Inflammatory biomarkers, cerebral microbleeds, and small vessel disease

Framingham Heart Study

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ABSTRACT

Objective: We investigated the association between circulating biomarkers of inflammation and MRI markers of small vessel disease.

Methods: We performed a cross-sectional study relating a panel of 15 biomarkers, representing systemic inflammation (high-sensitivity C-reactive protein, interleukin-6, monocyte chemotactic protein-1, tumor necrosis factor α, tumor necrosis factor receptor 2, osteoprotegerin, and fibrinogen), vascular inflammation (intercellular adhesion molecule 1, CD40 ligand, P-selectin, lipoprotein-associated phospholipase A_2 mass and activity, total homocysteine, and vascular endothelial growth factor), and oxidative stress (myeloperoxidase) to ischemic (white matter hyperintensities/silent cerebral infarcts) and hemorrhagic (cerebral microbleeds) markers of cerebral small vessel disease (CSVD) on MRI in 1,763 stroke-free Framingham offspring (mean age 60.2 ± 9.1 years, 53.7% women).

Results: We observed higher levels of circulating tumor necrosis factor receptor 2 and myeloperoxidase in the presence of cerebral microbleed (odds ratio [OR] 2.2, 95% confidence interval [CI] 1.1–4.1 and OR 1.5, 95% CI 1.1–2.0, respectively), higher levels of osteoprotegerin (OR 1.1, 95% CI 1.0–1.2), intercellular adhesion molecule 1 (OR 1.7, 95% CI 1.1–2.5), and lipoprotein-associated phospholipase A_2 mass (OR 1.5, 95% CI 1.1–2.1), and lower myeloperoxidase (OR 0.8, 95% CI 0.7–1.0) in participants with greater white matter hyperintensity volumes and silent cerebral infarcts.

Conclusions: Our study supports a possible role for inflammation in the pathogenesis of CSVD, but suggests that differing inflammatory pathways may underlie ischemic and hemorrhagic subtypes. If validated in other samples, these biomarkers may improve stroke risk prognostication and point to novel therapeutic targets to combat CSVD. *Neurology*® 2015;84:825–832

GLOSSARY

CAA = cerebral amyloid angiopathy; **CI** = confidence interval; **CMB** = cerebral microbleed; **CSVD** = cerebral small vessel disease; **ICAM-1** = intercellular adhesion molecule 1; **ln** = natural logarithm; **Lp-PLA$_2$** = lipoprotein-associated phospholipase A_2; **OR** = odds ratio; **ROC** = receiver operating characteristic; **SCI** = silent cerebral infarct; **TNF-α** = tumor necrosis factor α; **TNFR2** = tumor necrosis factor receptor 2; **WMH** = white matter hyperintensity.

Cerebral small vessel disease (CSVD) assessed using brain MRI is characterized by 3 main findings that include cerebral microbleeds (CMBs), lacunes of presumed vascular origin (silent cerebral infarcts [SCIs]), and white matter hyperintensity (WMH). The vascular changes underlying ischemic and hemorrhagic CSVD likely include activation of the inflammatory cascade with endothelial failure and resulting neurovascular unit dysfunction.[1–3] However, the specific mediators implicated in ischemic and hemorrhagic CSVD may differ.

The Framingham Offspring Cohort provides a large, middle-age, community-based sample in which to investigate the association between systemic biomarkers of inflammation and MRI markers of CSVD. Given the complexity of inflammatory pathways, it is likely that several molecules within the inflammatory cascade are involved. Furthermore, the specific biomarkers that are associated with ischemic and hemorrhagic CSVD is unclear, and such knowledge could lead to more precise risk prediction and identification of novel preventive and therapeutic targets.

Supplemental data at Neurology.org

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Accordingly, we aimed to assess the association of a comprehensive panel of systemic inflammatory markers with MRI measures of CSVD.

METHODS

Sample. The Framingham Offspring Cohort was enrolled in 1971, and participants have been examined every 4 to 8 years since.[4] In 1999, participants underwent brain MRI, and sequences allowing for CMB detection were added in the year 2000. Among offspring participants attending examination cycle 7 (1998–2001; n = 3,539 participants), a comprehensive list of inflammatory biomarkers was measured as part of the broader aim of investigating biomarkers of cardiovascular disease. In the present analysis, we excluded participants who did not have biomarker data (n = 209), did not undergo MRI (n = 1,021), did not have MRI data for the imaging markers of interest (n = 468, all missing CMB data because they had MRI without gradient echo sequences), or had prevalent TIA/stroke or other neurologic disease (severe head injury, multiple sclerosis, brain tumors, hemicraniectomy, etc.) that could affect the estimation of the MRI measures (n = 78).

Standard protocol approvals, registrations, and patient consents. The institutional review board of Boston University Medical Center approved the study protocol and we obtained informed consent from all subjects.

Clinical characteristics. Clinical and demographic characteristics were measured at examination cycle 7. Hypertension was defined using JNC-7 (Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure) criteria as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medications. We defined prevalent diabetes mellitus as a fasting blood glucose ≥126 mg/dL or use of oral hypoglycemic agents/insulin and current smoking as self-reported smoking of at least one cigarette per day within the year preceding examination. We ascertained medication use by self-report. Nonstroke cardiovascular disease was defined as coronary heart disease, peripheral arterial disease, and/or heart failure. Some participants lacked data on certain variables of interest (e-Methods on the *Neurology*® Web site at Neurology.org).

Biomarkers. We investigated a set of 15 biomarkers representing various components of the inflammatory cascade, including systemic inflammation (high-sensitivity C-reactive protein, interleukin-6, monocyte chemotactic protein-1, tumor necrosis factor α [TNF-α], tumor necrosis factor receptor 2 [TNFR2], osteoprotegerin, and fibrinogen), vascular inflammation/endothelial dysfunction (intercellular adhesion molecule 1 [ICAM-1], CD40 ligand, P-selectin, lipoprotein-associated phospholipase A_2 [Lp-PLA_2] mass and activity, total homocysteine, and vascular endothelial growth factor), and oxidative stress (myeloperoxidase).

Fasting morning samples were collected and plasma and serum aliquots were stored at −70°C. Several biomarkers were measured with commercially available ELISA kits from R&D Systems (Minneapolis, MN) (ICAM-1, interleukin-6, monocyte chemotactic protein-1, P-selectin, TNFR2, TNFα, vascular endothelial growth factor), Bender MedSystems (Vienna, Austria) (CD40 ligand), and Oxis (Beverly Hills, CA) (myeloperoxidase). C-reactive protein was measured using high sensitivity assay (BN100 nephelometer; Dade Behring, Deerfield, IL), and fibrinogen by the Clauss method (Diagnostica Stago Inc., Parsippany, NJ). Lp-PLA_2 activity was measured using a colorimetric activity method (diaDexus Inc., San Francisco, CA). Lp-PLA_2 mass was measured using a commercially available sandwich enzyme immunoassay (diaDexus Inc.). Total homocysteine was measured with high-performance liquid chromatography with fluorometric detection.

Intra-assay coefficients of variation (all <10% as previously reported)[5,6] are listed in e-Methods.

Outcomes: Brain MRI markers of CSVD. The MRI markers of CSVD of interest were CMBs, SCIs, and WMH. MRI acquisition, measurement techniques, and interrater reliability have been described previously.[3,7] Operators blinded to participants' demographic, clinical, and biomarker data rated the images of interest. We determined the volume of WMH according to previously published methods,[7] and defined extensive WMH where the natural log of the ratio of WMH volume to total cranial volume was >1 SD above the age-adjusted mean WMH volume.[8] We manually determined SCIs on the basis of their size (>3 mm, <15 mm), location, and imaging characteristics, as previously described.[8] MRI markers of CSVD were divided into hemorrhagic (CMBs) and ischemic (SCIs and/or extensive WMH). We dichotomized the presence or absence of ischemic CSVD as MRI evidence of SCIs, extensive WMH, or both. We graded CMBs as per previously described methods.[9] In addition, we performed secondary analysis on CMB burden and topography. CMB burden was assessed to minimize the potential of confounding from CMB mimics in cases with only one lesion, and to explore the potential additive role of increasing disease severity. Accordingly, CMB burden within individuals was graded as absent, present (≥1 CMBs), ≥2 CMBs, or ≥3 CMBs. These thresholds were influenced by the distribution of CMB number in our sample. CMB topography was divided into lobar only (confined to cortex and subcortical white matter), deep only (internal capsule/external capsule, thalamus, basal ganglia, brainstem), or any deep (deep only or concurrent lobar and deep location, including cerebellum).

