Author's response to reviews

Title: Estimation of Glomerular Filtration Rate from Serum Creatinine and Cystatin C in Octogenarians and Nonagenarians

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Author's response to reviews: see over
Dear Editor

Thank you for your considerations and the comments of the reviewers on our manuscript.
We send you the revised version of the manuscript and included the modifications (highlighted) suggested by the reviewers.
In this letter are the response to your question and the point-by-point answers to the reviewers’ comments.
Thank you for your comments and your attention.
Sincerely,

Ricardo Sesso, MD

Editorial Point:
‘Ethical Approval - In the methods section, can you please include the full name of the ethical committee that granted approval for your study’
The name of the ethical committee was included on pg. 6, 1st paragraph.
Reply to reviewer: Anders Larsson

1. ‘Both creatinine and cystatin C methods have changed over time, thus the time of assay is of importance. The elderly were identified 2010-2011, however there may be a time span between identification and testing??? I would appreciate if the authors also added approximately when the creatinine and cystatin C assays were performed’

Answer: Patients were initially identified in 2010-2011; were reassessed and enrolled between August 2011 and May 2012. They study samples were obtained within one week of the day of the interview, when the data on clinical and sociodemographic characteristics were obtained (pg 5). Serum creatinine was analyzed during the same day of the collection. All plasma cystatin C samples were frozen at -70°C and analyzed within 7 days in May 2012. Added to Methods, pg. 5 and 6.

2. ‘Under the result section, second paragraph please add mean and SD after 55.9’

We have added ‘mean±SD’ to the 2nd parag. of the Results (pg. 9).

3. ‘Under background, line 1-2 the authors say: that the prevalence is rising, particularly in the elderly. I agree that the prevalence is rising but I believe that it is mainly due to the fact that life expectancy is increasing… If I would be the authors I would add a few words saying that this is mainly due to increased life expectancy.’

Answer: We have added a short explanation for the increased prevalence of CKD in the elderly in the first sentence of the Background (pg. 4).
1. There is already the study (Bevc et al. Ther Apher Dial 2011; 15:261-8) comparing different equations (MDRD, CKD-EPI creatinine, CKD-EPI creatinine-cystatin C and simple cystatin C equation) in elderly (over 65). It is true that the population in the study by Bevc is not so old but should at least be mentioned in discussion. Very interesting in study by Bevc et al. is the usage of simple cystatin C equation which is usable in everyday clinical practice without complex calculation. It will be interesting if authors include this equation in their work and check its usefulness in the really old population.’

Answer: The study by Bevc et al was included in the Discussion, pg. 14, 1st parag.

2. ‘Authors are using »….tendency towards… p.9« , »….reclassification was favorable, albeit not statistically significant…« p.10, »….tended to improve classification…« - it should be clearly stated if the difference is statistically significant or not (p < 0.05; if not: »the trend« can also be coincidence’

Answer: We corrected or deleted the terms ‘tendency towards’, ‘albeit not statistically significant’, ‘trend to improve classification’ used on pg. 10 and 11, and in the Conclusion on pg 16, and tried to make clearer the comparisons that were statistically significant or not.
Reply to reviewer: Jonas Bjork

1. ‘It is unclear whether the used cystatin C assay was calibrated to the recent IFCC standardization. The underestimation of the cystatin C-alone equation CKD-EPI Cystatin C, which was developed based on standardized cystatin C, in the present cohort could indicate that this was not the case. Using cystatin C has less relevance and results low generalizability unless a standardized assay is used.’

Answer: The cystatin C assay was not calibrated against the international certified reference material ERM-DA471/IFCC for cystatin C. However, cystatin C values were reexpressed by the formula -0.105 + 1.13 X cystatin C to account for the standardization of cystatin C with ERM-DA471/IFCC prior to their use in GFR estimating equations, as suggested by Inker and colleagues [10]. Although the calculations had been performed with this adjustment, this information was missing in the initial version; this explanation was added to the new version (Methods pg. 6-7).

2. ‘Given the uncertainty about the cystatin C standardization, it is hard to assess the benefit of combining creatinine with cystatin C from this study. The analysis least affected by differences in standardization is probably the area under the ROC curve, something which is worth stressing. However, the author put too much emphasis on the improved classification (which does not reach statistical significance even focus is shifted to AUC) in the conclusion, given the statistical uncertainty that is present in a small study like this.’

Answer: Please, see the above answer/comment 1. In addition, we have reinforced the results of the ROC analyses in the Discussion (pg.:14, 2nd parag.)

3. ‘The authors conclude in the abstract that both CKD-EPI creatinine and BIS creatinine are satisfactory alternatives if cystatin C is not available. However, CKD-EPI creatinine has P30-accuracy (64%) that is below 75% when mGFR<60. By contrast, BIS creatinine has a more stable performance with respect to P30 in the two investigated GFR-groups (mGFR<60 and mGFR>=60). Thus, the study rather provides some evidence that BIS creatinine
is superior to CKD-EPI creatinine at decreased mGFR-levels, mainly due to increased precision.’

Answer: We do agree with the reviewer. We emphasized the superior results of the BIS_cr equation at measured GFR<60 and made modifications in the Results, Discussion/Conclusion and Abstract. (pg. 10 1st parag., pg 12 at bottom, pg 13 1st parag, pg 17 last sentence of the Discussion, and Abstract pg 2-Results, and pg 3-Conclusions)

4. ‘On the other hand, it should also be noted that BIS creatinine yields substantial underestimation in the present study in patients with mGFR>=60. Possible reasons for this should be discussed in more detail. Are there residual differences in creatinine standardization (which would be more influential at high mGFR-levels, i.e. low creatinine levels)? Differences in ethnicity?’

Answer: We noted the underestimation of the BIS-cr equation at higher GFR levels; we made some modifications in text and speculate possible reasons for these findings, as suggested by the reviewer (Discussion, pg 13 1st parag.).

5. ‘There is a recent validation study of BIS creatinine in comparison with MDRD and CKD-EPI from France that ought to be cited (J Nephrol 2013;26:716-723). There are also studies from Sweden suggesting that MDRD and, to a lesser extent, CKD-EPI overestimate GFR with decreased accuracy as a result (Scandinavian Journal of Clinical & Laboratory Investigation, 2011; 71: 129–138; Scandinavian Journal of Urology and Nephrology 2012;46:212-222). By contrast, another French study recently reported no such overestimation for MDRD and CKD-EPI among elderly (Am J Kidney Dis. 2012;60(5):847-849). Among cited work, I do not quite agree with the authors regarding the results of Murata et al. (cited on page 11). Among patients older than 70 years with CKD, the overestimation was only 5% for CKD-EPI and 9% for MDRD.’

Answer: The study from France by Koppe et al was cited and the results contrasted with ours in the Discussion. (pg. 13, 1st parag.).

We added the mentioned reference from Sweden suggesting that MDRD and the CKD-Epi overestimate GFR (pg. 12, 2nd parag.).
6. ‘No data on the non-participants (i.e. persons enrolled in the study that did not undergo the renal function study) are presented. In what respects did the participants and non-participants of the renal function study differ?’

Answer: There were no sociodemographic or clinical characteristics that were significantly different between the participants (n=97) and the remaining 103 individuals enrolled in the epidemiologic Geriatric study that did not undergo the renal function studies (added to pg. 5, last parag.).
Reply to reviewer: Pierre Delanaye

1. ‘The major limitation is the absence of calibration for the cystatin C measurement. This is a very important limitation (White CA, CJASN, 2011 or Delanaye P, NDT, 2008) (Grubb A, CCLM, 2010). It could be also important to know when the cystatin C measurements have been performed (Larsson A Clin Chem, 2011).’

Answer: The cystatin C assay was not calibrated against the international certified reference material ERM-DA471/IFCC for cystatin C. However, cystatin C values were reexpressed by the formula -0.105 + 1.13 X cystatin C to account for the standardization of cystatin C with ERM-DA471/IFCC prior to their use in GFR estimating equations, as suggested by Inker and colleagues [10]. Although the calculations had been performed with this adjustment, this information was missing in the initial version; this explanation was added to the new version (Methods pg. 6-7).

They study samples were obtained within one week of the day of the interview, when the data on clinical and sociodemographic characteristics were obtained (pg 5). Serum creatinine was analyzed during the same day of the collection. All plasma cystatin C samples were frozen at -70°C and analyzed within 7 days in May 2012. (Added to Methods, pg. 6).

2. ‘We thus think the conclusions about the cystatin C (and combined)-based equations are questionable. Moreover, the authors should better separate the discussion between creatinine-based equations on one part and cystatin C- or combined equations on the other parts (and once again, the results of this second part are more difficult to interpret because this lack of cystatin C calibration). In this view, it appears that the BIS equation (based on creatinine only) outperforms both the MDRD and the CKD-EPI equations, especially in term of precision. This important result should be more emphasized’

Answer: In this version, the Discussion initially focused on creatinine-based equations, then the cystatin C alone and finally the combined equations. We emphasized the increased precision of the BIS_cr equation compared with the MDRD and the CKD-Epi equations in the Results, pg 10 1st parag. and in
the Discussion: ‘Our results suggest that the BIS_cr equation outperforms the MDRD and the CKD-Epi_cr equations in terms of precision. Notably, we found in the present study that the BIS-cr equation had a superior performance compared with the CKD-Epi_cr and the MDRD at decreased GFR levels, mainly due to increased precision;...’ pg12, last sentence – pg. 13, 1st parag.

3. ‘Page 5, last line: the authors write that creatinine was measured with kinetic Jaffe colorimetric method” and Page 5, line 3 they write “Total CV of the enzymatic method...”. Please verify and correct’
Answer: The creatinine method was corrected (pg. 6).

4. ‘Measurement of iohexol with the capillary electrophoresis method is not the most accurate (Bird NJ, Nephrol Dial Transplant, 2008, 23, p4078).’
Answer: Regarding the iohexol measurement, although the most reliable method to analyze plasma iohexol is with high performance liquid chromatography, previous studies have shown that iohexol measured by capillary electrophoresis had an excellent correlation with measurements by high-performance liquid chromatography [13]. (added to the study limitations, pg 16, 1st parag.).

5. ‘Please avoid terms like “tendency towards a statistically significant...”. Results are significant or not. Do not “overinterpret”.’
Answer: In the Results section, we corrected or deleted the terms ‘tendency towards’, ‘albeit not statistically significant’, ‘trend to improve classification’ used on pg. 10 and 11, and in the Conclusion on pg 16, and tried to make clearer the comparisons that were statistically significant or not.

6. ‘Page 11, first paragraph about high prevalence of CKD (defined as eGFR<60 mL/min/1.73 m²). The major issue is not the high prevalence of CKD with the different equations. The major issue could be the definition of CKD (<60 mL/min/1.73 m²) itself in the elderly (Glassock RJ, NDT, 2008).’
Answer: We have added a sentence to the 1st parag. of the Discussion better explaining the controversy around the cutoff value used for the definition of CKD (pg. 11).
7. ‘The following references should be cited and briefly discussed: Flamant M, AJKD, 2013, Koppe L, J Nephrol 2013.’

Answer: The references mentioned, Flamant et al., and Koppe et al were cited and briefly discussed (pg. 13).

8. ‘Table 1. Regarding the MDRD equation: if an IDMS creatinine has been used, the correct equation is the one with the coefficient “175” not “186”. This is crucial.’

Answer: The correct coefficient of the MDRD equation is ‘175’ as you indicated and was indeed used in the calculations; the coefficient ‘186’ was inadvertently placed in Table 1. The correction was made.

9. ‘We recommend that eGFR should be reported and rounded to the nearest whole number.’

Answer: eGFR was reported with one decimal place as in many previous articles in the field. We would appreciate it if we could maintain as it is.

10. ‘Are the results still the same if the 7 black patients are excluded from the analysis?’

Answer: The results of the performance of the equations did not change significantly with the exclusion of the 7 black participants from the analysis.

11. ‘Compared to the CKD-EPI cohort and even to the BIS cohort, these Brazilian subjects have lower BSA. What could be the consequences for the performance of the equations (as mGFR is indexed to BSA)?’

Answer: The correction for BSA increases the GFR in subjects with lower BSA values as in our group. We understand that as the estimating equations came from populations with greater BSA this could influence the estimations in samples with lower BSA such as ours. We added to the Discussions that differences in BSA between our group and the CKD-Epi and the BIS cohorts could also play a role in the comparisons (pg. 15).