, duration and type of symptoms, baseline characteristics and time of first seeking medical attention. All cases were subsequently reviewed by the study senior neurologist (PMR) and classified as stroke or other condition using standard definitions.²² Assessments were made for severity of event using the National Institute of Health Stroke Scale (NIHSS)²³ and clinical features. Events were classified as minor stroke if there was a focal neurological deficit lasting more than 24 hours and an NIHSS score ≤ 3 at time of assessment by a study physician. Vascular evaluation was performed using CT, MR or catheter angiography as clinically appropriate. Recurrent vascular events were identified by face-to-face follow-up at 1, 6 and 12 months and by the ongoing case ascertainment in the OXVASC study.²¹

All patients were started on appropriate secondary preventive treatment on the day of the initial clinic assessment or after hospital admission, which generally included antithrombotic treatment, a statin (usually simvastatin 40mg daily) and antihypertensive medication (usually an ACE-inhibitor and indapamide initially) according to the EXPRESS study protocol.²⁴ Antithrombotic treatment was aspirin (300mg loading dose followed by 75mg daily) in all cases not requiring anticoagulation, with the addition of clopidogrel (300mg loading dose followed by 75mg daily) in patients with an ABCD score ≥ 4 or with recently symptomatic large artery stenosis, as per the EXPRESS study protocol.²⁴

Non-fasting blood samples were taken at the time of first assessment and included serum, 3.2 % buffered tri-sodium citrate plasma and lithium heparin (Li Hep) plasma (Vacutainer tubes^R, Becton Dickinson UK). Samples were centrifuged at 3000g for ten minutes and aliquots of serum and plasma were stored at -80°C prior to analysis when they were thawed for use at 37°C for ten minutes. All times from sampling to freezing were documented, typically within four hours of taking.

Assay Methods

A Randox Evidence Investigator Biochip multiple immunoassay system (Randox Laboratories Ltd, Co Antrim, UK) using Li Hep plasma simultaneously measured biomarkers via two panels: Cerebral Array I consisted of IL-6, hFABP and BDNF and Cerebral Array II consisted of CRP, NGAL, sTNFR-1, NSE and TM. All assays underwent

reproducibility studies using an internal control of pooled normal Li Hep plasma in addition to manufacturers control materials. Levels of fibrinogen by the Clauss clotting method and D-dimer and VWF antigen by immunoturbidometric assays were measured in citrated plasma using an automated coagulation analyser STA (Sta-Liatest VWF and Sta-Liatest D-Dimer, Diagnostica Stago, Asniers, France). These assays had well defined internal quality control and external control through participation in national quality assurance schemes. Commercial ELISA kits were used to assay human P-selectin (R&D Systems, UK) and PZ (Diagnostica Stago, France). Serum samples were used for anti PC antibodies (CVDefine^R, Athera Biotechnologies AB, Stockholm, Sweden), adhering to manufacturers recommendations. The intra- and inter coefficients of variation (CV %) of each assay are shown in Table 1. All assays were performed blind to study status.

Statistical Analysis

Data were analysed using SPSS for windows version 20. Spearman's rank correlations were calculated to determine the correlation between variables. Cox Regression was used to determine the Hazard Ratio (HR) and corresponding 95 % Confidence Interval (CI) for the risk of each decile of biomarker level (compared to the first/lowest) to recurrent ischaemic stroke in all patients within 90 days. Any interaction between prognostic value and severity of baseline event was explored by dividing the cohort into TIA versus stroke. The effect of time from the event to sampling on the predictive value of biomarker levels was assessed by performing the analysis on patients in whom the sample was taken within 7 days of the initial event. The decile trend was adjusted for age and sex and any biomarkers that remained significant were further adjusted for hypertension, current smoking, diabetes and body mass index. The area under the receiver operating characteristic (ROC) curve was determined for biomarkers with statistically significant univariate risk associations and the linearity of associations was assessed by hazard ratios across tertile groups.

The risk of the biomarkers for a combined outcome of ischaemic stroke and death in TIA and minor stroke patients was derived but not for major strokes deaths to avoid competing risks.

