Title: Determination of the best method to estimate glomerular filtration rate from serum creatinine in adult patients with sickle cell disease: a prospective observational cohort study.

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Author's response to reviews: see over
Dear Dr Henderson,

Please find enclosed the revised version of our manuscript and a cover letter with our point-by-point response. The modifications appear in yellow in the revised manuscript. We have added a figure.

We appreciate the close reading of our manuscript by the reviewers and we hope the changes we have made have addressed their concerns.

Best regards,

Dr Marie COURBEBAINSE
Response to the Editor

In addition to the Referees' comments, could you please also address the following editorial points

1. Ethical Approval
Can you please include the full name of the ethical committee that approved your study. Please include a reference number if obtained.

The study has been approved by a local ethic committee: Comité de Protection des Personnes, Ile de France II. This point has been added in the section Methods, Patients (page 7).
The reference number of the study is 2011531-RCEB.

2. Copyedit
We recommend that you copyedit the paper to improve the style of written English.

We tried to improve the language editing with the help of a native English speaker. Please, find below her certificate:

_I, Genevieve Arnaud-Vincent, associate professor of English at Paris Descartes University, hereby certify that I have edited for language the manuscript entitled “Determination of the best method to estimate glomerular filtration rate from serum creatinine in adult patients with sickle cell disease: a prospective observational cohort study” written by Drs Jean-Benoît Arlet, Marie Courbebaisse et al._

Genevieve Arnaud-Vincent,
Professeur agrégé d’anglais at the Paris Descartes University Language Center
E-mail address: genevieve.arnaud-vincent @parisdescartes.fr

3. General Formatting
You now have an opportunity to ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

We have carefully checked that the revised manuscript conformed to the journal style.
Response to reviewer 1

The study has compared Cockcroft-Gault, MDRD and CKD-EPI with the standard measurement of GFR using iohexol plasma clearance in Sickle cell disease. The manuscript is interesting, but need minor revision.

We thank the reviewer for this comment and for his careful review.

1. In the KIDGO recommendation, high GFR is suggest to be more than 105 mL/minute/1.73 m2. Arlet JB and colleague define glomerular hyperfiltration as mGFR higher than 110 mL/min/1.73 m2, as did Haymann and coll mentioned in the paper. What is the "coll"? A author?

« Coll » has been replaced by « et al. » throughout the revised manuscript.

2. Serum and urine creatinine were measured using an alkaline picrate assay with calibration traceable to IDMS. How to standardise it traceable to IDMS?

We have specified this point in the section Methods, Biological measurements (page 8) as follows:

“Serum and urine creatinine were measured by using an alkaline picrate rate-blanked compensated kinetic assay (Hitachi 917 analyzer; Roche Diagnostics) with standardization to isotope dilution mass spectrometry...”

3. In addition to the mean difference, ±1.96 SD are needed to represented by the dashed line in the Bland and Altman analysis.

This modification has been performed (see figure 1) and was added in Figures legends (page 25).
Response to reviewer 2

In current report Arlet et al assess performance of 5 GFR estimation equations in subjects with sickle cell disease (SCD). The results are interesting and carry potential of clinical implications. I have the following comments and suggestion.

We thank the reviewer for this comment and for his careful review.

1) Given the low number of patients mean values would be especially sensitive to outliers and Gaussian distributional assumption may not be met. I recommend to present in a sensitivity analysis Bias and precession assessed by median differences and the corresponding interquartile range in addition to the provided mean differences and 95% CIs. With current results of mean differences, I’m not totally convinced whether the made conclusions are indeed supported by the data.

As requested by the Reviewer we have added median and interquartile range for the differences between the various methods of estimation and the measured glomerular filtration rates (GFR) in the Table 3 (page 28). Median and mean were quite close. The graphical method by Bland and Altman (Figure 1) that uses as recommended mean difference and 95% CIs (Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986, 8:307-10) adds valuable information for the assessment of the agreement between estimated and measured GFR throughout the range of GFR.

2) In addition to the Bias and precision, quantification of the accuracy may be valuable (for an example, see Levey et al. Ann Intern Med. 2009; 150(9): 604–612).

