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Chronic kidney disease, cardiovascular risk markers and total mortality in older men: cystatin C vs creatinine

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We certify that this work is novel

Abstract

It remains uncertain whether cystatin C is a superior marker of renal function than creatinine in older adults. We have investigated the association between estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology (CKD-EPI) equations based on creatinine (CKD-EPIcr) and cystatin C (CKD-EPIcys), and cardiovascular risk markers and mortality in older adults.

Methods

Cross sectional and prospective study of 1639 British men aged 71-92 years, followed up for an average 5 years for mortality. Cox's survival model and receiving operating characteristic (ROC) analysis were used to assess the associations.

Results

The prevalence of CKD was similar using the two CKD-EPI equation although cystatin C reclassified 43.9% of those with stage 3a CKD (eGFR 45-59ml/min/1.73^2, moderate damage) to no CKD. However, CKD stages assessed using both CKD-EPIcr and CKD-EPIcys were significantly associated with vascular risk markers and with all-cause and CVD mortality. In all men with CKD (eGFR < 60ml/min/1.73^2), the hazard ratio (HR) (95% confidence interval) for all-cause mortality after adjustment for cardiovascular risk factors compared to those with no CKD were 1.53 (1.20, 1.96) and 1.74 (1.35, 2.23) using CKD-EPIcr and CKD-EPIcys respectively. Comparisons of the two CKD equations showed no significant difference in their predictive ability for mortality (difference in AUC $p = 0.46$).

Conclusion

Despite reclassification of CKD stages, assessment of CKD using CKD-EPIcys did not improve prediction of mortality in older British men >70 years. Our data does not support the routine use of CKD-EPIcys for identifying CKD in the elderly British male population.

Summary Box

What is already known on this subject?

Chronic kidney disease (CKD) is a significant worldwide public health problem associated with excess mortality and morbidity. CKD diagnosis relies on the estimated glomerular filtration rate (eGFR), traditionally based on equations using serum creatinine levels. It remains uncertain whether cystatin C is a superior marker of renal function than creatinine in older adults. We therefore investigated the association between estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) equations based on creatinine (CKD-EPIcr) and cystatin C (CKD-EPIcys), and cardiovascular risk markers and mortality in older adults.

What does this study add?

We found that the prevalence of CKD (eGFR < 60 ml/min/1.73^2) was similar irrespective of CKD equation used although cystatin C reclassified a large proportion of men with stage 3 CKD (eGFR 30-59 ml/ min/1.73^2) based on creatinine to no CKD and these men did not show increased mortality risk. However, CKD stages assessed using both equations were significantly associated with vascular risk markers (inflammation, endothelial and cardiac dysfunction) and with all-cause and cardiovascular mortality. Comparisons of the two CKD equations showed no significant difference in their predictive ability for mortality. Despite reclassification of CKD stages, assessment of CKD using CKD-EPIcys did not improve prediction of mortality in white British men older than 70 years. Our data does not support the routine use of CKD-EPIcys for identifying CKD in the elderly British white male population.

Introduction

Chronic kidney disease (CKD) is a significant worldwide public health problem associated with excess mortality and morbidity [1, 2]. CKD diagnosis relies on the estimated glomerular filtration rate (eGFR), traditionally based on equations using serum creatinine levels. Whilst the Modification of Diet in Renal Disease (MDRD) equation had been used for many years, more recent evidence suggests the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation performs better in predicting mortality [3, 4].

Many population studies have shown the prevalence of CKD increases with increasing age [5]. Using creatinine in the older population to calculate eGFR may lead to an overestimation of the GFR as serum creatinine is influenced by muscle mass and dietary protein intake, both of which reduce with increasing age [6]. In view of the high prevalence of CKD in older adults and the growing ageing population, there is a need for measures of eGFR which can more accurately predict death in this population. Cystatin C is an alternative GFR marker less influenced by exogenous factors [6]. The National Institute for Health and Care Excellence (NICE) guidelines for the diagnosis of CKD in those with CKD Stage 3a (eGFR 45-59ml/min/1.73m^2) and no proteinuria recommends the use of cystatin C based equations to calculate eGFR [7].

There is conflicting evidence in the literature regarding the use of CKD-EPI cystatin C (CKD-EPIcys) over CKD-EPI creatinine (CKD-EPIcr) based equations in the elderly population [8-11]. Whilst a recent meta-analysis of sixteen studies has shown use of the CKD-EPIcys equation strengthens the association between the eGFR and the risks of death and end-stage renal disease, the majority of those included were younger individuals [12]. There is some evidence to suggest that there is no benefit in using the CKD-EPIcys equation in the elderly population [8, 9].

Moreover, advanced CKD is associated with excess cardiovascular morbidity and mortality [1, 2] and whether eGFR calculated using CKD-EPIcys equations are better indicators of CVD risk than CKD-EPIcr has been less studied. Therefore, to address these issues we have 1)

compared the predictive value of CKD-EPIcr and the CKD-EPIcys equations for all-cause and CVD mortality; 2) compared mortality by stages of CKD and 3) examined the association between these CKD-EPI equations with cardiovascular risk markers including arterial stiffness and cardiac markers in a cohort of older men in primary care.

Methods

The British Regional Heart Study is a prospective study which recruited a socioeconomically and geographically representative cohort of 7735 men from 24 British towns between 1978 and 1980. This present study is an analysis of the data from the 2010-2012 re-examination when all surviving men (n = 3137), aged between 71–92 years, were sent a postal questionnaire and invited for a 30th year re-examination with the men being followed up until June 2016. The population studied was socio-economically representative and comprised predominantly white Europeans (>99%). 2137 (68%) men completed the postal questionnaire and 1722 (55%) men attended the re-examination [13]. Blood samples were collected after fasting for a minimum of six hours and were stored at −70°C. The National Research Ethics Service (NRES) Committee London provided ethical approval for the data collection. All men provided written informed consent to the investigations, which were carried out in accordance with the Declaration of Helsinki. The presence of co-morbidity was based on the men self-reporting a doctor made diagnosis.

CVD risk factors

Physical examination included blood pressure (BP), height and weight, from which body mass index (BMI) was calculated [14]. Details of measurement and classification for smoking status and physical activity in this cohort have been previously described [15]. The use of antihypertensive medication was based on self-reported medication history. Measurements of metabolic, inflammatory and endothelial markers were taken as described previously [14, 16,

17]. C-reactive protein (CRP) (marker of inflammation) was assayed by ultra sensitive nephelometry (Dade Behring, Milton Keynes, UK). Interleukin-6 was assayed using a high-sensitivity ELISA (R & D Systems, Oxford, UK). NT-pro brain natriuretic peptide (BNP) and cardiac troponin T (cTnT) were measured using the Elecsys 2010 electrochemiluminescence method (Roche Diagnostics, Burgess Hill, UK). Prevalent diabetes included men with doctor-diagnosed diabetes and men with fasting blood glucose ≥7 mmol/L. Femoral PWV was assessed by two vascular technicians as previously described [18, 19].

CKD assessment

1639 men had at least one renal function blood measurement (83 missing). Cystatin C was measured using an automated method on a biochemistry analyser (c311, Roche Diagnostics, Burgess Hill, UK) and was calibrated and quality controlled using the manufacturers reagents. Creatinine was measured using an automated analyser and GFR was estimated using the CKD-EPIcr [20] and CKD-EPIcys [21] equations:

$$\text{CKD EPI creatinine} = 141 \times \min\left(\frac{\text{creatinine}}{0.9}, 1\right)^{-0.411} \times \max\left(\frac{\text{creatinine}}{0.9}, 1\right)^{-1.209} \times 0.993^{\text{age}}$$

where "min" indicated the minimum of creatinine/0.9 or 1 and "max" indicates the maximum of creatinine/0.9 or 1.

$$\text{CKD EPI cystatin C} = 133 \times \min\left(\frac{\text{cystatin C}}{0.8}, 1\right)^{-0.499} \times \max\left(\frac{\text{cystatin C}}{0.8}, 1\right)^{-1.328} \times 0.996^{\text{age}}$$

where "min" indicated the minimum of cystatin C/0.8 or 1 and "max" indicates the maximum of cystatin C/0.8 or 1.

