Circulating inflammatory biomarkers are related to cerebrovascular disease in older adults

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Abstract

Objective
This investigation aimed at examining whether circulating inflammatory biomarkers C-reactive protein (CRP), interleukin-6 (IL6), and alpha 1-antichymotrypsin (ACT) were related to cerebrovascular disease (CVD) assessed by MRI.

Methods
The study included nondemented elderly participants of a community-based, multiethnic cohort, who received baseline MRI scans and had CRP (n = 508), ACT (435), and IL6 (N = 357) measured by ELISA. Silent brain infarcts and white matter hyperintensities (WMH) were derived from all available MRI scans at baseline, approximately 4.4 years after blood sample collection for inflammatory biomarkers. Repeated assessments of infarcts and WMH, as well as microbleeds assessment, were performed at follow-up MRI visits around 4.5 years later. Cross-sectional and longitudinal relationship between inflammatory biomarkers and CVD were analyzed using appropriate logistic regression models, generalized linear models, or COX models.

Results
After adjusting for age, sex, ethnicity, education, APOE genotype, and intracranial volume, 1 SD increase in log10IL6 was associated with infarcts on MRI (odds ratio [OR] [95% confidence interval [CI]] = 1.28 [1.02–1.60], p = 0.033), and 1 SD increase in log10CRP and log10ACT was associated with microbleeds (OR [95% CI] = 1.46 [1.02–2.09], p = 0.041; and 1.65 [1.11–2.46], p = 0.013; respectively). One SD increase in log10ACT was also associated with larger WMH at the follow-up MRI (b = 0.103, p = 0.012) and increased accumulation of WMH volume (b = 0.062, p = 0.041) during follow-up. The associations remained significant after additional adjustment of vascular risk factors and excluding participants with clinical stroke.

Conclusions
Among older adults, increased circulating inflammatory biomarkers were associated with the presence of infarcts and microbleeds, WMH burden, and progression of WMH.
Subclinical cerebrovascular diseases (CVDs), including primarily lacunar infarcts or silent brain infarcts, white matter hyperintensities (WMH), and cerebral microbleeds, are frequent findings on MRI scans of normal elderly people.1–3 “Silent” changes in the brain reflect individuals carrying subclinical CVD without clinical symptoms, but they are not benign because of the strong association with increased risk of recurrent strokes,4 dementia,5 and mortality.

Strong evidence suggests that chronic inflammation plays a crucial role in the development of stroke, cardiovascular disease, dementia,6 and atherosclerosis.7 Therefore, it is reasonable to hypothesize that chronic inflammation might also be involved in arteriolosclerosis damage of brain vessels, manifested radiologically as localized ischemic infarcts, diffuse WMH, or microbleeds.8 However, the association between inflammatory biomarkers and CVD remains unclear.9–27 Some existing studies were based on clinical convenience samples, some included middle-aged participants, and few studies included Hispanics or African-Americans.10,11,18,19 who have a higher frequency of vascular risk factors.28 Therefore, the results may not be applied to clinically normal, ethnically diverse, elderly populations who could be the key target population for potential intervention. In addition, there are few longitudinal studies9,15,22,23 and few studies13,19–21 that explored microbleeds. Most studies measured 2 key inflammatory markers c-reactive protein (CRP) and interleukin-6 (IL6), although alpha 1-antichymotrypsin (ACT), which has long been implicated in AD,29 has never been examined.

We examined whether circulating levels of inflammatory markers (CRP, IL6, and ACT) were associated with the presence and progression of CVD (i.e., infarcts, WMH, and microbleeds) among nondemented elderly participants of a community-based, multiethnic cohort.

Methods

Study participants

The current study included participants from an ongoing prospective study of aging and dementia, the Washington Heights-Hamilton Height-Inwood and Columbia Aging Project (WHICAP), who were elders identified from Medicare beneficiaries residing in northern Manhattan.30 The original cohort for this study included 2,776 participants. At baseline, participants were asked about medical and neurologic history, received assessments of health and function, and were assessed using a neuropsychological battery. Participants were followed approximately for 18 months, repeating the baseline examinations. The diagnosis of dementia or its absence was based on standard research criteria using all available information at a consensus conference.

A total of 769 WHICAP participants received MRI scans including T1-weighted images, fluid-attenuated inversion recovery (FLAIR)-weighted images, and proton density and T2-weighted double-echo images.31 Among the 680 nondemented (n = 45 demented) participants who had baseline MRI data available and analyzed by FreeSurfer (n = 44 without FreeSurfer processing),30 a total of 508 (75%) participants had CRP, 435 (64%) had ACT, and 357 (53%) had IL6 detected (figure 1). Participants who had measured biomarkers were younger, had fewer African-Americans, had fewer vascular risk factors than those who did not have biomarker data sample.30 Repeated MRI scans were completed for 310 participants 4.5 (SD = 0.9) years after their baseline scans, and 230 of them also received gradient echo (GRE) scans for microbleeds assessment. Compared with the neuroimaging project in which participants did not have repeated scans, those with repeated scans were younger at baseline (79.3 vs 80.7, p = 0.002) and had more hypertension (90% vs 83%, p = 0.08).30

Standard protocol approvals, registrations, and patient consents

The Columbia University Institutional Review Board has reviewed and approved this project. All individuals provided written informed consent.

MRI protocol

All scans were acquired on a 1.5T Philips Intera scanner at Columbia University. T1-weighted images were acquired with a repetition time of 20 milliseconds (ms); echo time, 2.1 ms; field of view (FOV), 240 cm; 256 × 160–pixel matrix with 1.3-mm section thickness, and voxel size 1 × 1 × 1.3 mm. Freesurfer (v5.1) (surfer.nmr.mgh.harvard.edu/) was used to analyze T1 images with visual quality control and manual correction. WMH quantification was based on T2-weighted FLAIR MRI scans (repetition time, 11,000 ms; echo time, 144 ms; inversion time, 2,800; and FOV 25 cm, 2 nex; 256 × 192 matrix with 3-mm slice thickness). An optimized, high-resolution three-dimensional T2*-weighted GRE image (repetition time, 45 ms; echo time, 31 ms; flip angle, 13; and slice thickness, 2 mm) was acquired at the follow-up MRI visit for microbleeds visualization and quantification. The imaging processing was blinded to any information about participants.

We used all the available images to determine whether a participant had brain infarction on MRI, which was defined as...
lesions 3 mm or larger. Signal void seen on the T2-weighted images was used to indicate a vessel. We also consider other necessary imaging characteristics such as CSF density on the T1-weighted image and distinct separation from the circle of Willis vessels and perivascular spaces.