Percent of estimates within 30% (± 30%) of the measured GFR (mGFR) are represented below:

<table>
<thead>
<tr>
<th></th>
<th>Cockcroft</th>
<th>CKD-EPI</th>
<th>CKD-EPI without ethnicity</th>
<th>MDRD-v4 with ethnicity</th>
<th>MDRD Without ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>n within 30% of the mGFR</td>
<td>25</td>
<td>32</td>
<td>50</td>
<td>18</td>
<td>46</td>
</tr>
<tr>
<td>% of patients within 30% of mGFR</td>
<td>39,1%</td>
<td>50,0%</td>
<td>78,1%</td>
<td>28,1%</td>
<td>71,9%</td>
</tr>
<tr>
<td>CI 95% low</td>
<td>27,1%</td>
<td>37,8%</td>
<td>68,0%</td>
<td>17,1%</td>
<td>60,9%</td>
</tr>
<tr>
<td>CI 95% high</td>
<td>51,0%</td>
<td>62,3%</td>
<td>88,3%</td>
<td>39,1%</td>
<td>82,9%</td>
</tr>
</tbody>
</table>

CI: confidence interval
We agree with the reviewer: there is actually no consensus to define glomerular hyperfiltration and the cut-off chosen in the present study may be too low. We have consequently modified the discussion as follows:

Page 16: “... As previously explained, we chose to define glomerular hyperfiltration as mGFR higher than 110 mL/min/1.73 m², as did Haymann et al [5]. Using this controversial definition, glomerular hyperfiltration seems to be a very frequent finding in young adult SCD patients...”

Page 17: “...Another limitation of our study is the definition of hyperfiltration. We chose to consider that a measured GFR higher than 110 mL/min/1.73 m² defines hyperfiltration for two reasons: first, this definition is the one given by Haymann et al. in their recent work about GFR in SCD patients [5] and we wished to compare our results to theirs; second, although this arbitrary level may be considered as too low, we observed that in our population, urinary albumin excretion was the lowest with mGFR between 96 and 112 mL/min/1.73 m², whereas it significantly increased with mGFR higher than 112 mL/min/1.73 m². Consequently a mGFR higher than 110 mL/min/1.73 m² may be reasonably considered as pathological in this population as it is more frequently associated with the presence of micro- or macroalbuminuria...”

4) Providing the distribution (eg., kernel density) of the eGFR that shows overlap of the 5 equations and measured GFR for visual assessment will be useful. Showing the mean difference with 95% CIs (table3) may be misleading in case of outliers.

We thank the Reviewer for this thoughtful comment. We have added in the revised manuscript a figure that displays the distribution (kernel density) and a brief description of the related statistical method.

Figure 2 representing “Distributions of measured GFR and estimated GFRs using a kernel density distribution” has been added (results page 11 and figures legends page 25).

The section statistical methods (page 9) has been modified as follows: “... Distributions were estimated using a kernel density distribution. Bandwith selection was done using the Sheather-Jones method [15]...”

5) The table 3 legend denotes that the tests are based on paired t-test, this require mentioning in the statistical methods section.

The t-test is now mentioned in the **statistical methods** *(page 10).*

6) There are several section of the paper that require language editing and correction of typographical errors:

   a. Methods section on patients, in the sentence “…patients with diabetes mellitus, hypertension or other diseases susceptible to…”, please list what “other disease” consisted of. I understand that the overall range could be long, however, the authors can provide the list of observed conditions that lead to exclusion in current study.

Three patients were excluded from the present study, including one with diabetes mellitus and two with hypertension *(section RESULTS, Description of the population and of SCD associated nephropathy, page 11)*

   b. The acronym BSA needs introduction at its first use, page 6 (glomerular filtration rate measurement section).

The acronym BSA (body surface area) has been specified **page 7.**

   c. Methods section on biological measurements: the first sentence “…..on single urinary plot expressed as mg/mmol…” needs revision. The authors probably mean measured in spot urine?

The word “plot” has been replaced by “spot” *(METHODS, section Biological measurements page 8).*

   d. Similarly the following sentence in the same section, “AER was defined as normoalbuminuria. …” should be changed to something like “AER was categorized as normoalbuminuria….”. It is the normo/micro/macro-albuminuria that are defined by AER, not the other-way around.

The correction has been performed as follows *(page 8): “…AER was categorized as normoalbuminuria (AER < 3 mg/mmol), microalbuminuria (AER from 3 to 30 mg/mmol), or macroalbuminuria (AER > 30 mg/mmol)...”*
e. The last sentence of the same paragraph ending with “…traceable to IDMS” needs revision to something like: “Serum and urine creatinine were measured by using an alkaline picrate rate-blanked compensated kinetic assay (Hitachi 917 analyzer; Roche Diagnostics) with standardization to isotope dilution mass spectrometry.

The correction has been performed as follows (page 8): “…Serum and urine creatinine were measured by using an alkaline picrate rate-blanked compensated kinetic assay (Hitachi 917 analyzer; Roche Diagnostics) with standardization to isotope dilution mass spectrometry.”

f. In the CKD-EPI equation formula on page 7 and 8, the alpha should be raised to the power.

