Author’s response to reviews

Title: Reference intervals for serum cystatin C and serum creatinine in an adult sub-Saharan African population

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Version: 1 Date: 22 Jan 2019

Author’s response to reviews:

TO THE EDITOR

Reference intervals for serum cystatin C and serum creatinine in an adult sub-Saharan African population

Dear Editor,
We are grateful to the reviewers for their time and very constructive comments on our manuscript. We have used their suggestions to improve the manuscript and wish to submit a revised version for further consideration. All modifications appear as track changes and we are
providing below a point-by-point response to explain how we have addressed each of the reviewers’ comments/criticisms. We look forward to the outcome of your evaluation.

Sincerely,

On behalf of the co-authors,

Dr. Jobert Richie Nansseu

REVIEWER 1

Luciano da Silva Selistre, Ph.D

Reviewer’s Comment 1

I am overwhelmed by ethnic division. The authors do not comment on the reason for this differentiation. Is there genetic evidence in the study for this division? I would not show the data in this ethnic division, unless there was strong genetic or other evidence for that division.

Authors’ Response 1

We thank you very much for the comment, and are also grateful for the question raised. Although it does not seem to exist any genetic difference between major ethnic groups in Cameroon, we were driven by the curiosity of investigating existence of differences in creatinine and cystatin C reference intervals among these ethnic groups. Indeed, we assumed that as creatinine values vary with anthropometric parameters, its reference intervals could differ between these ethnic groups which present some linguistic and cultural particularities. Moreover, we aimed to investigate factors susceptible of influencing these biomarkers’ reference intervals, so much so that their interpretation would take into account these factors. This statement was added at the end of our introduction section as it reads on page 5: “Besides, we aimed to identify potential factors likely influencing these reference intervals”.

On the other hand and considering your comment, data were no more presented with regard to ethnic groups. Additionally, the title was revised as follows: “Reference intervals for serum cystatin C and serum creatinine in an adult sub-Saharan African population”.

Reviewer’s Comment 2

I particularly associated that only inulin could be used as a measure of GFR (page 11). We know that other measures such as iohexol are accepted as a real measure of GFR.

Authors’ Response 2

We thank you for the comment. Indeed, we cited inulin as the only biomarker considered as gold-standard for the measurement of GFR, other measures being surrogates.

Reviewer’s Comment 3

I substitute the graph (creatinine and cystatin C) for graphs representing the logarithm of creatinine, cystatin C (2 graphs) by age and sex. This would be better representative of the variability of these markers in the sample studied.

Authors’ Response 3

We thank you very much for this suggestion according to which we removed the initial graph presenting the correlation between serum cystatin C and serum creatinine, which we replaced by 4 graphs depicting the variability of each biomarker’s values with respect to sex (see Figures 1.a, 1.b, 2.a and 2.b).

Reviewer’s Comment 4

Additionally, we compared estimated Glomerular Filtration Rate with and without indexing for body surface area (BSA) to investigate whether potential sex and "ethnics" differences are due to differences in body size.

Authors’ Response 4

We kindly appreciate this suggestion. Accordingly, we calculated each participant’s body surface area (BSA) using the Mosteller RD’s calculation procedure [1]. In addition, we performed a quantile regression analysis to investigate potential factors influencing serum cystatin C and serum creatinine reference values. We included in the model the age, sex and BSA and found that “across the various models, the sex remained the only factor likely influencing both serum
cystatin C and serum creatinine values. The age seemed to contribute in explaining serum cystatin C values in the 75th percentile quantile regression model, which was identical for serum creatinine values. The BSA was contributive in explaining serum creatinine values only in the 50th percentile quantile regression model (Table 4).” This statement appears on page 9.

Accordingly, our methods were revised as one can read on page 6: “We used the simplified calculation procedure from Mosteller RD to derive each participant’s body surface area (BSA)” and on page 7: “Furthermore, we used a 25th, 50th and 75th percentile quantile regression analysis to identify any factor likely influencing serum cystatin C or serum creatinine reference intervals in a model including the age, sex, and BSA”.


Reviewer’s Comment 5

Biochemical Assays

Are the creatinine and cystatin C IDMS reference? This information is very important. The authors must describe.

Authors’ Response 5

We thank you very much for pointing out this issue. The method used to measure serum cystatin C had been standardized against the ERM-DA471/IFCC reference material while we used the IDMS reference modified Jaffe kinetic method to measure serum creatinine. Precisions were added in this regard in the revised manuscript as it reads on page 6: “Serum cystatin C was measured by particle-enhanced turbidimetric immunoassay using Tina-quant® Cystatin C reagent kits (Roche Diagnostics, USA). The method applied was standardized against the ERM-DA471/IFCC reference material. Meanwhile, serum creatinine was determined by the Isotope Dilution Mass Spectrometry (IDMS) reference modified Jaffe kinetic method using Creatinine Jaffe Cobas® reagent kits (Roche Diagnostics, USA)”. 
Reviewer’s Comment 6

Statistical methods

The description of the methods is difficult to understand and little justification is offered for the choices made. There are methods used in the article which are not described in the methods and conversely the methods described do not always appear in the results section.

