Author's response to reviews

Title: Proteinuria and hematuria are associated with acute kidney injury and mortality in critically ill patients: a retrospective observational study

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Version: 2
Date: 14 March 2014

Author's response to reviews: see over
Dear Editor

We have revised our manuscript entitled “Proteinuria and hematuria are associated with acute kidney injury and mortality in critically ill patients: a retrospective observational study” by SS Han, et al. (MS: 7938794351166889) submitted for consideration for publication in BMC Nephrology.

The reviewers’ comments were very helpful and made this manuscript more fruitful. We have addressed critiques as follows (in blue). Also, we have revised manuscript extensively and significantly as reviewers recommended (using blue-colored character).

We hope that after these corrections, our manuscript will be acceptable for publication in BMC Nephrology.

March-14, 2014

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Reviewer's report

Title: Proteinuria and hematuria are associated with acute kidney injury and mortality in critically ill patients: a retrospective observational study

Version: 1
Date: 25 January 2014
Reviewer: Meghan Sise

Reviewer's report:

This is a large retrospective observational study of patients admitted to the intensive care unit (ICU) which show that proteinuria and hematuria detected at the time of admission to the ICU increase the risks of AKI and mortality. The study includes a large number of patients; exclusions are few and well reasoned. The methods are well described, including the relative excess risk index (RERI). The authors showed a “dose-dependent” effect of proteinuria and hematuria on the likelihood that AKI is diagnosed and influenced the prediction of mortality at 3 years. There is excellent longitudinal follow-up, with little loss of patient data. The title accurately reflects what their data has shown, that there is an association between proteinuria/hematuria detected at ICU admission with diagnosis of AKI and mortality. The fact that hematuria/proteinuria interact with AKI and increase the predictability of mortality in the AKI model (with an improved AUC) is a novel finding.

Major Compulsory Revisions

1. The issue of causality vs. association needs to be acknowledged. The authors compare their findings to the findings of hematuria as causal in the pathogenesis of warfarin induced nephropathy or the pathologic effects of glomerular hematuria in patients with IgA nephropathy. However, it is unproven in AKI that hematuria is leading to more kidney damage, rather it may just be a sign of kidney damage. In fact, it’s more likely that more
severe case of AKI/ATN causes hematuria, which is associated with mortality. For example, the authors state in the discussion “For the first time, the present study demonstrates the effect of hematuria on AKI and mortality in ICU patients.” This statement implies hematuria has causality, however its merely an association in this study and the wording needs to refer to this as an association throughout the text, i.e. In the above sentence rather “For the first time, the present study demonstrates association of hematuria detected on ICU admission with the diagnosis of AKI and mortality in critically ill patients.”

The reviewer’s comments are exactly right. As in all observational studies, associations do not prove causality. Furthermore, hematuria may be just a sign, a leading cause, of kidney damage. We have discussed the above-mentioned issue in the Limitation section and described the association rather than the effect (or the words implying causality) throughout the manuscript.

2. The question posed by the authors is not well defined. It is unclear if they are looking for urinary abnormalities (namely hematuria/proteinuria) as a “biomarker” of AKI or whether they are looking at proteinuria/hematuria as a risk factor or causal in the pathway of AKI. They state “we aim to verify whether proteinuria or hematuria increase the risks of AKI”, however the measurements are taken at ICU admission, likely at the time of the onset of AKI at the time of ICU admission, and there is no way to tell if hematuria/proteinuria precedes AKI or is just a mediator – i.e. Patient has acute tubular necrosis (ATN) and ATN causes hematuria and proteinuria.