Total WMH volume was determined following the procedures as previously described. First, FLAIR images were skull stripped, and then a threshold and seed-growing algorithm was applied to identify voxels that fell within an a priori–determined distribution of hyperintense signal. Total WMH volume was then calculated by multiplying labeled voxels by voxel dimensions. We calculated the ratio of WMH over total intracranial volume (ICV) to adjust the head size differences. We log-transformed WMH/ICV ratio value in the analyses.

Microbleeds were visually inspected and rated according to the research criteria. We classified microbleeds into “lobar” (in the frontal, temporal, parietal, and occipital lobes) and “deep” (in basal ganglia, thalamus, and infratentorial regions).

The number of microbleeds was also recorded. A dichotomous microbleeds status, presence (at least one microbleed) vs absence (as reference group), was used in the main analysis.

**Biomarker measurement**

The circulating levels of CRP, IL6, and ACT were measured in previous studies of WHICAP. Nonfasting plasma (CRP) and serum (IL6 and ACT) samples, collected 4.4 years before the first MRI scan (figure 1), were used in all analyses. Briefly, blood samples were collected in BD Vacutainer Ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged at 2,000 g for 15 minutes at room temperature and then stored at −80°C in polypropylene cryotubes until analysis. For serum samples, collected blood samples were allowed to sit for 30 minutes before they were centrifuged, aliquoted, and stored at −80°C. The CRP levels were measured using a high-sensitivity (1.6 ng/mL) ELISA kit ( Diagnostic systems laboratories, Inc., Webster, TX, with the intra-assay and interassay coefficient of variations (CVs) of 4.6% and 11.7%, respectively. The IL-6 levels were measured using a high-sensitivity (0.11 pg/mL) ELISA kit (R&D Systems, Minneapolis, MN; intra-assay CV = 7.4% and
inter-assay CV = 7.8%). The ACT levels were measured using an immunoperoxidase assay kit (Immunology Consultants Laboratory, Inc., Newberg; sensitivity of 2.518 ng/mL; both intra-assay and interassay CVs <10%). Laboratory personnel were blinded as to the demographic and clinical status of the study participants.

**Covariates**

Ethnicity, including Hispanic, non-Hispanic black, non-Hispanic white, or Other, based on self-report using the format of the 2000 US census, was used as a dummy variable with non-Hispanic white or other as the reference. Sex (male as the reference), smoking status (nonsmoker [as the reference] or ever smoked [including current smokers and past smokers who had ≥1 cigarette/day for ≥1 year]), and apolipoprotein (APOE) status (presence of either 1 or 2 vs absence of e4 alleles [as the reference]), were used as dichotomous variables. Body mass index (BMI; kg/m²) was calculated as weight over height squared. The presence of depression was defined as ≥4 on the 10-item version of the Center for Epidemiologic Studies Depression scale. Presence or absence of heart disease, diabetes mellitus, and hypertension, as well as the use of medication for the conditions, were based on self-reported information, and clinical stroke was determined by self-report, neurologic examination, or a review of medical records. A vascular comorbidity burden score (range 0–4) was calculated by summing these 4 dichotomized vascular comorbidity variables. Fasting plasma lipids were determined on baseline blood samples using standard techniques.

**Statistical analyses**

**Characteristic variables of the participants**

Circulating levels of inflammatory biomarkers were transformed using logarithm and then standardized ([log10(Biomarker) – mean of log10(Biomarker)]/SD of log10(Biomarker)). The resulting standardized values were approximately normally distributed. Demographics, clinical characteristics, vascular and lifestyle factors, and brain imaging findings of participants by biomarker tertiles were compared using analysis of variance for continuous variables and the χ² test for categorical variables.

**Cross-sectional associations between the inflammatory biomarkers and cerebrovascular disease**

Generalized linear models were used to test the associations between the inflammatory biomarkers and baseline WMH, and logistic regression models were used for the inflammatory biomarkers and infarcts or microbleeds status. All the models were adjusted for age at time of scan, sex, education, ethnicity, and APOE e4 status. In the fully adjusted models, we additionally adjusted for health and vascular risk factors, including BMI, depression, plasma lipids level, smoking status, and vascular comorbidity burden. All covariates were treated as time-independent variables.

**Longitudinal data analysis**

The changes of WMH were calculated as \([\log_{10}(\text{follow-up WMH/ICV}) – \log_{10}(\text{baseline WMH/ICV})]\). Multiple regression models were used to test whether inflammatory biomarkers were associated with differential WMH change, adjusted for time from baseline scan to the follow-up scan in addition to all the other covariates. Cox proportional hazard models were used to examine whether baseline inflammatory biomarkers were associated with the risk of developing incident infarcts among those without infarcts at baseline.

**Supplementary analyses**

We excluded 102 (20.1% of 508) participants with clinical stroke and repeated the analyses only among participants without clinical stroke. We also excluded 24 participants who developed incident dementia during follow-up and repeated the analysis for microbleeds and infarcts at follow-up. In addition, we examined whether certain regions were particularly vulnerable to inflammatory effects by examining WMH in the frontal, parietal, occipital, and temporal regions separately and by examining lobar and deep microbleeds separately. Finally, we examined the relationship between inflammatory biomarkers and CVD by race/ethnic groups, sex, and APOE status.

All analyses were performed using PASW Statistics 17.0 (formerly SPSS Inc., Chicago, IL). All p values were based on two-sided tests with the statistical significance level set at 0.05.

**Data availability**

The data that support the findings of this study are available from the corresponding author, yg2121@columbia.edu. Some access restrictions apply to the data used for the current study because of privacy concerns including the following: (1) the data contain elements that are considered Protected Health Information under HIPAA regulations; and (2) there is a potential risk for linkage of data made publicly available in conjunction with multiple studies based on the same cohort. Although the data on which the manuscript is based are not publicly available, a limited data set is available under a standard HIPAA Data Use Agreement, subject to review and approval by the Columbia University Privacy Officer.

**Results**

**Demographic and cerebrovascular disease characteristics according to levels of inflammatory biomarkers**

The 3 inflammatory markers positively correlated with each other, with correlation coefficients between CRP and IL6, between CRP and ACT, and between IL6 and ACT being 0.51, 0.39, and 0.21, respectively, all p < 0.0001.

