ARIC Manuscript Proposal #3723

PC Reviewed: 10/13/20	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Neutrophil Function and Cardiovascular Disease in Community-Dwelling Adults: the Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length 26 characters): Neutrophils and CVD

2. Writing Group:

Writing group members: Leo Buckley, Amil Shah, Pranav Dorbala, Bing Yu, Brian Claggett, Peter Libby, Josef Coresh, Weihong Tang, Christie Ballantyne, Ron Hoogeveen [Others Welcome]

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

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3. Timeline:

We will begin statistical analysis after manuscript proposal approval. We expect to complete analysis within 3-4 months of the manuscript approval date (February 2021). We anticipate submitting an abstract for presentation at the American College of Cardiology Scientific Sessions in March 2021, and the manuscript for publication in April 2021.

4. Rationale:

Soluble inflammatory mediators and innate immune cells have emerged as potential therapeutic targets for the prevention and treatment of cardiovascular disease. Canakinumab, an interleukin-1beta monoclonal antibody, and colchicine, an anti-inflammatory agent with pleiotropic effects, each significantly reduced the risk of atherosclerotic cardiovascular events in patients with coronary artery disease.[1-3] The role of inflammation in other cardiovascular diseases, such as heart failure, remains under intense investigation.[4,5]

In contrast to cytokines, chemokines and monocytes/macrophages, there is minimal epidemiologic knowledge on the associations between neutrophils and cardiovascular disease. Neutrophils are the most abundant circulating blood cell and the earliest responders to injury and microbial invasion. Neutrophils are the major source of potent antimicrobial enzymes, such as myeloperoxidase and proteinase-3, that contribute to atherogenesis. Once considered short-lived, transcriptionally inactive and phenotypically homogeneous, it is now appreciated that neutrophil lifespan, function and diversity can be fine-tuned by a variety of factors to respond to various insults.

Serum and plasma concentrations of myeloperoxidase predicted incident coronary artery disease in case-control studies of American and European cohorts.[6-8] Additionally, myeloperoxidase blood concentrations as well as circulating DNA concentrations (a surrogate of neutrophil extracellular traps) associate with subclinical atherosclerosis.[9,10] Whereas intracellular monocyte myeloperoxidase did not predict incident coronary heart disease or heart failure in an ARIC nested cohort study, blood myeloperoxidase concentrations predicted an increased risk of myocardial infarction, stroke, revascularization or cardiovascular death in 1302 adults within the EISNER study.[11,12] Little is known about other biomarkers of neutrophil activity and atherosclerotic cardiovascular disease.

Previous studies have identified associations between neutrophil-associated enzymes and heart failure. Plasma myeloperoxidase concentration predicted incident heart failure in the Cardiovascular Health Study and correlated with heart failure severity in cross-sectional cohorts.[13-15] In a study of 900 patients with acute myocardial infarction, plasma concentrations of proteinase-3 (PRTN3), but not myeloperoxidase (MPO), predicted incident heart failure.[16] Higher serum and plasma concentrations of neutrophil gelatinase-associated lipocalin may associate with worse functional status and risk of in patients with heart failure.[17,18] While these studies are consistent with an association between heightened neutrophil activity and heart failure, several questions remain unanswered, including the association between neutrophil function and cardiac structure and function and the relationship between other biomarkers of neutrophil activity and heart failure.

The combination of available echocardiography, adjudicated cardiovascular events and several candidate neutrophil biomarkers through the Somalogic platform in the ARIC cohort provides the opportunity to comprehensively address these questions about neutrophil function and cardiovascular disease. Specifically, we propose to estimate the association between neutrophil function biomarkers and cardiovascular disease and changes in cardiac structure and function in community-dwelling adults.

We selected biomarkers of interest based upon known neutrophil-derived mediators.[19,20] In particular, we pre-specify several proteins as primary exposures of interest based upon their responses to an established anti-inflammatory agent (colchicine) in patients with coronary artery disease (PRTN3, CEACAM8, AZU1, MPO, U-PAR) or obesity and metabolic syndrome (alpha-1 antichymotrypsin, bactericidal permeability-increasing protein, CD177, matrix metalloproteinase-9, S100A12).[21,22]

5. Main Hypothesis/Study Questions:

Our overall hypothesis is that heightened neutrophil activity predicts incident atherosclerotic cardiovascular events and heart failure as well as longitudinal worsening in cardiac structure and function in community-dwelling adults without established cardiovascular disease. We aim to:

1. Estimate the association of protein biomarkers of neutrophil function at Visit 3 with adjudicated coronary heart disease and stroke after Visit 3.
2. Estimate the association of protein biomarkers of neutrophil function at Visit 3 with any incident heart failure after Visit 3. In addition, we will estimate the association of protein biomarkers of neutrophil function at Visit 5 with adjudicated incident heart failure with reduced and preserved ejection fraction after Visit 5.
3. Estimate the association of protein biomarkers of neutrophil function at Visits 3 and 5 with cardiac structure and function at Visit 5, and with longitudinal changes in cardiac structure and function from Visit 5 to Visit 7.

