ARIC Manuscript Proposal # 3106

PC Reviewed: 1/9/2018	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Inflammatory markers and future risk of peripheral artery disease

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

2. Writing Group:

Writing group members: Ning Ding, Shoshana H. Ballew, Corey Andrew Kalbaugh, Ron C. Hoogeveen, Josef Coresh, Elizabeth Selvin, Christie M. Ballantyne, Kunihiro Matsushita

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

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3. Timeline: Data to be used in this proposal are already available, except cytokines/matrix metalloproteinases (MMPs). Analyses and manuscript preparation will be performed within 6 months. Analysis of cytokines/MMPs will start when relevant data become available.

4. Rationale:

Inflammation plays an important role in the development and progression of atherosclerosis [1, 2]. Moreover, chronic systematic inflammation has been shown to be associated with increased risk of cardiovascular disease [3, 4]. Indeed, several inflammatory markers, e.g. white blood cells (WBC), high-sensitivity C-reactive protein (hsCRP), interleukin-

6 (IL-6), tumor necrosis factor (TNF-α) have been reported to be related to cardiovascular disease, such as coronary artery disease and stroke [5-10].

However, the association of inflammatory markers with incident peripheral artery disease (PAD) is not fully characterized. Although there are a number of cross-sectional studies [11-19], prospective studies were limited to a few inflammatory markers such as hsCRP [20-24], WBC [24], and IL-6 [20]. Also, those prospective studies investigated specific populations such as only whites [20, 21], only male [22] or female [23], or patients with chronic kidney disease [24].

Therefore, to comprehensively quantify the association of various inflammatory markers (hsCRP, WBC, IL-1β, IL-6, IL-10, IL-18, TNF-α, GDF-15, MMP-1, MMP-2, MMP-7, TIMP-1) with incident PAD/CLI in general population, we will study longitudinal data in the Atherosclerosis Risk in Communities (ARIC) Study.

5. Main Hypothesis/Study Questions:

Hypothesis 1: Inflammatory markers are associated with an increased risk of incident PAD/CLI.
Hypothesis 2: Inflammatory markers are cross-sectionally associated with a lower ankle-brachial index (ABI).

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).

Inclusions:

- All black and white ARIC participants who provided data on inflammatory markers (see Table) and had outcome information of ABI (see Table) or incident PAD.

Table. Available information on inflammatory markers and ABI at visit 1-5

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Longitudinal with incident PAD	WBC	WBC hsCRP	WBC	WBC hsCRP	hsCRP cytokines* MMPs**
Cross-sectional with ABI	WBC	NA (ABI not measured)	WBC (ABI in subsample)	WBC hsCRP (ABI in subsample)	hsCRP cytokines* MMPs**

* IL-1β, IL-6, IL-10, IL-18, TNF-α, GDF-15; ** MMP-1, MMP-2, MMP-7, TIMP-1

Exclusions:

- Ethnicity other than black or white
- Missing data on inflammatory markers of interest
- Preexisting PAD at baseline defined as a history of leg revascularization, ABI ≤0.9, or intermittent claudication (for longitudinal analysis)
- Missing data on outcomes of interest and relevant baseline covariates

Exposures (independent variables):

See Table above

Outcomes (dependent variables):
- PAD and critical limb ischemia (CLI): PAD-related hospitalizations will be identified according to the following ICD codes based on previous literature [25, 26]: atherosclerosis of native arteries of the extremities, unspecified (440.20); atherosclerosis of native arteries of the extremities with intermittent claudication (440.21); atherosclerosis of native arteries of the extremities with rest pain (440.22); atherosclerosis of native arteries of the extremities with ulceration (440.23); atherosclerosis of native arteries of the extremities with gangrene (440.24); other atherosclerosis of native arteries of the extremities (440.29); atherosclerosis of bypass graft of the extremities (440.3); atherosclerosis of other specified arteries (440.8); peripheral vascular disease, unspecified (443.9); leg artery revascularization (38.18, 39.25, 39.29, 39.50). Of these, 440.22, 440.23, and 440.24 will be considered CLI. Also, we will consider any cases with the above code as CLI when the following codes coexist: leg amputation (84.1x), lower extremity ulcer (707.1x), and gangrene (785.4).
- ABI as a categorical (e.g. ABI<0.9) or continuous variable

Covariates:
- Sociodemographics: age, race, gender, education level
- Physical information: body mass index, systolic blood pressure, diastolic blood pressure
- Lifestyle: smoking and alcohol habit
- Comorbidities: obesity, dyslipidemia, diabetes, hypertension, antihypertensive medication use, cholesterol-lowering medication use, kidney function, and history of coronary heart disease or stroke

Statistical Analysis:
- For those inflammatory markers that were not normally distributed (e.g., hsCRP), the log-transformation will be performed.
- The inflammation markers will be divided into quartiles according to the distribution among study population. The inflammatory markers will also be categorized based on clinical cutoffs whenever available (e.g., hsCRP will be categorized to <1, 1-3, ≥3 mg/L [4]).
- For longitudinal analysis of inflammatory markers of interest and incident PAD/CLI, we will use Cox proportional hazards models to quantify the associations between inflammatory markers and incident PAD/CLI. Associations will be expressed as hazard ratios both by quartile using the lowest quartile as reference category, and by per SD increment of inflammatory markers.
- For cross-sectional analysis of inflammatory markers and ABI, odds ratios both by quartile using the lowest quartile as reference category, and by per SD increment will be obtained using of logistic-regression models. We will also use linear regression models to quantify the association between inflammatory markers and ABI.
- We will take into account high ABI (e.g., >1.3) as a sensitivity analysis by including as PAD together with ABI <0.9 or excluding those with high ABI.
- We will adjust for the covariates listed above.
- We will use C-statistics to examine the additive predictive value of inflammatory markers to traditional risk factors model in discriminating participants into those who develop or not develop a PAD event during the follow-up.
- We will use likelihood ratio test to test for interaction by key demographic and clinical factors (e.g., age, sex, race, smoking and diabetes).

- As a sensitivity analysis, we will exclude people with high values of inflammatory markers indicative of acute inflammatory disease (e.g., hsCRP >10mg/L [4]).
- The data will be analyzed in Stata 14.

Limitations:
- There is only single measurement of most inflammatory markers, which may vary considerably over time.
- Cytokines/MMPs were only measured at visit 5, and thus there might be limited number of PAD cases (particularly CLI) during follow-up.
- We will not be able to eliminate the possibility of residual confounding as is the case in any observation study.
- ARIC predominantly included whites and blacks, so the results may not be generalizable to races other than whites and blacks.

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? ____ 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 file ICTDER03 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/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)?
#1832: A risk prediction model for incident PAD in the ARIC cohort includes WBC as a potential predictor, and #2939: Galectin-3 and the risk of peripheral artery disease deals with another inflammatory marker, galectin-3 and PAD. However, the lead author of these proposals, Dr. Matsushita is the last author for the current proposal and will be in charge of avoiding any overlap.

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

___ A. primarily the result of an ancillary study (list number* _2014.05_____)

___ 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 ____ No.

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