ARIC Manuscript Proposal #2170

PC Reviewed: 7/9/13	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: sRAGE, progression of subclinical cardiac damage, and risk of heart failure

b. Abbreviated Title (Length 26 characters): sRAGE, subclinical CVD & HF

2. Writing Group:

Writing group members: Mariana Lazo; Lu Shen; Marc K. Halushka; Brad Astor; Andreea Rawlings; Ron C. Hoogeveen; Tina E Brinkley; Christie M. Ballantyne; Elizabeth Selvin; others welcome

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

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3. Timeline: All data are available. We anticipate a rapid timeline for this manuscript and plan to have a manuscript submitted to the ARIC Publications Committee ~6 months from approval of the proposal.

4. Rationale:

Advanced glycation end products (AGEs) are of interest as novel biomarkers because they are the postulated etiologic link between hyperglycemia and diabetic complications. AGEs can also bind a variety of receptors. When stimulated by AGEs, the receptor for AGEs (RAGE) induces inflammation and is thought to fuel progression of chronic disease through nuclear factor (NF)-kB mediated signaling[1-3]. Circulating AGE and RAGE levels are influenced by a number of endogenous (glucose, inflammation) and exogenous (smoking, diet) factors known to play a role in risk of cardiovascular disease and diabetes. Nonetheless, it is unclear if circulating AGE receptor levels provide useful information regarding risk of long-term complications and there are virtually no data linking AGEs to measures of subclinical cardiovascular disease in the general population.

RAGE is a transmembrane-spanning receptor for AGEs, S100/calgranulins, amyloid-β peptide and other molecules. RAGE is found on inflammatory cells and endothelial cells. The C-terminus of the protein is located on the extracellular surface. The N-terminus of RAGE is essential in activating pro-inflammatory NF-κB mediated signaling. The soluble receptor for advanced glycation end products (sRAGE) is the isoform of RAGE found in serum and is formed by proteolytic cleavage of RAGE. sRAGE has been described as a "sponge" for AGEs and may have protective functions as it lacks the N-terminus and cannot activate NF-κB signaling. Levels are dependent on cell surface RAGE levels [4].

Recent studies have demonstrated epidemiological associations of circulating levels of sRAGE with cardiovascular disease in persons with diabetes or kidney disease [5-14]. We have previously shown robust associations of plasma sRAGE with incident diabetes, coronary heart disease, stroke and all-cause mortality in the ARIC Study [15]. However, there are scant data on the possible association of sRAGE with risk of heart failure and measures of subclinical cardiovascular disease, particularly in a general population. We propose to examine the association of plasma sRAGE with 1) biomarkers of subclinical cardiac damage (high-sensitivity cardiac troponin T and NT-proBNP); and 2) incident heart failure as determined from hospitalizations identified during over 20 years of follow-up of ARIC participants. This manuscript will use data from ARIC Ancillary Studies 2006.16, 2008.10, and 2009.16. We will utilize the sRAGE measurements available in a subsample of participants at visit 2 (Ancillary 2006.16. PI: Astor), the hs-cTnT and NT-proBNP measurements at visit 2 (Ancillary 2009.16, PI: Selvin), and the hs-cTnT and NT-proBNP available at visit 4 (Ancillary 2008.10, PI: Ballantyne).

5. Main Hypothesis/Study Questions:

There is evidence from laboratory studies that soluble circulating RAGE (sRAGE) counteracts the detrimental effects of RAGE by binding serum AGEs [15-17], suggesting that low levels of sRAGE may be a marker of long-term chronic disease risk. Thus, the objectives of this study were:

Aim 1: To characterize the cross-sectional and longitudinal associations of sRAGE with concentrations of hs-cTnT and NT-proBNP measured at two time points, 6 years apart, among persons with normal kidney function and no history of cardiovascular disease at baseline.

Hypothesis 1.1: sRAGE will be inversely and independently associated with hs-cTnT and NT-proBNP.
Hypothesis 1.2: sRAGE will also be inversely and independently associated with 6-year change in hs-cTnT and NT-proBNP.
Hypothesis 1.3: sRAGE will also be inversely and independently associated with incident detectable cardiac damage as assessed by cardiac troponin T (detectable concentrations at visit 4 among persons with no detectable concentrations at visit 2).

Aim 2: To characterize the association of sRAGE with risk of incident heart failure among persons with normal kidney function and no history of cardiovascular disease at baseline.

Hypothesis 2: sRAGE will be inversely and independently associated with risk of heart failure.

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 population: sRAGE measurements are available for a random sample of 1289 ARIC participants ages 47-68 (visit 2, 1990-92) with estimated GFR >60 mL/min/1.73 m^2 who were included in Ancillary Study # 2006.16. The basis of this study population is the random sub-cohort of participants selected for the parent case-cohort study of incident chronic kidney disease. We will exclude those persons missing covariates of interest and those with prevalent cardiovascular disease by visit 2. The final sample size will be approximately 1200 participants.

Study Design: We will conduct cross-sectional, longitudinal, and prospective cohort analyses with visit 2 as baseline.