Those with a higher level of inflammatory biomarkers are more likely to be women and more likely to be African-Americans (table 1). Those in the highest tertiles were less likely to be APOE e4 positive (CRP), had higher BMI (CRP and IL6), had worse lipids profiles (CRP and IL6), had more vascular comorbidities (CRP), and had higher education.
|                          | Lowest | Middle | Highest | Total |
|--------------------------|--------|--------|---------|-------|
| CRP tertile              |        |        |         |       |
| CRP, mg/L, median (IQR)  | 2.03 (1.22–2.89) | 5.55 (4.44–7.51) | 22.3 (15.7–37.3) | 5.59 (2.87–15.8) |
| Age at blood collection, mean (SD) | 72.95 (5.87) | 72.05 (5.83) | 72.53 (5.42) | 72.51 (5.71) |
| Age at baseline MRI scan, mean (SD) | 80.49 (5.84) | 79.41 (5.6) | 79.87 (5.57) | 79.92 (5.68) |
| Age at follow-up, mean (SD) | 84.55 (5.54) | 83.62 (5.11) | 84.05 (5.36) | 84.07 (5.33) |
| Education, mean (SD)     | 10.92 (4.8) | 10.84 (4.88) | 10.6 (4.55) | 10.78 (4.74) |
| Female, N (%)            | 102 (60) | 113 (67) | 119 (70) | 334 (66) |
| Whites, N (%)            | 64 (38) | 50 (30) | 42 (25) | 156 (31) |
| African-American, N (%)  | 43 (25.4) | 53 (31.4) | 63 (37.1) | 159 (31.3) |
| Hispanics, N (%)         | 61 (36.1) | 61 (36.1) | 62 (36.5) | 184 (36.2) |
| Others, N (%)            | 1 (0.6) | 5 (3) | 3 (1.8) | 9 (1.8) |
| APOE ɛ4 positive, N (%)  | 54 (32) | 40 (24) | 33 (19) | 127 (25) |
| BMI, kg/m², mean (SD)    | 25.65 (4.6) | 27.7 (5.23) | 29.89 (6.45) | 27.77 (5.74) |
| Triglycerides (TG), mg/dL, mean (SD) | 134.5 (75.6) | 144 (68.6) | 169.7 (111.2) | 149.3 (88.1) |
| Total cholesterol, mg/dL, mean (SD) | 199.2 (38.7) | 200.8 (37) | 198.1 (41.4) | 199.3 (39) |
| HDL-cholesterol, mg/dL, mean (SD) | 51.8 (14.1) | 50.5 (15.9) | 44.6 (12.6) | 49 (14.6) |
| LDL-cholesterol, mg/dL, mean (SD) | 121.2 (33.7) | 121.5 (31.9) | 119.8 (33) | 120.8 (32.8) |
| Ratio of TG to HDL-cholesterol, mean (SD) | 3.03 (2.47) | 3.4 (2.57) | 4.4 (4.12) | 3.61 (3.19) |
| Heart disease, N (%)     | 61 (36) | 95 (55) | 74 (43) | 230 (45) |
| Hypertension, N (%)      | 135 (79) | 145 (85) | 149 (88) | 429 (84) |
| Diabetes mellitus, N (%) | 28 (16) | 45 (26) | 62 (36) | 135 (26) |
| Clinical stroke, N (%)   | 31 (18) | 33 (19) | 38 (22) | 102 (20) |
| Vascular score, mean (SD) | 1.51 (0.96) | 1.87 (0.98) | 1.91 (1.01) | 1.76 (1.00) |
| Infarct presence at baseline, N (%) | 54 (32) | 49 (29) | 66 (39) | 169 (33) |
| Infarct presence at follow-up, N (%) | 37 (52) | 37 (49) | 38 (59) | 112 (53) |
| WMH ‡ at baseline, mean (SD) | −6.02 (0.71) | −6.05 (0.64) | −5.97 (0.67) | −6.02 (0.67) |
| WMH ‡ at follow-up, mean (SD) | −5.53 (0.61) | −5.48 (0.45) | −5.34 (0.56) | −5.45 (0.55) |
| Increased WMH † mean (SD) | 0.63 (0.37) | 0.60 (0.45) | 0.64 (0.38) | 0.62 (0.4) |
| Microbleeds at follow-up, N (%) | 13 (20) | 18 (30) | 15 (33) | 46 (27) |
| Lobar microbleeds at follow-up, N (%) | 9 (14) | 12 (20) | 14 (31) | 35 (20) |
| Deep microbleeds at follow-up, N (%) | 4 (6) | 7 (12) | 4 (9) | 15 (9) |
| No. of microbleeds, mean (SD) | 0.23 (0.52) | 0.42 (0.79) | 0.82 (1.95) | 0.45 (1.16) |
| No. of lobar microbleeds, mean (SD) | 0.17 (0.48) | 0.3 (0.74) | 0.73 (1.8) | 0.36 (1.08) |
| No. of deep microbleeds, mean (SD) | 0.06 (0.24) | 0.12 (0.32) | 0.09 (0.29) | 0.09 (0.28) |

IL6 tertile

|                          | Lowest | Middle | Highest | Total |
|--------------------------|--------|--------|---------|-------|
| IL6, pg/mL, median (IQR) | 1.41 (1.18–1.65) | 2.43 (2.04–2.87) | 4.55 (3.85–6.19) | 2.44 (1.65–3.91) |

