Author's response to reviews

Title: Estimated glomerular filtration rate as an independent predictor of atherosclerotic vascular disease in older women

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Version: 2 Date: 9 May 2012

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

Please find enclosed the revised manuscript entitled “Estimated glomerular filtration rate is an independent predictor of atherosclerotic vascular disease in older women”, by Lewis et al., for consideration of publication in BMC Nephrology as a research article.

This paper is not under consideration elsewhere and none of the papers contents have been previously published. All authors have read and given final approval for the manuscript to be submitted to the journal. The study was supported by Kidney Health Australia grant S07 10, Healthway Health Promotion Foundation of Western Australia and by project grants 254627, 303169 and 572604 from the National Health and Medical Research Council of Australia. None of the funding agencies had any role in the conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript. The authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The authors have no disclosures to declare.

Sincerely Yours,


Corresponding author: Dr Joshua Lewis
We would like to thank the reviewers for their time and effort in reviewing the manuscript.

Reviewer's report 1
Title: Estimated glomerular filtration rate as an independent predictor of atherosclerotic vascular hospitalization in older women

Version: 1  Date: 10 February 2012

Reviewer: Kevan Polkinghorne

Reviewer's report:
Many thanks for asking me to review this paper by Dr Lewis and colleagues entitled ”Estimated glomerular filtration rate as an independent predictor of atherosclerotic vascular hospitalization in older women”. The paper is interesting, well written and assesses an important issue for the GFR estimating equations where there has been controversy on the utility as a prognostic marker in the elderly age group.

I have some major comments that I think need addressing.

Major Compulsory Revisions
1. A central issue to the study is the standardisation or calibration of the serum creatinine measurements to produce “reliable” eGFR estimates. The authors state that “the serum creatinine analysed using an isotope dilution mass spectrometry (IDMS) traceable Jaffe kinetic assay”. I have to question this statement. The recommendations and technique for creatinine standardisation to a IDMS reference material were published in 2005-2006 (eg Myers GL, Miller WG, Coresh J, et al. Recommendations for improving serum creatinine measurement: A report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem 2006;52:5-18), well after the baseline creatinine measurements in 1998. While I don’t doubt that the current Roche assay is IDMS traceable the assay at the time would not have been. Therefore their statement cannot be correct. This becomes very important as standardisation makes a big difference to the creatinine values in the normal range and thus the GFR estimates in the 50 to 60 range. It would likely have a big effect on the eGFR results. I am assuming there is not any stored serum to go back and measure a sub sample in order to standardise the creatinines. If it is not possible to ensure calibrated creatinines then the author should use the old 186 MDRD forumula. CKD-Epi was develop with IDMS creatinines so it will remain a big limitation. This needs to be satted as a clear limitation to the analysis.

The baseline samples were tested in late 2005 using isotope dilution mass spectrometry (IDMS) traceable Jaffe kinetic assay as stated. We have now clarified this by stating in the renal function assessment methods section “Baseline renal function was determined in 1,239 women. Serum was collected after an overnight fast and serum creatinine analysed in 2005 using an isotope dilution mass spectrometry (IDMS) traceable Jaffe kinetic assay for creatinine on a Hitachi 917 analyzer (Roche Diagnostics GmbH, Mannheim Germany).”

2. Statistical Analysis. The authors change the primary outcome (mortality) as it did not obey the proportional hazards assumption. I am assuming by this they are referring to the eGFR results and mortality. This to me is intriguing and I am surprised that is has not been explored in a different way. Did they try stratification? What is the exact issue with the data - is the loss of the proportional hazards present
in both males and females? Using logistic regression is not ideal as it takes away any issue of the time which seems here to be important. This needs to be explored more fully.

The primary outcome of the study was atherosclerotic vascular disease and as we had two separate datasets (hospitalizations and deaths) we tested the proportional hazards assumption for both before combining the datasets. We identified that eGFR for ASVD death did not obey the Cox proportional hazard criteria of proportionality.

We have now combined the hospitalizations and deaths and used logistic regression throughout the manuscript to avoid confusion and included Kaplan Meier Survival curves to demonstrate the relationship of eGFR with ASVD over time.

3. Figure 1. Using the bar graphs with numbers side by side makes it difficult to compare the two equations. Can they produce a smoothed density plot with both equation on the same plot which makes it easier to compare the two equations.

We have now removed this figure.

4. Recent work suggests the the CKD-Epi equation produces lower eGFR values in the elderly compare to MDRD (eg: van den Brand, J. A. J. G., etal .Introduction of the CKD-EPI equation to estimate glomerular filtration rate in a Caucasian population. NDT 2011). This seems at odds to the current data - mean EGF for CKD-Epi is higher (table 1). While this could be related to issue as discussed above, could the authors provide more information of the eGFR distribution in the cohort.

