ARIC Manuscript Proposal # 3011

PC Reviewed: 7/11/17	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Systemic inflammation and brain amyloid deposition: The ARIC-PET Study

b. Abbreviated Title (Length 26 characters): Inflammation and brain amyloid

2. Writing Group:

Writing group members: Keenan Walker (first and corresponding author); Beverly Gwen Windham; Charles Brown; David Knopman; Cliff Jack; Elizabeth Selvin; Dean Wong; Thomas Mosley; Timothy M. Hughes; Yun Zhou; Rebecca Gottesman (last author); Others welcome

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]**

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

4. Rationale:

The amyloid cascade hypothesis proposes that the accumulation of amyloid β (Aβ) or Aβ oligomers and the deposition of cortical Aβ plaques are key causative processes in the pathophysiology of Alzheimer's disease[1]. There is also considerable evidence suggesting that immune mediated pathways play a central role in driving the neurodegenerative changes that

occur in Alzheimer's disease[2]. Polymorphisms in genes involved in immune and microglial functioning[3–5] are known to confer risk for late-onset Alzheimer's disease, and elevations in inflammatory markers have been documented in the CSF[6] and blood[7,8] of individuals with mild cognitive impairment (MCI) and Alzheimer's dementia. Based on reports which show that repeated peripheral immune challenges enhance neuroinflammation and cerebral Aβ production in mice [9–11], it has been postulated that systemic inflammation may perpetuate or even drive the deposition of Aβ in the brains of elderly individuals. Although systemic inflammation has been associated with cognitive decline[12], dementia risk[8], and MRI-defined neurodegenerative changes[13], it remains unclear whether systemic inflammation during late-life, or during the preceding decades, is associated with cortical Aβ deposition in older adults.

In this study, we will determine whether there is an association between circulating levels of high-sensitivity C-reactive protein (CRP), a non-specific marker of systemic inflammation, and brain amyloid deposition, as measured by positron emission tomography (PET), within a biracial, non-demented group of participants in the Atherosclerosis Risk in Communities (ARIC) Study. CRP was measured 21 and 14 years before and concurrent with PET imaging, allowing us to test the hypothesis that individuals with chronically elevated systemic inflammation are at greatest risk for cortical Aβ deposition in late-life. In light of evidence for a higher burden of cortical amyloid among elderly African Americans[14] and among those with one or more *APOE* ε4 alleles[14], we will also examine whether the relationships between systemic inflammation and cortical Aβ deposition is stronger among African American and *APOE* ε4-positive individuals if sample size permits.

5. Main Hypothesis/Study Questions:

H1. Greater late-life systemic inflammation, as measured by CRP levels at Visit 5, will be associated with greater global cortical Aβ deposition by PET.

H2. Greater midlife systemic inflammation, as measured by CRP levels at Visits 2 and 4, will be associated with greater global cortical Aβ deposition by PET.

H3. Individuals who show a longitudinal pattern of elevated systemic inflammation (CRP > 3 mg/L) from mid- to late-life (Visits 2 or 4 to Visit 5) will have greater levels of Aβ deposition by PET.

H4. Associations observed in #1, 2, and 3 will be independent of vascular risk factors.

H5. Associations observed in #1 and 2 will be stronger among African American participants and among individuals with one or more *APOE* ε4 allele.

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).

Participants

We will include all participants who completed the ARIC-PET study in this analysis (*n*= 346 completed scans). One additional participant was unable to complete the scan, so her data will not be used.

Inclusion criteria: Only participants who met inclusion criteria for ARIC-PET will be included in this analysis. To exclude likely cases of dementia, ARIC-PET participants had a CDR of 3 or lower, an FAQ of 5 or lower, and a brain MRI (from ARIC-NCS) within 12 months of recruitment. All participants were required to be able to give their own consent.