Statistical analyses. We obtained descriptive statistics for the clinical, demographic, and biomarker variables. We performed natural logarithmic (ln) transformation on inflammatory biomarkers that had a skewed distribution, as well as on WMH volume to total cranial volume ratios to avoid undue influence of extreme observations. We used logistic regression to obtain odds ratios and 95% confidence intervals for the association of each biomarker and each outcome (CMBs, SCIs/extensive WMH). For both primary outcomes, our primary model was adjusted for age, sex, and time interval between examination 7 and MRI acquisition. Sensitivity analysis confined to participants without any missing biomarker data was also performed. In additional secondary models, we chose clinical covariates previously associated with the neuroimaging markers of interest within the Framingham Offspring Cohort.[10,11] For the CMB outcome, model 2a was adjusted for our primary model covariates plus hypertension, statin use, and antithrombotic use, and model 3a was adjusted for our primary model covariates plus MRI markers of CSVD (SCIs and ln WMH volume). For the SCIs and extensive WMH outcome, model 2b was adjusted for the primary model covariates plus hypertension, diabetes mellitus, current smoking, and history of cardiovascular disease, and model 3b was adjusted for primary model covariates plus the presence of any CMB (yes vs no). Receiver operating characteristic (ROC) curves were constructed for the notable associations within our primary analysis to determine whether the grouping of biomarkers was more predictive than individual biomarkers alone. All analyses were performed using SAS version 9.2 (Cary, NC). A p value <0.05 (uncorrected) was considered statistically significant.

The threshold for statistical significance after Bonferroni correction for multiple comparisons (primary analysis: 15 biomarkers × 2 outcomes [1 = all CMBs, 2 = composite of SCI and/or extensive WMH volume]) was set at a p value <0.0017.

RESULTS Of the 3,539 Framingham offspring participants attending examination cycle 7, 1,763 participants met our eligibility criteria for analysis within the current study. Baseline characteristics of the sample are shown in table 1. Baseline characteristics of participants excluded are presented in table e-1. Results of our primary analyses are presented in tables 2 and 3, e-2, and e-3.

Cerebral microbleeds. CMBs were present in 8% (n = 141) of the sample. In our primary analysis, we

Table 1 Baseline characteristics of the study sample (n = 1,763)

Clinical characteristics (continuous), mean (SD)		
Age, y		60.2 (9.1)
Time between biomarker measurement and MRI, y		5.4 (2.7)
Clinical characteristics (categorical), n (%)		
Women		946 (53.7)
Hypertension		679 (38.5)
Current smoking		195 (11.1)
Diabetes mellitus		173 (10.0)
History of nonneurologic cardiovascular disease[a]		151 (8.6)
Statin use		288 (16.3)
Antithrombotic use		576 (32.7)
Hypertension treatment		488 (27.7)
MRI markers of CSVD, n (%)		
Any cerebral microbleed[b]		141 (8.0)
Silent cerebral infarcts		222 (12.6)
Extensive white matter hyperintensity		370 (21.0)
Biomarkers, median (25th, 75th percentile)		**Ln scale**
Interleukin-6, pg/mL	2.5 (1.7, 4.0)	—
C-reactive protein, mg/L	1.9 (0.9, 4.5)	0.64 (−0.08, 1.50)
Tumor necrosis factor α, pg/mL	1.2 (0.9, 1.6)	0.17 (−0.09, 0.44)
Tumor necrosis factor receptor 2, pg/mL	1,931 (1,639, 2,310)	7.57 (7.40, 7.75)
Fibrinogen, mg/dL	366 (324, 414)	—
Osteoprotegerin, pmol/L	5.2 (4.3, 6.2)	—
Monocyte chemotactic protein-1, pg/mL	308 (252, 379)	5.73 (5.63, 5.94)
CD40 ligand, ng/mL	1.3 (0.5, 4.0)	0.28 (−0.61, 1.39)
Intercellular adhesion molecule 1, ng/mL	238 (209, 277)	5.47 (5.34, 5.62)
P-selectin, ng/mL	35.9 (28.2, 44.5)	3.58 (3.34, 3.79)
Total homocysteine, μmol/L	7.7 (6.5, 9.5)	—
Lp-PLA_2 activity	141 (118, 166)	4.95 (4.77, 5.11)
Lp-PLA_2 mass	283 (228, 353)	5.65 (5.43, 5.87)
Vascular endothelial growth factor, ng/mL	278 (159, 427)	—
Myeloperoxidase, ng/mL	39.8 (27.8, 59.2)	3.68 (3.32, 4.08)

