Author’s response to reviews

Title: How to estimate glomerular filtration rate in sub-Saharan Africa: design and methods of the African Research into Kidney Diseases (ARK) study

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Author’s response to reviews:

I read with interest the article submitted by Kalyesubula and colleagues. The authors described the protocol of a future study. The goal is to study the accuracy of current equations to estimate...
GFR, the topic is certainly important and of interest, especially in this part of the world. I have the following remarks.

1) The main article on the topic is neither cited nor discussed by the authors: Justine B. Bukabau, Kidney Int, 2019, 95(4), p896.

We have now included this paper and discussed its merits, particularly the multi-country nature and the use of a new equation - the Full Age Spectrum (FAS) equation which apparently performs better than CKD-EPI for GFR &lt; 60 mL/min/1.73m². Please see introduction for these details.

2) In the mentioned article, 494 subjects (or patients) have been included. Even if this sample is lower than the predicted sample of the authors, I think that the following sentence should be tempered: "Previous studies using iothalamate to measure GFR in sub-Saharan Africa have been relatively small and have included few people with impaired renal function".

We have adjusted the statement to highlight that the studies have included few people with CKD.

3) I strongly recommend that the authors studied the performance of the Full Age Spectrum equations (Pottel H, Nephrol Dial Transplant, 2017, 32(3), p497+ see Bukabau JB, Kidney Int) and the Lund-Malmö/CAPA equations (Björk J, Pediatr Nephrol, 2019, 34, p1087)

Thank you for the recommendation which we have included.

4) The last timing is 240 minutes. I think a later timing would be of interest for patients with low GFR.

Thank you for this suggestion which we have now included this as a possible limitation to our study protocol. We chose the timing to minimise participant burden which is already substantial for participants who are predominantly low-income farmers.

5) Regarding capillary sample, I suggest the authors to read the following reference: Luis-Lima S, Nephrol Dial Transplant, 2018, 33(9), p1597

Thank you for this suggestion. We plan to adopt the proposed protocol for validation of the DBS method. See section of methods under laboratory investigations on page 10.

6) The authors will measure serum creatinine with a Jaffe method. Even if the assay is claimed to be IDMS traceable, I recommend to consider an enzymatic method.
To make this study feasible, creatinine is measured in each country where all three laboratories use the Jaffe method traceable to IDMS. There is no major difference in the accuracy of using an enzymatic method and the Jaffe method and we reviewed this topic in our recently published systematic review (June Fabian et al 2019, Clinical Kidney Journal https://doi.org/10.1093/ckj/sfz089). We will adopt the recommended estimation and reporting format proposed in this paper and have updated the section of laboratory investigations to reflect this.

7) As for iohexol, I recommend creatinine and cystatin C to be measured in the central laboratory.

Please see above for our response regarding creatinine. For Cystatin C, samples will be collected and analysed at the end of the study from one central laboratory for Uganda and Malawi while the samples in South Africa will be analysed from the central laboratory in South Africa. We will cross validate samples between Uganda and South Africa. This detail has been added to the paper in the methods section under the subheading of laboratory investigations.

8) How will cystatin C be measured? Which assay? Standardization?

Cystatin C will be measured using the standardized Roche Gen2 assay at the end of the study. Please see point 7 for further details.

9) Description of statistics should be improved.

We have added more details to the statistics section detailing how the planned analysis will be performed. In particular, we have added a section on the determination of the measured GFR from Iohexol and added comparisons for the combined equations for cystatin c with and without creatinine. We have added the details of how bias and precision will be determined with the adoption of the methods used by Bukabua et al 2018 in Congo. However, our study is methodological and somewhat data exploratory, and harder therefore to specify exactly all analyses apriori as one might do in a statistical analysis plan for a trial.

We have also included a component on how the qualitative data will be analysed.

We have also included another author, Christian Hansen (CH) with PhD bio-statistics who was part of the initial study design team and has reviewed and updated the statistical aspects of the manuscript.

10) Iohexol will be measured in a central laboratory. Which method? An important question is to know if this laboratory is participating to the iohexol external control (by Equalis, Sweden).
All the Iohexol samples will be shipped for measurement to the National Health Laboratory, Johannesburg, South Africa to be measured by liquid chromatography-tandem mass spectrometry. The laboratory is accredited for the ISO standard and also has participated in the interlaboratory external quality controls with Equalis since 2017. This has been added to the methods section under laboratory measurements.

11) The way of calculating GFR from iohexol results should be better explained.

Many thanks: we have now clarified this and added more detail

We say “

in the analysis section of the revised paper. To determine the measured GFR (iohexo-GFR); we will calculate the slope from the 3 samples at 120, 180 and 240 minutes using the exact time of collection turn these into a GFR normalised to BSA using standard methods (Peters AM 1992; Bakabua et al 2018). We will also put in the R value of the fit, which will be used to exclude ones where the fit is particularly poor.

Responses to Reviewer 2

There are some issues which have to be addressed in this paper. I have the following comments, questions and suggestions:

1. The study aims to compare different eGFR formulas with iohexol excretion for GFR measurement. The study design is interesting. Investigators will include 3000 adult participants from three different African countries. Authors had given primary and secondary objectives, which are adequately addressed. The study will have a cross-sectional design with inclusion participants from Malawi, Uganda and South Africa. They have a working committee for a review of the protocols, data collection and plan of analysis, and they will have many meetings to update the study. I think that this is good for such type of research.

Thank you!

2. Please provide the process of selection of participants in each country.

We have now included greater details regarding participant selection in each country. Please see the methods section under ‘participant selection’.

3. It will be interesting to know the reason for bioimpedance measurements in this study. Authors should explain the connection between eGFR measurements and bioimpedance analysis.
We have added further explanation of the rationale for inclusion of this to the discussion section. We now state: Bioimpedance measurement was included in this study for measurement of lean mass and total body water and we hope to use it to optimize GFR estimation on an individual patient basis, for example in a patient with significant oedema. While this has not proven useful in high-income settings, such technologies may be useful in low-resource settings.

4. Please provide the time-frame for four urine analysis.

We have revised the section on urine sample collection in ‘Laboratory investigations’ to reflect the time for sample collection and the time frame for analysis.

The statement now reads ‘We will perform four urine tests. An early morning spot urine sample will be taken for quantitative urine albumin: creatinine ratio, an early morning urine dipstick analysis to qualitatively assess haematuria, leucocyturia and proteinuria and microscopy will be performed on the centrifuged urine to screen for urinary schistosomiasis within 1-2 hours of collection. A 24-hour urine collection for urinary albumin excretion, sodium and 24-hour creatinine clearance will be done in a sample of 300 participants from Uganda during the second phase of the study.’

5. Which statistical program will be used? Analysis of variance test between groups should be used.

All statistical analyses will be performed using STATA 15 SE (Stata Corp, Texas, USA). We will use Dedoose, qualitative data analysis software for the qualitative research. We have added more detail to the Statistical Analysis section.

6. I suggest that a native English speaker read the whole manuscript.

Our team has edited the full manuscript in detail and we hope this is now satisfactory.