Cystatin C estimated glomerular filtration rate and all-cause and cardiovascular disease mortality risk in the general population: AusDiab study

Running heading: Mortality and CVD risk: eGFR cystatin-C

Elizabeth L.M. Barr (PhD)[1,2]

Anne Reutens (PhD, FRACP)[1,3]

Dianna J. Magliano (PhD)[1]

Rory Wolfe (PhD)[3]

Zhong X Lu (PhD)[4]

Ken A Sikaris (BSc(Hons), FAACB)[4]

Stephanie K. Tanamas (PhD)[1]

Robert Atkins (DSc, FRACP)[1, 3]

Steve Chadban (PhD, FRACP)[5]

Jonathan E. Shaw (MD, MRCP (UK), FRACP)[1]

Kevan R Polkinghorne (PhD, FRACP)[3,6,7]

[1]Baker IDI Heart and Diabetes Institute, Melbourne, Australia

[2]Menzies School of Health Research, Darwin, Australia

[3]Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia

[4]Melbourne Pathology, Melbourne, Australia

[5]Sydney University, Sydney, Australia

[6]Department of Medicine, Monash University, Melbourne, Australia

[7]Department of Nephrology, Monash Medical Centre, Melbourne, Australia

Corresponding author: Elizabeth L.M Barr

Baker IDI Heart and Diabetes Institute

PO Box 6492, Melbourne 3004, Australia

Telephone: +61 (0)3 8532 1827 Fax: +61 (0)3 8532 1100

E-mail: Elizabeth.Barr@bakeridi.edu.au

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Abstract

Aims: Uncertainties about the role of cystatin C based estimated glomerular filtration rate (eGFR) in the prediction of cardiovascular disease (CVD) beyond traditional CVD risk factors remain. We assessed contributions of eGFR to CVD and mortality in the general population.

Methods: Using 14 year follow-up data on 9353 adults without a reported history of CVD from the Australian Diabetes, Obesity and Lifestyle study, we assessed the contributions of eGFR (assessed by cystatin C ($eGFR_{cysC}$) and serum creatinine ($eGFR_{cr}$)) and albuminuria (uACR) to total and CVD mortality.

Results: After adjusting for age, sex, CVD risk factors and uACR, compared to an $eGFR_{cysC}$ >90 ml/min per 1.73m^2, $eGFR_{cysC}$ <60 ml/min per 1.73m^2 was associated with 56% and 73% increases in the risks for all-cause and CVD mortality, respectively. The respective changes for the c-statistic when $eGFR_{cysC}$ was added to a risk prediction model were 0.003 (95% CI: 0.001 to 0.005) and 0.002 (-0.001 to 0.006). The net proportion of non-events assigned a lower risk category significantly improved with the addition of eGFR (non-event NRI $eGFR_{cr}$: 1.0%, and $eGFR_{cysC}$:1.5%) for all-cause mortality, but for CVD mortality, improvements were only significant when eGFR was combined with uACR. The net proportion of events assigned a higher risk category was not significantly improved.

Conclusion: In our community-based cohort, reduced $eGFR_{cysC}$ was associated with all-cause and CVD mortality. The addition of CKD measures to risk prediction models improved

overall risk stratification among those at low risk as opposed to those at high baseline risk of mortality.

(Words: 250)

Key Words: Cystatin C, estimated glomerular filtration rate, cardiovascular diseases, mortality, risk factors

Recently a large meta-analysis of studies from the Chronic Kidney Disease Prognosis Consortium (CKD-PC) demonstrated that creatinine based estimated glomerular rate ($eGFR_{cr}$) and albuminuria significantly contribute to absolute cardiovascular disease (CVD) risk prediction beyond established CVD risk factors within the general population for the primary prevention of CVD.[1] However, glomerular filtration rate estimated with cystatin C ($eGFR_{cysC}$) has been proposed as an alternative marker of kidney function to that estimated with serum creatinine ($eGFR_{cr}$). $eGFR_{cysC}$ possesses several characteristics that make it a superior marker of glomerular filtration rate.[2].

Population based cohorts show a strong association between $eGFR_{cysC}$ and all-cause mortality [3-7] and CVD [3-9] and some studies have assessed the contribution of $eGFR_{cysC}$ to absolute risk prediction of CVD.[3, 4, 6-9] However, these prior investigations are limited by (i) not adjusting for urinary albumin,[3, 5] which is independently associated with increased risk of mortality and CVD,[10] (ii) being conducted in select populations[5, 8] and (iii) the inclusion of individuals with prevalent CVD[3, 4, 6] which makes it difficult to generalise the findings to the primary prevention setting.

We hypothesized firstly that $eGFR_{cysC}$ is a strong predictor of all-cause and CVD mortality, independent of uACR, and secondly that the addition of $eGFR_{cysC}$ and uACR to a CVD risk prediction model with established CVD risk factors would improve discrimination for future mortality within a primary prevention population. Accordingly, we aim to investigate within a community-based study, free of reported myocardial infarction or stroke at baseline, (i) whether cystatin C-derived eGFR equations ($eGFR_{cysC}$ and $eGFR_{cysC/cr}$) are associated with

all-cause and CVD mortality after considering other CVD risk factors and uACR, and (ii) whether the addition of cystatin C-derived eGFR equations improve absolute all-cause and CVD risk prediction beyond traditional CVD risk factors.

