Systemic inflammation during midlife and cognitive change over 20 years
The ARIC Study


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Objective To examine the association between systemic inflammation measured during midlife and 20-year cognitive decline.

Methods Within the Atherosclerosis Risk in Communities cohort study, inflammatory biomarkers were measured during middle adulthood. We created an inflammation composite score using 4 blood biomarkers measured at visit 1 (fibrinogen, white blood cell count, von Willebrand
factor, and factor VIII); we measured C-reactive protein (CRP) at visit 2. Cognition was assessed over 3 visits spanning 20 years using measures of memory, executive function, and language.

**Results** A total of 12,336 participants (baseline age 56.8 [5.7], 21% black, 56% women) were included. After adjusting for demographic variables, vascular risk factors, and comorbidities, each standard deviation (SD) increase in midlife inflammation composite score was associated with an additional 20-year decline of $-0.035$ SD (95% confidence interval: $-0.062$ to $-0.007$) on the cognitive composite score. We found a similar association between each SD increase in midlife CRP level and additional 20-year cognitive decline ($-0.038$ SD, 95% confidence interval: $-0.057$ to $-0.019$). Participants with a midlife inflammation composite score in the top quartile had a 7.8% steeper cognitive decline, compared to participants in the lowest quartile; CRP in the top quartile was associated with an 11.6% steeper cognitive decline. In cognitive domain-specific analyses, elevated midlife inflammatory markers were most consistently associated with declines in memory. Results were similar after adjusting for attrition using inverse probability weighting.

**Conclusions** Our findings highlight what may be an early pathogenic role for systemic inflammation as a driver of cognitive decline in the decades leading up to older adulthood.

**Glossary**
In light of the growing body of evidence implicating aberrant immune functioning in the pathophysiology of Alzheimer disease and related dementia, understanding the relationship between inflammation and neurocognitive functioning has become a priority in recent years. The majority of studies linking systemic inflammation to cognitive impairment have been cross-sectional, and have therefore been unable to clarify the temporal relationship between systemic inflammation and cognitive outcomes. While several prospective studies have found greater rates of cognitive decline among individuals with higher levels of circulating inflammatory markers, the majority of these studies measure inflammatory markers only during older adulthood and have brief follow-up periods (≤10 years). Although age-related cognitive decline begins to accelerate during midlife, it is currently unclear to what degree systemic inflammation may contribute to such declines. Similarly, it is unknown which aspects of cognition may be most vulnerable to the effects of systemic inflammation.

It is essential to examine the long-term effects of midlife systemic inflammation because it is during the midlife period when many of the pathologic processes underlying age-related cognitive impairment and dementia are thought to begin and to perhaps be most responsive to intervention. Using a large, biracial, community-based sample, we tested
the hypothesis that elevated systemic inflammation during midlife is associated with greater cognitive decline over a 20-year period spanning from mid- to late-life. Given previous findings that suggest sex, race, and APOE ε4 status modify the association between systemic inflammation and neurologic outcomes, we examined the moderating effects of each of these demographic factors.

Methods

Study population
The Atherosclerosis Risk in Communities (ARIC) Study, an ongoing population-based, prospective cohort study, enrolled 15,792 adults between the ages of 45 and 65 years from communities within the United States: Washington County, MD; Forsyth County, NC; northwestern suburbs of Minneapolis, MN; and Jackson, MS, from 1987 to 1989. As shown in figure 1, participants were evaluated in person over 5 visits until 2011–2013 and contacted annually via telephone. Participants received a serial cognitive assessment at visits 2, 4, and 5. Of the 14,348 participants who attended visit 2 (1990–1992), we excluded participants on the basis of missing baseline cognitive data (n = 115), nonwhite or nonblack race (n = 42), black race living in Minneapolis or Washington County (n = 49), unavailable inflammatory biomarkers (n = 29), missing covariates (n = 700), clinical stroke before visit 2 (n = 272), or a score below the 5th percentile on any cognitive test at visit 2 (n = 805). The ARIC Study
protocols were approved by the institutional review boards at each participating center. All participants gave written informed consent at each study visit.

Figure 1
Study flow diagram
Study visits, assessments, and participant numbers are tabulated. aWe excluded 42 participants for nonwhite or nonblack race, and 49 black participants living in Minneapolis or Washington County. VWF = von Willebrand factor; WBC = white blood cell count.
Inflammatory biomarkers were measured in blood samples collected at visits 1 and 2. Four biomarkers were measured at visit 1: fibrinogen (mg/dL), von Willebrand factor (% of standard), and white blood cell count, from blood stored at −70°C using standardized protocols that have been described previously. A visit 1 inflammation composite score was created by converting each of the 4 inflammatory markers to a standardized \( z \) score (based on the visit 1 population) and calculating the mean of the 4 \( z \) scores for each participant.

