1.a. **Full Title**: Galectin-3 and the risk of peripheral artery disease

**b. Abbreviated Title (Length 26 characters)**: Galectin-3 and PAD

2. **Writing Group**:
   Writing group members: Kunihiro Matsushita, Chao Yang, Shoshana Ballew, John W. McEvoy, Maya Salameh, David Aguilar, Ron C. Hoogeveen, Vijay Nambi, Elizabeth Selvin, Aaron Folsom, Gerardo Heiss, Josef Coresh, Christie M. Ballantyne

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. KM

[please confirm with your initials electronically or in writing]

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**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**:  
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3. **Timeline**: Data to be used in this proposal are available. Analyses and manuscript preparation will be performed over the next 12 months.

4. **Rationale**:
Galectins are highly conserved lectins in mammals and regulate inflammation. Indeed, galectin-3 is involved in immune cell proliferation, neutrophil extravasation, oxidative stress, macrophage chemotaxis, apoptosis, and angiogenesis. In line with these properties, galectin-3 has been linked to atherosclerosis in animal models and humans. However, to our knowledge, there are no studies investigating prospective associations of
galectin-3 with incidence of lower-extremity peripheral artery disease (PAD). Since the risk factor profile is not necessarily the same across different vascular beds, it is important to specifically study PAD. Therefore, using data from the Atherosclerosis Risk in Communities (ARIC) Study, we will quantify the prospective association between galectin-3 and the risk of PAD beyond traditional cardiovascular risk factors.

5. **Main Hypothesis/Study Questions:**
Galectin-3 will be independently associated with risk of PAD.

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 subjects with variables of interest at visit 4, at which galectin-3 was measured.

_Exclusions:_
- Ethnicity other than black or white
- Missing data on variables of interest
- Participants with prevalent PAD at visit 4 (determined by ankle-brachial index <0.9, self-report of intermittent claudication, or leg artery revascularization at visit 1 or a PAD-related hospitalizations between visits 1 and 4 [details of diagnostic codes related to PAD are described below])

_Exposures:_
In ARIC, galectin-3 was measured using a chemiluminescent microparticle immunoassay on an Architect i 2000sr instrument (Abbott, Abbott Park, IL) in EDTA-plasma samples collected at visit 4 and stored at –70°C. The measurements were performed between July 2015 and February 2016.

_Outcomes:_
PAD-related hospitalizations will be identified according to the following ICD codes based on previous literature: 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 critical limb ischemia (CLI) when the following codes coexist: leg amputation (84.1x), lower extremity ulcer (707.1x), and gangrene (785.4) with any PAD cases.
Other variables of interest and covariates:

- Sociodemographics: age, race, gender, education, and insurance
- Physical information: body mass index, blood pressure including, presence/absence of left ventricular hypertrophy by electrocardiogram, and carotid atherosclerosis by ultrasound
- Lifestyle: smoking status/amount and alcohol habit
- Comorbidities: diabetes, dyslipidemia, hypertension, kidney function, coronary heart disease, stroke, heart failure, atrial fibrillation, cancer
- Laboratory values: fasting glucose, high-sensitivity C-reactive protein, NT-proBNP, and high-sensitivity cardiac troponin T

Statistical analysis plan:

We will use Cox proportional hazards models adjusting for the covariates listed above to quantify the association between galectin-3 and incident PAD- and CLI-related hospitalizations over time. We will implement a few models to account for the impact of potential confounders for the galectin-PAD relationship. Model 1 will be crude. Model 2 will be adjusted for demographic variables (age, gender, race, and center). Model 3 will further adjust for other potential confounders, education levels, smoking, and physical activity, diabetes, lipids, blood pressure, antihypertensive medications, kidney function, and other comorbidities listed above. Model 4 will include other biomarkers of inflammation and cardiac damage/overload. To evaluate whether galectin-3 has a uniquely strong association with CLI, we will compare hazard ratio for PAD without CLI vs. that for CLI using seemingly unrelated regression.

We will conduct sensitivity analyses by stratifying the study sample into key clinical subgroups by age, gender, race, smoking status, and clinical conditions (presence vs. absence of diabetes, hypertension, kidney dysfunction, or history of cardiovascular disease). The interaction will be evaluated using the likelihood ratio test. Finally, given the potential impact of the competing risk of death for estimating PAD and CLI risk, we will run Fine and Gray’s proportional subhazards models. In a subsample of data on ankle-brachial index at visit 4, we will also cross-sectionally evaluate the association between galectin 3 and ankle-brachial index.

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.csc.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)?  
There are a few ARIC proposals exploring the associations of galectin-3 with different cardiovascular phenotypes, but none of them tackle PAD.

#2771: Galectin-3 and Cardiovascular Outcomes  
#2805: Galectin-3 and Atrial Fibrillation Incidence  
#2882: Galectin-3 and Venous Thromboembolism Incidence

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* 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.csc.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.csc.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.
References: