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Filtration Markers as Predictors of ESRD and Mortality in Southwestern American Indians With Type 2 Diabetes

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Abstract

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Background—A growing number of serum filtration markers are associated with mortality and end-stage renal disease (ESRD) in adults. Whether β-trace protein (BTP) and β_2-microglobulin (B2M) are associated with these outcomes in adults with type 2 diabetes is not known.

Study Design—Longitudinal cohort study.

Setting & Participants—250 Pima Indians with type 2 diabetes (69% women; mean age, 42 years; mean diabetes duration, 11 years).

Predictors—Serum BTP, B2M, and glomerular filtration rate measured by iothalamate clearance (mGFR) or estimated using creatinine ($eGFR_{cr}$) or cystatin C ($eGFR_{cys}$).

Outcomes & Measurements—Incident ESRD and all-cause mortality through December 2013. HRs were reported per interquartile range decrease of the inverse of BTP and B2M (1/BTP and 1/B2M) using Cox regression. Improvement in risk prediction with the addition of BTP or B2M to established markers ($eGFR_{cys}$ with mGFR or $eGFR_{cr}$) was evaluated using C-statistics, continuous net reclassification improvement (NRI), and relative integrated discrimination improvement (rIDI).

Results—During median follow-up of 14 years, 69 participants developed ESRD and 95 died. Both novel markers were associated with ESRD in multivariable models. BTP remained statistically significant after further adjustment for mGFR (1/BTP, 1.53 [95% CI, 1.01-2.30]; 1/B2M, 1.54 [95% CI, 0.98-2.42]). B2M was associated with mortality in multivariable models and after further adjustment for mGFR (HR, 2.12; 95% CI, 1.38-3.26). The addition of B2M to established markers increased the C statistic for mortality but only weakly when assessed by either continuous NRI or rIDI; none were improved for ESRD by the addition of these markers.

Limitations—Small sample size, single measures of markers.

Conclusions—In Pima Indians with type 2 diabetes, BTP, and to a lesser extent B2M, was associated with ESRD. B2M was associated with mortality after adjustment for traditional risk factors and established filtration markers. Further studies are warranted to confirm whether inclusion of B2M in a multi-marker approach leads to improved risk prediction for mortality in this population..

Keywords

Beta-trace protein (BTP); beta-2 microglobulin (B2M); end-stage renal disease (ESRD); type 2 diabetes mellitus; diabetic kidney failure; mortality; filtration markers; glomerular filtration rate (GFR); kidney function; Pima Indians; CKD Biomarkers Consortium

Diabetes is the leading cause of end-stage renal disease (ESRD) in the United States, and diabetic kidney disease is associated with increased overall mortality risk.[1] Several markers of kidney function, such as serum creatinine and cystatin C, are widely recognized predictors of ESRD and death in persons with diabetes.[2-4] More recently, the predictive value of novel markers, such as β-trace protein (BTP) and β_2-microglobulin (B2M), for these health outcomes has been examined in the general population,[5,6] but the utility of these markers specifically in persons with diabetes is uncertain.

Epithelial cells in the central nervous system produce BTP, and all nucleated cells produce B2M. Both markers are filtered by the kidneys and accumulate in persons with chronic kidney disease (CKD). Associations of BTP and/or B2M with kidney disease, cardiovascular disease, and all-cause mortality are now reported in both high-risk[7-12] and general population or community-based cohorts.[5,6] Few studies, however, have investigated both BTP and B2M in the same population[11,12] or in combination with measured glomerular filtration rate (mGFR)[7,8,11] to identify associations that may be attributed to non-GFR determinants. No prior work has specifically evaluated these markers for prognostic value in the setting of diabetes.

The aims of this study are to evaluate associations of BTP and B2M with ESRD and all-cause mortality and to compare associations of these markers with established filtration markers in Pima Indians with type 2 diabetes. Kidney disease is common in this population and is characterized by early glomerular hyperfiltration followed by declining kidney function.[13] We hypothesized that BTP and B2M would predict adverse events in this high-risk population, independent of adjustment for standard CKD risk factors and further for estimated GFR (eGFR) or mGFR.

METHODS

Study Population

Study participants were drawn from a longitudinal population-based cohort study of diabetes and its complications in Pima Indians from the Gila River Indian Community, conducted from 1965 through 2007. Members of the community who were at least 5 years old were invited to have a research examination approximately every 2 years. Diabetes was diagnosed by a 2-hour post-load plasma glucose concentration ≥200 mg/dl at these biennial examinations, or when the diagnosis was documented in the medical record. We selected participants from this study who had type 2 diabetes and also participated in one of two longitudinal studies of kidney function (n=260).[13,14] Participants in whom measures of filtration markers or other covariates were missing were excluded from the analysis, resulting in a final sample size of 250 participants. This study was approved by the Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and each participant provided informed consent.

Exposure Assessment

Baseline assessment of serum filtration markers and covariates of interest was performed during a kidney function study visit conducted in the April 1989 through June 2007 time period. Urine and serum samples collected during the examination were stored at −80°C prior to assays. Except for creatinine, cystatin C, B2M, and BTP, all measurements used in this study were performed within 30 days of the sample collection. Concentrations of these four analytes were measured in 2012 in stored serum samples that had undergone a maximum of two prior freeze-thaw cycles. Serum creatinine concentration was measured using the Roche enzymatic method on a Roche Modular P Analyzer (Roche Diagnostics; inter-assay laboratory coefficient of variation [CV], 2.3%). This method was calibrated using an isotope-dilution mass spectrometry standard traceable to the National Institute of

Standards and Technology Standard Reference Material. Serum cystatin C, BTP, and B2M (inter-assay laboratory CVs of 4.7%, 6.0%, and 2.7%, respectively) were measured using the Siemens Dade Behring ProSpec nephelometer (Siemens Healthcare Diagnostics). These measures showed good reproducibility in a sub-sample of 50 participants with repeated measurements; intraclass correlation coefficients were high for creatinine (0.97), cystatin C (0.91), and B2M (0.91) and lower for BTP (0.74). Cystatin C measurements were calibrated to the international reference material ERM DA-417/IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) using the following equation: calibrated cystatin C = 1.14* cystatin C – 0.01.

Creatinine-based eGFR ($eGFR_{cr}$) was estimated using the 2009 CKD-EPI (CKD Epidemiology Collaboration) creatinine equation.[15] Cystatin C-based eGFR ($eGFR_{cys}$) was estimated using the 2012 CKD-EPI cystatin C equation.[16] Measured GFR (mGFR) was assessed using urinary iothalamate clearance, as described previously,[17] and indexed to body surface area. Briefly, four 20-minute urine collections, bracketed by the collection of blood samples, were made after a water load and a 60-minute equilibration period. A high performance liquid chromatography system with a sensitive UV light detector was used to assay iothalamate at 236 nm (Instrumentation Shimadzu #6A, Kyoto, Japan).

Outcome Assessment

Participants were followed up through December 31, 2013, for ESRD events and all-cause mortality. Surveillance for ESRD and death were conducted independently of the research examinations. For this study, ESRD was defined as the initiation of renal replacement therapy or an underlying cause of death due to diabetic kidney failure (International Classification of Disease, Ninth revision [ICD-9] code 250.4). All-cause mortality was ascertained through community surveillance, contact with participants during examinations, review of local newspaper obituaries, and regular requests to the National Death Index.

Covariate Assessment

Baseline covariates of interest included age, sex, duration of diabetes, hypertension, total cholesterol, urinary albumin-creatinine ratio (ACR), and hemoglobin A1c. Hypertension was defined as a systolic blood pressure ≥140 mmHg, a diastolic blood pressure ≥90 mmHg, or the use of antihypertensive medicine. Urinary albumin concentration was measured at the time of sample collection by nephelometric immunoassay and urinary creatinine concentration by a modified Jaffé reaction.[18,19] Urinary albumin excretion was estimated by computing the ACR in units of mg/g. The ACR was considered normoalbuminuria if <30 mg/g, moderately elevated albuminuria if 30-299 mg/g, and severely elevated albuminuria if ≥300 mg/g. Urinary albumin concentrations below the threshold detected by the assay (6.8 mg/L) were set to this value for analyses.

Statistical Analyses

Baseline characteristics were evaluated in the overall sample and by mGFR tertile. Associations between filtration markers were assessed using Pearson correlations. Inverse transformations were applied to BTP and B2M (i.e., 1/BTP and 1/B2M) since these markers are reciprocally associated with GFR. Kaplan-Meier survival curves were used to evaluate

the unadjusted associations of filtration markers with ESRD and mortality. Survival curves were plotted for tertiles of each filtration marker and a log-rank test was used to compare survival curves across marker tertiles. Cox proportional hazards regression was used to estimate the hazard ratio (HR) for ESRD or mortality associated with each filtration marker. Because vital status was established for all study participants through December 31, 2013, those who did not experience a mortality event were censored on December 31, 2013. In ESRD analyses, those who did not experience an ESRD event were censored at death or December 31, 2013, whichever came first. Markers were modeled continuously, with the HR reported per interquartile range decrease in each filtration marker. Models were initially adjusted for age and sex with further adjustment for hypertension, total cholesterol, natural logarithm of urinary ACR, hemoglobin A1c, and duration of diabetes (multivariable-adjusted model). Finally, the multivariable models were adjusted separately for $eGFR_{cr}$ and mGFR to determine whether associations were independent of GFR. In sensitivity analyses, models were performed with mGFR not indexed to body surface area and the effect of the competing risk of death on ESRD events was evaluated using a Fine and Gray model.[20]

We evaluated improvement in model discrimination for ESRD or all-cause mortality associated with adding novel markers individually to the established kidney function marker $eGFR_{cys}$ in combination with either mGFR or $eGFR_{cr}$ by comparing Harrell's C statistics and likelihood ratio tests for the nested multivariable-adjusted Cox proportional hazards models. Improvement in risk prediction associated with the novel markers was assessed by continuous net reclassification improvement (NRI) and relative integrated discrimination improvement (rIDI), computed for the 10-year risk of ESRD or all-cause mortality using multivariable-adjusted Poisson regression models. Statistical analyses were performed in Stata/SE, version 12.1 (StataCorp LP).

RESULTS

Characteristics of Study Participants

The mean age of study participants at baseline was 42.4 years and the median duration of diabetes was 11.2 years; the majority of participants were women (69.2%) (Table 1). Elevated albuminuria was common, with moderately elevated albuminuria present in 36.4% and severely elevated albuminuria present in 18.4% of participants. The mGFR averaged 129 mL/min/1.73m^2 in the cohort. When stratified by mGFR tertiles, participants in the lowest tertile (22-114 mL/min/1.72m^2) were older, had a longer duration of diabetes, and higher prevalence of severely elevated albuminuria. Both BTP and B2M were higher and $eGFR_{cr}$ and $eGFR_{cys}$ lower across decreasing mGFR tertiles. Scatter plots demonstrating associations of mGFR with $eGFR_{cr}$, $eGFR_{cys}$, 1/BTP, and 1/B2M are presented in Figure S1 (provided as online supplementary material). Correlations among mGFR and the four filtration markers (Table 2) ranged from 0.32 (1/BTP and mGFR) to 0.80 ($eGFR_{cr}$ and $eGFR_{cys}$; all $P<0.001$). Measured GFR was more highly correlated with $eGFR_{cr}$ and $eGFR_{cys}$ than with 1/B2M or 1/BTP. Correlations were weaker with 1/BTP than with the other markers.

End-Stage Renal Disease

Over a median follow-up of 13.5 years, 69 participants (27.6%) developed ESRD. In unadjusted analyses, adults in the lowest tertile for mGFR, eGFR, $eGFR_{cys}$, and 1/BTP (log-rank $P \leq 0.03$), but not 1/B2M ($P=0.08$), had a greater likelihood of progression to ESRD (Figure 1). After multivariable adjustment, an interquartile range decrease in each of the five markers was associated with a significantly increased risk of developing ESRD (Table 3). The highest multivariable-adjusted associations with ESRD were observed for $eGFR_{cys}$ and mGFR (Table 3). Associations were attenuated with additional adjustment for $eGFR_{cr}$; only $eGFR_{cys}$ remained statistically significant (Table 3, $P<0.001$). When the multivariate models were adjusted for mGFR, both $eGFR_{cys}$ and 1/BTP remained independently associated with ESRD whereas 1/B2M was of borderline significance (Table 3). Results did not change substantially in analyses with mGFR not indexed to body surface area. Fifty-one participants in our cohort died before developing ESRD. Adjusted sub-HRs from Fine and Gray models accounting for mortality as a competing risk for ESRD were similar in magnitude to HRs from Cox proportional hazards regression (Table S1).

Model discrimination was not improved when either 1/BTP (C statistic with 1/BTP 0.842 *vs*. 0.843 without; likelihood ratio test $P=0.4$) or 1/B2M (C statistic with 1/B2M 0.843 *vs*. 0.843 without, likelihood ratio test $P=0.4$) were added to the multivariable Cox models with mGFR and $eGFR_{cys}$; results were similar when 1/BTP or 1/B2M were added to multivariable Cox models with $eGFR_{cr}$ and $eGFR_{cys}$ (Table 4 and Table S2). Based on the continuous NRI and rIDI using risk estimates from multivariable Poisson regression models, neither the addition of 1/BTP nor 1/B2M improved the 10-year risk classification or the relative integrated discrimination for ESRD beyond mGFR and $eGFR_{cys}$ or beyond $eGFR_{cr}$ and $eGFR_{cys}$ (Table 4)

All-Cause Mortality

Over a median follow-up of 13.9 years, 95 participants (38.0%) died. In unadjusted analyses, adults in the lowest tertile for mGFR, $eGFR_{cr}$, $eGFR_{cys}$, and 1/B2M–but not 1/BTP–had faster progression to mortality (Figure 2). After multivariable adjustment, B2M and $eGFR_{cys}$ remained significantly associated with mortality (Table 3). These associations were stronger after additional adjustment for $eGFR_{cr}$ or mGFR. There was not an association of BTP with mortality (Table 3). Results did not change substantially in analyses with mGFR not indexed to body surface area.

Model discrimination was improved beyond established risk factors when 1/B2M was added to the multivariable Cox model with mGFR and $eGFR_{cys}$ or to the multivariable model with $eGFR_{cr}$ and $eGFR_{cys}$ (C statistics with 1/B2M of 0.735 vs 0.724 without and 0.733 *vs*. 0.719 without, respectively; likelihood ratio test $P=0.007$ for both; Table 4 and Table S2). In a multivariable Poisson regression model, the addition of 1/B2M led to improvements in the RIDI but did not substantially improve the 10-year mortality risk classification beyond mGFR and $eGFR_{cys}$ or beyond $eGFR_{cr}$ and $eGFR_{cys}$ (Table 4).

DISCUSSION

Higher BTP and B2M were associated with increased risk of incident ESRD in Pima Indians with type 2 diabetes, similar to mGFR, $eGFR_{cr}$, and $eGFR_{cys}$. Associations were attenuated with additional adjustment for $eGFR_{cr}$ or mGFR. Only higher serum concentrations of B2M were associated with increased mortality risk in this population, independent of other known risk factors and measures of kidney function. Our findings suggest that in this population BTP and B2M may contribute modestly to ESRD risk and B2M more strongly to mortality risk through mechanisms not related to kidney function. A multiple filtration marker approach incorporating B2M along with $eGFR_{cys}$, either mGFR or $eGFR_{cr}$, and other risk factors may lead to improvements in discrimination in mortality risk prediction models. These improvements, however, were weak when assessed by either continuous NRI or rIDI. These observations highlight the importance of evaluating new markers in important high risk subgroups, as associations observed in general populations (*vide infra*) may not be generalizable to specific groups based on disease status.

Associations of BTP (but not B2M) with ESRD have been investigated in patients with CKD; these studies have consistently shown that higher BTP is associated with increased risk of kidney failure, independent of mGFR, with effect sizes similar to our findings.[7,8,11] In the community-based Atherosclerosis Risk in Communities (ARIC) Study, both BTP and B2M were strong predictors of ESRD (HRs of 12-17 comparing participants in the upper 6.7^{th} percentile [upper tertile of upper quintile] to the lowest quintile for each marker) in multivariable adjusted models.[5] While risks were substantially greater in magnitude in the ARIC Study, they were directionally consistent with our findings. However, in contrast with our findings, inclusion of cystatin C, BTP, and B2M with $eGFR_{cr}$ led to substantial improvements in risk classification, suggesting that a combination of filtration markers shows greater promise for improving ESRD risk prediction in the general population than specifically among adults with type 2 diabetes. Perhaps mGFR and elevated albuminuria are such strong risk factors for ESRD in type 2 diabetes that the novel markers provide little additional risk information in prediction models, whereas in the general population or in other causes of progressive CKD factors other than GFR contribute more prominently to risk, and BTP and B2M capture this additional risk information.

In both the community-based ARIC Study and the general population-based Third National Health and Nutrition Examination Survey (NHANES III), higher BTP and B2M were associated with a similarly increased all-cause mortality risk.[5,6] In addition, higher B2M levels were associated with increased mortality over a median follow-up of 7.9 years in a study of Japanese adults aged 65 years and older[10] and in adults with asymptomatic carotid atherosclerosis.[9] While higher B2M was consistently and independently associated with increased all-cause mortality risk, the association with BTP was essentially null in all models in Pima Indians with type 2 diabetes. These findings suggest that B2M may be a more promising marker of mortality risk than BTP in this high-risk group than in the general population. Differences in the associations of BTP and B2M with mortality in this study suggest that B2M provides unique risk information unrelated to kidney function although, to our knowledge, the mechanisms underlying these differences have not been identified.

Our study has unique characteristics that add to the current literature. First, earlier studies investigating associations of BTP and B2M with ESRD and/or mortality focused predominately on Caucasian and African American adults from the general population in the United States.[5,6] The present analysis focuses on Pima Indians with type 2 diabetes – a population with substantially increased risk of ESRD and mortality as well as a greater degree of glomerular hyperfiltration (47.6% with mGFR >154 mL/min[21,22]) than observed in the general United States population. Thus, prior risk estimates from the general population may not be generalizable to this cohort. Of note, we did observe differences in risk associations with BTP in this population when compared to prior studies, which suggests that these filtration markers do indeed provide different risk information across populations. Second, we measured GFR in addition to filtration markers in our study, allowing us to evaluate whether risk associations are independent of measured GFR and therefore whether non-GFR determinants may contribute to risk associations.

Potential mechanisms underlying independent associations of BTP and B2M levels with adverse events are not well established. Our observed attenuations for BTP and B2M with ESRD and B2M with mortality after accounting for either $eGFR_{cr}$ or mGFR suggest that pathways related to both kidney function and non-GFR determinants play a role. In NHANES III, elevated BTP and B2M were associated with several factors that can also increase ESRD or mortality risk, including higher age, higher body mass index, hypertension, higher C-reactive protein, and lower HDL cholesterol for elevated B2M and higher age, male sex, race/ethnicity, hypertension, higher C-reactive protein, and lower HDL cholesterol for elevated BTP.[23] Serum B2M is also elevated in multiple myeloma and is part of risk stratification guidelines for this condition.[24] Additional potential non-GFR determinants for elevated BTP include genetic factors, cardiovascular disease, pregnancy, muscle mass, and inflammation.[25] Future work is needed to fully elucidate non-GFR determinants of BTP and B2M and their influence on risk associations with ESRD and mortality.

Our study has several strengths. Participants were drawn from a well-characterized cohort of Pima Indians with mGFR and extended follow-up for ESRD and mortality events. While serum samples were collected at a wide range of examinations for the baseline measurement, we assayed all four filtration markers at the same laboratory in 2012, minimizing potential drift in laboratory assays. Our study also has limitations. Although event rates were high in this high-risk cohort, the number of events was relatively small because the study sample was small. These factors may limit power to detect more modest effect sizes and increase the risk of over fitting in multivariable regression models. Accordingly, we took model parsimony into account when selecting covariates to reduce the impact of over fitting while still controlling for confounding bias from established risk factors. Prior work in the ARIC Study suggests there is a genetic component to BTP that may be related to racial/ethnic differences in serum levels, with genome-wide significant locus for BTP that explained about one-third of the difference in BTP levels in European and African Americans;[26] it is not clear how potential genetic components would affect the generalizability of our findings to other populations. Limited data are available on long-term stability of BTP and B2M in stored samples. Prior work in NHANES indicates that both B2M and BTP are robust to a single freeze-thaw cycle after long-term storage.[27] While it is

not clear how an additional freeze-thaw cycle would affect marker stability, we anticipate that results would be biased towards the null if this resulted in non-differential exposure misclassification with respect to the outcomes. Finally, our continuous NRI estimates may be unstable due to instability in our small sample size. We included comparisons based on the C statistics as more powerful tests of model discrimination, but work is needed in larger cohorts to confirm our findings for B2M and mortality.

In summary, BTP and B2M are associated with ESRD, similar to traditional filtration markers, and B2M is associated with all-cause mortality in Pima Indians with type 2 diabetes. Even though this study suggests that the inclusion of B2M in a multi-marker approach may lead to improved risk prediction for mortality in this population, further studies are warranted to confirm these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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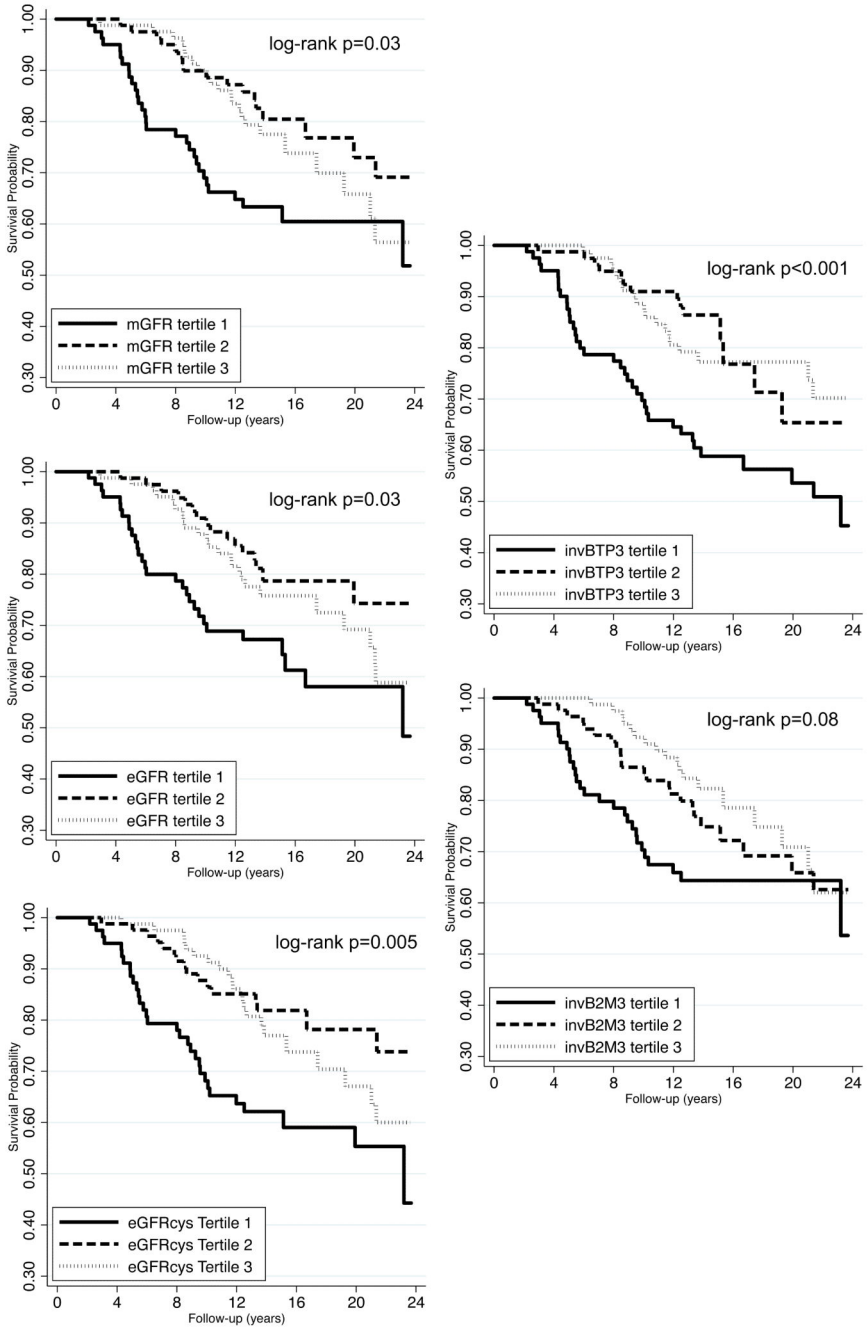

Figure 1.
Kaplan-Meier Curves for End-Stage Renal Disease by Tertiles of Filtration Markers

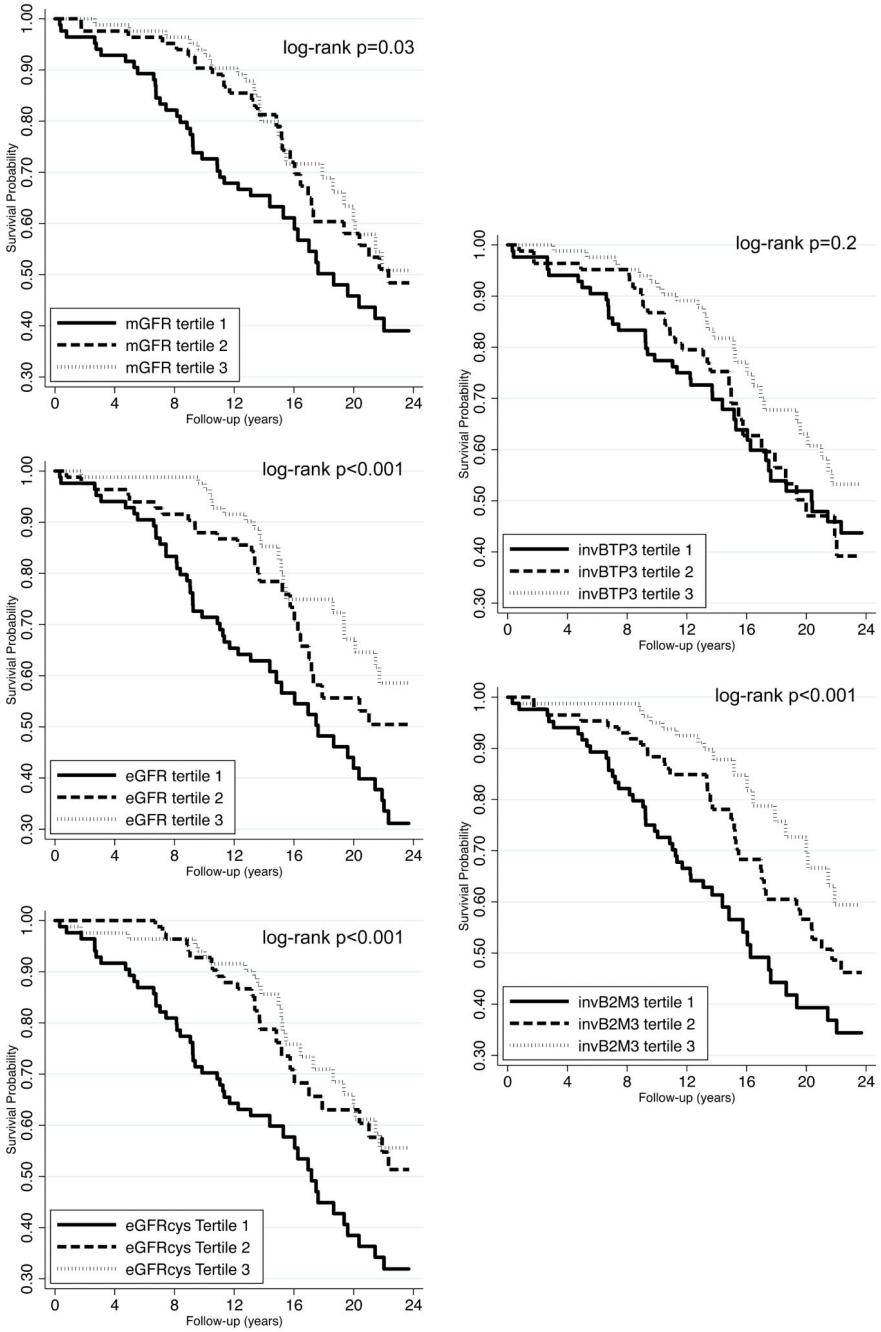

Figure 2.
Kaplan-Meier Curves for All-Cause Mortality by Tertiles of Filtration Markers

Table 1

Baseline Study Sample Characteristics by Tertiles of mGFR

Characteristic	Overall (N=250)	Low: mGFR 22-114 (n=84)	Middle: mGFR 115-146 (n=83)	High: mGFR 147-235 (n=83)
Age at sample collection (y)	42.4 ±10.5	48.4 ±9.6	42.5 ±9.8	36.1 ±8.5
Female Sex	173 (69.2%)	56 (66.7%)	53 (63.9%)	64 (77.1%)
BMI (kg/m^2)	35.4 (8.5)	36.6 (9.5)	34.2 (7.2)	35.5 (8.6)
Age at diabetes diagnosis (y)	31.2 (9.5)	35.4 (9.0)	32.6 (8.6)	25.6 (8.1)
Duration of diabetes (y)	11.2 (6.6)	13.0 (7.1)	9.9 (6.0)	10.5 (6.3)
Hemoglobin Alc	9.4 (2.3)	8.9 (2.3)	8.9 (2.3)	10.2 (2.2)
Insulin use	62 (24.8%)	25 (29.8%)	17 (20.5%)	20 (24.1%)
Oral hypoglycemic medicine use	110 (44.0%)	46 (54.8%)	36 (43.4%)	28 (33.7%)
Systolic BP (mm Hg)	120 (15)	125 (15)	119 (15)	116 (13)
Diastolic BP (mm Hg)	75 (9)	77 (10)	75 (9)	75 (8)
Antihypertensive medicine use	32 (12.8%)	16 (19.0%)	9 (10.8%)	7 (8.4%)
Hypertension	54 (21.6%)	27 (32.1%)	17 (20.5%)	10 (12.0%)
Serum cholesterol (mg/dL)	172 (42)	171 (39)	166 (39)	179 (46)
Serum triglycerides (mg/dL)	136 [101-216]	131 [105-214]	125 [98-192]	158 [106-230]
Urinary ACR (mg/g)	37 [14-169]	48 [13-718]	30 [11-128]	34 [17-89]
Albuminuria status				
Normoalbuminuria	113 (45.2%)	34 (40.5%)	42 (50.6%)	37 (44.6%)
Moderately elevated	91 (36.4%)	25 (29.8%)	31 (37.3%)	35 (42.2%)
Severely elevated	46 (18.4%)	25 (29.8%)	10 (12.0%)	11 (13.3%)
mGFR (mL/min)	149 (45)	104 (29)	148 (19)	195 (26)
mGFR (mL/min/ 1.73m^2)	129 (37)	89 (20)	129 (9)	169 (20)
Creatinine (mg/dL)	0.58 (0.24)	0.73 (0.34)	0.54 (0.10)	0.47 (0.12)
$eGFR_{cr}$ (mL/min/1.73m^2)	118 (20)	102 (21)	121 (10)	132 (14)
Cystatin C (mg/L)	0.83 (0.25)	1.05 (0.31)	0.78 (0.11)	0.68 (0.12)
$eGFR_{cys}$ (mL/min/1.73 m^2)	101 (23)	79 (22)	107 (13)	119 (14)
B2M (mg/L)	2.10 (0.70)	2.57 (0.89)	1.99 (0.41)	1.73 (0.33)
BTP (mg/L)	0.47 (0.24)	0.61 (0.31)	0.43 (0.17)	0.36 (0.15)

Note: mGFRs expressed in mL/min/1.73 m^2. Values for categorical variables are given as number (percentage); values for continuous variables are given as mean ± standard deviation or median [interquartile range]. Conversion factors for units: creatinine in mg/dL to μmol/L, ×88.4; cholesterol in mg/dL to mmol/L, ×0.02586; triglycerides in mg/dL to mmol/L, ×0.01129.

Abbreviations: BP, blood pressure; B2M, β_2-microglobulin; BTP, P-trace protein;; BMI, body mass index; $eGFR_{cr}$, creatinine-based estimated glomerular filtration rate; $eGFR_{cys}$, cystatin C–based estimated glomerular filtration rate; mGFR, measured glomerular filtration rate; ACR, albumin-creatinine ratio.

Table 2

Pearson Correlations Between Filtration Markers

	mGFR	$eGFR_{cr}$	$eGFR_{cys}$	1/BTP	1/B2M
mGFR	1.00				
$eGFR_{cr}$	0.76	1.00			
$eGFR_{cys}$	0.79	0.80	1.00		
1/BTP	0.32	0.37	0.39	1.00	
1/B2M	0.65	0.61	0.80	0.35	1.00

Abbreviations: B2M, β_2-microglobulin; BTP, β-trace protein; $eGFR_{cr}$, creatinine-based estimated glomerular filtration rate; $eGFR_{cys}$, cystatin C–based estimated glomerular filtration rate; mGFR, measured glomerular filtration rate.

Note: All *P*-values for correlations are <0.001.

Table 3

Adjusted Hazard Ratios for Incident ESRD and All-Cause Mortality by Filtration Marker

	Age-sex		Multivariable[a]		Multivariable[a] + $eGFR_{cr}$		Multivariable[a] + mGFR	
	HR[b] (95% CI)	*P*	HR[b] (95% CI)	*P*	HR[b] (95% CI)	*P*	HR[b] (95% CI)	*P*
ESRD (n=69 events; median follow-up, 13.5 y)								
mGFR	1.61 (1.07, 2.43)	0.02	2.01 (1.31, 3.11)	0.002	1.55 (0.90, 2.69)	0.1	-[d]	-
$eGFR_{cr}$	1.82 (1.33, 2.49)	<0.001	1.75 (1.26, 2.45)	0.001	-[c]	-	1.40 (0.91, 2.17)	0.1
$eGFR_{cys}$	2.20 (1.55, 3.12)	<0.001	2.86 (1.94, 4.22)	<0.001	3.37 (1.94, 5.87)	<0.001	3.28 (1.90, 5.67)	<0.001
1/BTP	1.68 (1.15, 2.44)	0.01	1.71 (1.14, 2.57)	0.01	1.48 (0.99, 2.23)	0.06	1.53 (1.01, 2.30)	0.04
1/B2M	1.70 (1.19, 2.44)	0.004	1.87 (1.28, 2.72)	0.001	1.53 (0.95, 2.46)	0.08	1.54 (0.98, 2.42)	0.06
All-Cause Mortality (n=95 events; median follow-up, 13.9 y)								
mGFR	1.16 (0.81, 1.66)	0.4	1.17 (0.80, 1.70)	0.4	1.01 (0.62, 1.65)	0.9	-[d]	-
$eGFR_{cr}$	1.28 (0.97, 1.70)	0.08	1.22 (0.89, 1.67)	0.2	-[c]	-	1.21 (0.80, 1.82)	0.4
$eGFR_{cys}$	1.44 (1.06, 1.94)	0.02	1.44 (1.04, 1.98)	0.03	1.54 (0.99, 2.41)	0.06	1.68 (1.07, 2.64)	0.02
1/BTP	1.05 (0.84, 1.32)	0.6	1.02 (0.81, 1.28)	0.9	0.98 (0.79, 1.23)	0.9	1.00 (0.80, 1.26)	0.9
1/B2M	1.78 (1.28, 2.47)	0.001	1.78 (1.25, 2.53)	0.001	2.02 (1.31, 3.11)	0.001	2.12 (1.38, 3.26)	0.001

Abbreviations: B2M, β_2-microglobulin; BTP, β-trace protein; CI, confidence interval; $eGFR_{cr}$, creatinine-based estimated glomerular filtration rate; $eGFR_{cys}$, cystatin C–based estimated glomerular filtration rate; ESRD, end-stage renal disease; HR, hazard ratio; mGFR, measured glomerular filtration rate.

[a] Adjusted for age, sex, duration of diabetes, hemoglobin A1c, hypertension, total cholesterol, and natural logarithm-transformed urinary albumin-creatinine ratio.

[b] Expressed per interquartile decrease in each marker.

[c] No HR reported because $eGFR_{cr}$ already in model for this row.

[d] No HR reported because mGFR already in model for this row.

Table 4

C statistics, Likelihood Ratio Test *P*-Values, and the 10-Year RIDI and Continuous NRI for Models with and without Biomarker Information

Outcome	Model	C statistic without Biomarker*	C statistic with Biomarker*	Difference in C statistic (95% CI)	Likelihood Ratio Test *P*-Value	RIDI (95% CI)	Continuous NRI (95% CI)
Base Model: mGFR+eGFR$_{cys}$ With Multivariable[a] Adjustment							
ESRD	+ 1/BTP	0.843	0.842	−0.001 (−0.006, 0.004)	0.4	−0.000 (−0.020, 0.019)	−0.050 (−0.390, 0.290)
	+ 1/B2M	0.843	0.843	0.000 (−0.004, 0.004)	0.4	0.002 (−0.019, 0.024)	0.189 (−0.156, 0.533)
All-cause Mortality	+ 1/BTP	0.724	0.724	−0.000 (−0.005, 0.005)	0.7	0.003 (−0.006, 0.013)	0.327 (−0.027, 0.681)
	+ 1/B2M	0.724	0.735	0.011 (−0.008, 0.030)	0.007	0.116 (0.007, 0.224)	0.186 (−0.176, 0.549)
Base Model: eGFR$_{cr}$+eGFR$_{cys}$ With Multivariable[a] Adjustment							
ESRD	+ 1/BTP	0.845	0.844	−0.001 (−0.007, 0.005)	0.4	−0.003 (−0.025, 0.018)	−0.029 (−0.371, 0.312)
	+ 1/B2M	0.845	0.845	0.000 (−0.004, 0.005)	0.4	−0.000 (−0.021, 0.021)	0.177 (−0.171, 0.524)
All-cause Mortality	+ 1/BTP	0.719	0.719	−0.000 (−0.004, 0.004)	0.7	0.003 (−0.007, 0.014)	0.011 (−0.335, 0.358)
	+ 1/B2M	0.719	0.733	0.014 (−0.004, 0.033)	0.007	0.108 (0.002, 0.215)	0.159 (−0.198, 0.515)

Abbreviations: B2M, β_2-microglobulin; BTP, β-trace protein; CI, confidence interval; eGFR$_{cr}$, creatinine-based estimated glomerular filtration rate; eGFR$_{cys}$, cystatin C–based estimated glomerular filtration rate; ESRD, end-stage renal disease; HR, hazard ratio; RIDI, relative integrated discrimination improvement; mGFR, measured glomerular filtration rate; NRI, net reclassification improvement.

*B2M or BTP.

[a]Adjusted for age, sex, hypertension, total cholesterol, natural logarithm-transformed urinary ACR, hemoglobin A1c, and duration of diabetes.

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