We measured high-sensitivity C-reactive protein (CRP) levels in 2011–2013 from blood collected at visit 2 and stored at −70°C. CRP was measured on the Roche Modular P chemistry analyzer (Roche Diagnostics, Indianapolis, IN) using an immunoturbidimetric assay. Interassay coefficients of variation were below 8% for fibrinogen, factor VIII, white blood cell count, and CRP, and 17%–19% for von Willebrand factor based on repeated testing.

### Cognitive change

Cognition was measured at visits 2, 4, and 5 using 3 standardized neuropsychological measures: the Delayed Word Recall Test, the Digit Symbol Substitution Test, and the Word Fluency Test, each of which has been described in detail previously. The Delayed Word Recall Test is a measure of verbal memory for which participants are asked to learn 10 common nouns by reading each noun and using it in a sentence. After a 5-minute distractor-filled delay period, participants were asked to recall each of the 10 nouns. Participant scores ranged from 0 to 10 based on the
number of correctly recalled words. The Digit Symbol Substitution Test is a measure of executive function and processing speed for which participants are asked to translate a series of numbers based on their corresponding nonsense symbols using a key that uniquely associates a number with a different symbol. The total score was calculated based on the total number of correctly completed symbols in 90 seconds. The Word Fluency Test is a measure of verbal fluency for which participants are asked to list as many words as possible that begin with the letter “F,” “A,” and “S” (excluding proper nouns) within three 1-minute word-naming trials. The total score was based on the number of words generated. We converted test scores from each visit to $z$ scores based on the visit 2 population mean (SD). A composite $z$ score was created as the sum of the 3 test-specific $z$ scores and standardized to the visit 2 composite $z$ score mean and SD for all participants.

**Assessment of covariates**

Participants reported race, education, and sex at visit 1. *APOE* was genotyped using the TaqMan assay (Applied Biosystems, Foster City, CA). Past long-term anti-inflammatory medication use (e.g., nonsteroidal anti-inflammatory drug, arthritis medication) was assessed at visit 5 by self-report. All other covariates were assessed at visits 1 and 2, concurrent with the inflammatory biomarker assessment. History of alcohol and cigarette use was assessed by self-report. Body mass index was calculated using measured height and weight ($\text{kg/m}^2$). The enzymatic method was used to measure total cholesterol. High-density lipoprotein (HDL) cholesterol was
measured following the precipitation of non-HDL lipoprotein.\textsuperscript{16} Hypertension was defined as systolic blood pressure $>140$ mm Hg, diastolic blood pressure $>90$ mm Hg, or use of hypertensive medication. Coronary heart disease was defined based on participant self-report at visit 1 and was adjudicated based on medical record evidence of previous myocardial infarction, coronary artery bypass graft or angioplasty, or ECG evidence of myocardial infarction for events occurring between visits 1 and 2. Heart failure was identified based on medical record evidence of heart failure-related hospitalization or self-reported heart failure medication use. Diabetes was defined as a fasting glucose $\geq 126$ mg/dL or nonfasting glucose of $\geq 200$ mg/dL, current use of diabetes medication, or self-report of physician-diagnosed diabetes. Cancer diagnoses were ascertained using information from state cancer registries; this was supplemented by abstraction of medical records and hospital discharge codes as part of ongoing hospital surveillance.\textsuperscript{17}

**Statistical analysis**

We used generalized estimating equations with an unstructured correlation matrix and robust variance to estimate the difference in population-averaged cognitive change over time according to midlife inflammatory biomarker level. We modeled midlife inflammatory biomarker levels as categorical (quartiles) and continuous parameters. For use as a continuous variable, CRP was log-transformed to correct for skewness. We additionally created a categorical variable based on the number of visit 1 inflammatory markers ($0, 1–2, 3–4$) in the top quartile. Time on study was
modeled with a 2-piece linear spline with a knot at year 6 (the approximate time of visit 4). Given evidence for steeper rates of cognitive decline after midlife, this spline term allowed us to model a nonlinear association between time and cognitive decline in a manner that fits our study design, while also allowing the effect of midlife inflammation to vary across time. An interaction term between inflammatory biomarker exposure and each of the 2 time spline terms was included in all models to examine whether rates of cognitive change differ by inflammatory biomarker level. All participants with cognitive data available at baseline (visit 2) were included in the analyses.

