Relation of Serum and Urine Renal Biomarkers to Cardiovascular Risk in Patients with Type 2 Diabetes Mellitus and Recent Acute Coronary Syndromes (From the EXAMINE Trial)

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A deeper understanding of the interplay between the renal axis and cardiovascular (CV) disease is needed in type 2 diabetes mellitus (T2DM). We aimed to explore the prognostic value of a comprehensive panel of renal biomarkers in patients with T2DM at high CV risk. We evaluated the prognostic performance of both serum (Cystatin C) and urine renal biomarkers (neutrophil gelatinase-associated lipocalin, kidney injury molecule-1 protein, and indices of urinary protein excretion) in 5,380 patients with T2DM and recent acute coronary syndromes in the EXAMINE trial. Patients requiring dialysis within 14 days were excluded. Single- and multimarker covariate-adjusted Cox proportional hazards models were developed to predict times to events. Primary endpoint was composite nonfatal myocardial infarction, nonfatal stroke, or CV death. Median age was 61 years, 68% were men, and mean baseline estimated glomerular filtration rate (eGFR) was 74 mL/min/1.73 m^2. During median follow-up of 18 months, 621 (11.5%) experienced the primary endpoint and 326 (6.1%) patients had died. All renal biomarkers were robustly associated with adverse CV events in step-wise fashion, independent of baseline eGFR. However, in the multimarker prediction model, only Cystatin C (per 1 SD) was associated with the primary endpoint (hazard ratio [HR] 1.28 [1.14 to 1.45]; $p \leq 0.001$), death (HR 1.51 [1.30 to 1.74]; $p \leq 0.001$), and heart failure hospitalization (HR 1.20 [0.96 to 1.49]; $p = 0.11$). Association between Cystatin C and the primary endpoint was similar in baseline eGFR above and below 60 mL/min/1.73 m^2 ($P_{interaction} > 0.05$). In conclusion, serum and urine renal biomarkers, when tested alone, independently predict long-term adverse CV events in high-risk patients with T2DM. In an integrative panel of renal biomarkers, only serum Cystatin C remained independently associated with subsequent CV risk. Renal biomarkers informing various aspects of kidney function may further our understanding of the complex interplay between diabetic kidney disease and CV disease.
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Patients with type 2 diabetes mellitus (T2DM) experience high rates of cardiovascular (CV) disease.[1] Diabetic kidney disease represents one of the strongest CV risk determinants[2] and its prevention and slowing have emerged as important targets of glucose-lowering therapies.[3−5] Although the link between chronic kidney disease (CKD) and CV disease is well-established, differentiating the relative contributions of glomerular filtration, tubular injury, and proteinuric disease is less clear. Measurement of renal biomarkers may facilitate broader understanding of this complex interplay.[6−9] Conventional renal biomarkers (e.g. serum creatinine) are commonly used in practice, but inform one axis of renal function and rise late during renal progression.[10] In patients with T2DM and recent acute coronary syndromes (ACS) in EXAMINE (Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care), we evaluated the prognostic performance of an integrative panel of renal biomarkers of filtration (serum Cystatin C), tubular injury (urinary neutrophil gelatinase-associated lipocalin [uNGAL], urinary kidney injury molecule [uKIM]-1 protein), and protein excretion in the prediction of CV events.

Methods

The design[11] and primary results[12] of EXAMINE have been previously published. In brief, EXAMINE was a

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global, multicenter, trial of adult patients with T2DM (glycated hemoglobin levels 6.5% to 11.0%) and recent ACS (within 15 to 90 days before randomization) who were randomly assigned to alogliptin, a dipeptidyl peptidase (DPP)-4 inhibitor, or matching placebo in 1:1 double-blinded fashion. Both treatment arms received standard of care for T2DM and secondary CV protection. Patients deemed unstable from a CV perspective or who required dialysis within 14 days of screening were excluded, but EXAMINE included patients with mild, moderate, and severe CKD. Due to renal-predominant drug clearance, alogliptin was renally dose adjusted (estimated glomerular filtration rate [eGFR] $\geq$ 60 mL/min/1.73 m^2: 25 mg; eGFR 30 to 60 mL/min/1.73 m^2: 12.5 mg; eGFR < 30mL/min/1.73 m^2: 6.25 mg).