Abbreviations: CSVD = cerebral small vessel disease; Lp-PLA_2 = lipoprotein-associated phospholipase A_2.
Missing data: All of the variables of interest were not available in all participants. Thirty-seven were missing data on their diabetes mellitus status, 5 interleukin-6, 6 C-reactive protein, 450 tumor necrosis factor α, 54 tumor necrosis factor receptor 2, 8 fibrinogen, 6 osteoprotegerin, 30 monocyte chemotactic protein-1, 18 CD40 ligand, 8 intercellular adhesion molecule 1, 5 P-selectin, 7 total homocysteine, 8 lipoprotein-associated phospholipase A_2 activity, 8 lipoprotein-associated phospholipase A_2 mass, 168 vascular endothelial growth factor, and 63 myeloperoxidase.
[a] Defined as history of peripheral artery disease, coronary heart disease, and/or heart failure.
[b] Eighty-eight participants had only lobar cerebral microbleeds (CMBs), 33 participants had only deep CMBs, 20 participants had CMB of mixed type (deep + lobar).

Table 2 Logistic regression results for the noteworthy association of biomarkers and prevalent CMB (n = 1,763)[a]

		Prevalent CMB (any vs none)											
		All CMB[c] (n = 141)			Lobar only (n = 88)			Deep only (n = 33)			Any deep (deep + mixed) (n = 53)		
Biomarker	Model[b]	OR[a]	95% CI	*p* Value	OR[a]	95% CI	*p* Value	OR[a]	95% CI	*p* Value	OR[a]	95% CI	*p* Value
Ln TNF-α	1	1.3	0.9–2.0	0.16	1.1	0.7–1.9	0.60	1.9	0.9–4.0	0.08	1.7	1.0–3.1	0.07
Ln TNFR2	1	2.2	1.1–4.1	0.02	2.0	0.9–4.5	0.08	3.9	1.2–12.4	0.02	2.7	1.0–7.3	0.05
Ln myeloperoxidase	1	1.5	1.1–2.0	0.02	1.4	0.9–2.0	0.13	1.4	0.8–2.7	0.26	1.7	1.0–2.8	0.04

Abbreviations: CI = confidence interval; CMB = cerebral microbleed; ln = natural logarithm; OR = odds ratio; TNF-α = tumor necrosis factor α; TNFR2 = tumor necrosis factor receptor 2.
[a] ORs for biomarkers designated as ln correspond to 1-unit increase on the ln scale.
[b] Model 1 is adjusted for age at examination 7, sex, and time between examination 7 and MRI.
[c] Primary analysis.

observed higher levels of circulating TNFR2 and myeloperoxidase in participants with CMBs (table 2). Secondary analysis revealed these associations to be most prominent in persons with only deep CMBs in the case of TNFR2 and any deep CMB in the case of myeloperoxidase. However, similar trends persisted in lobar locations. Grouping of biomarkers was not significantly more predictive of CMB in comparison to the individual biomarkers alone (area under the ROC curve: 0.76 for the composite of TNF-α, TNFR2, and myeloperoxidase vs 0.74 for TNF-α, $p = 0.73$; 0.74 for TNFR2, $p = 0.69$; and 0.75 for myeloperoxidase, $p = 0.78$).

The relation of some of the biomarkers with CMBs increased with greater CMB burden (table 4). Ln TNFR2 related to a 3.3-fold increase in odds of having ≥2 CMBs and 5.7-fold increase in odds of having ≥3 CMBs, each as compared with having no CMBs. In the case of myeloperoxidase, each additional unit increase (ln scale) resulted in a 1.9-fold increase in the odds of ≥2 CMBs and a trend toward a 1.6-fold increase in the odds of having ≥3 CMBs. In addition, a trend existed between higher levels of circulating Lp-PLA_2 mass and increasing levels of CMB burden.

