Midlife systemic inflammatory markers are associated with late-life brain volume: The ARIC study

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ABSTRACT

Objective: To clarify the temporal relationship between systemic inflammation and neurodegeneration, we examined whether a higher level of circulating inflammatory markers during midlife was associated with smaller brain volumes in late-life using a large biracial prospective cohort study.

Methods: Plasma levels of systemic inflammatory markers (fibrinogen, albumin, white blood cell count, von Willebrand factor, and Factor VIII) were assessed at baseline in 1,617~~633~~ participants (mean age ~~53~~52(8) years, ~~60~~61% female, ~~27~~26% African American) enrolled in the Atherosclerosis Risk in Communities Study (ARIC). Using all five inflammatory markers, an inflammation composite score was created for each participant. We assessed episodic memory and regional brain volumes, using 3 Tesla MRI, 24 years later.

Results: Each standard deviation increase in midlife inflammation composite score was associated with ~~1,788 mm^3 greater ventricular (p=.013), 110~~ 42 mm^3 smaller hippocampal (p=.~~013~~08), ~~519~~ 204 mm^3 smaller occipital (p=.~~009~~07), and ~~532~~ 197 mm^3 smaller Alzheimer's disease Signature Region (p=.~~008~~010) volumes ~~, and reduced episodic memory (p=.046)~~ 24 years later. Compared to participants with no elevated (4th quartile) midlife inflammatory markers, participants with elevations in three or more markers had significantly smaller ~~, on average, 5% smaller hippocampal and~~ Alzheimer's disease Signature Region (-751mm^3; p=.038) and hippocampal (-148mm^3; p=.038) volumes, and reduced episodic memory (p=0.049). The association between midlife inflammation and late-life brain volume was modified by age ~~and race~~, whereby younger participants ~~and white participants~~ with higher levels of systemic inflammation during midlife ~~were more likely to show~~demonstrated significantly reduced late-life brain volumes subsequently.

Conclusions: Our prospective findings provide evidence for what may be an early contributory role of systemic inflammation in neurodegeneration and cognitive aging.

INTRODUCTION

Although elevated levels of inflammatory markers have been found in the blood[1], CSF[2], and brain parenchyma[3] of individuals with cognitive impairment and Alzheimer's disease, it remains unclear whether this heightened inflammatory state is driving neurodegenerative changes. If low-grade systemic inflammation does play a causal role in Alzheimer's and other neurodegenerative diseases, a heightened inflammatory response during midlife would be expected to increase one's risk for pathological brain changes much later. Although cross-sectional studies have demonstrated a link between elevated inflammatory markers and reduced brain volume in older adults[4–7], it remains unclear whether systemic inflammation during midlife, before the onset of significant age- and disease-related neurological changes, is associated with brain volume loss later in life.

The goal of the current study was to examine how midlife plasma markers of inflammation relate to late-life brain volume among a biracial community sample of older adults. To this end, we examined the relationship between five markers of systemic inflammation measured during midlife and MRI measures of regional brain volume 24 years later in the Atherosclerosis Risk in Communities (ARIC) Study cohort. We tested the hypothesis that greater midlife systemic inflammation is associated with smaller brain volumes in regions most susceptible to Alzheimer's-related atrophy and reduced episodic memory in older adulthood. Based on cross-sectional evidence suggesting that race, sex, and age may modify the association between inflammatory markers and brain volume[5,8,9], the current study also examined the modifying effects of each of these demographic characteristics.

METHODS

Study population

The ARIC study, an ongoing community-based prospective study, enrolled 15,792 middle-aged adults (45-65 years of age at baseline)[10]. Participants were selected by probability sampling in four U.S. communities: Washington County, MD; Forsyth County, NC; northwestern suburbs of Minneapolis, MN; and Jackson, MS. Following the baseline visit in 1987-89 (Visit 1), participants were seen at three more visits, approximately three years apart until 1996-1998 (Visit 4), and at a fifth visit in 2011-2013 (Visit 5).

At Visit 5, a subset of 1,978 participants was selected to undergo brain MRI scans[11]. Participants were selected to undergo a brain MRI based on previous participation in the ARIC Brain MRI Ancillary Study and standard safety exclusion criteria. Additionally, all participants with evidence of cognitive impairment at Visit 5 and an age-stratified random sample of participants without evidence of cognitive impairment were recruited. The participation rate among eligible individuals selected to undergo brain MRI was approximately 81%. A detailed description of the MRI sampling strategy is provided in the supplemental methods. We excluded participants with missing or poor ~~imaging~~ quality neuroimaging (n=20~~6~~), neurological disease (i.e., stroke, multiple sclerosis) (n=80), missing inflammatory biomarker data (n=38), missing covariates (n=~~215~~210), and race other than white or African American (n=~~6~~5) or African American race at a primarily white study site (n=8). Participants who met criteria for dementia or had unknown cognitive status (5%, n=~~83~~81) ~~-~~were excluded from the primary analyses.

Standard protocol approvals, registrations, and patient consents.

The ARIC study protocol has been approved by the Institutional Review Boards at each participating center. All participants gave written informed consent at each study visit.

Inflammatory markers

Plasma levels of four acute-phase reactants – fibrinogen, albumin, von Willebrand factor (VWF), and Factor VIII (FVIII) – and white blood cell (WBC) count were used to measure systemic inflammation[12]. Using standard protocols, study technicians drew fasting blood, centrifuged samples, and froze plasma blood samples at -70°C until the samples were analyzed[13]. Fibrinogen (mg/dL), albumin (g/dL), VWF (% of standard), and FVIII activity (% of standard) measured at Visit 1 were analyzed in an ARIC research laboratory in accordance with a standardized protocol[13,14]. WBC count was determined from whole anti-coagulated blood using an automated particle Coulter Counter within 24 hours of venipuncture. Repeated testing revealed inter-assay coefficients of variation below 8% for fibrinogen, albumin, FVIII, and WBC, and 17-19% for VWF[15,16].