The collection and analysis of these renal biomarkers, including serum creatinine, were protocol-specified and run at a central laboratory.[13] The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[14] Urine creatinine was measured using a kinetic colorimetric assay on a Roche Automated Analyzer (Indianapolis, IN). Urine KIM-1 and NGAL were indexed to urine creatinine to account for dilutional effects.[7,8] Biomarker assays for Cystatin C, uKIM-1, and uNGAL were run together in batch using the same reagent. Urine-based biomarkers were stored at -80°C.

Serum Cystatin C, a small nuclear protein, which is freely filtered by the glomerulus and not actively secreted, serves as an important marker of glomerular function. Cystatin C was run using a Cobas analyzer (Roche Diagnostics Indianapolis, IN) with the Randox assay (Bardane Industrial Park, Kearneysville, WV) with detection range of 0.4 to 10 mg/L. Inter- and intra-assay coefficients of variation (CV) were 2.18% at 0.78 mg/L and 1.6% at 3.42 mg/L. Intra-assay CVs were 4.2% at 0.78 mg/L and 2.6% at 5.35 mg/L. *uKIM-1*, a tubular luminal-surface protein, was measured using a microbead enzyme linked immunosorbent assay (ELISA) (R&D Systems). The analytic sensitivity for KIM-1 was 0.06 ng/mL and the detection range was 0.16 to 2.37 ng/mL with intra- and inter-assay CVs of 4.4% and 7.8%, respectively. *uNGAL*, small complexes, was measured with an enzyme immunoassay (EIA) kit (R&D Systems). The analytic sensitivity was 0.16 ng/mL and the reference range was 1.4 to 78.1 ng/mg creatinine. Intra- and inter-assay CVs were 2.7 to 3.2% and 0.0 to 6.7%, respectively. *Baseline urine albumin/creatinine ratio* (mg/g) was calculated as urine albumin (mg/dL)/urine creatinine (mg/dL) $\times$ 1,000.

The primary endpoint was composite nonfatal myocardial infarction (MI), nonfatal stroke, or death from cardiovascular causes. Other endpoints included all-cause mortality and hospitalization for heart failure (HF). All endpoints were prospectively adjudicated by an independent clinical events committee blinded to treatment assignment (C5, Cleveland, Ohio).

We included all enrolled patients in both treatment arms of the EXAMINE trial with available baseline renal biomarker data. We first evaluated baseline clinical profiles in patients who did and did not experience the primary composite endpoint. We then described the renal biomarker distributions by treatment status. Nonparametric Wilcoxon signed-rank tests were used for comparisons related to the renal biomarkers and Cochran-Mantel-Haenszel tests were used for comparison of categorical ordinal eGFR categories. Given the right-skewed distributions of all biomarkers and the lack of validated cut-points in this cohort, we analyzed Kaplan-Meier estimates of event rates by renal biomarker quartiles (Supplemental Table 1).

Renal biomarkers, as quartiles and as a continuous function, were sequentially tested in single biomarker Cox proportional hazards models. Given wide variability in ranges and distributions, each renal biomarker was standardized and expressed per 1 standard deviation (SD) increase in the biomarker concentration. All analyses were stratified by treatment arm (alogliptin or placebo). Models were also adjusted for a standard covariate set, used in recent biomarker analyses of EXAMINE,[9,15,16] including age, sex, history of hypertension, history of HF, qualifying ACS event (MI or unstable angina), and baseline CKD-EPI eGFR. A multimarker prediction model was also created with all renal biomarkers tested simultaneously in patients with available data on all 5 renal biomarkers. We tested the interaction between renal biomarkers and CKD-EPI eGFR $\geq$ or <60 mL/min/1.73 m^2.

We conducted 3 sensitivity analyses. First, we constructed multimarker risk models fully adjusted for a complete list of clinically relevant covariates (beyond the initial prespecified set). Second, we evaluated Cystatin C-derived eGFR, rather than Cystatin C itself, in risk prediction models. Finally, given significant missing data (Supplemental Table 2) for urine albumin/creatinine ratio and to minimize collinearity with urine protein/creatinine ratio, we included all patients with available data for 4 renal biomarkers (excluding urine albumin/creatinine ratio). Missing data for the other 4 renal biomarkers were imputed using Markov chain Monte Carlo methods (based on the other covariates).[17]

We finally tested the incremental value of each renal biomarker over established clinical predictors. We used 3 separate models: a base model (with the 6 covariates listed above including baseline eGFR), the base model with serum Cystatin C, and the base model with serum Cystatin C and the 4 urinary renal biomarkers. We assessed model performance in 3 dimensions (discrimination, calibration, and reclassification). Model discrimination, the ability to discriminate between patients with or without an outcome of interest, was determined using C-statistics (based on Harrell method assuming an uncensored policy for handling ties (14)). Model calibration, the degree of agreement between observed and predicted events, was tested using the Hosmer-Lemeshow goodness-of-fit test. The potential of a renal biomarker to appropriately reclassify risk (either upward or downward) was tested using continuous net-reclassification improvement (NRI) and integrated discrimination improvement (IDI). The CI associated with NRI and IDI was determined by the bootstrap method with 1,000 replications.[18] SAS version 9.4 (SAS Institute Inc., Cary, NC) was used for all computations.

Results

Of the 5,380 patients enrolled in EXAMINE, all patients had a calculated CKD-EPI eGFR, 5,213 (97%) had serum

Cystatin C values, 4,875 (91%) had uKIM-1/Cr, 4,962 (92%) had uNGAL/Cr, 4,049 (75%) had urine protein/Cr ratio, and 3,135 (58%) had an albumin/Cr ratio measured at randomization (median 44 days from qualifying ACS) and available for analysis.

EXAMINE enrolled older adults (mean age, 61 years) who were predominantly men (68%) and White (73%). Patients commonly had co-morbid hypertension (83%) and 28% carried a history of HF. Index ACS events were MIs in most patients (77%) as opposed to unstable angina. Compared with patients who did not experience the primary endpoint, patients who did were more likely to be older, women, Black, enrolled from North America, and had longer durations of T2DM ($p \leq 0.007$ for all comparisons; Table 1).

Mean baseline eGFR was 74 ± 22 mL/min/1.73 m^2 and 75% of patients had eGFR ≥60 mL/min/1.73 m^2. Renal biomarkers were not significantly different between alogliptin and placebo-treated patients (Supplemental Table 3), except baseline urine protein/Cr ratio was lower in alogliptin arm (median 0.12 [0.08 to 0.24] mg/mg Cr) compared with placebo (median 0.13 [0.08 to 0.26] mg/mg Cr; $p = 0.02$). Given limited differences by treatment assignment, all analyses were performed in the total cohort.

During median follow-up of 18 months, 621 (11.5%) experienced the primary endpoint, 326 (6.1%) patients had died, and 164 (3.0%) experienced hospitalization for HF (as a first occurrence of a cardiovascular endpoint). Kaplan-Meier rates of each of these events at 24 months increased significantly in graded fashion across quartiles of baseline renal biomarkers (log-rank $p \leq 0.006$ for trend and log-rank $p \leq 0.002$ for comparisons of Q4 vs. Q1; Figure 1 and Supplemental Table 4). Rates increased most steeply from Q1 to Q4 for serum Cystatin C for each of the endpoints: primary endpoint (7.9% to 23.6%), all-cause mortality (2.2% to 14.7%), and hospitalization for HF (0.9% to 9.7%); all log-rank $p < 0.001$. Declining quartiles of Cystatin C-derived eGFR were similarly step-wise associated with each of the endpoints: primary endpoint (9.2% to 21.3%), all-cause mortality (3.4% to 13.6%), and hospitalization for HF (1.8% to 8.8%); all log-rank $p < 0.001$.

After stratification by treatment status and accounting for key demographic and clinical covariates and baseline eGFR, each of the renal biomarkers (including Cystatin C-derived eGFR) demonstrated strong, graded, and independent associations with each of the endpoints (Figure 1). Point estimates of HRs were consistently the highest for prediction of hospitalization for HF (adjusted HRs ranging from 1.82 for uNGAL/Cr to 5.14 for serum Cystatin C).