The alpha has been raised to the power in the CKD-EPI equation formula (see pages 8 and 9).

g. The final sentence of the “Glomerular filtration rate measurements section” on page 6&7 “Furthermore, we observed that a mGFR….. at least in our population” should be moved to results section or deleted as that is already mentioned in the results.

This sentence has been deleted.

h. Page 10 results section, 2nd paragraph, last sentence “mGFR comprised” needs rephrasing. Saying the 2nd quartile instead of the range for GFR may make the sentence more easier to understand.

The correction has been performed as follows (page 11): “…As shown in Table 2, when measured GFR is divided into quartiles, the median urinary albumin/creatinine ratio was the lowest for the second quartile of mGFR and significantly increased for mGFR above 112 mL/min/1.73m^2 (p=0.029)….”

i. Results section page 11, there is no figure 2d and 2e. Probably 1d and 1e is meant by that?

We agree with the Reviewer. This typographical error has been corrected (page 12).

j. The correlation coefficient of r=0.43 in the last sentence on page 11 should be negative? Otherwise the sentence does not make sense to me.

The Reviewer is right and the typographical error has been corrected. r= 0.43 has been replaced by r = -0.43 (page 12).

k. Please provide the correlation coefficient for all 5 equations rather than selecting some. It seems that also for the CKD-EPI with race adjustment the bias decreases with higher mean differences, given the figure?

The correlation coefficients have been added for the CKD-EPI equation and for the MDRD equation without ethnicity as follows:

- “…The difference between estimated GFR calculated with the CKD-EPI equation and mGFR decreases with increasing GFR values (r = -0.23, p = 0.06)…” (RESULTS,
section Determination of the best equation to estimate GFR from plasma creatinine in adult patients with SCD, page 12)

- “…whereas this difference increased for the MDRD equation without adjustment for ethnic group ($r = 0.538$, $p<0.001$)…” (RESULTS, section Determination of the best equation to estimate GFR from plasma creatinine in adult patients with SCD, pages 12 and 13)

I. Discussion section, page 12, paragraph 2, “…GFR <60 ml/min/1.73m² and substantially more accurate in the subgroup with estimated GFR >60 ml/min/1.73m² [12-16,17].” Not all references address this issue. Please list only the references addressing the mentioned point.

This point has been modified as follows (page 14): “…[12,17,18]…”

m. In the discussion section on page, the word “indexation for BSA” is used in several sentences, I suggest rephrasing that to something like “expressed per BSA”.

This modification has been performed throughout the manuscript.

n. The discussion is lengthy and have some repetitions. Please shorten the overall discussion and add a paragraph on limitation of the study. There is no study without limitations!

As requested by the Reviewer the Discussion section has been substantially shortened and a paragraph concerning limitations of the study has been added as follows (pages 16 and 17):

“One of the limitations of our study could lie in the lack of homogeneity of our population since we chose to pool the SCD patients with SS and non-SS genotypes. However, although the patients with hemoglobin SS had a more severe disease than those with other sickling hemoglobinopathies, the measurement properties of the five equations tested were similar in SCD patients with or without the SS genotype. Ideally, the validity of the CKD-EPI equation without the adjustment for African-American ethnicity should have been assessed in a control group comprising individuals of the same ethnic origin but with an AA genotype test to allow us to claim that hyperfiltration was specific to SCD but this last point was not the main goal of our study. Moreover, Thompson et al. already have already shown that SCD patients have higher GFR as well as higher urinary albumin to creatinine ratio than controls [29].

Another limitation of our study is the definition of hyperfiltration. We chose to consider that a measured GFR higher than 110 mL/min/1.73 m² defines hyperfiltration for two reasons: first, this definition is the one given by Haymann et al. in their recent work about GFR in SCD patients [5] and we wished to compare our results to theirs; secondly, although this arbitrary level may be considered as too low, we observed that in our population, urinary albumin excretion was the lowest when mGFR was between 96 and 112 mL/min/1.73 m², whereas urinary albumin excretion significantly increased when mGFR was higher than112 mL/min/1.73 m². Consequently a mGFR higher than 110 mL/min/1.73 m² may be considered as pathological in this population as it is more frequently associated with the presence of micro or macroalbuminuria.”
7) Finally, the paper will greatly benefit from language editing by a native English speaker.

We appreciated the close reading of the manuscript by the Reviewer and we tried to improve the language editing with the help of a native English speaker. Please, find below her certificate:

I, Genevieve Arnaud-Vincent, associate professor of English at Paris Descartes University, hereby certify that I have edited for language the manuscript entitled “Determination of the best method to estimate glomerular filtration rate from serum creatinine in adult patients with sickle cell disease: a prospective observational cohort study.” written by Drs Jean-Benoît Arlet, Marie Courbebaisse, et al.

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