Author's response to reviews

Title: Chronic Asymptomatic Pyuria Precedes Overt Urinary Tract Infection and Deterioration of Renal Function in Autosomal Dominant Polycystic Kidney Disease

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Author's response to reviews: see over
Oct. 17th, 2012

Dear Dr. Henderson,

Re: manuscript MS:1403982156752251 - Chronic Asymptomatic Pyuria Precedes Overt Urinary Tract Infection and Deterioration of Renal Function in Autosomal Dominant Polycystic Kidney Disease

Thank you very much for reviewing our manuscript. We do greatly appreciate the editor and reviewers for their insightful and constructive comments. We have gone through each comment and amended the manuscript accordingly. The changes that have been made are detailed below.

Thank you again for your kind consideration.

Yours sincerely,

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Reviewer 1:

Major points:

1. Groups A and B differ relevantly with respect to duration of follow up and logically, the group with a longer follow up ended up with a lower eGFR. The authors try to adjust for this by adjusting for follow up duration in multiple regression analysis. However, I am still concerned that there is a major confounding factor, which has not been corrected for: assuming that advanced disease stage or reduced GFR is a risk factor for pyuria, the chance to detect recurrent or persistent pyuria would then be increased in patients at late stages of disease. These are likely the patients with longer follow up, that end up with time, although the decline of GFR might have happened mainly late during follow up. This bias can be eliminated by a different design of the analysis (which also makes much more sense since the authors aimed to test whether pyuria is a risk factor for progression): They should divide the patients into two groups A and B based on only the initial 6 or 12 months of follow up and then assess whether pyuria in this phase was associated with future GFR decline.

Thank you very much for your considerate comments and suggestions. We agree that group B (chronic pyuria group) may have had faster decline of eGFR since they had longer duration of follow up. According to your advice, we divided the patients into new prospective pyuria groups: Group of patients with no pyuria in the first year (Group \( \text{no pyuria/1st year} \)) and group of patients with 1-4 pyuria episodes in the first year of follow up (Group \( \text{1-4pyuria/1st year} \)). As a result, neither initial eGFR, final eGFR, nor GFR change were significantly different among groups (Suppl. Table 1 and Suppl. Table 2). On the other hand, similar to our original data, the incidences of overt UTI and upper UTI were significantly higher in pyuria group in the first year compared to those without pyuria (Suppl. Figure 1).

In summary, the presence of pyuria did not affect subsequent renal function in this new analysis. We assume that one year may not be enough to show the impact of recurrent or persistent pyuria on eGFR. The relationship between the occurrence of pyuria and renal function decline is a dynamic and on-going process that is not easy to evaluate the causal relationship. Therefore, the frequency of pyuria in only the first year may not be enough to evaluate their relationship. In addition, overt UTI occurred more frequently in chronic pyuria group that may act as a ‘third hit’ to provoke renal cyst growth. Therefore, causal relationship between chronic pyuria and renal function decline may be elucidated in a study with a longer duration of follow up. As we have mentioned in the discussion(Page 14, Line 8-10), a well-designed long-term prospective study is needed.
2. Another factor for confounding (detection bias) is the frequency of follow up visits of patients. How often were patients seen in the clinic? Were all patients seen in similar intervals or was the interval for visits based on clinical need? In the latter case, patients with more severe disease would have more clinical visits and therefore have a higher chance for the detection of pyuria.

It is true that we have seen the patients more frequently as their renal function declined. In fact, the patients were seen in the clinic as follows: CKD stage I-II: q 3-6 months; CKD stage III: q 2-3 months; CKD stage IV: q 6-8 weeks. In addition to the disease severity, we followed up patients more frequently when the following acute problems happened. After the UTI episodes, there was a short term follow up period (average q 2-4 weeks, for 1-6 months until the condition gets better according to the patient's condition (high blood pressure, new onset hematuria, etc.). However, when we re-evaluated the proportion of group A (no or transient pyuria) and B (chronic pyuria) according to CKD stages based on initial eGFR, there were no significant differences among groups in advanced CKD stages (CKD II-IV) (Figure below).

3. Likewise, it should be defined whether patients were regularly questioned for intercurrent UTI symptoms on every visit. The rate of cystitis in this study was very low – much lower than for non-ADPKD women. Also, there was no gender difference in the risk of overt UTI in this study, which would be very unusual if all cystitis episodes were reported. Hence, I suspect that many cystitis episodes went undiagnosed. This point needs to be clarified and discussed.

All PKD patients were regularly questioned for intercurrent lower UTI symptoms on every visit. Even though it is true that the rate of cystitis and little gender difference in our study is apart from the previous studies, we do not think patient experienced several UTI events between outpatient visits.
4. A major limitation of the study is that urine culture was performed in very few cases. Hence, it is unclear whether most asymptomatic pyuria episodes were asymptomatic infections (and thus potentially treatable) or whether these were cases of sterile pyuria, which has been suggested to be frequent in ADPKD (ref. 7 of the manuscript; in contrast, a study evaluating the frequency of asymptomatic bacteriuria in ADPKD did not find an increased incidence compared to healthy controls: Pietrzak-Nowacka M, Pol Merkur Lekarski. 2010 Sep;29(171):173-6). At least, the authors should report in how many of the urine cultures performed there were no microorganism identified.

We admit that the absence of urine culture data is the main pitfall in our study. As we mentioned in the result section (Page 9, Line 10), about 4.6% of asymptomatic pyuria cases were followed by urine culture study and microorganisms were identified in 93% among them. We mentioned our limitation in the discussion part (Page 14, Line 13-15).

5. Please report in the methods section how cyst infection was diagnosed/defined. Comparison of APN and cyst infection with regards to clinical and laboratory characteristics (page 10) makes only sense if these characteristics were not used to identify cyst infections. CT/MRI and ultrasound evaluation (see discussion section of manuscript) are not reliable for diagnosing cyst infection. I would consider to omit this paragraph on the comparison cyst infection vs. APN since it is not the main focus of the manuscript.

We added the diagnostic criteria of APN and cyst infection in the method section (Page 7/Line 15-22). We also omitted the paragraph describing the clinical characteristics of upper UTI (page 10) and Table 6 because they are out of the main focus in this paper.

6. The CKD-EPI formula should be preferably used rather than the MDRD formula, since many patients had a preserved eGFR.

As you have recommended, we re-analyzed our data based on estimated GFR using CKD-EPI formula. All the data has been changed according to CKD-EPI formula throughout the main body and Tables/Figures. The main result did not change even after the amendment.
Minor points:

7. **Table 6:** “urine nitrogen positive” should probably mean “urine nitrite positive”

   Thank you for the comment. In fact, we decided to delete the Table 6 from the article because it is not the main point of this paper.
Reviewer2

Major Compulsory Revisions

1. Did the patients go regularly for exams when they haven’t clinical manifestation? If yes, how often the patients did perform the urinanalysis? In this point, may the patients of group B have been underestimated?

As we have answered to the first reviewer's comment, the patients were seen in the clinic based on their eGFR as follows: CKD stage I-II: q 3-6 months; CKD stage III: q 2-3 months; CKD stage IV: q 6-8 weeks. In addition to the disease severity, we followed up patients more frequently when the following acute problems happened. After the UTI episodes, there was a short term follow up period (average q 2-4 weeks, for 1-6 months until the condition gets better according to the patient's condition (high blood pressure, new onset hematuria, etc.). However, when we re-evaluated the proportion of group A (no or transient pyuria) and B (chronic pyuria) according to CKD stages based on initial eGFR, there were no significant differences among groups in advanced CKD stages (CKD II–IV).

2. In section PATIENTS AND METHODS, I suggest correcting the last sentence in the first paragraph after definition of pyuria – this word is repeated three times within a sentence.

We changed the sentence (page 6, Line 20) as follows: The duration of pyuria episode was from detection to resolution of pyuria in a subsequent urinalysis.

3. In section PATIENTS AND METHODS, the authors need to differ the APN and cyst infection in clinical and laboratory point of view (usually, in cyst infection the microorganisms are absent in the urine). I suggest including the criteria for cyst infection.

We added the diagnostic criteria of APN and cyst infection in the method section (Page 7/Line 15-22). Since the first reviewer recommended to omit the part describing clinical characteristics of overt UTI because it is not the main point of this study, we also omitted the paragraph describing the clinical characteristics of upper UTI (page 10) and Table 6.

4. Antibiotic therapy need to be better defined in the section PATIENTS AND METHODS and not only in the Table 6. How the antibiotics are given for renal cyst infection and APN: orally or parenterally?