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).

This study will be a longitudinal cohort study of ARIC participants across Visits 3, 5 and 7. We will use echocardiographic data from Visits 5 and 7, adjudicated atherosclerotic cardiovascular events after Visit 3, adjudicated heart failure events after Visit 5 and Somalogic-derived protein measurements from Visits 3 and 5.

Aim 1:

- Population: Aim 1 will include all participants who were free from coronary heart disease and stroke through Visit 3 and had the necessary protein measurements at Visit 3.
- Exposures:
    - Primary exposures include neutrophil function-related proteins significantly reduced by colchicine in patients with coronary artery disease or obesity and metabolic syndrome[21]: proteinase-3 (PRTN3, UniProt P24158), carcinoembryonic antigen-related cell adhesion molecule-8 (CEACAM8, UniProt P31997), azurocidin (AZU1, UniProt P20160), myeloperoxidase (MPO, P05164), urokinase plasminogen activator surface receptor (suPAR, UniProt Q03405), alpha-1 antichymotrypsin complex (UniProt P01011), Alpha-1 antichymotrypsin complex (UniProt P01011), Bactericidal permeability-increasing protein (BPI, UniProt P17213), CD177 (UniProt Q8N6Q3), Matrix metalloproteinase-9 (MMP9, UniProt P14780), S100A12 (UniProt P80511)

    - Secondary exposures include additional proteins related to neutrophil function: neutrophil collagenase (MMP-8, UniProt P22894), neutrophil elastase (elastase, UniProt P08246), lactoferrin (UniProt P02788), lysozyme C (UniProt P61626), neutrophil gelatinase-associated lipocalin (UniProt P80188), cathelicidin (UniProt P49913), arginase-1 (ARGI1, UniProt P05089)
- Outcomes: the composite of incident coronary heart disease or stroke; incident coronary heart disease; incident stroke
- Covariates: We will sequentially adjust regression models for Visit 3 demographic characteristics (model 1: age, gender, race/ethnicity and field center), clinical characteristics (model 2: diabetes mellitus, hypertension, smoking, BMI, eGFR [visit 2, unavailable at visit 3]) and other risk factors (pulse pressure, non-HDL cholesterol, statin use, blood pressure lowering medication use) . Model 4 will additionally adjust for levels of key proteins of reflective of the NLRP3 inflammasome (IL-18, IL-6), another putative inflammatory pathway that has been implicated in CHD.
- Analysis: We will use Cox proportional hazards regression models to estimate the association of each exposure to the specified outcomes. We will use restricted cubic splines to assess for non-linear associations. We will employ multiple approaches to model our exposures of interest. First, we will relate each protein value to each outcome using multivariable Cox proportional hazards model employing Bonferroni correction for multiple testing. Second, to identify proteins that – in aggregate – predict outcomes, we will enter all primary and secondary exposure proteins into a single model using LASSO regression.

Aim 2:

- Population: (1) For analysis of post-Visit 3 HF, all participants who were free from HF through Visit 3 and had the necessary protein measurements at Visit 3 will be included; (2) For analysis of post-Visit 5 HF, all participants who were free of HF through Visit 5 and had the necessary protein measurements at Visit 5 will be included.
- Exposures:
    - Same as listed for Aim 1 above
    - We will also model changes in protein levels from Visit 3 to Visit 5
- Outcomes: Primary outcomes of interest will be (1) incident HF post-Visit 3 (using proteomic measures at Visit 3 as primary exposures); and (2) incident adjudicated heart failure post-Visit 5, and incident HFrEF (<50%) or HFpEF (≥50%) (using proteomic measures at Visit 5 as primary exposure). We will also assess associations with the composite of incident any heart failure or all-cause death.
- Covariates: Model covariates will be similar to those specified in Aim 1 above, with the exception that Model 2 will additionally adjust for prevalent CHD and visit 5 analyses will use visit 5 eGFR.
- Analysis: We will use Cox proportional hazards regression models to estimate the association of each exposure to the specified incident heart failure outcomes. We will use restricted cubic splines to assess for non-linear associations. For analysis of incident HFpEF, we will censor patients at the time of incident HFrEF or HF with unknown LVEF. For analysis of incident HFrEF, we will censor patients at the time of incident HFpEF or HF with unknown LVEF. We will repeat analyses with censoring after

incident myocardial infarction. We will employ multiple approaches to model our exposures of interest as described in Aim 1 above.

