Author's response to reviews

Title: Total protein, albumin and low-molecular-weight protein excretion in HIV-positive patients

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Author's response to reviews: see over
Dear Editor

We thank the reviewers for their detailed feedback and the opportunity to submit a revised manuscript. We have incorporated the suggested changes and addressed the critique as follows:

**Reviewer 1’s report:**

Major concerns:

> This study showed prevalence of proteinuria and albuminuria, and urinary concentrations of LMWPs including RBP, cystatin C and NGAL as markers for subclinical tubular dysfunction or structural damage. They were also compared among 4 groups, that is, groups receiving no cART, cART/no TFV, TFV/NNRT and TFV/PI. The results could be lack of novelty. Numerous articles have already reported prevalence (extent) of proteinuria and albuminuria in multiethnic HIV populations, with special reference to cardiovascular disease and mortality.

We agree that there already are several data sets on proteinuria and albuminuria in HIV positive patients and have removed major sections of the manuscript that refer to proteinuria and albuminuria per se.

> The authors likely want to emphasize that RBPCR could be a potential marker for early renal tubular injury in HIV-infected individuals receiving cART/TFV. However, similar significance of the urinary concentrations of LMWPs including RBP has been suggested in the HIV-infected individuals with incipient glomerular defects by Kabanda A et al (AJKD 27; 803-8, 1996) and Hall AM et al (AJKD 54; 1034-42, 2009). In addition, Ando M et al studied prevalence of tubular damage in the absence of apparent glomerular defects in HIV-infected patients on HAART using multiple urinary concentrations of LMWPs, and showed clinical associations of tubular damage with a near-term decline in eGFR and higher incidence of proteinuria (NDT 26; 3224-29, 2011). This paper is rather descriptive in nature, and thus it is unclear whether the data shown is clinically significant or simply epiphenomena with comorbidities including CKD, diabetes, and hypertension.

We agree that the clinical significance cannot be determined from a cross sectional dataset. We do not merely describe epiphenomena of comorbidities as the prevalence of CKD, DM and HPT was low and considered in the multivariate analysis. We have included the paper by Ando et al in the references.

Minor concerns:

> 1. Nobody can determine that individuals with upper quartile RBPCR have proximal tubular injury without renal biopsy findings or tubular functional tests.

We agree with the reviewer and have been careful not to infer this in our manuscript.

> 2. Readers may want to know the reference values for urinary concentrations of NGAL, cystatin C and RBP to see that they are elevated or not in the HIV cohort.

We initially did not supply this information as the assays to quantify LMWP in urine are poorly standardised and the reference ranges for persons without kidney disease have not been validated. We now have added these data for RBP and NGAL to the results section, using conversions of the reference ranges supplied by the manufacturers.

> 3. Readers may want to see the data on dipstick test for proteinuria.
Unfortunately we do not have complete data on dipstick findings for the cohort

>4. The reviewer wonders why you took the value of eGFR <75 ml/min/1.73 m2 as a cutoff value?

We chose eGFR <75 as we had insufficient numbers of patients with eGFR <60. We have previously shown that eGFR <75 is a predictor of ARF and TFV-associated kidney disease (refs 10 and 15 in the manuscript)

>5. Duration of cART (especially TFV) and cumulative use of other nephrotoxic drugs should be clarified.

We found no relationship between duration of TFV exposure and RBPCR ($R^2=0.03$) and have inserted this in the text.

>6. Habit of smoking and drugs should be considered as well.

Unfortunately we do not have accurate data on smoking or co-medications for the cohort.

**Reviewer 2’s report**

Major Compulsory Revisions

>1) The authors understandably focus on TFV combinations in these analyses. But it would also be helpful to combine the TFV/NNRTI and TFV/PI groups to compare TFV vs. non-TFV more directly.

We agree and have added an additional column to Tables 1 and 2 to describe the characteristics of patients exposed to TFV

>2) The authors might wish to highlight in the discussion the fact the albuminuria was a small component of total proteinuria in this cohort. This would be of interest to the general readership.

We agree and have added the median ratio of ACR/PCR to the results section.

Minor Essential Revision

>1) A minor issue is that an interquartile range is the distance between quartiles 1 and 3; the data are listed as (Q1, Q3), which is somewhat different.

The reviewer is correct. Reflecting the IQR as (Q1, Q3) is common practice and in our view enhances the ease of reading of the data. We would prefer to maintain the data as presented, but would be happy to oblige if the editor insists.

**Reviewer 3’s report**

1) Major Compulsory Revisions

>Abstract: Authors should define "elevated protein", "elevated albumin excretion", and "elevated RBP" mean. It would also be of interest to provide the OR estimates from the multivariate analyses. The last conclusion statement is over-reaching since no statistically significant difference was noted among the drug groups with regards to RBP.
We have rewritten the abstract, removed the term “elevated, and inserted the OR.

>Methods - Definitions: The authors chose an usual cutoff of 150 mg/g to define proteinuria; this cutoff should be justified in some way. Also, "albuminuria" and "microalbuminuria" diverge from the conventional cutoffs of >300 mg/g and 30-300 mg/g, respectively. "Microalbuminuria" in this study actually represents individuals w/ normal levels of albumin excretion. The cutoffs they used should also be justified. Also, it's unclear why the authors chose sex-specific cutoffs for "albuminuria", but not "microalbuminuria".

In the submitted version of the manuscript, we used cut offs for ACR and PCR as per the recommendations of the British Renal Association – we have revised to use PCR >200 mg/g to define proteinuria, ACR >300 mg/g to define albuminuria, and ACR 30-300 mg/g to define microalbuminuria, as suggested by the reviewer.

>Methods - Statistical analyses: The authors need to clarify how covariates were chosen for inclusion in the multivariate models.

As stated in the manuscript, variables were tested for interaction and included in the multivariate models if p was <0.05 in univariate analysis.

>Results: The authors need to clarify whether the logistic models for NGALCR and CCR were multivariate. If not, why were they not adjusted models.

Only a single factor was significantly associated with UQ NGALCR and UQ CCR. Consequently, no multivariate analysis was conducted. We have clarified this in the results section and removed the univariate analyses for NGALCR and CCR from Table 3 and instead describe the observed significant associations in the results section.

>Discussion: The authors findings are difficult to interpret, esp. in light of previous studies, given their definition of microalbuminuria. The authors should re-analyze their data using more conventional cutoffs for microalbuminuria.

We have re-analyzed our data using the criteria as suggested. However, as per reviewer 1, we have removed most data regarding ACR and PCR from the Tables, results section and discussion as our findings have been well documented in previous studies.

>The authors observed poor correlation between NGAL and RBPCR which they purport are both markers of tubular function; however, they did not provide an explanation as to why they found such a poor correlation in the discussion section.

Thanks you – we have now included a section on this phenomenon in the discussion.

2) Minor Essential Revisions

>Discussion: The authors should be consistent in the units they use for the various labs (e.g. RBPCR ug/g or ug/mmol?)

We have done as suggested.

>Table 4 - Authors should clarify whether the NGALCR and the CCR logistic models are crude or adjusted.
Only a single factor was significantly associated with UQ NGALCR and UQ CCR. Consequently, no multivariate analysis was conducted. We have removed the univariate analyses from Table 3 and inserted a single sentence in the results section.

**Reviewer 4's report**
>Overall the article is well written and clear. Objectives and methods are clearly described. Results section it would be helpful to clarify if known the mode of transmission of HIV.

These data have been added to the results section

>Instead of reporting the % of Female should use % Male which is larger.

This has been done

>Also, the mean age and range of age should be reported.

The mean age was already provided in Table 1 – we have added the age range

>In page 8 of the Result Section there are too many numbers given and it is hard to read. May be reference to Tables or Figures might help the reader.

We have simplified this section as suggested.

>The Discussion Section is a bit too long, but covers well most other important issues including the limitations of the study.

We have shortened the discussion

>Table 1 should clarify the p-value as comparison of which groups

We have amended the Table as suggested

>Figure 1 is not clear the significant difference when comparing group 150.5-343.4 vs 343.4 black bars are similar...

We have clarified the legend in Figure 1

>Could some of the results be simplified in correlation figures?

We agree and have added the correlations in a separate figure

We hope that the manuscript is now acceptable for on-line publication in BMC Nephrology.

Yours sincerely

Frank Post