Reviewer's report

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

Version: 2
Date: 9 January 2015

Reviewer: Austin Stack

Reviewer's report:

Summary
The authors test the hypothesis that inflammation is associated with the severity and presence of chronic kidney disease in a case-control study from the region. Comparing 201 cases with CKD, and 201 community controls without CKD, they report significant inverse association of TNF and IL6 with eGFR, but do not find any evidence linking CRP levels with GRF or albuminuria. The authors adjust for traditional cardiovascular risk factors and co-existing cardiovascular disease (all causes of inflammation), and several other covariates that might act as confounders. They conclude that IL6 and TNF are associated with CKD, while CRP is not.

The manuscript is well written and with appropriate statistical analyses. The contribution of inflammation to the onset and progression of CKD is of major importance, and qualifying the contribution of various components of the inflammatory cascade is essential. While the manuscript has several strengths, there are several areas of weakness, which I feel need to be further addressed. These include the relatively small sample size, the lack of a matching strategy in the matching of cases with controls, the lack of information of whether cases had recent episodes of acute illness and the degree of adjustment in the multivariate models.

Major Revision

Threats to validity (Internal and External)

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).

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.
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

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.

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

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?

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.

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.

Level of interest: An article of importance in its field

Quality of written English: Acceptable
**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

'I declare that I have no competing interests'