# ARIC Manuscript Proposal #2775

**PC Reviewed:  7/12/16**        **Status: <u>A</u>**        **Priority: <u>2</u>**
**SC Reviewed:  _____**        **Status:  _____**        **Priority: ____**

**1.a.  Full Title**: High-sensitivity troponin I and incident heart failure hospitalization, myocardial infarction, stroke and cardiovascular disease mortality in ARIC

   **b.**  Abbreviated Title (Length 26 characters): Troponin I and CVD risk in ARIC

**2.    Writing Group**:
     Writing group members: **Christie M. Ballantyne, Eric Boerwinkle, Kenneth Butler, David Couper, James de Lemos, Aaron Folsom, Gerardo Heiss, Ron Hoogeveen, Kunihiro Matsushita, Vijay Nambi, Elizabeth Selvin, Scott Solomon (in alphabetical order)**

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

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**3.    Timeline**: Abstract to AHA, publication fall of 2016

**4.    Rationale**:
Heart failure (HF) is a major cardiovascular disorder that is increasing in incidence, prevalence, and lethality. The transition from asymptomatic cardiac structural and functional abnormalities to symptomatic heart failure is associated with extensive remodeling of the muscular, collagenous, and vascular compartments of the myocardium.[1] HF involves the progressive loss of myocytes,

which is mediated by various neurohormonal and hemodynamic alterations and leads to progressive cardiac dysfunction and left ventricular (LV) remodeling.[2]

Troponins are proteins found in the myofibrils of cardiac and skeletal muscle tissues. Cardiac troponin T and I are the preferred markers for the diagnosis of myocardial injury because of the recent development of standardized assays with high sensitivity and specificity.[3,4] In the Dallas Heart Study, elevated cardiac TnT level ($\geq$0.01 µg/L) using the standard 4th-generation commercially available assay was present in 0.7% of the general population and was associated with HF or LV dysfunction, LV hypertrophy, diabetes mellitus, and moderate chronic kidney disease (estimated glomerular filtration rate <60 ml/min/1.73m$^2$); without these conditions, the probability of troponin elevation was almost 0, and the number of these conditions present had an additive association with TnT elevation.[5] Of note, using standard assays, elevated troponin is very rarely seen in patients without cardiovascular disease (CVD) or major CVD risk factors and is therefore not a normal occurrence. However, common cardiac conditions and risk factors are often associated with measurable troponin levels even without cardiac symptoms.[6]

Recently, new very high sensitivity troponin assays have shown that levels of troponin T can be measured in a much higher proportion of individuals with prevalent CVD, such as HF, and that levels are linearly associated with risk.[7] In the ARIC study, 66.5% of the population without CVD has a level of hs-TnT with the Roche assay that was above the limit of measure of 3 ng/L, and approximately 50% were above the limit of detection of 5 ng/L. The Abbott hs-TnI assay has a limit of detection of 1.2 pg/ml, and the diagnostic cutoff representing the 99th percentile in the general population is 15.6 pg/ml in women and 34.2 pg/ml in men.[8] With this assay, hs-TnI could be measured in 98% of patients with CHD[9] and 95% of an elderly population.[10] Furthermore, by Pearson's correlation, logarithmically transformed hs-TnI levels were only moderately correlated with hs-TnT (r = 0.44). TnT had stronger associations with age, gender, diabetes, and obesity, whereas TnI had stronger associations with prior MI. In patients with CHD, levels of hs-TnI were associated with incident CV death and HF independently of hs-TnT levels, and individuals who had high levels of both markers had the worst outcomes. Levels of both TnI and TnT have been associated with incident MI, stroke, and CVD mortality in ARIC and in other studies.

Levels of hs-TnI were measured in 2015–2016 from visit 4 plasma samples of 11,539 ARIC participants using a standardized assay on an automated chemistry analyzer (Abbott Architect).

5.    **Main Hypothesis/Study Questions**:
Levels of hs-TnI will be associated with risk for incident HF hospitalization, CHD events, stroke, CVD mortality, and total mortality among individuals in the population without a prior history of CVD. Levels of hs-TnI will be additive to hs-CRP in multivariable models.

**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 cohort analysis

**Exposure:** Measurement of hs-TnI was performed in visit 4 samples from the entire ARIC cohort. The new hs-TnI assay method is a double chemiluminescent immunoassay using a capture antibody directed against amino acids 24–40 of the TnI protein and a chimeric detection antibody directed against amino acids 41–47. The level of detection for this assay is 1.2 pg/ml (range: 0–50,000 pg/ml), with a coefficient of variation of 10% observed at a concentration of 3.0 pg/ml, and the diagnostic cutoff representing the 99th percentile in the general population is 15.6 pg/ml in women and 34.2 pg/ml in men.[8] Levels lower than the detection limit will be assigned a value of 1.2 pg/ml.