Aim 3:

- Population: (1) For cross-sectional association with echocardiographic measures at Visit 5, participants free of HF through Visit 5 with echocardiography measures at Visit 5 and protein measurements at Visit 5 will be included; (2) For association with change in echocardiographic measures from Visit 5 to Visit 7, participants also undergoing echocardiographic at Visit 7 will be included.
- Exposures will be the same as for Aim 2 above.
- Outcomes: Echocardiographic measures of cardiac structure and function (1) at Visit 5; and (2) their change from Visits 5 to 7. Primary measures of interest will be those reflective of LV structure (left ventricular wall thickness, dimensions, mass, RWT), LV diastolic function (E wave, e', E/e', LA diameter, LA volume index), left ventricular systolic function (LVEF, longitudinal strain, circumferential strain), PASP, RV measures (area, fractional area change, tricuspid annular s')
- Covariates: Model covariates will be similar to those specified for Aim 2 above. In addition, all models will be adjusted for systolic blood pressure and heart rate at Visit 5. Models with change in echo measures as an outcome will also adjust for systolic blood pressure and heart rate at Visit 7.
- We will use multivariable linear regression models to assess for linear associations, and will use restricted cubic splines to assess for non-linear associations. We will employ multiple approaches to model our exposures of interest parallel to the approach outlined in Aim 1 above.

Detailed Statistical Analysis Plan: Continuous and categorical data will be summarized using means and standard deviations, medians and interquartile ranges and numbers and percentages, respectively.

We will estimate the association of protein biomarker levels to incident cardiovascular outcomes using Cox proportional hazards regression models as described above in Aim 1. We will estimate associations between protein biomarker levels and echocardiographic measures using linear regression as described above in Aim 2.

We will test key assumptions of the linear and Cox proportional hazards regression models and adjust (e.g., time-dependent covariates, stratify cohort) as appropriate. We will conduct sensitivity analyses using inverse probability of attrition weights to account for non-random Visit 7 attendance. We will conduct separate analyses according to gender, race/ethnicity and in patients with and without ischemic heart disease at Visit 5. We will use quality control samples to assess assay drift from Visit 3 to Visit 5.

There are certain limitations to this analysis. Missing LVEF data from time of incident heart failure hospitalization will reduce the number of classifiable HFpEF and HFrEF events. We will perform sensitivity analysis by assigning all incident HF cases with missing LVEF as either HFpEF or HFrEF and re-evaluating our models. Non-random non-attendance at Visit 7 due to survivor bias and attendance bias may bias the results of our echocardiographic analyses towards

the null since we expect Visit 7 non-attendees to be at higher risk of adverse remodeling and to have more severe CKD than attendees. We will adjust for non-random non-attendance at Visit 7 using inverse probability of attrition weights as described above. It is possible that HFpEF cases were underreported or other conditions were misclassified as HFpEF due to the challenges associated with diagnosing this condition. We do not expect the number of misclassified cases to alter our estimates since all events have been adjudicated by an independent committee according to standardized definitions. Regardless, underreporting of HFpEF cases may bias estimates towards the null. Absolute quantification of protein biomarkers is not provided but we will compare aptamer-based relative quantification to ELISA-based absolute quantification for select biomarkers.

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/mantrack/maintain/search/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)?

#3673: Agha A, et al. The Role of Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Matrix Metalloproteinases (TIMPs) in Predicting Cardiovascular Outcomes and CardiacDysfunction: Atherosclerosis Risk in Communities (ARIC) Study

#1219: Matijevic N, et al. Peripheral blood monocyte myeloperoxidase (MPO) and cyclooxygenase-2 (COX-2) levels and carotid artery plaque presence/progression (ARIC CAR MRI Study)

#3065: Folsom A, et al. Association of Monocyte Myeloperoxidase and CVD

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* 2015.34; 2017.27)

___ 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 https://www2.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.

The writing group agrees to complete the manuscript within this timeframe.

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.

The writing group agrees to upload the manuscript to PubMed Central.

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