For the cross-sectional analyses, we will examine the associations between sRAGE and subclinical myocardial damage markers (hs-cTnT and NT-proBNP) measured at visit 2. For the longitudinal analyses, we will examine the association between sRAGE, 6-year changes in subclinical myocardial damage markers (hs-cTnT and NT-proBNP). For the prospective cohort analyses, we will examine the association of sRAGE with incident heart failure using the most recent follow-up data available (currently through 2010).

Exposure: sRAGE measured by ELISA (R&D Systems, CV<3%) from stored plasma samples at ARIC visit 2.

Outcomes: Levels of hs-cTnT and NT-proBNP at visits 2 and 4, 6-years changes in hs-cTnT and NT-proBNP (absolute, percent, categorical) and incident CHF events.

Visit 2: NT-proBNP and hs-cTnT levels were measured from stored (visit 2) serum samples using two sandwich immunoassay methods (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.

Visit 4: NT-proBNP and hs-cTnT levels were measured from stored (visit 4) plasma samples using two electrochemiluminescent immunoassays on an automated Cobas e411 analyzer (Roche Diagnostics) in 2009[?] at the Baylor College of Medicine as part of Dr. Ballantyne's ancillary study.

Calibration of visit 2 and 4 measurements: We are currently conducting a calibration study to evaluate the comparability of different laboratory assays between different ARIC visits, including the Roche NT-proBNP and hs-cTnT assays at visit 2 (serum, Roche Elecsys2010 at UMN) and visit 4 (plasma, Cobas e411 at Baylor). We expect that NT-proBNP will need re-calibration and all of our analyses will incorporate a statistical correction to correct for the methodological differences between the two measurements.

Other variables of interest: To examine the independent association of sRAGE with subclinical myocardial damage markers (hs-cTnT and NT-proBNP) and incident CHF, we will adjust for potential confounders (primarily measured at visit 2) including: age, sex, race-center, body mass index, total cholesterol, systolic and diastolic blood pressures, blood pressure-lowering medication use, smoking status, drinking status, diabetes status (self-reported history, medication use, glucose, HbA1c), C-reactive protein, kidney function (as measured by eGFR), physical activity level (Baecke index, visit 1), and education level (visit 1).

Statistical Analysis:

We will use linear and logistic regression models to investigate the cross-sectional associations between hs-cTnT and NT-proBNP measured at visit 2 and low sRAGE levels (first quartile vs the other four). We will conduct sensitivity analyses examining the associations of hs-cTnT and NT-proBNP with very low sRAGE levels (first decile). Given the large racial differences in the levels of sRAGE we will also conduct race stratified analyses. We will use linear and restricted cubic splines to characterize the shapes of the continuous associations between these biomarkers.

For the longitudinal analyses of 6-year change in hs-cTnT and NT-proBNP, we will first characterize the association of sRAGE (modeled continuously and in categories) at visit 2 (baseline) with incidence and progression of myocardial injury, as indicated by change in hs-cTnT and NT-proBNP from visit 2 to 4. Among persons with no detectable levels of hs-cTnT at visit 2, we will examine the association of sRAGE with "incident" detectable hs-cTnT at visit 4 (binary variable). Among persons with detectable levels of hs-cTnT or

NT-proBNP at visit 2, we split the population into approximate thirds as per previous analyses of hs-cTnT in ARIC [18]. We will characterize change across these categories (i.e., movement across categories of detactable and thirds of detectable hs-cTnT at visit 2 to these categories at visit 4). For simplicity, we may also compare the association of sRAGE across categories of change from undetectable, detectable, and elevated at visit 2 to visit 4 (3x3 grid). All covariates will be assessed at visit 2.

To evaluate the prospective association of baseline sRAGE with risk of heart failure, we will use Cox proportional hazards models to with adjustment for potential confounders (listed above). The proportional hazards assumption will be examined using log-(-log) plots and by testing risk factor-by-time interactions; if the assumption is violated the interactions term(s) will be kept in the model and the time-dependent nature of the risk will be interpreted accordingly. sRAGE will be divided into quartiles in initial analyses and we will generate linear splines with knots corresponding to the quartiles of the distribution of sRAGE. Restricted cubic splines will also implemented to obtain a smoother fit to the data.

Sensitivity analyses: Because we have previously shown substantial differences in the distribution of sRAGE in blacks compared to whites[15], we will conduct sensitivity analyses to explore the possibility of effect modification by race. We will formally test for race interactions and conduct analyses stratified by race. We will also conduct sensitivity analyses to address the possibility of a small number of incident clinical cases of cardiovascular disease that may occur between baseline (visit 2) and the follow-up visit (visit 4). We will also address the potential impact of the small number of deaths that may have occurred after visit 2 but before the visit 4.
For the prospective analyses we will model important covariates as time-varying covariates.

Limitations:

- Single measurement of sRAGE at visit 2
- Measurements of hs-cTnT and NT-proBNP at only two time points, 6 years apart.
- As with all observational studies, we will not be able to eliminate the possibility of residual confounding despite rigorous adjustment for known cardiovascular disease 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 ICTDER03 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)?

MSP# 1890 Determinants of sRAGE and its Association with Cardiovascular Disease, Diabetes, and Mortality in a Community based Population

MSP# 1905 The Association of Lifestyle Factors with circulating levels of the Soluble Receptor for Advanced Glycation End Products

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* 2006.16, 2008.10, 2009.16)
_X_ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* 2006.15)

*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.
_ES_

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.
_ML_

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