Continued
| Demographic, clinical, and dietary characteristics | Lowest | Middle | Highest | Total | p Value | (H vs L) |
|--------------------------------------------------|--------|--------|---------|-------|---------|---------|
| **Lowest**                                       | 63     | 62     | 74      | 74    | 0.085   | 0.042   |
| **Middle**                                       | 73     | 75     | 63      | 63    | 0.790   | 0.629   |
| **Highest**                                      | 89     | 107    | 45      | 45    | 0.880   | 0.629   |
| **Total**                                        | 237    | 237    | 237     | 237   | 0.880   | 0.629   |
| **BMI, kg/m², mean (SD)**                        | 26.4   | 27.6   | 28.5    | 27.5  | 0.006   | 0.001   |
| **Triglycerides (TG), mg/dL, mean (SD)**         | 136.7  | 157.2  | 155.5   | 149.6 | 0.006   | 0.001   |
| **Total cholesterol, mg/dL, mean (SD)**          | 205.5  | 200.8  | 199.7   | 202   | 0.006   | 0.001   |
| **HDL-cholesterol, mg/dL, mean (SD)**            | 53.6   | 50.0   | 46.3    | 50.1  | 0.006   | 0.001   |
| **LDL-cholesterol, mg/dL, mean (SD)**            | 125    | 118.2  | 123.3   | 122.2 | 0.006   | 0.001   |
| **Ratio of TG to HDL-cholesterol, mean (SD)**    | 3.08   | 3.65   | 4.02    | 3.58  | 0.006   | 0.001   |
| **Heart disease, N (%)**                         | 52     | 51     | 49      | 52    | 0.006   | 0.001   |
| **Hypertension, N (%)**                          | 99     | 102    | 99      | 102   | 0.006   | 0.001   |
| **Diabetes mellitus, N (%)**                     | 31     | 30     | 33      | 33    | 0.006   | 0.001   |
| **Clinical stroke, N (%)**                       | 18     | 20     | 32      | 32    | 0.006   | 0.001   |
| **Vascular score, mean (SD)**                    | 1.68   | 1.71   | 1.79    | 1.76  | 0.006   | 0.001   |
| **Infarct presence at baseline, N (%)**          | 33     | 40     | 47      | 40    | 0.006   | 0.001   |
| **Infarct presence at follow-up, N (%)**         | 26     | 31     | 28      | 28    | 0.006   | 0.001   |
| **WMH† at baseline, mean (SD)**                  | −6.05  | −6.02  | −5.95   | −6.01 | 0.006   | 0.001   |
| **WMH† at follow-up, mean (SD)**                 | −5.59  | −5.41  | −5.34   | −5.45 | 0.006   | 0.001   |
| **Increased WMH,‡ mean (SD)**                    | 0.56   | 0.66   | 0.59    | 0.61  | 0.006   | 0.001   |
| **Microbleeds at follow-up, N (%)**              | 12     | 13     | 10      | 10    | 0.006   | 0.001   |
| **Lobar microbleeds at follow-up, N (%)**        | 10     | 10     | 8       | 8     | 0.006   | 0.001   |
| **Deep microbleeds at follow-up, N (%)**         | 4      | 4      | 5       | 5     | 0.006   | 0.001   |
| **No. of microbleeds, mean (SD)**                | 0.54   | 0.43   | 0.74    | 0.56  | 0.006   | 0.001   |
| **No. of lobar microbleeds, mean (SD)**          | 0.44   | 0.34   | 0.61    | 0.45  | 0.006   | 0.001   |
| **No. of deep microbleeds, mean (SD)**           | 0.10   | 0.09   | 0.13    | 0.10  | 0.006   | 0.001   |
| **ACT tertile**                                   |        |        |         |       |         |         |
| **Number**                                       | 145    | 145    | 145     | 435   | 0.006   | 0.001   |
| **ACT, mg/L, median (IQR)**                      | 158    | 188    | 232     | 188   | 0.006   | 0.001   |
| **Age at blood collection, mean (SD)**           | 72.55  | 72.72  | 73.56   | 72.95 | 0.006   | 0.001   |
| **Age at baseline MRI scan, mean (SD)**          | 79.86  | 79.84  | 80.28   | 79.99 | 0.006   | 0.001   |
| **Age at follow-up, mean (SD)**                  | 84.12  | 84.64  | 83.71   | 84.16 | 0.006   | 0.001   |

Continued
Those with a higher tertile of any of the inflammatory biomarker tended to have more CVD (table 1). Comparing the inflammatory marker levels according to the CVD status revealed a similar relationship, i.e., participants with infarcts at baseline had higher IL6 levels than those without, those with higher WMH at follow-up had higher ACT levels than those with lower WMH, and those with microbleeds had higher ACT levels than those without microbleeds (table 2 and figure 2).

Table 1  Demographic, clinical, and dietary characteristics in relation to CRP, IL6, and ACT (continued)

|                        | Lowest  | Middle | Highest | Total          | *p Value | *p (H vs L) |
|------------------------|---------|--------|---------|----------------|----------|------------|
| Education, mean (SD)   | 10.25 (4.71) | 10.53 (4.89) | 11.37 (4.36) | 10.72 (4.67) | 0.096   | 0.038  |
| Female, N (%)          | 84 (58)  | 105 (71) | 101 (71) | 290 (67)       | 0.035   | 0.030   |
| Whites, N (%)          | 42 (29.2) | 41 (27.7) | 52 (36.4) | 135 (31)       | 0.046   | 0.048   |
| African-American, N (%)| 46 (31.9) | 42 (28.4) | 53 (37.1) | 141 (32.4)     |         |         |
| Hispanics, N (%)       | 54 (37.5) | 61 (41.2) | 33 (23.1) | 148 (34)       |         |         |
| Others, N (%)          | 2 (1.4)  | 4 (2.7)  | 5 (3.5)  | 11 (2.5)       |         |         |
| APOE e4 positive, N (%)| 38 (26)  | 25 (17)  | 38 (27)  | 101 (23)       | 0.081   | 0.972   |
| BMI, kg/m², mean (SD)  | 27.68 (5.65) | 26.95 (4.95) | 27.52 (6.2) | 27.38 (5.63) | 0.501   | 0.815   |
| Triglycerides (TG), mg/dL, mean (SD) | 145.3 (82.3) | 144.3 (61.1) | 151 (108.7) | 146.9 (86.4) | 0.789   | 0.583   |
| Total cholesterol, mg/dL, mean (SD) | 196.7 (40.8) | 201.1 (40.5) | 200.8 (37.1) | 199.5 (39.5) | 0.581   | 0.380   |
| HDL-cholesterol, mg/dL, mean (SD) | 50.2 (16.7) | 50.2 (14.3) | 49.4 (14) | 49.9 (15) | 0.861   | 0.627   |
| LDL-cholesterol, mg/dL, mean (SD) | 116.4 (30.4) | 122 (35.9) | 122.4 (32.8) | 120.3 (33.1) | 0.253   | 0.138   |
| Ratio of TG to HDL-cholesterol, mean (SD) | 3.52 (2.93) | 3.31 (2.12) | 3.66 (3.84) | 3.5 (3.05) | 0.644   | 0.715   |
| Heart disease, N (%)   | 56 (38)  | 64 (44)  | 64 (44)  | 184 (42)       | 0.547   | 0.340   |
| Hypertension, N (%)    | 115 (79) | 124 (85) | 127 (87) | 366 (84)       | 0.133   | 0.058   |
| Diabetes mellitus, N (%)| 31 (21)  | 46 (31)  | 41 (28)  | 118 (27)       | 0.131   | 0.174   |
| Clinical stroke, N (%) | 29 (20)  | 28 (19)  | 32 (22)  | 89 (20)       | 0.832   | 0.666   |
| Vascular score, mean (SD) | 1.59 (1.04) | 1.81 (1.00) | 1.82 (0.96) | 1.76 (1.00) | 0.095   | 0.053   |
| Infarct presence at baseline, N (%) | 47 (33)  | 46 (32)  | 50 (35)  | 143 (33)       | 0.873   | 0.709   |
| Infarct presence at follow-up, N (%) | 32 (46)  | 28 (54)  | 36 (62)  | 96 (54)        | 0.210   | 0.077   |
| WMH† at baseline, mean (SD) | −6.04 (0.7) | −5.95 (0.67) | −5.95 (0.73) | −5.98 (0.7) | 0.476   | 0.288   |
| WMH† at follow-up, mean (SD) | −5.6 (0.62) | −5.4 (0.53) | −5.34 (0.58) | −5.46 (0.59) | 0.028   | 0.013   |
| Increased WMH,‡ mean (SD) | 0.54 (0.39) | 0.62 (0.38) | 0.70 (0.42) | 0.61 (0.4)    | 0.085   | 0.027   |
| Microbleeds at follow-up, N (%) | 10 (20)  | 7 (15)   | 23 (49)  | 40 (28)        | <0.0001 | 0.003   |
| Lobar microbleeds at follow-up, N (%) | 8 (16)   | 7 (15)   | 18 (38)  | 33 (23)        | 0.008   | 0.013   |
| Deep microbleeds at follow-up, N (%) | 2 (4)    | 2 (4)    | 10 (21)  | 14 (10)        | 0.005   | 0.010   |
| No. of microbleeds, mean (SD) | 0.30 (0.76) | 0.21 (0.54) | 1.13 (1.97) | 0.54 (1.31) | 0.001   | 0.001   |
| No. of lobar microbleeds, mean (SD) | 0.26 (0.75) | 0.17 (0.43) | 0.91 (1.84) | 0.44 (1.20) | 0.004   | 0.002   |
| No. of deep microbleeds, mean (SD) | 0.04 (0.20) | 0.04 (0.20) | 0.21 (0.41) | 0.10 (0.30)  | 0.004   | 0.004   |