Estimated GFR is expressed in millilitres per min per 1.73 meters squared, weight in kilograms, age in years, cystatin C and creatinine in milligrams per decilitre. The men were initially classified into 6 CKD stages 1, 2, 3a, 3b, 4 and 5. Stage 1 and 2 indicates normal or mild CKD. Patients with CKD stage 3 had moderate CKD, stage 4 represents severe CKD and

stage 5 indicates kidney failure. In subsequent analyses three categories were defined: those with eGFR ≥ 60ml/min/1.73^2 (CKD stages 1 and 2), eGFR ≥ 30 and < 60ml/min/1.73^2 (CKD stages 3a [eGFR 45-59ml/min/1.73^2] and 3b [eGFR 30-44ml/min/1.73^2]), eGFR < 30ml/min/1.73^2 (CKD stages 4 and 5). Of the 1639 men, 3 men had missing data on creatinine and 35 men had missing data on cystatin C.

Mortality follow-up

All men were followed prospectively for CVD and all-cause mortality from re-examination (2010-2012). In the present analyses, total mortality events are based on follow-up from re-screening in 2010-2012 to 30th June 2016, a mean follow-up period of 5 years. Information on death was collected through the established "tagging" procedures provided by the National Health Service central registers. In order to obtain death notifications, details were supplied to the centre which enabled them to identify and tag the relevant cases [22]. Cardiovascular deaths included all those with International Classification of Diseases (ICD-9th Revision) codes 390–459.

Statistical analysis

Distributions of HbA1c, glucose, insulin, C-reactive protein (CRP), interleukin 6 (IL-6), NT-pro BNP and cTnT were highly skewed and log transformation was used. Comparisons of baseline characteristics between the CKD groups were carried out using the chi squared test for categorical variables and analysis of variance for continuous variables.

Cox proportional hazards models were used to assess hazard ratios (HRs) for the three CKD definitions compared to subjects without CKD (i.e. stages 1 and 2) at baseline with respect to cardiovascular and all-cause mortality during follow up. Receiving operating characteristic (ROC) analysis was used to compare the predictive ability of the two equations. We also evaluated how all-cause mortality for each eGFR category changed depending on the

equation used. The ability of the CKD-EPIcys equation to reclassify mortality risk was assessed using methods suggested by Pencina *et al.* [23] by calculating the net reclassification improvement (NRI). All analyses were performed using SAS version 9.3 (SAS, Cary, North Carolina).

Results

This study was based on 1639 men aged 72-91 years who attended the 2010-2012 re-examination and who had at least one renal blood measurement. Of these 1601 men had both renal blood measurements. Table 1 shows the prevalence of CKD stages using the CKD-EPIcr and the reclassification of CKD stages when using CKD-EPIcys in comparison to CKD-EPIcr. 32% were classified as having CKD using the creatinine based equation compared to 32.4% using the cystatin C based equation. The CKD-EPIcys reclassified 15.9% of men with no CKD (stages 1 and 2) using the CKD-EPIcr to having CKD and 32.4% of those with CKD (eGFR < 60ml/min/1.73^2) to no CKD. The largest reclassification was seen in men with CKD stage 3a; use of CKD-EPIcys resulted in the reclassification of 43.9% of those with CKD stage 3a to CKD stages 1 and 2 (i.e. no CKD) and 17.5% to a more advanced CKD stage.

Cohort characteristics

Men with no CKD, regardless of which equation was used, were the youngest and had the lowest prevalence of diabetes, inactivity, manual workers, use of anti-hypertensives, myocardial infarction (MI), heart failure (HF), and stroke (Table 2).

Vascular risk markers and subclinical atherosclerosis

Table 3 shows the association between CKD stages and age adjusted mean levels of cardiovascular risk markers. Both CKD-EPIcr and CKD-EPIcys showed significant associations with systolic blood pressure, HDL-C, inflammation, vWF, and cardiac markers

(NT-proBNP and cTnT). Only CKD-EPIcr was associated with mean levels of insulin and triglycerides. No association was seen with arterial stiffness.