Brain MRI

MRI scans were conducted using a 3T MRI scanner[11]. MP-RAGE, Axial T2*GRE, Axial T2 FLAIR, and Axial DTI sequences were obtained. Freesurfer (http://surfer.nmr.mgh.harvard.edu) was used to measure brain volume from MP-RAGE sequences[17]. Total brain and ventricular volume, lobar volume (frontal, temporal, parietal, occipital), Alzheimer's disease (AD) Signature Region volume (i.e., the combined volume of the parahippocampal, entorhinal, inferior parietal lobules, hippocampus, and precuneus)[18], hippocampal volume, and total intracranial volume

were evaluated for the current study. We applied a square root transformation to ventricular volume to correct for skewness.

Episodic memory

Episodic memory was assessed at Visit 5, concurrent with the brain MRI, using the delayed word recall test (DWR). DWR is a test which requires participants to learn and recall a list of 10 words following a delay period[19]. Participants were scored based on the total number of words correctly recalled.

Covariates

Race, sex, years of education attained (less than high school; high school/GED/vocational school; or any college), cigarette smoking status (current/former/never), average weekly alcohol consumption (grams), and previous cancer diagnosis were self-reported. A random zero sphygmomanometer was used to calculate sitting diastolic and systolic blood pressure. Second and third blood pressure measurements were averaged for the current analyses. Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or use of hypertensive medication. Body mass index (BMI) was calculated using recorded height and weight (kg/m^2). Coronary heart disease was defined as self-reported coronary bypass, balloon angioplasty, angioplasty of one or more coronary artery, or myocardial infarction. Medications used in the previous two weeks was recorded. The presence of chronic inflammatory conditions (e.g., arthritis, lupus, gout) was assessed by patient self-report of physician diagnosis at Visit 4. History of regular anti-inflammatory medication use (e.g., nonsteroidal anti-inflammatory drug [NSAID], arthritis medication) was assessed at Visit 5. All other variables were assessed at Visit

1. Dementia diagnosis was adjudicated at Visit 5 by an expert committee using cognitive, imaging and functional data[20].

Total cholesterol and triglycerides were measured using enzymatic methods[21,22], and low-density lipoprotein (LDL), using the Friedewald equation[23]. Serum glucose was measured using the hexokinase method. Diabetes was defined as a fasting glucose ≥126 mg/dl or a non-fasting glucose ≥ 200 mg/dl, current use of diabetes medication or insulin, or participant report of physician-diagnosed diabetes. *APOE* genotype (0, 1, or 2 ε4 alleles) was assessed using the TaqMan assay (Applied Biosystems, Foster City, CA).

Statistical analysis

We examined systemic inflammation as both a continuous and categorical exposure parameter. A continuous inflammation composite Z-score was created using the five inflammatory markers. WBC count was log-transformed to correct for skewness. Each inflammatory biomarker was converted to a standardized Z-score such that the group mean was zero with a standard deviation of one. The mean of the five Z-scores was calculated to generate an inflammation composite Z-score; this composite score was then standardized. Because albumin decreases in response to inflammation, albumin values were multiplied by -1 before being included in the composite Z-score. With few exceptions, the inter-correlations between inflammatory markers were within an optimal range, between .2 and .4; composite score item-test correlations, principal component factor loadings, and Cronbach's alpha (0.61) were satisfactory for our purposes (table e-1). For each participant, we also created a categorical measure of systemic inflammation by computing

the number of inflammatory marker z-scores in the highest quartile (≥75%tile) and trichotomizing this number (0, 1-2, or 3-5).

Participant characteristics were compared using an ANOVA or chi-square tests. Multivariable linear regression was used to assess the association between continuous and categorical inflammation variables and measures of brain volume and episodic memory. Brain volume analyses were adjusted for total intracranial volume, and all analyses included the covariates described in the previous section. Interaction terms or stratification were used to evaluate the modifying effects of age, race, and sex.

Sensitivity analyses were performed excluding participants who reported regular anti-inflammatory medication use during follow-up and including participants who met criteria for dementia. For all analyses, sampling weights were incorporated to account for the ARIC brain MRI sampling strategy. Thus, all results represent estimates for the entire ARIC Visit 5 study population. Because the associations between inflammation markers and specific ROIs are correlated, we did not adjust for multiple comparisons. A two-sided p value < .05 designated statistical significance. All analyses were conducted using Stata Version 14 (StataCorp, College Station, Tex., USA).

RESULTS

Study population characteristics

A total of 1,617~~633~~ participants (baseline mean age 52.8 [5.3], ~~27~~26% African American, ~~60~~61% women, 46% college or professional degree) were included in the study sample. The time

between baseline assessment and follow-up MRI scan was 24 (1) years; the average age at follow-up was 76.~~5~~ 4 (5.4). As shown in table 1, a higher inflammation composite score at baseline was associated with older age, female sex, African American race, and increased levels of a number of cardiovascular risk factors.

Inflammatory markers and brain volume

We did not find a statistically significant association between midlife inflammation composite score and late-life brain volume in the full analytic sample. Each standard deviation (SD) increase in inflammation composite score at baseline was associated with a ~~532~~ 197 mm^3 smaller AD Signature Region volume (95% confidence interval [CI]: -~~922~~ 431 to ~~-141~~39; *p*=.10), a ~~519~~ 204 mm^3 smaller occipital lobe volume (CI: -427 ~~-906~~ to ~~-~~18; *p*=.07~~132~~), a ~~110~~ 42 mm^3 smaller hippocampal volume (CI: -~~196~~ 88 to -5; *p*=.08~~24~~), and a ~~1,788~~1.87 ~~mm^3~~ larger squared ventricular volume (CI: ~~371~~ -.29 to ~~3,205~~4.03; *p*=.09) at follow-up (table 2). ~~We found the estimated effect of a one SD increase in inflammation composite score during midlife on occipital lobe, ventricular, and hippocampal volume to be similar to the effect associated with possession of a single *APOE* ε4 allele in our multivariable regression analyses.~~ No association was found for total brain, frontal lobe, temporal lobe, or parietal lobe volume (p's >.~~071~~72). Our findings did not change meaningfully after excluding participants who regularly used anti-inflammatory medication during the follow-up period (table e-2) and after including participants who met criteria for dementia at Visit 5 (table e-3). For descriptive purposes, we explored the associations between individual inflammatory markers and AD Signature Region volume (~~are provided in a~~ table e-4). Only midlife WBC count was significantly associated with AD

Signature Region volume: each SD increase in midlife WBC count was associated with 292mm^3 smaller late-life AD Signature Region volume (CI: -564, -20; p=0.036).