When all 5 renal biomarkers were simultaneously tested together in a multimarker integrative model (Figure 2), only Cystatin C (per 1 SD) was associated with the primary endpoint (adjusted HR 1.28; 95% confidence interval 1.14, 1.45; $p \leq 0.001$), along with age, history of hypertension, and history of HF. Similarly, only Cystatin C (per 1 SD) was independently associated with all-cause mortality (adjusted HR 1.51; 95% confidence interval 1.30, 1.74; $p \leq 0.001$), along with age, qualifying ACS event, and history of HF. For hospitalization for HF, no biomarker was independently predictive of risk (Figure 3). Interaction terms between CKD-EPI-derived eGFR and all 5 renal biomarkers were negative in predicting the primary endpoint in prespecified and fully adjusted models ($P_{interaction} > 0.05$).

In a fully adjusted analysis including clinical variables beyond the initial prespecified model, Cystatin C remained the only renal biomarker associated with clinical events (Supplemental Table 5). When Cystatin C-derived eGFR was substituted for Cystatin C, it was only associated with risk of hospitalization for HF in the multimarker model (adjusted HR 0.71 [0.51, 0.99]; $p = 0.04$). Given the missing baseline biomarker data for urine albumin/Cr ratio (n = 2,245, 42%), sensitivity analysis was performed with a fully-adjusted multimarker model with the 4 remaining renal biomarkers. Cystatin C (per 1 SD) remained independently and robustly associated with the primary endpoint (adjusted HR 1.28 [1.16, 1.42]; $p \leq 0.001$), all-cause mortality (adjusted HR 1.40 [1.23, 1.59]; $p \leq 0.001$), and hospitalization for HF (adjusted HR 1.24 [1.04, 1.49]; $p = 0.02$). uKIM-1/Cr (per 1 SD) became weakly associated with clinical endpoints: primary endpoint (adjusted HR 1.05 [0.99, 1.12]; $p = 0.10$), all-cause mortality (adjusted HR 1.07 [0.999, 1.15]; $p = 0.054$), and hospitalization for HF (adjusted HR 1.09 [1.00, 1.18]; $p = 0.048$).

Models with key clinical variables demonstrated modest discrimination of subsequent cardiovascular events (C-statistics of 0.66, 0.72, and 0.79 for the primary endpoint, all-cause mortality, and HF hospitalization, respectively). The addition of Cystatin C minimally improved model C-statistics (0.68, 0.74, 0.79), respectively, and did not improve reclassification ability with continuous NRI inclusive of the null value. The sequential addition of each of 4 urinary renal biomarkers to a base model with the clinical parameters and Cystatin C did not improve discrimination or reclassification of risk based on continuous NRI. Although IDI did suggest significant discrimination/reclassification potential for each of the renal biomarkers, overall C-statistics remained modest and qualitatively similar to those of base models (Table 2).

Discussion

In a large, well-characterized cohort of patients with T2DM, 4 urinary biomarkers and 1 serum renal biomarker measured early after index ACS were robustly associated with risk of subsequent CV events and death in step-wise manner, independent of baseline eGFR. The highest quartiles of these renal biomarkers informing various axes of renal function identified patients who face the highest risks of major adverse CV events (18% to 25% at 2 years). However, when tested together, only serum Cystatin C—a glomerular filtration marker—maintained strong, independent association in a multimarker integrative model, but it did not appear to be useful in reclassifying CV risk beyond clinical variables. Novel renal biomarkers of tubular injury (uKIM-1 and uNGAL) and measures of protein excretion did not improve discrimination or reclassification potential over serum Cystatin C. These data do not support routine measurement of urinary renal biomarkers of tubular damage in patients with T2DM, CV disease, and largely preserved eGFR with respect to risk prediction of subsequent CV events.