Mortality

During a median follow-up time of 5 years, 300 of the 1639 men died. Of these, 99 men (33.0%) died of a cardiovascular cause. Table 4 shows the hazard ratio (HR) for all-cause and CVD mortality for the three main CKD stages (stages 1+2, 3a+3b and 4+5). In the age adjusted analysis, irrespective of CKD equations those with CKD stages 3, 4 and 5 showed significantly higher risk of all-cause and CVD mortality. Adjustment for major CVD risk factors (Table 4) slightly attenuated the association but the relationships with all-cause and CVD mortality remained significant. In particular, those classified as stage 3 CKD irrespective of CKD equations showed significantly higher risk of CVD and all-cause mortality even after adjustment than those with no CKD. Comparisons of ROC-AUC for the two CKD equations showed no significant difference in their predictive ability for mortality. In the multivariate analysis the AUC for CKD-EPIcr vs CKD-EPIcys were 0.733 (0.029) vs 0.737 (0.029) (p = 0.46 for difference) for all-cause mortality and AUC = 0.746 (0.028) vs 0.754 (0.029) (p = 0.22 for difference) for CVD mortality.

We also examined the association between the two CKD equations and all-cause mortality separately in men with and without prevalent CVD or diabetes adjusting for major CVD risk factors. The associations were seen in both groups. In men with no prevalent CVD or diabetes (n = 1080), compared to those with no CKD, those with any CKD (stages 3, 4 or 5) had an adjusted HR (95% CI) of 1.39 (1.02, 1.89) and 1.50 (1.09, 2.05) using CKD-EPIcr and CKD-EPIcys respectively. In the subset of participants with CVD or diabetes (n = 559), the corresponding HRs were 1.81 (1.19, 2.75) and 1.99 (1.30, 3.04) respectively.

We also examined the all-cause mortality risk in those categorised as any CKD (stages 3a+3b+4+5) and no CKD based on the EPIcr and EPIcys equation and 4 groups were used: No CKD (both equations; n = 916) (reference group), CKD (using CKD-EPIcr only; n = 166), CKD (using CKD-EPIcys only; n = 173) and those classified as CKD for both equations (n = 346). In multivariate analyses those with CKD based on creatinine who were reclassified downwards on the basis of cystatin C showed similar risk to those with no CKD. Those who were reclassified as CKD on the basis of cystatin C showed higher risk than those with no CKD but this difference was not significant. The HR for the four groups were 1.00, 1.03 (0.66, 1.59), 1.31 (0.89, 1.92) and 1.99 (1.48, 2.67) respectively.

Finally, we assessed the NRI for all-cause mortality using the CKD-EPIcys equation for the three CKD-EPIcr CKD categories (1+2, 3a+3b and 4+5). The reclassification of men who died and who did not die is shown in Table 5. Among men who died 49 men (16.8%) were reclassified to a higher CKD stage using the CKD-EPIcys equation and 28 men (9.6%) were reclassified to a lower CKD stage with the CKD-EPIcys equation. The net gain in reclassification in those who died was 0.087 ($p < 0.01$). Among men who did not die 77% were classified as the same CKD stage (149 were reclassified up and 144 were reclassified down). The net gain in those who did not die (NRI = 0.004) was not significant. The overall NRI was 0.07 ($p = 0.02$).

Discussion

Our study has shown that in a representative sample of British men aged 71–92 years drawn from primary care, use of the CKD-EPIcys equation to calculate eGFR resulted in a similar prevalence of CKD compared to the CKD-EPIcr equation. However, cystatin C reclassified, in particular, a large proportion of those with CKD stage 3 to no CKD and these men showed similar risk to those with no CKD. However, the majority of men classified as having no CKD

using the creatinine based equation were also classified as having no CKD using the cystatin C based equation. Both equations predicted all-cause and CVD mortality and overall CKD-EPIcys was not a significantly better predictor of all-cause or CVD mortality than CKD-EPIcr as measured by the AUC. The overall net reclassification improvement for total mortality although statistically significant was largely due to the events component. No improvement was seen for the non-events component. A recent study reported that use of eGFR calculated using CKD-EPIcys did not improve prediction of mortality in older men with CKD stage 3a in primary care [8]. This study only looked at participants with CKD stage 3; however, they were followed up, similar to our study, for 5 years. Survival models in this study were adjusted for age, sex, previous CVD as well as urine albumin-to-creatinine ratio, haemoglobin, albumin and bicarbonate which differed from our study. Our study extends the findings of Shardlow *et al*. to a general older population with and without CKD in primary care. We also investigated the association between CKD (assessed using the CKD-EPIcys and CKD-EPIcr equations) and a wide range of metabolic, cardiac, inflammatory and non-invasive markers of arterial disease not previously reported.

CKD and vascular risk

Our findings confirm the known association between CKD and vascular risk factors including blood pressure, lipids, cardiac markers (NT-proBNP, cTnT) and inflammation [24-29]. With the exception of insulin, glucose and triglycerides both measures of eGFR showed significant associations with vascular risk markers. However, only, the CKD-EPIcr equation showed significant association with insulin. In contrast we found no association between CKD and arterial stiffness which is consistent with previous reports [30]. Overall both equations showed significant associations with most of the major vascular risk markers.

CKD and mortality

In this study of older men, the use of CKD-EPIcys did not improve the prediction of mortality in the general elderly male cohort overall as measured by the AUC. The findings indicate that the use of CKD-EPIcr is sufficient as an initial screening tool. Only 16% of men were reclassified as having CKD on the basis of cystatin C and these men showed a slightly higher mortality risk than those with no CKD but the difference was not significant. The non-significant findings may be due to the small numbers in the study and larger studies are needed to confirm this. However, our findings that those classified as having CKD on the basis of creatinine but not on cystatin C based equation did not show higher risk than those with no CKD supports NICE guidelines in the use of cystatin C in those with CKD stage 3a to rule out CKD [7].

Few studies have compared the association between CKD-EPIcr and CKD-EPIcys based equations with mortality in the general older adult population and the findings have been inconsistent. Our findings confirm previous reports from a prospective cohort study of 1165 elderly women aged over 70 years in Western Australia followed up for 10 years which found the CKD-EPIcys equation was not superior in predicting all-cause mortality or CVD events compared to the CKD-EPIcr equation [9]. In this study, similarly to ours, the findings were adjusted for age, BMI, smoking history, diabetes, systolic blood pressure, use of antihypertensive medications, prevalent renal disease and CVD. Our adjustments also included cholesterol and prevalent heart failure.

By contrast one study has found CKD-EPIcr is associated with a U-shaped increased HR of mortality with both high and low eGFR being associated with increased mortality whilst using the CKD-EPIcys equation showed a linear association between eGFR and all-cause mortality [31]. Of note, participants in this study had a mean age of 85 years, which is older than our cohort and had a shorter follow up of 2.6 years. A further study looking at 2994 community dwelling men with a mean age of 76.4 years showed that the CKD-EPIcr equation was inferior to the CKD-EPIcys equation in predicting all-cause mortality [10]. The reasons for the differences in findings are not clear but this study included a multi-ethnic population who were

on average slightly younger and included men under 70 years of age and had a longer follow up time of 7.3 years compared to 5 years in our study.

Strengths and limitations

The strengths of our study include the fact that it is a study of older men, a group who are at high risk of CKD and vascular disease and a group in whom there has been limited evidence for the validity of cystatin C based eGFR equations. However, the BRHS includes a predominantly older white male population of European extraction. This limits generalisability to women, middle-aged adults and ethnic minority groups. The response rate for the baseline assessment in this study was 55%, and, therefore, the issue of survival bias cannot be overlooked. Although men who participated in our re-examination study were healthier (younger, more active, lower BMI, less disabled, lower prevalence of CVD and diabetes) at the previous examination 10 years earlier than those who did not, there was little difference in prevalence of CKD between responders and non-responders. Although healthy survival bias may underestimate the prevalence of CKD in older adults and the incident mortality rates may be lower than the total population, this should not bias the nature of the association between CKD and mortality. Moreover, we have shown that the two equations showed broadly similar significant associations with mortality in men with and without CVD or diabetes. Whilst we have focused on CKD in this study, acute kidney injury (AKI, i.e. a rapid decline in GFR), is an important clinical problem encountered on a day-to-day basis. Studies have shown that compared to creatinine, cystatin C is an earlier marker of AKI which we have not discussed in this paper [6]. In the elderly, using a cystatin C based equation has been shown to estimate larger declines in kidney function than a creatinine based equation [32]. We have used single measures of creatinine and cystatin C to calculate eGFR and we have not looked at changes in eGFR or compared this to the gold standard measured GFR as these data were not available. Another potential limitation was the lack of albuminuria measurement in this cohort.

Conclusion

Estimating GFR using CKD-EPIcys leads to a similar prevalence of CKD compared to CKD-EPIcr although a large proportion of stage 3a CKD were reclassified as having no CKD. Assessment of CKD using CKD-EPIcys does not significantly improve prediction of all-cause and CVD mortality in older white British men when compared to CKD-EPIcr. Both CKD equations showed similar associations with inflammatory and cardiac risk markers and markers of subclinical atherosclerosis. Creatinine measurement is inexpensive and widely available. Data from our study supports the use of CKD-EPIcr as an initial screening tool for the assessment of eGFR at least in older white British men although the CKD-EPIcys equation may be useful in reclassifying those with CKD stage 3a.

Contributions

SGW initiated the concept and design of the paper. SZ analysed the data with help from OP and drafted the manuscript. SGW, JPJH, EAE, PHW contributed to the interpretation of data. EAE, JPJH, LL, PHW and SGW contributed to the acquisition of the data. All authors assessed the manuscript critically for important intellectual content and approved the final version.

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Competing Interests

The authors report no conflicts of interest.

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Ethics statement

The National Research Ethics Service (NRES) Committee London provided ethical approval for the data collection. All men provided written informed consent to the investigations, which were carried out in accordance with the Declaration of Helsinki.

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Table 1. Reclassification of CKD stages using CKD-EPIcys in comparison to CKD-EPIcr

CKD stages		CKD-EPIcys							Total (% of all men)
		1	2	3a	3b	4	5		
CKD-EPIcr	**1**	29 (90.6%)	3 (9.4%)	0	0	0	0	100%	32 (2.0%)
	2	348 (32.9%)	536 (50.7%)	156 (14. 8%)	16 (1.5%)	1 (0.1%)	0	100%	1057 (66.0%)
	3a	31 (9.2%)	117 (34.7%)	130 (38.6%)	57 (16.9%)	2 (0.6%)	0	100%	337 (21.0%)
	3b	3 (2.0%)	15 (10.1%)	35 (23.5%)	73 (49.0%)	23 (15.4%)	0	100%	149 (9.3%)
	4	0	0	1 (4.2%)	5 (20.8%)	17 (70.8%)	1 (4.2%)	100%	24 (1.5%)
	5	0	0	0	0	1 (50%)	1 (50%)	100%	2 (0.12%)
Total (% of all men)		411 (25.7%)	671 (41.9%)	322 (20.1%)	151 (9.4%)	44 (2.8%)	2 (0.1)		1601

CKD, chronic kidney disease

CKD stage 1 – eGFR > 90ml/min/1.73^2; CKD stage 2 - eGFR 60-90ml/min/1.73^2; CKD stage 3a - eGFR 45-59ml/min/1.73^2; CKD stage 3b - eGFR 30-44ml/min/1.73^2; CKD stage 4 - eGFR 15-29ml/min/1.73^2; CKD stage 5 – eGFR < 15ml/min/1.73^2

Table 2. Baseline characteristics according to CKD categories by CKD-EPI equations

		CKD-EPI Cr			p	CKD-EPI Cys			p
		1 + 2 n = 1112[a]	3a + 3b n = 497[a]	4 + 5 n = 27[a]		1 + 2 n = 1084[a]	3a + 3b n = 474[a]	4 + 5 n = 46[a]	
Age	Mean±std	77.5 ± 4.2	80.3 ± 5	79.1 ± 4.8	**<.0001**	77.5 ± 4.2	80.31 ± 5	79.4 ± 5	**<0.0001**
Obese	%	19.1	19.2	26.9	0.60	18.9	18.1	31.84	0.08
Smoker	%	3.1	4.5	3.9	0.38	2.5	4.9	11.1	**0.001**
Inactive	%	34.0	47.3	55.6	**<0.0001**	33.1	47.5	63.0	**<0.0001**
Manual Social Class	%	42.6	49.7	55.6	**0.04**	43.2	48.8	52.1	0.09
Diabetes	%	15.0	19.3	25.9	**0.04**	15.6	18.2	21.7	0.28
Using Antihypertensives	%	48.8	64.0	66.8	**<0.0001**	47.7	65.0	73.0	**<0.0001**
MI	%	12.8	20.4	30.4	**0.0002**	13.0	19.2	31.6	**0.0002**
HF	%	1.2	4.4	15.8	**<0.0001**	1.1	4.1	18.2	**<0.0001**
Stroke	%	8.0	15.2	8.0	**<.00001**	8.2	14.7	9.5	**0.0006**

Table 2. Cohort demographics

a, maximum n in group, varies slightly with missing co-variate data

MI, myocardial infarction; HF, heart failure; PVD, peripheral vascular disease

CKD stage 1 – eGFR > 90ml/min/1.73^2; CKD stage 2 - eGFR 60-90ml/min/1.73^2; CKD stage 3a - eGFR 45-59ml/min/1.73^2; CKD stage 3b - eGFR 30-44ml/min/1.73^2; CKD stage 4 - eGFR 15-29ml/min/1.73^2; CKD stage 5 – eGFR < 15ml/min/1.73^2

Table 3. Association between CKD stages assessed using cystatin C vs creatinine and metabolic risk factors, inflamma[...]
markers and non-invasive vascular measurements

	CKD-EPIcr								p		1 + 2 n = 1084[b]	
	1 + 2 **n = 1112[b]**		**3a + 3b** **n = 497[b]**		**4 + 5** **n = 27[b]**		**p**				**1 + 2** **n = 1084[b]**	
Metabolic risk factors (age adjusted mean (95% CI))												
SBP (mmHg)	147.8	(146.7, 149.0)	143.8	(142.1, 145.5)	138.5	(131.3, 145.8)	**<0.0001**				148.1	(146.9, 149.2)	14[...]
HDL-C (mmol/L)	1.50	(1.47, 1.52)	1.36	(1.32, 1.40)	1.25	(1.10, 1.41)	**<0.0001**				1.49	(1.47, 1.52)	
Triglycerides (mmol/L)	1.23	(1.19, 1.27)	1.43	(1.37, 1.49)	1.67	(1.44, 1.91)	**<0.0001**				1.28	(1.24, 1.32)	
HbA1c (%)	5.79	(5.75, 5.8)	5.95	(5.89, 6.00)	6.13	(5.87, 6.39)	**<0.0001**				5.81	(5.77, 5.75)	5[...]
Glucose[a] (mmol/L)	5.61	(5.53, 5.70)	5.66	(5.53, 5.75)	5.80	(5.37, 6.30)	0.33				5.64	(5.53, 5.70)	5[...]
Insulin[a] (Mu/L)	7.78	(7.46, 8.08)	9.00	(8.50, 9.58)	9.00	(6.96, 11.59)	**0.0004**				7.39	(7.77, 8.33)	8[...]
Inflammatory and endothelial markers (age adjusted mean (95% CI))												
CRP[a] (mg/L)	1.23	(1.15, 1.32)	1.64	(1.47, 1.84)	3.46	(2.18, 4.57)	**<0.0001**				1.12	(1.04, 1.20)	
IL-6[a] (pg/mL)	2.94	(2.83, 3.09)	3.32	(3.10, 3.56)	6.93	(5.23,9.51)	**<0.0001**				2.80	(2.69, 2.92)	3[...]
vWF (IU/dL)	130.4	(126.0, 134.7)	142.1	(135.7, 148.9)	181.0	(153.4, 208.6)	**<0.0001**				126.9	(122.4, 131.3)	14[...]
Cardiac markers (age adjusted mean (95% CI))												
NT-pro BNP[a] (pg/mL)	122.7	(114.4, 134.3)	177.7	(157.6, 200.5)	871.3	(523.2, 1480.3)	**<0.0001**				115.6	(106.7, 125.21)	19[...]
cTnT[a] (pg/ml)	10.07	(9.68, 10.38)	13.32	(12.56, 14.15)	26.0	(20.49, 33.12)	**<0.0001**				9.68	(9.30, 10.07)	14[...]
Non-invasive vascular measurements (age adjusted mean (95% CI))												
PWV (m/s)	10.19	(10.09, 10.28)	10.11	(9.95, 10.26)	9.47	(8.82, 10.12)	0.09				10.15	(10.04, 10.25)	10[...]

a, geometric mean; b, maximum n in group, varies slightly with missing co-variate data

SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high density lipoprotein cholesterol; HbA1c, glyca[...]
protein; IL-6, interleukin 6; vWF, von Willebrand factor; FEV1, forced expiratory volume in 1s; NT-pro BNP, N-terminal [...]
cardiac troponin T;; PWV, pulse wave velocity;

CKD stage 1 – eGFR > 90ml/min/1.73^2; CKD stage 2 - eGFR 60-90ml/min/1.73^2; CKD stage 3a - eGFR 45-59ml/min/1[...]
44ml/min/1.73^2; CKD stage 4 - eGFR 15-29ml/min/1.73^2; CKD stage 5 – eGFR < 15ml/min/1.73^2

Missing data SBP (n=3); triglycerides (n=1); HDL-C (n=1); Hba1c (n=42); glucose (n=103); insulin (n=27); CRP (n=38); [...]
(n=37); cTnT (n=37); PWV (n=29)

Table 4. Hazard ratio for all-cause and CVD mortality according to CKD categories based on eGFR equations derived from serum creatinine (CKD-EPIcr) and from cystatin C (CKD-EPIcys)

		Mortality Hazard Ratio (95% confidence interval)			
		All-Cause		CVD	
		Age-adjusted	Adjusted +	Age-adjusted	Adjusted +
CKD-EPIcr CKD Stages	**1+2**	1	1	1	1
	3a+3b	1.60 (1.26, 2.05)	1.45 (1.13, 1.87)	2.02 (1.32, 3.11)	1.65 (1.05, 2.58)
	4+5	3.88 (2.23, 6.74)	3.51 (1.99, 6.17)	6.62 (2.94, 14.88)	4.72 (2.03, 11.01)
	3a+3b+4+5 (CKD)	1.70 (1.34, 2.16)	1.53 (1.20, 1.96)	2.21 (1.46, 3.36)	1.77 (1.14, 2.75)
CKD-EPIcys CKD Stages	**1+2**	1.00	1.00	1.00	1.00
	3a+3b	1.75 (1.36, 2.25)	1.66 (1.28, 2.15)	2.33 (1.48, 3.65)	2.01 (1.26, 3.20)
	4+5	3.63 (2.32, 5.68)	2.85 (1.75, 4.64)	6.45 (3.26, 12.78)	3.92 (1.82, 8.45)
	3a+3b+4+5 (CKD)	1.88 (1.48, 2.40)	1.74 (1.35, 2.23)	2.62 (1.70, 4.05)	2.13 (1.35, 3.37)

CKD, chronic kidney disease

CKD stage 1 – eGFR > 90ml/min/1.73^2; CKD stage 2 - eGFR 60-90ml/min/1.73^2; CKD stage 3a - eGFR 45-59ml/min/1.73^2; CKD stage 3b - eGFR 30-44ml/min/1.73^2; CKD stage 4 - eGFR 15-29ml/min/1.73^2; CKD stage 5 – eGFR < 15ml/min/1.73^2

+Adjusted for age, smoking, cholesterol, systolic blood pressure, BMI, prevalent diabetes, prevalent CVD (stroke, MI), prevalent heart failure, and BP lowering drugs.

Table 5. Reclasssification of men using the CKD-EPIcys equation among men who died and those who did not die on follow-up

	CKD-EPI Cys				
	1+2	**3a+3b**	**4+5**	**Total**	
CKD-EPIcr	**Number of men who died (n = 291)**				
1+2	110 (75.3%)	36 (24.7%)	0	**146**	
3a+3b	26 (19.7%)	93 (70.5%)	13 (9.8%)	**132**	**49 classified up** **28 classified down**
4+5	0 (0%)	2 (15.4%)	11 (84.6%)	**13**	
	NRI = 0.07 *p* = 0.01				
	Number of men who did not die (n = 1310)				
1+2	806 (85.4%)	136 (14.4%)	1 (0.1%)	**943**	**149 classified up** **144 classified down**
3a+3b	140 (39.5%)	202 (57.1%)	12 (3.4%)	**354**	
4+5	0	4 (30.8%)	9 (69.2%)	**13**	
	NRI = 0.004 *p* = 0.39				
	Overall NRI = 0.068 *p* = 0.02				

CKD, chronic kidney disease; NRI, net reclassification improvement

CKD stage 1 – eGFR > 90ml/min/1.73^2; CKD stage 2 - eGFR 60-90ml/min/1.73^2; CKD stage 3a - eGFR 45-59ml/min/1.73^2; CKD stage 3b - eGFR 30-44ml/min/1.73^2; CKD stage 4 - eGFR 15-29ml/min/1.73^2; CKD stage 5 – eGFR < 15ml/min/1.73^2