1.a. **Full Title**: Comparison of novel markers of kidney function and prediction of cardiovascular events, mortality, and kidney failure: the Atherosclerosis Risk in Communities (ARIC) Study

b. **Abbreviated Title (Length 26 characters)**: Markers of kidney function

2. **Writing Group**:
   Writing group members: Brad Astor (lead), Josef Coresh, Christie Ballantyne, Ron Hoogeveen, others welcome

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

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**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).

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3. **Timeline**: Assays on of Visit 4 specimens are expected to begin within 3 months and be completed within 9 months. We anticipate that the manuscript will be prepared within six months after receiving the data.

4. **Rationale**:

   Individuals with decreased kidney function are at a substantially higher risk of kidney failure and mortality than the general population.1-3 Early detection of decreased
Kidney function is important to tailor therapy to slow progression of kidney disease and minimize the incidence of these outcomes. Serum creatinine is the most commonly used marker of kidney function. Creatinine is a byproduct of muscle breakdown and, therefore, serum levels are affected by an individual’s muscle mass.\textsuperscript{4,5} Equations accounting for age, race and sex improve the estimation of glomerular filtration rate (GFR) by accounting for average differences in muscle mass across these factors.\textsuperscript{6,7} Estimating equations, however, cannot account for individual differences in muscle mass. Muscle wasting due to chronic illness is associated with lower creatinine generation, leading to an overestimation of GFR in such individuals. As these same individuals are at an elevated risk of mortality, this systematic bias would result in an underestimate of the association between decreased GFR and mortality risk.

Cystatin C is less affected by muscle mass than serum creatinine and is thought to be a better marker of kidney function.\textsuperscript{5,8} Some reports, however, have shown that cystatin C levels are affected by non-renal factors, including smoking and inflammation, independent of kidney function.\textsuperscript{5,9-11}

Additional analytes, including beta trace protein (BTP) and $\beta_2$ microglobulin ($\beta_2$M), have recently been examined as alternative markers of kidney function. Serum levels of beta-trace protein (BTP) levels were strongly correlated with GFR in a study of kidney transplant patients and in a small study (n=60) of individuals with various types of kidney diseases.\textsuperscript{12-16} In a combined analysis using data from the Modification of Diet in Renal Disease (MDRD) and African American Study of Kidney Disease and Hypertension (AASK), GFR estimated by an equation including serum creatinine, cystatin C and $\beta_2$M levels correlated with directly-measured GFR more closely than equations based on any single marker, and was nearly as highly correlated as a repeated GFR.\textsuperscript{[Coresh, unpublished data]} Higher $\beta_2$M levels predict early onset atherosclerosis and mortality in hemodialysis patients.\textsuperscript{17-19} Data are limited in other populations. It is currently unknown whether other factors affect BTP and/or $\beta_2$M levels.

As novel markers are investigated for use in estimating GFR, it is important to understand how the potential biases of GFR estimates based on these markers may impact the ability of these estimates to predict future outcomes, such as CVD, ESRD and death. To our knowledge, no studies have compared the associations of GFR estimates based on serum creatinine, cystatin C, BTP, and $\beta_2$M with the risk of CVD, ESRD and death.

5. **Main Hypothesis/Study Questions:**

a. Do estimates of GFR based on serum creatinine, cystatin C, BTP, and $\beta_2$M independently predict CVD events, ESRD and death?

b. Does risk prediction differ by marker for these outcomes?

c. Does the predictive ability of these markers differ by age, race, and sex subgroups?

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).

Cystatin C and high sensitivity CRP (hsCRP) were measured on Visit 4 samples as part of Ancillary Study 2006.16, “Longitudinal Study of Predictors and Consequences of Chronic Kidney Disease.” BTP and β2M assays will also be performed on Visit 4 samples as a part of this ancillary study. The most recent follow-up data will be used to assess the association between these Visit 4 measures and subsequent outcomes.

All analyses will be performed locally. Estimates of GFR will be calculated using equations based on data from the AASK and MDRD studies. Participants with missing values for these variables or with severely decreased kidney function (<15 ml/min/1.73m²) will be excluded from analyses.

Correlation between markers and between estimated GFR (eGFR) values using different markers will be assessed. Baseline characteristics will be examined in the overall population and stratified by quartiles of each GFR estimate. T-tests and chi-square tests were used to test differences between continuous and categorical covariates, respectively.

Associations between baseline eGFR measurements and outcomes will be assessed with Cox proportional hazards regression models. Multivariate models will adjust for race, sex, age, urinary albumin:creatinine ratio (ACR), diabetes, prevalent coronary heart disease, hypertension, smoking status and hsCRP. Additional covariates will be used in other models. Overall hazard ratios, and hazard ratios stratified by eGFR categories (≥ or <60 ml/min/1.73m²), will be compared across markers. Receiver operator characteristic (ROC) curves for all markers and GFR estimates will be used to examine the diagnostic performance (AUC) of each. Model discrimination also will be assessed by estimating the c-index, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) scores.

An important component of the analyses will be an attempt to define the risk of events across the entire range of eGFR for each marker. This analysis will use nonlinear models, and the shape of these curves will be qualitatively compared.

Additional variables required for analyses include demographic factors (age, race, sex, center), comorbid conditions (blood pressure, diabetes status, prior CHD events), anthropometric data (waist circumference, waist:hip ratio, BMI), smoking status, medication use (antihypertensives, lipid-lowering medications), and laboratory variables (lipids, blood glucose).

7.a. Will the data be used for non-CVD analysis in this manuscript?  ___X___ Yes  ____ 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?  ____X___ 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

8.c. If yes, is the author aware that the participants with RES_DNA = ‘not for profit’ restriction must be excluded if the data are used by a for profit group?  
   ____ 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)?

MS1123: Albuminuria and Kidney Function as Predictors of Cardiovascular Events and Mortality (lead author: Astor).

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)  
   ____  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/

12. 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.

Agreed
Reference List