~~An assessment of linear trend revealed that~~Next, we compared the brain volumes of~~to~~ individuals with 0 elevated (≥ 75th %tile) inflammatory biomarkers at midlife ~~baseline~~ (reference)~~,~~ to those with 1-2 and 3-5 elevated biomarkers at midlife ~~had lower AD Signature Region (p-trend=.001), occipital lobe (p-trend=.007), and hippocampal volume (p-trend=.041) 24 years later~~ (figure 1). Compared to the reference group, participants with ~~three or~~1-2 elevated midlife inflammatory biomarkers had significantly smaller late-life AD Signature region volume (-790mm^3; CI: -1,320, -260; p=.003) and occipital lobe volume (-727mm^3; CI: -1,228, -226; p=.004). Participants with 3 or more elevated markers demonstrated ~~5.3%~~ smaller AD Signature Region volumes (-751mm^3; CI: -1,461, -41; p=.038) and~~, 5.7% smaller occipital lobe volumes, and 4.6% smaller~~ hippocampal volumes (-148mm^3; CI: -288, -8; p=.038).~~, on average. However, this pattern was not statistically supported for total brain, ventricular, frontal lobe, temporal lobe, and parietal lobe volume (p-trends >.072).~~

The modifying effects of age, race, and sex

An~~ significant~~ age-by-inflammation composite score interaction was found for ~~AD Signature Region,~~ occipital lobe, and hippocampal volume (table 2). Because a reversal of association was observed at age 60 (figures 2~~, e-1, e-2~~), we stratified the sample into young-midlife and old-midlife subgroups (<60/≥ 60). As displayed in table 2, we found a significant~~the~~ associations between higher midlife inflammation composite score~~-~~ and lower AD Signature Region (young-midlife: -262mm^3; CI: -509, -15 vs. old-midlife: 455mm^3; CI: -281, 1,190; interaction-p=0.06),

occipital lobe (young-midlife: -256mm^3; CI: -491, -21 vs. old-midlife: 316mm^3; CI: -343, 975; interaction-*p*=0.009), and hippocampal volume (young-midlife: -51mm^3; CI: -100, -2 vs. old-midlife: 56mm^3; CI: -98, 212; interaction-*p*=0.03) ~~at follow-up were significantly stronger~~ among participants who were 60 or younger at baseline but not among participants older than 60. In this younger group we found the estimated effect of a one SD increase in inflammation composite score during midlife on hippocampal volume to be similar to the effect associated with possession of a single *APOE ε4* allele in our multivariable regression analyses. Notably, the associations among ~~compared to~~ those who were older than 60, although non-significant, were nearly all reversed in terms of direction~~. A marginal race-by-inflammation composite score interaction was found for occipital lobe volume, whereby a higher midlife inflammation composite score was associated with lower occipital lobe volume among white, but not African American, participants (table 3)~~. We found no evidence for effect modification by ~~No interactions with~~ race or sex ~~were found~~ (table 3 and table e-5).

Inflammatory markers and episodic memory

Late-life episodic memory~~, which~~ was associated with hippocampal ~~and AD Signature Region~~ volume after controlling for age (partial ~~*r's >*~~ = 0.221, ~~*p's*~~ <.001)~~., was reduced among participants wit~~Although~~h~~h~~ higher levels of the inflammation composite score~~. Each SD increase in inflammation composite score was~~ were not associated with ~~a -0.08 SD performance~~ decrement on the DWR after adjusting for covariates ~~(CI: -0.15 to 0.00, *p*=.046). Similarly~~, ~~a higher number~~participants with three or more ~~of~~ elevated inflammatory biomarkers, compared to those with no elevated inflammatory markers, showed lower episodic memory performance (ß=-

0.27; CI: -0.54, 0.00; *p*=.049; ~~at baseline was associated with reduced DWR performance (p-trend=.009;~~ figure 1).

DISCUSSION

Using a large community sample, we ~~demonstrated that~~examined whether a higher level of systemic inflammatory markers measured during midlife ~~is~~ was independently associated with lower regional brain volume and reduced episodic memory 24 years later among non-demented older adults. We demonstrated ~~Similarlt~~hat~~y,~~ participants who had elevations in a larger number of five inflammatory markers during midlife ~~were found~~had ~~to have~~ lower regional brain volumes (particularly in the AD signature region and hippocampus) and reduced episodic memory in late-life ~~in a dose-response manner~~. Although not associated with lower brain volume in the total sample, a higher systemic inflammation composite score was associated with lower late-life brain volume in a subset of younger participants, (i.e., those age 60 or younger at the time of inflammatory marker measurement). ~~For several brain regions, including the hippocampus~~For these participants, the effect of a one SD increase in midlife inflammation composite score on hippocampal volume was comparable that of possessing a single *APOE* ε4 allele during late-life. Whereas age ~~and race were~~was found to ~~modestly~~ modify the relationship between midlife inflammation and late-life regional brain volume, ~~the previously reported~~a modifying effect of race and sex was not supported.

Although cross-sectional evidence from the Framingham[5] study and several other population-based[8,9] studies suggests an association between brain volume and inflammation in older adults, the temporal relationship between inflammation and brain volume loss is still not well

understood. As a result, whether heightened systemic inflammation constitutes a potential cause or consequence of neurodegeneration and brain atrophy remains unclear. Because the pathophysiological processes driving neurodegeneration and brain volume loss begin decades before the onset of frank cognitive decline[24], it is essential to determine how biological processes that take place during middle adulthood relate to neurological outcomes later in life. By demonstrating that an elevation in plasma inflammatory markers during midlife is independently associated with smaller regional brain volumes ~~, larger ventricular volume,~~ and reduced episodic memory in late-life, the current findings provide support for a potential causal, rather than associative, role of systemic inflammation in late-life neurodegeneration (i.e., atrophy) and resulting cognitive decline. The current findings align closely with those from the neuro-cardiovascular literature which have found associations between midlife blood pressure[25], cholesterol[26], and diabetes[27], and adverse neurological and cognitive outcomes in older adulthood. The contributing role of systemic inflammation to subsequent neurodegenerative processes has been previously demonstrated by animal studies[28], but had not yet been supported by a large prospective MRI study.