Table 1
Baseline characteristics by primary endpoint status in the EXAMINE trial

Variable	Primary endpoint (n = 621)	No primary endpoint (n = 4,759)	p	All EXAMINE patients (n = 5,380)
Age (years)				
Mean ± SD (n)	63.8 ± 9.8 (621)	60.5 ± 9.9 (4759)	≤0.001	61 ± 10 (5380)
Median (Q1, Q3)	64.0 (57.0,71.0)	60.0 (54.0,68.0)		61 (54,68)
Range (min, max)	(39.0,89.0)	(26.0,91.0)		(26,91)
Age ≥65 years	46.7% (290/621)	34.0% (1617/4759)	≤0.001	35.4% (1907/5380)
Men	63.1% (392/621)	68.5% (3259/4759)	0.007	67.9% (3651/5380)
Duration of diabetes mellitus (years)				
Mean ± SD (n)	11.5 ± 9.1 (619)	8.8 ± 8.0 (4740)	≤0.001	9.2 ± 8.2 (5359)
Median (Q1, Q3)	10.1 (4.8,16.3)	6.8 (2.5,13.3)		7.1 (2.8,13.7)
Range (min, max)	(0.0,49.9)	(0.0,48.5)		(0.0,49.9)
Baseline HbA1c concentration (%)				
Mean ± SD (n)	8.0 ± 1.1 (621)	8.0 ± 1.1 (4758)	0.965	8.0 ± 1.1 (5379)
Median (Q1, Q3)	7.9 (7.2,8.6)	7.9 (7.2,8.7)		7.9 (7.2,8.7)
Range (min, max)	(5.7,12.8)	(4.9,12.7)		(4.9,12.8)
Body weight (kg)				
Mean ± SD (n)	81.4 ± 20.5 (621)	82.3 ± 19.0 (4759)	0.305	82.2 ± 19.2 (5380)
Median (Q1, Q3)	79.2 (67.0,93.4)	80.1 (68.9,93.6)		80.0 (68.5,93.5)
Range (min, max)	(35.5,171.9)	(36.0,196.3)		(35.5,196.3)
Body mass index (kg/m^2)				
Mean ± SD (n)	29.7 ± 6.1 (621)	29.4 ± 5.5 (4758)	0.371	29.5 ± 5.6 (5379)
Median (Q1, Q3)	28.8 (25.4,33.2)	28.7 (25.6,32.5)		28.7 (25.6,32.6)
Range (min, max)	(15.6,56.8)	(15.7,68.3)		(15.6,68.3)
Race			0.002	
American Indian or Alaska native	2.6% (16/621)	2.0% (94/4759)		2.0% (110/5380)
Asian	17.2% (107/621)	20.6% (982/4759)		20.2% (1089/5380)
Black	6.8% (42/621)	3.7% (174/4759)		4.0% (216/5380)
Native Hawaiian or Other Pacific Islander	0.0% (0/621)	0.2% (11/4759)		0.2% (11/5380)
White	72.6% (451/621)	72.7% (3458/4759)		72.7% (3909/5380)
Multiracial	0.8% (5/621)	0.8% (40/4759)		0.8% (45/5380)
Region of world			0.004	
United States, Canada	19.2% (119/621)	15.4% (734/4759)		15.9% (853/5380)