We examined 3 models. The first model adjusted for a set of potential confounders: baseline age and age squared, sex, race/center (white-MD/white-MN/white-NC/black-NC/black-MS), education (less than high school; high school/GED/vocational school; any college), APOE ε4 status (0/1/2 ε4 alleles), body mass index, total cholesterol, HDL, cigarette and alcohol use (current/former/never), cholesterol-lowering medication use (yes/no), and anti-inflammatory medication use (yes/no/missing). A second fully adjusted model additionally adjusted for variables that may lie in the causal pathway between inflammation and cognitive decline: hypertension, diabetes, coronary heart disease, heart failure, and cancer. We examined a third model that used inverse probability of attrition weighting (IPAW) to account for the effects of differential study dropout (informative missingness). IPAW is a weighting procedure used to correct for sampling bias by applying larger weights to participants with
characteristics associated with study dropout. This application of IPAW incorporated demographic, physiologic, and clinical variables listed in table 1 to derive attrition weights; methods are described in detail in data available from Dryad (appendix e-1): doi.org/10.5061/dryad.vn806pd. All models were also adjusted for the interaction between time spline terms and the demographic and clinical variable covariates. Covariates that varied with time (e.g., age, comorbid disease) were updated to be concurrent with inflammatory marker assessment. To evaluate whether the association between midlife inflammatory biomarkers and cognitive decline was subject to effect modification by race (white/black), sex (male/female), and APOE ε4 genotype (positive/negative), we used 3-way multiplicative interaction terms. Estimates of additional total cognitive change over a 20-year period are presented for all analyses.

Table 1

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<td>Baseline (visit 2) participant characteristics stratified by visit 1 inflammation composite score</td>
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We conducted several sensitivity analyses. We repeated analyses using only participants who were assessed at all 3 visits to ensure our inferences regarding 20-year cognitive change were reflected among participants with complete 20-year follow-up. To determine whether our results occurred independent of stroke, we repeated analyses after censoring participants following clinical stroke up to the time of visit 5. To isolate the effect of low-grade systemic inflammation, we repeated analyses after removing
cases with abnormally elevated inflammatory marker levels, suggestive of an acute inflammatory response (i.e., visit 1 inflammation composite score >2 SD or visit 2 CRP >10 mg/L). A 2-sided $p$ value <0.05 was used as the cutoff for statistical significance. All analyses were conducted using Stata version 14 (StataCorp, College Station, TX).

Data availability

On inquiry, the ARIC Study data used here can be made available. For information on how to access available data and study protocols, see www2.cscc.unc.edu/aric/.

Results

A total of 12,336 participants were included in the current study (21% black, 56% women). Participants were 56.8 years of age (5.7 SD) at the time of baseline cognitive assessment (visit 2: 1990–1992) and 75.8 years of age (5.3 SD) at the time of the final cognitive assessment (visit 5: 2011–2013). Participants’ characteristics are presented stratified according to visit 1 inflammation composite score (table 1) and visit 2 CRP level (data available from Dryad [table e-1]: doi.org/10.5061/dryad.vn806pd). Participants with higher levels of midlife inflammatory markers were older, more likely to be female and black, have greater rates of cardiovascular risk factors, and have lower baseline cognition. Of the participants who were assessed at visit 2, 83% had at least 1 follow-up visit and 46% of participants were assessed at the final visit, visit 5 (figure 1). Thirty-seven percent of participants contributed data to only visits 2 and 4 (6 years of follow-up), whereas 17%
of participants had only visit 2 data available and thus only contributed to the estimate of baseline differences in cognition. The mean and median time between baseline and final cognitive assessment was 14.2 years (7.4 SD) and 19.4 years (Q1 to Q3: 6.0–21.0 years), respectively.

