Reviewer’s report

Title: Serum klotho: a potential predictor of cerebrovascular disease in hemodialysis patients

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Reviewer: Javier Neyra

Reviewer's report:

Wei et al report their work on "Serum klotho and cerebrovascular disease in hemodialysis patients". I consider that their work is relevant because of the limited available risk-stratification tools for the prediction of cerebrovascular disease in prevalent HD patients. In addition, Klotho is a novel protein with evolving experimental evidence and limited human studies. I congratulate the authors for their work. However, I have some observations and comments.

Major comments:

- Methods: The authors describe as one of the inclusion criterion the presence of head CT or MRI within 1 year of enrollment. Is it possible that most of these patients have prevalent cerebrovascular disease? Please clarify if the outcome of interest is recurrent events, de novo events or both.

- Methods: Was the outcome determined by neurology evaluation of clinical symptoms and imaging studies? Please clarify.

- Methods: Please report the time relationship between the Klotho measurement and the cerebrovascular disease event (mean/median time between measurement and event).

- Methods: Please provide reference(s) regarding the Klotho assay used and the intra-assay or inter-assay CVs if available. Have the authors utilized this assay before? This is important as there is no established assay for serum Klotho in humans.

- Methods: Was the variable "diabetes" utilized in the analysis as a categorical, ordinal or continuous variable using A1C or serum glucose? Please clarify as Spearman correlation performs better for continuous/ordinal variables.

- Methods: Data pertaining to hypertension, prior stroke, diabetes, and tobacco smoking are relevant but not included in Table 1 or tested in the multivariable models. In
particular, "diabetes" was not included in the multivariable models despite it was found to be significantly correlated with Klotho levels. Please clarify.

- Methods: Please report Model 3 for serum Klotho and cognitive impairment in the subgroup of patients with cerebral infarction.

- Methods: Was dividing serum Klotho levels in tertiles explored (rather than dichotomizing levels by the mean)? Any specific reason for dichotomizing Klotho levels in the multivariable models?

- Results: Please provide some characteristics of the ESRD/HD such as etiology of ESRD and HD vintage (months or years). I suggest adding these parameters to Table 1 if data are available.

Minor comments:

- Stats paragraph: Add median (IQR) to continuous data reporting.

- Discussion: Please summarize second paragraph of the Discussion which is a little extensive.

- References: I suggest the authors to add these 2 references to the manuscript (PMID:21115613 for Klotho pathobiology in vascular calcification; PMID:28115282 for Klotho candidacy as a biomarker in renal disease; and PMID 19419323 for Klotho variants and mortality in ESRD pts).

**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.

Yes

**Are the conclusions drawn adequately supported by the data shown?**  
If not, please explain in your comments to the authors.

Yes
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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None.

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