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ORIGINAL RESEARCH

Multiple Blood Biomarkers and Stroke Risk in Atrial Fibrillation: The REGARDS Study

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BACKGROUND: Atrial fibrillation is associated with increased stroke risk; available risk prediction tools have modest accuracy. We hypothesized that circulating stroke risk biomarkers may improve stroke risk prediction in atrial fibrillation.

METHODS AND RESULTS: The REGARDS (Reasons for Geographic and Racial Differences in Stroke) study is a prospective cohort study of 30 239 Black and White adults age ≥45 years. A nested study of stroke cases and a random sample of the cohort included 175 participants (63% women, 37% Black adults) with baseline atrial fibrillation and available blood biomarker data. There were 81 ischemic strokes over 5.2 years in these participants. Adjusted for demographics, stroke risk factors, and warfarin use, the following biomarkers were associated with stroke risk (hazard ratio [HR]; 95% CI for upper versus lower tertile): cystatin C (3.16; 1.04–9.58), factor VIII antigen (2.77; 1.03–7.48), interleukin-6 (9.35; 1.95–44.78), and NT-proBNP (N-terminal B-type natriuretic peptide) (4.21; 1.24–14.29). A multimarker risk score based on the number of blood biomarkers in the highest tertile was developed; adjusted HRs of stroke for 1, 2, and 3+ elevated blood biomarkers, compared with none, were 1.75 (0.57–5.40), 4.97 (1.20–20.5), and 9.51 (2.22–40.8), respectively. Incorporating the multimarker risk score to the CHA_2DS_2VASc score resulted in a net reclassification improvement of 0.34 (95% CI, 0.04–0.65).

CONCLUSIONS: Findings in this biracial cohort suggested the possibility of substantial improvement in stroke risk prediction in atrial fibrillation using blood biomarkers or a multimarker risk score.

Key Words: atrial fibrillation ■ biomarkers ■ prospective studies ■ risk factors ■ stroke

Patients with atrial fibrillation (AF) have twice the mortality as those without AF.[1] This is chiefly attributable to the 5-fold increased risk of stroke associated with AF[2] and the greater severity of AF-related strokes,[3] as reflected in the 2-fold higher 30-day mortality after an AF-related stroke compared with non-AF–related ischemic stroke.[4] Anticoagulation is the mainstay of therapy and decreases the risk of stroke and systemic embolism by 67% to 75%[5,6]; however, this approach involves an increased risk of bleeding.[7] Clinical decisions about anticoagulant therapy hinge on a given patient's risk of stroke. Currently used stroke risk estimation models provide rather modest predictive accuracy, with the CHA_2DS_2VASc score offering a C-statistic of only 0.606.[8] Prior work in select populations at risk for stroke have demonstrated that incorporation of individual blood biomarker levels can provide an incremental increase in the prediction of stroke, with C-statistics of >0.70.[9–16] Multi-blood-biomarker approaches to risk prediction might have more discriminatory ability[17–19] to predict a patient's risk of stroke and, by extension, the efficacy and safety of anticoagulation. A subset of participants from the REGARDS (Reasons for Geographic and Racial Differences in Stroke) study underwent measurement of blood biomarkers selected because of hypotheses on their relationships with ischemic stroke.[15,20–29] We hypothesized that blood biomarkers associated with risk of stroke might identify high stroke risk in patients with AF.

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CLINICAL PERSPECTIVE

What Is New?

- Among participants in the REGARDS (Reasons for Geographic and Racial Differences in Stroke) study with atrial fibrillation, baseline levels of several biomarkers were associated with risk of incident ischemic stroke: cystatin-C, factor VIII antigen, interleukin-6, and NT-proBNP (N-terminal B-type natriuretic peptide).
- A novel biomarker-based stroke risk score including these biomarkers improved stroke risk prediction above and beyond the CHA_2DS_2VASc score.

What Are the Clinical Implications?

- Biomarker-based stroke risk stratification may substantially improve prediction of ischemic stroke among patients with atrial fibrillation.
- Findings have implications for considering prevention.

Nonstandard Abbreviations and Acronyms

REGARDS	Reasons for Geographic and Racial Differences in Stroke study

METHODS

Qualified researchers trained in human subject confidentiality protocols may request access to the data that support the findings of this study by contacting the REGARDS Operations Center at regardsadmin@uab.edu.

Subjects

The REGARDS study is a prospective cohort study designed to better understand the regional and racial disparities in stroke mortality in the United States. REGARDS recruited 30 239 participants from the contiguous United States between 2003 and 2007.[30] The cohort intentionally oversampled Black individuals (41%), women (55%), and residents of the stroke-belt in the southeastern United States (56%; includes Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee). After an extensive baseline telephone-administered questionnaire, participants underwent an in-home study visit, including measuring anthropometric parameters and blood pressure, and phlebotomy in the fasting state. Study methods were approved by the institutional review boards at participating institutions and all participants provided written informed consent.

The REGARDS investigators selected 646 participants with stroke and a stratified random sample of 1104 other participants to form a nested case-cohort study sample of 1750 participants.[13] Extensive blood biomarker measurements were made in this sub-study of participants.

For this analysis, we excluded both cases and cohort participants without baseline AF (1567), who experienced a hemorrhagic stroke during follow-up (7), or who had a stroke before the first in-home visit (1). The remaining analysis sample was composed entirely of AF participants with baseline blood biomarker data (n=175).

Stroke Identification

Details on stroke event identification and adjudication were previously described.[31] Briefly, report of a possible stroke or transient ischemic attack, or a positive response to the stroke symptoms on the Questionnaire for Verifying Stroke-Free Status,[32] resulting in hospitalization generated a request for retrieval of medical records that were centrally adjudicated by a panel of stroke expert physicians. Incident stroke cases were included if they were reported by August 1, 2011, adjudicated by January 1, 2012, and met the World Health Organization stroke definition or REGARDS clinical stroke definition. Incident cases that were identified through death adjudication were included only if medical records were reviewed. Participants were not eligible to become incident stroke cases if at baseline they reported a history of physician-diagnosed stroke.[31]

Laboratory Methods

Participants of the case-cohort study sample contributed blood samples at the baseline in-home study visit. Samples were centrifuged near participants' homes, and serum and plasma were shipped overnight to the University of Vermont core laboratory, where they were re-centrifuged, and then stored at −80°C.[33] Blood samples from participants with and without stroke were analyzed together in random order so that technicians were masked to their status. Blood biomarkers measured were adiponectin, CRP (C-reactive protein), interleukin-6 (IL-6), interleukin 8, interleukin-10, resistin, pro-enkephalin, soluble CD14, D-dimer, fibrinogen, hepatocyte growth factor, leptin, lipoprotein (a), NT-proBNP (N-terminal pro-B-type natriuretic peptide), cystatin C, dehydroepiandrosterone, galectin-3, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, and coagulation factors VIII, IX, and XI. Details on the measurement methods for these blood biomarkers are provided elsewhere.[33]

Atrial Fibrillation Identification

AF was defined in REGARDS by ECGs recorded during the baseline in-home visit or self-report of a physician diagnosis. Study staff were trained in standard procedures of ECG recording using centrally trained supervisors, web-based education, and continuous quality feedback to individual examiners. The ECG tracings were sent to a central ECG reading center at Wake Forest School of Medicine (Winston Salem, NC) where they were read by electrocardiographers masked to clinical data. Self-reported history of AF was defined as a positive response to the question: "Has a physician or a health professional ever told you that you had atrial fibrillation?" AF by both ECG and self-reported history of AF are similarly predictive of stroke in REGARDS.[34]

Covariates

Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medications. Diabetes mellitus was defined as fasting blood glucose >126 mg/dL, non-fasting blood glucose >200 mg/dL, or oral hypoglycemic or insulin use. Prior coronary artery disease was defined as self-reported history of prior myocardial infarction, coronary artery bypass surgery, coronary angioplasty, or stenting as well as evidence of myocardial infarction on the study-scheduled ECGs recorded during baseline. Prior heart failure was defined by self-reported history of diagnosis of heart failure or symptoms suggestive of heart failure as previously described.[35]

