ARIC Manuscript Proposal #4050

PC Reviewed: 5/17/22 **Status: _____** **Priority: 2**
SC Reviewed: ___/___/___ **Status: _____** **Priority: ____**

1.a. Full Title: Inflammatory markers and calcification of coronary arteries, aorta and cardiac valves in older adults: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Inflammatory markers and cardiac calcification

2. Writing Group:

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

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3. Timeline: Analyses and manuscript preparation will be performed over the next 6 months.

4. Rationale:

Inflammation plays an important role in the pathophysiology of atherosclerosis [1]. For example, atherosclerotic plaques have inflammatory infiltrate [2], and often inflammation occurs in conjunction with lipid accumulation in the arteries [3]. Several molecules such as vascular cell adhesion molecule-1 [1] and monocyte chemoattractant protein-1 contribute to leukocytes

penetration into the intima of the artery [1], and leukocytes initiate a local inflammatory response [4]. Indeed, a number of epidemiological studies have demonstrated a strong link between inflammatory markers (e.g., C-reactive protein [CRP], erythrocyte sedimentation rate, and procalcitonin) and the pathophysiology of atherosclerotic disease risk [5-11].

However, surprisingly, the relationship between inflammatory markers (often CRP) and a representative measure of subclinical atherosclerosis, coronary artery calcium (CAC) [12, 13], has been found to be weak [14-20]. More specifically, a systematic review of 12 studies showed that in the relationship was attenuated after adjusting for traditional risk factors such as body mass index (BMI) [16]. For example, a cross-sectional study of 914 asymptomatic participants at a United States preventive cardiology clinic found that plasma CRP levels and CAC had a weak association in women (odds ratio [OR] = 1.1 [95% CI 1.04 – 1.17]) but no association in men and that this association in women was lost after adjustment for BMI (OR = 1.02 [95% CI 0.94 – 1.08]) [20]. Two studies from the Multi-Ethnic Study of Atherosclerosis (MESA) have reported basically similar patterns (i.e., non-significant results once accounting for traditional atherosclerotic risk factors) [14, 15]. Although it is not entirely clear, these weak/lack of associations could reflect the observation in some animal studies that inflammation may be more strongly related to rupture and thrombosis rather than the development of atherosclerosis [21, 22].

Moreover, the existing literature does not greatly explore the relationship between inflammatory markers such as CRP and extra coronary calcium (ECC), which may have a different association than CAC. Of importance, there is a growing body of literature reporting the additional prognostic value of ECC beyond CAC [23-30]. Moreover, different vascular beds may have differing pathophysiological mechanisms. For example, calcification of the aorta has been observed to occur in its various segments (ascending thoracic, descending thoracic, and abdominal) at different rates and with different associations with cardiovascular events and mortality [31, 27, 24, 32-34]. Valvular calcification, such as in the mitral and aortic valve, has also been thought to be a manifestation of generalized atherosclerosis but can also be accelerated when there is mechanical stress on the valve such as in hypertension [35, 36]. Thus far, ECC in relation to inflammatory markers has been studied by only 2 studies [37, 38], with one using data from MESA showing a weak association of interleukin-6 with the presence and severity of descending thoracic aorta calcification [37] and the other demonstrating CRP associated with aortic valve calcification. However, to the best of our knowledge, no studies have explored multiple biomarkers and their associations with CAC and ECC in a single study population.

To fill these gaps, we seek to evaluate the associations of a few inflammatory markers (i.e., high-sensitivity CRP [hs-CRP] and galectin-3) with CAC and ECC (aortic valve, thoracic aorta, mitral valve) in the Atherosclerosis Risk in Communities (ARIC) study. This study will give us a unique opportunity to study inflammatory markers at both mid-life and late-life and their contributions to vascular and valvular calcification at ~75 years or older.

5. Main Hypothesis/Study Questions:

- Inflammatory markers (i.e., hs-CRP and galectin-3) measured in middle and late life will be associated with CAC measured later in life independently of traditional atherosclerotic risk factors.
- Inflammatory markers (i.e., hs-CRP and galectin-3) will be associated with ECC independently of traditional atherosclerotic risk factors.