**Study population:** The proposed analyses will focus on individuals who do not have any evidence of CVD including a prior history of heart failure at the time of visit 4 (1996–1998).

**Statistical methods: Cox Proportional Hazards Model**
For each of the following Cox proportional hazards models, the estimated parameter, standard errors, p-value of the Chi-square tests, and the proportional hazards of the 10-year follow-up outcome with corresponding 95% confidence intervals will be reported.

1) **Unadjusted Cox Proportional Hazards Model**
   i) **Model 1:** Cox proportional hazards model will be performed using hs-TnI categories (men and overall: low risk <6 ng/L, moderate risk 6–12 ng/L, at risk >12 ng/L; women: low risk <4 ng/L, moderate risk 4–10 ng/L, at risk >10 ng/L).
   ii) **Model 2:** Cox proportional hazards model will be performed using hs-CRP categories (low risk <1.0 mg/L [reference group], average risk 1.0–3.0 mg/L, high risk >3.0 mg/L).

2) **Adjusted Cox Proportional Hazards Models**
   Any analysis below including *Framingham risk score* will be done using Framingham score as a categorical variable (two sets of cutoffs: <10%, 10–20%, >20%; and <6%, 6–20%, >20%) as well as using all components of the Framingham risk score. Analyses using the pooled cohort equations will be done using the equations as a categorical variable (<5%, 5–7.5%, >7.5%) as well as using all components of the score.

   i) **Model 1:** Cox proportional hazards model will be performed using *Framingham risk score.*
   **Model 1b:** Cox proportional hazards model will be performed using AHA/ACC pooled cohort equations.

   ii) **Model 2:** Cox proportional hazards model will be performed including both hs-CRP categories and hs-TnI categories.
   iii) **Model 3:** Cox proportional hazards model will be performed using hs-TnI categories, adjusting with *Framingham risk score.*
   **Model 3b:** Cox proportional hazards model will be performed using hs-TnI categories, adjusting with AHA/ACC pooled cohort equations.

   iv) **Model 4:** Cox proportional hazards model will be performed using hs-CRP categories, adjusting with *Framingham risk score.*

> **Model 4b:** Cox proportional hazards model will be performed using hs-CRP categories, adjusting with AHA/ACC pooled cohort equations.

v) **Model 5:** Cox proportional hazards models will be performed using both hs-CRP categories and hs-TnI categories, adjusting with *Framingham risk score*.
**Model 5b:** Cox proportional hazards models will be performed using hs-CRP categories and hs-TnI categories, adjusting with AHA/ACC pooled cohort equations.

Hazard ratios for biomarker categories will be compared between models incorporating hs-TnI vs hs-CRP, and models including both biomarkers. A higher hazard ratio for one biomarker over the other suggests stronger prediction performance. A statistically significant hazard ratio for hs-TnI in a model that also contains hs-CRP demonstrates incremental risk prediction for hs-TnI over hs-CRP.

To find the 'best' model in terms of goodness of fitting and prediction performance, the Akaike's Information Criterion (AIC) will be calculated for each model. The smaller the AIC value, the better the model.

Calculate the 10-year risk using the above fitted model for each participant as follow:

$$\text{10-year Risk} \quad = \quad 1 - S_{10}^{exp(\beta_1 x_1 + \beta_2 x_2 + \beta_2 x_2 + \cdots + \beta_k x_k)}$$

$S_{10:}$ the baseline survival rate at 10 years
$\beta_{i:}$ estimated coefficient from the model
$x_{i:}$ covariates values

The following SAS codes will be used to perform the above analysis:

```
ods output ParameterEstimates = ParameterEstimates;
ods output Type1 = Type1;

proc phreg data = indata;

class hsTnI (and other covariates used as categorical variables);

model survival * status(0) = hsTnI (or hsCRP)

                        + (other covariates)  /Type1 risklimits = pl;
```

*survival:* Time between specimen collection and last contact, or, if event occurred, time between collection and event occurrence
*status:* 1 if event occurred, 0 otherwise

To evaluate the improvement in risk prediction of hs-TnI, the net reclassification improvement (NRI) will be calculated using the 10-year risk estimates for each participant from the above fitted models. The risk categories with respective to different endpoints for NRI analysis are as follows:

i) Global cardiovascular composite outcome: 0-<7.5%, 7.5% - 10%, >10% .
ii) CAD: 0-<6%, 6%-20%, >20% and 0-<10%, 10% - 20% and >20%. Only applies to analyses using the *Framingham risk score.*
iii) ASCVD: 0%-<5%, 5% - 7.5%, >7.5%. Only applies to analyses using the AHA/ACC pooled cohort equations.

