Author's response to reviews

**Title:** Creatinine-or cystatin C-based equations to estimate glomerular filtration in the general population: impact on the epidemiology of chronic kidney disease

**Authors:**

- Pierre Delanaye (pierre_delanaye@yahoo.fr)
- Etienne Cavalier (etienne.cavalier@chu.ulg.ac.be)
- Olivier Moranne (moranne.o@chu-nice.fr)
- Laurence Lutteri (laurence.lutteri@chu.ulg.ac.be)
- Jean-Marie Krzesinski (jm.krzesinski@chu.ulg.ac.be)
- Olivier Bruyère (olivier.bruyere@ulg.ac.be)

**Version:** 2  **Date:** 8 February 2013

**Author's response to reviews:** see over
Dear Editor,

We would like to thank you for giving us the opportunity to submit a revised version of our manuscript. The remarks raised by the reviewers were very helpful and we think the manuscript is now largely improved. You will find hereafter our point-by-point responses to the Reviewers.

Reviewer: Jan AJG van den Brand

The report details a comparison of four glomerular filtration rate (GFR) estimating equations used to assess the prevalence of eGFR lower than 60 ml/min (CKD stage III or higher) in the general population. The authors show that agreement between the creatinine and cystatin C based equations is limited. On average, the CKD-EPI equations result in higher eGFR than the MDRD equation, and thus in lower estimated CKDIII+ prevalence. The CKDIII+ prevalence increases with age, but the within age category differences in prevalence by eGFR remain the same. The authors conclude that the introduction of cystatin C based equations would reduce the estimated prevalence of CKDIII+ by half.

Overall, the report is well written and presented clearly.

We thank Dr van den Brand for this comment.

However, there are a few point I would like to see addressed prior to publication.

Major Revision:
I would like to see some more details on the participant inclusion. How many persons were invited? How were community visits announced? A what times of day did the measurements take place? Could there be an inverse health worker effect if measurement took place during daytime, healthier persons are more likely to be preoccupied at least among those aged under 65.

We fully agree with the reviewer that it is a very important point. We have provided more information about the participant inclusion. “Between June 2008 and March 2010, 112,000 subjects without any other restriction that being aged between 40 and 75 years were invited through a personal letter to participate in a CKD screening. All individuals wanting to voluntary participate had to register and make an appointment by giving a phone call to the Department of Health of the Province of Liège. The time slot to visit the screening bus was from 9am to 7.45pm so that active working people could participate.”. Consequently, we do not believe that our screening strategy could have caused an inverse health worker effect.

Minor Revisions:
The term CKD is somewhat misleading, as the authors use it interchangibily to refer to chronic kidney disease as the entire disease spectrum and the subset of patients with
CKD stage 3 or worse. I would like to advise to authors to use the term CKD stage 3 when referring to their estimates of prevalence. We have modified the text as required by the reviewer.

Table 6 was difficult to read. Furthermore, I could not view it completely. Please change the page format to landscape so that the table fits. Additionally, the authors may want to use the actual names of the estimating equations rather than binary designation in the table header. We have modified the table as required by the reviewer.

The authors used the kappa statistic to assess concordance of the estimated CKDIII+ prevalence by eGFR equations. However, this statistic has its limitations. First, it depends on the number of categories used. The higher the number, the lower the kappa and vice versa. Secondly, the kappa depends on the prevalence of the outcome. The higher the prevalence, the more likely concordance is due to chance alone. As a result the the kappa statistic by itself, without the classification tables on which it was based is hard to interpret correctly. Perhaps the original classification tables could be added to the (supplementary) results.

We agree with the reviewers. Kappa statistics are not perfect but this type of statistics remains helpful and, especially, relatively easy to understand for non-statisticians. Moreover in our analysis, we only used two categories (eGFR<60 mL/min/1.73 m² or not). This has been added in the methodology: “Agreement or reliability between GFR estimations was assessed by Cohen’s kappa statistics to discriminate GFR greater and less than 60 mL/min/1.73 m² (only two possible categories)”. Moreover, conclusions drawn from the kappa statistics are the same as those drawn from the Bland and Altman analysis (and from the ICC, even if the last ones are now deleted). We have also added a table with different results of CKD staging according to the equations used.