We agree with the reviewer’s comments. When we designed our study at the beginning, we hypothesized that the urinary abnormalities are risk factors of AKI. There is little knowledge about this issue. As we have discussed, proteinuria and hematuria each play a role (directly or indirectly) in the pathogenesis of AKI. However, as the reviewer precisely
commented, the roles of the urinary abnormalities in the pathogenesis of AKI could not be determined in the present study alone. We defined AKI when it developed on the day of admission to 15 days in the ICU. However, most of the AKI cases developed at the time of ICU admission; 74.7% of the total AKI cases were diagnosed at the time of ICU admission; other cases (25.3%) developed 2 to 15 days of ICU admission. However, after excluding the AKI cases with onset at day 0, proteinuria and hematuria were also associated with the AKI cases with onset on day 1 to 15, as shown in table that follows:

Table. Odds ratios for acute kidney injury not at the time of admission

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th></th>
<th>Multivariate *</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Proteinuria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- (n = 333)</td>
<td>1 (Reference)</td>
<td>&lt; 0.001 †</td>
<td>1 (Reference)</td>
<td>0.037 †</td>
</tr>
<tr>
<td>+/- (n = 127)</td>
<td>1.88 (1.245–2.843)</td>
<td>0.003</td>
<td>1.69 (1.088–2.624)</td>
<td>0.020</td>
</tr>
<tr>
<td>1+ (n = 178)</td>
<td>1.89 (1.310–2.737)</td>
<td>0.001</td>
<td>1.49 (1.003–2.212)</td>
<td>0.047</td>
</tr>
<tr>
<td>2+ (n = 113)</td>
<td>1.92 (1.249–2.959)</td>
<td>0.003</td>
<td>1.58 (0.991–2.514)</td>
<td>0.053</td>
</tr>
<tr>
<td>3+ (n = 25)</td>
<td>3.36 (1.410–8.011)</td>
<td>0.006</td>
<td>2.53 (0.987–6.463)</td>
<td>0.052</td>
</tr>
<tr>
<td>Hematuria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- (n = 232)</td>
<td>1 (Reference)</td>
<td>&lt; 0.001 †</td>
<td>1 (Reference)</td>
<td>0.010 †</td>
</tr>
<tr>
<td>+/- (n = 116)</td>
<td>1.04 (0.658–1.633)</td>
<td>0.877</td>
<td>1.02 (0.621–1.660)</td>
<td>0.951</td>
</tr>
<tr>
<td>1+ (n = 108)</td>
<td>1.47 (0.926–2.323)</td>
<td>0.103</td>
<td>1.47 (0.901–2.409)</td>
<td>0.122</td>
</tr>
<tr>
<td>2+ (n = 137)</td>
<td>2.34 (1.518–3.603)</td>
<td>&lt; 0.001</td>
<td>2.10 (1.315–3.356)</td>
<td>0.002</td>
</tr>
<tr>
<td>3+ (n = 183)</td>
<td>1.79 (1.212–2.654)</td>
<td>0.003</td>
<td>1.65 (1.076–2.528)</td>
<td>0.022</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, body weight, primary diagnosis, underlying chronic kidney disease, diabetes mellitus, history of malignancy, the need for mechanical ventilation, the use of vasoactive drugs, and APACHE II score.

† P for trend
Both proteinuria and hematuria were also associated with the risk for AKI despite its onset after the time of ICU admission. Based on this result, we may mention that ICU patients with proteinuria and hematuria are at risk of developing AKI. We have described and discussed the aforementioned results in the revised version of the manuscript.

3. The discussion focuses on hematuria/proteinuria as a biomarker that can be easily assayed. If it is a biomarker then the appropriate test must be conducted: sensitivity/specificity/positive predicted value/negative predictive value need to be calculated and discussed.

As mentioned earlier, we have considered proteinuria and hematuria as risk factors of AKI or mortality in ICU patients. We do not consider proteinuria or hematuria as a biomarker for AKI (or mortality) because neither parameter is a conclusive marker (unlike serum creatinine level) or an early marker (unlike NGAL). We try to address these urinary abnormalities as risk factors of AKI or mortality like several other parameters such as underlying cardiovascular disease, increased age, preexisting renal disease, or use of contrast dye. We have measured the sensitivity and specificity of proteinuria and hematuria for the AKI.

Table. Sensitivity and specificity of urinary abnormalities in predicting acute kidney injury

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>Proteinuria</th>
<th>Hematuria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>+/-</td>
<td>0.723</td>
<td>0.507</td>
</tr>
<tr>
<td>1+</td>
<td>0.567</td>
<td>0.652</td>
</tr>
<tr>
<td>2+</td>
<td>0.282</td>
<td>0.853</td>
</tr>
<tr>
<td>3+</td>
<td>0.071</td>
<td>0.980</td>
</tr>
</tbody>
</table>

However, if possible, we would like to exclude the data on sensitivity/specificity/PPV/NPV.
from the report. We think these values do not contribute much to the present study results or our study objective.

