1.a. **Full Title**: Risk factors for Progression of Subclinical Myocardial Injury: Six-year change in highly-sensitive troponin T in a community-based population study

1.b. **Abbreviated Title (Length 26 characters)**: Exploring delta hscTnT in ARIC.

2. **Writing Group**: John W. McEvoy; Mariana Lazo; Lu Shen; Christie M. Ballantyne; Ron C. Hoogeveen; Vijay Nambi; Roger S. Blumenthal; Josef Coresh and Elizabeth Selvin. Others welcome.

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

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3. **Timeline**: We aim to submit this manuscript to the ARIC publications committee in <12 months from this date.

4. **Rationale**: 
New ultra high-sensitivity troponin T (hs-cTNT) assays have recently been developed that have a number of potentially important clinical applications. These assays can detect concentrations of troponin 10-fold lower than conventional assays and have been shown to independently predict all-cause mortality, cardiovascular death, heart failure, stroke and sudden cardiac death.

An important consideration in the evaluation of a new biomarker is an understanding of temporal change in that biomarker. In the case of hs-cTNT, such temporal change may reflect progression or regression of subclinical myocardial injury. Investigation of demographic variables most strongly associated with change in hs-cTNT could provide mechanistic insight regarding risk-factors that influence subclinical myocardial injury. In addition, this line of investigation may yield targets for pharmacologic interventions designed to reverse myocardial injury, as well as informing the utility of hs-cTNT as a primary or safety endpoint in trials and other clinical studies.

However, the assessment of temporal changes in biomarker levels is often complex. Various factors contribute to change: intra-individual variability (e.g. physiologic changes), inter-individual variability (e.g. heterogeneity in populations), and measurement variability (e.g. methodological variability). Furthermore, change can be expressed in numerous ways. For example, as absolute change, percent proportional change, changes in transformed variables, and proportional change in pre-defined categories (e.g., clinically-relevant categories or more arbitrarily defined categories such as tertiles).

Modeling change is further complicated when there is a high proportion of individuals with non-detectable levels of a biomarker at baseline. In this setting, assessment of temporal change is often separated into two defined categories; incident abnormality (e.g. zero level at measurement 1 and non-zero level at measurement 2) and non-zero progression (e.g. non-zero level at both measurements).

To address these issues, we plan to perform an analysis of change in hs-cTnT at two time points, 6 years apart, in the ARIC Study. We will focus on describing change in the cohort, as well as investigating clinical factors associated with hs-cTNT change.

5. Main Hypothesis/Study Questions:

Aim 1: To document the natural history of progression of subclinical myocardial damage as assessed by 6 year change in hs-cTnT in a community-based population. We will use multiple methodological approaches to modeling change, focusing on both absolute and relative change.

Aim 2: To evaluate the associations of traditional cardiovascular risk factors, Framingham Risk Score categories, and new 2013 AHA/ACC ASCVD risk thresholds with 6-year change in hs-cTnT in the community. We will compare risk factor associations across different methodological formulations of 6-year change in hs-cTnT.

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: Prospective analysis to characterize change in hs-cTnT measured at two time points: visit 2 (1990-1992) and visit 4 (1996-1998). We will also examine the independent associations of traditional cardiovascular risk factors and clinical risk score categories with change in hs-cTnT modeled in different ways.

Hs-cTnT: Cardiac troponin T was measured at two time points using the same high sensitivity (pre-commercial) Roche assay. Visit 2: cardiac troponin T concentrations were measured from stored (visit 2) serum samples using a sandwich immunoassay method (Roche Diagnostics) implemented on a Roche Elecys 2010 Analyzer in 2012-2013 at the University of Minnesota as part of Dr. Selvin’s ancillary study (#2009.16). Visit 4: cardiac troponin T concentrations were measured from stored (visit 4) plasma samples using the same sandwich immunoassay method implemented on a Cobas e411 analyzer in 2010 at the Baylor College of Medicine as part of Dr. Ballantyne’s ancillary study (#2008.10). ARIC investigators conducted a formal crossover study (N=200 paired samples) to evaluate possible differences in hs-cTnT values across specimen type and laboratory. The difference between the two measurements was not statistically significantly different from zero; thus statistical correction for differences in hs-cTnT assessed at the two laboratories was not indicated (Ref Parrinello et al ARIC MSP #2243).

Various metrics of temporal change in hs-cTNT will be analyzed. These will include

A- Categorical Change in hs-TNT. Hs-cTnT will be categorized as: undetectable (<5 ng/L), detectable (5-14 ng/L), and elevated (≥14 ng/L). Upwards change will be defined as progression from either undetectable to detectable/elevated or from detectable to elevated. We will conduct sensitivity analyses using categories of (<3, 3-14, and ≥14 ng/L) since, while 5 ng/L is defines the lower limit of detection, 3 ng/L is the lower limit of measurability of this assay.