Results

A total of 1292 consecutive consenting eligible patients were recruited. Table 2 shows the baseline characteristics at the time of the event for all patients classified as TIA and stroke. Mean(SD) age was 73.1(13.3) years, 48%(n=617) were men, and hypertension was the most prevalent risk factor being found in 59%(n=767). The median (IQR) days from event to sampling was 5(3-11) days. A maximum of 1207 samples were analysed by the Randox panels comprising of 401 TIA and 806 stroke. Numbers were limited by inadequate sample volumes in 79 patients and assay failure in 6. Thrombotic markers were measured in a maximum of 1072 patients after exclusion of cases with inadequate sample volumes (n=152) and/or anticoagulant therapy (n=68).

The correlations between biomarker levels are shown in Table 3. There was good cross-correlation within the subset of inflammatory markers, the greatest was between CRP and IL-6 (0.58). Within the thrombotic subset fibrinogen and VWF correlated with each other

(0.41) but to a lesser degree to D-dimer and none of these factors correlated with P-selectin, TM or PZ. Moderate correlations ($p < 0.0001$) existed between the biomarker subsets, the greatest was for CRP and fibrinogen (0.61), CRP and VWF (0.47). Markers of neurone cell damage NSE and BDNF correlated with each other (0.48) and the inflammatory marker NGAL (0.39 and 0.45 respectively), but not to the same degree with hFABP which itself correlated well with the inflammatory markers, particularly sTNFR-1 (0.54). Anti PC did not correlate with any other biomarker.

After sampling and within 90 days of follow up there were 53 recurrent ischaemic strokes. The hazard ratio per decile increase in biomarker (HR 95 % CI) for a recurrent stroke for all patients, TIA and all stroke patients adjusted for age and sex is shown in Table 4. For all patients, two inflammatory biomarkers were predictive for a recurrent stroke, IL-6 (HR 1.12, 95% CI 1.01-1.24, $p=0.035$) and CRP (1.16, 1.02-1.30, $p=0.019$). On analyses limited to patients with samples ($n=829$) taken within 7-days of the event (Web Table 1) predictive value for 90-day risk of stroke was again limited to CRP (1.20, 1.03-1.39, $p=0.018$) and IL-6 (1.12, 0.99-1.26, $p=0.06$). When adjusted for age, sex, smoking, diabetes and BMI the associations remained for both IL-6 (1.12, 1.00-1.26, $p=0.041$) and CRP (1.17, 1.02-1.34, $p=0.028$). Across tertiles, these two biomarkers were the only ones to have a significant risk association; the top two tertiles compared to the lowest for CRP was 2.69, 1.26-5.75, $p=0.010$ and for IL-6 was 1.77, 1.0-3.12, $p=0.050$. The area under the receiver operating characteristic (ROC) area under the curve for CRP was 0.60 (95%CI 0.52-0.68, $p=0.015$) and for IL6 0.59 (0.51-0.67, $p=0.029$) (data not shown).

For no biomarker was there a statistically significant difference in predictive value between patients with TIA and stroke, but P-selectin was predictive of risk of recurrent stroke in patients with TIA (age/sex adjusted HR= 1.31, 1.03-1.66, $p=0.028$; fully-adjusted HR =1.28, 1.01-1.62, $p=0.036$) and CRP was most predictive in patients with stroke (age/sex adjusted HR=1.16, 1.01-1.34, $p=0.042$; fully-adjusted HR =1.21, 1.02-1.43, $p=0.03$).

For the combined outcome at 90 days for ischaemic stroke and death in TIA and minor stroke patients, the deaths were non vascular, the majority due to cancer and respiratory infection. All biomarker associations were non significant except for borderline P-selectin (1.12, 1.00-1.21, $p=0.05$) and significant for IL-6 (1.21, 1.09-1.34, $p=0.0003$) and CRP (1.24, 1.10-1.41, $p=0.0006$). (data not shown?)

Discussion

In the largest published study to date in patients with TIA and stroke, no single biomarker stood out as being a strong independent predictor of the early risk of recurrent stroke. Although our data on correlations between biomarkers should be helpful in refining biomarker selection in future studies and in panel designs, we did not find that the biomarkers that we chose were likely to be clinically useful in routine practice. The inflammatory biomarkers IL-6 and CRP were weakly predictive of recurrent stroke but the marginally statistically significant results must be interpreted with caution due to the large number of biomarkers studied.

Our data adds to previous reports of associations with severity of prior stroke.^{25,26} In one previous study in 194 patients with TIA, CRP was weakly predictive of the 2-year risk of stroke,¹⁵ but did not predict 90-day risk in another study of 167 TIA patients, although this was based on only five recurrent strokes.¹⁶ CRP has been used for the prediction of cardiovascular events in primary prevention settings, but has not been considered to be a sufficiently strong independent predictor to be useful in clinical practice.^{13,25}

Several studies have related inflammatory markers to short-term outcome after acute stroke.^{14,17,27} Although some associations with poor outcome have been reported, these appear mainly to reflect reverse-causation (i.e. more severe strokes have greater acute phase inflammatory responses), rather than any association with risk of recurrent events. To avoid such competing risks, mortality analysis was confined to the outcome for TIA and minor stroke combined; the deaths in this cohort were of a non-vascular nature. All the biomarkers remained non-significant predictors for death except for (borderline P-selectin) CRP and IL6. That these are also predictive for recurrent stroke suggests they are more informative than other biomarkers used in this study. There is some evidence that biomarker profiles differ between lacunar stroke and other ischemic stroke subtypes,²⁸ that IL-6 is elevated in cardio-embolic stroke compared to lacunar stroke²⁹ and CRP may be a long term prognostic indicator after lacunar stroke.¹² However, all of these studies and our current study were underpowered to stratify predictive values by stroke subtype.

P-selectin levels were possibly predictive of recurrent stroke in TIA patients in our study, but this finding should be interpreted cautiously and requires further validation. P-selectin has been associated with CVD, hypertension and stroke risk in atrial fibrillation,^{30,31} but there are few studies of stroke prognosis. A systematic review found fibrinogen and D-dimer but not P-selectin were associated with poor outcome after stroke.¹⁰

In contrast to some of the previous literature, mainly on stroke outcome and long-term risk prediction, we found no associations with early recurrent risk for anti PC,^{32,33} hFABP,¹⁷ fibrinogen,^{11,34,35} D-dimer³⁶ or VWF.³⁷ PZ appears to be associated with ischaemic stroke,¹⁸ but had not previously been validated as a predictor of recurrent stroke. As a cofactor in the coagulation cascade, subject to feedback mechanisms, any deficiency of PZ is feasibly compensated for in the regulation of thrombin, thereby reducing its significance for early stroke.

We consider our findings to be valid, but our study did have some shortcomings. First, although to our knowledge it is the largest study of its kind, statistical power was limited by the 53 recurrent stroke outcomes at 90-days. We were therefore unable to determine predictive power stratified by TOAST subtype. However, our findings on correlations between biomarkers were very highly powered. Second, patients were sampled as soon as possible after clinical presentation, but it is possible that peak levels of some markers may have been missed,⁹ although a previous work showed that delay to sampling will not have affected findings for IL-6 or CRP.⁷ When the analysis was limited to samples from patients within 7 days, the associations of biomarker levels were similar (slightly weaker for IL-6); thus we are confident that our data for the whole cohort is truly representative. Antiplatelet and statins treatment could have affected the levels of inflammatory and thrombotic markers

and the number of recurrent strokes but given the high rate of compliance in our cohort we were not able to determine the predictive value of biomarkers in patients not on medication.

In conclusion, we were unable to validate previous suggestions that blood biomarkers might aid prediction of early recurrent stroke. Larger studies would be required to determine predictive values within specific ischaemic stroke subtypes, but routine measurement of the biomarkers that we studied is not currently justified in routine clinical practice.

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References

1. Giles MF, Rothwell PM. Systematic review and pooled analysis of published and unpublished validation of the ABCD and ABCD² transient ischaemic attack risk scores. *Stroke*. 2010; 41:667–663. [PubMed: 20185786]
2. Giles MF, Albers GW, Amarenco P, Arsava MM, Asimos A, Ay H, Calvet D, et al. Addition of brain infarction to the ABCD2 Score (ABCD2I): a collaborative analysis of unpublished data on 4574 patients. *Stroke*. 2010; 41:1907–13. [PubMed: 20634480]
3. Merwick A, Albers GW, Amarenco P, Arsava EM, Ay H, Calvet D, et al. Addition of brain and carotid imaging to the ABCD score to identify patients at early risk of stroke after transient ischaemic attack: a multicentre observational study. *Lancet Neurol*. 2010; 9:1060–9. [PubMed: 20934388]
4. Zakai NA, Katz R, Jenny NS, Psaty BM, Reiner AP, Schwartz SM, et al. Inflammation and haemostasis biomarkers and cardiovascular risk in the elderly: the cardiovascular health study. *J Thromb Haemost*. 2007; 5:1128–35. [PubMed: 17388967]
5. Blankenberg S, Zeller T, Saarela O, Havulinna AS, Kee F, Tunstall-Pedoe H, et al. Contribution of 30 biomarkers to 10-year cardiovascular risk estimation in 2 population cohorts. The MONICA, Risk, Genetics, Archiving and Monograph(MORGAM) biomarker project. *Circulation*. 2010; 121:2388–97. [PubMed: 20497981]
6. Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, Gao P, et al. C-reactive protein, fibrinogen and cardiovascular disease prediction. Emerging Risk Factors Collaboration. *N Eng J Med*. 2012; 367:1310–20.
7. Whately W, Wardlaw J, Dennis M, Lowe G, Rumley A, Sattar N, et al. The Use of Blood Biomarkers to Predict Poor Outcome After Acute Transient Ischaemic Attack or Ischaemic Stroke. *Stroke*. 2012; 43:86–91. [PubMed: 22020034]
8. Maas MB, Furie KL. Molecular biomarkers in stroke diagnosis and prognosis. *Biomark Med*. 2009; 3:363–83. [PubMed: 20160867]
9. Saenger AK, Christenson RH. Stroke biomarkers: Progress and challenges for diagnosis, prognosis differentiation and treatment. *Clinical Chemistry*. 2010; 56:21–33. [PubMed: 19926776]
10. Hasan N, McColgan P, Bentley P, Edwards RJ, Sharma P. Towards the identification of blood biomarkers for acute stroke in humans: a comprehensive systematic review. *Br J Clin Pharmacol*. 2012; 74:230–40. [PubMed: 22320313]
11. Welsh P, Lowe GDO, Chalmers J, Campbell DJ, Rumley A, Neal BC, et al. Associations of Proinflammatory cytokines with the risk of recurrent stroke. *Stroke*. 2008; 39:2226–30. [PubMed: 18566306]
12. Elkind MSV, Luna JM, McClure LA, Zhang Y, Coffey CS, Roldan A, et al. C-Reactive protein as a prognostic markers after lacunar stroke. *Stroke*. 2014; 45:1–10.

13. Whitely W, Chong WL, Sengupta A, Sandercock P. Blood markers for the prognosis of ischaemic stroke: A systematic review. *Stroke*. 2009; 40:e380–389. [PubMed: 19286602]
14. Selvarajah JR, Smith CJ, Hulme S, Georgiou R, Sherrington C, Staniland J, et al. Does inflammation predispose to recurrent vascular events after recent transient ischaemic attack and minor stroke? The North West of England transient ischaemic attack and minor stroke (NORTHSTAR) study. *Int J Stroke*. 2011; 6:187–94. [PubMed: 21557802]
15. Corso G, Bottacchi E, Brusa A, Lia C, Cordera S, Giardini G, et al. Blood C-Reactive protein concentration with ABCD² is a better prognostic tool than ABCD² alone. *Cerebrovasc Dis*. 2011; 32:97–105. [PubMed: 21709407]
16. Cucchiara BL, Messe SR, Sansing L, MacKenzie L, Taylor RA, Pacelli J, et al. Lipoprotein-Associated Phospholipase A2 and C-Reactive Protein for risk stratification of patients with TIA. *Stroke*. 2009; 40:1–5. [PubMed: 19008466]
17. Park SY, Kim J, Kim O, Kim J, Song J, Shin D, Oh S. Predictive value of circulating interleukin-6 and heart-type fatty acid binding protein for three months clinical outcome in acute cerebral infarction: multiple blood markers profiling study. *Critical Care*. 2013; 17:R45. [PubMed: 23497639]
18. Sofi F, Cesari F, Abbate R, Gensini GF, Broze G jr, Fedi S. A meta-analysis of potential risks of low levels of protein Z for diseases related to vascular thrombosis. *Thromb Haemost*. 2010; 103:749–56. [PubMed: 20076855]
19. Fiskesund R, Stegmayr B, Vikstrom M, Weinehall L, de Faire U, Frostegard J. Low levels of antibodies against phosphorylcholine predict development of stroke in a population-based study from northern Sweden. *Stroke*. 2010; 41:607–12. [PubMed: 20150554]
20. Rothwell PM, Coull AJ, Giles MF, Howard SC, Silver LE, Bull LM, et al. Change in stroke incidence, mortality, case-fatality, severity, and risk factors in Oxfordshire, UK from 1981 to 2004(Oxford Vascular Study). *Lancet*. 2004; 363:1925–33. [PubMed: 15194251]
21. Coull AJ, Silver LE, Bull LM, Giles MF, Rothwell PM. Direct assessment of completeness of ascertainment in a stroke incidence study. *Stroke*. 2004; 35:2041–45. [PubMed: 15256682]
22. Rothwell PM, Coull AJ, Silver LE, Fairhead JF, Giles MF, Lovelock CE, et al. Population-based study of event-rate, incidence, case fatality, and mortality for all acute vascular events in all arterial territories (Oxford Vascular Study). *Lancet*. 2005; 366:1773–83. [PubMed: 16298214]
23. Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke*. 1989; 20:864–70. [PubMed: 2749846]
24. Rothwell PM, Giles MF, Chandratheva A, Marquardt L, Geraghty O, Redgrave JNE, et al. Effect of urgent treatment of transient ischaemic attack and minor stroke on early recurrent stroke(EXPRESS study): a prospective population-based sequential comparison. *Lancet*. 2007; 370:1432–42. [PubMed: 17928046]
25. Di Napoli M, Schwaninger M, Cappelli R, Ceccarelli E, Di Gianfilippo G, Donati C. Evaluation of C-Reactive protein measurement for assessing the risk and prognosis in ischaemic stroke. *Stroke*. 2005; 36:1316–29. [PubMed: 15879341]
26. Whiteley W, Jackson C, Lewis S, Lowe G, Rumley A, Sandercock P, et al. Inflammatory markers and poor outcome after stroke: A prospective cohort study and systematic review of interleukin-6. *Plos Medicine*. 2009; 6:1–12.
27. Welsh P, Barber M, Langhorne P, Rumley A, Lowe GDO, Stott DJ. Associations of inflammatory and haemostatic biomarkers with poor outcome in acute ischaemic stroke. *Cerebrovasc Dis*. 2009; 27:247–53. [PubMed: 19176958]
28. Tuttolomondo A, Di Sciacca R, Di Raimondo D, Serio A, D'Aguzzo G, La Placa S, et al. Plasma levels of inflammatory/fibrinolytic markers in acute ischemic strokes: Relationship with TOAST subtype, outcome and infarct site. *J of NeurImmunology*. 2009; 215:84–89.
29. Wiseman S, Marlborough F, Doubal F, Webb DJ, Wardlaw J. Blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non lacunar stroke and non- stroke: systematic review and meta-analysis. *Cerebrovasc Dis*. 2014; 37:64–75. [PubMed: 24401164]

30. Carter AM, Anagnostopoulou M, Mansfield MW, Grant PJ. Soluble P-selectin levels, P-selectin polymorphisms and cardiovascular disease. *J Thromb Haemost.* 2003; 1:1718–23. [PubMed: 12911583]
31. Kornej J, Apostolakis S, Bollmann A, Lip GY. The emerging role of biomarkers in atrial fibrillation. *Can J Cardiol.* 2013; 29:1181–93. [PubMed: 23962731]
32. Gronlund H, Hallmans G, Hakan Jansson J, Boman K, Wikstrom M, de Faire U, et al. Low levels of IgM antibodies against phosphorylcholine predict development of acute myocardial infarction in a population-based cohort from northern Sweden. *Eur J Cardiovasc Prev Rehabil.* 2009; 16:382–86. [PubMed: 19369878]
33. Sjoberg B, Su J, Dahlbom I, Gronlund H, Wikstrom M, Hedblad B, et al. Low levels of IgM antibodies against phosphorylcholine- a potential risk marker for ischaemic stroke in men. *Atherosclerosis.* 2009; 203:528–32. [PubMed: 18809177]
34. Danesh J, Lewington S, Thompson SG. Plasma fibrinogen and the risk of major cardiovascular diseases and non -vascular mortality: an individual participant meta-analysis. *JAMA.* 2005; 294:1799–1809. [PubMed: 16219884]
35. Rothwell PM, Howard SC, Power DA, Gutnikov SA, Algra A, van Gijn J, et al. Fibrinogen concentration and risk of ischemic stroke and acute coronary events in 5113 patients with transient ischemic attack and minor ischemic stroke. *Stroke.* 2004; 35:2300–05. [PubMed: 15345800]
36. Barber M, Lanthorne P, Rumley A, Lowe GDO, Stott DJ. Haemostatic function and progressing ischaemic stroke. *Stroke.* 2004; 35:1421–30. [PubMed: 15073383]
37. Hanson E, Jood K, Karlsson S, Nilsson S, Blomstrand C, Jern C. Plasma levels of von Willebrand factor in the etiologic subtypes of ischemic stroke. *J Thromb Haemost.* 2011; 9:275–81.