Methods

Study design and population: The Australian Diabetes, Obesity and Lifestyle (AusDiab) study is a national community based longitudinal observational study. From 1999 to 2000 11,247 adults aged ≥25 years underwent baseline examinations.[11] Participants provided informed consent, and the Human Research Ethics Committees of the International Diabetes Institute, Alfred Hospital and the Australian Institute of Health and Welfare approved the study.

Participants were excluded if mortality status could not be confirmed (n=21), a history of myocardial infarction or stroke were reported at baseline (n=640), they had not fasted for ≥8 hours (n=14), were pregnant (n=60) or had missing values for cystatin C, creatinine or uACR (n=677) or other covariates included in the analyses (n=482).

Baseline measurements: In 2010, thawed frozen sera (-80 degrees Celsius) collected at baseline measured cystatin C using an immunoturbidimetric assay (Roche/Hitachi 917, MODULAR P analyzer, Roche Diagnostics) and serum creatinine using a Roche IDMS aligned enzymatic method (Roche Modular, Roche Diagnostics). Cystatin C remains stable over several freeze-thaw cycles.[12] We calculated eGFR based on serum creatinine, cystatin C or a combination of both measures using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations.[13, 14] Cystatin C values were standardised according to

the IFCC.[6] Urine creatinine from a spot sample was measured using the modified kinetic Jaffé reaction (Olympus AU600 autoanalyzer, Olympus Optical Co Ltd.), and urine albumin was measured using rate nephelometry (Beckman Array, Beckman Coulter Inc.). Data on age, sex, use of anti-hypertensive and lipid-lowering medications, self-reported history of doctor (or nurse) diagnosis of heart attack (including 'coronary', coronary occlusion, coronary thrombosis, or myocardial infarction) or stroke and smoking (never, ex- or current smoker) were collected by interview at baseline. Fasting and two-hour plasma glucose, serum total cholesterol, triglycerides and HDL-cholesterol were measured using an analyser at a central laboratory (AU600; Olympus Optical, Tokyo, Japan). Seated blood pressure (BP) was measured (Dinamap semi-automatic oscillometric recorder or manual mercury sphygmomanometer) after five minutes rest, and the mean of at least two measures, taken one minute apart, was recorded. Height was recorded on a stadiometer to the nearest 0.5cm and weight recorded on a beam balance to the nearest 0.1 kg. For each of waist (horizontal mid-point between lower ribs and iliac crest) and hip (over the buttocks) circumferences, two measurements to the nearest 0.5 cm were recorded. A third measurement was taken for variations greater than 2 cm,. The mean of the two closest measurements was calculated. ,A 75g oral glucose tolerance test was undertaken. Diabetes was classified when fasting plasma glucose value was ≥7.0 mmol/l or two-hour plasma glucose was ≥11.1 mmol/l or participants reported physician-diagnosed diabetes.[15]

Follow-up and outcomes: Mortality status and cause(s) of death were obtained from the National Death Index (NDI).[16] Follow-up for all-cause mortality was to the date of death or 9th January 2014, and for CVD mortality was 30th November 2011 (as causes of death data

were only available from the NDI until 2011). CVD mortality was defined by an underlying cause of death coded I10-I25, I46.1, I48, I50-I99 or R96 according to the 2006 International Classification of Diseases 10th revision (ICD-10, www.who.int/classifications/icd/en/). In cases where the underlying cause of death was uncomplicated diabetes (E109, E119 or E149) or unspecified hyperlipidaemia (E785), CVD was considered to be the cause of death (n=5) if any of the above CVD codes were the first listed cause on the death certificate.

Statistical analysis: To test differences in means and proportions for baseline CVD risk factors between categories of decreasing $eGFR_{cysC}$ (≥90, 60 to 90 and <60 ml/min per $1.73m^2$), one-way analysis of variance and χ^2 tests were used, respectively. Cox proportional hazards regression was used to estimate the all-cause and CVD mortality hazard ratios (HRs) and 95% confidence intervals (CIs) comparing eGFR 60 to 90 ml/min per$1.73m^2$ and <60 ml/min per $1.73m^2$ with eGFR ≥90 ml/min per $1.73m^2$. Regression analyses were based on fewer eGFR categories to ensure sufficient numbers of outcomes in each strata. Age was used as the time scale. Models were adjusted for sex, smoking (never, ex- and current smoker), diabetes, self-reported use of lipid-lowering medication, hypertension (BP ≥140/90 mmHg or self-reported anti-hypertensive use), and linearly modelled total cholesterol, HDL-C, and log-uACR. There was no evidence of multicollinearity between covariates for any of the fitted models (variance inflation factor ≤3 for all independent variables).[17]. First-order interactions between each of $eGFR_{cysC}$ and $eGFR_{cysC/cr}$ with sex, age (25-64 *vs.* ≥65 years), diabetes (no diabetes *vs.* diabetes) and microalbuminuria (no microalbuminuria *vs.* uACR ≥2.5mg/mmol for men and ≥3.5mg/mmol for women) were tested using log-likelihood ratio tests of models containing the variables' main effects nested within models including the interactions.