Additional adjustment for clinical covariate and neuroimaging markers of ischemic CSVD did not considerably alter the above findings. There were no noteworthy observations to report with the other biomarkers (table e-2).

SCIs and large WMH volume. SCIs and/or extensive WMH volume occurred in 30% (n = 522) of the sample. Associations of biomarkers with ischemic CSVD defined as presence of SCIs and/or extensive WMH are listed in table 3. We observed higher levels of circulating osteoprotegerin, ICAM-1, and Lp-PLA_2 mass, and lower levels of circulating myeloperoxidase in persons with ischemic CSVD. Grouping of biomarkers was not significantly more predictive of SCIs and/or extensive WMH volume in comparison to the individual biomarkers alone (area under the ROC curve: 0.61 for the composite of osteoprotegerin, ICAM-1, Lp-PLA_2 mass, and myeloperoxidase vs 0.58 for osteoprotegerin, $p = 0.20$; 0.58 for ICAM-1, $p = 0.19$; 0.58 for Lp-PLA_2 mass, $p = 0.24$; and 0.58 for myeloperoxidase, $p = 0.15$).

Additional adjustment for clinical covariates and presence of CMBs did not considerably alter the above findings. There were no noteworthy observations to report with the other biomarkers (table e-3).

Only the correlation between higher levels of circulating osteoprotegerin and ischemic markers of CSVD withstood Bonferroni correction for multiple comparisons. Sensitivity analysis confined to participants without any missing biomarker data did not substantially alter our findings.

Table 3 Logistic regression results for the noteworthy association of biomarkers and prevalent silent cerebral infarcts and/or extensive white matter hyperintensities

		Prevalent silent cerebral infarcts and/or extensive white matter hyperintensities (n = 522)		
Biomarker	Model[a]	OR[b]	95% CI	*p* Value
Osteoprotegerin, pmol/L	1	1.1	1.0–1.2	0.0009
Ln ICAM-1	1	1.7	1.1–2.5	0.02
Ln Lp-PLA_2 mass	1	1.5	1.1–2.1	0.01
Ln myeloperoxidase	1	0.8	0.7–1.0	0.06

Abbreviations: CI = confidence interval; ICAM-1 = intercellular adhesion molecule 1; ln = natural logarithm; Lp-PLA_2 = lipoprotein-associated phospholipase A_2; OR = odds ratio.
[a] Model 1 is adjusted for age at examination 7, sex, and time between examination 7 and MRI.
[b] ORs for biomarkers designated as ln correspond to 1-unit increase on ln scale.

DISCUSSION Our results suggest higher levels of various circulating markers of inflammation in persons with MRI markers of CSVD and may support the hypothesis that endothelial failure contributes to the pathogenesis of CSVD.

Table 4 Logistic regression results for the noteworthy association of biomarkers and CMB burden

		CMB burden								
		≥1 CMB vs 0 CMBs (same as all CMB in table 2) (n = 141)			≥2 CMBs vs 0 CMBs (n = 48)			≥3 CMBs vs 0 CMBs (n = 26)		
Biomarker	Model[a]	OR[b]	95% CI	*p* Value	OR[b]	95% CI	*p* Value	OR[b]	95% CI	*p* Value
Ln TNFR2	1	2.2	1.1–4.1	0.02	3.3	1.2–9.3	0.03	5.7	1.48–21.7	0.01
Ln myeloperoxidase	1	1.5	1.1–2.0	0.02	1.9	1.1–3.3	0.01	1.6	0.8–3.1	0.20
Ln Lp-PLA_2 mass	1	1.2	0.7–2.1	0.62	1.6	0.6–4.2	0.34	3.1	0.8–11.7	0.09

Abbreviations: CI = confidence interval; CMB = cerebral microbleed; ln = natural logarithm; Lp-PLA_2 = lipoprotein-associated phospholipase A_2; OR = odds ratio; TNFR2 = tumor necrosis factor receptor 2.
[a] Model 1 is adjusted for age at examination 7, sex, and time between examination 7 and MRI.
[b] ORs for biomarkers designated as ln correspond to 1-unit increase on ln scale.