B- Continuous Change in hs-cTNT. For the purposes of this analysis, imputation of values (1.5 ng/L) will be performed for those with values below the limit of measurability at baseline or follow-up.

C- In keeping with prior literature⁶, relative change in hs-cTNT in those with non-zero baseline hs-cTNT will also be analyzed. Change will be defined in this group as:
- Percent proportional change from baseline (with a focus on categories with >50% decrease in hs-TNT, <50% change, and >50% increase.

We will also generate histograms and Bland-Altman plots of hs-cTnT to evaluate the variability of hs-cTnT across the two visits. Bland-Altman plots (difference of the two measurements against their mean) on the absolute, log and square-root scales will be performed to evaluate whether the variance is stabilized by transformation. We also plan to add regression lines to these Bland-Altman plots to more formally test whether the slopes are significant.

Clinical subgroups: We will also stratify the ARIC sample based on known cardiac risk estimating algorithms. In particular, we will focus on subgroups based on baseline Framingham
Risk Score (FRS) for CHD, a commonly used clinical metric\textsuperscript{13}. Subgroups will be created based on categories of FRS estimated 10yr CHD risk (low risk \(<10\%\), intermediate risk 10-20\%, and high risk \(>20\%\)). Recent guidelines released by the AHA/ACC now recommend a new ASCVD (atherosclerotic cardiovascular disease) risk score. The threshold for consideration of statin therapy in the group is 7.5\% (groups will be defined as \(\geq7.5\%\) or \(<7.5\%\) 10yr risk). Therefore we will also create subgroups using these thresholds. Thus, in addition to standard cardiovascular risk factors, the influence of these risk categories on change in hs-cTNT will be analyzed.

**Exclusions:** Persons who did not attend visits 2 and 4, were missing information on hs-cTnT or covariates of interest, who had a history of cardiovascular disease (coronary heart disease, stroke, or heart failure).

**Statistical analyses:**

Statistical description of the absolute continuous change in hs-cTNT will be performed in the whole sample as well as within each FRS-based subgroup (mean change, median change, and standard deviation of change).

Similarly, statistical description of the other metrics of change in hs-cTNT (A and C, above) will also be performed.

Multivariable analyses will be conducted for both the categorical and continuous measures of change. For categorical change, Poisson regression will be conducted in order to compare the probabilities of upwards change in hs-cTNT category by baseline levels of traditional cardiovascular risk factors FRS and ASCVD risk categories. Three models will be constructed, Model 1- age and sex adjusted, Model 2- inclusive of the risk categories, and Model 3- including multiple known cardiac risk factors (age, gender, race, Hypertension, LVH, hypercholesterolemia, HDL-C, Diabetes, BMI \(\geq30\mathrm{kg/m^2}\), eGFR \(\leq60\mathrm{ml/min}\), hsCRP \(\geq2\mathrm{mg/L}\), and smoking).

Robust linear regression will be performed for the continuous change analysis. For those with undetectable hs-cTNT at baseline, imputation with a value of 1.5ng/L will be conducted.

The purpose of these various models will be to identify variables that are robustly associated with upwards hs-cTNT change. In addition, the use of FRS/ASCVD categories will allow us to report expected change based on these categories, and relative change compared to low risk individuals. This may allow the identification of high risk individuals who are demonstrating change in hsTNT which is more pronounced than would be expected based on their average calculated cardiovascular risk score.

**Sensitivity analyses:**

Sensitivity analyses will include:

1. Based on controlling for hs-cTNT at baseline.
2. Using either annualized or time independent data.
3- Excluding persons with events between visits 2 and 4.
4- Including persons with fatal events by visit 4 as cases (“elevations in hs-cTnT”).
   Specifically, for the categorical analysis, individuals with interval cardiovascular events between visits 2 and 4 will be allocated to the elevated hs-cTnT group unless they have an available hs-cTnT value at follow-up. Sensitivity analyses will be conducted using logistic regression for comparison. Similarly, those with interval cardiovascular events and without available hs-cTnT in follow-up will be allocated a value of 14ng/L.

Limitations:

- Measurements of hs-cTnT at only two time points, 6 years apart.
- The measurements of hs-cTnT were conducted by different laboratories at different time points. We have information from the rigorous direct calibration study to inform comparability of these measurements but it is always possible that systematic differences (due to laboratory methods, Roche reagents, etc) may remain.
- As with all observational studies, we will not be able to eliminate the possibility of residual confounding despite rigorous adjustment for known risk factors.

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)?


Selvin et al. ARIC MSP 2128

Parrinello et al ARIC manuscript proposal #2243, “Calibration of analytes over twenty-five years in the Atherosclerosis Risk in Communities Study: The impact of calibration on chronic kidney disease prevalence and 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* __2009.16 and 2008.10_______)

____ 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.

References