1a. **Full Title**: High sensitivity cardiac troponin-T (cTnT) and amino terminal pro-brain natriuretic peptide (NT-proBNP) and the relationship with retinopathy and incident cardiovascular disease

b. **Abbreviated Title (Length 26 characters)**: TnT, BNP and Retinopathy

2. **Writing Group**:
   Writing group members: Natalie Bello, Susan Cheng, Angela B S Santos, Amil Shah, Deepak Gupta, David Aguilar, Christie Ballantyne, Brian Claggett, Tien Yin Wong, Barbara Klein, Ronald Klein, A. Richey Sharrett, Scott Solomon, OTHERS.

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

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3. **Timeline**:
Analysis will begin immediately following proposal approval with the aim of completing analyses and submitting an abstract to the American College of Cardiology Annual Meeting (November deadline). The subsequent aim will be to complete a manuscript within 6 months of proposal approval.
4. **Rationale:**
Images of the retina provide a window into the microcirculation and allow a non-invasive assessment of microvascular dysfunction and cardiovascular risk. In ARIC, the presence of retinopathy has been shown to add incremental risk prediction to the traditional Framingham criteria.\(^1\) Narrowed retinal artery diameters are associated with hypertension,\(^2,^3\) as well as incident diabetes,\(^4\) coronary heart disease (CHD),\(^5,^6\) and congestive heart failure (CHF).\(^7,^8\) These findings are especially pronounced in women and diabetics; two groups who are particularly susceptible to microvascular dysfunction.\(^9,^{10}\)

In diabetic retinopathy, chronic exposure to hyperglycemia is believed to initiate a cascade of biochemical and physiological changes that ultimately lead to microvascular damage and retinal dysfunction. Local hypoxia, inflammation, increased oxidative stress, and intraocular up-regulation of the renin-angiotensin system also play a pathophysiological role.\(^11\) Importantly, the development of retinopathy is not limited to individuals with diabetes. It has been shown that the traditional cardiovascular risk factors of hypertension, dyslipidemia and obesity are associated with the presence of retinopathy in non-diabetic individuals as well,\(^12\) suggesting a shared systemic pathophysiology of microvascular and macrovascular disease.

Similar to the visualization of retinopathy on physical examination, the presence of measurable levels of cardiac biomarkers in plasma offers a non-invasive tool to identify persons at heightened risk of future cardiovascular events. Troponin T is a structural protein found in cardiac myocytes which is released into the circulation when the myocyte cell membrane is disrupted through cell injury or death. Levels of troponin T are used clinically as a marker of myocardial damage due to ischemia in coronary artery disease,\(^13\) and correlate with incident heart failure\(^14\) and mortality in congestive heart failure.\(^15\) Amino terminal brain natriuretic peptide (NT-proBNP) is the biologically inert cleavage product generated during the formation of BNP from its prohormone pro-BNP. Physiologically, BNP has diuretic and natriuretic effects, and similar to troponin its release into the circulation is magnified in heart failure as well as following myocardial infarction.\(^16\)

While troponin is a marker of cardiac injury and BNP is more closely related to volume overload, both have been shown to predict future cardiovascular events in otherwise asymptomatic individuals.\(^17,^{18,19}\) Recently, much attention has been focused on the presence and significance of measurable levels of high sensitivity cardiac TnT and NT-proBNP in the absence of obstructive coronary disease or overt heart failure. Mechanisms analogous to those central to the development of retinopathy including inflammation, oxidative stress, and hypoxia from supply demand mismatch due to subendocardial ischemia or increased myocardial oxygen demand have been put forth as potential sources of circulating biomarkers.\(^20\) The association between elevated levels of these cardiac biomarkers and retinopathy is not known, though potentially both occur as a result of microvascular damage. We propose a primary analysis to determine if a correlation exists between high sensitivity cardiac troponin T, NT-proBNP and retinopathy and a secondary analysis of whether the combination of troponin T, NT-proBNP and retinopathy predicts the incidence of cardiovascular diseases and congestive heart failure.
5. **Main Hypothesis/Study Questions:**
We hypothesize that retinopathy is independently and positively associated with elevations in the cardiac biomarkers high sensitivity cardiac troponin-T (cTnT) and amino-terminal pro-brain natriuretic peptide (NT-proBNP). Specifically, we aim to determine the associations of:

1. Retinal microvascular abnormalities including retinopathy, arteriovenous nicking, focal arteriolar narrowing, and retinal arteriolar and venular caliber, with the presence and magnitude of cTnT.

2. Retinal microvascular abnormalities including retinopathy, arteriovenous nicking, focal arteriolar narrowing, and including retinopathy, arteriovenous nicking, focal arteriolar narrowing, and retinal arteriolar and venular caliber, with the presence and magnitude of NT-proBNP.

3. The combination of troponin T, NT-proBNP and retinal microvascular abnormalities and the incidence of cardiovascular diseases (CHD, stroke and CHF).

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:**
This will have a cross-sectional analysis of the participants who had interpretable retinal photography at the time of the third examination and had high sensitivity cardiac troponin T (cTnT) and/or amino terminal brain natriuretic peptide (NT-proBNP) assays obtained from blood drawn at visit four, and a prospective component examining the combination of cTnT, NT-proBNP and retinal microvascular changes and the incidence of coronary heart disease, stroke, and congestive heart failure.

**Inclusion/Exclusion Criteria:**
After standard ARIC exclusions, participants will be eligible if they have interpretable retinal photography at visit 3 as well as cTnT and/or NT-proBNP measured at visit 4. Participants with missing retinal photography at visit 3, and those missing cTnT and NT-proBNP assays at visit 4 will be excluded.

**Predictor/Outcome variables** (dependent and independent variables):
1. High sensitivity cardiac troponin T (cTnT)
2. Amino terminal brain natriuretic peptide (NT-proBNP)

**Predictor variables** (independent variables):
1. Retinal microvascular variables (visit 3): Retinopathy, focal arteriolar narrowing, arterio-venous nicking, arteriolar and venular diameters, AV ratio.
2. Age-related maculopathy (ARM) variables (visit 3): any ARM, early ARM, late ARM and specific ARM lesions (drusen, pigmentary changes)
**Outcome variables** (dependent variables):

1. Incident cardiovascular disease
2. Incident congestive heart failure

**Statistical Analysis:**
All non-normally distributed variables will be natural logarithmically transformed, as needed. cTnT and NT-proBNP will be used as both continuous and categorical variables. For continuous models, undetectable levels of cTnT and NT-proBNP will be assigned a value of half the lower limit of detection. For the categorical analysis, cTnT levels will be divided into 4 groups: cTnT undetectable (<0.003ug/L) and 3 groups based on tertiles of detectable cTnT levels. For the categorical analysis of NT-proBNP, values will be divided into quartiles. Retinal microvascular variables, ARM variables, and clinical covariates will be described by cTnT and NT-proBNP categories. Linear regression analyses will be performed to identify univariable and multivariable retinal correlates of cTnT and NT-proBNP after adjusting for the effects of known or potential confounders. Regression model 1 will adjust for demographic variables (age, gender, race/ethnicity, education level, study center), systolic, diastolic and mean arterial blood pressure, smoking status, body mass index, total cholesterol, HDL cholesterol, LDL cholesterol, total triglyceride, plasma fibrinogen, factor VIII, diabetes (defined as a fasting glucose ≥126 mg/dL or taking glucose lowering medication.), HOMA-IR, eGFR (using the MDRD formula), electrocardiographic left ventricular hypertrophy, history of coronary artery disease, heart failure, prior MI, prior stroke or TIA, and peripheral arterial disease. Model 2 will adjust for variables in model 1 and use of antihypertensive or lipid lowering medications, and the physical activity index. In secondary analyses, we will test for effect modification by age, sex, race, and diabetes status. Logistic regression analyses will be performed for the categorical analysis of highest tertile of detectable cTnT and highest quartile NT-proBNP.

**Limitations:**
A limitation of this study is the cross-sectional design, which precludes the ability make any inferences regarding causality. Additionally, the retinal examinations and laboratory analyses of cardiac biomarkers were obtained at different study visits (3 and 4), separated by a period of approximately 3 years. Work by Wong et al.\textsuperscript{21} in non-diabetics suggests a 2.9% incidence of retinopathy over 3 years and a 64% resolution rate of retinopathy in the same eye at 3 years, with similar incidence and resolution rates over 10 years of follow-up.\textsuperscript{22} Although the presence of retinopathy may vary in both non-diabetics and diabetics over time, any history of retinopathy has been shown to be a marker of future cardiovascular risk in the ARIC cohort and is therefore of interest.\textsuperscript{2-8}

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
8.a. Will the DNA data be used in this manuscript?

____ Yes   _X___ No

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)?


MS#1564 (Saunders) Correlation of high sensitivity troponin-T (hs-cTnT) and amino terminal pro-brain natriuretic peptide (NT-proBNP) with renal function parameters; and association with mortality and adverse cardiovascular events. Circulation 2011;123:1367-76.

MS#1757 (Nambi) The association of high sensitivity troponin with heart failure, mortality and recurrent coronary heart disease (CHD) in individuals with prevalent CHD.

MS#1811 (Oluleye) Association of high sensitive troponin T (hs-cTnT),N- Terminal pro- brain natriuretic peptide (NT-proBNP) and high sensitivity C- reactive protein (hs-CRP) with cause-specific mortality: ARIC study.
11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?

___X___ Yes    ____ No

ARIC Ancillary Study #2008.10, “Measurement of NT-pro-BNP and troponin T at visit 4 for the full ARIC cohort”

11.b. If yes, is the proposal

___X__  A. primarily the result of an ancillary study (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.cscu.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.cscu.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:


