

Using Ultrasound and Inflammation to Improve Prediction of Ischemic Stroke: A Secondary Analysis of the Multi-Ethnic Study of Atherosclerosis

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Keywords

Cerebrovascular disease/stroke · Carotid ultrasound · Atherosclerosis

Abstract

Introduction: Current ischemic stroke risk prediction is primarily based on clinical factors, rather than imaging or laboratory markers. We examined the relationship between baseline ultrasound and inflammation measurements and subsequent primary ischemic stroke risk. **Methods:** In this secondary analysis of the Multi-Ethnic Study of Atherosclerosis (MESA), the primary outcome is the incident ischemic stroke during follow-up. The predictor variables are 9 carotid ultrasound-derived measurements and 6 serum inflammation measurements from the baseline study visit. We fit Cox regression models to the outcome of ischemic stroke. The baseline model included patient age, hypertension, diabetes, total cholesterol, smoking, and systolic blood pressure. Goodness-of-fit statistics were assessed to compare the baseline model to a model with ultrasound and inflammation predictor variables that remained significant when added to the baseline model. **Results:** We included 5,918 participants. The primary outcome of ischemic stroke was seen

in 105 patients with a mean follow-up time of 7.7 years. In the Cox models, we found that carotid distensibility (CD), carotid stenosis (CS), and serum interleukin-6 (IL-6) were associated with incident stroke. Adding tertiles of CD, IL-6, and categories of CS to a baseline model that included traditional clinical vascular risk factors resulted in a better model fit than traditional risk factors alone as indicated by goodness-of-fit statistics. **Conclusions:** In a multiethnic cohort of patients without cerebrovascular disease at baseline, we found that CD, CS, and IL-6 helped predict the occurrence of primary ischemic stroke. Future research could evaluate if these basic ultrasound and serum measurements have implications for primary prevention efforts or clinical trial inclusion criteria.

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Introduction

Because of the high morbidity and mortality from stroke and cerebrovascular disease, identifying those who may be at highest risk is of great public health importance. In order to identify those who may benefit from more aggressive risk reduction, clinicians often rely on risk pre-

diction scores, such as the Framingham risk score [1, 2]. Many of these methods of risk scoring rely solely on clinical factors, without utilizing relevant early imaging or laboratory findings that may point to intermediate vascular phenotypes such as atherosclerotic disease [3, 4].

Many carotid imaging findings are associated with increased stroke risk beyond well-established carotid stenosis, including increased carotid intima-media thickness (CIMT), carotid artery stiffness, and certain carotid plaque features such as intraplaque hemorrhage [5–8]. Of these, only later-stage markers of disease such as severe carotid stenosis currently drive changes in clinical management based on NASCET, CREST, and other trials [9–12]. Despite the established association of early sonographic imaging features with future cerebrovascular disease, there is conflicting data regarding the usefulness of earlier-stage markers of disease such as CIMT [13–15] to traditional risk prediction models [16, 17]. In addition to imaging findings, there is compelling evidence that some markers of systemic inflammation are associated with stroke [18–20]. Prior studies have shown that serum inflammatory markers, including interleukin-6 (IL-6) and C-reactive protein (CRP), may improve cerebrovascular risk prediction and prognostication of stroke outcomes [21–23]. Since early imaging and laboratory markers are not routinely included in stroke risk prediction scores, we sought to evaluate whether including them would improve future stroke risk prediction. In a secondary analysis of the Multi-Ethnic Study of Atherosclerosis (MESA), we investigated whether findings on the baseline carotid ultrasound and serum inflammatory markers improved ischemic stroke risk prediction.

Materials and Methods

Subjects

MESA is a prospective epidemiologic study designed to identify clinical factors which may predict the development of ischemic symptoms in previously asymptomatic individuals [24]. With a local Institutional Review Board waiver, we obtained the MESA dataset from the NHLBI Biologic Specimen and Data Repository Information Coordinating Center. In brief, MESA participants included 6,814 men and women aged 45–84 years at baseline who were free of cerebrovascular disease, defined as no history of physician-diagnosis of a cerebrovascular event (ischemic or hemorrhagic stroke or TIA). The participants are from 4 racial/ethnic groups (White, Black, Hispanic, and Asian) and from households in 6 US population centers (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles, CA; New York, NY; and St Paul, MN).

The dates for examinations in the MESA study were as follows: examination 1 between July 2000 and August 2002, exam-

ination 2 from September 2002 to February 2004, examination 3 from March 2004 to September 2005, examination 4 from September 2005 to May 2007, and examination 5 from April 2010 to December 2011 [24–26]. Each participating site had approval from their respective Institutional Review Boards and all participants provided informed consent. Inclusion criteria were participants who had a carotid ultrasound examination and serum laboratory markers of inflammation at the baseline study visit.

Ultrasound Examination

Details of how carotid artery ultrasounds were obtained have been previously published [27]. Carotid stenosis was identified on Doppler ultrasound using peak systolic velocities in the internal carotid artery (ICA) and common carotid artery (CCA) by MESA investigators [24]. In an effort to include earlier stages of carotid stenosis, we created a binary variable with 0–24% stenosis and >25% stenosis [28]. The intima-media thickness (IMT) of both the CCAs and ICAs were measured and reported in millimeters. Blinded replicate scans were performed on a subset of participants with intraclass correlation coefficients of 0.92 for CCA IMT and 0.88 for ICA IMT [27]. Interreader reproducibility was good with intraclass correlation coefficients of 0.81 for CCA IMT and 0.88 for ICA IMT [27]. Carotid plaque was defined as a discrete, focal wall thickening ≥ 1.5 cm or focal thickening at least 50% greater than the surrounding intima media (see online suppl. materials; see www.karger.com/doi/10.1159/000514373 for all online suppl. material) [29].

Arterial Stiffness Measures

All carotid stiffness measures were recorded with a B-mode ultrasound in the distal CCA using a Logiq 700 ultrasound machine (GE Medical Systems, Milwaukee, WI, USA) by the MESA investigators [30]. Briefly, carotid distensibility was defined as a ratio of the relative change in the cross-sectional area of the CCA over the cardiac cycle by the pulse pressure at the brachial artery. Peterson's elastic modulus was calculated as pulse pressure divided by the relative change in diameter of the carotid artery between the systole and diastole. Young's elastic modulus is a similar calculation of distensibility, but accounts for wall thickness by including a wall thickness term. It was calculated by dividing Peterson's elastic modulus by the wall thickness of the aorta. All of these indices are calculations of arterial stiffness, with lower values indicating a greater degree of arterial stiffness. The previously reported intraobserver and interobserver class correlation coefficient was 0.71 and 0.85 for carotid distensibility, respectively, and 0.69 and 0.84 for Young's modulus, respectively [30].

Total brachial artery reactivity, or dilatation of the brachial artery after the release of an occlusive blood pressure cuff, was also measured with a B-mode ultrasound using a Logiq-700 [31]. Intraobserver correlation coefficients for this method ranged from 0.50 to 0.90 [31]. Large and small artery elasticity, indicative of arterial stiffness, were measured noninvasively (see online suppl. materials for details) [32].

Measurement of Laboratory Biomarkers of Inflammation

We analyzed inflammatory markers of fibrinogen, CRP, plasmin-antiplasmin complex, factor VIII, D-dimer, and IL-6 measured at baseline for their association with future ischemic stroke (see online suppl. materials).

Table 1. Baseline demographics shown for the full cohort and those with versus without ischemic stroke during follow-up ($n = 5,918$, except where noted otherwise)

	Full cohort ($n = 5,918$)	No ischemic stroke ($n = 5,813$)	Ischemic stroke ($n = 105$)	p value
Age, years	62.0 (10.3)	61.9 (10.2)	68.1 (9.9)	<0.001
Female	3,090, 52.2	3,041, 52.3	49, 46.7	0.251
White	2,264, 38.3	2,225, 38.3	39, 37.1	0.143
Black	1,563, 26.4	1,530, 26.3	33, 31.4	0.239
Hispanic ethnicity	1,349, 23.2	1,322, 23.1	27, 26.5	0.428
Hypertension	2,610, 44.1	2,531, 43.5	79, 75.2	<0.001
Systolic blood pressure	126.4 (21.4)	126.1 (21.3)	141.8 (22.2)	<0.001
Diastolic blood pressure	72.0 (10.3)	71.9 (10.3)	76.3 (9.9)	<0.001
Diabetes	654, 11.1	627, 10.8	27, 25.7	<0.001
Atrial fibrillation	1, 0.02	1, 0.02	0, 0	0.893
Weight	172.7 (37.6)	172.6 (37.6)	178.0 (36.8)	0.142
Body mass index	28.2 (5.3)	28.2 (5.3)	29.1 (4.9)	0.101
Total cholesterol	194.3 (35.7)	194.2 (35.7)	196.9 (35.0)	0.444
Low-density lipoprotein cholesterol	117.3 (31.4)	117.2 (31.5)	120.3 (31.1)	0.324
High-density lipoprotein cholesterol	50.8 (14.6)	50.9 (14.6)	47.8 (12.8)	0.034
Statin use ($n = 5,915$)	847, 14.3	829, 14.3	18, 17.3	0.380
Intentional exercise (MET), min/week	1,553.0 (2,345.4)	1,557.4 (2,356.3)	1,308.3 (1,608.8)	0.283
Family history of heart attack ($n = 5,550$)	2,355, 42.4	2,307, 42.3	48, 48.0	0.256
Completed high school or less education ($n = 5,904$)	2,148, 36.4	2,102, 36.2	46, 44.2	0.093
Current smoker	745, 12.6	729, 12.5	16, 15.2	0.409
Current alcohol use ($n = 4,744$)	3,272, 69.0	3,218, 69.0	54, 67.5	0.774
Left ventricular hypertrophy on electrocardiogram ($n = 5,883$)	58, 1.0	56, 1.0	2, 1.9	0.336
Aspirin use ($n = 5,662$)	1,111, 19.6	1,083, 19.5	28, 29.5	0.015

Binary variables are presented as n , % and interval variables as the mean (SD). p values are shown for the stroke vs. no stroke group and calculated with the Student t test for interval variables and the χ^2 test for binary variables. MET, metabolic equivalent.

Cerebrovascular Disease Follow-Up

The process for adjudicating cerebrovascular events in MESA has been previously published [24]. Ischemic strokes, TIAs, and hemorrhagic strokes were adjudicated by a MESA committee including neurologists and physician epidemiologists using data from a combination of medical records and in-person interviews with participants and family members of participants. Ischemic strokes were defined as fatal or nonfatal strokes due to ischemic brain infarction.

Statistical Analysis

We fit Cox proportional hazard models to ischemic stroke. Because there were complex interactions between covariates, we used least absolute shrinkage and selection operator (LASSO) regression analysis to select variables for the baseline model, which included 4 decades of patient age (44–54 vs. 55–64 vs. 65–74 vs. 75–84 years), 3 ordinal categories of total cholesterol (65–199 vs. 200–239 vs. ≥ 240 mg/dL), and binary variables (yes/no) of baseline hypertension, diabetes, current and former smoking, and baseline systolic blood pressure ≥ 160 mm Hg. We then calculated a likelihood ratio test and Akaike's information criterion to compare a baseline model for predicting ischemic stroke using standard clinical predictors including age, systolic blood pressure, cholesterol,

diabetes, current smoking, and hypertension to a new model which added the predictor variables that remained significant from our regression analyses. For the new prediction model, we evaluated models using interactive variable selection and cutpoint determination and backward stepping with an α -error criterion of 0.05. We also calculated the net reclassification improvement (NRI) and integrated discrimination improvement (IDI) before and after adding the new markers in the model. The NRI reflects the sum of the percent improvement for events and non-events between the new and baseline model while the IDI evaluates the ability of additional biomarkers to predict an outcome of interest by estimating the percent change in prediction of the new model compared to the baseline model.

Results

After excluding 896 patients due to incomplete imaging and serum inflammatory marker data, we included 5,918 participants with an average age of 62.0 years at baseline (52.2% female; Table 1). With a mean follow-up

Table 2. The baseline multivariate Cox proportional hazards model fit to the outcome of ischemic stroke

Variable	Hazard ratio ¹	95% CI	<i>p</i> value
Age (years)	1.60	1.30–1.98	<0.001
Hypertension	2.52	1.56–4.08	<0.001
Diabetes	2.30	1.48–3.60	<0.001
Total cholesterol	1.40	1.07–1.84	0.014
Current smoking	1.99	1.15–3.45	0.014
Systolic blood pressure	2.03	1.24–3.32	0.005

¹ Adjusted for baseline age, diabetes, hypertension, total cholesterol, smoking, and systolic blood pressure ≥ 160 mm Hg.

time of 7.7 years (1.9 years), the subjects had a total of 105 ischemic strokes at a mean of 4.1 years of follow-up. We found that the ultrasound measures of carotid distensibility and carotid stenosis, and the laboratory marker of IL-6 were significantly associated with incident ischemic stroke when added to the baseline model with patient age, hypertension, diabetes, total cholesterol, smoking, and blood pressure (Table 2–4). The other evaluated imaging and laboratory values, including data on carotid plaque, were not significantly associated with incident ischemic stroke (Table 3, 4).

For ease of use in future risk prediction scores, we divided carotid distensibility and IL-6 into tertiles and then compared the baseline model to a new model with the addition of tertiles of carotid distensibility, IL-6, and categories of carotid stenosis. Goodness-of-fit of the baseline and new model was assessed by evaluating results from a likelihood ratio test and Akaike information criterion (AIC) of the 2 models. The likelihood ratio test resulted in a significant test statistic, suggesting that the new model provides a better fit to the data ($p < 0.001$). To further strengthen this claim, a decrease in AIC was observed from the baseline to model (1,720.0 to 1,703.2), indicating a better fit of the new model to the dataset. In addition, the new model that includes tertiles of carotid distensibility, IL-6, and categories of carotid stenosis shows better predictive capability than the baseline model based on concordance statistics (0.78 compared to 0.76, respectively). We found positive values for both NRI and IDI with an NRI estimate of 0.198 ($p < 0.001$, 95% CI 0.084–0.338) and an IDI estimate of 0.012 ($p < 0.001$, 95% CI 0.002–0.065), indicating the addition of new biomarkers were beneficial in improving performance of the model.

Discussion

In a secondary analysis of a multiethnic cohort free of cerebrovascular disease at baseline, we found that carotid distensibility and $>25\%$ carotid stenosis on ultrasound and a serum marker of inflammation predicted the occurrence of ischemic stroke over an average of an almost 8-year period. Furthermore, we found that the addition of these basic sonographic parameters and serum measurements to a baseline ischemic stroke prediction model improved the model's ischemic risk prediction. While many traditional risk prediction scoring systems assess future risk for both hemorrhagic and ischemic strokes, we focused on the future prediction of only ischemic strokes because there are risk factors unique to ischemic strokes, including large artery atherosclerosis. We found that with the addition of some imaging and serum markers not usually included in many standard risk prediction modeling techniques, we were able to significantly improve ischemic stroke risk prediction.

Imaging markers such as those seen on carotid ultrasound can more directly visualize subclinical atherosclerosis and be helpful in improving stroke risk prediction. Though CIMT is strongly associated with future cerebrovascular ischemia, other studies, including a large meta-analysis, have found mixed utility of the addition of CIMT to standard stroke risk calculators [14, 33]. Similarly, our analysis did not find the addition of measures of CIMT to be significant in improving stroke risk prediction. However, we found that carotid distensibility was significantly associated with incident stroke. Other studies have shown only marginal benefit in improving risk prediction models using arterial stiffness measurements [17]. Having increased arterial stiffness is thought to affect the pulsatility of small cerebral vessels and is associated with chronic microvascular changes and cerebral microbleeds [34]. Differences in utility of arterial stiffness measurements in the prediction of future stroke may be related to technique in measurement, for example more direct carotid stiffness measures, such as what was used in our cohort, may be more predictive compared to less direct assessment of arterial stiffness using peripheral brachial measurements. Also, decreased distensibility may be a marker of endothelial dysfunction, which can impair nitric oxide production and vascular reactivity leading to plaque production [35, 36].

Since carotid atherosclerotic disease is a direct cause of large artery atherosclerotic stroke, we expected to see increased risk of ischemic stroke in participants with carotid atherosclerotic plaque and stenosis. Other studies

Table 3. Effect of adding individual ultrasound variables to the baseline Cox proportional hazards model

Ultrasound variable	Hazard ratio ¹	95% CI	<i>p</i> value
Total brachial reactivity (%)	0.98	0.95–1.01	0.206
Large artery elasticity index	0.99	0.94–1.03	0.463
Small artery elasticity index	0.91	0.82–1.02	0.101
Total vascular impedance	1.00	1.00–1.00	0.745
Carotid distensibility coefficient (×100)	<i>0.05</i>	<i>0.00–0.75</i>	<i>0.030</i>
Carotid Youngs modulus	1.00	1.00–1.00	0.752
Common CIMT (mm)	0.92	0.33–2.53	0.871
Internal CIMT (mm)	1.17	0.90–1.53	0.244
Z-score for maximum IMT	1.07	1.21–3.27	0.463
Maximum carotid stenosis (0–24 vs. >25%)	<i>1.98</i>	<i>1.29–3.04</i>	<i>0.002</i>
Carotid plaque surface irregularity	0.97	0.90–1.04	0.353
Carotid plaque echotexture	1.12	0.96–1.30	0.146

Italicized variables are statistically significant. CIMT, carotid intima-media thickness.

¹ Adjusted for baseline age, diabetes, hypertension, total cholesterol, smoking, and systolic blood pressure ≥160 mm Hg.

