ARIC Manuscript Proposal # 2865

PC Reviewed: 10/11/16 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Inflammatory biomarkers at midlife and late-life and brain atrophy in older adults: The ARIC Study

b. Abbreviated Title (Length 26 characters): Inflammation and MRI volume

2. Writing Group:

Writing group members: Keenan Walker (first and corresponding author); Ron Hoogeveen; Aaron Folsom; Christie Ballantyne; David Knopman; Beverly Gwen Windham;Cliff Jack; Rebecca Gottesman (last author)

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __KW___ **[please confirm with your initials electronically or in writing]**

First author: **Keenan Walker, PhD**
Address: Phipps 446; 600 North Wolfe Street; Baltimore, MD 21287

Phone: 626-840-6216 Fax: 410-955-0672
E-mail: kwalke26@jhmi.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).
Name: **Rebecca Gottesman, MD PhD**
Address: Phipps 446D; 600 North Wolfe Street; Baltimore, MD 21287

Phone: 410-614-2381 Fax: 410-955-0672
E-mail: rgottesm@jhmi.edu

3. Timeline: 3-6 months; manuscript submission winter 2017.

4. Rationale:

Both peripheral and central nervous system (CNS) inflammation have been identified as risk factors for cognitive decline and neurodegenerative disease in older adults. Blood derived markers of inflammation increase with age, and the levels of several proinflammatory proteins

have been linked to late-life cognitive functioning and dementia risk [1–4]. The peripheral immune response, which regulates inflammation outside of the CNS, can communicate with the brain through both neural and humoral routes, triggering changes in glial and neuronal functioning [5–7]. Several studies have demonstrated that blood, CSF, and parenchymal levels of proinflammatory molecules are elevated in individuals with cognitive impairment, Alzheimer's disease (AD), and vascular dementia [2,3,8–11]. Although older adults with mild cognitive impairment or dementia express higher levels of inflammatory molecules in blood, CSF, and brain parenchyma, it remains unclear whether this heightened innate immune response is driving neurodegenerative changes, or if it simply constitutes a secondary response to the accumulation of misfolded protein and the degeneration of neural cells.

Brain volume loss, a marker of brain atrophy that has been associated with histopathological lesions, is used as a biomarker of the neurodegeneration that occurs in the preclinical phase of Alzheimer's disease and vascular dementia [12–14]. Despite what is known about the association between peripheral inflammation and dementia risk, few studies have evaluated the relationship between inflammation and brain volume loss in older adults. Previous findings have demonstrate that elevated levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and other inflammatory mediators (CRP, VCAM-1, plasminogen activator inhibitor 1) are associated with reduced whole brain and regional gray matter volume [3,15–17]. Because of the cross-sectional nature of previous studies, it remains unclear whether systemic inflammation is a cause or a consequence of the neurodegenerative changes observed on the MRI in late-life.

The cascade of pathological processes that take place preceding the most common form of dementia, Alzheimer's disease, begins as early as two to three decades before the onset of clinically defined dementia [18–20]. Thus, the pathophysiological processes driving neurodegeneration may begin many years before the onset of frank cognitive decline [21]. If the peripheral inflammatory response does play a causal role in the onset and progression of Alzheimer's and other neurodegenerative disease, peripheral inflammation during midlife would likely be a strong predictor of disease onset and progression. To date, no study has examined whether heightened peripheral inflammation in midlife, before the onset of disease-related neurodegenerative changes, predicts subsequent brain volume loss in late-life. Without this understanding of the temporal relationship between peripheral inflammation and cerebral atrophy, it is difficult to determine whether peripheral inflammation has a causal or a bystander role in the neurodegenerative processes that precede the onset of dementia.

The goal of the current study is to improve the understanding of the temporal relationship between systemic inflammation and neurodegeneration by examining how plasma markers of inflammation measured at midlife and late-life relate to reductions in brain volume among older adults. Associations between peripheral inflammation and the volume of brain regions most susceptible to *Tau* and amyloid-β accumulation and Alzheimer's-specific atrophy will be assessed to help inform whether peripheral inflammation may be tied to Alzheimer's disease pathology. Given the established link between inflammation and metabolic syndrome, an additional goal will be to examine whether there is an interaction between peripheral inflammation and metabolic syndrome at midlife on brain volume reductions in late life. Race-based differences in the strengths of these associations will be examined as well to explore

whether inflammation-related brain changes are more pronounced among black participants who, on average, experience higher rates of metabolic syndrome.

5. Main Hypothesis/Study Questions:
1). Higher levels of peripheral inflammatory markers measured in midlife (visit 1 and visit 2) and late-life (visit 5/NCS) will be associated with reduced whole brain volume on brain MRI in late-life (visit 5/NCS).

2). Higher levels of peripheral inflammatory markers measured in midlife (visit 1 and visit 2) and late-life (visit 5/NCS) will be associated with reduced regional brain volume in regions vulnerable to Alzheimer's disease pathology.

3). Compared to late-life CRP levels, midlife CRP levels will be a stronger predictor of brain volume in analyses #1 and #2.

4). There will be an interaction between plasma inflammatory markers at midlife and metabolic syndrome on measures of whole and regional brain volume examined in analyses #1 and #2 whereby those with metabolic syndrome and highest level of peripheral inflammation at visit 1 will demonstrate the greatest reductions of global and regional volume on MRI.

5). The associations between plasma inflammatory markers and MRI measures of brain volume examined in #1 and #2 will be stronger in black participants compared to white participants.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Inclusion/Exclusion Criteria: Participants will be included on the basis of 1) having received a brain MRI at visit 5 as part of the ARIC-NCS, and 2) having inflammatory biomarkers collected during midlife (visit 1 or visit 2) and/or late-life (visit 5). Participants who have documented neurological conditions (e.g., clinical stroke, TBI with residual cognitive impairment) or received treatment (e.g., radiation or chemotherapy) that is likely to alter brain MRI volumes will be excluded from the analyses. Participants will also be excluded on the basis of heavy alcohol use at visit 5 (defined as more than 14 drinks per week; NIAAA, 2007).

Outcome Variables
Whole brain volume (WBV): WBV will be used as an overall measure of parenchymal volume loss. WBV will be extracted from the ARIC-NCS MRI scans obtained at visit 5/NCS; all analyses using this variable will include adjustment for total intracranial volume.

Regional gray matter volume: Several gray matter structures known to be susceptible to atrophic changes in the earliest stages of Alzheimer's disease will be specified as regions of interest (ROIs). ROIs will include the hippocampus, medial temporal lobe, inferior temporal cortex, medial prefrontal cortex, anterior cingulate cortex, posterior cingulate cortex, the inferior parietal lobule, and the primary sensory and motor cortex (as control regions). In addition, we will evaluate the composite variable, "Alzheimer's Disease Signature Region" which has already

been created in ARIC. Regional gray matter volumes will also be extracted from ARIC-NCS MRI scans obtained at visit 5/NCS. Gray matter volume of will be calculated using a semi-structured parcellation program.

Additional Variables

Plasma Inflammatory Markers: plasma levels of inflammatory biomarkers will be extracted from ARIC visits 1, 2, and 5 for each participant. The list of inflammatory markers to be extracted at each visit is provided in the table below.

Available in Full Cohort		
Visit 1 (87-89)	**Visit 2 (90-92)**	**Visit 5 (11-13)**
WBC		
Fibrinogen		
Albumin		
vWF		
Factor VIII		
	CRP	CRP
	LpPLA2	

Note: Lp-PLA2 = Lipoprotein-associated phospholipase A2; WF = von Willebrand factor

Demographic variables, including race, sex, age, APOE genotype, and center will be extracted from ARIC visit 1, visit 2, and visit 5/NCS. Additionally, cardiovascular risk factors including hypertension, systolic and diastolic blood pressures, diabetes diagnosis, hypercholesterolemia diagnosis, smoking status, BMI, and prior cardiovascular disease will be assessed from ARIC visit 1, visit 2, and visit 5/NCS. Based on findings from previous studies, the following variables will also be extracted for potential use as covariates: total/high density lipoprotein cholesterol, triglycerides, fasting glucose, hemoglobin A1C, homocysteine, use of hormone replacement therapy, and use of lipid lowering drugs. Variables that may affect inflammatory status, including the presence of specific autoimmune disease, chronic inflammatory diseases (e.g., rheumatoid arthritis), and use of anti-inflammatory drugs will be extracted as well.

Data Analysis.

Hypotheses 1, 2, 3, and 5: To examine the relationship between individual biomarkers and MRI variables, each biomarker from visit 1, visit 2, and visit 5 will be categorized into quartiles (Q1, lowest; Q2, lower middle; Q3, upper middle; Q4 highest). The lowest category will serve as the reference group to which the individual upper categories will be compared on MRI outcome variables. To examine the effect of multiple heightened inflammatory markers, patients will be classified into three groups based on the number of inflammatory markers classified into the highest quartile at visit 1 (T1, 0; T2, 1-2; T3, 3-5). The lowest category (T1) will serve as the reference group to which the individual upper categories will be compared on MRI outcome measures. To examine the effect of overall inflammatory burden, an inflammatory composite score will be created using the five inflammatory biomarkers available at Visit 1 (i.e., WBC, fibrinogen, albumin, von Willebrand factor, factor VIII). The inflammatory composite score will be created by summing the biomarker levels after each is rescaled to a z-scores based on the sample mean. Covariate-adjusted linear regression and logistic regression will be used to compare groups on continuous and categorical MRI outcome variables, respectively. The following covariates will be included as covariates in the initial multivariable regression model: age, sex, education, race-center, APOE e4 status, systolic and diastolic blood pressures, diabetes

diagnosis, fasting glucose, homocysteine, BMI, lipid lowering treatment, and cardiovascular disease burden. To examine the role of midlife vs. late-life inflammation on MRI metrics, the main effect of visit 2 CRP and visit 5 CRP on MRI outcome variables will be compared. The sample will then be stratified based on race and these analyses will be repeated.

Hypotheses 4: To examine the inflammatory marker by metabolic syndrome interaction on interaction on brain volume, participants will be stratified into four groups according to the presence or absence of metabolic syndrome (defined using the American Heart Association criteria; Grundy, Cleeman, Daniels, & Donato, 2005) and presence of low vs. high visit 1 inflammatory composite score (median split). The interaction between metabolic syndrome and low vs. high inflammatory composite score on whole brain volume and Alzheimer's Disease Signature Region volume will be examined using covariate-adjusted regression models. For all analyses, covariates from the visit during which the inflammatory markers were derived will be used.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __X__ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? ____ Yes ____ No
(This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? __X__ Yes ____ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? __X__ Yes ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.
ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php

___X___ Yes _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

# 2551	Midlife and late life vascular risk factors and white matter integrity assessed using diffusion tensor imaging: the ARIC-NCS study
# 2351	Association of blood pressure with neurodegenerative and cerebrovascular changes on brain MRI
#1771	Cognitive, vascular risk factor and APOE genotype predictors of hippocampal volume
# 2266	Associations between brain vascular imaging features and regional volumetrics
#1735	Inflammation mediates the impacts of fatty acids on CHD and ischemic stroke incidence: the Atherosclerosis Risk in Communities (ARIC) Study
#2203	Chronic inflammation and race-ethnic disparities in ischemic stroke: the ARIC study

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? _X___ Yes ____ No

11.b. If yes, is the proposal

__X_ A. primarily the result of an ancillary study (list number* _2008.06_)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.
Understood

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes _X___ No.

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