Author’s response to reviews

Title: Association of C-reactive protein, tumor necrosis factor-alpha, and interleukin-6 with chronic kidney disease

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Author’s response to reviews: see over
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Dr. Hayley Henderson
Executive Editor Hayley Henderson
BMC Nephrology

RE: Association of C-reactive protein, tumor necrosis factor-alpha, and interleukin-6 with chronic kidney disease

Dear Dr. Henderson,

Attached please find our revised manuscript for your review. We appreciate the thoughtful comments provided by the editors and reviewers. We have revised our manuscript according to the reviewers’ comments and believe that our manuscript has been greatly strengthened after revision. The point-by-point responses to the reviewer’s comments are attached. All changes have been highlighted in blue color in the revised manuscript. In addition, we have included a completed STROBE checklist with our resubmission and formatted the revised manuscript to the journal style.

My co-authors and I would like to thank you for the opportunity to resubmit our work to BMC Nephrology for potential publication.

Sincerely,

Jing Chen, MD, MSc
Associate Professor of Medicine
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Responses to Editorial Comment

1. The manuscript while interesting has a number of deficits and not acceptable for publication in its current form. However, the Editorial Committee would be willing to review this manuscript again and consider it for publication if it specifically addresses the following concerns”

Thank you.

2. “the relatively small sample size and lack of power to detect clinically meaningful associations”

Thank you. Our study was designed to have an 80% statistical power to detect an odds ratio of 1.77 at a 2-sided significance level of 0.05 when the cases and controls were compared by the tertile of biomarkers. In addition, we have an 80% statistical power to detect a mean difference of 0.3 standard deviations of biomarkers between the cases and controls at a 2-sided significance level of 0.05. These differences are clinically meaningful. We have added this information from page 8, line 20 to page 9, line 2.

3. “the cross-sectional nature of the design and difficulty in inferring causality”

Thank you. We have discussed this as a limitation: “First, this study cannot establish a temporal relationship or make causality inferences due to its cross-sectional, observational nature” (page 11, lines 15-16).

4. “lack of novelty”

Thank you for this comment. The association between inflammatory biomarkers and chronic kidney disease has been examined previously in several epidemiological studies. However, these studies have reported inconsistent findings on the associations of C-reactive protein, tumor necrosis factor-alpha, and interleukin-6 with chronic kidney disease. Our study contributes additional information on inflammatory biomarkers and chronic kidney disease.

5. “selection bias and inclusion criteria and whether patients with acute illness were excluded”

All patients with chronic kidney disease and controls without chronic kidney disease were recruited from the greater New Orleans area in Louisiana. There is no evidence that patients or controls were selected based on their levels of inflammatory biomarkers. We have now added the following information in page 6, lines 5-7: “CKD was defined as eGFR <60 ml/min/1.73 m\(^2\) or the presence of albuminuria (≥30 mg/24-hours) for three or more months. Patients with acute kidney injury were excluded.”

6. “the matching strategy for cases and controls, and the potential for residual confounding due to the limited covariate adjustment”

We did not use an individual matching method to select controls because it is not feasible to match for multiple co-variables. Instead, we adjusted for age, gender, race, high-school education, physical activity, current cigarette smoking, weekly alcohol drinking, body-mass index, LDL-cholesterol, plasma glucose, systolic blood pressure, history of cardiovascular disease, antihypertensive treatment, antidiabetic treatment, lipid lowering treatment, as well as aspirin use in the multivariable models (see page 8, lines 8-11). Multivariable modeling methods are more advantageous compared to the matching
method because they can control for multiple confounding factors.

7. “Finally the authors need to temper their conclusion and provide a more comprehensive discussion on the inherent limitations of this study”

    We agree. We have rewritten the conclusion section: “Our study findings suggest that TNF-α and IL-6, but not CRP, are associated with the prevalence and severity of CKD independent of established CKD risk factors, history of CVD, and use of antihypertensive, antidiabetic, and lipid-lowering agents and aspirin. Further studies are warranted to investigate the role of inflammatory biomarkers in the etiology of CKD and their predictive value for advanced CVD and ESRD events among patients with CKD” (page 12, lines 4-8 form the top). In addition, we provide a more comprehensive discussion on limitations of this study from page 11, line 15 to page 12, line 2.
Responses to Reviewer 1

“The largest limitation in my mind is an issue which may not necessarily be something the authors can adjust for but I believe must be acknowledged in the discussion section. The association of chronic kidney disease (CKD) with various biomarkers is fraught by the fact that these markers may just be co-linear with reduced GFR, or may be associated with the underlying disease process causing CKD rather than CKD itself. For example an underlying autoimmune inflammatory disease causing both CKD and the elevation in these biomarkers. That said, I think the authors should report the breakdown of causes of kidney disease in the CKD group, so that the reader can see the frequency of traditionally systemic inflammatory diseases (ex Lupus Nephritis) versus non inflammatory (ex ADPKD). I also believe the authors should compare these inflammatory markers across the causes of CKD in the cohort to see if certain causes are associated with higher levels. For instance if those with Lupus nephritis have significantly higher levels than those with polycystic kidney disease then perhaps within the CKD cohort the association is being driven by the underlying systemic disease rather than CKD per se.”

Thank you for the insightful comments. We agree that our cross-sectional, observational study cannot establish a temporal relationship and causality. We have now discussed this limitation in page 11, lines 15-16: “First, this study cannot establish a temporal relationship or make causality inferences due to its cross-sectional, observational nature.”