After adjusting for demographic and cardiovascular risk factors, participants with a higher visit 1 inflammation composite score had a steeper 20-year decline on the composite cognitive score (table 2). Having an inflammation composite score in the 2nd, 3rd, and 4th quartile was associated with cognitive declines that were 7.5%, 7.7%, and 8.9% steeper, respectively, than that of the 1st quartile. These findings were similar after additionally adjusting for potentially mediating disease variables, and after additionally incorporating IPAW weights to account for attrition (table 2). In domain-specific analyses, a higher inflammation composite score was associated with steeper declines in memory, but not executive function or language (table 3). There was an interaction between the inflammation composite score (specified as a continuous variable) and race; however, this interaction was not reflected in our results, which examined inflammation composite score quartiles (table 4). We found no evidence for effect modification by sex or APOE ε4 status (p-interactions >0.108). Findings were similar in sensitivity analyses that examined only participants with 20 years of complete cognitive data (table 5), censored cases after incident stroke, and excluded participants with abnormally elevated inflammatory markers (data available from Dryad [tables e-2 and e-3]: doi.org/10.5061/dryad.vn806pd).
Relatedly, we found that participants with a higher number of elevated (top quartile) visit 1 inflammatory markers had a steeper decline on the composite cognitive score in a dose-dependent manner. Similar findings
were observed for measures of memory and language in domain-specific analyses (figure 2).
Figure 2
Number of elevated inflammatory markers and additional 20-year cognitive decline
Estimates and 95% confidence intervals of additional 20-year cognitive decline from generalized linear models fit using covariate-adjusted generalized estimating equations for composite and domain-specific cognitive decline among participants with 0, 1–2, and 3–4 elevated inflammatory markers. Findings were observed for the composite cognitive score (A), memory (B), executive function (C), and language (D) in domain-specific analyses. Inflammatory marker levels were classified as elevated if they were in the top quartile (≥75th %tile). Models are adjusted for baseline age and age squared, sex, race-center, education, APOE ε4 status, body mass index, total cholesterol, high-density lipoprotein, cigarette and alcohol use status, hypertension, diabetes, coronary heart disease, heart failure, cancer, cholesterol-lowering medication use, and anti-inflammatory medication use, and interactions of demographic variables and medical comorbidity with time spline terms (model 2). DSST = Digit Symbol Substitution Test; DWRT = Delayed Word Recall Test; WFT = Word Fluency Test.

Elevated visit 2 CRP was also associated with a steeper 20-year cognitive decline after adjusting for demographic and cardiovascular risk factors (table 2). A CRP level in the top 2nd, 3rd, and 4th quartile was associated with 9.7%, 8.5%, and 12.3% steeper decline on the cognitive composite score, respectively, compared to participants with CRP in the 1st quartile. Results were similar after additionally adjusting for disease variables, and after incorporating IPAW weights to account for attrition (table 2). We found no evidence for effect modification by race (table 4), sex, or APOE ε4 status (p-interactions >0.094). In domain-specific analyses, a higher CRP level was associated with steeper declines on a measure of memory, and to a lesser extent, a measure of language (table 3). Findings were not substantively changed in sensitivity analyses that included only participants with 20 years of complete cognitive data (table 5), censored cases after incident stroke, and omitted cases with atypically high CRP levels (data available from Dryad [tables e-2 and e-3]: doi.org/10.5061/dryad.vn806pd).
Based on our findings that suggest CRP levels in the top 3 quartiles are associated with similar levels of additional cognitive decline, we performed a secondary analysis to determine whether a CRP threshold could be determined. Our results, which examined 10 candidate CRP cutpoints (CRP deciles), indicate a CRP threshold near 1.05 mg/L, above which is most strongly associated with subsequent cognitive decline (data available from Dryad [table e-4 and figure e-1]: doi.org/10.5061/dryad.vn806pd).

**Discussion**

Using a large community sample, we have demonstrated that a midlife elevation of circulating inflammatory markers is associated with greater cognitive decline over the decades leading up to older adulthood. Specifically, participants with an elevated inflammation composite score, an elevated CRP level, or a greater number of elevated inflammatory markers during midlife experienced steeper cognitive decline over a 20-year period. Each of these associations occurred independent of potentially confounding variables and incident stroke and did not differ according to sex or APOE ε4 genotype. A comparison of our results to previously published findings from the ARIC Study suggests that the effect on 20-year cognitive decline of midlife inflammation in the upper 3 quartiles is greater than that of midlife hypertension.21

Although systemic inflammation has been associated with cognitive decline among older adults,3,22,23 few studies have investigated the effects of systemic inflammation during middle adulthood on later cognitive
Accordingly, the degree to which midlife systemic inflammation contributes to declines in cognition over the decades spanning from mid- to late-life, when cognitive change is thought to accelerate,\(^4,5\) has remained unknown. Here, we demonstrate that midlife systemic inflammation may constitute a risk factor for accelerated cognitive decline in the decades leading up to older adulthood. Our findings are supported by those from the Honolulu-Asia Aging Study, which demonstrated that individuals with higher levels of CRP during midlife are at greater risk of dementia 25 years later.\(^25\) Given that neuropathologic processes associated with dementia, such as β-amyloid deposition, are believed to begin during midlife, decades before the onset of clinical symptoms,\(^26\) the current findings provide support for the idea that systemic inflammation may have an early pathologic role in driving or accelerating some of the pathologic processes underlying late-life cognitive decline.\(^1\)

However, there are several alternative explanations that should be considered in the context of the current findings. For example, it is possible that systemic inflammation during midlife is a marker, not a cause, of neurodegenerative disease or neuronal or glial injury.\(^27\) In addition, inflammation may serve as a compensatory response to the emergence of neural proteopathies, such as β-amyloid, which have been shown in previous studies to generate a local inflammatory response.\(^28,29\)

Because most studies have used cognitive screening tests to explore inflammation-related cognitive decline,\(^2,22,24\) the degree to which systemic inflammation is associated with decline in discrete cognitive domains is
unclear. Our results indicate that episodic memory is more vulnerable to the effects of systemic inflammation, relative to language, executive function, and processing speed. These findings stand in contrast to the results from studies of midlife vascular risk factors, such as hypertension\textsuperscript{21} and elevated lipid levels,\textsuperscript{20} which are more strongly associated with declines in executive function and processing speed. Thus, inflammation- and vascular-related cognitive decline may result from pathologically or neuroanatomically distinct processes. Consistent with the current findings, systemic inflammation in rodent models has been found to lead to changes in hippocampal structure and function.\textsuperscript{30,31} Through multiple routes (e.g., vagal nerve signaling, circumventricular organs), activation of the peripheral inflammatory response can generate a neuroinflammatory response, which can, in turn, disrupt neural and glial functioning.\textsuperscript{32}

The additional absolute level of cognitive change associated with systemic inflammation was modest overall, but comparable to that which has been associated with vascular risk factors such as hypertension and diabetes.\textsuperscript{19,7,21} Our analysis of the total sample did not reveal a linear dose-response increase in rate of cognitive decline with higher levels of midlife inflammation. Rather, groups in the top 3 quartiles displayed similar rates of cognitive change. However, in race-stratified analyses, we observed the expected dose-response increase in cognitive decline among white, but not black, participants. A potential explanation for the absence of a dose-response increase among black participants is a heightened rate of attrition due to death and dropout among participants with high levels of systemic
inflammation during midlife (data available from Dryad [table e-5]: doi.org/10.5061/dryad.vn806pd), as black participants were overrepresented in the groups with higher levels of midlife inflammation (table 1 and data available from Dryad [table e-1]: doi.org/10.5061/dryad.vn806pd). Although we found evidence for an interaction between race and the linear specification of midlife inflammation composite score on cognitive change, this finding was neither reflected in our analysis of inflammation composite score quartiles nor observed in our analyses that examined CRP. Taken together, our results did not show strong support for a modifying effect of race on the association between midlife systemic inflammation and cognitive decline. However, additional studies using alternative markers of systemic inflammation will be needed to confirm this finding.

The current study has several strengths, including the use of a large, biracial, population-based sample, the long follow-up period, and the careful ascertainment of and adjustment for potentially confounding variables. However, there are several limitations. First, potential bias related to selective attrition represents a major limitation of the current study. Fifty-four percent of the sample dropped out or died before the final cognitive assessment and 17% of participants had only a baseline cognitive assessment. Accordingly, only data from a subset of participants who attended visit 5 contributed to inferences about cognitive decline during late-life. Given that participants with higher levels of systemic inflammation at baseline were more likely to drop out or die before the
final follow-up visit (data available from Dryad [table e-5]: doi.org/10.5061/dryad.vn806pd), it is possible that a survivorship effect may have biased our results, especially for participants with higher levels of systemic inflammation during midlife. However, our findings, which incorporated IPAW to mitigate the effect of differential attrition, suggest that our results are robust to selection bias. We should also note that the absence of more than 2 time points for the majority of the sample limits the study's ability to characterize the dynamic trajectory of cognitive decline related to midlife systemic inflammation. Future studies with more frequent cognitive assessments will be informative in this regard. Second, because our serial cognitive battery was limited to 3 measures, we were unable to provide a precise assessment of specific domains or a comprehensive assessment of all relevant cognitive domains. Third, although our analyses were adjusted for demographic confounders, vascular risk factors, and medical comorbidity, we are unable to fully rule out the possibility of bias related to residual confounding from unmeasured covariates, such as subclinical disease. Lastly, the current study used a panel of acute-phase proteins, which are upregulated following an inflammatory event, to assess systemic inflammation. The use of a broader assay of inflammatory markers, including inflammatory cytokines and chemokines, in future studies may facilitate a more nuanced understanding of pro- and anti-inflammatory signaling as it relates to cognitive decline. Despite these limitations, the current study provides support for an association between
midlife systemic inflammation and subsequent cognitive decline, and in doing so, provides additional evidence for an early pathogenic role of systemic inflammation in late-life neurocognitive impairment.

**Author contributions**


**Study funding**

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