Exclusion criteria: ARIC PET excluded individuals with history of: (1) radiation therapy, chemotherapy, or surgery in the 6 weeks preceding the ARIC-PET visit; (2) clinically significant liver or renal dysfunction; (3) prolonged QT interval; (4) drug or alcohol abuse. Participants with a "low" MMSE (<19 for African-Americans and <21 for Caucasians) at the time of Visit 5/ NCS were also excluded, in addition to those meeting criteria for dementia based on the CDR and FAQ as described above. Participants taking anticholinergic medications or memantine were included only if their dose was stable for ≥3 months preceding the PET study. Inclusion and exclusion criteria has been described in greater detail previously[14].

Only participants with one or more available CRP levels will be included in this analysis. Analyses which examine the longitudinal pattern of CRP will only include participants with CRP levels available for Visits 2, 4, and 5.

Outcome

Standardized Uptake Volume Ratio (SUVR): SUVR is a measure of florbetapir (amyloid) in pre specified regions of interest derived from the ARIC-PET study. Global mean cortical SUVR is a weighted average (based on region-of-interest (ROI) volumes) of regions known to be typically impacted in Alzheimer's disease. The SUVR's will be evaluated at a cut-point of 1.2, with values >1.2 considered positive. Other cut-points used in the literature, including 1.1 and 1.11, will also be explored in sensitivity analyses. SUVR may also be examined as a continuous variable after correcting for skewness using a transformation. The image processing protocol used for the ARIC-PET has been described in detail previously[14].

Exposure

Plasma Inflammatory Markers: We will use CRP levels, previously measured, from Visits 2, 4, and 5 for all participants included in the ARIC-PET study. We will also evaluate several additional inflammatory biomarkers that were assessed from blood at Visit 4 in a subset of participants for exploratory analyses: interleukin 1 beta (IL1-β), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and intercellular adhesion molecule-1 (ICAM-1).

Inflammatory Biomarkers		
Visit 2 (90-92)	**Visit 4 (96-99)**	**Visit 5 (11-13)**
CRP	CRP	CRP
	IL1-β	
	IL-6	
	TNF-α	
	ICAM-1	

Other Variables

Demographic variables, including race, sex, age, *APOE* genotype (0, 1, or 2 ε4 alleles), and center will be extracted. Additionally, laboratory and physiologic data, including systolic and diastolic blood pressures, total/high density lipoprotein cholesterol, triglycerides, and BMI

(kg/m^2) will be extracted from study Visits 2, 4, and 5. Cardiovascular risk factors and disease information (i.e., diabetes, hypertension, coronary heart disease, cigarette smoking and alcohol use), previous cancer diagnoses, and medication use (i.e., anti-inflammatory medication and statins) will also be extracted from Visits 2, 4, and 5. Information about chronic inflammatory disease diagnoses (i.e., lupus, gout, and arthritis) will be extracted from Visit 4. Cognitive status at the time of PET imaging (normal/MCI) will be extracted from Visit 5.

Data Analysis

We will examine **H1** and **H2** using logistic and linear regression models with SUVR as a binary and continuous dependent variable, respectively. We will evaluate a model which adjusts for potentially confounding demographic variables (model 1) and a second model which will additionally adjust for physiologic/laboratory variables, and cardiovascular and inflammatory risk factors (model 2; **H4**). Multiplicative interaction terms and stratification will be used to examine effect modification by race and *APOE* ε4 allele status (**H5**). We recognize that our ability to assess effect modification may be limited due to the relatively small sample size. Values for covariates that change with time will be derived from the visit concurrent with inflammatory biomarker assessment, when available.

To examine the association between the longitudinal pattern of CRP levels and SUVR (**H3**), each participant will be categorized as having "low" or "high" CRP levels at each visit using a cut-off of 3 mg/L. A CRP level above 3 mg/L is suggestive of ongoing low-grade systemic inflammation[15,16,17]. Using this "low" versus "high" CRP dichotomization, participants will be categorized into one of six categories based on their patterns of CRP over three Visits (Figure 1).

- *Stable low*: low CRP levels at all three visits
- *Early ascending*: low CRP at Visit 2, and high CRP at Visits 4 and 5
- *Late ascending:* low CRP at Visits 2 and 4, and high CRP at Visit 5
- *Early descending:* high CRP at Visit 2, and low CRP at Visits 4 and 5
- *Late descending:* high CRP at Visits 2 and 4, and low CRP at Visit 5
- S*table high:* high CRP at Visits 2, 4, and 5.