Since differentiating cyst infection from other renal infection in ADPKD patients is not the main point of this study, we
decided to omit this part and Table 6. Just to answer to your question, all patients diagnosed with either cyst infection or APN received parenteral antibiotics initially and subsequently changed to oral antibiotics when clinical symptoms and signs improved.

5. In section Results, first paragraph, I suggest for not repeating in the text the data of Table 1.

We omitted some sentences and concisely summarized the clinical characteristics of the patients (pages 8-9).

6. I think the authors need to correct the data in the Table 2: if we do the sum of patients result totally 291 instead of 256 patients, so and the percentage is 113.1%, instead of 100%.

Thank you for your detailed examination through our paper. We made a change in Table 2. In summary, a total number of patients are 256 but not 291. Some patients experienced both APN, cyst infection, and cystitis sequentially. Therefore, a total number of patients (n = 256) is the sum of no pyuria group (n = 80) and asymptomatic pyuria group (n = 176). To avoid misunderstanding, we have omitted the number of patients who had experienced UTI (cystitis, APN, and cyst infection). Indeed, overt UTI is one of the primary measures we used to analyze the outcome of pyuria.

7. When presenting demographic data in the tables, BMI would be present (if the authors have data registered).

Unfortunately, we do not have BMI data calculated from height and weight of the patients.

8. In section TABLES, I’m a few confused as regard the data in the table 3: to which group are the data as regard the age, HTN, stones. Are these data for group B which is analyzed in this table? The same explanation is needed and for the Table 4 and 5.

Table 3 is the result of chi-square test comparing those who developed overt UTI and those who did not developed overt UTI during follow up. We tried to assess associated factor with the development of UTI in this univariate analysis and demonstrated that Group B (chronic pyuria) is one of the associated factor with the development of overt UTI.

Table 4 is the result from Cox regression analysis (both univariate and multivariate model) to assess risk factors associated
with the development of overt UTI. After adjustment for gender, HTN, and initial eGFR, Group B was still the independent risk factor for the occurrence of overt UTI.

Table 5 is the result from Cox regression analysis (both univariate and multivariate model) to assess risk factors associated with the development of upper UTI. Similar to the overt UTI results, Group B was the independent risk factor for the occurrence of upper UTI.

9. **Instead of values of P > 0.05 in the tables I would suggest to put NS (not significant).**

Thank you for your considerate comments. We have made changes in the tables.
Supplementary Table 1.

<table>
<thead>
<tr>
<th>Frequency of Pyuria in the 1st year of follow up</th>
<th>N</th>
<th>Mean ΔeGFR/yr</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>129</td>
<td>-1.2132</td>
<td>4.23743</td>
<td>-8.30</td>
<td>24.70</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
<td>-2.3063</td>
<td>6.67659</td>
<td>-29.30</td>
<td>21.10</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>-1.8919</td>
<td>6.03602</td>
<td>-14.80</td>
<td>20.90</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>-3.3500</td>
<td>6.31710</td>
<td>-13.20</td>
<td>6.40</td>
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<tr>
<td>4</td>
<td>2</td>
<td>-2.4000</td>
<td>2.68701</td>
<td>-4.30</td>
<td>-0.50</td>
</tr>
<tr>
<td>Total</td>
<td>256</td>
<td>-1.7269</td>
<td>5.42397</td>
<td>-29.30</td>
<td>24.70</td>
</tr>
</tbody>
</table>

Supplementary Table 2.

<table>
<thead>
<tr>
<th></th>
<th>0 Pyuria/1st Year (N=129)</th>
<th>1-4 Pyuria/1st Year (N=127)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial eGFR(\text{mL/min/1.73m}^2)</td>
<td>91.3 ± 26.7</td>
<td>90.9 ± 31.6</td>
<td>0.931</td>
</tr>
<tr>
<td>Final eGFR(\text{mL/min/1.73m}^2)</td>
<td>78.9 ± 33.0</td>
<td>75.7 ± 37.6</td>
<td>0.471</td>
</tr>
<tr>
<td>eGFR change(\triangle \text{GFR /yr})</td>
<td>-1.21 ± 4.24</td>
<td>-2.25 ± 6.38</td>
<td>0.127</td>
</tr>
</tbody>
</table>
Supplementary Figure 1

A. Overt UTI

B. Upper UTI