Table 1

Biomarker measurement methods and intra- and inter- coefficient of variation. * total samples analysed differ due to constraints on sample size and *[†] where warfarin therapy may have affected protein synthesis and [‡]according to the materials available.

Biomarker	n	Measurement system	Intra- CV %	Inter- CV%
<i>Inflammatory markers</i>				
Interleukin-6 pg/mL	1207	Randox microchip	21.0	13.4
CRP mg/L	1207	Randox microchip	5.7	11.1
NGAL ng/mL	1207	Randox microchip	4.7	9.0
TNF receptor-1 ng/mL	1205	Randox microchip	7.8	14.1
<i>Cardiac or Neuronal function/injury</i>				
hFABP ng/mL	1205	Randox microchip	14.6	10.8
NSE ng/mL	1207	Randox microchip	7.5	20.0
BDNF pg/mL	1207	Randox microchip	9.6	11.1
<i>Thrombotic markers</i>				
Thrombomodulin ng/mL	1207	Randox microchip	10.1	14.1
Fibrinogen g/L	1030*	Stago analyser	2.3	5.7
P-selectin ng/mL	1072*	ELISA	4.9	9.0
D-dimer µg/L	915*	Stago analyser	4.3	4.3
VWF %	1040*	Stago analyser	4.6	4.6
Protein Z ng/mL	1028* [†]	ELISA	4.9	14.8
<i>Anti atherogenic antibody</i>				
Anti PC U/mL	896 [‡]	ELISA	6.7	8.2

Table 2

Pre Stroke Baseline characteristics of OXVASC patients n (%) unless stated otherwise plus antiplatelet, antihypertensive and statin treatments at one month. CE = Cardio embolic, LAA = Large artery, SMV = small vessel, UND = undefined, UNK = unknown. PVD = peripheral vascular disease, MI = myocardial infarction.* n = 1159 all patients, n = 416 TIA, n = 743 stroke, *†n=1124, n=396 TIA, n=728 stroke.

Baseline Characteristics	All patients n = 1292	TIA n = 435	Stroke n = 857
Age years mean(SD)	73.07(13.25)	71.97(13.16)	73.07(13.25)
Male sex	617 (48)	201 (46)	416 (49)
TOAST*			
CE	258 (22)	69 (17)	189 (25)
LAA	143 (12)	50 (12)	93 (13)
SMV	191 (17)	40 (10)	153 (21)
UND	482 (42)	231 (56)	251 (34)
UNK	82 (7)	26 (6)	56 (8)
Incident Event	1059 (82)	332 (76)	727 (85)
Previous Stroke	170 (13)	45 (10)	125 (15)
Previous TIA	176 (14)	74 (17)	102 (12)
Atrial Fibrillation	227 (18)	65 (15)	162 (19)
Antiplatelet *†	457 (41)	148 (37)	309 (42)
<i>At one month</i>	<i>1176 (91)</i>	<i>418 (96)</i>	<i>754 (88)</i>
Antihypertensive*†	652 (59)	215 (55)	437 (60)
<i>At one month</i>	<i>965 (75)</i>	<i>313 (72)</i>	<i>654 (76)</i>
Statin n/total *†	256 (23)	103 (26)	153 (21)
<i>At one month</i>	<i>949 (74)</i>	<i>320 (74)</i>	<i>629 (73)</i>
PVD	103 (8)	30 (7)	73 (9)
Smoker	207 (16)	60 (14)	147 (17)
Previous MI	128 (10)	42 (10)	86 (10)
Hypertension	767 (59)	221 (51)	546 (64)
Diabetes	155 (12)	55 (13)	100 (12)
Hyperlipid	413 (32)	143 (33)	270 (32)
Previous angina	212 (16)	68 (16)	144 (17)