The intra class correlation coefficient (ICC) depends strongly on the range of the tested variables. The broader the range, the higher the correlation. Essentially, the Bland-Altman plots and calculated bias and limits of agreement address the agreement between two continuous variables more appropriately. Thus the ICCs can be omitted in my opinion. As required by the reviewer, we have omitted the data with ICC.

Perhaps the authors could calculate and plot 95% confidence intervals around the point estimates in figure 2 to give the reader an indication of the precision of the estimated CKDIII+ prevalence by age. This is an important point raised by the reviewer. We have modified the figure accordingly.

Discussion, page 13: use cannot rather than can't We have modified the text as required.

Discretionary Revisions
Out of curiosity, did the standard deviation of the bias (Blant-Altman analysis) depend upon the level of eGFR? It would be usefull from a clinical point of view to know if a estimated eGFR is always within x% of the 'true' GFR regardless of eGFR level. We thank the reviewer for this interesting remark. We looked for a correlation between differences in eGFR and mean of eGFR. Such a correlation was found between all equations (except between MDRD and CKD-EPI Cys). As clearly shown by figure 1, interpretation...
must be different when the MDRD study equation is considered. We have modified the paragraph of the discussion as followed:

“Bias and precision results must be interpreted with caution in our study. Indeed, the systematic difference between the MDRD and the CKD-EPI equations seems low (3 mL/min/1.73 m²). It can be suggested from Figure 1 that this bias is however very dependent on the GFR level. The systematic difference between the MDRD and the CKD-EPI equation increases with increasing GFR values especially at high values. Comparing the three CKD-EPI equations, we also found a positive correlation between the difference in equations and the mean of the two equations, which means that the differences between equations slightly increases with eGFR levels. This correlation is however low ($r^2$ between 0.02 and 0.04, $p<0.001$) and linear although a clear non linear relationship with a knot join-point around 80 mL/min/1.73 m² does exist when MDRD is compared to CKD-EPI and CKD-EPI Mix. This fact also means that discrepancies between the MDRD and the CKD-EPI are systematic and almost in the same direction, i.e. MDRD giving a positive screening result and CKD-EPI giving a negative one. Such a systematic deduction cannot be done when discrepancies are found between the CKD-EPI Cys and the CKD-EPI mix equations. “

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, and I have assessed the statistics in my report.

Reviewer: Martin Flamant

In this study, Delanay et al. compare 4 GFR-estimating equations based on creatinine and/or cystatin C plasma concentrations. This issue had aroused considerable interest since recent publications showed that GFR estimates based on the combination of creatinine and cystatin C displayed the best performances of all estimates, at least in total population. A very large number of volunteers have been included in this study, which clearly demonstrates that GFR estimation differs significantly depending on the equation used, hence leading to different epidemiological analysis. This work thus answers the question raised in the title in a convincing manner, with however no possibility to assess which equation gives the best performances in the absence of a reference measurement of GFR.

We thank Dr Martin Flamant for these comments.

A few methodological questions appear upon reading this manuscript: exclusion of patients older that 50 yo is surprising as it does not correspond the any particular pre-defined threshold.

We suppose the Reviewer means “exclusion of patients younger than 50y”. This is simply explained by the fact that screening was only proposed to patients older than 40y but the sample between 40 and 50y was little. We agree that this choice is questionable. We added in the methodology “We arbitrary limited our analysis to subjects older than 50 years”

The lack of information regarding ethnicity is also a matter of concern. It would have made more sense to either collect the information or exclude patients of African origin. We agree but the reviewer must admit that we have clearly stated this limitation in the discussion: “
As the ethnicity factor for each equation is different, this could be source of bias. But in the Province of Liège, Caucasians are by far the dominant ethnic group. Therefore, it is doubtful that the differences observed in our study are due to ethnic factors.” Searching a posteriori the ethnicity of the population won’t be accepted from an ethical point of view in Belgium.

The method to measure plasma creatinine concentration is also an issue; as the different equations have been established with different measurement methods, relative performances might have differed with an enzymatic dosage of creatinine. As the main message of this work is to highlight the epidemiological consequences of the method used to estimate GFR, it would have been of great interest to simultaneously measure plasma creatinine concentration with an enzymatic method. Indeed, the method used to measure creatinine is probably as important as the equation itself in terms of epidemiological consequences.

