1. Full Title: Evaluation of novel circulating biomarkers in the prediction of adverse cardiovascular events including heart failure

b. Abbreviated Title (Length 26 characters): Novel biomarkers & ASCVD risk

2. Writing Group:
   Writing Group Members:
   *Vijay Nambi MD PhD
   *Michael Wesley Milks MD
   Aaron Folsom MD, MPH
   Elizabeth Selvin PhD, MPH
   Wensheng Sun PhD
   Salim S Virani MD PhD
   Eric Boerwinkle PhD
   Ron C Hoogeveen PhD
   Joe Coresh MD PhD
   Scott Solomon MD
   Christie Ballantyne MD

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

First author: Vijay Nambi
Address:
   Baylor College of Medicine,  
   One Baylor Plaza  
   Houston, TX 77030  
   Phone: 713-798-7545  
   Fax: 713-798-7885  
   E-mail: vnambi@bcm.tmc.edu

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):

3. Timeline: Analysis is anticipated to begin as soon as approval is obtained. The manuscript is to be prepared as soon as analyses are available. The analysis and
manuscript preparation is anticipated to take place within one year of approval of the proposal.

4. Rationale: Several biomarkers continue to be evaluated for their association with and for their incremental predictive ability in the estimation of cardiovascular (CV) event risk. While a clinical evaluation of traditional risk factors, history, and physical exam together represent the mainstay of patients CV risk evaluation and treatment, a risk-based treatment decision such as the decision whether to pursue primary preventive statin therapy can be further enhanced by the addition of biomarkers (Goff DC et al., Circ 2014;129:S49-73). Several biomarkers have been evaluated separately and some in concert for their ability to improve the prediction of CV risk in several studies including ARIC. In the 4th visit in ARIC several novel biomarkers were measured. We propose to evaluate the additional value of these biomarkers when used in concert with traditional risk factors and when compared with traditional risk factors in the prediction of CV disease. As coronary heart disease (CHD), ischemic stroke (CVA), and heart failure (HF) share some common pathophysiologic pathways in addition to looking at these individual outcomes a composite endpoint including these 3 entities will also be assessed.

Relevant biomarkers generally have a basis in the detection of underlying processes such as high sensitivity CRP (hs-CRP) to detect inflammation, high sensitivity cardiac troponin T (hs-cTnT) to detect cardiomyocyte damage or neurohormonal N-terminal pro B-type natriuretic peptide (NT-proBNP) to detect evidence of ventricular wall stress. A recent analysis of the Multi-Ethnic Study of Atherosclerosis (MESA) and Phase 1 of Dallas Heart Study (DHS) suggests that different markers (imaging and biomarkers) associated with CVD when abnormal, can each provide non-redundant incremental cardiovascular risk stratification beyond a base model risk estimate derived from the pooled cohort equations (de Lemos JA, Circ 2017;135(17): doi: 10.1161/CIRCULATIONAHA.117.027272. [Epub ahead of print]).

While generally one or several biomarkers are tested for predictive discrimination beyond traditional risk factors in each individual study, there is relative paucity of evidence that compares and combines many circulating biomarkers simultaneously. The basis for the collective consideration of established and emerging biomarkers for the current proposal follows.

Cardiac troponins I and T. Highly sensitive troponin assays that detect lower levels of circulating troponin are becoming well-established as predictive biomarkers for incident coronary heart disease (CHD), heart failure (HF), and mortality in a general population without previously defined cardiovascular disease (Saunders JT, Circ 2011;123:1367-1376) expanding the potential value of troponin beyond its ability to evaluate for a myocardial infarction.

Natriuretic peptides. Natriuretic peptides and their precursors were first developed as neurohormonal surrogates for the degree of ventricular wall stress and have proven to be of great clinical utility in the management of patients with HF. Analysis of numerous
prospective studies has shown the incremental predictive capacity of B-type natriuretic peptide (BNP) and NT-proBNP in the estimation of CVD risk (Di Angelantonio Circ 2009;120:2177-2187). Subsequent analyses of the ARIC study confirmed a role of NT-proBNP in predicting HF risk both in individuals with and without obesity, the presence of which potentially affects the level of natriuretic peptides (Nambi V Clin Chem 2013;59:1802-1810; Ndumele CE Circ 2016;133:631-638).

**Galectin-3.** The growing body of evidence that guides the understanding of HF pathophysiology has elucidated the role of galectin-3 as a mediator of cardiac remodeling and fibrosis, with predictive implications for mortality as well as unplanned HF rehospitalization among hospitalized subjects (Meijers Am Heart J 2014;167:853-860.e4). Even in community-dwelling individuals, galectin-3 has been shown to be an independent predictor of CVD mortality even after correction for NT-proBNP (Daniels Am Heart J 2014;167:674-682.e1). Its value in the ARIC study has been reported (McEvoy J J Am Heart Assoc. 2016 May 13;5(5)) and through another manuscript proposal.

