Author's response to reviews

Title: Urinary Phosphorus Excretion per Creatinine Clearance as A Prognostic Marker for Progression of Chronic Kidney Disease: A Retrospective Cohort Study

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Version: 3 Date: 20 May 2015

Author's response to reviews: see over
May 20, 2015

Dear Dr. Hayley Henderson
Executive Editor, BMC Nephrology

Authors: Tomoki Kawasaki et al.

We are grateful to reviewer A for the critical comments and useful suggestions that have helped us to improve the quality of our paper considerably. As indicated in our responses as follows, we have taken all of your comments and suggestions into account in the revised version of our paper.
The new text is underlined.

I hope that the revised manuscript is accepted for publication in BMC nephrology.

Sincerely,

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The manuscript entitled “Urinary phosphorus excretion per creatinine clearance as a prognostic marker for progression of chronic kidney disease: A retrospective cohort study” by Kawasaki et al. describes a study investigating the prognostic value of urinary phosphorus excretion per creatinine clearance to predict progression of CKD. In a cohort of 191 patients (single center, Japanese population), the authors observed a gradually increased risk of CKD progression with higher values of urinary phosphorus excretion per creatinine clearance. Therefore, it is suggested that urinary phosphorus load is a direct contributor to CKD progression, possibly due to tubular injury.

The rationale behind the study is interesting and the results of the study are fairly well presented. Of course, the study design precludes causal inferences. Although the findings seem quite robust, I still have some comments that the authors need to address before the manuscript can be considered for publication.

1. Major Compulsory Revisions

- The authors suggest that 24h urinary excretion of phosphorus is a surrogate for the tubular load. This would certainly be true if phosphorus excretion is mainly driven by tubular secretion. In contrast, 24h urinary excretion of phosphorus is the composite of glomerular filtration minus tubular absorption. As tubular absorption rate goes down when renal function declines, I’m not sure that 24h urinary excretion is a good estimate of the tubular load. I suggest that the authors also calculate the tubular absorption rate (= (GFR x serum phosphorus) – 24h urinary excretion)), also corrected for GFR, and link this parameter to
outcome. If there is no relationship between tubular load and outcome, this may suggest that is not the intracellular load of phosphorus, but the external phosphorus cell burden that possibly mediates renal injury.

We quite agree with reviewer’s comment.

24h urinary excretion of phosphorus per creatinine clearance is not a surrogate for the intracellular load of phosphorus.

In the previous animal experiment, phosphate excretion per nephron over 1µg/day caused tubular damage. Therefore, to evaluate actual phosphate burden to residual nephrons, we would have suggested that 24h urinary excretion of phosphorus is a surrogate for the nephron load.

We have changed the following text from p4 line 7-8 to

To evaluate actual phosphate load on each nephron, the association between, a newly proposed index that is a surrogate for the nephron load, 24-hour urinary phosphorus excretion per creatinine clearance (24h U-P/CCr) and CKD progression was examined.

- As serum phosphorus increase is prevented in earlier stages of CKD due to higher urinary phosphorus excretion, there is no good correlation between serum phosphorus and urinary phosphorus excretion/creatinine clearance in stage 3b, while there is a nice correlation in stage 5. I’m not sure whether the same is true if you correct serum phosphorus for creatinine clearance as well? If there is a good correlation, could the authors perform additional analyses with serum phosphorus/creatinine clearance as marker for outcome and demonstrate whether or not this is inferior to the urinary excretion/creatinine clearance? A possible way to investigate is to use logistic regression (outcome yes or no) and compare AUCs.
As the reviewer indicated, we have examined for correlation between serum phosphorus / creatinine clearance and urinary phosphorus excretion /creatinine clearance in CKD stageG3b and stageG5.

Pearson’s correlation coefficient (r) and the corresponding P values for these correlations were $r = 0.35$ and $0.38$, $P = 0.08$ and $< 0.001$ respectively. Significant statistical correlation was not observed in CKD stage3b, and low correlation was observed in CKD stageG5. But r in CKD stageG3b is similar to r in CKD stageG5. So the same is not true if we correct serum phosphorus for creatinine clearance as well.

-Discussion p14 line 14: I believe the authors suggest the possible superiority of U-P/eGFR to U-P/CCr and not U-P/CCr to U-P/eGFR? Nevertheless, although you may have the advantage that you are not obligated to measure urinary excretion of creatinine, you still have to measure excretion of phosphorus, thus needing urinary collections.

In accordance with reviewer’s comment, we have changed the text.

-Furthermore, the authors also hope that phosphorus per creatinine excretion in spot urine could replace their proposed marker of 24 U-P/CCr (or U-P/eGFR), but that’s not correct. Even assuming negligible circadian change of phosphorus per creatinine excretion in spot urine, this would be a surrogate of 24h phosphorus excretion, thus you still need to adjust for eGFR (= spot U-P/U-Creat//eGFR).

In accordance with reviewer’s comment, we have changed the text.

-In this study the authors define CKD progression as evolution to ESRD, 50% reduction of eGFR and death. As the rationale behind the study is to link renal burden of phosphate to CKD progression, I would leave out evolution to death
and just focus to ESRD or 50% reduction, also because there are only 5 deaths, making this endpoint less important.

**We have left out evolution to death as reviewer indicated.**

-Limitation: single center study in Japanese population. Care must be taken to extrapolate these findings to other populations. This should be added in the limitation section.

**We have added the following text as one of the limitation of the study as reviewer indicated.**

**Firstly, our study is a single center, retrospective observational, not interventional study in Japanese population.**

2. Minor Essential Revisions

- As a marker of phosphorus excretion the authors use “urinary phosphorus excretion per 24h creatinine clearance”. As creatinine clearance is expressed as ml/min, I don’t think it’s necessary to add “24h”. In contrast, phosphorus excretion is referring to its 24h excretion, so the correct statement would be: “24h urinary phosphorus excretion per creatinine clearance (24h U-P/CCr)”.

**We have changed from U-P/24hCCr to 24h U-P/CCr as reviewer indicated.**

-MM study population p7 line 14-16: Please add units for biochemistry measurements.

**We have added units for biochemistry measurements as reviewer indicated.**

- MM study population p8 line 2: Please give reference for this formula.

**In accordance with reviewer’s comment, We have added the following reference.**

In accordance with reviewer’s comment, we have added the following formula to calculate TRP:

\[ TRP = 1 - \left( \frac{\text{Urinary phosphate} \times \text{Serum creatinine}}{\text{Serum phosphate} \times \text{Urinary creatinine}} \right) \]

- Results p12 line 14: I suggest adding the result of analyses performed with eGFR to the manuscript.

In accordance with reviewer’s comment, we have added the result of analyses performed with eGFR to the manuscript.

- Results p12 line 5: I suggest adding the correlation coefficient and P-value for correlation between TRP and eGFR.

In accordance with reviewer’s comment, we have added the correlation coefficient and P-value for correlation between TRP and eGFR.

P12 line 13-14: TRP and eGFR were correlated \((r = 0.68, p < 0.001)\).

- Grammar and spelling:
  * Abstract line 11: I suggest “only patients” instead of “inpatients”.
  * Introduction line 7: I suggest “causes tubular damage” instead of “cause”.
  * Introduction line 11: I suggest “24h urinary excretion” instead of “urinary phosphorus”.
  * Introduction line 13 to 15: This sentence needs rephrasing.
  * MM study population p7 line 5: Please add “who WERE admitted”.

* MM study population p7 line 10: Please add “who WERE lost to FU”.

* MM study population p7 line 13: Please add “by REVIEW of medical records”.

* MM study population p9 line 16: Please change into “primary” instead of “parimary”.

* MM study population p10 line 1: Please change into “,” instead of “and (eight)”. 

* MM study population p10 line 1: I suggest “evidence of” instead of “evidence with”.

* MM study population p10 line 2: I suggest “, excluding them” instead of “, they were excluded”.

* MM study population p10 line 4: I suggest “analysis” instead of “analyze”. *

Discussion p14 line 10: I suggest “minimally” instead of “minimamllly”.

* Discussion p15 line 4: This sentence needs rephrasing.

We have changed the following text as reviewer indicated.

From Abstract line 11 to 
only patients on the educational program for CKD.

From Introduction line 7 to 
causes tubular damage.

From Introduction line 11 to 
24h urinary phosphorus excretion per creatinine clearance (24h U-P/CCr)

From Introduction line 13 to 15 to 
only patients on the educational program for CKD with fixed diet regimen were 

included and the association between 24h U-P/CCr and CKD progression in
these patients was examined.

From MM study population p7 line 5 to who were admitted to the hospital
From MM study population p7 line 10 to who were lost to follow-up
From MM study population p7 line 13 to by review of medical records.
From MM study population p9 line 15 to p9 line 16 to primary hyperparathyroidism eight

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests: I declare that I have no competing interests
Reviewer's report
Title: Urinary Phosphorus Excretion per Creatinine Clearance as A Prognostic Marker for Progression of Chronic Kidney Disease: A Retrospective Cohort Study
Version: 2 Date: 2 April 2015
Reviewer: Nadine Kaesler

Reviewer's report:

Major Compulsory Revisions

The manuscript by Kawasaki et al. describes the impact of urinary phosphorous load per creatinine clearance on the progression of chronic kidney disease. Having an early marker for phosphate load is of interest, but the authors need to point out the importance of their established marker. Balancing phosphorous on creatinine is a simple method and creatinin clearance it is already considered in established markers, i.e. fractional excretion or tubular reabsorption. Recently, Bech et al reported the impact of fractional excretion on progression of CKD (Bech AP, J Nephrol, 2015) in relation to FGF 23. Here, corresponding FGF23 levels in serum and fractional excretion of phosphate are missing. The source of protein is not included, although it is a direct link to the bioavailability of alimentary phosphorous and its excretion. Phosphorous excretion is dependent on CKD stages (as shown by the authors) but the number of observations in CKD 2 and 3 are low. Data of CKD progression of individuals is missing. Therefore the evidence for U-P/24h-CCr being a valid marker for CKD progression in all CKD stages is low. Using creatinine clearance as marker for nephron number needs to be confirmed. The discussion of the advantage for using U-P/24h-CCr is contradictory.

Abstract:

1) Lines 6-8: How does the ratio of U-P/24h-CCr reflect the phosphate loss for each nephron? A proof that 24h-CCr is a marker for nephron number is missing. Why is it important to normalize to creatinine clearance?
In animal experience, renal biopsy and magnetic resonance imaging were used to estimate glomerular number. Fulladosa et al reported that a positive correlation between GFR and Glomerular number in stable renal transplants. (Fulladosa X, Moreso F, Naráez JA, Grinyó JM, Serón D. Estimation of total glomerular number in stable renal transplants. J Am Soc Nephrol 2003; 14: 2662-8).

Because 24h-CCr can be measured less invasively, we used 24h-CCr as marker for nephron number.

We have therefore added the following text as part of introduction (p6-7 line 12-1).

In animal experience, renal biopsy and magnetic resonance imaging were used to estimate glomerular number. Previous study reported that a positive correlation between GFR and Glomerular number in stable renal transplants. (11). Therefore, we used 24h-CCr as marker for nephron number.

We have also added the following reference.


2) Many studies deal with negative outcome of high levels of phosphate in serum.

What is the advantage of this established marker than for example phosphate in serum? Can you show a graph that it is an earlier marker than phosphate in serum in these patients by a graph (results section)?

We have added new the figure that shows U-P/CCr elevated earlier than serum
phosphorus in CKD as reviewer indicated.

3) Usually FGF23 is one marker which increases early in serum. How is the association of U-P/24-CCr with FGF 23 in serum?

FGF23 is a hormone that increases phosphate excretion per nephron by increasing the fractional excretion of phosphate and increasing GFR. So we have assumed there is a positive correlation between 24h-CCr and FGF23 in serum.

4) What is the key result for the conclusion? Material & Methods:

The key result for the conclusion is that higher U-P/24h-CCr showed a higher risk for the composite outcomes.

5) The source of protein is of importance, for example for association of phosphate with protein rich foods has reduced bioavailability from plant origin.

Phosphate intake is not mentioned but excretion is highly dependent on phosphate intake.

In our study, the fixed diet regimen was served for the patient. The proportion of animal protein in the diet regimen was 50-60 percent of total. Therefore phosphate intake of each patient is fixed about 0.8 to 1.0 g / kg, standard body weight.

We have added the following text as reviewer indicated.

The proportion of animal protein in the diet regimen was 50-60 percent of total. Therefore, phosphate intake of each patient is also fixed about 0.8 to 1.0 g / kg, standard body weight.
6) Table 1: An average BMI of 59.8 is highly obese- is that the correct figure? Please give a more detailed description of CKD patients in the results section.

That is not the correct figure. Table 1 have been changed as reviewer detected.

7) Table 1: 86% of the patients were in CKD stage 5. Were all CKD stages analysed all together? Was there an adjustment for CKD stages?

There was not an adjustment for CKD stages but for eGFR, because highly significant statistical correlation between CKD stage and eGFR was observed.

8) How war the tubular reabsorption calculated? Usually it considers GFR and Creatinine.

Tubular absorption of phosphate (TRP) was calculated using the formula as follows: TRP = 1 - (U-phosphate × S-creat) / (S-phosphate × U-creat)

We have added the following text.

TRP was calculated using the following formula: TRP = 1 - (Urinary phosphate × Serum creatinine) / (Serum phosphate × Urinary creatinine).

Results:

9) Figure1 a) and b): The impact of showing the regression of both approaches is not clear. Graphs need more detailed descriptions. Are all CKD stages included?
Figure 1 shows assessing the assumption in Cox regression. Hazard ratio in each patient was calculated, and then patients were divided into two groups in the median of hazard ratio. The graph of the log(-log(survival)) versus log of survival time graph should resulted in parallel curves if the Cox regression models was acceptable.

We have change the following figure legend (Figure 1.)

Figure 1.

Proportional hazards with the applied predictors in the Cox model was assessed by plotting a negative logarithm of the Kaplan Meier Survivor estimate. Hazard ratio in each patient was calculated, and then patients were divided into two groups in the median of hazard ratio. a) A graph assessed the proportionality of hazards in Model 2 divided into high and low hazard. b) A graph assessed the proportionality of hazards in Model 3 in the same way.

10) Urinary phosphate increase by high protein intake might due to the increased uptake, as it is associated with protein rich foods. Can the authors comment?

There is a strong correlation between amount of urinary phosphate and protein intake. Thus in our study, fixed amount of protein was served to avoid potential confounds.

Discussion:
11) Why is phosphate in serum only increased in Quartile 4?

Is this the reason that U-P/24h-CCr is not associated with serum phosphorous in CKD stage 3?
In early stage of CKD, serum phosphorus was reported to be kept in normal range due to an increase in phosphate excretion by fibroblast growth factor-23 (FGF-23). But as CKD progresses, FGF-23 cannot enhance urinary phosphate excretion, thus leading to the development of hyperphosphatemia. In this study, we assume serum phosphorus is kept in normal range in CKD stage G3b by increasing U-P/24hCCr. In Quartile 4, because they had lower eGFR than other groups, they had hyperphosphatemia.

12) What is the outcome of an association of fractional phosphorous excretion with CKD progression?

The work of Bech 2015 shows no impact on CKD progression by fractional phosphate excretion, which needs to be discussed here.

14) Which information provides U-P/CCr that is described by the established maker, i.e. fractional excretion, TRP and phosphate in serum? As no data of fractional phosphate excretion is provided here, it is difficult to evaluate the better impact of U-P/CCr on CKD progression.

Answer to 12) & 14)

The outcome of an association of fractional phosphorus excretion (FeP) with serum phosphorus was shown as follows:

Table. Hazard Ratio (HR) and 95% Confidence Interval (CI) of the Composite Outcome of ESKD or 50% Reduction of eGFR Associated with fractional phosphorus excretion
<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95%CI)</td>
<td>P Value</td>
<td>HR (95%CI)</td>
<td>P Value</td>
<td>HR (95%CI)</td>
</tr>
<tr>
<td>FeP Quartile 1</td>
<td>1.00 (reference)</td>
<td></td>
<td>1.00 (reference)</td>
<td></td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>FeP Quartile 2</td>
<td>2.82 (130 – 6.77)</td>
<td>0.01</td>
<td>1.57 (0.65 – 4.35)</td>
<td>0.33</td>
<td>1.72 (0.65 – 5.10)</td>
</tr>
<tr>
<td>FeP Quartile 3</td>
<td>7.06 (3.43 – 16.44)</td>
<td>&lt; 0.001</td>
<td>2.65 (1.05 – 7.65)</td>
<td>0.03</td>
<td>3.51 (1.23 – 11.4)</td>
</tr>
<tr>
<td>FeP Quartile 4</td>
<td>7.69 (3.63 – 18.20)</td>
<td>&lt; 0.001</td>
<td>2.46 (0.85 – 7.92)</td>
<td>0.09</td>
<td>2.27 (0.69 – 8.27)</td>
</tr>
</tbody>
</table>

The association between FeP and the outcome was observed in Model 1, but in Model 2 and 3, higher FeP did not show a higher risk for the outcomes. The work of Bech 2015 shows no impact on CKD progression by FeP. In this study, patients were categorized by FGF23 and FeP. Whereas there was not adjustment for FGF23 in our study. U-P/24h-CCr would be a confounding factor for FGF23. Although U-P/24h-CCr would be a reflecting FGF23, We think that U-P/24h-CCr could be a surrogate marker of FGF23 in clinical practice, because FGF23 cannot be measured in clinical use in Japan.
We have changed the following text from p10 line 2 to
To compare 24h U-P/CCr with common markers of urinary phosphorus excretion, we examined for the association of fractional phosphate excretion (FeP).

We have also added the following text p13 line 12
A previous study showed no impact on CKD progression by FeP(). In this study, patients were categorized by value of FGF23 and FeP. In our study, there was no adjustment for FGF23. 24h U-P/CCr would be a confounding factor for FGF23. Even though 24h U-P/CCr would be reflecting FGF23, we think that U-P/24h-CCr could be a surrogate marker of FGF23 in clinical practice, because FGF23 cannot be measured in clinical use in Japan. Moreover, the association between FeP and the outcome was observed in Model 1, but in Model 2 and 3, higher FeP did not show a higher risk for the outcomes.

We have added the following reference.

13) The alternate model which normalises to GFR showed similar results. Why is U-P/CCr preferred to normalisation to GFR?

It is difficult to collected 24-hour urine accurately because of residual urine. The unit of U-P/24h-CCr and U-P/eGFR are presented as follows: (mg/24 hours) / (ml/min/24 hours) and (mg/24 hours) / (ml/min).

If collected urine is sampled only 23 hours, the value of U-P/24h-CCr is not change greatly, because U-P and 24h-CCr were collected during the same time. In this case, the unit of U-P/24h-CCr is (mg/23hours) / (ml/min/23hours). But when normalizes eGFR, each urine sample is required to be collected
24-hours accuracy.

We have changed the following text p15-16 line11-2
One possible superiority of 24h U-P/CCr to 24h U-P/eGFR is described in the following. It is difficult to collected 24-hour urine accurately because of residual urine. The unit of U-P/24h-CCr and U-P/eGFR are presented as follows: (mg/24 hours) / (ml/min/24 hours) and (mg/24 hours) / (ml/min). If collected urine is sampled only 23 hours, the value of 24h U-P/CCr is not change greatly, because 24h U-P and CCr were collected during the same time. In this case, the unit of 24h U-P/-CCr is (mg/23hours) / (ml/min/23hours). But when normalizes eGFR, each urine sample is required to be collected 24-hours accuracy.

15) Page 14 Line 14-16 The study was performed with U-P/24h-CCr (U-P/CCr in line 14? U-P/Cr in line 16). Suitability of spot urine is specultaive and contradictory to the strength section where the circadian change of phosphate excretion is mentioned. Here in the discussion section it is pointed out as only advantage of using this marker.

We have changed the text as reviewer indicated.

Minor Essential Revisions Material & Methods:

16) Page 9-10 Lines 16-2: There are several typos in one sentence: Primary hyperparathyroidism, evidence for, missing values

We have changed the following text as reviewer indicated.

17) Page 10, Lines 2 and 5: Missing values of patients are mentioned twice.
We have changed the text as reviewer indicated.

Strength & Limitations:
18) Page 15 lines 4-5 First sentence seems to be incomplete
We have changed the text as reviewer indicated.

19) Page 15 line 7: Referring to phosphate binder or phosphate restriction studies should not be part of this section and could be moved to the general discussion for importance of phosphate metabolism.
We have moved the text to the part of general discussion as reviewer indicated.

29) Page 15 line 8: Phosphate in serum or urine?
We have changed the following text as reviewer indicated.

But it is certain that urinary phosphate is a surrogate marker reflecting protein intake that is associated with CKD progression

Discretionary Revisions

Abstract/Introduction:

21) A short overview of the different factors influencing the phosphate excretion might be helpful.
We have added a short overview of influencing the phosphate excretion as reviewer indicated.

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

**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.
In accordance with editorial comment,
We have changed the following text from p16 line 5-12 to

One possible superiority of 24h U-P/CCr to 24h U-P/eGFR is described in the following. It is difficult to collected 24-hour urine accurately because of residual urine. The unit of U-P/24h-CCr and U-P/eGFR are presented as follows: (mg/24 hours) / (ml/min/24 hours) and (mg/24 hours) / (ml/min). If collected urine is sampled only 23 hours, the value of U-P/24h-CCr is not change greatly, because U-P and 24h-CCr were collected during the same time. In this case, the unit of U-P/24h-CCr is (mg/23hours) / (ml/min/23hours). But when normalizes eGFR, each urine sample is required to be collected 24-hours accuracy.

We have also changed the following text from p17 line 6-12 to

Compared with spot urine, to measure 24-h collected urine samples takes time and effort. Urinary phosphorus excretion increase from morning to the middle of the night in healthy subjects (24). But in CKD patients, circadian rhythm of urinary phosphorus excretion along with U-P/Cr remains unclear.

Using 24-h collected urine can measure phosphorus excretion more accurate
than using spot urine. Spot urine could be a alternative prognostic marker if
circadian change of U-P/Cr was resolved.