4. The timing of admission to ICU (and measurement of proteinuria/hematuria) and the onset of AKI diagnosis should be reported. It should be reported as mean ± standard deviation. It would be helpful to know if most cases were diagnosed early (i.e. at the time of admission to ICU) or if they were delayed. Does the relationship of hematuria/proteinuria change based on the timing of AKI?

The reviewer’s thought is very impressive. We further collected and reported the onset of AKI (until 15 days of ICU admission). As stated earlier, most AKI cases (74.7%) developed at the time of ICU admission. The distribution of the onset time of AKI development was not normal. Therefore, we have reported the distribution of the onset of AKI between the time of admission and after admission (2 to 15 days), instead of the mean and SD values. Regardless of whether the AKI cases were divided into these time frames, both proteinuria and hematuria were associated with the risk for AKI. We have described the results in the revised Result section.

5. Table 3 doesn’t actually add to the overall message of the paper. The distinction can be made with a 1-2 sentence explanation in the results rather than dedicate a full figure to it.

We agree with the reviewer’s thought. Therefore, we have deleted Table 3 and have described the results in the revised Result section.

6. Lacking from the literature review is a discussion of the known prevalence of hematuria and proteinuria in patients with acute tubular necrosis, which is the etiology of AKI most likely to affect the patients in their ICU based sample. The prevalence of hematuria and proteinuria
in this setting has been published in the literature and should be discussed.

We have described the prevalence of proteinuria and hematuria (> 50%) among the patients with acute tubular necrosis (ATN) in the revised version of the manuscript. We agree that ATN is the most common form of intrinsic renal failure that occurs in the ICU. However, other forms such as postrenal obstruction may be an AKI-related cause. If so, proteinuria or hematuria may rarely occur. Glomerular injury can also lead to proteinuria, which may be included as a cause of AKI in ICU patients. We have described this issue in the revised version of our manuscript.

Minor Essential Revisions

1. It should be stated in the methods that the patients were consecutively recruited if this is indeed the case.

   The study patients were consecutively recruited, but some patients were excluded according to the exclusion criteria. We have described this in the Method section.

Discretionary Revisions

1. The writing is acceptable, but could be improved in the following places.

   a) The first sentence of the second paragraph of the introduction, which ends with “and so on”, needs to be revised; this term is too casual for a scientific paper.

   b) The second paragraph of the discussion, the sentence “This is mainly because proteinuria has an impact on mortality outcome with a comparable power to smoking” should be re-written, a suggestion being “This is mainly because proteinuria has an impact on mortality, with an effect on mortality similar to smoking.”

   We have changed those phrases in accordance with the reviewer’s recommendations.
**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests
Reviewer's report

Title: Proteinuria and hematuria are associated with acute kidney injury and mortality in critically ill patients: a retrospective observational study

Version: 1 Date: 8 February 2014

Reviewer: Byung Ha Chung

Reviewer's report:

General comments

This study investigated the role of proteinuria and hematuria in the development of acute kidney injury and mortality. This topic is interest and manuscript is well written and the patient number is impressive. However, I have some major and minor comments.

Major Compulsory Suggestions

1. Authors should describe more in detail about the role of hematuria on mortality. The increased risk of the development of AKI or the progression of CKD in patients with hematuria has been reported in many previous reports. However, there has been few report to prove the role of hematuria on the development of mortality. Authors should discuss the proposed mechanism or relationship between hematuria and mortality.

   We agree with the reviewer's comments. Transient hematuria may be attributable to conditions such as urogenital irritation or inflammation, urinary infection, menstrual blood, or recent heavy exercise. None of these conditions are expected to be significantly associated with high mortality risk. No reports or commentaries have described a direct correlation between hematuria and mortality; that is, no observational study has been conducted on the direct correlation between hematuria and mortality. However, hematuria is known to increase the patients’ risk for AKI or CKD development. Based on the current evidence, we could
propose only the following mechanism: hematuria is a marker of kidney injury and is thus related with mortality via advanced (or severe) kidney damage. We have discussed this issue in the revised version of the manuscript.

