Circulating biomarkers and incident ischemic stroke in the Framingham Offspring Study

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ABSTRACT

Objective: We related a panel of inflammatory biomarkers to risk of incident ischemic stroke (IIS) in a community-dwelling sample.

Methods: Stroke-free Framingham offspring attending examination cycle 7 (1998–2001) had 15 circulating inflammatory biomarkers measured. Cox proportional hazard models were used to calculate the hazard ratios (HRs) of IIS per SD increment of each biomarker. Model 1 included age and sex. Model 2 additionally adjusted for systolic blood pressure, hypertension treatment, current smoking, diabetes, cardiovascular disease, and atrial fibrillation. The continuous net reclassification improvement was used to assess the improvement in IIS risk prediction of statistically significant biomarkers from our main analysis over traditional stroke risk factors.

Results: In 3,224 participants (mean age 61 ± 9 years, 54% women), 98 experienced IIS (mean follow-up of 9.8 [±2.2] years). In model 1, ln-C-reactive protein (ln-CRP) (HR 1.28, 95% confidence interval [CI] 1.04–1.56), ln-tumor necrosis factor receptor 2 (ln-TNFR2) (HR 1.33, 95% CI 1.09–1.63), ln-total homocysteine (ln-tHcy) (HR 1.32, 95% CI 1.11–1.58), and vascular endothelial growth factor (VEGF) (HR 1.25, 95% CI 1.07–1.46) were associated with risk of IIS. All associations, except for ln-CRP, remained significant in model 2 (ln-TNFR2: HR 1.24, 95% CI 1.02–1.51; ln-tHcy: HR 1.20, 95% CI 1.01–1.43; and VEGF: HR 1.21, 95% CI 1.04–1.42). The addition of these 4 biomarkers to the clinical Framingham Stroke Risk Profile score improved stroke risk prediction (net reclassification improvement: 0.34, 0.12–0.57; $p < 0.05$).

Conclusions: Higher levels of 4 biomarkers—CRP, tHcy, TNFR2, and VEGF—increased risk of IIS and improved the predictive ability of the Framingham Stroke Risk Profile score. Further research is warranted to explore their role as potential therapeutic targets. ***Neurology*® 2016;87:1206–1211**

GLOSSARY

CE = cerebral embolus; **CI** = confidence interval; **CRP** = C-reactive protein; **HR** = hazard ratio; **IIS** = incident ischemic stroke; **NRI** = net reclassification improvement; **tHcy** = total homocysteine; **TNFR2** = tumor necrosis factor receptor 2; **VEGF** = vascular endothelial growth factor.

Inflammatory cascades are believed to contribute to ischemic stroke pathogenesis. Risk stratification of persons at risk of future vascular events can separate subpopulations that would benefit most from established and emerging primary stroke preventative therapies. Accordingly, we related a comprehensive panel of inflammatory biomarkers to risk of incident ischemic stroke (IIS) in a community-dwelling sample. We hypothesized that inclusion of circulating inflammatory biomarkers would refine the predictive ability of the Framingham Stroke Risk Profile score.

METHODS The Framingham Offspring Cohort was enrolled in 1971, and participants have been examined every 4 to 8 years since.[1] Among offspring participants attending examination cycle 7 (1998–2001; n = 3,539 participants), we measured a broad list of inflammatory biomarkers. In the present analysis, we excluded participants without biomarker data (n = 209) or available follow-up (n = 7), and those with TIA/stroke (n = 99), resulting in a sample size of 3,224 participants.

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Supplemental data at Neurology.org

Standard protocol approvals, registrations, and patient consents. The Boston University Medical Campus reviewed the study, and all participants provided informed consent.

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Clinical characteristics. Components of the Framingham Stroke Risk Profile were used as baseline covariates.[2] These include age, sex, systolic blood pressure, antihypertensive therapy, diabetes mellitus, smoking status, cardiovascular disease, and atrial fibrillation. Clinical variables were measured at examination cycle 7. Prevalent diabetes mellitus was defined as a fasting blood glucose ≥126 mg/dL or use of oral hypoglycemic agents/insulin. Current smoking was defined as self-reported smoking of ≥1 cigarette per day within the year preceding examination. Medication use was ascertained by self-report. Nonstroke cardiovascular disease was defined as coronary heart disease, peripheral arterial disease, and/or heart failure.