Proportional hazards assumptions were satisfied for eGFR variables as assessed with graphs of log-log plots of the relative hazards by time and by scaled Schoenfeld residuals. Overall the models generally showed adequate calibration by inspection of the cumulative hazard of Cox-Snell residuals (see Supplementary Figure 1). There was no indication of systematic bias to under- or over-estimate survival. There were deviations at the upper end of the cumulative hazard, but these are consistent with estimation uncertainty being at its greatest, so these deviations are not a major concern. [18, 19]

We summarised the incremental value of eGFR variables to risk prediction models of traditional CVD risk factors using Harrell's c-statistics, relative integrated discrimination improvement and the net reclassification improvement.[20] In these analyses, the eGFR variables were modelled as a continuous variable (linear spline with 4 knots at 45, 60, 90, 105 ml/min per 1.73m^2) to accommodate the non-linear relationship of eGFR with the log-hazard of all-cause and CVD mortality. The incremental addition of (i) $eGFR_{cr}$, $eGFR_{cysC}$, $eGFR_{cysC/cr}$ or log-uACR and (ii) the joint addition of $eGFR_{cr}$ + log-uACR, $eGFR_{cysC}$ + log-uACR, or $eGFR_{cysC/cr}$ + log-uACR to a multivariate model of traditional CVD risk factors (as outlined above) were assessed. We estimated the difference in Harrell's C statistics between these prediction models.[21] The net reclassification improvement was used to evaluate the extent to which different models reclassified individuals across three *a priori* categories (0 - <10%, 10 to <20% and ≥20%) of all-cause and CVD death risk. The relative integrated discrimination improvement examines the standardised change in estimated predicted probabilities as a continuous measure and was used to evaluate predicted all-cause and CVD death risks. We specifically reported event and non-event net reclassification improvement to

assess the changes in both the true and false-positive rates, respectively, when assessing the incremental value of kidney markers to traditional CVD risk factors.[22, 23] Bootstrap methods were used to derive 95% CIs for the net reclassification improvement and relative integrated discrimination estimates, and were based on 1000 replications.[22, 23] Analyses were conducted with Stata Statistical Software version 14 (StataCorp, College Station, TX, USA).

Results

Characteristics of participants according to $eGFR_{cysC}$

Of the 11,247, 9,353 (83%) had complete data for this analysis. In comparison to participants who were excluded from this analysis, participants included in the analysis had a lower CVD risk profile (data not shown), which is consistent with the exclusion of participants who reported having had a prior myocardial infarction or stroke at baseline. Lower $eGFR_{cysC}$ was observed with older age, women, higher uACR, and other traditional CVD risk factors (Table 1).

Comparison of the associations of $eGFR_{cysC}$, $eGFR_{cr}$ and $eGFR_{cysC/cr}$ with all-cause and CVD mortality

There were 918 deaths after a median follow-up of 13.8 years, and 206 CVD deaths of which 112 (54%) were attributable to ischaemic heart disease, and 54 (26%) attributable to stroke, after a median follow-up of 11.7 years. The unadjusted cumulative incidence of all-cause (figure 1A) and CVD mortality (figure 1B) increased with worsening $eGFR_{cr}$, $eGFR_{cysC}$ and $eGFR_{cysC/cr}$. In adjusted models, eGFR <60 ml/min per $1.73m^2$ based on cystatin C and a

combination of cystatin C and creatinine were associated with respectively 69% and 59% increased risks for all-cause mortality and 98% and 90% increased risks for CVD mortality. Estimates of mortality risk increases for $eGFR_{cr}$ <60 ml/min per 1.73m^2 were not as large and confidence intervals included one. Although additional adjustment for uACR attenuated the observed associations, $eGFR_{cysC}$ and $eGFR_{cysC/cr}$ <60 ml/min per 1.73m^2 remained significantly associated with outcome (Tables 2 and 3). We observed no significant interactions for $eGFR_{cysC}$ or $eGFR_{cysC/cr}$ with sex, diabetes or microalbuminuria for any outcome (data not shown).

The contribution of the components of CKD to the absolute risk prediction of all-cause and CVD mortality

Table 4 shows small but statistically significant incremental increases in c-statistics when cystatin C based eGFR variables or uACR were added to a multivariate all-cause mortality risk model. There were no such significant improvements in c-statistics for CVD risk prediction. Relative integrated discrimination improvement results indicated that the addition of eGFR measures, uACR or a combination of both to a model with established CVD risk factors improved the discrimination property of the model for the prediction of overall mortality. Similar results were observed for CVD mortality but the greater uncertainty attached, in the form of wide confidence intervals, precluded a conclusion of enhanced prediction with cystatin C based eGFR, unless it was also combined with uACR.

Table 4 shows that for overall mortality, the net proportion of non-events assigned a lower risk category significantly improved with the addition of eGFR measures to traditional risk

factor models (NRI $eGFR_{cr}$: 1.0%, $eGFR_{cysC}$:1.5%, $eGFR_{cysC/cr}$: 1.2%). However, for CVD mortality the proportion of non-events assigned a lower risk category was only improved for eGFR variables when added to models in conjunction with uACR. The net proportion of events assigned a higher risk category was not significantly improved for either all-cause or CVD mortality. Table 5 shows that whilst the addition of CKD markers correctly increased the proportions of non-events labelled as low risk (<10%) for CVD and all-cause mortality, the same degree of correct reclassification was not evident among events.

Discussion

Our study of Australian adults, free of CVD at baseline and followed for 14 years, showed that compared to an $eGFR_{cysC}$ >90 ml/min/1.73m^2, $eGFR_{cysC}$ < 60 ml/min/1.73m^2 was associated with 56% and 73% increases in the risks of all-cause and CVD mortality, respectively, independent of traditional CVD risk factors and uACR. Weaker associations with mortality and CVD were observed for $eGFR_{cysC/cr}$ and $eGFR_{cr}$. We observed small improvements in c-statistics when any renal parameter was included in a conventional risk factor model, however, significant differences were only observed for all-cause mortality. Risk reclassification as assessed with the relative integrated discrimination improvement revealed that addition of renal parameters to risk models significantly improved all-cause mortality risk prediction, but the addition of cystatin C based eGFR only significantly improved CVD mortality prediction when combined with uACR. Further inspection of the impact of renal parameters on risk reclassification among cases and non-cases showed that

correct reclassification was predominately among those classified as low-risk for all-cause and CVD mortality, compared to those classified as being at high risk for mortality.

Our findings support existing evidence [3, 4, 6, 8] that demonstrate a stronger association between eGFR based on cystatin C compared to one based on creatinine for all-cause and CVD mortality. We also show that these relationships for $eGFR_{cysC}$ are independent of albuminuria. Only few studies in select populations have also shown this for cystatin C.[4, 6, 8] This provides further evidence that albuminuria and impaired GFR may be markers of different CVD pathophysiology pathways.

In contrast to other studies,[4, 6] we did not observe a strong relationship between mild reductions in eGFR (60 to 90 ml/min/1.73m^2) and risk of mortality. A meta-analysis reported that the risk of all-cause mortality or CVD increased significantly if $eGFR_{cysC}$ or $eGFR_{cysC/cr}$ fell below approximately 85 ml/min/1.73m^2, which was higher than the level observed for $eGFR_{cr}$. However, individuals with prevalent CVD were included in this analysis, which may explain these differences[6]. Nerpin et al.[8] revealed that CVD risk prediction was optimized at an $eGFR_{cysC}$ of 45 ml/min/1.73m^2 when men with prevalent CVD were excluded. Our study may not have had sufficient power to detect elevations in mortality risk at moderately reduced eGFR if the effect size is small. Intervention studies that assess the impact of moderate improvements of eGFR on mortality and CVD will help to elucidate the potential contributions of less severe reductions in $eGFR_{cysC}$ to mortality and CVD.

We found that the association between $eGFR_{cysC/cr}$ of <60 ml/min/1.73m^2 and the mortality outcomes was weaker compared to the associations observed for reduced $eGFR_{cysC}$. This

concurs with Astor et al.[3] and Shlipak et al.,[6] and suggests that serum cystatin C may be directly associated with other physiological processes, such as insulin resistance and inflammation, that are related to the development of CVD independent of reduced GFR.[24]

Whilst a recent meta-analysis indicated that $eGFR_{cr}$ and uACR improved five year CVD mortality prediction in the general population,[1] uncertainty remains as to whether the addition of $eGFR_{cysC}$ to models of traditional risk factors improves CVD risk stratification. One study showed that the addition of $eGFR_{cysC}$ or urine excretion rate resulted in minimal improvements for the c-statistic and *categorical* net reclassification index.[8] Others demonstrate significant improvements in the *continuous* net reclassification index for the addition of $eGFR_{cysC}$ to multivariate risk prediction models for all-cause mortality and CHD risk.[4, 9] However, *continuous* net reclassification index does not capture the incremental effects of the parameter on both the true- and false-positive rates [22, 23]. Our study showed statistically significant but small improvements in the c-statistic for adding renal parameters to traditional risk factor models for all-cause mortality. However, since the c-statistic is a rank based measure it does not necessarily account for reclassification of individuals between clinically important risk categories. Further analysis showed that adding renal measures to risk prediction models correctly increased the proportions of individuals classified as low risk among participants *not experiencing events*, whereas, the same degree of correct reclassification was not observed among high risk participants *experiencing events.* Given that there are more non-events than events in our study population, a larger proportion of individuals were correctly assigned a risk category with the addition of CKD parameters.

Our study is strengthened by being based on a large community-based sample with a spectrum of baseline renal function. We also used standardised cystatin C values[6] and the most recent eGFR equations for creatinine and cystatin C.[13, 14]

However, limitations exist. We were unable to capture the CVD risks associated with evolving renal function, as renal parameters were measured once at baseline. uACR was measured from a spot sample, and as such this may have introduced some excess variability in the baseline measures. However, any measurement error most likely would have been random, leading to attenuation of study findings. We did not measure glomerular filtration rate directly, as it is time consuming and invasive, and not a cost effective approach for large epidemiological studies or clinical practice. There is some evidence that cystatin C level may be affected by thyroid function and high doses of glucocorticoids,[2] however, we were not able to assess this. Exclusion of participants at baseline with a previous CVD event was based upon self-report; a validation study has revealed that self-reported myocardial infarction and stroke overestimated true disease in our study, and hence our exclusion criterion was conservative.[25] The AusDiab cohort consists of individuals with a predominant European ancestry and thus findings may not be generalisable to other populations. Finally, as this analysis preferentially selected participants without a known history of CVD, participants with advanced CKD are not well represented. Hence, our findings are not generalisable to this high risk patient group, but rather to the general population.

In conclusion, our study provides additional supportive evidence that eGFR based on cystatin C may play an important role in the pathophysiology of CVD within the general population,

as evidenced by the moderate associations observed with mortality from all-causes and CVD. This relationship is independent of albuminuria and other established CVD risk factors. We conclude that given further formal cost effectiveness evaluations on the clinical utility of cystatin C based eGFR, this measure may be useful in the identification of individuals for further screening and investigation.

Disclosures

None

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References

1 Matsushita K, Coresh J, Sang Y, Chalmers J, Fox C, Guallar E, *et al.* Estimated glomerular filtration rate and albuminuria for prediction of cardiovascular outcomes: a collaborative meta-analysis of individual participant data. *Lancet Diabetes Endocrinol*. 2015; **3**: 514-25.

2 Filler G, Bokenkamp A, Hofmann W, Le Bricon T, Martinez-Bru C, Grubb A. Cystatin C as a marker of GFR--history, indications, and future research. *Clin Biochem*. 2005; **38**: 1-8.

3 Astor BC, Levey AS, Stevens LA, Van Lente F, Selvin E, Coresh J. Method of glomerular filtration rate estimation affects prediction of mortality risk. *J Am Soc Nephrol*. 2009; **20**: 2214-22.

4 Waheed S, Matsushita K, Sang Y, Hoogeveen R, Ballantyne C, Coresh J, *et al.* Combined association of albuminuria and cystatin C-based estimated GFR with mortality, coronary heart disease, and heart failure outcomes: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Kidney Dis*. 2012; **60**: 207-16.

5 Peralta CA, Lee A, Odden MC, Lopez L, Zeki Al Hazzouri A, Neuhaus J, *et al.* Association between chronic kidney disease detected using creatinine and cystatin C and death and cardiovascular events in elderly Mexican Americans: the Sacramento Area Latino Study on Aging. *J Am Geriatr Soc*. 2013; **61**: 90-5.

6 Shlipak MG, Matsushita K, Arnlov J, Inker LA, Katz R, Polkinghorne KR, *et al.* Cystatin C versus creatinine in determining risk based on kidney function. *N Engl J Med*. 2013; **369**: 932-43.

7 Svensson-Farbom P, Ohlson Andersson M, Almgren P, Hedblad B, Engstrom G, Persson M, *et al.* Cystatin C identifies cardiovascular risk better than creatinine-based estimates of glomerular

filtration in middle-aged individuals without a history of cardiovascular disease. *J Intern Med*. 2014; **275**: 506-21.

8 Nerpin E, Ingelsson E, Riserus U, Sundstrom J, Larsson A, Jobs E, *et al.* The combined contribution of albuminuria and glomerular filtration rate to the prediction of cardiovascular mortality in elderly men. *Nephrol Dial Transplant*. 2011; **26**: 2820-7.

9 Waheed S, Matsushita K, Astor BC, Hoogeveen RC, Ballantyne C, Coresh J. Combined association of creatinine, albuminuria, and cystatin C with all-cause mortality and cardiovascular and kidney outcomes. *Clin J Am Soc Nephrol*. 2013; **8**: 434-42.

10 Cirillo M, Lanti MP, Menotti A, Laurenzi M, Mancini M, Zanchetti A, *et al.* Definition of kidney dysfunction as a cardiovascular risk factor: use of urinary albumin excretion and estimated glomerular filtration rate. *Arch Intern Med*. 2008; **168**: 617-24.

11 Dunstan DW, Zimmet PZ, Welborn TA, Cameron AJ, Shaw J, de Courten M, *et al.* The Australian Diabetes, Obesity and Lifestyle Study (AusDiab)--methods and response rates. *Diabetes Res Clin Pract*. 2002; **57**: 119-29.

12 Finney H, Newman DJ, Gruber W, Merle P, Price CP. Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the Behring nephelometer systems (BNA, BN II). *Clin Chem*. 1997; **43**: 1016-22.

13 Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009; **150**: 604-12.

14 Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, *et al.* Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012; **367**: 20-9.

15 The World Health Organization and International Diabetes Federation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. Geneva: The World Health Organization 2006.

16 Barr EL, Zimmet PZ, Welborn TA, Jolley D, Magliano DJ, Dunstan DW, *et al*. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation*. 2007; **116**: 151-7.

17 Glantz S, Slinker B. *Primer of Applied Regression and Analysis of Variance*. New York: McGraw-Hill 1990.

18 Cox D, Snell, EJ,. A general definition of residuals (with discussion). *Journal of the Royal Statistical Society, Series B*. 1968; **30**: 248-75.

19 Box-Steffensmeier JM, Jones BS. *Event History Modeling: A guide for social scientists*: Cambridge University Press 2004.

20 Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med*. 2008; **27**: 157-72.

21 Newson RB. Comparing the predictive powers of survival models using Harrell's C or Somers' D. *The Stata Journal*. 2010; **10**: 339-58.

22 Kerr KF, Wang Z, Janes H, McClelland RL, Psaty BM, Pepe MS. Net reclassification indices for evaluating risk prediction instruments: a critical review. *Epidemiology*. 2014; **25**: 114-21.

23 Leening MJ, Vedder MM, Witteman JC, Pencina MJ, Steyerberg EW. Net reclassification improvement: computation, interpretation, and controversies: a literature review and clinician's guide. *Ann Intern Med*. 2014; **160**: 122-31.

24 Mathisen UD, Melsom T, Ingebretsen OC, Jenssen T, Njolstad I, Solbu MD, *et al.* Estimated GFR associates with cardiovascular risk factors independently of measured GFR. *J Am Soc Nephrol*. 2011; **22**: 927-37.

25 Barr ELM, Tonkin AM, Welborn TA, Shaw JE. Validity of self-reported cardiovascular disease events in comparison to medical record adjudication and a statewide hospital morbidity database: the AusDiab study. *Internal Medicine Journal*. 2009; **39**: 49-53.

Figure legend

Figure 1. Unadjusted cumulative incidence of (A) all-cause mortality and (B) CVD mortality

Supplementary Figure 1: Supplementary Figure 1. Cumulative hazard of Cox-Snell residuals

Table 1. Baseline characteristics according to categories of estimated glomerular filtration rate based on cystatin C ($eGFR_{cysC}$) CKD-Epi

	$eGFR_{cysC}$ categories (ml/min per 1.73m^2)			
	≥90	**60 to 90**	**<60**	**Total**
N	6863	2024	466	9353
Age (years)	46 (11)	61 (11)	73 (9)	50 (14)**
Men (%)	3133 (46%)	829 (41%)	164 (35%)	4126 (44%)**
$eGFR_{cr}$ CKD-Epi (ml/min per 1.73m^2)	102.8 (94.3, 111.0)	85.1 (75.4, 93.5)	65.6 (54.5, 76.9)	98.4 (86.9, 108.0)**
Urine albumin/creatinine ratio (mg/mmol)	0.5 (0.4, 0.8)	0.6 (0.4, 1.2)	1.1 (0.6, 3.0)	0.5 (0.4, 0.9)**
Cigarette smoking				
-ex-smoker (%)	1893 (28%)	599 (30%)	143 (31%)	2635 (28%)
-current smoker (%)	1130 (16%)	324 (16%)	49 (11%)	1503 (16%)*
Hypertension (%) (≥140/90mmHg or anti-hypertensive medication use)	1427 (21%)	999 (49%)	342 (73%)	2768 (30%)**
Diabetes (%)	256 (4%)	192 (9%)	70 (15%)	518 (6%)**
HbA_{1c} (%)	5.0 (4.9, 5.2)	5.2 (5.0, 5.4)	5.3 (5.1, 5.5)	5.1 (4.9, 5.3)**
Fasting Plasma Blood Glucose (mmol/L)	5.3 (5.0, 5.7)	5.5 (5.1, 5.9)	5.5 (5.2, 6.0)	5.4 (5.0, 5.7)**
Post Load Plasma Glucose (mmol/L)	5.6 (4.7, 6.7)	6.2 (5.2, 7.7)	7.2 (5.8, 9.0)	5.8 (4.9, 7.0)**
Waist to hip ratio	0.85 (0.09)	0.88 (0.09)	0.89 (0.09)	0.86 (0.09)**
BMI value (kg/m^2)	26.5 (4.7)	28.0 (5.4)	28.0 (5.3)	26.9 (4.9)**
Reported lipid lowering medication use	313 (5%)	241 (12%)	71 (15%)	625 (7%)**
Cholesterol (mmol/L)	5.6 (1.1)	5.9 (1.1)	5.9 (1.1)	5.7 (1.1)**
High Density Lipoprotein (mmol/L)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
Triglycerides (mmol/L)	1.2 (0.8, 1.8)	1.4 (1.0, 2.0)	1.6 (1.1, 2.1)	1.3 (0.9, 1.9)**

Data are: n (%) for categorical variables, and mean (sd) or median (25^{th}, 75^{th} percentile) for continuous variables

CKD-Epi – Chronic Kidney Disease Epidemiology Collaboration; $eGFR_{cysC}$ – glomerular filtration rate estimated with cystatin C; $eGFR_{cr}$ –glomerular filtration rate estimated with serum creatinine; HbA_{1c} - Haemoglobin A_{1c}.

* p< 0.05; ** p <0.001

Table 2. Risk of all-cause mortality according to different eGFR equations

		Model 1			Model 2			Model 3		
eGFR categories	Deaths, n (%)	HR	[95% Conf.	Interval]	HR	[95% Conf.	Interval]	HR	[95% Conf.	Interval]
$eGFR_{cr}$										
>90	287 (4)	1.0	-	-	1.0	-	-	1.0	-	-
60 to 90	500 (19)	0.84	0.71	1.00	0.85	0.71	1.01	0.87	0.73	1.04
<60	131 (56)	1.09	0.85	1.40	1.11	0.87	1.43	1.10	0.86	1.42
$eGFR_{cysC}$										
>90	274 (4)	1.0	-	-	1.0	-	-	1.0	-	-
60 to 90	391 (19)	1.18	0.98	1.41	1.09	0.91	1.31	1.09	0.91	1.31
<60	253 (54)	1.86	1.51	2.30	1.69	1.36	2.09	1.56	1.26	1.94
$eGFR_{cysC/cr}$										
>90	279 (4)	1.0	-	-	1.0	-	-	1.0	-	-
60 to 90	457 (21)	1.08	0.90	1.29	1.04	0.87	1.24	1.05	0.88	1.26
<60	182 (60)	1.67	1.33	2.10	1.59	1.26	2.01	1.46	1.16	1.84

$eGFR_{cr}$ –glomerular filtration rate estimated with serum creatinine; $eGFR_{cysC}$ – glomerular filtration rate estimated with cystatin C; $eGFR_{cysC/cr}$ – glomerular filtration rate estimated with cystatin C and serum creatinine.

Model 1: Adjusted age (time scale) and sex
Model 2: Model 1 plus smoking, diabetes, total cholesterol, high density lipoprotein cholesterol, self-reported lipid lowering medication use and hypertension (≥40/90mmHg or anti-hypertensive medication).
Model 3: Model 2 plus urine albumin/creatinine ratio

Table 3. Risk of CVD mortality according to different eGFR equations

		Model 1			Model 2			Model 3		
eGFR categories	CVD, n (%)	HR	[95% Conf.	Interval]	HR	[95% Conf.	Interval]	HR	[95% Conf.	Interval]
$eGFR_{cr}$										
≥90	42 (0.7)	1.0	-	-	1.0	-	-	1.0	-	-
60 to 90	122 (5)	0.92	0.61	1.39	0.91	0.60	1.39	0.96	0.63	1.46
<60	42 (18)	1.28	0.76	2.14	1.25	0.74	2.13	1.27	0.75	2.14
$eGFR_{cysC}$										
≥90	43 (0.6)	1.0	-	-	1.0	-	-	1.0	-	-
60 to 90	81 (4)	1.06	0.70	1.62	0.98	0.64	1.49	0.97	0.64	1.49
<60	82 (18)	2.22	1.41	3.51	1.98	1.25	3.15	1.73	1.09	2.75
$eGFR_{cysC/cr}$										
≥90	41 (0.6)	1.0	-	-	1.0	-	-	1.0	-	-
60 to 90	106 (5)	1.15	0.75	1.75	1.10	0.72	1.68	1.12	0.74	1.71
<60	59 (19)	2.05	1.25	3.36	1.90	1.15	3.13	1.64	1.00	2.71

$eGFR_{cr}$ –glomerular filtration rate estimated with serum creatinine; $eGFR_{cysC}$ – glomerular filtration rate estimated with cystatin C; $eGFR_{cysC/cr}$ – glomerular filtration rate estimated with cystatin C and serum creatinine.

Model 1: Adjusted age (time scale) and sex
Model 2: Model 1 plus smoking, diabetes, total cholesterol, high density lipoprotein cholesterol, self-reported lipid lowering medication use, and hypertension (140/90 mmHg or anti-hypertensive medication).
Model 3: Model 2 plus urine albumin/creatinine ratio

Table 4. Discriminatory ability of eGFR equations and uACR in addition to common risk factors for all-cause mortality and CVD mortality

	Difference in c-statistic (95% CI)	P-value*	rIDI	NRI(event) (95% CI)	NRI(non-event) (95% CI)
All-cause mortality					
Traditional model (ref)	0.872	-	-	-	-
+ uACR	0.003 (0.001, 0.005)	0.001	2.8% (0.5%, 5.2%)	-1.0% (-2.5%, 1.0%)	0.6% (-0.02%, 1.2%)
+ $eGFR_{cr}$	0.001 (-0.001, 0.003)	0.395	2.2% (0.03%, 4.5%)	-2.1% (-3.9%, -0.3%)	1.0% (0.2%, 1.9%)
+ $eGFR_{cysC}$	0.003 (0.001, 0.005)	<0.001	3.6% (0.9%, 6.3%)	-0.7% (-2.8%, 1.5%)	1.5% (0.6%, 2.0%)
+ $eGFR_{cysC/cr}$	0.001 (0.0003, 0.003)	0.015	3.0% (0.6%, 5.4%)	-0.9% (-2.9%, 1.2%)	1.2% (0.2%, 2.2%)
+ $eGFR_{cr}$ and uACR	0.003 (0.001, 0.005)	0.007	6.7% (2.1%, 11.3%)	-1.9% (-3.9%, 0.2%)	1.4% (0.5%, 2.3%)
+ $eGFR_{cysC}$ and uACR	0.005 (0.003, 0.008)	<0.001	8.5% (3.5%, 13.4%)	-1.3% (-3.7%, 1.0%)	1.9% (0.8%, 3.0%)
+ $eGFR_{cysC/cr}$ and uACR	0.004 (0.002, 0.006)	<0.001	7.6% (2.8%, 12.4%)	-2.2% (-4.5%, 0.1%)	1.6% (0.5%, 2.6%)
CVD mortality					
Traditional model (ref)	0.916	-	-	-	-
+ uACR	0.004 (-0.001, 0.009)	0.114	9.5% (0.2%, 18.7%)	-1.0% (-7.5%, 5.6%)	0.7% (0.2%, 1.2%)
+ $eGFR_{cr}$	0.004 (-0.003, 0.012)	0.282	9.0% (-1.8%, 36.1%)	0% (-5.2%, 5.2%)	0.3% (-0.2%, 0.8%)
+ $eGFR_{cysC}$	0.002 (-0.001, 0.006)	0.190	10.3% (-1.3%, 21.8%)	0% (-6.4%, 6.4%)	0.5% (-0.1%, 1.0%)
+ $eGFR_{cysC/cr}$	0.001 (-0.004, 0.005)	0.728	10.8% (-2.7%, 24.4%)	1.0% (-5.3%, 7.2%)	0.4% (-0.1%, 1.0%)
+ $eGFR_{cr}$ and uACR	0.007 (-0.002, 0.016)	0.112	15.9% (-12.1%, 43.8%)	-0.5% (-7.2%, 6.2%)	1.0% (0.5%, 1.6%)
+ $eGFR_{cysC}$ and uACR	0.005 (-0.0004, 0.011)	0.069	16.8% (2.5%, 31.1%)	1.9% (-5.1%, 8.9%)	1.1% (0.5%, 1.7%)
+ $eGFR_{cysC/cr}$ and uACR	0.004 (-0.003, 0.010)	0.260	17.8% (0.9%, 34.7%)	3.4% (-3.7%, 10.5%)	0.8% (0.2%, 1.3%)

uACR – urinary albumin to creatinine ratio; $eGFR_{cr}$ –glomerular filtration rate estimated with serum creatinine; $eGFR_{cysC}$ – glomerular filtration rate estimated with cystatin C; $eGFR_{cysC/cr}$ – glomerular filtration rate estimated with cystatin C and serum creatinine; rIDI – relative integrated discrimination index; NRI – net reclassification index among deceased (events) and survivors (non-events). P-value for comparing c-statistic of the model with the renal parameters compared to the multivariate model without the renal parameters. Multivariate model adjusted for age and sex, smoking, diabetes status, total cholesterol, high density lipoprotein cholesterol, self-reported lipid lowering medication use and hypertension.

Table 5. Reclassification (n and % change) of participants with the addition of uACR, $eGFR_{cr}$ or $eGFR_{cysC}$ to a model with traditional CVD risk factors according to risk categories and events and non-events for all-cause and CVD mortality.

	Base multivariate model		**Multivariate model plus uACR**		**Multivariate model plus $eGFR_{cr}$**		**Multivariate model plus $eGFR_{cysC}$**	
All-cause mortality risk categories								
	Non-events (n=8,435)	Events (n= 918)	Non-events (n=8,435)	Events (n= 918)	Non-events (n=8,435)	Events (n= 918)	Non-events (n=8,435)	Events (n= 918)
0-<10%	6866	161	6885 (0.3%)	161 (0%)	6918 (0.8%)	168 (4.3%)	6944 (1.1%)	160 (-0.6%)
10-<20%	759	122	768 (1.2%)	128 (4.9%)	743 (-2.1%)	127 (4.1%)	731 (-3.7%)	129 (5.7%)
≥20%	810	635	782 (-3.5%)	629 (-0.9%)	774 (-4.4%)	623 (-1.9%)	760 (-6.2%)	629 (-0.9%)
CVD mortality risk categories								
0-<10%	8590	73	8624 (0.4%)	68 (-6.8%)	8618 (0.3%)	69 (-5.5%)	8625 (0.4%)	72 (-1.4%)
10-<20%	305	48	295 (-3.3%)	60 (25%)	277 (-9.2%)	52 (8.3%)	274 (-10.2%)	49 (2.1%)
≥20%	252	85	228 (-9.5%)	78 (-8.2%)	252 (0%)	85 (0%)	248 (-1.6%)	85 (0%)

uACR – urinary albumin to creatinine ratio; $eGFR_{cr}$ –glomerular filtration rate estimated with serum creatinine; $eGFR_{cysC}$ – glomerular filtration rate estimated with cystatin C

Data are n and % change of individuals reclassified with the addition of uACR or eGFR according to creatinine or cystatin C to a multivariate model. Multivariate model adjusted for age and sex, smoking , diabetes status, total cholesterol, high density lipoprotein cholesterol, self-reported lipid lowering medication use and hypertension .