ARIC Manuscript Proposal #3598

PC Reviewed: 4/14/20	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a.Full Title: Change in Prevalence of Elevated High Sensitivity Cardiac Troponin-T and NT-proBNP with Age: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Change in hs-cTnT and NT-proBNP over long-term follow-up

2.Writing Group:

Writing group members:

Aliza Hussain
Olive Tang
Xiaoming Jia
Elizabeth Selvin
Vijay Nambi
Caroline Sun
Aaron Folsom
Thomas Mosley
Salim S. Virani
Eric Boerwinkle
Bing Yu
Ron C. Hoogeveen
Josef Coresh
Christie Ballantyne

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

First author: Aliza Hussain

Phone: 412-8886615
E-mail: alizah@bcm.edu

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: Christie Ballantyne

Address: 1 Baylor plaza, Baylor College of Medicine
P**hone**:7137985034

E-mail: cmb@bcm.edu

3.Timeline:

The data needed for this analysis are currently available; we plan to submit for publication within 1 year.

4.Rationale:

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), can result in acute respiratory disease syndrome (ARDS). Current data indicates that older adults, men and those with underlying comorbidities such as cardiovascular disease (CVD), hypertension, diabetes and chronic respiratory disease are at particularly higher risk for severe COVID-19 associated illness and death[1-3]. Furthermore, adults with COVID-19 mediated cardiac injury exhibit poor prognosis and higher risk of mortality[4-5].

In a recent study of 416 patients with confirmed COVID-19 infections, patients with cardiac injury as defined by elevated high-sensitivity troponin above the 99th percentile were more likely to be older and have prevalent comorbidities such as coronary heart disease (CHD), cerebrovascular disease and hypertension. Furthermore, patients with cardiac injury demonstrated four-fold increased risk of death compared to those without cardiac injury (hazard ratio, 4.26; 95% CI, 1.92 - 9.49), after adjustment for age, comorbidities (CHD, diabetes, chronic obstructive pulmonary disease, renal failure, cancer), ARDS, creatinine elevation and NT-proBNP levels[4]. Concentrations of high-sensitivity cardiac troponin-T (hs-cTnT) increase with age and are higher in men than women[6]. We have previously shown in Atherosclerosis Risk in Communities (ARIC) that a high proportion of older adults (mean age 75) had detectable hs-cTnT >6 ng/L (~64%) and elevated NT-proBNP > 100 pg/mL (~57%). Moreover, both NT-proBNP and hs-cTnT were independent predictors of CVD and improved short-term risk prediction in older adults[7]. We hypothesize that the changing prevalence of subclinical CVD as

measured by elevated levels of hs-cTnT and NT-proBNP is related to the higher mortality rates seen with COVID-19 in older male individuals.

The renin-angiotensin-aldosterone system (RAAS) is an important hormone system involved in regulation of blood pressure and fluid/electrolyte balance. SARS-CoV-2 interacts with RAAS by entering human cells through angiotensin converting enzyme 2 (ACE2) receptors[8,9]. ACE2 is a crucial counter-regulatory enzyme of RAAS and opposes the activity of angiotensin II. ACE-inhibitors (ACEi) and angiotensin-receptor blockers (ARB), which inhibit the RAAS system, may theoretically modulate the activity of ACE2, raising concern for potential increased virulence of SARS-CoV-2 in patients being treated with these medications[10,11].

The beneficial effects of ACE2 are well-established. In animal models, ACE2 deficiency has been shown to result in cardiac hypertrophy, adverse ventricular remodeling and cardiac fibrosis[12]. Furthermore, ACE2 is protective against severe acute lung injury. Whereas, ACE and angiotensin II induce pulmonary edema and impair lung function[13]. SARS-CoV-2 appears to down-regulate ACE2 expression in lungs, and it has been postulated that unabated angiotensin II activity may in part be responsible for organ injury. Preclinical studies have shown that ACE2 levels decrease with age[14], with higher levels found in older females than males[14,15]. However, there is limited clinical data of the association of ACE2, ACE, renin and angiotensin levels with either subclinical cardiac injury or those with prevalent cardiovascular disease.

Pharmacological inhibition of RAAS has a definitive role in prevention of heart failure, kidney disease, coronary artery disease and cardiovascular mortality in high-risk patients[16-18]. As such, withdrawal of these agents may increase risk for adverse cardiovascular events, especially in adults with hypertension or cardiovascular disease who are already susceptible to severe COVD-19. On the other hand, animal models have shown inconsistent findings regarding

effects of ACEi or ARBs on ACE2 levels or activity with some showing increase in RNA expression or proteins levels of ACE2 in tissue[19,20] and others showing no effect[21]. Few studies have evaluated effects of RAAS inhibition on ACE2 in humans. We hypothesize that unopposed activity of the renin-angiotensin system may in fact be associated with increased risk for heart failure and cardiovascular disease.

In this study, we plan to 1) trend the change in hs-cTnT and NT-proBNP across visits serial visit 2 (1990-1992) to visit 7 (2018-2019) 2) assess associations of ACE2, ACE, renin and angiotensinogen with elevated hs-cTnT, NT-proBNP and CVD in the ARIC study.

The ARIC study is ideal for this analysis given the comprehensive assessment of cardiovascular risk factors and cardiovascular events and availability of Somalogic data to measure ACE2, ACE, renin and angiotensinogen levels at visit 3 and visit 5. Measurement of hs-cTnT and NT-proBNP using the same assay were performed from visit 2 (1990-1992, mean age 57 years), visit 4 (1996-1998, mean age 63 years), visit 5 (2011-2013, mean age 76), visit 6 (2016-2017, mean age 79) and visit 7 (2018-2019) allows for serial assessment over long term follow-up of 18 years. This allows us to examine the prevalence of elevated levels of hs-cTnT and NT-proBNP in the same cohort over almost 2 decades in the population that has shown the greatest risk for COVID19.

5. Main Hypothesis/Study Questions:

Aim 1: Evaluate the prevalence of elevated levels of hs-cTnT and NT pro BNP from middle aged to older age adults

Hypothesis 1a: The prevalence of “detectable” and “elevated” hs-cTnT will be higher in older adults

Hypothesis 1b: The prevalence of elevated NT-proBNP levels will be higher in older adults

Aim 2: Examine the association of ACE2, ACE, renin and angiotensinogen levels (measured using SomaScan) with elevated markers of subclinical cardiac injury i.e. hs-cTnT and NT-proBNP and prevalent CVD

Hypothesis 2: ACE2 levels, ACE, renin and angiotensinogen levels are associated with elevated levels of cardiac markers of subclinical injury or prevalent global CVD (CHD, ischemic stroke or heart failure).

Aim 3: Examine the association of ACE2, ACE, renin and angiotensinogen levels (measured using SomaScan) with incident global CVD (CHD, ischemic stroke or heart failure) at visit 3 (mid-life) and visit 5 (late-life).

Hypothesis 3: ACE2, ACE, renin and angiotensinogen levels (measured using SomaScan) are associated with incident CHD, ischemic stroke or heart failure events.

Aim 4: If ACE2 levels or any of the other biomarkers of the RAAS axis in aim 2 and 3 show any significant associations with markers of subclinical cardiac injury, we will also conduct an exploratory aim to examine the biological pathways associated with elevated plasma ACE2 levels in the participants in ARIC visit 5 using proteomic data available in ARIC.

Hypothesis 4: There are unique biological pathways associated with levels of ACE2 in those with subclinical cardiac injury or prevalent CVD and may help explain the higher mortality seen in older adults and may provide insights into why these individuals may exhibit increased risk of complications with SARS-CoV2 virus.

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).

Aim 1:

Study Design: cross- sectional analysis at ARIC visit 2 (1990-1992), 4 (1996-1998), 5 (2011-2013), 6 (2016-2017), 7 (2018-2019)

Exclusions:

- Participants with prior ASCVD or HF hospitalization will be excluded

Exposure:

- hs-cTnT measured at each visit

hs-cTnT at each time point (visit) will be categorized as: "undetectable" (hs-cTnT <6 ng/L), detectable" (hs-cTnT ≥6 and <14 ng/L) or "elevated" (hs-cTnT ≥14ng/L). The level of 6 ng/L was chosen as it is the limit of quantification for the assay in the manufactures package insert and 14 ng/L reflects the 99th percentile upper reference limited as recommended by the Joint European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Heart Federation Task Force for the Universal Definition of Myocardial Infarction[22].

- NT-proBNP measured at each visit

NT-proBNP will be categorized as low risk (<100 pg/mL), intermediate risk (≥100 and <300 pg/mL) and high risk (≥300 pg/mL). These cut-point were chosen based on previous ARIC study evaluating risk prediction of NT-proBNP in older adults[7].

Statistical Analyses:

Baseline characteristics will be tabulated by categories of both biomarkers, hs-cTnT and NT-proBNP at visit 2, visit 4, visit 5, visit 6 and visit 7. Continuous variables will be reported using mean (SD) or median (IQR) depending on normality of the data, while categorical variables will be expressed as count (percentage). Differences will be tested using ANOVA, non-parametric testing, or chi-square testing as appropriate.

For NT-proBNP and hs-cTnT, original assays were conducted at Baylor College of Medicine (BCM) in 2010 using stored plasma from visit 4, at BCM in 2011-13 using stored plasma from visit 5, and at UMN in 2011-13 using stored serum from visit 2. Plasma assays conducted at BCM from 2010 through 2013 were shown to be stable, and thus the original measurements from visits 4 and 5 were considered equivalent. To address the methodological difference between visit 2 and visits 4 and 5, we will re-calibrate based on prior study from ARIC[23].

The prevalence (SD) of undetectable, detectable and elevated hs-cTnT levels will be calculated for each time point (i.e. visit 2, 4, 5, 6, 7). Similarly, prevalence of low, intermediate or high risk NT-proBNP levels will be calculated at each visit. Results will be stratified by sex and race/ethnicity.

Aim 2:

Study Design: cross-sectional analysis at ARIC visit 5 (n= 6,538)

Exclusions:

- Standard race-center exclusions
- Missing measurements of covariates

Exposure: Natural log-transformed ACE2, ACE, renin and angiotensinogen levels (measured using SOMAScan)

Outcomes:

Markers of subclinical cardiac injury

- hs-cTnT, categorized as "undetectable" (hs-cTnT <6 ng/L), detectable" (hs-cTnT ≥6 and <14 ng/L) or "elevated" (hs-cTnT ≥14ng/L).
- NT-proBNP categorized as low risk (<100 pg/mL), intermediate risk (≥100 and <300 pg/mL) or high risk (≥300 pg/mL)

Prevalent cardiovascular events:

- Prevalent CHD[24]
- Prevalent ischemic stroke[25]
- Prevalent ASCVD (cumulative of CHD, ischemic stroke)
- Prevalent heart failure [26]
- Prevalent global CVD (cumulative coronary heart disease, ischemic stroke and heart failure)

Statistical Analyses:

Multivariable logistic or linear regression models will be used to assess cross-sectional association between log-transformed ACE2, ACE, renin or angiotensinogen levels (measured using SomaScan) with clinical and biochemical variables: diabetes, hypertension, use of ACE/ARBi, hs-cTnT (per 1 SD) and NTproBNP (per 1 SD)

<u>ACE2, ACE, renin and angiotensinogen and prevalent outcomes</u>

Logistic regression models will be used to assess cross-sectional association between log-natural transformed ACE2, ACE, renin and angiotensinogen and tertile of hs-cTnT and NT-proBNP or prevalent CVD.

Models will be sequentially adjusted for age, sex, race/ethnicity (Model 1) and hypertension, diabetes mellitus, dyslipidemia, obesity, cigarette smoking, alcohol use, BMI, SBP, LDL-C, HDL-C, HbA1c, lipid-lowering medication use, antihypertensive medication use, ACEi/ARB use, glucose-lowering medication use, eGFR (Model 2).

Sensitivity analysis:

Results will be stratified by sex, age (categorized as <60, 60-70, 70-80, ≥80 years), presence of hypertension, presence of diabetes, use of ACEi/ARB. Multiplicative interaction testing will be

performed between ACE2, ACE, renin or angiotensinogen and each of the and each of the aforementioned groups and tested for significance in multivariable models (Models 1 and 2 as described above).

Aim 3:

Study Design: prospective analysis at ARIC visit 3 (n=12887) and visit 5 (n= 6,538)

Exclusions:

- Standard race-center exclusions
- Missing measurements of covariates

Exposure: Natural log-transformed ACE2, ACE, renin and angiotensinogen levels (measured using SOMAScan)

Outcomes:

- Incident CHD
- Incident ischemic stroke
- Incident ASCVD (CHD + ischemic stroke)
- Incident heart failure hospitalization
- Incident global CVD (CHD + ischemic stroke + heart failure hospitalization)

Statistical analysis:

ACE2, ACE, renin and angiotensinogen and incident global CVD:

Multivariable Cox regression models will be used to study the association of log-transformed ACE2, ACE, renin or angiotensinogen levels (per 1 SD increase) and incident CVD events at visit 3 (mid-life) and visit 5 (late-life).

Models will be sequentially adjusted for age, sex, race/ethnicity (Model 1) and hypertension, diabetes mellitus, dyslipidemia, obesity, cigarette smoking, alcohol use, BMI, SBP, LDL-C,

HDL-C, HbA1c, lipid-lowering medication use, antihypertensive medication use, ACEi/ARB use, glucose-lowering medication use, eGFR (Model 2).

Sensitivity analysis:

Results will be stratified by sex, age (categorized as <60, 60-70, 70-80, ≥80 years), presence of hypertension, presence of diabetes, use of ACEi/ARB, prevalent CVD. Multiplicative interaction testing will be performed between ACE2, ACE, renin or angiotensinogen and each of the and each of the aforementioned groups and tested for significance in multivariable models (Models 1 and 2 as described above). Analyses of incident ASCVD will account for competing risk of non-cardiovascular death using the method of Fine and Gray.

Aim 4

Exposures: SOMAScan proteins, excluding non-human proteins detected due to cross-reactivity (n ~ 5,000)

Outcome: Log-transformed ACE2

Analysis:

1) Cross-sectional analysis of plasma proteins measured in the SomaLogic proteomics assay and ACE2 levels at visit 5. Proteins (as measured by SomaLogic assay) that are significantly associated with ACE2 level based on p-values for the β coefficient from linear regression analysis with model 2 (see above) adjustment will be identified. P-values will be corrected for multiple testing using the Benjamini–Hochberg false discovery rate procedure. A false discovery rate <0.05 will be used as a threshold for statistical significance.
2) Exploratory pathway using Ingenuity Pathway Analysis (using Qiagen, Hilden, Germany) based on proteins/corresponding genes significantly associated with ACE2 to 1) construct a network of interacting proteins 2) elucidate potential upstream regulators (direct or non-

direct mediator genes) among proteins significantly associated with ACE2. Proteome-wide significance will be defined based on a Bonferroni-corrected p-value of 9.46e-. The pathway analysis will use all proteins in the study as a background/reference to account for possible selection biases of proteins used in the aptamer assay

Limitations:

- There is the potential for residual confounding.
- Ideally, we would have ACE2, ACE, renin and angiotensinogen levels measured by immunoassay at visit 5. However, we have renin and ACE2 levels measured using Olink proteomic assay at visit 5(n=476) which we will use to validate renin and ACE2 levels measured using SomaScan (n=476)

7.a.Will the data be used for non-CVD analysis in this manuscript? 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?NA
(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? 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"?NA

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

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)?**

No relevant proposals have been identified.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? Yes

11.b. If yes, is the proposal

_X_ A. primarily the result of an ancillary study (list number*

___ 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

1. Remuzzi A, Remuzzi G. COVID-19 and Italy: what next? *Lancet*. 2020. pii: S0140-6736(20)30627-9. doi: 10.1016/S0140-6736(20)30627-9.
2. Severe Outcomes Among Patients with Coronavirus Disease 2019 (COVID-19) — United States, February 12–March 16, 2020. MMWR Morb Mortal Wkly Rep. Published online March 2020. doi: http://dx.doi.org/10.15585/mmwr.mm6912e2
3. Onder G, Rezza G, Brusaferro S. Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *JAMA.* Published online March 23, 2020. doi:10.1001/jama.2020.4683

4. Shi S, Qin M, Shen B, et al. Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China. *JAMA Cardiol.* Published online March 25, 2020. doi:10.1001/jamacardio.2020.0950
5. Guo T, Fan Y, Chen M, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA Cardiol. Published online March 27, 2020. doi:10.1001/jamacardio.2020.1017Gore MO, Seliger SL, deFilippi CR, et al. Age- and Sex-Dependent Upper Reference Limits for the High-Sensitivity Cardiac Troponin T Assay. *J Am Coll Cardiol*. 2014;63 (14):1441-1448.
6. Saeed S, Nambi, V, Sun W, et al. Short-Term Global Cardiovascular Disease Risk Prediction in Older Adults. *J Am Coll Cardiol.* 2018;71(22): 2527-2536.
7. Zhou, P., Yang, X., Wang, X. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273 (2020). https://doi.org/10.1038/s41586-020-2012-7
8. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020. pii: S0092-8674(20)30229-4. doi: 10.1016/j.cell.2020.02.052. [Epub ahead of print]
9. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? Lancet Respir Med 2020 March 11 (Epub ahead of print).
10. Sommerstein R, Gräni C. Preventing a COVID-19 pandemic: ACE inhibitors as a potential risk factor for fatal COVID-19. BMJ 2020;368:m810-m810 (https://www.bmj.com/content/368/bmj.m810/rr-2. opens in new tab).

11. Crackower MA, Sarao R, Oudit GY, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature. 2002;417(6891):822-8.
12. Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. 2005;436(7047):112-6.
13. Xie X, Chen J, Wang X, et al. Age- and gender-related difference of ACE2 expression in rat lung. Life Sci. 2006;78(19):2166-71.
14. Fernández-Atucha A, Izagirre A, Fraile-Bermúdez AB, et al. Sex differences in the aging pattern of renin-angiotensin system serum peptidases. Biol Sex Differ. 2017;8:5. doi:10.1186/s13293-017-0128-8
15. ACE Inhibitor Myocardial Infarction Collaborative Group. Indications for ACE inhibitors in the early treatment of acute myocardial infarction: systematic overview of individual data from 100,000 patients in randomized trials. Circulation. 1998 Jun 9;97(22):2202-12.
16. Jafar TH, Schmid CH, Landa M, et al. Angiotensin-converting enzyme inhibitors and progression of nondiabetic renal disease. A meta-analysis of patient-level data. Ann Intern Med. 2001 Jul 17;135(2):73-87.
17. Flather MD, Yusuf S, Køber L, et al. Long-term ACE-inhibitor therapy in patients with heart failure or left-ventricular dysfunction: a systematic overview of data from individual patients. ACE-Inhibitor Myocardial Infarction Collaborative Group. Lancet. 2000 May 6;355(9215):1575-81.
18. Ferrario CM, Jessup J, Chappell MC, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. 2005;111(20):2605-1

19. Ishiyama Y, Gallagher PE, Averill DB, et al. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. Hypertension 2004;43:970-976.
20. Burchill LJ, Velkoska E, Dean RG, Griggs K, Patel SK, Burrell LM. Combination renin-angiotensin system blockade and angiotensin-converting enzyme 2 in experimental myocardial infarction: implications for future therapeutic directions. Clin Sci (Lond) 2012;123:649-658.
21. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. J Am Coll Cardiol 2012;60:1581-98.
22. Parrinello CM, Grams ME, Couper D, Ballantyne CM, Hoogeveen RC, Eckfeldt JH, Selvin E, Coresh J. Recalibration of blood analytes over 25 years in the atherosclerosis risk in communities study: impact of recalibration on chronic kidney disease prevalence and incidence. Clin Chem. 2015;61(7):938-47. doi: 10.1373/clinchem.2015.238873.
23. White AD, Folsom AR, Chambless LE, et al. Community surveillance of coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study: methods and initial two years' experience. J Clin Epidemiol 1996; 49: 223–33.
24. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol 1989; 129: 687–702.
25. Rosamond WD, Chang PP, Baggett C, et al. Classification of heart failure in the atherosclerosis risk in communities (ARIC) study: a comparison of diagnostic criteria. Circulation Heart failure 2012;5:152-9.