- The associations of each inflammatory marker will be different across CAC and ECC (aortic valve, thoracic aorta, and mitral valve)

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).

Study design: We will evaluate whether the inflammatory markers, hs-CRP and galectin-3 at middle age (visit 4, at which galectin-3 was first measured in ARIC but secondary visit 2 for hs-CRP) are associated with CAC and ECC at the age of 75 or older.

- Secondarily, we will also evaluate if the inflammatory markers at late (visits 5 and 6) life are associated with CAC and ECC at the age of 75 years or older. Since the development of calcification is a lengthy process, we will apply a lag time and thus are not planning to use inflammatory marker data from visit 7 in this study.

Inclusion

- All ARIC participants for whom information on hs-CRP, galectin-3, CAC, and ECC is available.
    - CT scan to evaluate CAC and ECC was done among participants without a history of coronary heart disease (CHD) prior to the end of 2015

Exclusion

- Missing CAC or ECC data
- Missing data on both hs-CRP and galectin-3
- Missing data on covariates

Exposures

hs-CRP was measured in a central laboratory on plasma stored at -70 degrees Celsius before analysis with an immunonephelometric assay on a BNII analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) according to the manufacturer's protocol. The reliability coefficient for the hs-CRP assay was 0.99. Galectin-3 levels were measured using a chemiluminescent microparticle immunoassay on an Architect I 2000sr instrument (Abbott Diagnostics, Abbott Park, IL) in EDTA-plasma samples collected at visit 4 and stored at -70 degrees Celsius before analysis. The measurements were performed between July 2015 and February 2016. The split sample reliability coefficient was 0.92.

Covariates of interest: socio-demographic characteristics (age, race, gender, education), alcohol intake, smoking status, family history of CHD, BMI, history of stroke, history of heart failure, blood pressure, use of antihypertensive medication, diabetes (fasting blood glucose ≥126 mg/dl, non-fasting glucose ≥ 200 mg/dl, reported a history of diabetes, or use of diabetes medication), lipid parameters (total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride), and lipid lowering therapy.

Outcomes

- Calcification of coronary artery and vascular beds other than coronary arteries

- CAC measured by non-contrast CTs were calculated using the Agatston method. CAC will be modeled as a binary outcome (ex. >0 vs. 0, >100 vs. ≤ 100, >400 vs. ≤400, >1000 vs. ≤ 1000, and >75th percentile vs ≤ 75th percentile) and as a continuous outcome (ln[CAC+1]).
- ECC includes calcification at five sites: aortic valve, aortic valve ring, mitral valve, ascending aorta, and descending aorta. This will also be modeled as a binary and continuous outcome.

Statistical Analysis:

1. We will summarize baseline characteristics according to quartiles of hs-CRP and galectin-3
2. We will quantify the association between hs-CRP and galectin-3 with CAC and ECC using logistic regression models with CAC and ECC as dichotomous dependent variables and linear regression models with CAC and ECC as continuous dependent variables. hs-CRP and galectin-3 will be modeled categorically (e.g., quartiles) and continuously (spline terms).
3. We will adjust our models for potential covariates and confounders including age, sex, race, smoking status, diabetes, blood pressure, total and HDL cholesterol, BMI, hypertension medication use, and cholesterol medication use (e.g., statin).
4. We will conduct a few sensitivity analyses such as taking the average of inflammatory marker levels across visits, time-varying analysis of the inflammatory markers, subgroup analyses by sex, race, and use of cholesterol lowering medications, and exclusion of those with a history of myocardial infarction or stroke.

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes __X__ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ____ Yes ____ No
(The 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? ____ Yes _X___ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the current derived consent file ICTDER05 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ____ 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/aricproposals/dtSearch.html

__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)?
To the best of our knowledge, there are no proposals exploring inflammatory markers and their associations with CAC and ECC in ARIC.

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

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* _____)
___ 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 https://sites.cscc.unc.edu/aric/approved-ancillary-studies

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.

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