Western Europe, Australia, New Zealand and Middle East	14.5% (90/621)	11.1% (526/4759)		11.4% (616/5380)
Central and South America, Mexico	24.0% (149/621)	26.1% (1244/4759)		25.9% (1393/5380)
Eastern Europe and Africa	26.6% (165/621)	28.2% (1343/4759)		28.0% (1508/5380)
Asia, Pacific Islands	15.8% (98/621)	19.2% (912/4759)		18.8% (1010/5380)
Cardiovascular risk factors and history				
Current smoker	11.9% (74/621)	13.9% (660/4759)	0.140	13.6% (734/5380)
Hypertension	92.1% (572/621)	81.9% (3897/4759)	≤0.001	83.1% (4469/5380)
Prior myocardial infarction*	58.6% (364/621)	0.0% (0/4759)	≤0.001	88.0% (4734/5380)
Prior percutaneous coronary intervention*	57.5% (357/621)	63.4% (3015/4759)	0.004	62.7% (3372/5380)
Prior coronary artery bypass graft surgery*	18.5% (115/621)	12.0% (573/4759)	≤0.001	12.8% (688/5380)
Prior cerebrovascular accident	10.8% (67/621)	0.0% (0/4759)	≤0.001	7.2% (388/5380)
Heart failure	40.1% (249/621)	26.3% (1252/4759)	≤0.001	27.9% (1501/5380)
Peripheral artery disease	16.7% (104/621)	8.6% (410/4759)	≤0.001	9.6% (514/5380)
Index ACS event			≤0.001	
Myocardial infarction	83.9% (521/621)	76.5% (3631/4745)		77.4% (4152/5366)
Unstable angina	16.1% (100/621)	23.5% (1114/4745)		22.6% (1214/5366)
Time from index ACS event to randomization (days)				
Mean ± SD (n)	44.8 ± 21.3 (621)	48.2 ± 22.0 (4745)	≤0.001	47.8 ± 22.0 (5366)
Median (Q1, Q3)	41.0 (28.0,60.0)	45.0 (30.0,64.0)		44.0 (30.0,64.0)
Range (min, max)	(10.0,101.0)	(8.0,141.0)		(8.0,141.0)
Baseline medical therapies				
Angiotensin-converting enzyme inhibitors or Angiotensin II receptor blockers	83.6% (519/621)	81.6% (3885/4759)	0.238	81.9% (4404/5380)
Aspirin	90.0% (559/621)	90.8% (4322/4759)	0.517	90.7% (4881/5380)
Beta-blocker	80.5% (500/621)	82.2% (3911/4759)	0.310	82.0% (4411/5380)
Insulin	38.0% (236/621)	28.8% (1369/4759)	≤0.001	29.8% (1605/5380)
Statin	88.9% (552/621)	90.6% (4314/4759)	0.160	90.4% (4866/5380)

