Reviewer’s report

Title: The Association Between Soluble Klotho and Cardiovascular Parameters in Chronic Kidney Disease: Results from the KNOW-CKD Study

Version: 1 Date: 05 Jan 2018

Reviewer: Annet Bouma - de Krijger

Reviewer’s report:

This study examines in a large cohort of CKD patients the relation between soluble Klotho and cardiovascular outcome parameters. This article contributes to previous research to elucidate the relation between CKD and cardiovascular disease, major health burdens. The revised manuscript processed the previous recommendations very well and has substantially improved, yet some adjustments are still recommended.

A. Essential revision concern statistical analysis (in addition to the already performed improvements):

1. The authors substantially improved their manuscript after the first recommendations. Yet their statistical analysis leaves an important question unanswered, namely if sKlotho is associated with LVH through all stages of CKD. Previous studies in other stages of CKD showed no relation between soluble klotho and LVMI. The difference between the finding of the present study and previous studies could be because the association between changes during the progression of CKD changes (possible diminish with the progression to ESRD).

The authors statement on page 12 in the discussion: 'our study included all stages of CKD patients and presented that sKlotho could be a marker of LVMI across all stages of CKD', is therefore not fully true. They did not answer the last part ('across all stages of CKD').

I would strongly recommend that the authors add a stratification for the different stages of CKD in their analysis on the relation between soluble sKlotho and LVMI and PWV (table 2 and 3). Since they have such a large cohort this must be possible. (So a table can be made with the different models on the y-axis and on the x-axis the results of the whole cohort followed by a stratification for the different stages of CKD).

Performing this additional analysis certainly would add to our knowledge on the subject of sKlotho and its association with CVD (parameters) and could bring a possible explanation for the discrepancy with previous studies, which the authors then subsequently can comment on in their discussion.

2. Another important issue is that the authors in their improved manuscript suddenly left out the adjustment for FGF23 in the analysis between sKlotho and LVMI and PWV (table 2 and 3). This
was not recommended by one of the reviewers and the correction for FGF23 is of importance, since there is a close connection between sKlotho and FGF23. Since the association between FGF23 and LVH is well established, FGF23 could be a confounder and is a factor which on pathophysiological grounds should be added to the model. (During progression of CKD FGF23 concentrations rise and sKlotho level diminish, the reader wants to know whether the association between sKlotho and LVMI is independent of FGF23).

B. Minor revisions:

p8 Methods, section 'statistical analysis': It is not clear which statistical test was used for analysis on the sKlotho quartiles. (p-values and p-for trend) depicted in fig 1 and fig 3. It is not described if the reference value was the lowest quartile? And is the p-value the difference between the highest and lowest quartile?

p9 Results; section 'comparison of baseline (...) to klotho levels'. The summing-up of different characteristics between the klotho quartiles is not easy readable. I recommend to leave out all the p-values in between since all this information is visible in table 1.

fig 1. legend states †p= <0.05 and †† p=>0.05; better use NS (not significant) for ††

fig 3. The mentioned p 0.127 and p 0.022 ; do these p-values concern the difference between the 1e quartile compared to 4e quartile? It helps to show this graphically (as is done in figure 1) with an connecting line figure (㊦)

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Unable to assess

Are the conclusions drawn adequately supported by the data shown?
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Yes

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