The current results suggest that several demographic factors modify the relationship between midlife inflammation and late-life brain volume. Younger individuals with elevated levels of inflammation (particularly participants in their 40s and early 50s) were more likely to display lower brain volumes decades later, supporting the idea that elevated systemic inflammation earlier in life may make individuals especially vulnerable to neurodegenerative brain changes as they age. Although we expected stronger effects would emerge within the African American group, given the greater burden of systemic disease[29] and dementia[30], the associations between

inflammation and brain volume were generally weaker among African Americans. A previous study which examined the moderating effects of race found similar results in a cross-sectional analysis of non-demented older adults[8].

Circulating levels of acute-phase reactants, such as those used in the current study, change in parallel with an inflammatory response as a result of signaling from inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α[12]. Cytokines in the periphery have the potential to induce a pro-inflammatory neurotoxic state within the CNS through multiple routes, including activation of endothelial cells of the blood brain barrier[31], activation of macrophage in circumventricular organs[32], and signaling of the afferent vagus nerve[33]. In addition to providing support for a pathogenic role of systemic inflammation in neurodegenerative disease, the present findings indicate that elevations in commonly assayed inflammatory proteins may serve as markers of risk for future neurodegenerative changes and cognitive decline. Although we did not examine all brain regions in our analysis, our assessment of seven representative ROIs suggests that brain regions vulnerable to atrophy, amyloid deposition, and metabolic abnormalities in the earliest phases of Alzheimer's disease may be more vulnerable to volume loss associated with heightened midlife inflammation. This pattern of neuroanatomical specificity has been supported by previous cross-sectional studies of non-demented older adults[4,7–9,34].

In the context of the current findings, several alternative explanations should be considered. First, it remains possible that elevated systemic inflammation may simply serve as a marker of another pathological process linked to neurodegeneration (e.g., oxidative stress). Second, it is possible that the biological processes causing brain atrophy trigger a protective neuro-immune

response, which increases peripheral inflammation. Third, the associations found here may be an effect of residual or unmeasured confounding. Despite these caveats, the contributory role of systemic inflammation has been supported by a sizable body of literature implicating peripheral inflammatory signaling in neurodegenerative processes such as neural apoptosis[35], amyloid-β formation[36], and neuronal tau phosphorylation[37].

Strengths of the current study include the prospective study design, length of follow-up, detailed assessment of potentially confounding variables, large sample size, and the inclusion of a large African American sample. However, the current findings should be interpreted within the context of several limitations. Although the acute-phase reactants used in the present study represent components of the innate immune system, several of these proteins are implicated in other closely related physiological process, such as hemostasis, which may also influence brain volume. Evaluating inflammatory biomarkers that have greater biological specificity in future prospective studies will allow for stronger inferences about the contributing role of systemic inflammation. Interpretation of the current findings is also limited by the measurement of inflammatory markers at a single time point, as it is unclear whether a single measurement can adequately capture inflammation chronicity. The relatively high inter-assay variability of VWF also increases the likelihood of exposure misclassification; however, this possibility is mitigated by the use of the inflammation composite score. We found that participants who dropped out and participants who died before Visit 5 had significantly higher levels of midlife inflammation, were older, had greater levels of medical comorbidity at baseline, and were more likely to be African American[38] (table e-6). As a result, selective attrition may have biased results in the direction of the null hypothesis, particularly for African American and older participants. Lastly, our

interpretation of the contributory role of inflammation in neurodegeneration rests on the assumption that brain volume loss occurred after inflammatory markers were assessed. Although evidence suggests that this is likely the case (brain volume loss accelerates after age 60[39]), this cannot be confirmed without the assessment of change over time.

Despite these limitations, the current study provides insights into the connection between midlife systemic inflammation and late-life brain volume loss. These findings provide support for inflammation's early pathogenic role in the development of neurodegenerative brain changes associated with late-life cognitive decline, Alzheimer's disease, and other forms of dementia.

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Figure Titles and Legends.