Statistical Analysis

Means or frequencies of baseline characteristics were compared between cases and controls using Student *t*-tests (continuous variables) and Chi-square tests (categorical variables). Cox proportional hazards models were used to calculate the hazard ratios (HRs) and 95% CI of incident stroke for each blood biomarker (highest versus lowest tertile). Models were adjusted for age, sex, race, age-race interaction (to reflect the Black-White disparity in stroke at younger but not older age[36]), warfarin use, and age-sex-adjusted Framingham Stroke Risk Score. The age and sex-adjusted Framingham Stroke Risk Score was calculated by subtracting the contribution to the raw risk score explained by the age and sex variable using residual analysis.[37] Given the a priori nature of the prespecified and physiologically plausible relationships, and exploratory nature of the study, results reported were not corrected for multiple comparisons. For the multi-blood-biomarker analysis, the 4 blood biomarkers that were associated with increased stroke risk were considered, and each participant was classified as having elevated levels of 0, 1, 2, or 3+ of the biomarkers. Utility in prediction was assessed using the net classification improvement, which reflects how the addition of an additional covariate influences the accuracy of a prediction model.[38] The continuous net reclassification improvement method was used, as there was a small sample size and the stroke risk score lacks intrinsic categories of risk strata.[39] All analyses were weighted using inverse probability weights to account for stratification factors for selection of the cohort random sample. Statistical analysis was conducted with SAS version 9.4 (Cary, NC).

RESULTS

There were 175 participants in the REGARDS case-cohort study with AF and no prebaseline stroke (63% women, 37% Black), including 81 participants with a stroke occurring for 5.2 years median follow-up. Those with AF and a subsequent stroke were more likely to be White, men, regular users of warfarin, and have a history of cardiovascular comorbidities, including hypertension, left ventricular hypertrophy, and heart failure (Table 1).

The median levels of baseline blood biomarkers and their individual associations with incident stroke are given in Table 2. In models adjusted for demographics and stroke risk factors, higher cystatin C, factor VIII, IL-6, and NT-proBNP were associated with substantial increases in stroke incidence, with HRs ranging from 2.77 to 9.64. Higher gamma-glutamyltransferase and resistin had suggestive associations, with HRs 2.80 (0.85–9.18) and 2.21 (0.82–6.00), respectively. In contrast, there were no substantial associations of adiponectin, interleukin-8, interleukin-10, CRP, pro-enkephalin, CD14, D-dimer, factor IX, factor XI, fibrinogen, hepatocyte growth factor, leptin, lipoprotein (a), dehydroepiandrosterone, galectin-3, alanine aminotransferase, or aspartate aminotransferase with stroke incidence in this AF population.

A multi-blood-biomarker stroke risk prediction score based on the number of elevated blood biomarkers (highest tertile) was developed that incorporated cystatin C, factor VIII antigen, IL-6, and NT-proBNP. After adjustment for age, race, age-by-race interaction, sex, warfarin use and the age-sex adjusted residuals from the Framingham Stroke Risk Score, this score was associated with increased risk of stroke with a dose-response, with adjusted HRs for 1, 2, and 3+ blood biomarkers of 1.75 (0.57–5.40), 4.97 (1.20–20.5), and 9.51 (2.22–40.8), respectively (Table 3 and Figure). Addition of the multimarker risk score to the CHA_2DS_2VASc improved prediction, with a net reclassification improvement[38] for 5-year prediction of stroke of 0.34 (95% CI, 0.04–0.65).

Table 1. Baseline Characteristics, Stratified by Incident Stroke

Characteristics	Total	Incident Stroke		*P* Value
		No	Yes	
Age (y, mean±SD)	66±9	66±9	72±8	0.13
White (%)	1723 (62.9%)	1662 (62.5%)	61 (75.3%)	0.02
Men (%)	1017 (37.1%)	975 (36.7%)	42 (51.9%)	0.005
Stroke Belt region (%)	1898 (69.3%)	1854 (69.7%)	44 (54.3%)	0.003
Education ≥ high school (%)	2424 (88.5%)	2352 (88.5%)	72 (88.9%)	0.91
Income ≥$35 000 (%)	756 (27.6%)	720 (27.1%)	36 (44.4%)	0.001
Smoking (ever; %)	1723 (62.9%)	1669 (62.8%)	54 (66.7%)	0.48
Hypertension (%)*	1725 (64.3%)	1662 (63.9%)	63 (77.8%)	0.01
Diabetes mellitus (%)†	994 (36.3%)	974 (36.6%)	20 (25.6%)	0.05
Hyperlipidemia (%)‡	1770 (66.0%)	1715 (65.9%)	55 (69.6%)	0.49
History of TIA (%)	211 (8.6%)	200 (8.5%)	11 (13.9%)	0.09
Left ventricular hypertrophy (%)	212 (8.0%)	198 (7.7%)	14 (17.7%)	0.001
Coronary artery disease (%)	543 (20.2%)	522 (20.1%)	21 (26.3%)	0.17
Prior heart failure (%)	971 (35.5%)	931 (35.0%)	40 (51.3%)	0.003
Statin use (%)	1182 (43.2%)	1142 (43.0%)	40 (49.4%)	0.25
Warfarin use (%)	536 (19.6%)	512 (19.3%)	24 (29.6%)	0.02
CHA_2DS_2VASc score >2 (%)	1643 (74.3%)	1568 (59.0%)	66 (81.5%)	<0.0001
Framingham Stroke Risk Score	22.9±16.0	22.6±15.8	32.0±18.5	0.20

Baseline characteristics of the 175 eligible participants from the REGARDS (Reasons for Geographic and Racial Differences in Stroke) study, stratified by incident stroke. CHA_2DS_2VASc score indicates stroke risk score; and TIA, transient ischemic attack.

*Defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of hypertension medications.

†Defined as fasting blood glucose >126 mg/dL, non-fasting blood glucose >200 mg/dL, or use of oral hypoglycemic or insulin.

‡Defined as total cholesterol ≥240 mg/dL, low-density lipoprotein cholesterol ≥160 mg/dL, high-density lipoprotein cholesterol ≤40 mg/dL, or on cholesterol-lowering medication.

DISCUSSION

In this analysis from the REGARDS study, we showed that 4 blood biomarkers identified people with AF at increased stroke risk, and that addition of blood biomarkers to the CHA_2DS_2VASc score might improve stroke risk stratification to personalize therapy among patients with AF. Stroke accounts for 1 of every 19 deaths in the United States, and every 40 seconds someone develops a stroke.[40] Similarly, up to 6.1 million currently have AF and this number is expected to double in the next decade.[41] These alarming estimates and the strong link between AF and stroke underscore the importance of stroke prevention among patients with AF. While appropriate use of anticoagulant therapy is effective in prevention of stroke among patients with AF, our ability to predict stroke risk and select patients with AF who may benefit from anticoagulant therapy remains modest, and these findings, if confirmed, hold promise that blood biomarkers might be helpful clinically.

We observed that higher levels of cystatin C, factor VIII, IL-6, and NT-proBNP were strongly associated with increased risk of stroke in AF, with HRs ranging from 2.77 to 9.64. In addition, a multi-blood-biomarker stroke risk score based on the number of elevated blood biomarkers exhibited a dose-response relationship with subsequent ischemic stroke, with HRs of 5 to 10 among participants with 2+ elevated blood biomarkers, and a net reclassification improvement of 34% over the CHA_2DS_2VASc score. While replication of these findings is needed in a larger study, results suggest a hypothesis that incorporation of these blood biomarkers in stroke risk prediction may more accurately quantify stroke risk among patients with AF.

If confirmed, this line of research could ultimately inform clinical practice and decrease the burden of stroke. This confirmation could be a challenge, as it requires blood samples collected in participants who have AF and stored blood samples before developing a stroke. Owing to the large size of the cohort, the fact that REGARDS had blood samples drawn on 81 such participants, and a similar number with AF who did not develop stroke, provided a sample size not previously available in other research to assess these associations.[20,42]

The net reclassification improvement of 0.34 observed here with incorporation of a simple multimarker risk score to the CHA_2DS_2VASc score[8] demonstrates the utility of blood biomarkers for further refining stroke risk assessment among those with AF. Refining stroke risk assessment in AF has progressed iteratively over

Table 2. Baseline Blood Biomarker Levels and Risk of Stroke

	Blood Biomarker Concentration (Median)		HR (95% CI) of Stroke by Blood Biomarker Tertiles*		
	Incident Stroke				
	No (n=94)	Yes (n=81)	Lower Tertile	Middle Tertile	Upper Tertile
Markers of inflammation					
Adiponectin, µg/mL	9.33	13.34	Reference	1.74 (0.49–6.19)	1.74 (0.53–5.72)
CRP, mg/L	2.34	2.71	Reference	1.33 (0.43–4.11)	1.64 (0.51–5.26)
Interleukin-6, pg/mL	2.65	3.78	Reference	5.45 (1.40–21.20)†	9.35 (1.95–44.78)†
Interleukin-8, pg/mL	2.83	2.79	Reference	0.51 (0.17–1.32)	0.65 (0.25–1.68)
Interleukin-10, pg/mL	9.64	10.12	Reference	1.76 (0.68–4.56)	1.91 (0.73–5.01)
Resistin, pg/mL	23.6	28.4	Reference	1.21 (0.48–3.03)	2.21 (0.82–6.00)
Pro-enkephalin, pg/mL	63.8	67.0	Reference	1.25 (0.35–4.47)	1.45 (0.37–5.73)
CD14, pg/mL	1964	1928	Reference	1.31 (0.48–3.59)	0.89 (0.32–2.44)
Lipoprotein (a), mg/dL	28	28	Reference	0.86 (0.27–2.72)	1.37 (0.49–3.79)
Markers of thrombosis					
D-Dimer, µg/mL	0.41	0.58	Reference	1.92 (0.75–4.89)	2.24 (0.72–6.96)
Factor VIII (%)	122	133	Reference	1.15 (0.38–3.47)	2.77 (1.03–7.48)†
Factor IX (%)	104	98	Reference	2.21 (0.71–6.89)	1.91 (0.54–6.77)
Factor XI (%)	114	105	Reference	1.245 (0.52–4.01)	1.91 (0.69–5.28)
Fibrinogen, mg/dL	404	417	Reference	0.76 (0.29–2.00)	1.55 (0.44–5.49)
Protein C, IU/dL	116	107	Reference	1.00 (0.28–3.54)	1.07 (0.29–3.88)
Markers of atrial fibrosis					
Hepatocyte growth factor, pg/mL	289	368	Reference	0.90 (0.34–2.38)	1.21 (0.42–3.49)
Leptin, µg/mL	22.9	14.0	Reference	0.39 (0.15–1.06)	1.65 (0.51–5.30)
Galectin-3, ng/mL	10.5	11.8	Reference	0.96 (0.35–2.65)	2.11 (0.65–6.88)
Marker of myocardial strain					
NT-proBNP, pg/mL	120	384	Reference	1.94 (0.75–5.05)	4.21 (1.24–14.29)†
Marker of renal function					
Cystatin-C, mg/mL	0.93	1.11	Reference	1.83 (0.68–4.93)	3.16 (1.04–9.58)†
Hormones					
Dehydroepiandrosterone, µg/dL	66.4	62.0	Reference	0.69 (0.24–1.94)	0.70 (0.21–2.35)
Liver enzymes					
ALT, U/L	14	13	Reference	0.49 (0.18–1.33)	1.40 (0.44–4.46)
AST, U/L	19	19	Reference	0.92 (0.30–2.80)	0.68 (0.24–1.89)
GGT, U/L	23	26	Reference	0.35 (0.12–1.05)	2.80 (0.85–9.18)

Baseline blood biomarker levels in cases and controls are provided, followed by the hazard ratio for stroke associated with having a blood biomarker level in the highest tertile. After multivariable adjustment, the following biomarkers were associated with an increased risk of stroke: interleukin-6, factor VIII antigen, NT-proBNP, and cystatin-C. ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; HR, hazard ratio; and NT-proBNP; N-terminal B-type natriuretic peptide.

*Model was adjusted for age, sex, race, age×race, warfarin use, and Framingham Stroke Risk Score.

†Significance at the level of $P<0.05$.

70 years. Starting with the first reports of associations between AF and stroke risk[43] and after confirmatory epidemiologic studies,[44] there were increasing calls for consideration of anticoagulant therapy to mitigate this risk.[45] The $CHADS_2$ score[46] allowed for selection of a low-risk cohort who might not benefit from anticoagulants and the CHA_2DS_2VASc score[8] further improved risk stratification. More recently, the age, biomarker, clinical history (ABC) stroke risk score, which incorporates NT-proBNP and troponin, yielded higher c-indices than the CHA_2DS_2VASc score alone.[47] Similarly, addition of the P-wave axis to the CHA_2DS_2VASc score gave net reclassification improvements of 0.25 for derivation and 0.51 for validation cohorts.[48] As more studies delineate which biomarkers, including laboratory-based and electrocardiographic, are most

Table 3. Multi-Blood-Biomarker Score and Risk of Stroke

	Incident Stroke		HR (95% CI)*		
	No (n=92)	Yes (n=78)	Model 1	Model 2	Model 3
No. of elevated blood biomarkers					
0 (n=59)	1194 (98.3%)	21 (1.7%)	Reference	Reference	Reference
1 (n=40)	769 (98.1%)	15 (1.9%)	1.22 (0.50–2.97)	1.07 (0.42–2.72)	1.75 (0.57–5.40)
2 (n=38)	324 (93.4%)	23 (6.6%)	4.31 (1.64–11.4)†	2.77 (0.96–7.99)	4.97 (1.20–20.5)†
3+ (n=33)	271 (93.4%)	19 (6.5%)	5.05 (1.88–13.6)†	3.24 (1.03–10.2)†	9.51 (2.22–40.8)†

The hazard ratio and 95% CI associated with having 0, 1, 2, or 3+ blood biomarkers in the highest tertile are provided, including the raw, partially adjusted, and fully adjusted models. Five participants with missing blood biomarker data for any of the blood biomarkers were excluded. HR indicates hazard ratio.

*Model 1 unadjusted; Model 2 adjusted for age, sex, race, and age×race; Model 3 adjusted for Model 2 plus warfarin use and Framingham Stroke Risk Score.

†Significance at the level of $P<0.05$.

strongly associated with subsequent stroke, replication studies on these biomarkers will be useful toward eventual incorporation into clinical practice.

Patients with AF have a hypercoagulable state, signified by substantially elevated procoagulant blood biomarkers in comparison with those without AF.[22] Prior studies suggest that patients with procoagulant blood biomarker levels have a greater risk of cardioembolic stroke[20] and that use of D-dimer in AF risk assessment can help refine stroke risk stratification beyond that achieved by using clinical factors alone.[29] Our study produced new results, with participants in the top tertile of factor VIII having a 2.77-fold increased risk of stroke; higher D-dimer doubled stroke risk but we had insufficient power for confidence in this finding. Just as D-dimer levels can guide clinical practice by risk-stratifying patients for recurrent venous thromboembolism,[24] venous thromboembolism with postmenopausal estrogen,[17] or refine risk estimation for intracardiac thrombus before cardioversion,[49] factor VIII may have a role in the future in decisions on the need for anticoagulation in AF.

The current findings build on prior literature, and suggest that IL-6 levels are tightly linked with propensity for stroke.[50] Inflammation is linked to thrombosis on a cellular level, and there appears to be a