Table 3

Spearman correlation coefficients (p value) between biomarkers

	VWF	Pselectin	PZ	DD	Anti PC	BDNF	IL-6	hFABP	CRP	NSE	NGAL	TNFR1	TM
Fib	0.41 (0.000)	0.09 (0.004)	-0.07 (0.037)	0.18 (0.000)	-0.02 (0.470)	0.17 (0.000)	0.43 (0.000)	0.27 (0.000)	0.61 (0.000)	0.13 (0.001)	0.31 (0.000)	0.33 (0.000)	0.01 (0.600)
VWF		0.11 (0.000)	-0.10 (0.001)	0.27 (0.000)	-0.05 (0.083)	0.13 (0.000)	0.45 (0.000)	0.43 (0.000)	0.47 (0.000)	0.15 (0.000)	0.33 (0.000)	0.42 (0.000)	0.15 (0.000)
Pselectin			-0.01 (0.580)	0.13 (0.000)	-0.01 (0.570)	0.14 (0.000)	0.18 (0.000)	0.14 (0.000)	0.15 (0.000)	0.14 (0.000)	0.24 (0.000)	0.20 (0.000)	0.08 (0.035)
PZ				-0.07 (0.035)	0.02 (0.460)	-0.08 (0.033)	-0.18 (0.000)	-0.11 (0.002)	-0.12 (0.001)	-0.03 (0.380)	-0.13 (0.000)	-0.15 (0.000)	-0.03 (0.410)
DD					-0.03 (0.320)	0.13 (0.001)	0.29 (0.000)	0.21 (0.000)	0.23 (0.000)	0.15 (0.000)	0.26 (0.000)	0.31 (0.000)	0.12 (0.002)
Anti PC						0.00 (1.000)	-0.04 (0.260)	-0.09(0.024)	-0.0 (0.150)	0.06 (0.130)	0.01 (0.820)	-0.10 (0.017)	0.00 (0.051)
BDNF							0.24 (0.000)	0.15 (0.000)	0.21 (0.000)	0.48 (0.000)	0.45 (0.000)	0.18 (0.000)	0.02 (0.450)
IL-6								0.36 (0.000)	0.58 (0.000)	0.22 (0.000)	0.46 (0.000)	0.45 (0.000)	0.13 (0.000)
hFABP									0.31 (0.000)	0.17 (0.000)	0.36 (0.000)	0.54 (0.000)	0.33 (0.000)
CRP										0.20 (0.000)	0.38 (0.000)	0.46 (0.000)	0.10 (0.000)
NSE											0.39 (0.000)	0.26 (0.000)	0.09 (0.003)
NGAL												0.55 (0.000)	0.26 (0.000)
TNFR1													0.42 (0.000)
TM													

Table 4

Risk of recurrent ischemic stroke at 90 days after presenting event adjusted for age and sex, Hazard Ratio, HR(95 % Confidence Intervals) per increase in decile.

Biomarkers	All Patients	TIA	Stroke
Inflammatory markers			
IL-6	1.12(1.01-1.24) p=0.035	1.07(0.85-1.35)	1.10(0.98-1.23)
CRP	1.16(1.02-1.30) p=0.019	1.02(0.78-1.33)	1.16(1.01-1.34) p=0.042
NGAL	1.02(0.92-1.13)	1.03(0.81-1.31)	0.99(0.88-1.12)
TNFR-1	1.01(0.91-1.12)	0.90(0.71-1.16)	1.01(0.90-1.13)
Cardiac or Neuronal function/injury			
hFABP	1.04(0.93-1.16)	0.89(0.68-1.18)	1.05(0.92-1.19)
NSE	1.04(0.95-1.15)	0.93(0.74-1.18)	1.05(0.94-1.17)
BDNF	1.02(0.93-1.12)	1.11(0.88-1.41)	0.98(0.89-1.09)
Thrombotic markers			
TM	1.01(0.91-1.12)	0.99(0.78-1.24)	1.02(0.91-1.14)
Fibrinogen	1.10(0.98-1.23)	0.99(0.76-1.28)	1.11(0.97-1.26)
P-selectin	1.04(0.94-1.14)	1.31(1.02-1.66) p=0.028	0.96(0.86-1.07)
D-dimer	1.01(0.89-1.15)	1.06(0.81-1.41)	0.97(0.84-1.13)
VWF	0.98(0.87-1.10)	0.96(0.73-1.26)	0.96(0.84-1.09)
Protein Z	0.98(0.88-1.10)	0.80(0.60-1.07)	1.03(0.91-1.17)
Anti atherogenic antibody			
Anti PC	0.91(0.79-1.05)	0.95(0.70-1.29)	0.90(0.77-1.06)