Using the *stable low* group as the reference, covariate-adjusted logistic and/or linear regression will be used to examine the relationship between group status and SUVR using model 1 and model 2. Covariates assessed at the time of the baseline CRP assessment (Visit 2) will be used. Given that the relatively small sample size, it may be necessary to collapse multiple groups. For example, we may combine the "early descending" and "late descending" groups and the "early ascending" and "stable high" groups.

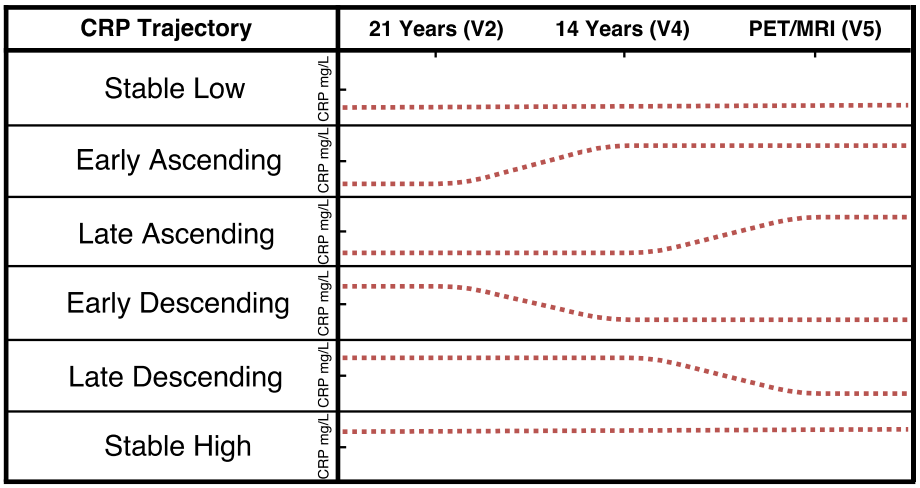

Figure 1. An illustration of the six longitudinal patterns of C-reactive protein using data from Visits 2, 4, and 5.

We will also consider using alternative approaches to model CRP levels longitudinally if sample size does not permit use of the categorical approach described above. We may use latent growth curve modeling to classify participants with similar longitudinal patterns of CRP into groups. These groups will then be compared on SUVR. Alternatively, we may examine the association between the average CRP level measured across three time points and late-life SUVR.

Covariates to be included in model 1 are age, sex, race, and education. The additional covariates to be included in model 2 will be cigarette smoking and alcohol use (current/former/never) status, BMI, hypertension, diabetes, total cholesterol, HDL, triglycerides, coronary heart disease, chronic inflammatory conditions, previous cancer, regular and current anti-inflammatory medication use, statin use, and *APOE* ε4 status.

A sensitivity analysis will be conducted using inverse probability of attrition weighting (IPAW) to assess the potential effects of differential attrition due to death or dropout. We may also consider sensitivity analyses to examine the effect of cognitive status (normal cognition vs. MCI) and use of alternative florbetapir cut-points. A two-sided p value < .05 will be used as the cutoff for statistical significance. Analyses will be conducted using Stata Version 14 (StataCorp, College Station, Tex., USA).

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)?

MP# 2544. Arterial stiffness and beta-amyloid deposition in the ARIC-PET study. Hughes et al.
MP# 2511. Vascular risk factors and brain amyloid deposition: The ARIC-PET Study. Gottesman et al.
MP# 2822. Subclinical cerebrovascular disease and brain amyloid deposition: The ARIC-PET Study. Gottesman et al.
MP# 2865. Inflammatory biomarkers at midlife and late-life and brain atrophy in older adults: The ARIC Study. Walker et al.
MP# 2866. The association of midlife and late-life inflammatory biomarkers with cerebral small vessel disease and white matter integrity in the elderly: The ARIC Study. Walker et al.

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* __2009.29__)
___ 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.

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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