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Novel Filtration Markers as Predictors of All-Cause and Cardiovascular Mortality in US Adults

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Abstract

Background—New filtration markers, including β-trace protein (BTP) and β_2-microglobulin (B2M), may, similar to cystatin C, enable a stronger prediction of mortality compared to serum creatinine-based estimated glomerular filtration rate ($eGFR_{cr}$). We sought to evaluate these mortality associations in a representative sample of US adults.

Study Design—Prospective cohort study.

Setting & Participants—6445 adults age ≥ 20 years from the Third National Health and Nutrition Examination Survey (1988–1994) with mortality linkage through December 31, 2006.

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Supplementary Material
Table S1: Range of marker values across weighted quantiles of $eGFR_{cr}$, cystatin C, BTP, and B2M.
Table S2: Multivariable-adjusted HRs of all-cause, cardiovascular disease, and coronary heart disease mortality, by quintile of kidney function.
Note: The supplementary material accompanying this article (doi:_____) is available at www.ajkd.org

Descriptive Text for Online Delivery
Hyperlink: Supplementary Table S1 (PDF)
About: Range of marker values across weighted quantiles of $eGFR_{cr}$, cystatin C, BTP, and B2M.
Hyperlink: Supplementary Table S2 (PDF)
About: Multivariable-adjusted HRs of all-cause, cardiovascular disease, and coronary heart disease mortality, by quintile of kidney function.

Predictors—Serum cystatin C, BTP, and B2M and $eGFR_{cr}$ categorized into quintiles, with the highest quintile (lowest for $eGFR_{cr}$) split into tertiles (sub-quintile Q5a–Q5c).

Outcomes—All-cause, cardiovascular disease, and coronary heart disease mortality.

Measurements—Demographic and multivariable adjusted Cox proportional hazard models.

Results—During follow-up, 2392 deaths (cardiovascular, 1079; coronary heart disease, 605) occurred. All four filtration markers were associated with mortality risk after adjusting for demographics (p-trend<0.02). Adjusted for mortality risk factors, compared to the middle quintile, the highest sub-quintiles for cystatin C (Q5c: HR, 1.94; 95% CI, 1.43–2.62), BTP (Q5c: HR, 2.14; 95% CI, 1.56–2.94), and B2M (Q5c: HR, 2.58; 95% CI, 1.96–3.41) were associated with increased all-cause mortality risk while the association was weaker for $eGFR_{cr}$ (Q5c: HR, 1.31; 95% CI, 0.84–2.04). Associations persisted for the novel markers and not for $eGFR_{cr}$ at $eGFR_{cr}$ ≥60 mL/min/1.73 m^2. Trends were similar for cardiovascular disease and coronary heart disease mortality.

Limitations—Single measurements of markers from long-term stored samples.

Conclusions—The strong association of cystatin C with mortality compared to serum creatinine estimates is shared by BTP and B2M. This supports the utility of alternative filtration markers beyond creatinine when improved risk prediction related to decreased GFR is needed.

Index Words

Cystatin C; β-trace protein; β2-microglobulin; estimated glomerular filtration rate; mortality; Third National Health and Nutrition Examination Survey

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A reduced estimated glomerular filtration rate (eGFR) is associated with increased risk of all-cause mortality and cardiovascular disease morbidity and mortality.[1–5] In epidemiologic studies, GFR is usually estimated from endogenous serum filtration markers, so associations with risk may be due to direct effects of markers or due to non-GFR determinants of their serum levels (generation, tubular secretion and reabsorption, and extra-renal elimination). Creatinine, an inert amino acid metabolite produced by muscle,[6] is influenced by muscle mass, diet, and tubular secretion.[5,7] Cystatin C is a low-molecular-weight serum protein that is filtered and metabolized by the kidney and increasingly recommended as an alternative filtration marker.[8] Cystatin C is also inert, with serum levels less influenced by muscle mass than creatinine and is associated more strongly with cardiovascular events and mortality than creatinine-based eGFR ($eGFR_{cr}$). [4,9,10] However, it is not known whether the stronger associations of cystatin C with outcomes reflects confounding with other non-GFR determinants.[9] The difficulty in measuring GFR in large population studies hampers the identification of non-GFR determinants of filtration markers and the study of their associations with outcomes. Comparisons of associations among multiple filtration markers in the same population can reveal similarities and differences in their role as risk predictors, enabling optimal evaluation of the relative contribution of GFR and non-GFR determinants as well as advantages or limitations of specific markers as risk predictors.

β-trace protein (BTP), a prostaglandin-D synthase produced in the central nervous system,[11] and β_2-microglobulin (B2M), a component of class I major histocompatibility molecules

found on the surface of nucleated cells,[12] are novel filtration markers that share some properties with cystatin C.[13–18] They are low molecular weight serum proteins that are freely filtered by the glomeruli, reabsorbed, and almost entirely metabolized by the renal tubules. Prior work suggests that, similar to cystatin C, BTP and B2M have high correlations with measured GFR and are associated with increased risk of mortality and kidney outcomes compared to $eGFR_{cr}$,[19–24] suggesting less confounding by non-GFR determinants than for creatinine. 1 However, prospective studies of BTP and B2M are few and limited to middle-aged or elderly populations[24,25] or those with cardiovascular or kidney disease.[21,23,26,27] The objective of this study was to determine whether BTP and B2M share the stronger associations with all-cause and cardiovascular mortality of cystatin C compared to $eGFR_{cr}$ and to evaluate whether novel filtration markers improved risk reclassification beyond $eGFR_{cr}$ in a nationally representative sample of adults in the United States.

METHODS

Study Sample

The Third National Health and Nutrition Examination Survey (NHANES III) is a multistage, stratified, clustered probability sample of the non-institutionalized civilian US population conducted between 1988 and 1994.[28] Our study sample was drawn from the NHANES III Cystatin C Project (n=7596);[29] participants who were <20 years of age (n=719), missing sufficient data for National Death Index linkage (n=5),[30,31] missing BTP or B2M measurements (n=63), or missing one or more multivariable covariates (n=364) were excluded, resulting in a final sample of 6445 participants. Protocols for conduct of this study were approved by the Institutional Review Boards of the National Center for Health Statistics (NCHS) and the Johns Hopkins Bloomberg School of Public Health. Informed consent was obtained from all participants.

Filtration Marker Measurement

Serum creatinine was measured in the original NHANES III protocol using a modified Jaffe reaction and standardized.[32] Serum cystatin C was measured using a particle-enhanced immunonephelometric assay[29,33] and standardized. BTP and B2M were measured from stored serum samples using N Latex BTP and B2M assays (Siemens Diagnostics, IL).[34] Short-term within-person variability was low for serum cystatin C (within-person coefficient of variation [CV_w], 6.8%), creatinine (CV_w, 7.6%) and B2M (CV_w, 8.4%) with slighter higher variability observed for BTP (CV_w=, 11.6%).[35] Serum BTP and B2M measurements were robust to storage and freeze-thaw cycles,[36] with inter-assay CVs of 8.6% and 3.8%, respectively. $eGFR_{cr}$ was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2009 creatinine equation.[37]

Outcome Assessment

Mortality status, underlying causes of death, and person-months of follow-up through December 31, 2006 was ascertained using the public-use NHANES III mortality linkage, which links participants to mortality data through the National Death Index. Underlying cause of death was assigned by the NCHS based on the 10th revision of the International Classification of Diseases (ICD-10) guidelines.[30,31,38] Outcomes of interest included all-

cause, cardiovascular (ICD-10, I00–I78), and coronary heart disease (ICD-10, I20–I25) mortality.

Additional Covariate Assessment

Body mass index was calculated from measured weight and height (kg/m^2). Current smoking status was based on self-report. Serum triglycerides, high-density lipoprotein (HDL) cholesterol, C-reactive protein (CRP) and plasma glucose were determined using blood samples collected during the Mobile Examination Center examination. Diabetes was defined as a self-reported physician diagnosis of diabetes, self-reported diabetes medication use, a non-fasting plasma glucose ≥ 200mg/dL, or a fasting plasma glucose ≥ 126mg/dL. Systolic blood pressure was measured during the Mobile Examination Center examination and the use of hypertension medication was based on self-report. Prevalent coronary heart disease was defined as a self-reported history of a physician-diagnosed heart attack. The urinary albumin-creatinine ratio (ACR, in mg/g) was determined using spot urine samples.

Statistical Analyses

Statistical analyses were performed in Stata Version 11.1 (StataCorp LP, http://www.stata.com/) using modified sampling weights approved by NCHS[29] and standard errors for estimates were obtained using the Taylor series (linearization) method. Serum cystatin C, BTP and B2M were compared to $eGFR_{cr}$ rather than serum creatinine to account for known associations of age, sex and race with non-GFR determinants of creatinine. Similar to previous work investigating cystatin C and mortality in the Cardiovascular Health Study[4] and comparing $eGFR_{cr}$, cystatin C, BTP and B2M in the ARIC (Atherosclerosis Risk in Communities) Study[24] and to provide a simple method to compare associations across markers measured on different scales, weighted quantiles (quintiles with quintile 5 split into tertiles) were created separately for each of the four filtration markers (category ranges presented in Table S1, available as online supplementary material). Quintile order was reversed for $eGFR_{cr}$ to have quintile 5 denote the lowest filtration level for all markers. Cox proportional hazards regression was used to assess the associations of $eGFR_{cr}$, cystatin C, BTP, and B2M separately with mortality outcomes. Due to possible non-linear associations, marker categories were modeled using indicator variables; quintile 3 was selected as the reference group to avoid undue influence of the lowest quintiles with few events. Models were initially adjusted for age, sex, and race and further in multivariable adjusted models for diabetes, current smoking, systolic blood pressure, hypertension medication use, HDL-cholesterol, natural log–transformed triglycerides, CRP (<0.22, 0.22–1.00, and >1.00 mg/dL), prevalent coronary heart disease, and natural log-transformed ACR. Regression coefficients from different models were compared using seemingly unrelated regression.[39] In a secondary analysis, BTP and B2M models were additionally adjusted for cystatin C. We conducted sensitivity analyses limited to participants with a baseline $eGFR_{cr}$ ≥60 mL/min/1.73m^2.

We used continuous and categorical net reclassification improvement (NRI)[40,41] to quantify the amount of correct and incorrect reclassification when cystatin C, BTP, and B2M are added to $eGFR_{cr}$ and when BTP and B2M are added to cystatin C and $eGFR_{cr}$ in multivariable-adjusted Poisson models to estimate 10-year predicted all-cause,

cardiovascular, and coronary heart disease mortality risk. The categorical NRI was based on 10-year predicted risk categories of <5%, 5%–20%, and >20%.

RESULTS

Baseline Characteristics

Baseline characteristics by $eGFR_{cr}$ category are presented in Table 1. In this general population sample, the cutoff for the lowest $eGFR_{cr}$ category (5c) was <65mL/min/1.73m^2, somewhat higher than the GFR threshold for CKD of 60 mL/min/1.73m^2. Within this largely normal $eGFR_{cr}$ range, adults in lower $eGFR_{cr}$ categories were older with a higher body mass index, systolic blood pressure, serum triglycerides, and urine ACR. Lower $eGFR_{cr}$ categories were also associated with a higher prevalence of diabetes, coronary heart disease, anti-hypertension medication use, higher CRP, and a lower prevalence of black race and current smoking. Modest overlap was observed across marker categories; among adults in $eGFR_{cr}$ Q5c, 61%, 55%, and 55% fall in Q5c for cystatin C, BTP, and B2M, respectively.

Correlation of Filtration Markers

After transformations to account for the reciprocal physiologic association of filtration markers with GFR, all four markers were positively correlated with one another (Table 2, all $p \leq 0.006$). The correlation between $eGFR_{cr}$ and 1/cystatin C ($r=0.52$) was intermediate between that with 1/B2M ($r=0.61$) and 1/BTP ($r=0.45$) with some of the novel filtration markers showing even stronger correlations with one another.

All-Cause Mortality

Over a median follow-up of 14.4 years, 2,392 deaths occurred. With adjustment for age, sex, and race, higher cystatin C, BTP, and B2M were associated with higher mortality risk (Figure 1, p-trend<0.001). Multivariable-adjusted hazard ratios (HR) for each filtration marker with all-cause mortality are presented in Table 3. For $eGFR_{cr}$, all-cause mortality risk was not significantly elevated within the lowest category (sub-quintile Q5c) when compared to the referent quintile 3 ($eGFR_{cr}$ 97–107mL/min/1.73m^2), with an HR of 1.31 (95% confidence interval [CI], 0.84–2.04). In contrast, all-cause mortality risk tended to increase with higher cystatin C, BTP, and B2M categories and was significantly increased in sub-quintile Q5c for cystatin C, BTP, and B2M (Table 3, HRs of 1.86, 2.07, and 2.44, respectively; all p<0.001). The associations of higher BTP and B2M, but not cystatin-C, with all-cause mortality were stronger than observed for $eGFR_{cr}$ (p=0.04, 0.01, and 0.09 respectively). When the multivariable BTP and B2M models were further adjusted for cystatin C, both BTP sub-quintile Q5c (HR, 1.60; 95% CI, 1.13–2.27) and B2M sub-quintiles Q5b (HR, 1.85; 95% CI, 1.27–2.71) and Q5c (HR, 2.42; 95% CI, 1.68–3.49) remained significantly associated with all-cause mortality. When compared to $eGFR_{cr}$ alone, using all four filtration markers improved risk classification based on both the continuous and categorical NRI, overall and in adults with normal $eGFR_{cr}$ (Table 4, p<0.05). The addition of cystatin C to $eGFR_{cr}$ improved risk classification although to a lesser extent for the continuous NRI for all-cause mortality in adults with normal $eGFR_{cr}$ while further addition of BTP and B2M only improved the continuous NRI (Table 4).

Cardiovascular Disease Mortality

Overall, 1,079 cardiovascular disease deaths occurred during follow-up. After multivariable adjustment, higher cystatin C, BTP, and B2M, but not lower $eGFR_{cr}$, were associated with significantly increased risk of cardiovascular disease mortality (Table 3), although the magnitude of these associations were not stronger than for $eGFR_{cr}$ based on seemingly unrelated regression. After further adjusting for cystatin C, the associations of higher BTP and B2M with cardiovascular mortality were no longer statistically significant. The use of $eGFR_{cr}$, cystatin C, BTP, and B2M compared to $eGFR_{cr}$ alone improved risk reclassification based on both the continuous and categorical NRI (Table 4). The addition of BTP and B2M to $eGFR_{cr}$ and cystatin C also improved continuous net risk classification, although the addition of these markers did not significantly improve categorical reclassification based on 10-year risk categories (Table 4).

Coronary Heart Disease Mortality

During follow-up, 605 coronary heart disease deaths occurred. Results were similar to those observed in multivariable-adjusted models for each filtration marker with all-cause and cardiovascular mortality, whereas the magnitude of the association for cystatin C with coronary heart disease mortality was greater than observed for BTP or B2M (HRs of 2.61, 2.33, and 2.15, respectively; Table 3). The associations of higher BTP and B2M with coronary heart disease mortality were attenuated and no longer significant when adjusted for cystatin C. Using all four markers improved risk classification when compared $eGFR_{cr}$ alone (Table 4, $p<0.001$). While the addition of BTP and B2M to $eGFR_{cr}$ and cystatin C improved risk classification based on the continuous NRI, the addition of these markers did not significantly improve risk prediction based on 10-year risk categories (Table 4).

Subgroup Analyses

In the sub-sample of 5,632 participants with baseline $eGFR_{cr} \geq 60$ mL/min/1.73m^2 (Table S2), $eGFR_{cr}$ was not a risk factor for all-cause, cardiovascular or coronary heart disease mortality (p-trend=0.3, 0.8, and 0.8, respectively). In contrast, all novel filtration markers showed strong associations with all-cause mortality (p-trend<0.001) and cardiovascular mortality (p-trend <0.002) and consistent but less statistically significant associations with coronary heart disease mortality. NRI values in this subsample comparing the four filtration markers to $eGFR_{cr}$ alone in a multivariable risk prediction models were similar in magnitude to those observed in the overall sample for both the continuous and categorical NRI (Table 4).

DISCUSSION

This is the first description of the risk associations of BTP and B2M in a nationally representative sample of US adults. The comparisons with creatinine and cystatin C provide clues about the association of GFR and the non-GFR determinants of filtration markers with mortality outcomes, which cannot be evaluated directly in large population studies. We observed that higher BTP and B2M were associated with an increased risk of all-cause, cardiovascular disease, and coronary heart disease mortality, and showed stronger associations than observed for lower $eGFR_{cr}$. Further, cystatin C, BTP, and B2M each

remained associated with all-cause and cardiovascular mortality among adults with $eGFR_{cr}$ ≥60mL/min/1.73m^2, where $eGFR_{cr}$ was largely unrelated to mortality. Finally, we observed that using all four markers led to modest improvements in 10-year risk prediction over $eGFR_{cr}$ in models adjusted for mortality and cardiovascular risk factors. These results suggest that the non-GFR determinants of serum creatinine may weaken the relationship of $eGFR_{cr}$ with mortality outcomes compared to alternative filtration markers whose estimates of GFR may allow more accurate risk predictions.

Serum levels of endogenous filtration markers are useful for estimating GFR and are expected to be related to prognosis. Required properties of an endogenous filtration marker are elimination largely by glomerular filtration and generation at a relatively constant rate, so that the marker serum level highly correlates with measured GFR after accounting for its known non-GFR determinants. Differences among filtration markers in the association of their serum levels with outcomes can reflect differences in direct effects of the markers or factors that affect their non-GFR determinants. Differences may also reflect differences in biological variation and measurement error. Prior studies have shown a strong correlation between serum levels of cystatin C, BTP and B2M with measured GFR[19–22] but other studies have shown marked differences among other low molecular weight serum protein concentrations in their correlation with GFR estimated from creatinine and cystatin C, potentially indicating differences in their non-GFR determinants.[42,43] Of note, other markers related to kidney disease, such as urinary albumin and hemoglobin, may also be associated with prognosis through other mechanisms, but are not strongly correlated with measured GFR. Consequently, filtration markers represent one class of prognostic markers in kidney disease. Distinguishing among prognostic markers according to their mechanism is important for understanding their utility in research and clinical practice.

Our findings are consistent with prior work comparing BTP and B2M to creatinine and cystatin C and substantially extend its conclusions. In the ARIC study, the combination of B2M, BTP and cystatin C, were more strongly associated than $eGFR_{cr}$ with all-cause mortality over 10 years follow-up among adults aged 54 years and older.[24] Our findings show that the stronger associations observed within this older population-based sample can be extended to a nationally representative sample with a broad range of age and ethnicity. In both the current study and ARIC study, the association persisted in adults with a baseline $eGFR_{cr}$ ≥60 mL/min/1.73m^2. Results from the ARIC study also indicated that a multi-marker approach incorporating cystatin C, BTP, B2M, and $eGFR_{cr}$ led to improvements in risk prediction when compared with $eGFR_{cr}$ alone.[24] Our results show that this approach also led to significant improvements in mortality risk prediction beyond $eGFR_{cr}$ and established cardiovascular risk factors in the general US adult population. Overall, a small but growing body of literature supports a consistent message that B2M and BTP share the advantages of cystatin C over $eGFR_{cr}$ as risk factors for mortality and cardiovascular disease.

The weaker mortality associations of $eGFR_{cr}$ than cystatin C, BTP and B2M in the present analysis may reflect the overestimation of $eGFR_{cr}$ in people with low muscle mass and low meat intake due to chronic illness, leading to higher risk in the highest $eGFR_{cr}$ quintile, and underestimation of $eGFR_{cr}$ in people with high muscle mass due to good health and higher

meat intake, leading to a lower risk of death in the lowest $eGFR_{cr}$ quintile. The alternative filtration markers that we studied are not known to be associated with muscle mass and diet, thus their risk associations are not confounded by these non-GFR determinants. Furthermore, they are produced by different tissues and are not part of a single metabolic pathway. However, we cannot rule out the possibility that the stronger mortality risk of the alternative filtration markers reflects confounding by factors associated with non-GFR determinants that potentially overestimate the contribution of higher serum levels to mortality risk. Several factors are associated with higher serum cystatin C, including current smoking, higher body mass index, lower HDL cholesterol, higher triglycerides, and higher CRP levels, a marker of inflammation.[9,29,44] Similarly in NHANES III, several factors are associated with higher serum BTP and B2M, including older age, hypertension, higher CRP, and lower HDL-cholesterol, whereas female sex and non-Hispanic black and Hispanic race/ethnicity are associated with lower BTP and lower body mass index is associated with lower B2M.[45] Some have suggested that BTP may play a role in cardiovascular disease, potentially through atherosclerotic pathways. BTP expression has been observed in heart tissue and BTP accumulation has been observed in atherosclerotic plaques.[46–48] Higher B2M has been associated with peripheral artery disease and arterial stiffness,[49,50] suggesting that B2M may influence mortality through atherosclerosis, tissue deposition, or other inflammatory-based mechanisms. The persistence of strong effect sizes after multivariable adjustment for these factors suggests that the observed associations are not likely due to the influence of the non-GFR determinants examined.

Unlike BTP and B2M, the lowest quintile of cystatin C was consistently protective for morality. This finding for cystatin C is consistent with previous reports.[4] The finding that the lowest quintiles of serum BTP and B2M are not consistently associated with lowest risk may suggest differences among these markers in non-GFR determinants at higher levels of GFR and needs to be replicated.

Prior work has shown that while GFR estimation equations based on either creatinine or cystatin C separately perform similarly well, the combination of these two markers can lead to more precise and accurate GFR estimates.[8,51] The results of our study and others suggest that BTP and B2M, in addition to cystatin C, may be useful as an adjunct to creatinine for GFR estimation and risk prediction across a broad range of clinical settings. We suggest that a panel with additional filtration markers has the potential to improve GFR estimation and prediction of adverse health outcomes over using only $eGFR_{cr}$. The growing literature about BTP and B2M suggests they provide promising avenues for developing a larger range of options for clinical testing in the future, although algorithms for combining filtration markers require further work, which may benefit from studies where measured GFR is available. Additionally, while assays for BTP and B2M are relatively low cost and available on automated analyzers and B2M is used in clinical practice (as a prognostic factor in multiple myeloma[52,53]), BTP is currently a research test and would require approval for clinical use. The current literature is most developed for cystatin C where clinical applications, including confirmation of CKD in patients with $eGFR_{cr}$ 45–59 mL/min/1.73m^2 without albuminuria or other markers of kidney damage.[8,54] The additional risk information provided by cystatin C appears to be shared by the other novel filtration markers examined in this study and does not appear to be a unique attribute specific to cystatin C. This

increases the confidence in cystatin C as a filtration marker as well as suggests that strategies for using multiple markers could result in better risk prediction.

Important strengths of our study include the measurement of four different filtration markers in a well-characterized, nationally representative population with over 15 years of follow-up for mortality. The filtration markers examined were measured using state-of-the-art methods and have high reliability.[36] The study also benefited from standardized measurement of covariates by trained clinic staff. There are limitations of this study that warrant mention. Serum levels of each filtration marker were based on a single measurement obtained after more than 20 years of storage. However, we have previously demonstrated that these measurements are reliable and robust to freeze-thaw cycles.[36] The use of single measurements does not account for potential within-person variability in measurements and may lead to exposure misclassification. However, part of the utility of combining multiple filtration markers in prediction is the reduction in misclassification based on single measurements for each marker. Finally, outcomes were assessed through death record linkage, so while we could examine cardiovascular or coronary heart disease mortality, we were unable to examine non-fatal cardiovascular or kidney events.

In summary, the increased mortality risk observed with elevated cystatin C was also shared by two other filtration markers, BTP and B2M, and extended to the normal range of $eGFR_{cr}$ ($\geq$60 mL/min/1.73 m^2) in a representative sample of the US adult population. Thus, the stronger mortality risk associated with cystatin C over $eGFR_{cr}$ is not unique to cystatin C and supports the utility of using cystatin C or other novel filtration markers beyond creatinine in situations where we need to improve risk prediction related to decreased GFR in US adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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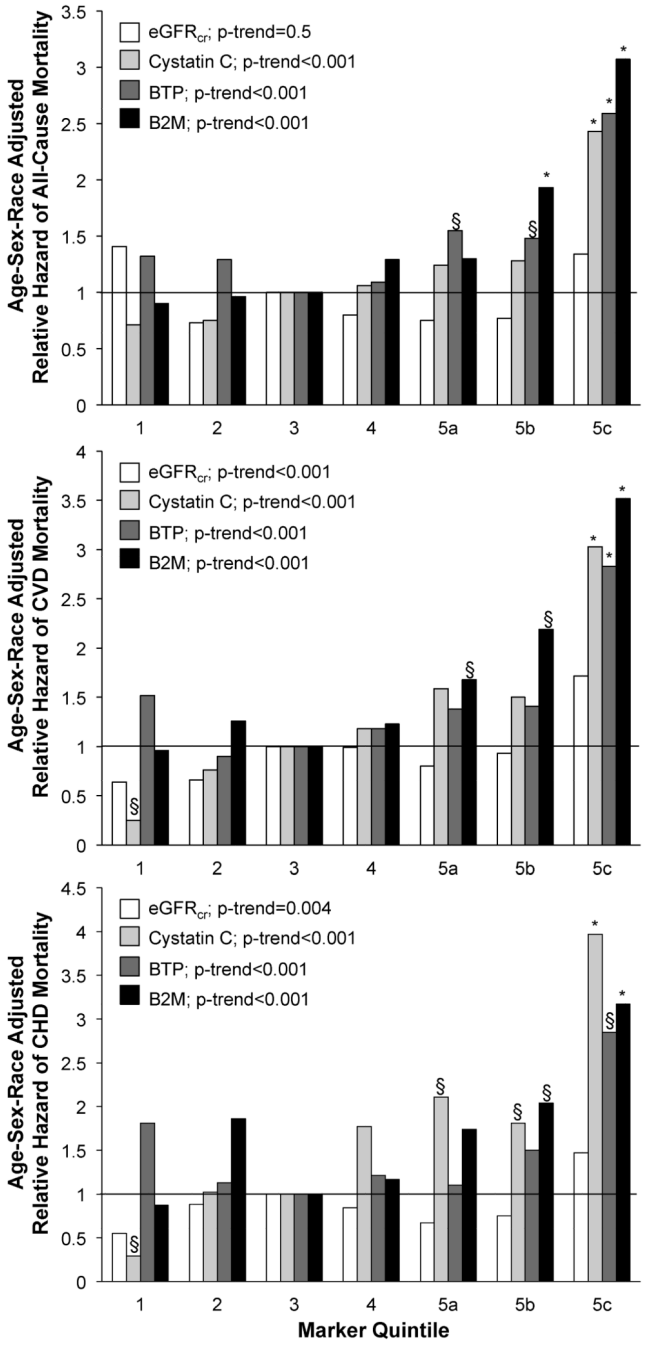

Figure 1.

Age-, sex-, and race-adjusted hazard ratios by filtration marker quintile of (a) all-cause mortality, (b) cardiovascular disease (CVD) mortality, and (c) coronary heart disease (CHD) mortality. Note that higher quintiles denote the lowest filtration level for all markers (highest levels for beta trace protein [BTP], beta-2 microglobulin [B2M], cystatin-C and lowest levels for creatinine based estimated glomerular filtration rate [$eGFR_{cr}$]) * denotes p-value

<0.001 for hazard ratio compared to Quintile 3. § denotes p-value <0.05 for hazard ratio compared to Quintile 3.

Table 1

Baseline characteristics by $eGFR_{cr}$ category, US population aged ≥ 20 years, NHANES III.

	Quintile 1 (>118)	Quintile 2 (107–118)	Quintile 3 (97–107)	Quintile 4 (82–97)	Quintile 5a (76–82)	Quintile 5b (65–76)	Quintile 5c (<65)
$eGFR_{cr}$ (mL/min/1.73 m^2)	126.2	112.7	102.1	90.1	79.1	70.8	52.9
Unweighted sample size	880	676	775	1574	617	805	1124
Weighted percentage	19.8	20.1	19.9	20.2	6.7	6.7	6.7
Age (y)	28.6	35.2	42.1	52.0	58.6	63.4	71.5
Female sex	57.3	50.5	50.0	50.2	54.8	54.0	58.6
Black	23.3	10.1	8.4	8.0	6.1	8.8	8.3
Current Smoking	38.4	36.8	30.3	23.6	17.6	15.6	12.1
Body Mass Index (kg/m^2)	25.7	25.7	26.5	27.2	28.0	27.7	27.3
Systolic blood pressure (mmHg)	113.2	116.7	120.5	125.2	133.1	134.9	141.8
Antihypertensive medication use	1.6	4.6	8.0	15.6	20.0	24.2	47.5
HDL-cholesterol (mg/dL)	51.1	49.5	50.8	50.3	51.1	50.3	49.4
Triglycerides (mg/dL)	93	94	112	125	123	137	154
C-reactive protein							
<0.22 mg/dL	74.7	77.3	74.2	69.7	61.1	69.4	56.3
0.22–1.00 mg/dL	19.0	16.3	21.0	23.2	33.6	25.1	30.4
>1.00 mg/dL	6.3	6.4	4.8	7.1	5.3	5.5	13.4
Diabetes	2.1	2.4	3.4	7.7	6.1	8.9	14.8
Coronary heart disease	0.1	0.6	1.4	3.1	4.9	7.8	14.4
Urinary ACR (mg/g)	5.41	5.74	5.17	5.62	6.64	7.57	12.09
Serum Creatinine (mg/dL)	0.70	0.77	0.82	0.87	0.92	0.99	1.25
Serum Cystatin C (mg/L)	0.71	0.74	0.78	0.84	0.90	0.96	1.29
Serum BTP (mg/L)	0.49	0.52	0.53	0.58	0.64	0.68	0.95
Serum B2M (mg/L)	1.59	1.64	1.77	1.93	2.17	2.31	3.37

Quintiles are of $eGFR_{Cr}$, express in mL/min/1.73 m^2.

Abbreviations: $eGFR_{Cr}$, creatinine-based estimated glomerular filtration rate, HDL, high-density lipoprotein. ACR, albumin-creatinine ratio; NHANES III, Third National Health and Nutrition Examination Survey; BTP, β-trace protein; B2M, β_2-microglobulin

Note: Estimates are weighted means, proportions, or median [interquartile range]. Conversion factors for units: serum creatinine in mg/dL to μmol/L, x88.4; HDL cholesterol in mg/dL to mmol/L, 0.02586; triglycerides in mg/dL to mmol/L, x0.01129.

Table 2

Pearson correlations for the filtration markers

	$eGFR_{cr}$	**1/Serum CysC**	**1/Serum BTP**	**1/Serum B2M**
$eGFR_{cr}$	1.00			
1/Serum CysC	0.52	1.00		
1/Serum BTP	0.45	0.43	1.00	
1/Serum B2M	0.61	0.69	0.52	1.00

Note: Transformation of the filtration markers was done to take into account the reciprocal physiologic associations between filtration and marker levels.

$eGFR_{CR}$, creatinine-based estimated glomerular filtration rate; CysC, cystatin C; BTP, β-trace protein; B2M, β2-microglobulin

Table 3

Multivariable-adjusted hazard ratios of all-cause, cardiovascular disease, and coronary heart disease mortality by quintile of filtration marker.

	Quintile 1*	Quintile 2*	Quintile 4*	Quintile 5*			P-trend
				Subquintile 5a	Subquintile 5b	Subquintile 5c	
All-cause Mortality (2392 deaths/6445 participants)							
$eGFR_{cr}$	1.33 (0.63–2.79)	0.68 (0.32–1.43)	0.88(0.60–1.31)	0.83 (0.5–1.31)	0.82 (0.53–1.26)	1.31 (0.84–2.04)	0.02
Cystatin C	0.76 (0.43–1.34)	0.80 (0.48–1.33)	1.02 (0.70–1.46)	1.19 (0.86–1.65)	1.10 (0.79–1.55)	1.94 (1.43–2.62)	<0.001
BTP	1.15 (0.72–1.85)	1.25 (0.87–1.78)	1.06 (0.75–1.50)	1.45 (0.98–2.14)	1.37 (0.93–1.99)	2.14 (1.56–2.94)	<0.001
B2M	0.90 (0.54–1.50)	0.96 (0.61–1.52)	1.26 (0.90–1.78)	1.22 (0.93–1.59)	1.79 (1.32–2.43)	2.58 (1.96–3.41)	<0.001
Cardiovascular Disease Mortality (1079 deaths/6445 participants)							
$eGFR_{cr}$	0.61 (0.08–4.68)	0.63 (0.14–2.78)	1.13 (0.50–2.56)	0.89 (0.40–1.98)	0.97 (0.43–2.18)	1.56 (0.71–3.43)	0.002
Cystatin C	0.27 (0.12–0.59)	0.83 (0.33–2.05)	1.11 (0.67–1.82)	1.47 (0.89–2.42)	1.20 (0.70–2.04)	2.10 (1.33–3.32)	<0.001
BTP	1.22 (0.53–2.81)	0.86 (0.41–1.80)	1.15 (0.70–1.89)	1.32 (0.70–2.50)	1.25 (0.71–2.17)	2.27 (1.34–3.85)	<0.001
B2M	0.91 (0.33–2.57)	1.27 (0.61–2.68)	1.17 (0.70–1.96)	1.50 (0.93–2.41)	1.83 (1.07–3.14)	2.59 (1.62–4.14)	<0.001
Coronary Heart Disease Mortality (605 deaths/6445 participants)							
$eGFR_{cr}$	0.53 (0.04–6.26)	0.89 (0.15–5.25)	1.05 (0.47–2.37)	0.84 (0.37–1.87)	0.85 (0.37–1.94)	1.40 (0.69–2.84)	0.1
Cystatin C	0.33 (0.13–0.82)	1.13 (0.35–3.71)	1.68 (0.90–3.15)	2.01 (1.11–3.63)	1.42 (0.77–2.64)	2.61 (1.43–4.78)	0.001
BTP	1.36 (0.47–3.88)	1.09 (0.39–3.03)	1.23 (0.65–2.31)	1.08 (0.51–2.30)	1.37 (0.66–2.84)	2.33 (1.16–4.68)	0.001
B2M	0.80 (0.16–3.94)	1.86 (0.84–4.08)	1.12 (0.71–1.76)	1.55 (0.94–2.55)	1.68 (0.92–3.09)	2.15 (1.30–3.56)	0.006

Note: The 95% confidence interval is shown in parentheses. Adjusted for age, sex, race, diabetes, current smoking status, systolic blood pressure, hypertension medication use, high-density lipoprotein cholesterol, natural log(triglycerides), prevalent coronary heart disease, C-reactive protein (<0.22 mg/dL, 0.22–<1.00 mg/dL, ≥1.00 mg/dL), and natural log(urinary albumin-creatinine ratio).

$eGFR_{CR}$, creatinine-based estimated glomerular filtration rate, HDL, high-density lipoprotein. ACR, albumin-creatinine ratio; NHANES III, Third National Health and Nutrition Examination Survey; BTP, β-trace protein; B2M, β_2-microglobulin

*Quintile 3 is the reference group.

Table 4

NRI values comparing multivariable adjusted models including a single filtration marker ($eGFR_{cr}$) to additional filtration markers

	Continuous NRI			Categorical NRI**		
	Event NRI	**Non-event NRI**	**Overall NRI (95% CI)**	**Event NRI**	**Non-event NRI**	**Overall NRI (95% CI)**
Adding Cystatin C, BTP, and B2M to $eGFR_{cr}$ and Risk Factors						
All participants						
All-cause mortality	0.226	0.222	0.448 (0.393, 0.504)‡	0.006	0.012	0.018 (0.005, 0.031)†
CVD mortality	0.278	0.098	0.376 (0.302, 0.450)‡	0.005	0.032	0.037 (0.009, 0.064)†
CHD mortality	0.312	0.112	0.424 (0.330, 0.518)‡	0.041	0.027	0.067 (0.027, 0.108)‡
Participants with $eGFR_{cr}$ ≥60mL/min/1.73m^2						
All-cause mortality	0.316	0.211	0.527 (0.473, 0.580)‡	0.012	0.002	0.014 (0.0004, 0.028)*
CVD mortality	0.392	0.058	0.449 (0.378, 0.521)‡	0.032	0.022	0.055 (0.027, 0.083)‡
CHD mortality	0.342	0.132	0.474 (0.381, 0.567)‡	0.051	0.018	0.070 (0.027, 0.113)†
Adding Cystatin C to $eGFR_{cr}$ and Risk Factors						
All-cause mortality	0.194	0.145	0.339 (0.281, 0.397)‡	0.002	0.008	0.010 (−0.001, 0.021)
CVD mortality	0.321	0.008	0.329 (0.255, 0.404)‡	0.001	0.023	0.025 (0.002, 0.047)*
CHD mortality	0.426	0.019	0.446 (0.357, 0.535)‡	0.046	0.010	0.056 (0.017, 0.095)†
Adding BTP and B2M to Cystatin C and $eGFR_{cr}$ and Risk Factors						
All-cause mortality	0.187	−0.0137	0.174 (0.120, 0.227)‡	0.004	0.004	0.008 (−0.004, 0.019)
CVD mortality	0.244	0.074	0.318 (0.243, 0.393)‡	0.002	0.010	0.012 (−0.010,0.033)
CHD mortality	0.130	0.142	0.273 (0.171, 0.374)‡	−0.002	0.017	0.015 (−0.016, 0.046)

Note: Adjusted for age, sex, race, diabetes, current smoking status, systolic blood pressure, hypertension medication use, high-density lipoprotein-cholesterol, natural log(triglycerides), prevalent CHD, C-reactive protein (<0.22 mg/dL, 0.22–<1.00 mg/dL, ≥1.00 mg/dL), and natural log(urinary albumin-creatinine ratio).

*p≤0.05,

†p≤0.01,

‡p≤0.001

** 10-year risk categories: <0.05, 0.05–0.20, >0.20.

NRI, net reclassification improvement; $eGFR_{Cr}$, creatinine-based estimated glomerular filtration rate; BTP, β-trace protein; B2M, β2-microglobulin; CVD, cardiovascular disease; CHD, coronary heart disease; CI, confidence interval