Biomarkers. We investigated a set of 15 biomarkers representing various components of the inflammatory cascade, including systemic inflammation (C-reactive protein [CRP], interleukin 6, monocyte chemotactic protein 1, tumor necrosis factor α, tumor necrosis factor receptor 2 [TNFR2], osteoprotegerin, and fibrinogen), vascular inflammation/endothelial dysfunction (intercellular adhesion molecule 1, CD40 ligand, P-selectin, lipoprotein-associated phospholipase A_2 mass and activity, total homocysteine [tHcy], and vascular endothelial growth factor [VEGF]), and oxidative stress (myeloperoxidase).

Methods of measurement and intra-assay coefficients of variation were all <10% as previously reported.[3]

Outcomes. The primary outcome of interest was IIS occurring between examination 7 and December 31, 2010. Stroke surveillance methods and protocol for determining the diagnosis and type of stroke have previously been published.[2,4]

Events were ascertained by ≥2 neurologists via consensus. Adjudicators were blinded to biomarker levels.

Stroke was defined as acute-onset focal neurologic deficit of vascular origin that persisted for >24 hours. All available clinical, laboratory, imaging, and autopsy data were used in the adjudication process. Using this information, it was possible to classify stroke subtypes as follows: atherosclerotic brain infarction, including large vessel atherothrombotic and lacunar infarction, and cerebral embolus (CE) from a documented cardiac source.

Statistical analyses. Descriptive statistics were obtained for all variables. Natural logarithmic (ln) transformation was performed on biomarkers that had skewed distribution. To facilitate comparisons, all biomarkers were standardized to a mean of 0 and an SD of 1. Cox proportional hazard models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between each biomarker and risk of IIS. HRs are presented per 1-SD increment of biomarker. Our primary model (model 1) was adjusted for age and sex. Model 2 additionally adjusted for the remaining Framingham Stroke Risk Profile variables (systolic blood pressure, hypertension treatment, current smoking, diabetes, cardiovascular disease, and atrial fibrillation). Additional exploratory models were used to assess the relationship between individual biomarkers and ischemic stroke subtypes.

To assess improvement in IIS risk prediction, we compared a baseline model that contained Framingham Stroke Risk Profile variables to a model that additionally contained all biomarkers that showed a statistically significant association with IIS in our main analysis. We calculated both the relative integrated discrimination improvement and the continuous net reclassification improvement (NRI) and their 95% bootstrap CIs for models predicting 10-year IIS risk. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). A p value <0.05 was considered statistically significant.

Table 1 Baseline characteristics

	N = 3,224
Clinical characteristics (continuous), mean (SD)	
Age, y	61.3 (9.4)
Follow-up time for stroke/TIA, y	9.3 (2.2)
Clinical characteristics (categorical), n (%)	
Women	1,736 (53.9)
Current smoking	416 (12.9)
Diabetes mellitus	389 (12.4)
History of CVD	332 (10.3)
History of AF	122 (3.8)
Hypertension treatment	1,057 (32.8)
Biomarkers, median (25th, 75th percentile)	
Interleukin 6 (n = 3,195), pg/mL	2.7 (1.8, 4.3)
CRP (n = 3,200), mg/L	2.2 (1.0, 5.1)
Fibrinogen (n = 3,198), mg/dL	371 (329, 422)
Tumor necrosis factor α (n = 2,461), pg/mL	1.2 (0.9, 1.6)
TNFR2 (n = 3,127), pg/mL	1,969 (1,661, 2,410)
Osteoprotegerin (n = 3,198), pmol/L	5.4 (4.4, 6.5)
Monocyte chemotactic protein 1 (n = 3,146), pg/mL	314 (254, 382)
CD40 ligand (n = 3,202), ng/mL	1.2 (0.5, 3.9)
Intercellular adhesion molecule 1 (n = 3,194), ng/mL	242 (211, 284)
P-selectin (n = 3,203), ng/mL	36.2 (28.4, 45.4)
tHcy (n = 3,197), μmol/L	7.9 (6.6, 9.6)
Lp-PLA_2 activity (n = 3,197)	140 (118, 165)
Lp-PLA_2 mass (n = 3,197)	288 (229, 361)
VEGF (n = 2,926), ng/mL	284 (162, 443)
Myeloperoxidase (n = 3,113), ng/mL	39.8 (27.7, 59.5)

Abbreviations: AF = atrial fibrillation; CRP = C-reactive protein; CVD = cardiovascular disease; Lp-PLA_2 = lipoprotein-associated phospholipase A_2; tHcy = total homocysteine; TNFR2 = tumor necrosis factor receptor 2; VEGF = vascular endothelial growth factor.

RESULTS Of the 3,539 Framingham offspring participants attending examination cycle 7, 3,224 participants met our eligibility criteria for analysis within the current study. Baseline characteristics of the sample are shown in table 1. The mean age of the sample was 61 ± 9 years and 54% were women. During a mean follow-up of 9.3 (±2.2) years, 98 participants (3%) experienced an IIS.

In our primary model, ln-CRP (HR 1.28, 95% CI 1.04–1.56), ln-TNFR2 (HR 1.33, 95% CI 1.09–1.63), ln-tHcy (HR 1.32, 95% CI 1.11–1.58), and VEGF (HR 1.25, 95% CI 1.07–1.46) were associated with risk of IIS (table 2). All associations, except for ln-CRP, remained statistically significant in model 2 (ln-TNFR2: HR 1.24, 95% CI 1.02–1.51; ln-tHcy: HR 1.20, 95% CI 1.01–1.43; and VEGF: HR 1.21, 95% CI 1.04–1.42). The HRs for these biomarkers were similar across individual ischemic stroke subtypes (table e-1 at Neurology.org).

Table 2 Cox proportional hazards model results for the association of each biomarker (per SD increment) and incident ischemic stroke (N = 3,224)

	Ischemic stroke outcome		
Biomarker (per SD increment)[a]	No. of events/no. of participants	HR (95% CI)	*p* Value
Interleukin 6, pg/mL			
Model 1	97/3,195	1.09	0.15
Model 2	96/3,113	1.07	0.33
CRP[b]			
Model 1	97/3,200	1.28 (1.04–1.56)	0.02
Model 2	96/3,118	1.13 (0.92–1.40)	0.25
Fibrinogen			
Model 1	97/3,198	1.11	0.33
Model 2	96/3,115	0.95	0.64
TNF-α[b]			
Model 1	76/2,461	1.13	0.24
Model 2	75/2,396	1.11	0.34
TNFR2[b]			
Model 1	92/3,127	1.33 (1.09–1.63)	0.005
Model 2	91/3,046	1.24 (1.02–1.51)	0.03
Osteoprotegerin, pmol/L			
Model 1	96/3,198	1.18	0.05
Model 2	95/3,115	1.12	0.23
Monocyte chemotactic protein 1[b]			
Model 1	97/3,146	1.12	0.26
Model 2	96/3,067	1.11	0.34
CD40 ligand[b]			
Model 1	96/3,202	1.01	0.91
Model 2	95/3,119	1.06	0.54
Intercellular adhesion molecule[b]			
Model 1	97/3,194	1.16	0.11
Model 2	96/3,114	1.05	0.65
P-selectin[b]			
Model 1	96/3,203	1.14	0.21
Model 2	95/3,120	1.06	0.58
tHcy[b]			
Model 1	97/3,197	1.32 (1.11–1.58)	0.002
Model 2	96/3,115	1.20 (1.01–1.43)	0.04
Lp-PLA_2 activity[b]			
Model 1	96/3,197	0.92	0.43
Model 2	95/3,114	0.95	0.67
Lp-PLA_2 mass[b]			
Model 1	96/3,197	0.98	0.88
Model 2	95/3,114	1.02	0.82
VEGF, ng/mL			
Model 1	89/2,926	1.25 (1.07–1.46)	0.005
Model 2	88/2,857	1.21 (1.04–1.42)	0.02