Data are median (IQR), mean ± SD, or number (%), as appropriate. The primary efficacy endpoint of EXAMINE was the composite of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke.

* Includes the index acute coronary syndrome event.ACS = acute coronary syndrome; IQR = interquartile range; SD = standard deviation.

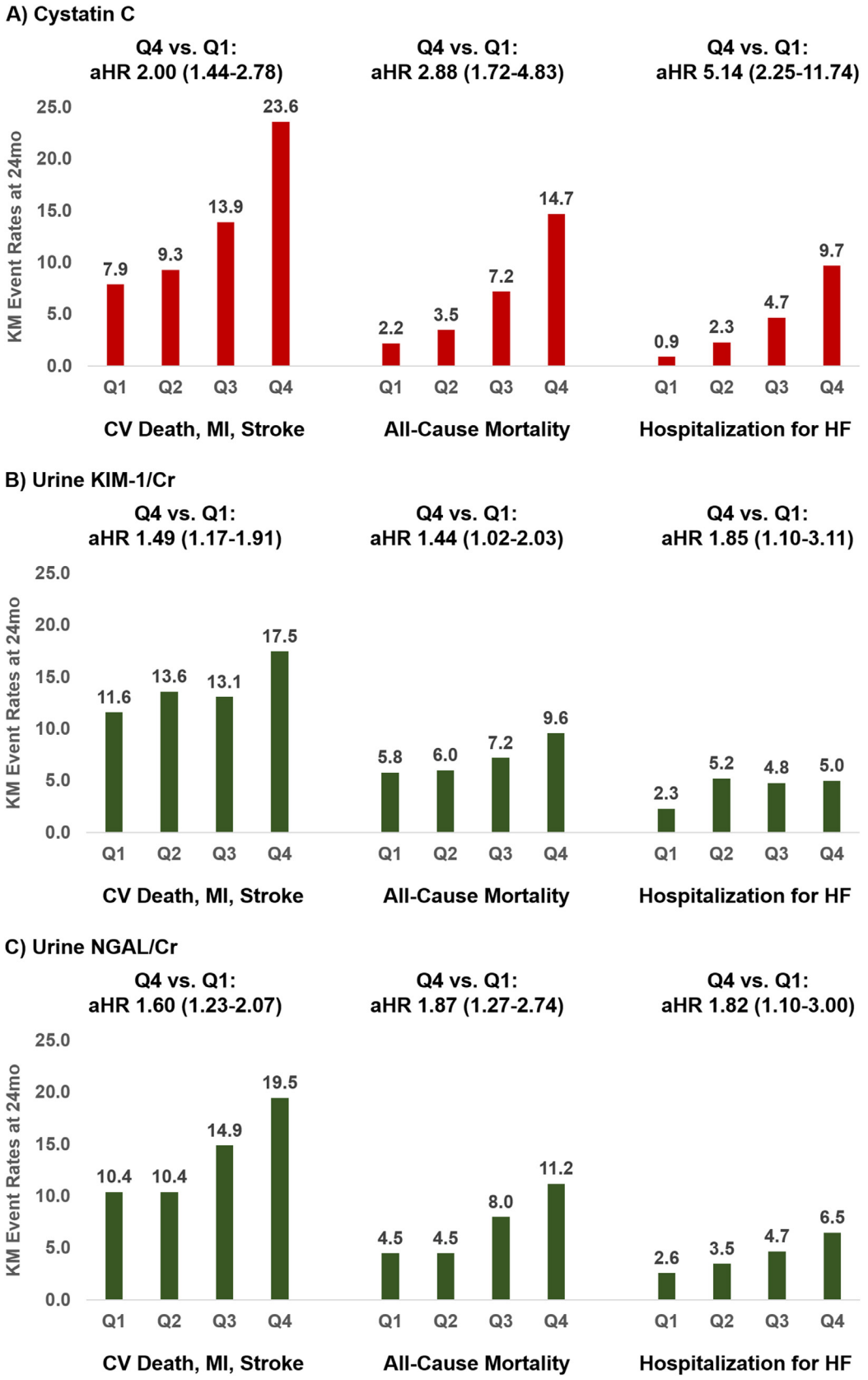

Figure 1. Twenty-four-month event rates across quartiles of the 5 baseline renal biomarker concentrations and single-biomarker prediction models. Time from randomization to endpoint was fit using the Cox proportional hazards model, stratified by randomized treatment with alogliptin versus placebo, and adjusted for age, sex, history of hypertension, history of heart failure, qualifying acute coronary syndrome event, baseline glomerular filtration rate (estimated using the Chronic Kidney Disease Epidemiology Collaboration equation). Only subjects with available data related to renal biomarker of interest were included in each analysis. aHR = adjusted hazard ratio; CV = cardiovascular; HF = heart failure; KM = Kaplan-Meier; MI = myocardial infarction; uKIM-1/Cr = urinary Kidney Injury Molecule-1/Creatinine; uNGAL/Cr = urinary Neutrophil Gelatinase Associated Lipocalin/Creatinine.