We agree with the Reviewer. The following sentence has been added in the limitations: “In the same vein, our Jaffe assay has lesser precision than enzymatic assays, although IDMS traceable.”

We could however add that the CKD-EPI and MDRD equations have been built from serum creatinine measured with Jaffe methods. In the same vein, the NHANES study used different Jaffe assays. At least and once again, we used an IDMS traceable assay.

Another matter of concern is related to part of the discussion. This study provides information on the differential modelling of age, sex, and plasma creatinine/cystatin C concentrations in the equations rather than on their respective performances. The authors outline this limitation several times in the manuscript, but try to overcome this intrinsic methodological issue by interpreting their results in the light of a thorough discussion of the literature. Evaluating GFR estimation equations without a gold standard measurement, by comparing them with each other is questionable: in the absence of a reference value, one cannot draw conclusion about the superiority of one method compared to another.

However, this review of the literature, which is very extensive and interesting, is particularly difficult in this field because of the heterogeneity of the studies, and the evolution of the performances of the equations, mainly MDRD, since creatinine is now frequently measured enzymatically. Because of this limitation, the discussion concerning the comparison of the equations should maybe emphasize more the potential epidemiological differences of the equations rather than their performances.

We agree with the Reviewer. Without GFR measurement, we can only illustrate discrepancies between the equations. We feel (and the Reviewer agreed) we have underlined this limitation several times throughout the text. I remind the last sentence: “Moving from strictly creatinine-based equations (MDRD or CKD-EPI) to cystatin C-based or combined equations will decrease prevalence of stage 3 CKD by half, which is highly significant from an epidemiological point of view. Additional studies are thus necessary before asserting we know the true prevalence of CKD in the general population.” Please also consider our reply to the next comment.

One particularly interesting result that could be further developed is the difference in prevalence of diabetes mellitus is subjects whose CKD is defined by equations based on cystatin C or equations based on creatinine. What is the opinion of the authors concerning this finding? It would be interesting to analyse separately the subgroup of diabetic patients. Indeed, if the difference of the GFR estimates between cystatin-derived equations and creatinine-derived equations is not the same in diabetic patients versus
non diabetics subjects, this may question the specificity of the cystatin C dosage in this subpopulation.

We thank the Reviewer for this interesting comment. As required by the Reviewer, we have analyzed the results in diabetic patients (n=498). In these patients, the prevalence of stage 3 CKD with the MDRD, CKD-EPI, CKD-EPI Cys and CKD-EPI mix were 17.1%, 14.5%, 10.2% and 10.4%, respectively. As expected, prevalence was higher than in non-diabetic patients. Interestingly, the difference between the prevalence of creatinine-based versus the cystatin C-based equations appears less important than in the general population. In the absence of measured GFR, we can only describe such differences. Moreover, we must be careful in our interpretation of this result, as diabetic patients have also different clinical characteristics than non-diabetics, like a higher mean BMI.

The following sentences have been added in the results: ”We also calculated the prevalence of stage 3 CKD in diabetic patients. In these patients, the prevalence with the MDRD, CKD-EPI, CKD-EPI Cys and CKD-EPI mix were 17.1%, 14.5%, 10.2% and 10.4%, respectively.”

The following sentences have been added in the discussion: ”Diabetes is a well-known risk factor associated with CKD. As expected, CKD prevalence was higher in diabetic patients than in non-diabetic patients. Interestingly, the difference between the prevalence with creatinine-based versus the cystatin C-based equations appears less important than in the general population. Once again, in the absence of measured GFR, we can only describe such differences. Moreover, we must be careful in our interpretation of this result, as diabetic patients have also different clinical characteristics than non-diabetics, like a higher mean BMI.”

One indirect way of addressing the issue of the respective performances of the equations would have been to have data regarding metabolic complications of CKD, and how they correlate with the estimated level of GFR.

Unfortunately, we don’t have to data to reply to this interesting comment. This study is cross sectional.

In conclusion, this large-scale study describes very well the relative differences of creatinine and/or cystatin C-derived GFR estimating equations, and their potential epidemiological consequences. An attempt to go further and compare the respective performances of the equations is made through an extensive discussion, even though the study itself, because of its design, is unable to bring new results concerning the superiority of one equation over the others.

We fully agree with the Reviewer.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

As required by the Editor, the manuscript has been revised by a native English speaker and the name of the institutional ethic committee has been added.