Abbreviations: ACT = alpha 1-antichymotrypsin; ANOVA = analysis of variance; BMI = body mass index; CRP = C-reactive protein; ICV = total intracranial volume; IL6 = interleukin-6; WMH = white matter hyperintensities.

*p Values were from the χ² test for categorical variables or the one-way ANOVA test for continuous variables. p (H vs L) is the p value corresponding to the comparison of the highest (H) to the lowest (L) tertile.

†"WMH" was calculated as log10(WMH volume/total intracranial volume).

‡"Increased WMH" was calculated as the increase from the first to the second WMH value [log10(2nd WMH/ICV) − s(baseline WMH/ICV)].

Bold values indicate statistical significant difference with p < 0.05.
| Table 2 Average CRP, IL6, and ACT levels according to the cerebrovascular disease status |
|----------------------------------------|--------|--------|--------|--------|--------|--------|--------|
|                                       | CRP    |         |        | IL6    |         |        | ACT    |         |
|                                       | N      | z-score, mean (SD) | Geometric, mean (SD) | p Value | N      | z-score, mean (SD) | Geometric, mean (SD) | p Value | N      | z-score, mean (SD) | Geometric, mean (SD) | p Value |
| Infarct at baseline                    |        |        |        |        |        |        |        |        |        |        |        |        |
| No                                     | 339    | −0.02 (0.98) | 6.06 (3.3) | 0.388 | 237    | −0.08 (0.98) | 2.41 (1.79) | 0.036 | 292    | −0.04 (0.99) | 190.7 (1.25) | 0.357 |
| Yes                                    | 169    | 0.06 (1.06) | 6.69 (3.6) | 1.20   | 120    | 0.15 (1.03) | 2.77 (1.84) | 0.036 | 143    | 0.06 (1.02) | 194.8 (1.26) |        |
| Total                                  | 508    | 0 (1.01) | 6.26 (3.4) | 357    | 0 (1) | 2.53 (1.81) | 0.036 | 435    | 0 (1) | 192.0 (1.25) |        |        |
| Infarct at follow-up                   |        |        |        |        |        |        |        |        |        |        |        |        |
| No                                     | 98     | −0.11 (0.95) | 5.48 (3.2) | 0.556 | 68     | −0.12 (0.93) | 2.35 (1.74) | 0.236 | 83     | −0.22 (0.94) | 182.9 (1.24) | 0.168 |
| Yes                                    | 112    | −0.03 (0.99) | 6.04 (3.3) | 0.85   | 85     | 0.07 (1.02) | 2.64 (1.83) | 0.236 | 96     | −0.01 (1.04) | 191.6 (1.27) |        |
| Total                                  | 210    | −0.07 (0.98) | 5.77 (3.3) | 1.53   | 153    | −0.02 (0.99) | 2.51 (1.79) | 0.236 | 179    | −0.11 (1) | 187.5 (1.25) |        |        |
| Total microbleeds                      |        |        |        |        |        |        |        |        |        |        |        |        |
| No                                     | 125    | −0.24 (1.03) | 4.66 (3.5) | 0.063 | 89     | −0.05 (0.96) | 2.45 (1.76) | 0.709 | 105    | −0.23 (0.97) | 182.3 (1.25) | 0.019 |
| Yes                                    | 46     | 0.09 (0.97) | 6.92 (3.2) | 0.35   | 35     | 0.02 (0.98) | 2.56 (1.79) | 0.709 | 40     | 0.20 (1.03) | 201.2 (1.26) |        |        |
| Total                                  | 171    | −0.15 (1.02) | 5.19 (3.4) | 1.24   | 124    | −0.03 (0.96) | 2.48 (1.77) | 0.709 | 145    | −0.11 (1.00) | 187.3 (1.26) |        |        |
| WMMH at baseline*                      |        |        |        |        |        |        |        |        |        |        |        |        |
| Low                                    | 256    | −0.05 (1.03) | 5.86 (3.5) | 0.104 | 170    | −0.03 (1) | 2.48 (1.81) | 0.581 | 208    | −0.08 (0.9) | 188.7 (1.23) | 0.084 |
| High                                   | 234    | 0.1 (0.99) | 7.00 (3.3) | 0.175  | 175    | 0.03 (0.98) | 2.57 (1.79) | 0.581 | 214    | 0.09 (1.1) | 196.1 (1.28) |        |        |
| Total                                  | 490    | 0.02 (1.01) | 6.38 (3.4) | 0.345  | 345    | 0 (0.99) | 2.52 (1.8) | 0.581 | 422    | 0 (1.01) | 192.4 (1.26) |        |        |
| WMMH at follow-up*                     |        |        |        |        |        |        |        |        |        |        |        |        |
| Low                                    | 115    | −0.15 (0.98) | 5.20 (3.3) | 0.189  | 83     | −0.15 (0.98) | 2.32 (1.79) | 0.166 | 98     | −0.31 (0.86) | 179.2 (1.22) | 0.005 |
| High                                   | 111    | 0.02 (0.97) | 6.39 (3.3) | 0.80   | 80     | 0.06 (0.93) | 2.63 (1.74) | 0.166 | 95     | 0.08 (1.05) | 195.9 (1.27) |        |        |
| Total                                  | 226    | −0.07 (0.98) | 5.75 (3.3) | 1.63   | 163    | −0.04 (0.96) | 2.46 (1.77) | 0.166 | 193    | −0.12 (0.98) | 187.3 (1.25) |        |        |
| Infarct during follow-up†              |        |        |        |        |        |        |        |        |        |        |        |        |
| No                                     | 98     | −0.11 (0.95) | 5.48 (3.2) | 0.88   | 68     | −0.12 (0.93) | 2.35 (1.74) | 0.674 | 83     | −0.22 (0.94) | 182.9 (1.24) | 0.995 |
| Yes                                    | 42     | −0.08 (0.89) | 5.66 (2.9) | 0.32   | 32     | −0.04 (0.93) | 2.48 (1.74) | 0.674 | 37     | −0.01 (1.04) | 182.8 (1.28) |        |        |

Continued
Cross-sectional association between inflammatory biomarkers and cerebrovascular disease

In adjusted models, a 1 SD increase in circulating log-transformed CRP level was associated with increased odds of having microbleeds (table 3), and the association remained significant in the fully adjusted model (odds ratio [OR] = 1.80, 95% confidence interval [CI] = 1.07–3.03, \( p = 0.026 \)).

One SD increase in circulating log-transformed IL6 level was associated with increased odds of having infarcts at initial (baseline) scan visit (table 3), and the association remained significant in the fully adjusted model (OR = 1.30, 95% CI = 1.01–1.66, \( p = 0.039 \)).

ACT seemed to be associated with increased odds of infarcts at the follow-up visit (table 3), but the association was no longer significant in the fully adjusted model. An increased ACT level was associated with increased odds of having microbleeds (table 3) and remained so in the fully adjusted model (OR comparing 3rd to 1st tertile = 3.74, 95% CI = 1.15–3.50, \( p = 0.015 \)) larger WMH at follow-up visit (b = 0.101, \( p = 0.044 \)), and greater accumulation of WMH (b = 0.119, \( p = 0.001 \)), all in the fully adjusted models.

Excluding 24 participants who developed dementia during the follow-up, adjusted models showed that higher log\(_{10}\)CRP and log\(_{10}\)ACT remained significantly associated with increased odds of microbleeds, with OR (95% CI) = 1.49 (1.03–2.14), \( p = 0.03 \), and 1.62 (1.08–2.42), \( p = 0.02 \), respectively, and log\(_{10}\)ACT was also associated with WMH burden at follow-up (b = 0.116, \( p = 0.006 \)) and accumulation (b = 0.063, \( p = 0.048 \)).

In the fully adjusted model, higher log\(_{10}\)ACT was associated with larger WMH at the follow-up visit in the frontal (b = 0.114, \( p = 0.017 \)), parietal (b = 0.127, \( p = 0.028 \)), and temporal (b = 0.140, \( p = 0.029 \)) regions and was associated with greater accumulation of WMH during follow-up in the frontal (b = 0.102, \( p = 0.037 \)), parietal (b = 0.117, \( p = 0.052 \)), and temporal (b = 0.133, \( p = 0.047 \)) regions.

One SD increase in log\(_{10}\)CRP level was associated with increased odds of lobar microbleeds (OR = 1.81, 95% CI = 1.12–2.94, \( p = 0.016 \); p-trend = 0.011). ACT was also associated with larger WMH at the follow-up visit (table 3) and remained associated in the fully adjusted model (b = 0.087, \( p = 0.041 \)).

Longitudinal association between inflammatory biomarkers and cerebrovascular disease

A higher ACT level was associated with greater accumulation of WMH during follow-up (table 3). In the fully adjusted model, a 1-SD increase in log\(_{10}\)ACT was associated with 0.083 (\( p = 0.011 \)) increase in log\(_{10}\)WMH during follow-up (i.e., approximately 1.21 times increase in WMH volume). None of the inflammatory biomarkers was associated with risk of incident infarcts (table 3).

Supplementary analyses

When participants with clinically diagnosed stroke were excluded, log\(_{10}\)CRP remained associated with microbleeds (OR = 1.77, 95% CI = 1.08–2.92, \( p = 0.024 \)), log\(_{10}\)IL6 with baseline infarcts (OR = 1.40, 95% CI = 1.05–1.87, \( p = 0.022 \)), and log\(_{10}\)ACT with microbleeds (OR = 2.01, 95% CI = 1.15–3.50, \( p = 0.015 \)), larger WMH at follow-up visit (b = 0.101, \( p = 0.044 \)), and greater accumulation of WMH (b = 0.119, \( p = 0.001 \)), all in the fully adjusted models.

In excluding 24 participants who developed dementia during the follow-up, adjusted models showed that higher log\(_{10}\)CRP and log\(_{10}\)ACT remained significantly associated with increased odds of microbleeds, with OR (95% CI) = 1.49 (1.03–2.14), \( p = 0.03 \), and 1.62 (1.08–2.42), \( p = 0.02 \), respectively, and log\(_{10}\)ACT was also associated with WMH burden at follow-up (b = 0.116, \( p = 0.006 \)) and accumulation (b = 0.063, \( p = 0.048 \)).

In the fully adjusted model, higher log\(_{10}\)ACT was associated with larger WMH at the follow-up visit in the frontal (b = 0.114, \( p = 0.017 \)), parietal (b = 0.127, \( p = 0.028 \)), and temporal (b = 0.140, \( p = 0.029 \)) regions and was associated with greater accumulation of WMH during follow-up in the frontal (b = 0.102, \( p = 0.037 \)), parietal (b = 0.117, \( p = 0.052 \)), and temporal (b = 0.133, \( p = 0.047 \)) regions.

One SD increase in log\(_{10}\)CRP level was associated with increased odds of lobar microbleeds (OR = 1.81, 95% CI = 1.12–2.94, \( p = 0.016 \); p-trend = 0.011). ACT was also associated with larger WMH at the follow-up visit (table 3) and remained associated in the fully adjusted model (b = 0.087, \( p = 0.041 \)).

The associations of inflammatory biomarkers with CVD in general did not vary by ethnic groups, sex, or APOE status. The only exception was that the association between ACT and WMH accumulation during the follow-up (b for interaction between ACT and Hispanic ethnicity = –0.143, \( p = 0.068 \)) was significant for whites (b = 0.124, \( p = 0.042 \)) and African-Americans (b = 0.131, \( p = 0.037 \)), but not for Hispanics (b = –0.018, \( p = 0.669 \)).

Discussion

In this multiethnic, nondemented, elderly population, we found that higher circulating proinflammatory biomarkers were associated with more CVD. Our finding that IL6 was associated with the presence of silent brain infarcts is consistent with some previous studies,\(^ {10,12} \) but not others.\(^ {14,22} \)
Although some studies found CRP to be associated with infarcts \cite{10,12,13}, our study, along with a few other studies \cite{9,14-16,19}, did not replicate this finding. Studies also found that CRP or intercellular adhesion molecule-1 (sICAM-1) levels were associated with incident lacunar infarcts. \cite{9,23} Overall, there is some evidence showing a cross-sectional relationship between inflammatory biomarkers and CVD, but longitudinal evidence is limited. The potential role of inflammatory markers other than CRP or IL6 in CVD is worth further exploration.

A few studies found that higher CRP, \cite{9,10,19} IL6, \cite{10,26} or both \cite{22} were associated with more white matter lesions. Two other studies found that the association between CRP and WMH
### Table 3: Association between inflammatory biomarkers and brain structural MRI measures

| Logistic regression models* | CRP | IL6 | ACT |
|-----------------------------|-----|-----|-----|
|                | OR (95% CI) | p Value | OR (95% CI) | p Value | OR (95% CI) | p Value |
| Infarcts at baseline | | | | | | |
| 1 SD increase | 1.10 (0.89–1.30) | 0.333 | 1.28 (1.02–1.60) | 0.033 | 1.12 (0.91–1.38) | 0.290 |
| Middle tertile | 0.90 (0.56–1.44) | 0.657 | 1.30 (0.74–2.27) | 0.361 | 1.00 (0.61–1.66) | 0.995 |
| Highest tertile | 1.42 (0.90–2.24) | 0.136 | 1.74 (1.00–3.03) | 0.052 | 1.12 (0.68–1.85) | 0.646 |
| p-trend | 0.130 | 0.051 | 0.646 |
| Infarcts at follow-up | | | | | | |
| 1 SD increase | 1.07 (0.80–1.44) | 0.642 | 1.17 (0.83–1.65) | 0.370 | 1.32 (0.96–1.81) | 0.089 |
| Middle tertile | 0.97 (0.50–1.89) | 0.932 | 1.07 (0.49–2.35) | 0.860 | 1.44 (0.68–3.07) | 0.341 |
| Highest tertile | 1.33 (0.65–2.70) | 0.432 | 1.22 (0.53–2.83) | 0.643 | 2.17 (1.03–4.57) | 0.042 |
| p-trend | 0.446 | 0.645 | 0.042 |
| Total microbleeds | | | | | | |
| 1 SD increase | 1.46 (1.02–2.09) | 0.041 | 1.18 (0.76–1.81) | 0.468 | 1.65 (1.11–2.46) | 0.013 |
| Middle tertile | 1.86 (0.81–4.32) | 0.146 | 0.99 (0.37–2.62) | 0.983 | 0.87 (0.29–2.59) | 0.798 |
| Highest tertile | 2.33 (0.93–5.85) | 0.071 | 1.00 (0.30–2.57) | 0.996 | 4.62 (1.78–11.99) | 0.002 |
| p-trend | 0.108 | 0.996 | 0.092 |

| Linear regression models* | CRP | IL6 | ACT |
|-----------------------------|-----|-----|-----|
|                | b | p Value | b | p Value | b | p Value |
| WMH at baseline | | | | | | |
| 1 SD increase | 0.026 | 0.368 | −0.002 | 0.962 | 0.060 | 0.072 |
| Middle tertile | −0.032 | 0.654 | 0.022 | 0.798 | 0.108 | 0.179 |
| Highest tertile | 0.004 | 0.950 | 0.028 | 0.753 | 0.090 | 0.262 |
| p-trend | 0.945 | 0.752 | 0.263 |
| WMH at follow-up | | | | | | |
| 1 SD increase | 0.052 | 0.151 | 0.054 | 0.245 | 0.103 | 0.012 |
| Middle tertile | 0.050 | 0.545 | 0.131 | 0.204 | 0.184 | 0.057 |
| Highest tertile | 0.116 | 0.190 | 0.158 | 0.157 | 0.218 | 0.025 |
| p-trend | 0.191 | 0.150 | 0.022 |
| WMH change† | | | | | | |
| 1 SD increase | 0.002 | 0.959 | 0.041 | 0.248 | 0.062 | 0.041 |
| Middle tertile | −0.031 | 0.639 | 0.086 | 0.282 | 0.081 | 0.260 |
| Highest tertile | 0.028 | 0.697 | 0.053 | 0.538 | 0.148 | 0.041 |
| p-trend | 0.728 | 0.519 | 0.040 |

| Cox proportional hazard models* | CRP | IL6 | ACT |
|-----------------------------|-----|-----|-----|
|                | HR (95% CI) | p Value | HR (95% CI) | p Value | HR (95% CI) | p Value |
| Incident infarcts‡ | | | | | | |
| 1 SD increase | 0.89 (0.64–1.24) | 0.491 | 0.82 (0.49–1.37) | 0.446 | 1.34 (0.98–1.83) | 0.070 |

Continued
was attenuated by the adjustment of cardiovascular or other factors. Our study and several others did not find CRP or IL6 to be associated with the severity of WMH volume. One previous study found that CRP was associated with more progression of white matter lesions. However, other studies did not find such a relationship. We found that ACT, but not CRP or IL6, was associated with WMH severity cross-sectionally at the follow-up MRI scan visit and with progression of WMH longitudinally. Two recent studies found that sICAM-1, but not CRP, was associated with the presence or progression of WMH. Therefore, inflammatory biomarkers other than CRP or IL6 may be relevant for WMH. Interestingly, both ACT and sICAM-1 play a role in cell adhesion and endothelial dysfunction, which may contribute to the pathogenesis of WMH. Similar to our study, other bi-racial studies found that there was no evidence for effect of inflammatory biomarkers on WMH to be different between white and black adults. More studies are needed to examine the effect of inflammatory biomarkers on WMH among Hispanics.

Only a few studies have examined the relationship between circulating inflammatory biomarkers with microbleeds, but the results are inconsistent. Two studies found that CRP was not associated with microbleeds, but a Japanese study found that CRP, IL6, and IL18 were all associated with the presence of microbleeds. The Framingham offspring study found that tumor necrosis factor receptor 2 and myeloperoxidase were associated with increased odds of having microbleeds, especially deep microbleeds. We found that CRP was associated with increased odds of lobar but not deep microbleeds, whereas ACT was associated with increased odds of deep but not lobar microbleeds. The distribution of microbleeds is believed to reflect 2 distinct underlying types of microangiopathy; lobar microbleeds are considered likely to be attributable to cerebral amyloid angiopathy (CAA), whereas deep microbleeds are considered to be due to hypertensive arteriopathy. Therefore, CRP and ACT may represent 2 different pathways toward small hemorrhagic lesions in the brain. This is in line with our previous findings that increased CRP, but not ACT, was associated with smaller gray matter volume, as it has been shown that CAA-related, but not CAA-unrelated, microbleeds are associated with gray matter atrophy. Nevertheless, such differential roles of CRP, ACT, or other inflammatory biomarkers in microbleeds need to be confirmed in future studies.

Growing evidence suggest that inflammatory cytokines are associated with increased risk of developing stroke, cardiovascular disease, and dementia. In addition, our study and other longitudinal studies found inflammatory biomarkers were associated with progression of CVD (particularly WMH), suggesting cytokines, acute-phase proteins, endothelial cell adhesive molecules, and other immune-related proteins may play an active role in building up or contributing to the vascular injuries in the brain. However, current evidence has been inconsistent. The exact reason for this is unknown, but it might be due to differences in age, prevalence of vascular risk factors, ethnicity, MRI acquisition techniques, and analytic strategies. In addition, there are few longitudinal studies and some existing ones that failed to establish this longitudinal relationship. Therefore, an alternative explanation of the results might be that elevated inflammatory biomarkers may be a marker of the inflammation as a result of vascular or other pathologic injury. Generation of cytokines can be upregulated in the brains of patients with stroke and CAA, probably representing an immune response stimulated by the vascular deposits of β-amyloid and other injuries. In addition, increased circulating levels of peripheral proinflammatory cytokines are found in patients with AD compared with controls. Overall, it is possible that both directions exist and create a vicious circle for a progressive accumulation of vascular and neurodegenerative damages, as well as elevated inflammatory responses. Either way, further understanding the role of inflammatory biomarkers in the presence and progression of CVD is important because it may help elucidate the pathogenesis of CVD and allow the development of immune-modulating intervention measures for CVD and related neurologic outcomes or it may help determine how inflammatory markers can be used to monitor disease progression.

Table 3 Association between inflammatory biomarkers and brain structural MRI measures (continued)

| Cox proportional hazard models* | CRP | IL6 | ACT |
|--------------------------------|-----|-----|-----|
|                                | HR (95% CI) | p Value | HR (95% CI) | p Value | HR (95% CI) | p Value |
| **Middle tertile**             |     |     |     |
| Highest tertile                | 0.92 (0.43–2.00) | 0.838 | 0.72 (0.30–1.76) | 0.473 | 1.84 (0.76–4.42) | 0.176 |
| **p-trend**                    |     |     |     |
|                                | 0.222 |     | 0.400 |     | 0.071 |     |

Abbreviations: ACT = alpha 1-antichymotrypsin; CI = confidence interval; CRP = C-reactive protein; ICV = intracranial volume; IL6 = interleukin-6; OR = odds ratio; WMH = white matter hyperintensity.
* All models adjusted for age at time of scan, sex, ethnicity/race, APOE status, and, for WMH only, also ICV.
† Additionally adjusted for duration between scans.
‡ Limit to 158 participants without infarcts at baseline only. Bold values indicate statistical significant difference with p < 0.05.
Although we adjusted for several key factors, we cannot completely exclude the possibility that our results are subject to residual confounding due to unmeasured factors. We only examined 3 of the many inflammatory markers in our study, whereas the role of many other inflammatory markers is also worthy of examination. We only measured the inflammatory markers once and they may not represent the long-term average level of the subject. Although WMH overall is strongly associated with vascular factors and viewed as a reflection of small vessel CVD, the pathogenesis of WMHs is not fully understood and could be multifactorial. Thus, another limitation of our study is that we did not subclassify WMHs by their putative etiologies. Our study might be underpowered to examine interactions, stratified analyses in subgroups, and the longitudinal relationship. We did not correct for multiple comparison as we consider our study to be exploratory in nature and need replication in an external sample.

Our study has many strengths. We added new evidence to this line of research by including several key inflammatory markers, both cross-sectional and longitudinal analyses, an elderly population with advanced age, and different cerebrovascular features. In addition, our study population comprised multiple ethnic groups, making the results more generalizable to the increasingly diverse US population. Finally, several key potential confounding factors were adjusted in the analyses.

Among the old participants of our study, higher levels of peripheral CRP and IL6 were associated with increased odds of microbleeds and infarcts, respectively, and increased ACT was associated with cerebrovascular events including increased odds of infarcts and microbleeds and larger burden and more accumulation of WMH. More studies, especially longitudinal studies with a wide range of inflammatory biomarkers, are needed to clarify whether inflammatory biomarkers play an etiologic role in CVD or represent an epiphenomenon of CVD.

Author contributions
Y. Gu: design and conceptualized the study; analysis and interpretation; and drafted the manuscript for intellectual content. J. Gutierrez-Contreras, I.B. Meier, V.A. Guzman, J.J. Manly, and N. Schupf: acquisition of data; interpretation; and revised the manuscript for intellectual content. R. Mayeux: design and conceptualized the study; acquisition of data; study supervision; and revised the manuscript for intellectual content.

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