Whilst we agree that the CKD-EPI may produce lower eGFR values in some elderly cohorts Van der Brand found that the median eGFR was lower by the CKD-EPI equation in women ~75 years but mean eGFR was higher. In our elderly cohort (mean age of 75) there was virtually no difference between the CKD-EPI and the MDRD equations (mean difference of -1.06 ml/min/1.73m^2). These findings are consistent with those reported by Van der Brand et al that the mean eGFR from the 2 equations intersect between 75-79 and 80-84 years.

5. Table 2 & 3. Table 2 shows unadjusted HR’s. Can the authors provide the fully adjusted data as well as that is more relevant.

Given that predictive risk models in the elderly are not well described the unadjusted OR are provided in Table 2 to enable the reader to compare the individual strength of the association of each variable per standard deviation to other well known risk. We therefore believe that table 2 with the unadjusted OR is more informative.

6. I am a little unclear on the table 3 data. Is the HR for the eGFR equations adjusted only by framingham risk score and nothing else?

The OR for eGFR is adjusted by the predicted Framingham risk using the equation for females which takes into account age, body mass index, systolic blood pressure, diabetes, and smoking. We have now included a separate paragraph in the methods section describing this.

*Framingham risk score*

The **10-year Framingham general cardiovascular disease risk score** was calculated using age, previous diabetes, body mass index, current smoking status and the untreated systolic blood pressure using the equation and estimated regression coefficients developed by D'Agostino et al 2008 [22].

The Framingham risk score (FRS) equation was:

\[
    \text{FRS} = 1 - 0.94833^{\exp(2.72107*\ln(\text{Age})+0.51125*\ln(\text{BMI})+2.81291*\ln(\text{SBP})+0.61868*(\text{Currentsmoker})+0.77763*(\text{Diabetes})-26.0145)}
\]
The individual risk scores were then confirmed using the online calculator prepared by R.B. D’Agostino and M.J. Pencina based on the publication by D’Agostino et al [22].”

7. If so they need to be fully adjusted for the other risk factors. We are unsure which other risk factors the reviewer is referring to. Because the Framingham risk score is the best recognised approach to combining risk factors we would contend that further adjustment is inappropriate.

While the score will I guess will account got some of the confounders will it account for all. We are again unsure what point the reviewer is making. The approach we adopted is to use Framingham as the “gold standard” of calculators and then see if eGFR adds more information which in a statistical sense it does.

6. NRI I IDI data. This data is very interesting. Again are the models fully adjusted as per above. Can they provide confidence intervals for the NRI (& IDI) and not just the p values.

Yes the NRI and the IDI models are all fully adjusted for all of the Framingham risk factors such as systolic blood pressure, age, BMI, diabetes and smoking. We have now included ± SEM for the IDI but as we based the NRI on the methods described by Pencina et al 2008 we are unable to provide 95%CI for the NRI without bootstrapping methods.

Reviewer's report 2
Title: Estimated glomerular filtration rate as an independent predictor of atherosclerotic vascular hospitalization in older women
Version: 1 Date: 22 February 2012
Reviewer: William McClellan
Reviewer's report:
Major Compulsory Revisions
Comments: The authors seek to determine if impaired eGFR is an independent risk factor for future occurrence of ASCVD among older women after accounting for risk predicted by the Framingham model. There are several general concerns that make it difficult to assess the manuscript.

First, the I don’t think the use of the Framingham risk scores is consistent with the literature. We are unsure as to the literature that the reviewer refers to. We used the constants and equation developed by D’Agostino et al 2008 Circulation. We selected Framingham as our comparator in view of the fact that it is the best recognised and investigated predictor available at the current time.

Second, excluding deaths from the analyses is problematic.
We agree and have now included deaths and used logistic regression throughout.

Third, it is difficult to follow much of the presentation of the results of joint adjustment of the FRS and eGFR on hospitalization.

1. Methods, outcome assessment. You state that ”The primary outcome was an atherosclerotic vascular disease event causing hospitalization this was because ASVD mortality did no obey the Cox proportional hazard assumption “. Does this mean that deaths ascribed to ASCVD that occurred out of hospital were excluded in the primary analyses?
Yes deaths were not counted in the hospitalizations outcome as they were derived from a different database. A separate outcome for ASVD deaths was presented in the manuscript however we have now combined hospitalizations and deaths and used logistic regression throughout to avoid confusion.

2. There are a number of approaches to modifying a Cox PHM to account for non-proportionality and you should consider using one. 

We have now provided Kaplan Meir plots (Figure 1)

![Kaplan Meir plots](image)

Figure 1: Kaplan Meir survival curves for ASVD hospitalizations and deaths dichotomized by a) K/DOQI chronic kidney disease categories of eGFR by the MDRD equation; blue line <45 mL/min/1.73m² (n = 71), green line 45-59 mL/min/1.73m² (n = 388), grey line 60-89 mL/min/1.73m² (n = 722) and black line ≥90 mL/min/1.73m² (n = 58) and b) Framingham predicted risk, blue line ≥30% 10-year risk (n=128), green line 15-29% 10-year risk (n = 649) and grey line <15% 10-year risk (n = 427).

This is particularly so as the Framingham prediction score is based on both deaths and nonfatal CHD events. How was mortality handled in the hospitalization analyses?

*Mortality was part of a separate dataset and not counted in the hospitalization analyses.*

3. Methods and Table 2. Are the HRs for the Framingham risk scores based on the predicted risks derived from tables in the cited reference (22) or are they empiric estimates based on the score added to a Cox PH model? If the former then this is an unusual means of using the Framingham risk score and it should be described in detail with supporting citations.

The OR for eGFR is adjusted for by the predicted Framingham risk using the equation for individual females which takes into account age, body mass index, systolic blood pressure, diabetes, and smoking. We have now included a separate paragraph in the methods section describing this.

**Framingham risk score**
The 10-year Framingham general cardiovascular disease risk score was calculated using age, previous diabetes, body mass index, current smoking status and the untreated systolic blood pressure using the equation and estimated regression coefficients developed by D'Agostino et al 2008 [22].

The Framingham risk score (FRS) equation was:

\[
FRS = 1 - 0.94833 \exp(2.72107 \ln(Age) + 0.51125 \ln(BMI) + 2.81291 \ln(SBP) + 0.61868 \times \text{Current smoker} + 0.77763 \times \text{Diabetes} - 26.0145)
\]

The risk scores were then confirmed using the online calculator prepared by R.B. D’Agostino and M.J. Pencina based on the publication by D’Agostino et al [22].

4. Methods, Framingham score. The Framingham score should be estimated for individuals free of ASCVD at baseline. Including prevalent cases of ASCVD in the follow-up may bias the comparisons between risk predicted by eGFR alone and that predicted by an equation developed for healthy adults.

We agree and have now included a sensitivity analysis for participants without prevalent ASVD in the Figure 2 which shows that the association remains significant however the OR per 10mL/min/1.73m² decline are reduced from 20-21% to 16-18%.

Minor Essential Revisions

5. Results. The presentation of the results in the paragraphs beginning “To put these findings in a clinical context the effect of eGFR on ASVD hospitalization…” is interesting but difficult to follow. One or more figures illustrating these points would be welcomed.

We have now included a further figure (Figure 2) to illustrate this point.
Whole cohort (n = 1,239)

ASVD hospitalizations (n = 307)
- MDRD OR 1.21 (1.10-1.34), P < 0.001
- CKD-EPI OR 1.22 (1.11-1.35), P < 0.001

ASVD deaths (n = 129)
- MDRD 1.16 (1.03-1.36), P = 0.016
- CKD-EPI OR 1.22 (1.06-1.40), P = 0.005

ASVD events (n = 369)
- MDRD OR 1.20 (1.09-1.32), P < 0.001
- CKD-EPI OR 1.21 (1.11-1.34), P < 0.001

Without previous ASVD (n = 1,089)

ASVD hospitalizations (n = 232)
- MDRD OR 1.17 (1.05-1.31), P = 0.004
- CKD-EPI OR 1.19 (1.06-1.33), P = 0.003

ASVD deaths (n = 95)
- MDRD 1.15 (0.98-1.34), P = 0.085
- CKD-EPI OR 1.19 (1.01-1.40), P = 0.033

ASVD events (n = 279)
- MDRD OR 1.16 (1.05-1.29), P = 0.004
- CKD-EPI OR 1.18 (1.06-1.34), P = 0.002

Figure 2: Framingham risk score-adjusted odds ratio and 95% confidence interval per 10 ml/min/1.73m² decrease in eGFR in the whole cohort and those free of ASVD at baseline. ASVD atherosclerotic vascular disease, eGFR estimated glomerular filtration rate, MDRD Modification of Diet in Renal Disease equation, CKD-EPI Chronic Kidney Disease Epidemiology equation.

6. Results, mortality. The presentation of the mortality results is not informative. What was the event rate? If you are going to separately analyze mortality there should be appropriate tables and detailed presentation of results. We have now included separate information for hospitalizations and deaths in figure 2 and 3 and table 3. Discretionary Revisions

6. Results, Figure 1. There is no legend for this figure that I can find; it doesn't seem particularly informative. We have now removed this figure
Results. Given the degree of missing data it is not clear that the inclusion of lipids strengthens your results.

We agree with have now removed the lipid data from the manuscript