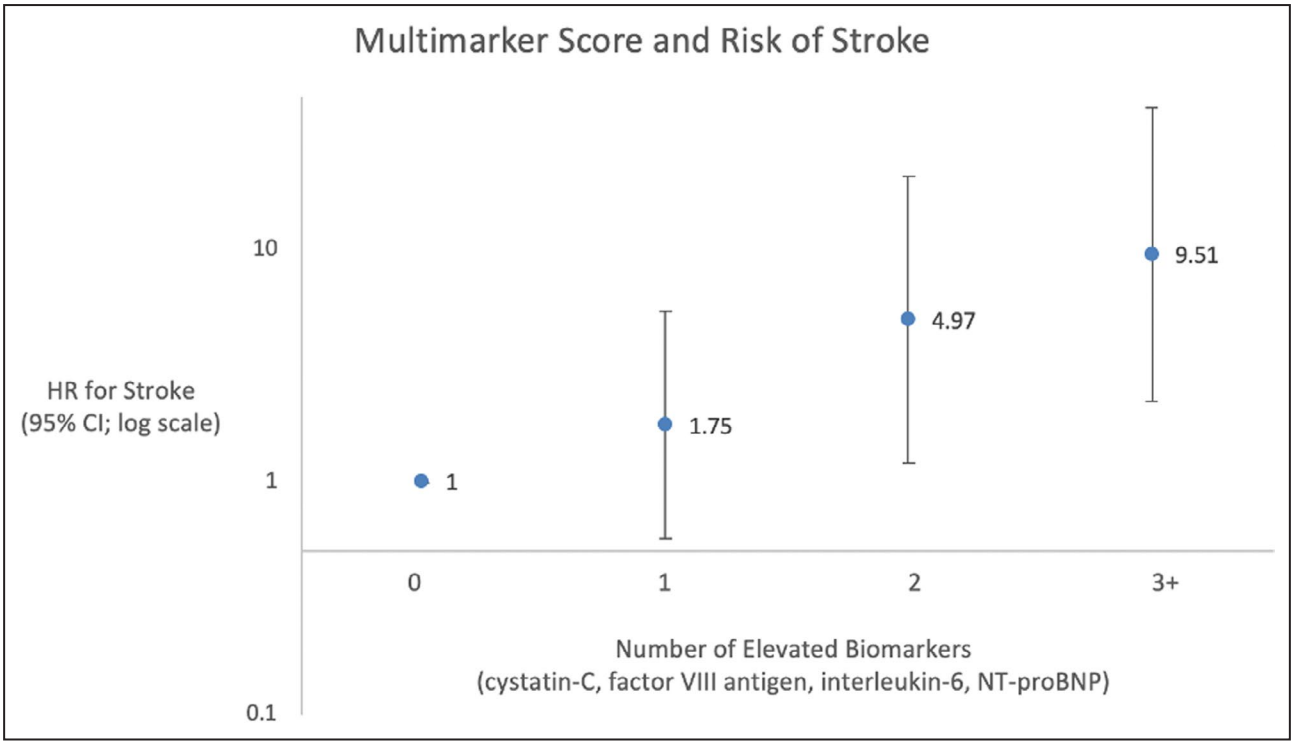

Figure 1. Multimarker score and risk of stroke.
The hazard ratios and 95% CI associated with having 0, 1, 2, or 3+ blood biomarkers in the highest tertile are graphically depicted. Higher numbers of elevated blood biomarkers are associated with an increased risk of stroke. From a prospective cohort of 78 cases and 92 controls, all with prevalent atrial fibrillation. HR indicates hazard ratio; and NT-proBNP; N-terminal B-type natriuretic peptide.

2-way relationship between them; that is, pathological thrombosis leads to subsequent elevations in markers of inflammation, while inflammation is associated with an increased risk of thrombosis.[23,25] IL-6 is an proinflammatory cytokine that changes leukocyte infiltration profiles and mediates the transition between acute and chronic inflammation.[27] It is associated with risk of ischemic stroke in general.[50] A prior study of 77 patients with AF, smaller than reported here, suggested that IL-6 may predict subsequent stroke.[26] The larger RE-LY (Randomized Evaluation of Long-Term Anticoagulation Therapy) study with 4893 clinical trial participants who had blood biomarkers measured, found similar results; patients with AF in the top compared with bottom quartile of IL-6 had a 50% increased risk of stroke or systemic embolism.[42] Results here demonstrated a stronger association of IL-6 with stroke in AF, and this might be because of the racial makeup of this study (as IL-6 is higher in Black individuals), and that it is a general population sample, not a clinical trial with selected patients included.