The strongest association occurred between TNF-α and CMBs. TNF-α is a key regulatory cytokine that is predominantly secreted by macrophages/microglia (the predominant cell type found underlying CMBs in pathologic/autopsy samples)[12] and can exert diverse regulatory functions through 2 cell membrane receptors, TNFR1 and TNFR2.[13] CMBs are believed to result from a state of increased vascular fragility/permeability and represent hemosiderin-laden macrophages. In addition, they are often associated with a surrounding degree of tissue necrosis.[12] Accordingly, their association with TNFR2 has several plausible explanations. TNF-α has been consistently observed to increase vascular permeability and blood-brain barrier dysfunction in animal models.[14] Both TNFR1 and TNFR2 seem to be involved in this process.[15] Hence, higher levels of TNFR2 may promote the pathogenesis of CMBs. Alternatively, CMB formation could be driving TNF activity through CMB-induced microglial and inflammatory cascade activation. TNFR2 expression may also be increased as a neuroprotective measure in the face of CMB-induced neuronal damage.[16]

Myeloperoxidase is an inflammatory oxidizing enzyme expressed predominantly by neutrophils in the first 3 days after neuronal injury, followed by macrophages/microglia, which peak within 1 week postinjury.[17] Activated leukocytes have been observed to secrete 10 times more myeloperoxidase than their nonactivated counterparts.[17] Myeloperoxidase activity results in the generation of hypochlorous acid, a strong oxidant that can cause local tissue damage and amplify the inflammatory cascade. Myeloperoxidase also has been associated with endothelial and blood-brain barrier dysfunction.[18] Accordingly, similarly to TNF, myeloperoxidase can either be a cause or effect of CMBs through myeloperoxidase-induced endothelial/blood-brain barrier dysfunction or CMB-induced microglial activation, respectively. Furthermore, myeloperoxidase also may be an active culprit in CMB-induced neuronal injury.

The observation that higher levels of TNFR2 and myeloperoxidase were most pronounced in deep or mixed CMB cases, thought to be consequent to hypertensive arteriopathy (arteriolosclerosis), in comparison to lobar CMBs, suggestive of cerebral amyloid angiopathy (CAA), possibly implies the presence of different inflammatory signaling cascades in association with CMBs in these 2 prevalent underlying microangiopathies. Previous radiographic-pathologic concordance studies have not investigated such differences in persons with CMBs,[12] and relevant in vivo circulating biomarker data in patients with CAA are lacking. However, pathologic and experimental studies have noted activated macrophages/microglia, reactive astrocytes, and T lymphocytes adjacent to cerebral vascular amyloid deposits, with a broad inflammatory response including complement, reactive oxygen species, and proinflammatory cytokines, such as interleukin-6, CD40L, and TNF-α.[19–22] Although we also observed a trend for elevated TNFR2 levels in persons with purely lobar CMBs, we did not observe an association with these other markers present in our panel. Consequently, alternatively, the aforementioned discrepancy between deep/mixed and lobar CMBs may be attributed to the systemic nature of hypertensive arteriopathy, which would result in a greater and more detectable change in circulating inflammatory biomarkers than CAA, which is confined to the cerebrum. To our knowledge, no other studies have previously investigated serum TNF and myeloperoxidase levels in patients with CMBs.

Lp-PLA_2, an enzyme secreted by circulating macrophages that hydrolyzes oxidized phospholipids, is involved in inflammation and the metabolism of low-density lipoprotein. It is reported to be atherogenic, and elevated levels previously have been observed in individuals with myocardial infarction

and ischemic stroke.[23] It has also been associated with MRI markers of CSVD.[3,24] Our findings further corroborate these observations. Although in keeping with a previous report,[3] we did not note an association between Lp-PLA_2 and any CMBs in our primary analysis, secondary exploratory analysis assessing CMB burden demonstrated higher levels of circulating Lp-PLA_2 in individuals with greater numbers of CMBs. Furthermore, we noted higher levels of circulating Lp-PLA_2 in persons with ischemic markers of CSVD. The particular association of Lp-PLA_2 with both ischemic and hemorrhagic markers of CSVD may be explained by its potential role in various forms of vascular pathogenesis, including atherogenesis and endothelial dysfunction.[24]