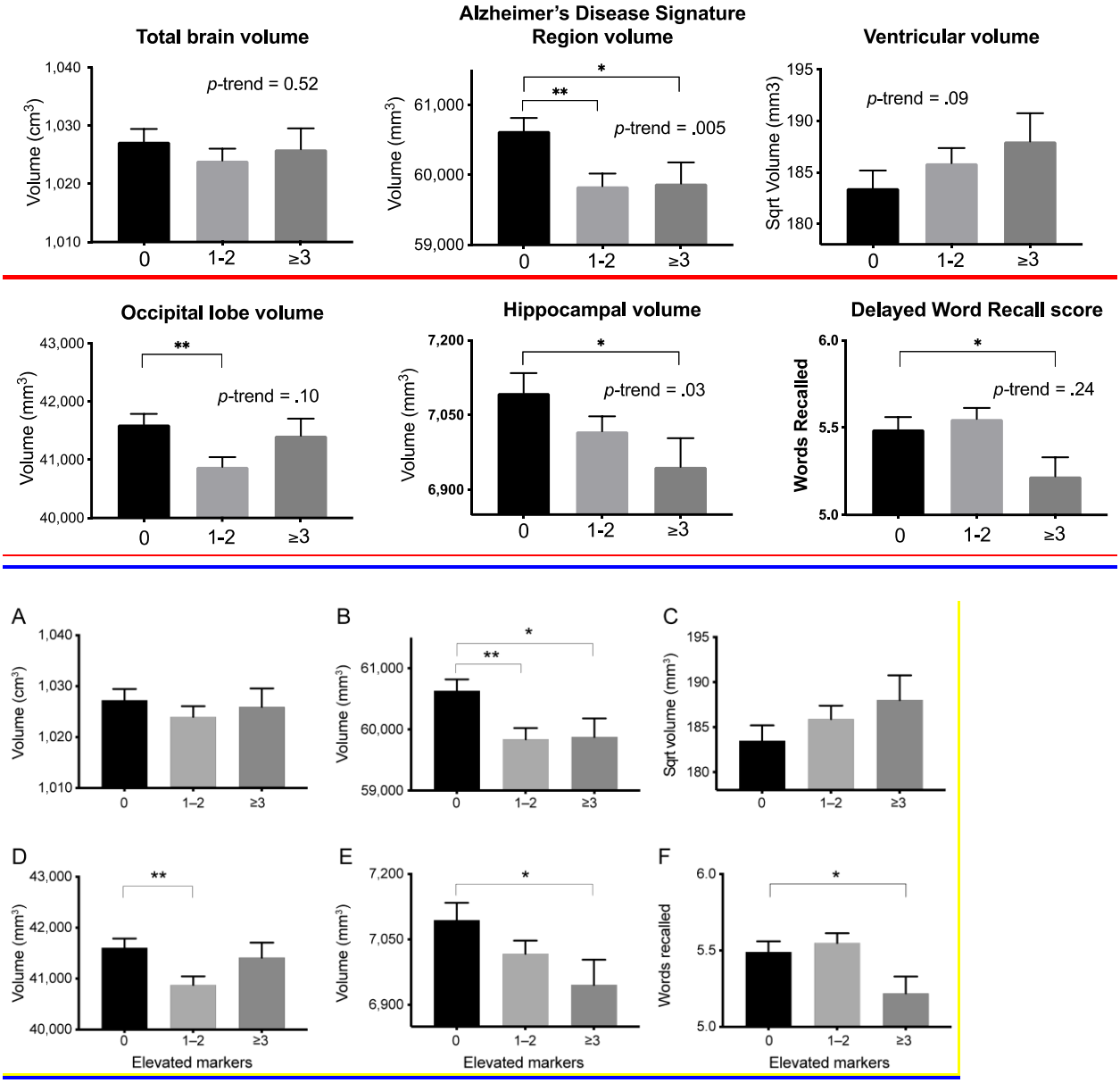
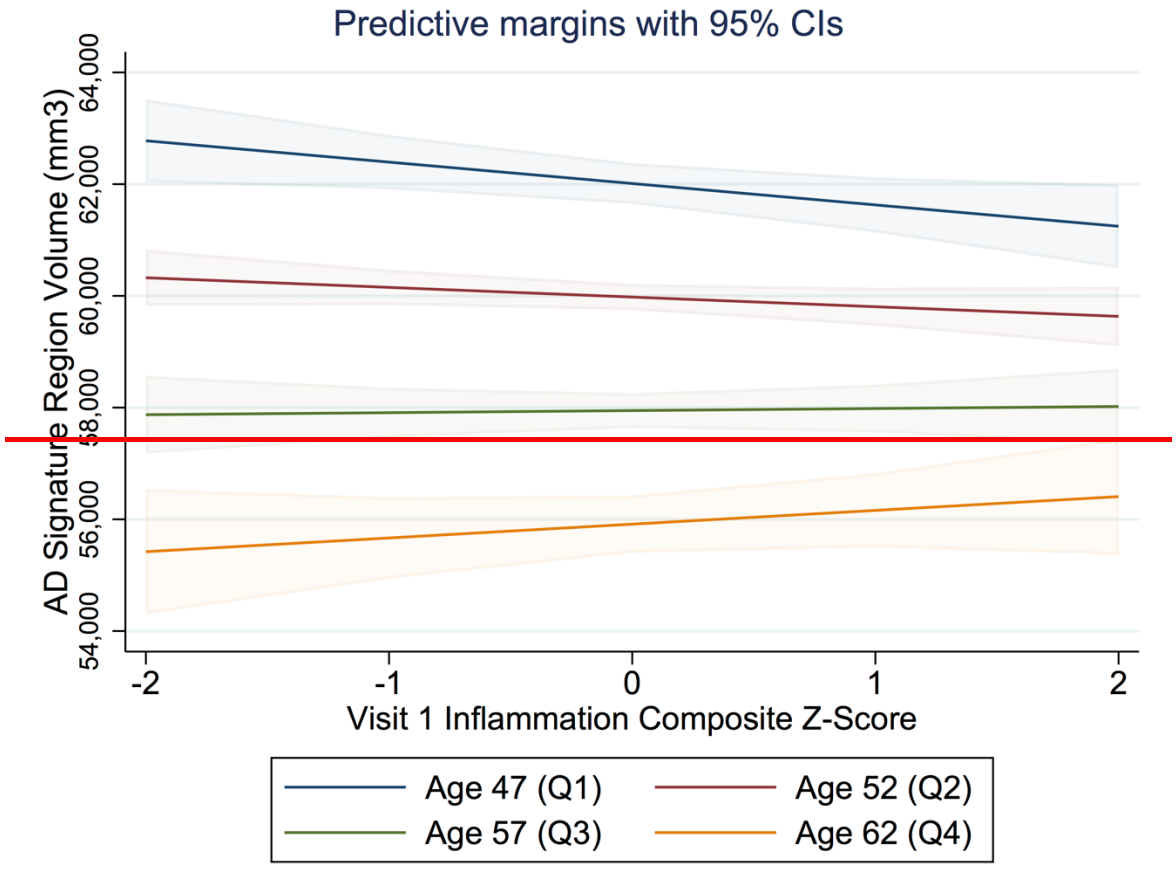


Figure 1. Number of elevated inflammatory markers, predicted brain volumes and episodic memory

Covariate-adjusted predicted brain volumes and delayed word recall test scores among participants with 0, 1-2, and ≥ 3 elevated inflammatory markers. Inflammatory marker levels were classified as elevated if they were ≥ 75th %tile based on the study sample.

*p < .05, **p < .01


Predictive margins with 95% CIs
AD Signature Region Volume (mm3)
54,000
56,000
58,000
60,000
62,000
64,000
-2
-1
0
1
2
Visit 1 Inflammation Composite Z-Score
Age 47 (Q1)
Age 52 (Q2)
Age 57 (Q3)
Age 62 (Q4)

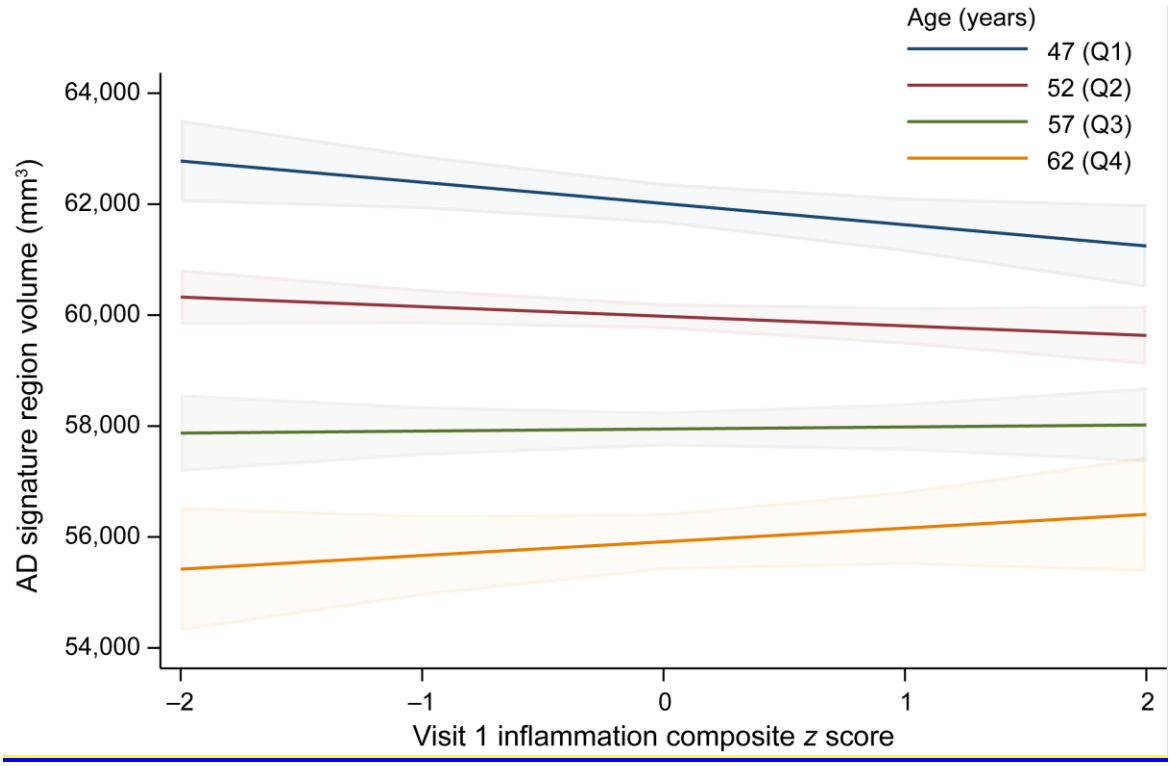


Figure 2. Association between midlife inflammation and late-life brain volume stratified by baseline age

Predicted values of Alzheimer's disease Signature Region volume across levels of the inflammation composite score using a covariate-adjusted regression. Estimates are derived for the mean age for each age quartile derived from the total ARIC sample.

Table 1. Baseline (Visit 1) participant characteristics stratified across inflammation composite score quartiles

	Midlife Inflammation Composite Score				
Characteristic	Low	Medium-Low	Medium-High	High	*p*
	n = 405 ~~408~~	n = 404 ~~408~~	n = 404 ~~410~~	n = 404 ~~407~~	
Demographic Variables					
Age	52.0 (4.8)	~~53.1 (5.3)~~ 53.0 (5.2)	~~53.0 (5.4)~~ 52.9 (5.4)	53.3 (5.5)	~~0.002~~ .006
Female, n (%)	~~213 (52.2)~~ 212 (52.4)	~~243 (59.6)~~ 241 (59.7)	~~243 (59.3)~~ 241 (60)	~~286 (70.3)~~ 285 (70.5)	< .001
White Race, n (%)	~~316 (77.5)~~ 317 (78.3)	~~310 (76.0)~~ 309 (76.5)	~~303 (73.9)~~ 296 (73.3)	~~265 (65.1)~~ 267 (66.1)	< .001
Education, n (%)					~~0.109~~ .11
Less than high school	~~43 (10.5)~~ 41 (10.1)	~~52 (12.8)~~ 51 (12.6)	~~51 (12.4)~~ 52 (12.9)	~~71 (17.4)~~ 69 (17.1)	
High school, GED, or vocational	~~163 (40.0)~~ 163 (40.3)	~~173 (42.4)~~ 173 (42.8)	~~171 (41.7)~~ 168 (41.6)	~~159 (39.1)~~ 155 (38.4)	
College, graduate, or professional	~~202 (49.5)~~ 201 (49.6)	~~183 (44.9)~~ 180 (44.6)	~~188 (45.9)~~ 184 (45.5)	~~177 (43.5)~~ 180 (44.6)	
Apolipoprotein E ε4 alleles, n (%)					~~0.959~~ .93
0	~~286 (70.1)~~ 285 (70.4)	~~298 (73.0)~~ 294 (72.8)	~~291 (71.0)~~ 286 (70.8)	~~292 (71.7)~~ 290 (71.8)	
1	~~109 (26.7)~~ 107 (26.4)	~~101 (24.8)~~ 100 (24.8)	~~109 (26.6)~~ 110 (27.2)	~~105 (25.8)~~ 104 (25.7)	