Continued

Table 2 Continued

Biomarker (per SD increment)[a]	Ischemic stroke outcome: No. of events/no. of participants	HR (95% CI)	p Value
Myeloperoxidase[b]			
Model 1	95/3,113	1.04	0.71
Model 2	93/3,029	0.97	0.76

Abbreviations: CI = confidence interval; CRP = C-reactive protein; HR = hazard ratio; Lp-PLA$_2$ = lipoprotein-associated phospholipase A$_2$; tHcy = total homocysteine; TNF-α = tumor necrosis factor α; TNFR2 = tumor necrosis factor receptor 2; VEGF = vascular endothelial growth factor.
[a] Model 1 is adjusted for age at examination 7 and sex. Model 2 is adjusted for age at examination 7, sex, systolic blood pressure, hypertension treatment, current smoking, history of diabetes, history of cardiovascular disease, history of atrial fibrillation.
[b] Natural log (ln) transformed.

Of all 4 individual biomarkers, the addition of VEGF to the clinical Framingham Stroke Risk Profile provided the greatest discriminatory ability (NRI: 0.27 [0.03–0.50], p value <0.05); however, inclusion of all 4 biomarkers led to the greatest improvement in ischemic stroke risk prediction (NRI: 0.34 [0.12–0.57], p value <0.05) (table 3).

Exploratory analyses investigating the association between biomarkers and individual ischemic stroke subtypes additionally demonstrated significant associations between ln-CRP (HR 1.31, 95% CI 1.03–1.69) and atherosclerotic brain infarction, as well as interleukin 6 (HR 1.01, 95% CI 1.06–1.33) and fibrinogen (HR 1.40, 95% CI 1.06–1.86) and CE, in model 1. Only the association between interleukin 6 and CE remained significant in model 2 (table e-1).

DISCUSSION Our results suggest that circulating biomarkers of inflammation and endothelial dysfunction are associated with IIS in stroke-free community-dwelling individuals and they can be used to refine stroke prediction models. Inclusion of 4 biomarkers (CRP, TNFR2, tHcy, and VEGF) in a model that contained the Framingham Stroke Risk Profile components resulted in an NRI of 0.34 (0.12–0.57).

VEGF was the circulating biomarker that had the greatest individual degree of discrimination for future ischemic stroke. Data from the Framingham Study have recently demonstrated an association between VEGF and IIS.[5] The relation between VEGF and ischemic stroke pathogenesis is, however, not well established. Upregulation of VEGF may be a protective measure in the face of advanced systemic/cerebral vascular disease by way of its neuroprotective and beneficial angiogenic properties.[5] Alternatively, it may be pathogenic, for instance through the potentiation of atherosclerotic disease. Of note, the use of VEGF inhibitors does not seem to influence stroke risk in patients with macular degeneration.[6] This observation would suggest an indirect rather than causal link between VEGF and future stroke.

Table 3 NRI and IDI statistics for the addition of various biomarkers to a stroke prediction model using 10-year follow-up

Baseline prediction model[a]	Variable(s) added to the baseline model	Ischemic stroke outcome (no. of events = 79, no. of participants = 2,773): Relative IDI (95% CI)	Continuous NRI (95% CI)
A	VEGF	0.0156 (−0.0642, 0.1128)	0.2672 (0.0349, 0.4970)
A	CRP[b]	0.0264 (−0.0119, 0.0702)	0.1856 (−0.0243, 0.3987)
A	TNFR2[b]	0.1465 (0.0483, 0.2574)	0.1412 (−0.0901, 0.3662)
A	tHcy[b]	0.0994 (0.0334, 0.1681)	0.0986 (−0.1217, 0.3252)
A	CRP,[b] TNFR2,[b] tHcy[b]	0.213 (0.0961, 0.3504)	0.2788 (0.0444, 0.5154)
A	VEGF, CRP,[b] TNFR2,[b] tHcy[b]	0.201 (0.0584, 0.3831)	0.3418 (0.1234, 0.5731)

Abbreviations: CI = confidence interval; CRP = C-reactive protein; IDI = integrated discrimination improvement; NRI = net reclassification improvement; tHcy = total homocysteine; TNFR2 = tumor necrosis factor receptor 2; VEGF = vascular endothelial growth factor.
[a] Model A contains the following variables: age, sex, systolic blood pressure, hypertension treatment, smoking, history of diabetes, history of cardiovascular disease, history of atrial fibrillation.
[b] Natural log (ln) transformed.

Total homocysteine and CRP are well-established markers of increased stroke risk, the former via its role in accelerated atherosclerotic disease,[7] and the latter marking systemic inflammation and plaque instability.[8] Elevated CRP levels and associated single-nucleotide polymorphisms were also recently reported to predict recurrent stroke in Vitamin Intervention for Stroke Prevention trial participants.[9] We demonstrated an independent association between TNFR2 and IIS risk. A prior observational study did not demonstrate an independent relationship between TNF-α and future stroke[10]; however, preliminary data have shown lower vascular events in patients with rheumatoid arthritis on TNF-α inhibitors, particularly in patients with longer duration of use,[11] and characterizing the protective effects of TNF inhibitors in stroke patients, as well as animal models of ischemic cerebral injury, is an active and promising area of research.[12]

Our study is not without limitations. Observational data, even when longitudinal, are prone to residual confounding and cannot establish direct causal relationships. However, establishing causation was not the primary aim of our study, but rather our interest was to define the predictive utility of circulating biomarkers. The predominant European descent of Framingham Heart Study participants limits generalization to other ethnic groups. We analyzed circulating biomarkers at only one point in time and we have not accounted for concurrent infection, renal impairment, chronic inflammatory diseases/rheumatologic disease, or malignancy that could have altered our results. Moreover, we have not accounted for stroke preventative therapies (such as statins), or underlying cerebral small vessel disease, which could have influenced the risk of IIS and inflammatory biomarker levels.

The exploratory analyses investigating the relationship between circulating biomarkers and ischemic stroke subtypes were largely limited by small sample size and multiple testing and should be considered hypothesis-generating. Nevertheless, the association between fibrinogen and CE is supported by prior observations from the Atherosclerosis Risk in Communities Study demonstrating greater risk of atrial fibrillation–related mortality and cardiovascular outcomes with higher fibrinogen levels,[13] as well as observations from the Vitamin Intervention for Stroke Prevention trial.[9] A final limitation is that we have not identified clinically significant threshold values that predict the risk of IIS.

Our study demonstrates improved predictive ability of the Framingham Stroke Risk Profile score for IIS through the addition of 4 circulating biomarkers of inflammation and endothelial dysfunction. Future research investigating whether any of these biomarkers could serve as therapeutic targets for primary stroke prevention is warranted.

AUTHOR CONTRIBUTIONS

Study concept/design: Ashkan Shoamanesh, Sarah R. Preis, Alexa S. Beiser, Jose R. Romero, and Sudha Seshadri. Analysis and interpretation of data: Ashkan Shoamanesh, Sarah R. Preis, Alexa S. Beiser, Jose R. Romero, and Sudha Seshadri. Drafting/revising the manuscript for content: Ashkan Shoamanesh, Sarah R. Preis, Alexa S. Beiser, Emelia J. Benjamin, Carlos S. Kase, Philip A. Wolf, Jose R. Romero, and Sudha Seshadri. Acquisition of data: Sarah R. Preis, Alexa S. Beiser, Ramachandran S. Vasan, Emelia J. Benjamin, Carlos S. Kase, Philip A. Wolf, Jose R. Romero, and Sudha Seshadri. Study supervision/coordination: Alexa S. Beiser, Carlos S. Kase, Philip A. Wolf, Jose R. Romero, and Sudha Seshadri.

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DISCLOSURE

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