Based on the above table, NRI will be calculated as follows:

$$NRI = (\hat{p}_{up,\ events} - \hat{p}_{down,\ events}) - (\hat{p}_{up,\ nonevents} - \hat{p}_{down,\ nonevents})$$

$$\hat{p}_{up,\ events} = \frac{Number\ of\ Events\ Move\ up\ to\ a\ higher\ risk\ category}{Number\ of\ Events}$$

$$\hat{p}_{down,\ events} = \frac{Number\ of\ Events\ Move\ down\ to\ a\ lower\ risk\ category}{Number\ of\ Events}$$

$$\hat{p}_{up,\ nonevents} = \frac{Number\ of\ No\ Events\ Moving\ up\ to\ a\ higher\ risk\ category}{Number\ of\ No\ Events}$$

$$\hat{p}_{down,\ nonevents} = \frac{Number\ of\ No\ Events\ Moving\ down\ to\ a\ lower\ risk\ category}{Number\ of\ No\ Events}$$

The integrated discrimination index (IDI) will also be calculated to evaluate the prediction improvement of hs-TnI as well. The IDI represents the improvement in average sensitivity minus any increase in (1 - specificity). It can be estimated as follow:

$$IDI = (\overline{\hat{p}}_{with\ hsTnI,\ events} - \overline{\hat{p}}_{without\ hs\ TnI,\ events}) - (\overline{\hat{p}}_{with\ hsTnI,\ events} - \overline{\hat{p}}_{without\ hsTnI,\ events})$$

$\overline{\hat{p}}_{with\ hsTnI,\ events}$ : mean of the predicted risks of an event for those who develop events from the model with hs-TnI

$\overline{\hat{p}}_{with\ hsTnI,\ events}$ : mean of the predicted risks of an event for those who develop events from the model without hs-TnI

$\overline{\hat{p}}_{without\ hsTnI,\ events}$ : mean of the predicted risks of an event for those who do not develop events from the model with hs-TnI

$\overline{\hat{p}}_{without\ hsTnI,\ events}$ : mean of the predicted risks of an event for those who do not develop events from the model without hs-TnI

An asymptotic test for NRI and IDI, respectively, will be performed to evaluate whether the improvement is significant at a 0.05 significance level [5]. The z test statistics is calculated as follow for NRI and IDI, respectively.

NRI:
$$H_0: \text{NRI} \leq 0$$
$$H_1: \text{NRI} > 0$$

$$z = \frac{\widehat{NRI}}{\sqrt{\dfrac{\hat{p}_{\text{up, events}} + \hat{p}_{\text{down, events}}}{Number\ of\ Events} + \dfrac{\hat{p}_{\text{up, nonevents}} + \hat{p}_{\text{down, nonevents}}}{Number\ of\ NonEvents}}} \sim N(0,1)$$

IDI:
$$H_0: \text{IDI} \leq 0$$
$$H_1: \text{IDI} > 0$$

$$z = \frac{\widehat{IDI}}{\sqrt{(\widehat{Se}_{events})^2 + (\widehat{Se}_{noevents})^2}} \sim N(0,1)$$

To evaluate the prediction improvement of h-sTnI, the following models will be compared by NRI and IDI:
- ✓ Model 3 (hs-TnI + Framingham) vs. Model 1 (Framingham), to evaluate the improvement of hs-TnI from using Framingham risk score only (three comparisons).
- ✓ Model 3b (hs-TnI + AHA/ACC) vs. Model 1b (AHA/ACC), to evaluate the improvement of hs-TnI from using score from AHA/ACC risk calculator only.
- ✓ Model 3 (hs-TnI + Framingham) vs. Model 4 (hs-CRP + Framingham), to evaluate the improvement by replacing hs-CRP with hs-TnI adjusted by Framingham risk score (three comparisons).
- ✓ Model 3b (hs-TnI + AHA/ACC) vs. Model 4b (hs-CRP + AHA/ACC), to evaluate the improvement by replacing hs-CRP with hs-TnI adjusted by score from AHA/ACC risk calculator.
- ✓ Model 5 (hs-TnI + hs-CRP +Framingham) vs. Model 4 (hs-CRP + Framingham), to evaluate the improvement from using hs-CRP with Framingham risks score (three comparisons).
- ✓ Model 5b (hs-TnI + hs-CRP + AHA/ACC) vs. Model 4b (hs-CRP + AHA/ACC), to evaluate the improvement from using hs-CRP with score from AHA/ACC risk calculator.