In this study, patients on immunotherapy in the past six months were excluded. Therefore, patients with active lupus were also excluded. The most common causes for chronic kidney disease in our study population were diabetes and hypertension with 49% of patients reporting a history of diabetes and 88% reporting a history of hypertension. However, it is difficult to define diabetic nephropathy or hypertensive nephropathy or other subtypes of kidney disease without renal biopsy. Therefore, we are not able to compare inflammatory markers across the causes of chronic kidney disease in our study. We have now discussed this limitation from page 11, line 22 to page 12, line 2: “Finally, we were not able to analyze the association between inflammatory markers and subtype of CKD by different causes because renal biopsy data were not available in our study”.

“Those in the CKD group were slightly older, had a higher frequency of smoking, lower exercise and higher prevalence of cardiovascular disease. Again, it is therefore still unclear whether these processes are driving the higher inflammatory markers rather than CKD itself. Since many of these covariates are generally correlated with CKD, the authors should report a correlation matrix between eGFR and ACR and these baseline characteristics.”

Thank you for this suggestion. In our multivariable models, age, gender, race, high-school education, physical activity, current cigarette smoking, weekly alcohol drinking, body-mass index, LDL-cholesterol, plasma glucose, systolic blood pressure, history of cardiovascular disease, use of antihypertensive, antidiabetic, and lipid-lowering agents, and aspirin were adjusted (see page 8, lines 8-11).

“Lastly, the interest of the nephrology community in this area will be focused mainly on whether these markers are superior to traditional markers of CKD (eGFR & ACR) in some way, or whether they are more sensitive or specific for the prediction of adverse events in patients with CKD. The CKD cohort in this study was likely too small to detect associations with adverse events, and therefore larger studies are necessary to discover the performance and potential application of these biomarkers, this should also be acknowledged in the discussion.”
We agree. We have now added the following sentence in conclusion section: “Further studies are warranted to investigate the role of inflammatory biomarkers in the etiology of CKD and their predictive value for advanced CVD and ESRD events among patients with CKD.”

Responses to Reviewer 2

1. Sample Size. The sample size is small and may have had reduced power to test significant associations. While CRP associations did not achieve conventional levels of significance, there was nonetheless, an association between CRP and the odds of CKD (OR of 1.8 in the multivariate model). I would be fairly certain that this may have achieved significance if the sample size were larger (as highlighted in previous publications).

Please see response to editors, point #2.

2. Lack of Matching Strategy between Cases and controls. The cases and control differ substantially across a wide range of demographic characteristics. It would have been desirable to have a matched strategy to reduce the confounding impact of these measured baseline characteristics.

Please see response to editors, point #6.

3. Cross Sectional Design. The study is cross-sectional in nature and repeats what other studies have explored with greater sample sizes. Causality is difficulty to prove. The language throughout the manuscript needs to be reflect this, especially the abstract. “Our data suggests that TNF–alpha and IL-6 but not CRP, are associated with risk and severity of CKD…. This needs to be changed to associated with.

Please see response to editors, point #3. We have now stated “TNF-α and IL-6, but not CRP, are associated with the prevalence and severity of CKD” in the abstract and conclusion sections.

4. Selection Bias and exclusion criteria. In studies of this type, it is important to qualify the status of the enrolled population at time of lab draws. Were the patients free of any recent acute illness- it does not state this in the manuscript? I would like to clear and categorical statement that all cases were free of an acute illness within month if study enrollment.

Please see response to editors, point #5.

5. Multivariate models. The multivariate analyses, lack some important covariates. There appears to be a limited adjustment for non-cardiovascular comorbidities and I wonder would such a strategy have given rise to the observed results. For example, I did not see the following covariates adjusted for history of diabetes-history of COPD etc.

We adjusted for fasting plasma glucose and anti-diabetic treatment in the multivariable models (see page 8, lines 8-11). We did not adjust for the history of COPD because there is no evidence that COPD causes CKD.

6. The average eGFR of the cases was 45 ml/min. I would like to have seen the breakdown of the CKD group by GFR, What % percent were in stage 3, 4 or 5?
Among 201 CKD patients, five were stage-1, 33 stage-2, 104 stage-3, 49 stage-4, and 10 stage-5. We have now presented this information in page 9, lines 7-8.

7. Fasting Creatinine levels. Creatinine levels were drawn in a fasting state, Might this have given rise to lower than expected GFR due to relative volume depletion? Would the authors please comment on this? I wonder also whether the authors considered evaluating the association of inflammatory factors with 24 hour urine creatinine clearance measurements. Concordance with the CKD EPI results might serve to strengthen their hypothesis.

Overnight fasting should not cause dehydration because patients were instructed to drink water as usual. On the other hand, the levels of non-fasting creatinine might vary greatly due to diet. The CKD-EPI equation has been validated in many populations and provides a more accurate estimation of GFR than 24-hour urine creatinine clearance measurements.

8. Discussion. The discussion does not adequately address the inherent limitations. The discussion needs to be further revised and refined according to the suggestions outlined above. The imbalances at baseline remain a major deficiency and contribute to a substantial selection bias. I would urge a more thorough discourse on the limitations of this study with attention to the design and external validity. The use of words that infer causality should be removed throughout the manuscript.

Thank you for this comment. In our study, control participants were recruited through mass mailings to the same community as patients which reduced potential selection bias. We adjusted for multiple important confounding factors in the multivariable models which should effectively deal with the co-variable imbalance in this observational study. We have discussed the limitations of this study from page 11, line 15 to page 12, line 2. We have now removed all words that infer causality throughout the manuscript.

Responses to Reviewer 3

The main limitation is its lack of novelty as the associations of these markers in patients with CKD (and cardiovascular disease) is very well demonstrated in the literature. This should be clearly mentioned in the discussion as a major limitation.

Please see response to editors, point #4.