ARIC Manuscript Proposal # 2866

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

1.a. Full Title: The association of midlife and late-life inflammatory biomarkers with cerebral small vessel disease and white matter integrity in the elderly: The ARIC Study

b. Abbreviated Title (Length 26 characters): Inflammation, WMH, and DTI

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; Susumo Mori; Melinda C. Power; Rebecca Gottesman

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.

Cerebral small vessel disease, which includes white matter hyperintensities, silent infarcts, and microbleeds, has been consistently associated with cognitive decline[12] and elevated dementia risk[13–15]. Longitudinal studies have demonstrated that patients with greater levels of cerebral small vessel disease display faster rates of cognitive decline independent of amyloid-β pathology[16]. Similarly, the presence of white matter microstructure abnormalities in patients with mild cognitive impairment increases risk for progression to dementia [19]. Together, these findings suggest that cerebral small vessel disease and white matter dysfunction play a key role in the pathogenesis of Alzheimer's and vascular dementia.

Currently, the relationship between peripheral inflammation, cerebral small vessel disease, white matter integrity, and the development of dementia is unclear. Several inflammatory mediators are known to have prothrombotic and proatherosclerotic effects [20–22], which may in turn promote cerebral small vessel disease and subsequent white matter damage. Multiple inflammatory mediators are also known to promote endothelial[23,24] and microcirculatory dysfunction[25,26], which may lead to blood brain barrier disruption, hypoperfusion, and subsequent disruption to neuronal and glial functioning [27]. On the basis of these findings, it is possible that peripheral inflammation contributes to the development of Alzheimer's and vascular dementia by promoting the development of cerebral small vessel disease and white matter dysfunction. Few studies have examined the relationship between cerebral small vessel disease, white matter integrity, and peripheral inflammation [28–32], and it remains unclear whether peripheral inflammation constitutes causes or consequence of these pathological brain changes. To date, no study has examined whether heightened peripheral inflammation in midlife, before the typical onset of small vessel disease, predicts the development of late-life WMH, silent infarcts, microbleeds, or white matter microstructural abnormalities.

The goal of the current study is to improve the understanding of the temporal relationship between peripheral inflammation, cerebral small vessel disease, and white matter integrity by examining how plasma markers of peripheral inflammation measured at midlife and late-life relate to WMH volume, the presence of silent infarcts and microbleeds, and white matter microstructure integrity among older adults. If peripheral inflammation does in fact play a causal role in the development of cerebral small vessel disease and white matter dysfunction, it's likely that measures of midlife peripheral inflammation will be most predictive of the development of these late-life neuroimaging abnormalities. Given the established link between inflammation and metabolic syndrome, an additional goal will be to examine whether there is an interaction between inflammation and metabolic syndrome at midlife on cerebral small vessel disease and

white matter integrity 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 at midlife (visit 1 and visit 2) and late-life (visit 5) will be associated with the presence of silent infarcts, cerebral microbleeds, and greater white matter hyperintensity volume (WMH) in late-life.

2). Higher levels of peripheral inflammatory markers at midlife (visit 1) and late-life (visit 5) will be associated with reduced white matter integrity as measured using MRI diffusion tensor imaging (DTI; lower fractional anisotropy and higher mean diffusivity).

3). Compared to late-life CRP levels, midlife CRP levels will be a stronger predictor of WMH volume, silent infarcts, cerebral microbleeds, and white matter integrity in #1.

4). There will be an interaction between plasma inflammatory markers at midlife and metabolic syndrome on measures of white matter integrity whereby those with the highest level of metabolic syndrome and highest levels of peripheral inflammation at visit 1 will demonstrate the greatest levels of late-life DTI-defined white matter damage and WMH volume.

5). Associations between plasma inflammatory markers and MRI measures examined in #1 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
White matter hyperintensity volume (WMH): WMH scores will be derived from proton density-weighted images extracted from the ARIC-NCS MRI scans obtained at visit 5/NCS. WMH burden will be determined using a quantitative computer-aided segmentation program which uses an algorithm to segment fluid-attenuated inversion recovery (FLAIR) images (FLAIR-histoseg) to measure the volumetric burden of leukoaraiosis [33]. All analyses using WMH will include adjustment for total intracranial volume.

Subclinical and lacunar infarction: The presence of subclinical infarction and lacunar infarction will be determined for each patients using the ARIC-NCS MRI scans obtained at visit 5/NCS.

Subclinical infarction will be defined as cortical and subcortical infarctions >3mm in size that do not correlate in time with the onset of neurological symptoms. Lacunar infarctions will be defined as subcortical infarctions between >3mm and <20mm in size [34].

Cerebral microbleeds: The presence of cerebral microbleeds will be determined for each patient using the T2* GRE MRI sequences from the ARIC-NCS MRI scans obtained at visit 5/NCS. Microbleeds will then be classified according to their location as cortical or subcortical.

White matter microstructure: Diffusion tensor imaging (DTI) will be used to evaluate axonal integrity. Measures of mean diffusivity (MD) and fractional anisotropy (FA) will be extracted for the following regions: uncinated fasciculus, superior lateral fasciculus, genu and splenium of the corpus callosum, and whole brain. DTI imaging will be extracted from the ARIC-NCS MRI scans obtained at visit 5/NCS.

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.

Hypothesis 4: To examine the inflammatory marker by metabolic syndrome interaction on WMH volume and whole-brain DTI defined FA and MD, participants will be stratified into one of 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 WMH and DTI defined FA and MD will be examined using covariate-adjusted linear 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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