2	13 (3.2)	~~9 (2.2)~~ 10 (2.5)	~~10 (2.4)~~ 8 (2.0)	10 (2.5)	
Physiological and Lab Variables					
Body mass index (kg/m^2)	~~25.7 (3.8)~~ 25.6 (3.7)	26.2 (4.0)	~~27.3 (4.8)~~ 27.3 (4.9)	~~28.7 (5.6)~~ 28.6 (5.6)	< .001
Systolic blood pressure (mm Hg)	~~115.8 (15)~~ 115.7 (14.9)	~~115.5 (16)~~ 115.4 (15.6)	~~115.7 (15)~~ 115.7 (14.5)	~~116.8 (16)~~ 116.9 (15.8)	~~0.633~~ .52
Diastolic blood pressure (mm Hg)	~~72.6 (10)~~ 72.5 (10.2)	~~72.7 (11)~~ 72.7 (10.8)	~~71.8 (9)~~ 71.8 (9.1)	~~73.1 (11)~~ 73.1 (11.1)	~~0.323~~ .35
Total cholesterol (mg/dl)	~~210.1 (42)~~ 210.0 (41.6)	~~210.6 (39)~~ 210.8 (38.6)	~~211.8 (36)~~ 211.8 (36.5)	~~215.0 (40)~~ 215.1 (40.5)	~~0.271~~ .27
HDL (mg/dl)	~~55.8 (19)~~ 55.9 (18.5)	~~56.6 (19)~~ 56.6 (18.5)	~~54.4 (16)~~ 54.5 (16.3)	~~53.5 (17)~~ 53.6 (17.1)	~~0.057~~ .07
LDL (mg/dl)	~~132.7 (40)~~ 132.5 (39.5)	~~131.4 (36)~~ 131.4 (36.4)	~~134.9 (35)~~ 134.8 (35.1)	~~137.3 (38)~~ 137.0 (37.8)	~~0.124~~ .15
Triglycerides (mg/dl)	~~107.8 (55)~~ 107.4 (55.1)	~~112.9 (62)~~ 113.6 (62.6)	~~112.5 (55)~~ 112.3 (55.5)	~~121.5 (59)~~ 121.2 (59.9)	~~0.009~~ .004
Cardiovascular Disease, n (%)					
Hypertension	~~80 (19.6)~~ 77 (19.0)	~~90 (22.1)~~ 88 (21.8)	~~88 (21.5)~~ 86 (21.3)	~~111 (27.3)~~ 109 (27.0)	~~0.057~~ .047
Diabetes mellitus	9 (2.2)	~~12 (2.95)~~ 11 (2.7)	~~14 (3.4)~~ 13 (3.2)	~~22 (5.4)~~ 24 (5.9)	~~0.079~~ .02
Coronary heart disease	~~12 (2.9)~~ 3 (0.7)	~~15 (3.7)~~ 5 (1.2)	~~13 (3.2)~~ 3 (0.7)	~~18 (4.4)~~ 3 (0.7)	~~0.674~~ .83
Heart Failure	6 (1.5)	8 (2.0)	~~10 (2.4)~~ 10 (2.5)	~~10 (2.5)~~ 9 (2.2)	~~0.728~~ .78
Inflammatory Conditions, n (%)					

Arthritis [a]	~~112 (27.5)~~	~~165 (40.4)~~	~~156 (38.1)~~	~~181 (44.5)~~	< .001
	112 (27.7)	163 (40.4)	155 (38.4)	180 (44.6)	
Lupus [a]	3 (0.7)	1 (0.3)	~~1 (0.2)~~	2 (0.5)	~~0.662~~
			1 (0.3)		.67
Gout [a]	13 (3.2)	~~22 (5.4)~~	~~20 (4.9)~~	26 (6.4)	~~0.199~~
		21 (5.2)	19 (4.7)		.20
Cancer	~~16 (3.9)~~	~~28 (6.9)~~	~~19 (4.6)~~	~~22 (5.4)~~	~~0.324~~
	16 (4.0)	27 (6.7)	19 (4.7)	21 (5.2)	.39
Medication, n (%)					
Anti-inflammatory (last 4 weeks)	~~142 (34.8)~~	~~142 (34.8)~~	~~158 (38.5)~~	~~190 (46.7)~~	.001
	140 (34.6)	140 (34.7)	155 (38.4)	189 (46.8)	
Anti-inflammatory (regularly use) [b]	~~48 (11.8)~~	~~70 (17.2)~~	~~67 (16.3)~~	64 (15.8)	~~0.139~~
	47 (11.6)	71 (17.6)	65 (16.1)		.10
Cholesterol lowering (last 2 weeks)	5 (1.2)	6 (1.5)	~~7 (1.7)~~	~~12 (3.0)~~	~~0.261~~
			7 (1.8)	11 (2.7)	.40
Cigarette Smoking Status, n (%)					~~0.004~~
					.001
Current	~~42 (10.3)~~	~~61 (15.0)~~	~~71 (17.3)~~	~~85 (20.9)~~	
	40 (9.9)	62 (15.4)	71 (17.6)	86 (21.3)	
Former	150 (36.7)	133 (32.6)	123 (30.0)	126 (31.0)	
	151 (37.3)	131 (32.4)	123 (30.5)	123 (30.5)	
Never	~~216 (52.9)~~	~~214 (52.5)~~	~~215 (52.4)~~	~~196 (48.2)~~	
	214 (52.8)	211 (52.2)	210 (52.0)	195 (48.3)	
Alcohol consumption, n (%)					< .001
Current	~~279 (68.4)~~	~~250 (61.3)~~	~~236 (57.6)~~	~~209 (51.4)~~	
	280 (69.1)	250 (61.9)	230 (56.9)	208 (51.5)	
Former	~~50 (12.3)~~	~~58 (14.2)~~	~~48 (11.7)~~	~~61 (15.0)~~	
	49 (12.1)	58 (14.4)	48 (11.9)	62 (15.4)	

Never	~~79 (19.4)~~	~~100 (24.5)~~	~~126 (30.7)~~	~~137 (33.7)~~	
	76 (18.8)	96 (23.8)	126 (31.2)	134 (33.2)	
Weekly Alcohol Intake (grams)	~~35.8 (70)~~	~~33.5 (73)~~	~~22.2 (51)~~	~~22.7 (67)~~	~~0.003~~
	41.6 (75.7)	36.8 (75.3)	26.2 (55.5)	30.6 (79.5)	.01

Values are displayed as means (SD) unless otherwise specified

[a] Assessed at Visit 4 (1996-1998)

[b] Assessed at Visit 5 (2011-2013)

Table 2. Association between midlife inflammation composite score and late-life MRI volumes among non-demented participants

	Total Sample (n = 1,536 ~~1,550~~)		Age < 60 at baseline (n = 1,357 ~~1,366~~)		Age ≥ 60 at baseline (n = 179 ~~184~~)		Age Interaction
Region	ß (95% CI) mm^3	*p*	ß (95% CI) mm^3	*p*	ß (95% CI) mm^3	*p*	*p*
Total Brain	~~21,976 (26,495, 2,541)~~	~~0.391~~	~~22,175 (26,927, 2,576)~~	~~0.369~~	~~5,647 (28,191, 19,484)~~	~~0.421~~	~~0.339~~
	-171 (-2,956, 2,614)	.90	-189 (-3,319, 2,740)	.88	2,313 (-14,942, 28,194)	.59	.46
AD Signature Region	~~2532 (2922, 2141)~~	~~0.008~~	~~2645 (21,056, 2235)~~	~~0.002~~	~~737 (2456, 1,929)~~	~~0.224~~	~~0.033~~
	-197 (-431, 39)	.10	-262 (-509, -15)	.038	455 (-281, 1,190)	.22	.06
Ventricular Volume*	~~1,788 (371, 3,205)~~	~~0.013~~	~~1,671 (173, 3,170)~~	~~0.029~~	~~1,871 (22,538, 6,281)~~	~~0.403~~	~~0.436~~
	1.87 (-0.29, 4.03)	.09	1.79 (-0.51, 4.09)	.13	3.05 (-3.31, 9.40)	.35	.48
Frontal Lobe	~~84 (2801, 969)~~	~~0.852~~	~~289 (21,016, 838)~~	~~0.850~~	~~2,650 (2444, 5,745)~~	~~0.180~~	~~0.829~~
	27 (-519, 573)	.92	-81 (-656, 493)	.78	1,343 (-612, 3298)	.18	.80
Temporal Lobe	~~2609 (21,271, 53)~~	~~0.071~~	~~2767 (21,467, 268)~~	~~0.032~~	~~1,126 (2788, 3,041)~~	~~0.342~~	~~0.051~~
	-273 (-672, 125)	.72	-359 (-782, 63)	.10	580 (-631, 1,792)	.35	.15
Parietal Lobe	~~2363 (21,032, 306)~~	~~0.287~~	~~2505 (21,210, 201)~~	~~0.161~~	~~1,674 (2481, 3,829)~~	~~0.132~~	~~0.095~~
	-18 (-424, 389)	.93	-102 (-530, 327)	.64	1,014 (-281, 2,308)	.12	.12
Occipital Lobe	~~2519 (2906, 2132)~~	~~0.009~~	~~2612 (21,021, 2201)~~	~~0.004~~	~~666 (2417, 1,630)~~	~~0.177~~	~~0.006~~
	-204 (-427, 18)	.07	-256 (-491, -21)	.033	316 (-343, 975)	.35	.009

Hippocampus	~~2110 (2196, 224)~~	~~0.013~~	~~2124 (2216, 233)~~	~~0.008~~	~~10 (2284, 305)~~	~~0.992~~	~~0.047~~
	-42 (-88, 5)	.08	-51 (-100, -2)	.042	56 (-98, 212)	.47	.03

Abbreviations: AD Signature Region = Alzheimer's disease Signature Region

Adjusted ß coefficients represent the change in late-life brain volume per one standard deviation increase in midlife inflammation composite score. Model adjusted for age, sex, center-race, APOE ε4 status, diabetes, hypertension, total cholesterol, LDL, triglycerides, BMI, coronary heart disease, cancer, chronic inflammatory disease, smoking status, weekly alcohol use, and anti-inflammatory medication use. * Values displayed as squared ventricular volume level.

Table 3. Association between midlife inflammation composite score and late-life MRI volumes among non-demented participants stratified by race

	White (n = 1,129 1,134)		African American (n = 407 416)		Race Interaction
Region	ß (95% CI) mm^3	p	ß (95% CI) mm^3	p	p
Total Brain	~~21,952 (27,618, 3,713)~~	~~0.499~~	~~1,785 (24,992, 8,562)~~	~~0.605~~	~~0.433~~
	-80 (-3,607, 3447)	.97	988 (-3,485, 5,439)	.67	.67
AD Signature Region	~~2554 (21,034, 274)~~	~~0.024~~	~~226 (2653, 602)~~	~~0.936~~	~~0.336~~
	-246 (-538, 45)	.10	65 (-337, 467)	.75	.50
Ventricular Volume*	~~2,027 (208, 3,845)~~	~~0.029~~	~~44 (21,822, 1,909)~~	~~0.963~~	~~0.455~~
	1.98 (-0.75, 4.70)	.16	0.54 (-2.76, 3.85)	.75	.74
Frontal Lobe	~~351 (2768, 1,471)~~	~~0.538~~	~~228 (21,181, 1,638)~~	~~0.750~~	~~0.852~~
	235 (-446, 917)	.50	-66 (-998, 866)	.89	.71
Temporal Lobe	~~2713 (21,528, 102)~~	~~0.087~~	~~119 (2959, 1,197~~	~~0.829~~	~~0.300~~
	-359 (-850, 132)	.15	104 (-574, 782)	.76	.38
Parietal Lobe	~~2244 (21,077, 588)~~	~~0.565~~	~~339 (2725, 1,402)~~	~~0.532~~	~~0.596~~
	-22 (-539, 494)	.93	155 (-522, 831)	.65	.84
Occipital Lobe	~~2529 (2997, 260)~~	~~0.027~~	~~120 (2436, 677)~~	~~0.671~~	~~0.056~~
	-247 (-528, 34)	.09	21 (-359, 401)	.91	.20
Hippocampus	~~2150 (2253, 248)~~	~~0.004~~	~~26 (2176, 163)~~	~~0.940~~	~~0.110~~
	-60 (-117, -2)	.041	29 (-49, 106)	.47	.27

Abbreviations: AD Signature Region = Alzheimer's disease Signature Region

Adjusted ß coefficients represent the change in late-life brain volume per one standard deviation increase in midlife inflammation composite score. Model adjusted for age, sex, center-race, APOE ε4 status, diabetes, hypertension, total cholesterol, LDL, triglycerides, BMI, coronary

heart disease, cancer, chronic inflammatory disease, smoking status, weekly alcohol use, and anti-inflammatory medication use. * Values displayed as squared ventricular volume level.