**Lipoprotein subtypes.** Beyond total, HDL cholesterol and CRP, other contemporary biomarkers for CVD risk prediction are rooted in the assessment of severity of an individual’s atherosclerotic and atheroinflammatory vascular milieu. Lipoprotein(a) [Lp(a)] has been shown to be associated with CV outcomes in the ARIC study (Virani SS Circulation. 2012 Jan 17;125(2):241-9) and to offer slight incremental improvement in the C-index of a predictive model based on the Reynolds Risk Score, with greatest reclassification noted among intermediate risk subjects in the Bruneck, Italy cohort (Willeit JACC 2014;64:851-60). In addition, Lp(a) level has been shown to predict sudden cardiac death risk in middle-aged Finnish men after adjustment for lipid and CRP levels (Kunutsor Int J Cardiol 2016;220:718-725). Remnant-like lipoprotein particles cholesterol (RLP-C) predict incident coronary heart disease (Joshi J Am Heart Assoc 2016;5:e002765 doi:10.1161) and may capture an aspect of the atherogenicity of an individual’s lipid biochemistry beyond routinely measured cholesterol subtypes (recent ARIC analysis have evaluated this in ARIC study). Lastly, there has been interest in whether concentration of triglycerides in LDL (LDL-TG) has the capacity to predict CVD events, although in a secondary analysis of the AIM-HIGH study, ultimately HDL subclass HDL3-C had the strongest relationship to cardiovascular events (Albers Atherosclerosis 2016;251:454-459).

Obtaining unique predictive data about an individual’s cardiovascular risk beyond relatively routinely available clinical variables including age, gender, race, systolic blood pressure (SBP), antihypertensive medication, antidiabetic medication, smoking, body-mass index (BMI), serum creatinine, total cholesterol, and HDL-C has the potential to refine a risk estimate to reach a risk-based treatment decision. The ARIC study is uniquely poised to explore novel circulating risk prediction factors.

5. **Main Hypothesis/Study Questions:**

   **Hypothesis:**
Beyond a risk estimate based on traditional risk factors, risk prediction of incident CVD and HF can be refined with use of novel biomarkers.

**Study questions:**
1. To what extent does a model that includes the novel biomarkers (discussed above) improve the prediction of incident CVD including HF beyond a model that includes the “traditional risk factors”?
2. How does a model that includes the novel biomarkers alone compare to a traditional risk factor model?
3. What is the optimal parsimonious model for the entire cohort to predict incident CVD or HF considering a subset of aforementioned variables?
4. What is the optimal parsimonious model for the intermediate risk (10-year ASCVD risk of ≥5 to <7.5% as estimated by the pooled cohort equations risk calculator) group to predict incident CVD or HF considering a subset of aforementioned variables?

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 methodological limitations or challenges if present).**

Traditional ARIC exclusions (race other than white/ black, blacks from centers other than Jackson) will be applied in this prospective cohort analysis, with ARIC visit 4 as baseline. All remaining individuals with available hsTnT, hsTnI, NT-proBNP, galectin-3, Lp(a), hs-CRP, RLP-C, LDL-TG and traditional risk factors (hypertension status [systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg, or antihypertensive medication use], systolic blood pressure, diabetes status [fasting blood glucose > 126 mg/dl, or antidiabetic medication use or any glucose value >200 mg/dL], cholesterol values, smoking-current, former or never) at the fourth ARIC visit will be eligible and individuals with missing data will be excluded. Individuals with prevalent CVD (definite or probable MI, revascularization, ischemic or hemorrhagic stroke) or HF will be excluded.

Using Cox-proportional hazards models, the association between the various biomarkers and adverse cardiovascular events (evaluated individually and as a composite) will be studied. Coronary heart disease will be defined as myocardial infarction [probable or definite], death from coronary heart disease and revascularization while stroke would be ischemic stroke; HF will include heart failure hospitalization; and death (total and CV) will be reported initially using minimally adjusted models (age, gender, race) and then after adjustment for extended variables including systolic blood pressure (SBP), antihypertensive medication, body-mass index (BMI), serum creatinine, total cholesterol, HDL-C, smoking status, hypertension and diabetes status. A comprehensive model also including adjustments for the other novel candidate
biomarker variables including hsTnT, hsTnl, NT-proBNP, galectin-3, Lp(a), RLP-C, or LDL-TG will then be generated.

Risk prediction will then be assessed, first for the entire cohort studied and then as applied to an intermediate risk group with 10-year ASCVD risk of ≥5 to <7.5% as estimated by the pooled cohort equations (PCE) risk calculator. Performance characteristics to assess the discrimination of each model will be described using the area under the receiver operating characteristic curve (AUC) and each successive model (minimally adjusted, extended, comprehensive) will be tested for incremental net reclassification improvement. Categorical and continuous net reclassification index in the whole cohort and those at intermediate risk and integrated discrimination index will be described. Model calibration will be assessed using Gronnesby-Borgan statistics for model fit. Selection of a final parsimonious model will be sought taking into account the AUC improvement and simplicity and ease of availability of candidate variables.

7.a. Will the data be used for non-CVD analysis in this manuscript?  ___ Yes  ___ 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  ___ 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

     ___ 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)?
     MS 1808, 1757, 1759, 1564

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  ___ Yes  ___ No
11.b. If yes, is the proposal
   ___  A. primarily the result of an ancillary study *
   ___  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.cscce.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
http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals
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