Table 4. Effect of adding individual serum inflammatory marker variables to the baseline Cox proportional hazards model

Serum inflammatory marker	Hazard ratio ¹	95% CI	<i>p</i> value
CRP	1.01	0.98–1.04	0.433
Fibrinogen antigen (mg/dL)	1.00	1.00–1.00	0.192
Plasmin-antiplasmin complex (nM)	1.03	0.97–1.10	0.324
D-Dimer (µg/mL)	0.95	0.71–1.26	0.704
Factor VIII (%)	1.00	1.00–1.01	0.608
IL-6 (pg/mL)	<i>1.21</i>	<i>1.08–1.36</i>	<i>0.001</i>

Italicized variables are statistically significant. CRP, C-reactive protein.

¹ Adjusted for baseline age, diabetes, hypertension, total cholesterol, smoking, and systolic blood pressure ≥160 mm Hg.

have shown improvements in stroke risk prediction with the addition of specific carotid plaque data [16, 17]. High-risk plaque features, such as echolucent plaque, are strongly associated with future cerebrovascular ischemia [6]. Though we showed that having some plaque leading to at least 25% stenosis is associated with incident ischemic stroke, our study did not demonstrate any improvements in stroke risk with the inclusion of specific carotid plaque characteristic data. Because of the low numbers of participants with high-risk plaque features, such as plaque surface irregularity, differences in stroke risk prediction were difficult to detect. Additionally, plaque volume was not accounted for in the analysis, which further limits the predictive ability of the included plaque data. This some-

what limits extrapolation to patients with advanced atherosclerotic disease; however, future studies can be designed that target patients with these early imaging and inflammatory markers to determine if aggressive medical therapy can prevent vulnerable plaque features from developing.

Serum markers of systemic inflammation have also been used to improve stroke risk prediction, with CRP the most commonly cited [37]. These inflammatory markers are thought to play a key role in atherosclerotic plaque rupture, leading to thrombosis and cerebrovascular ischemia [38]. IL-6 specifically stimulates synthesis of acute phase proteins, such as CRP and fibrinogen, and also stimulates release of white blood cells. IL-6 also has strong

evidence demonstrating its utility in predicting outcomes in the setting of acute stroke [18, 23, 39] and is associated with cardiovascular morbidity [40], but has less evidence in predicting future cardiovascular events [38]. Our findings show that there may be a role for IL-6 in predicting future stroke.

Our study has several limitations. First, we used data from the baseline visit for included participants so we did not account for changes in vascular risk factors over the entire study period. While this limits evaluation of changes to vascular risk factors over time, it is similar to how other risk prediction systems are structured and is an inherent limitation to risk prediction schemes. Another limitation is the general applicability of the findings to all populations. Since the included cohort were free of cerebrovascular disease at baseline, the risk prediction scoring may be different than those with more vascular risk factors at baseline. Additionally, atrial fibrillation did not contribute to any of the ischemic strokes included in our cohort, which limits the applicability of our findings to those who suffer from ischemic stroke secondary to cardioembolic causes. Furthermore, there are inherent limitations with the use of ultrasound in evaluating carotid plaque, including limited ability to evaluate specific plaque components, such as intraplaque hemorrhage. Another inherent limitation in the data is the lack of baseline brain imaging. Future studies including baseline brain MRIs to evaluate for markers of cerebral small vessel disease, including white matter hyperintensities, covert brain infarctions, and cerebral microbleeds, may allow for improved stroke risk prediction. Lastly, adding markers of carotid artery disease and serum inflammatory markers improved stroke risk prediction modestly, perhaps in part due to the relatively young and cardiovascular risk factor-free cohort. Future studies in cohorts with higher baseline risk will be helpful to confirm our findings.

We found that sonographic measures of atherosclerosis including carotid stenosis and carotid distensibility, and a serum inflammatory marker, IL-6, were significantly associated with increased risk of ischemic stroke in a population free from cerebrovascular disease. We found that including these markers in risk prediction scoring systems also significantly improved risk prediction. With further validation, adding these basic measurements to prediction models could improve primary prevention efforts with more focused primary prevention strategies and could be used to enroll patients in prospective clinical trials.

Statement of Ethics

This research complied with the guidelines for human studies and should include evidence that the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. With a local Institutional Review Board waiver, we used de-identified data.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

H.B. and A.D.H.: substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; drafting the work or revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. A.D., K.-H.W., N.S., J.S.M., M.A., and J.J.M.: substantial contributions to the conception or design of the work; revising the work critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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