All the above analysis will be repeated using hs-TnI values and hs-CRP as a continuous variable.

A larger NRI for one biomarker vs the other suggests stronger performance in risk reclassification over the base model. A statistically significant value for NRI for hs-TnI in a

model that also contains hs-CRP demonstrates that hs-TnI improves risk classification compared with traditional risk models + hs-CRP.

## Kaplan-Meier Curve

Kaplan-Meier curves for time to event will be graphed for each hs-TnI category. The results of the log-rank test, the mean and median survival time with corresponding 95% confidence intervals, and the summary of censored and uncensored data for each hs-TnI group will be reported. If the number of subjects that have event is less than 50%, then report the proportion of events for each hs-TnI group instead of the mean and median survival times.

## Area Under the ROC Curve (AUC)

The area under the ROC curve (AUC) is the average sensitivity of the biomarker over the range of specificities. The empirical AUC is calculated via the 'trapezoidal' rule. The sum of the areas of the trapezoids is the AUC.

The traditional time-independent ROC curve and AUC will be calculated from unadjusted and adjusted logistic models.

The AUCs will be compared as follows:

✓ Model with (hs-TnI + hs-CRP) vs. Model with (hs-CRP)
✓ Model with (hs-TnI + Framingham) vs. Model with (Framingham)
✓ Model with (hs-CRP+Framingham) vs. Model with (Framingham)
✓ Model with (hs-TnI + AHA/ACC) vs. Model with (AHA/ACC)
✓ Model with (hs-CRP + AHA/ACC) vs. Model with (AHA/ACC)

P-value for the AUC comparison will also be reported to evaluate whether there is statistically significant improvement with addition of hs-TnI or hs-CRP to the base models.

### d.  Level of Significance/Confidence Statement
Two-sided 95% confidence interval will be provided.

## Additional Analysis

We will examine whether the levels of hs-TnI provide additional information as compared to levels of hs-TnT and NT-proBNP. Several approaches will be taken. First, we will examine the correlation between levels of hs-TnI and hs-TnT using regression analyses. If the R value is less then 0.6, then we will perform tertile analyses and examine the hazard ratios of elevated levels of hs-TnI and hs-TnT, with the reference group being individuals with both hs-TnI and hs-TnT levels in the lowest tertile. We will also repeat some of the Cox proportional-hazards models described above to see what happens to the hazard ratios if levels of hs-TnT are added to the models.

### a.  Analysis Variables
- Low Risk: hs-TnI values < 6 ng/L
- Low Risk: hs-CRP values 1.0 mg/L

- Composite outcome (i.e., all cause and cardiovascular mortality, incident HF, MI, stroke, and coronary revascularization)

b. **Statistical Method**

Incidence rates of the low risk groups defined by hs-TnI/ hs-CRP will be calculated based on the following formula:

$$\text{Incidence Rate} = \frac{\text{Number of Subjects with an Event}}{\text{Total person-time at risk in Low Risk Group}} \times 1000 \text{ person years}$$

Total person-time at risk: sum of each subject's time at risk (i.e., the length of time they were followed up or the length of time they were free of an event).

The Incidence Rate difference between the two low risk groups defined by hs-TnI and hs-CRP values, respectively, can be calculated as follow.

$$\text{Difference} = \text{Incidence Rate}_{\text{hs-TnI low risk}} - \text{Incidence Rate}_{\text{hs-CRP low risk}}$$

To construct the confidence intervals for difference of the incidence rates between the low risk groups defined by hs-TnI/hs-CRP, bootstrapping method will be used.

    i. Draw a random sample with replacement from each low risk group, the sample size will be equal to that of each low risk group, respectively.

    ii. Calculate the incidence rate for each random sample, and then calculate the difference between the two incidence rates.

    iii. Repeat the above steps for 1,000 times.

    iv. Use the 2.5 percentile (lower limit) and 97.5 percentile (upper limit) from the 1,000 calculated differences to construct the 95% confidence intervals.

f. **Level of Significance / Confidence Statement**

Two-sided 95% confidence interval (CI) will be provided.

**References:**
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**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)?**

**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\* 2013.20)**
    **___ 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 __x__ No.