Our findings that cystatin C in the top versus bottom tertile conveyed a 3-fold increased risk of stroke are in accord with previously published research. Cystatin C is synthesized at a constant rate by all nucleated cells and is cleared renally, making it one of several markers of glomerular filtration. Prior analyses of the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE)[51] and RE-LY[42] trials demonstrated that cystatin C was associated with a heightened risk of stroke. In addition, cystatin C may have value in predicting bleeding risk when incorporated into the age, biomarker, clinical history bleeding risk score.[52]

NT-proBNP secretion is stimulated by increased atrial wall tension and is elevated in patients with AF.[28] Our results of a 4-fold increased risk of stroke among participants with NT-proBNP in the top tertile among those with AF agree with other findings. Among atherosclerosis risk in communities) study participants (with and without AF), those in the highest compared with the lowest quintile of NT-BNP had an HR for stroke of 2.6, similar to a prior REGARDS finding.[13,53] Participants with AF in the ARISTOTLE and RE-LY trials who were in the highest quartile of BNP had more than double the risk of stroke in comparison with the lowest quartile.[8,12] The underlying mechanism for the association between NT-proBNP and stroke in AF is likely multifactorial; individuals with higher BNP may have more persistent AF,[54] which may confer a heightened risk of stroke,[55] greater degrees of diastolic dysfunction,[56] and more severe atrial dilation.[57]

Findings here add to information from previously published research. First, our study evaluated blood biomarkers from diverse molecular and physiologic pathways and incorporated multiple blood biomarkers into a stroke risk score. This advancement holds the promise to improve the precision of risk estimates for ischemic stroke in practice, as we found that incorporation of the multimarker risk score offered improved prediction in comparison with the CHA_2DS_2VASc score alone. This may improve our ability to accurately classify patients as truly low-risk versus moderate- and high-risk, facilitating judicious prescription of anticoagulants and decreasing the burden of stroke without unnecessarily increasing the population burden of bleeding. Second, REGARDS over-recruited Black participants because they have a large stroke disparity compared with White, so 41% of study participants were Black. In contrast, the populations upon which prior analyses of blood biomarkers and risk of stroke were largely White, limiting their external validity. Black individuals comprised only a small minority of participants; <1% in RE-LY[58] and 1.2% in ARISTOTLE.[59] While we cannot rule out the possibility that race acts as an effect modifier for the interaction between blood biomarkers and risk of stroke, our study provides evidence that the blood biomarker–stroke-risk relationship holds for Black and White individuals. Third, other studies were based upon clinical trial participants, while the current study is from a population-based sample, allowing greater generalizability of results.

There are several limitations of our study. First, we had a limited sample size of participants with both AF and comprehensive blood biomarker evaluation in comparison with prior studies from participants enrolled in randomized clinical trials of direct anticoagulants, though the broad inclusion criteria of the REGARDS study allowed for a more representative study population than in randomized trials. In addition, blood biomarkers were measured at only a single point in time (study enrollment). Serial measurements of blood biomarkers could allow even better determination of their associations with stroke. The biomarkers analyzed were chosen based on known or suspected associations with incident ischemic stroke. Other measures like troponin may[60] have added value and merit inclusion in future studies. Analysis of genomic and transcriptomic markers is also needed. Though we address the utility of blood biomarkers in improving prediction of stroke, the clinical implications of this refinement depend on balancing the risks of stroke and the risks of anticoagulant-induced bleeding. Unfortunately, the risk factors for each are highly colinear, with the CHA_2DS_2VASc score performing as well as the HAS-BLED score in prediction of major bleeding.[61] The utility of a multimarker score for refinement of risk is greatest in those patients with borderline-indication for therapeutic anticoagulation or a relatively balanced risk-benefit profile. Unfortunately, our study only included 26 participants with CHA_2DS_2VASc score of 0, 1, or 2, so this analysis will require future larger studies of AF populations. The use of the net

reclassification improvement for assessing the additive value of a novel biomarker has limitations, including the possibility of statistical significance without clinically meaningful changes in management.[60] Despite these concerns, the net reclassification improvement is widely used within the contemporary cardiovascular literature for evaluating the additive value of a novel biomarker and provides incremental value beyond Cox models.[62–65] Finally, all presented findings require validation in subsequent studies.

In conclusion, this study provides proof of concept that measuring circulating blood biomarkers may have utility in improving stroke risk prediction in patients with AF, beyond the presently used risk prediction models. Specifically, a multi-blood-biomarker risk score based upon higher levels of cystatin C, factor VIII, IL-6, and NT-proBNP was strongly associated with increased risk of stroke in AF and offered an improvement in prediction when added to the CHA_2DS_2VASc score.

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Disclosures

None.

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