Minor Essential Suggestions

1. I recommend to present the number of patients who showed both proteinuria and hematuria in table 1. In addition, how about the outcome of patients with both hematuria and proteinuria compared to patients who showed solely proteinuria or hematuria? I am curious whether hematuria and proteinuria have synergistic effect on AKI or mortality.

As the reviewer recommended, we have presented the numbers of patients with both proteinuria and hematuria. We found a trend of interaction between AKI and proteinuria (or hematuria) for mortality. Therefore, we used the RERI method to evaluate a synergistic effect for mortality between AKI and proteinuria or hematuria. However, there was no interaction for AKI or mortality between proteinuria and hematuria. Accordingly, we have performed stratified analyses according to 4 groups (no PU and HU, only PU, only HU, and both PU and HU). The results are as follows:

Table. Risks for AKI or 3-year mortality according to the presence of proteinuria and hematuria

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>AKI</th>
<th>3-year mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI) *</td>
<td>P</td>
</tr>
<tr>
<td>PU (-) HU (-) (n = 363)</td>
<td>1 (Reference)</td>
<td></td>
</tr>
<tr>
<td>PU (+) HU (-) (n = 303)</td>
<td>1.37 (0.950–1.985)</td>
<td>0.092</td>
</tr>
<tr>
<td>PU (-) HU (+) (n = 251)</td>
<td>1.44 (0.985–2.105)</td>
<td>0.060</td>
</tr>
<tr>
<td>PU (+) HU (+) (n = 966)</td>
<td>2.86 (2.102–3.901)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
* Adjusted for age, sex, body weight, primary diagnosis, underlying chronic kidney disease, diabetes mellitus, history of malignancy, the need for mechanical ventilation, the use of vasoactive drugs, and APACHE II score.

The subjects are divided into four groups with following references: non-proteinuria vs. trace or more for proteinuria; non-hematuria and trace vs. 1+ or more for hematuria.

AKI, acute kidney injury; OR, odds ratio; CI, confidence interval; PU, proteinuria; HU, hematuria.

As a result, the patients with both proteinuria and hematuria had greater ORs for acute kidney injury and 3-year mortality compared with the patients without them. We have described the results in the revised manuscript.

2. I recommend to include DM, which has been proved as strong prognostic factor for AKI and mortality in many previous reports, as covariate in multivariate analysis in table 2-4.

   We agree with the reviewer’s comment. Accordingly, we have collected data on the presence of diabetes mellitus among subjects of the present study and included the data throughout the multivariate analyses.

3. I recommend to add ROC curve about hematuria or proteinuria for the prediction of AKI. Addition of it would help readers to understand easily the role of hematuria or proteinuria in the development of AKI.

   As the reviewer’s recommendation, we have analyzed the AUCs for the proteinuria and/or hematuria groups for the prediction of AKI. The results are as follows: proteinuria, 0.639 (0.609–0.669) \( (P < 0.001) \); hematuria, 0.630 (0.599–0.661) \( (P < 0.001) \); and proteinuria +
hematuria, 0.648 (0.618–0.678) \((P < 0.001)\). The \(P\) values are calculated when compared with the reference line \((\text{AUC} = 0.5)\).

Discretionary Suggestions

1. I recommend to move some sentences in ‘Conclusions’ to ‘Discussion’ part or to omit some of them because they are repeated. For example, you already mentioned about the limitation of this study in the last paragraphs of discussion, but you describe it again in conclusion.

   **We agree in part with the reviewer’s comments. Some sentences in the conclusion section may have overlapped. However, the meanings of those sentences (reducing proteinuria or hematuria / cost-effectiveness of the test) were not repeated, but were first mentioned in the Conclusion section. Therefore, if possible, we would like to retain the original Conclusion section.**

2. I do not understand why author present both table 2 and table 3. I think many contents of them overlapped.

   **We agree with the reviewer’s comment. Therefore, we have deleted Table 3 and described its contents in the Method section of the revised manuscript.**

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests'