Author’s response to reviews

Title: Association of Kidney Function with Inflammatory and Procoagulant Markers in a Diverse Cohort: A Cross-Sectional Analysis from the Multi-Ethnic Study of Atherosclerosis (MESA)

Authors:

Christopher Keller (Christopher.Keller@ucsf.edu)
Ronit Katz (RKatz@u.washington.edu)
Mary Cushman (Mary.Cushman@uvm.edu)
Linda F Fried (Linda.Fried@va.gov)
Michael G Shlipak (Michael.Shlipak@ucsf.edu)

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Author’s response to reviews: see over
Date: June 19, 2008

To: Melissa Norton, MD
Editor-in-Chief, BMC Nephrology

From: Christopher R. Keller, MD
Michael G. Shlipak, MD MPH

Re: Resubmission of Research Article Manuscript: Association of Kidney Function with Inflammatory and Procoagulant Markers in a Diverse Cohort: A Cross-Sectional Analysis from The Multi-Ethnic Study of Atherosclerosis (MESA)

Dear Dr. Norton,

Thank you very much for the comments on the manuscript. We have taken great care to address all comments from the three reviewers. Attached is a copy of the manuscript with tracked changes for review. Here are our responses to all comments:

Comments:
Reviewer #1 (Anders Larsson)
Page 6: CVs are provided for cystatin C. CV should also be provided for creatinine or even more appropriate for MDRD estimated GFR. The CV for estimated GFR is higher than for creatinine concentration due to the non-linear relationship between creatinine and eGFR.

➢ The CV for creatinine was ≤2%. This has been added to the manuscript, as requested.

Friedewalds equation can only be used if the TG values are below a certain level. If there was a substantial number of high TG values that made it impossible to calculate LDL this should be commented.

➢ In the cohort, only 87 out of 6750 subjects (1%) had a triglyceride level > 400 mg/dL. As this number was quite small, we did not comment on it in the manuscript.

Chronic kidney disease was defined as eGFR < 60…. The authors should add that this was MDRD eGFR as there are also cystatin C eGFR.

➢ The manuscript has been edited to add “MDRD” or “creatinine-based” eGFR where possible to clarify that eGFR was always based from creatinine in our analysis.
The authors should provide the detection levels for CRP IL-6 and TNFR1 as high sensitivity assays were used.

- The detection levels have now been included in the manuscript.

Page 7, end of second paragraph: divided by height (m²). This could be misinterpreted (?) as m² usually describes an area. height (m)/². ?

- BMI was calculated in each subject by taking weight in kilograms and dividing by the square of height (m²). This has been clarified in the manuscript.

One interesting aspect of this study is that it includes several ethnic groups. There was a difference in cystatin C levels in the different ethnic populations but this could be due to differences in age or sex (?). If the authors adjust for age and sex, is there still an ethnic difference for cystatin c and creatinine.

- In table 4, the beta coefficients listed by ethnicity have been adjusted for multiple covariates, including age and sex. A legend at the bottom of the table now more clearly lists the variables used in adjustment.

Reviewer #2 (Radovan Hojs)     No comments

Reviewer #3 (Richard MacIssac)
The authors need to clearly reflect on what they are trying to show in this study. In the abstract, it is stated that the study investigates the association of kidney function with multiple biomarkers in a diverse cohort. However, in the background to the paper, the authors state ‘Inflammation in a potential mediator of the association between cystatin C and cardiovascular disease’. The authors need to make it clear to readers of this paper as to what they think serum cystatin C levels reflect. Do they think that cystatin C levels simply reflect GFR levels but represent a far more accurate way of estimating try GFR when compared to creatinine based methodology? Or do the authors feel that cystatin C levels measure something more than GFR alone. i.e. that subclinical inflammation also influences cystatin C levels. It is appreciated that this type of question can only truly be answered in studies that include a reference GFR measurement and that in a large population study such as the ‘MESA’ study this approach is not practical. Nevertheless, I do not believe that the paper as presented in its current format helps to sort out this issue or is indeed even designed to answer this question.

- We greatly appreciate the above comment by Dr. MacIssac. The purpose of the manuscript was to demonstrate associations between two markers of kidney function and multiple markers of inflammation in a cohort characterized by ethnic diversity. We believe strongly, based on mounting evidence from prior studies, that cystatin C primarily reflects changes in kidney function. However, it may be slightly influenced by inflammatory markers themselves, which is not completely known at this time and is a limitation to our analysis. As a result of the comment above, we have made changes to the introduction and the discussion section that more clearly document the goal of the study and its limitations. For example, in the introduction we deleted the misleading comment on “inflammation as a mediator between cystatin C and cardiovascular disease,” and added supporting comments on the role of cystatin C as a marker for kidney function in subjects with mild to moderate kidney disease. In the limitations section, we specifically discuss the possibility that cystatin C may be influenced by inflammation, independent of kidney function. We believe that these changes make the information presented in the manuscript more straightforward and cohesive.
Why have the authors investigated the relationship between cystatin C and biomarkers with the relationship between creatinine and biomarkers with eGFR > 60 given the obvious insensitivities of creatinine to detect GFR levels above 60 ml/min? If the authors really want to look at the relationship between kidney function (GFR) and the biomarkers measured in this study a comparison between eGFR measurements, instead of creatinine, versus the relationship between cystatin C and the biomarkers should have at least been made. I would suggest that a useful approach to looking at the data that the authors have collected would be to ask the question as to whether an early decline in renal function is associated with markers of inflammation and a procoagulant state. In the introduction, the authors state that in subjects with kidney disease but not on haemodialysis, kidney function has been associated with markers of inflammation for GFR levels < 60 ml/min whereas above this threshold other studies have not been able to demonstrate an association using createine-based estimates of GFR. This may be because creatinine-based estimates of GFR lack the accuracy to pick up early decline in true GFR. GFR estimates based solely on cystatin C levels have clearly been shown to out-perform creatinine based estimates of GFR and to be an excellent predictor of true GFR levels in the high to normal GFR range (Perkins et al JASN 2005, 16, 1404). Why not compare the relationship between biomarkers and a GFR based on cystatin C levels with an eGFR based on the MDRD equation? As mentioned above, I can see little point in comparing biomarkers with creatinine levels alone.

We agree very much with your comment above. The point of stratifying at an eGFR of 60, as you suggest, is to separate the known associations of kidney dysfunction and inflammation (persons with GFR<60) from the novel findings that earlier declines in kidney function are also associated with higher inflammatory markers when the kidney function is more appropriately characterized by cystatin C. We have tried to improve the presentation to reflect your comments. We have changed the analysis to compare cystatin C with MDRD-based eGFR instead of serum creatinine. This change primarily affects table 2, which lists the partial correlations between the markers of kidney function and the markers of inflammation. However, the correlations do not significantly change whether creatinine-based eGFR or creatinine is used, and our conclusions do, in fact, remain the same.

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Minor points
Abstract
Background: the authors have not given us a background to the study but have simply stated the aim of the study.

We have changed the abstract background to address the comment.

Main body
Results: In the results section, the authors state the main cystatin C and creatinine levels but don’t mention what the mean eGFR level was. Could they please add the mean eGFR level to the results section?

We have changed the mean eGFR level has been added to the first paragraph of the results section.

Thank you again for your comments. We look forward to hearing from you soon.

Sincerely,
Christopher R. Keller, MD
Michael G. Shlipak, MD MPH