Many of the comparisons between groups are made without formal tests of interactions between group and method but rely on informal tests. This is not really acceptable. Some sort of overall model as a preliminary would seem preferable to treating the dataset as a number of separate substudies. Of course readers are going to be interested in specific groups but moving too quickly to them magnifies the problem of multiple comparisons.

More thought needs to be given to the measures being used here. I would consider whether some composite of bias and precision like mean square error might be better. As it stands we know which method is on average closer to being unbiased but we have recognised at least since Maurice Kendall's poem about Hiawatha and his archery (Amer Stat 1959 13(5) 23-24) that lack of bias is not everything.

Authors’ Response 6

We thank you very much for having brought these points to our attention. Our statistical procedures were revised and ameliorated. First, we used the Kolmogorov Smirnov test to assess how were our continuous variables distributed, and found that none had a Gaussian distribution. Therefore, we applied non-parametric tests for bivariate comparisons (the U-test of Mann-Whitney and the H-test of Kruskal-Wallis) and for correlations (the Spearman correlation test). Furthermore, a quantile regression analysis was performed in line with your suggestion. Corresponding statements were introduced or amended in the revised manuscript and unnecessary information was cancelled, as it reads on page 7: “Data were coded and entered using the Census and Survey Processing System version 7.1. Statistical analysis was performed using the Statistical Package for Social Sciences version 23.0 (IBM SPSS Inc., Chicago, Illinois, USA) and STATA version 12.0 (STATACORP, Texas, USA). Categorical variables are presented using frequency (percentage) while continuous variables are summarized with their median [interquartile range, IQR]. The Kolmogorov Smirnov test was used to assess the normality of continuous variables’ distributions. Reference intervals (RIs) were determined by the nonparametric method as described in the IFCC guidelines [11]. This method was used to determine the 2.5 and 97.5 percentiles and the respective 90% confidence intervals (CI) around these estimates. The Mann–Whitney U-test and the Kruskal-Wallis H-test were used for bivariate analyses, to compare the distributions of continuous variables, considering that these variables did not follow a Gaussian shape. For the same reason, it is the Spearman correlation
test (with its rho (\(\rho\)) coefficient) that was used to investigate existence of any correlation between continuous variables including serum cystatin C, serum creatinine and age. Furthermore, we used a 25th, 50th and 75th percentile quantile regression analysis to identify any factor likely influencing serum cystatin C or serum creatinine reference intervals in a model including the age, sex, and BSA. Statistical significance was set at a p-value lower than 0.05”.

Reviewer’s Comment 7

Given the overlap in CIs, please confirm how this is consistent with the small P-values.

Authors’ Response 7

We thank you for having raised this point. Indeed, the overlaps in CIs have been observed, but it is worth remembering that the non-parametric tests we have used do not compare the medians, but the distributions in a whole, of the continuous variable among the various modalities of the categorical variable, here the sex. Therefore, it can happen that the p-values might be small despite these overlaps as it is the case in the present study.

Reviewer’s Comment 8

Why the authors used Spearmann correlation? The creatinine and cystatin C are measures parametric.

Authors’ Response 8

We thank you very much for the question. After performing the Kolmogorov-Smirnov test, it happened that the distributions of serum creatinine and serum cystatin C values were skewed. In this respect, we had to use a non-parametric estimator, which is the Spearman correlation coefficient in this case. In fact, the Pearson correlation coefficient may exaggerate or dampen the strength of relationship with skewed variables; therefore, it is inappropriate when either or both variables to be included in the correlation test are not normally distributed.

Reviewer’s Comment 9

We suggested to compared median and IQR used regression quantile with covariate adjustments.
Authors’ Response 9

We thank you ever so much for this suggestion, which we took into consideration in our revised manuscript; Indeed, a quantile regression was applied with cystatin C or creatinine being the dependent variable, and age, sex and BSA being the explicative variables introduced in the model.

Reviewer’s Comment 10

Summary

This is an impressive dataset which should be able to answer the question posed but as it stands the article left me in some doubt about what the authors did and why they chose to do what they did.

Authors’ Response 10

Humbly, we appreciate all the comments and suggestions made and have undertaken corresponding improvements in the revised manuscript. We thank you very much as we now submit a clearer draft and hope to have dissipated your doubts and brought more information for a better understanding by the journal’s readership.

REVIEWER 2

Reviewer’s Comment 1

Hans Pottel

This article wants to establish reference intervals for serum creatinine and serum cystatin C in a Cameroonian adult population.

It has been shown, for Caucasians, that creatinine is different between males and females. Therefore, when establishing reference intervals, the authors should present RIs separately for males and females, for both biomarkers. As this is clearly presented in Table 2, I wonder why they did not present these results in the abstract. I would recommend to present RIs separately for males and females already in the abstract!!
Authors’ Response 1

We thank you for this suggestion. The RIs for males and females were introduced in the revised abstract as it reads on page 2: “The RIs for SCysC varied between 0.57 (90%CI 0.50-0.60) and 1.03 mg/L (90%CI 1.00-1.10) for females, and from 0.70 (90%CI 0.60-0.70) to 1.10 mg/L (90%CI 1.10-1.20) for males. Concerning SCr, its RIs ranged from 0.58 (90%CI 0.54-0.61) to 1.08 mg/dL (90%CI 1.02-1.21) for females, and from 0.74 (90%CI (0.70-0.80) to 1.36 mg/dL (90%CI 1.30-1.45) for males”.

Reviewer’s Comment 2

Is the modified Jaffe kinetic method equivalent to the gold standard IDMS method? Is the cystatin C assay calibrated against the international certified standard? Please specify!

Authors’ Response 2

We thank you for pointing out these issues. Accordingly, clarifications were added in the revised manuscript as one can read on page 6: “Serum cystatin C was measured by particle-enhanced turbidimetric immunoassay using Tina-quant® Cystatin C reagent kits (Roche Diagnostics, USA). The method applied was standardized against the ERM-DA471/IFCC reference material. Meanwhile, serum creatinine was determined by the Isotope Dilution Mass Spectrometry (IDMS) reference modified Jaffe kinetic method using Creatinine Jaffe Cobas® reagent kits (Roche Diagnostics, USA)”.

Reviewer’s Comment 3

Table 3 and 4 should be split up into males/females. I know that it will reduce the sample size, but the differences between males and females are too important and do not allow pooling of the data.

Authors’ Response 3

We thank you ever so much for this suggestion. These tables (now Table 2 and Table 3) have been split up and we present now reference intervals for cystatin C and creatinine by age and sex, as it was reported in Pottel et al’s study [1]. Since the sample size in each substratum was not always enough to determine the 90% confidence intervals around the 2.5th and 97.5th percentiles, we chose to represent the 2.5th, 50th and 97.5th percentiles for serum cystatin C and
serum creatinine values. We observed that men had significantly higher serum cystatin C values than women, except in the 50+ age group where serum cystatin C values were higher than in all younger age groups (Table 2). Concerning serum creatinine values, men still had significantly higher titres than women, here across all age groups (Table 3). These statements have been added in the revised manuscript on page 8.

However, it is worth mentioning that according to the other reviewer’s comments, all data referring to ethnic groups were removed from the manuscript, as we did not have evidence of a genetic difference between these ethnic groups.


Reviewer’s Comment 4

The differences between genders are more important than between ethnicities. As the male/female ratio is inversed between Sudanese and Semi-Bantu samples, with much more males in the Sudanese sample compared to the Semi-Bantu sample, this may explain the results of Table 4, where the median creatinine is much higher for the Sudanese sample compared to the other ethnicities. This is because males have higher creatinine values than females. The authors should present medians and RIs for the different ethnicities separately for males and females. Only in case they do not find significant differences, the data can be pooled. E.g. the authors can perform two-way anova for creatinine as continuous variable, using gender and ethnicity as the categories.

Authors’ Response 4

We thank you ever so much for having raised this issue, to which we totally align. As mentioned above, data by ethnic groups were no more presented in the paper, in line with the other reviewer’s comment and considering that there seems not to exist any genetic difference between these major ethnic groups in the country. Our initial ambition was to see whether the interpretation of serum cystatin C or serum creatinine would require taking into account some factors including the patient’s age, sex and/or ethnic origin.

Reviewer’s Comment 5

It has also been seen in Caucasians that creatinine and cystatin C start to rise with age, after the age of 65 years. There can be referred to relevant articles (see below).
Authors’ Response 5

We thank you for having brought these points to our attention. We added some points in the discussion concerning the relation between these biomarkers and age, as it reads on page 12: “On the other hand, subjects aged 50 years and over had 11% higher serum cystatin C levels compared to lower age groups (0.90 vs. 0.80; \( p < 0.001 \)). Concurring with these results, several other studies have demonstrated an increase in cystatin C values above a threshold age varying from 40 to 70 years \([12,14,28–31]\). The higher levels of serum cystatin C in older subjects could be due to the physiological decrease in GFR which starts from 40 years \([32]\).

Serum creatinine levels are also expected to increase around the same age (\( \geq 50 \) years); however, we observed that the distributions of serum creatinine values were similar across the various age groups (\( p=0.491 \)). Likewise, Pottel et al. using a Caucasian population noticed that between 20 and 70 years old, the mean serum creatinine level was stable \([15]\)."

Reviewer’s Comment 6

Page 8 lines1-5: the difference between Sudanese and Bantus or Semi-Bantus can be due to the different male/female ratio. Please check whether the difference is due to this or due to the different ethnicity. Provide RIs for each ethnicity separately for males and females.

Authors’ Response 6

We thank you for having pointed out this issue. Data on ethnic groups were no more presented.

Reviewer’s Comment 7

It would be interesting to compare the results of this Cameroonian population with known reference intervals of Caucasians. The authors should consider referring to two important articles (not mentioned here) concerning reference intervals for serum creatinine, especially the article of Pottel et al, CCA 2008; 396: 48-55 who presents median values and RIs for all ages/sexes and describes that serum creatinine increases with age (see also Ceriotti et al, Clin Chem 2008; 54:559-566). For cystatin C, they may refer to Pottel et al, NDT 2017; 32: 497-507 where he describes the rationale for normalizing cystatin C with the median value of 0.82mg/L. It is also shown that cystatin C increases with age, after the age of 70 years.
We thank you ever so much for these suggestions in the respect of which these statements were added in the revised discussion as one can read on page 10: “By contrast, the reference intervals for serum creatinine obtained in this study (0.61-1.3 mg/dL) seem to differ from that of Caucasians. Indeed, Pottel et al. found reference intervals around 0.48-0.93 mg/dL in women and 0.63-1.16 mg/dL in men within a healthy adult Caucasian population [15]. These intervals concur with those of Ceriotti et al. obtained in a multicenter analysis of three studies based on Caucasian adults. In this study indeed, the reference intervals for serum creatinine varied between 0.45-0.92 mg/dL in women and 0.59-1.05 mg/dL in men [16]. These differences could be explained by the fact that the measurement of serum creatinine used enzymatic methods in the two studies just cited, which could give slightly lower values than colorimetric assays that were used in our study. Additionally, evidence has accumulated that black people have a more important lean tissue mass and a lower GFR compared to Caucasians [3,17].”

Correlation between biomarkers has been reported before, see e.g. Pottel et al 2017; Data in Brief vol 14: 763-772. In this article, cystatin C reference intervals are presented according to age decades. Appropriate scaling of the biomarkers resulted in Pearson correlation coefficients as high as 0.85-0.90.

We thank you very much for figuring our this point, according to which a statement was added in the revised discussion as one can read on page 13: “We found a positive and significant correlation between serum cystatin C and serum creatinine, both in males (ρ= 0.39, p< 0.001), in females (ρ= 0.55, p< 0.001) and in the total population (ρ = 0.58; p < 0.001). These findings mirror those from Pottel et al. who reported a positive correlation between these two biomarkers in a Caucasian population of 8584 subjects (r = 0.87; p<0.0001). Potter et al.’s correlation coefficient was higher than ours, perhaps because they used the Pearson correlation test and rescaled their biomarkers”.

Another point is that we have rescaled biomarkers values according to the rescaling done by Pottel et al [1]. Rescaling factor (Qcrea) used for creatinine was 0.90 mg/dL for men and 0.70 mg/dL for women. Rescaling factor (QcysC) used for cystatin C was 0.82 mg/L in subjects aged less than 70 years and 0.95 mg/L in subject aged 70 and above (only one in our sample). Then, we performed a correlation analysis between SCysC/ QcysC and SCr/ Qcrea and obtained a spearman coefficient correlation of -0.056 (CI [-0.149-0.047], p= 0.279) which is lower than the
one we have obtained with non-rescaled biomarkers. Definitely, we did not present these data in our paper, thinking that this rescaling could be not adapted for our study population or that some factors may not have been taken into account concerning the rescaling methodology in our study.


Reviewer’s Comment 9

Other important references to be considered in the discussion are: Bukabau J. et al Plos One 2018; 13(3): e0193384, where mean Scr-values are presented for a healthy Congolese population.

Authors’ Response 9

We thank you very much for this suggestion. However and humbly, we felt embarrassed to include this citation in the discussion as the study only reports serum cystatin C and serum creatinine mean values with corresponding standard deviations whereas we sought for studies which have identified the two biomarkers reference intervals to be compared to ours.

Reviewer’s Comment 10

Discussion: it is not because the minimum of 120 subjects is recommended by the IFCC that you should pool the data when it is clear that there important differences between males and females. It is more important to present the data separately than to fulfill the IFCC requirements in this case. Only the 90%CIs on the limits will be wider.

Authors’ Response 10

We thank you very much for the remark. Accordingly, data were mostly presented by sex and by age groups in the revised manuscript.