Osteoprotegerin is a cytokine secreted by vascular endothelial and smooth muscle cells and regulates processes involved in vascular injury and inflammation. There exists a strong association between osteoprotegerin and large vessel atherosclerosis, but there are conflicting reports as to whether the association is causal or protective.[25] Although higher osteoprotegerin levels have been previously reported in persons with lower total brain volume and stroke secondary to atherosclerotic large artery disease,[5,26] our findings are the first, to our knowledge, to suggest a relationship between osteoprotegerin and direct neuroimaging markers of CSVD. Alternatively, because we did not account for cervical or intracranial large artery disease within our study, our observations may be secondary to small vessel orifice atheroma or possibly chronic hypoperfusion from proximal large artery stenosis.

ICAM-1 is an adhesion molecule that facilitates the adhesion of leukocytes to the endothelium and microvessel occlusion, as well as their transendothelial migration. Elevated levels, indicating endothelial cell activation, have been reported as risk factors for the presence and progression of SCIs, WMH, and lacunar stroke.[27] However, a recent neuropathologic study did not find any relation between CSVD and ICAM-1, or any suggestion of endothelial activation.[28] The authors concluded that previously observed elevated levels of ICAM-1 in the setting of CSVD originated from the peripheral vasculature. It is uncertain whether their postmortem tissue analysis is reflective of the in vivo antemortem state. Nevertheless, their report stresses the need for exercising caution when drawing firm conclusions from cross-sectional biomarker studies.

The mechanism underlying the lower levels of circulating myeloperoxidase observed in persons with ischemic markers of CSVD, in contrast to higher levels in those with CMBs, is uncertain. If validated, in keeping with studies demonstrating more pronounced rotenone-induced neuronal injury in myeloperoxidase-deficient mice,[29] and worsening cognitive decline in human participants with the myeloperoxidase AA genotype (leading to lower myeloperoxidase levels),[30] myeloperoxidase deficiency may promote ischemic neuronal injury. An alternative explanation is that chronically progressing large volumes of ischemic neuronal and glial injury, and the ensuing inflammatory response, promotes microglial senescence (the dysfunction and dystrophy of microglia with advancing age).[31]

It is of interest that, irrespective of myeloperoxidase, we observed different inflammatory biomarker profiles between hemorrhagic and ischemic MRI markers of CSVD. Although this observation requires replication to ensure validity, if validated, it lends support to the involvement of different inflammatory pathways in the pathogenesis of ischemic vs hemorrhagic MRI markers or possibly the manner by which the 2 pathologies elicit cerebral tissue injury and activate the inflammatory cascade.

Our findings have a number of limitations. Our cross-sectional analysis does not allow for causal inferences, and we cannot exclude the possibility of residual confounding. Our secondary analysis relating markers to CMB burden was limited by small sample size. The predominant European descent of Framingham Heart Study participants limits generalization to other ethnic groups. We analyzed circulating biomarkers at only one point in time and we have not accounted for concurrent infection, rheumatologic disease, or malignancy that could have altered our results. It is uncertain whether systemic circulating biomarkers are adequate proxy measures of cerebrovascular inflammation and endothelial dysfunction. Although the accumulation of MRI markers of CSVD is considered an ongoing chronic process, they have been observed to occur rapidly within acute/subacute time periods[32–34]; accordingly, the time interval between serology and acquisition of MRI also limits our findings, despite our attempts to account for it as a covariate. Most notably, because all but one of our associations did not withstand Bonferroni correction, our findings could have occurred merely by chance, should be considered exploratory in nature, and require replication in an external sample to ensure validity. It is reassuring, however, that the association of some of these markers, such as TNFR2, withstood several models and analyses.

Our study further supports the view that inflammatory cytokines may be involved in the pathogenesis of CSVD and suggests the possibility of differing inflammatory pathways between ischemic and hemorrhagic manifestations of CSVD. Further research is required to ensure the validity of our findings and to determine whether any of these molecules might serve as prognostic markers or therapeutic targets to combat CSVD and related neuronal injury.

AUTHOR CONTRIBUTIONS

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

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DISCLOSURE

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