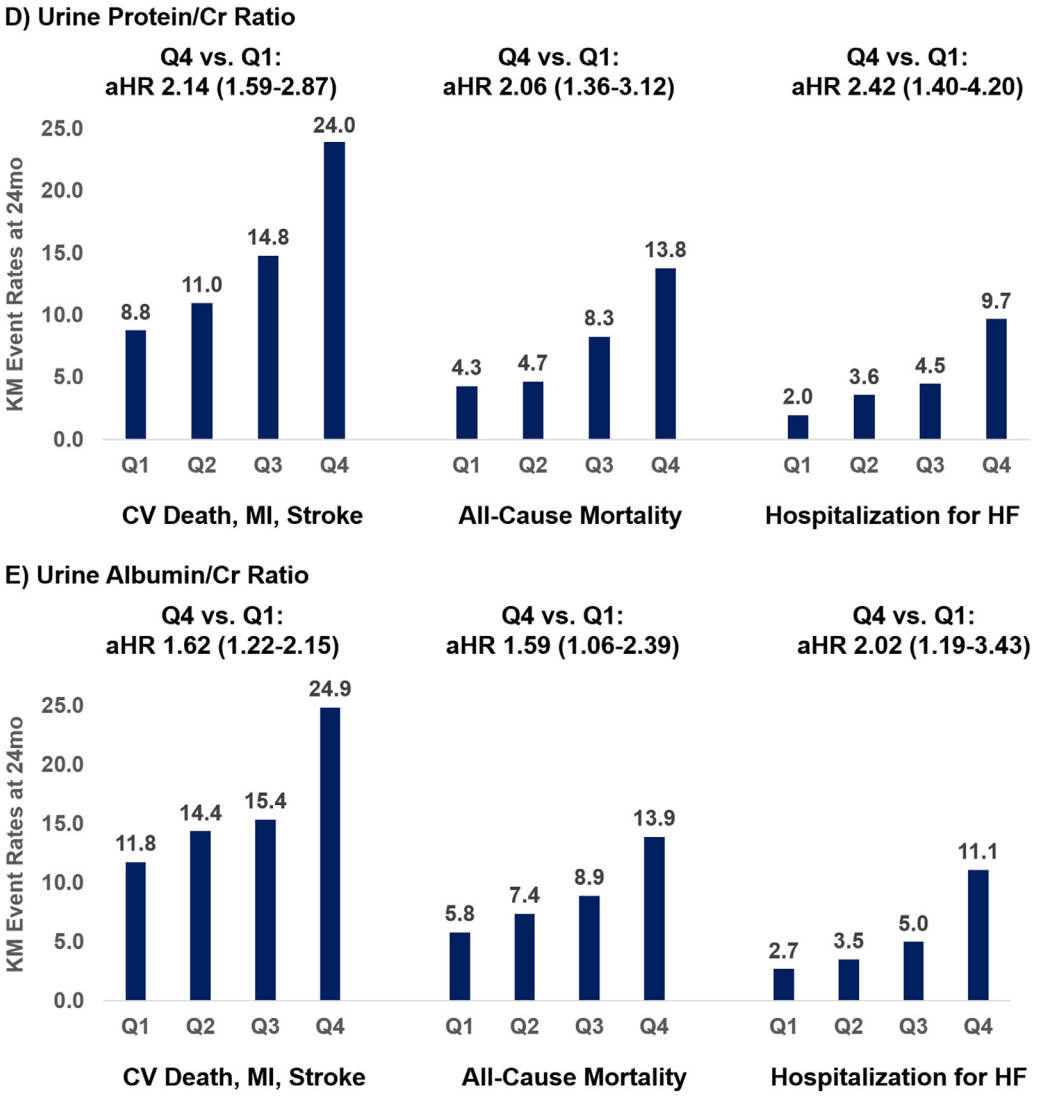

Figure 1 Continued.

EXAMINE is well-suited to explore the interplay between diabetic kidney disease and CV disease. Aside from the requirement of dialysis at the time of randomization, the trial did not exclude patients based on kidney function and enrolled a full range of eGFRs. Despite this, the majority (75%) had normal or mildly impaired renal function, and the prognostic utility of individual renal biomarkers appears consistent even in patients with eGFR above 60 mL/min/1.73 m^2. Complete renal biomarker data were available in over 3,000 patients, representing the largest experience to rigorously assess this comprehensive panel of renal biomarkers in patients with type 2 DM at high CV risk.

Contemporary guidelines suggest routinely evaluating measures of renal function (eGFR based on serum creatinine) and urine albumin in patients with T2DM to help inform risks of development and progression of diabetic kidney disease. Compared with serum creatinine, Cystatin C may be less subject to influence by age, sex, race, and muscle mass, and may more closely vary with inflammatory inputs observed in T2DM,[19] potentially providing a more discriminant estimate of underlying glomerular function.[20,21] These data from EXAMINE provide further support for the adjunctive use of Cystatin C or Cystatin C-based eGFR calculations[22] in this setting, however its role in reclassifying risk and its clinical utility require further study. Indeed, the Cystatin C-based CKD-EPI equation appears to perform better than the Modification of Diet in Renal Disease equation in the setting of MI.[23] Similar to our current findings in CV risk prediction, we recently demonstrated that although serum Cystatin C appears to be associated with risk of renal progression in the EXAMINE trial, it does not appear to reclassify risk beyond common clinical and laboratory factors.[9]

Limited data are available characterizing the prognostic value of urinary kidney injury biomarkers. In a recent study of 2,466 patients with established CKD (50% with co-morbid DM) followed for median 6.5 years, uKIM-1/Cr and other select urinary renal biomarkers were independently associated with CV events

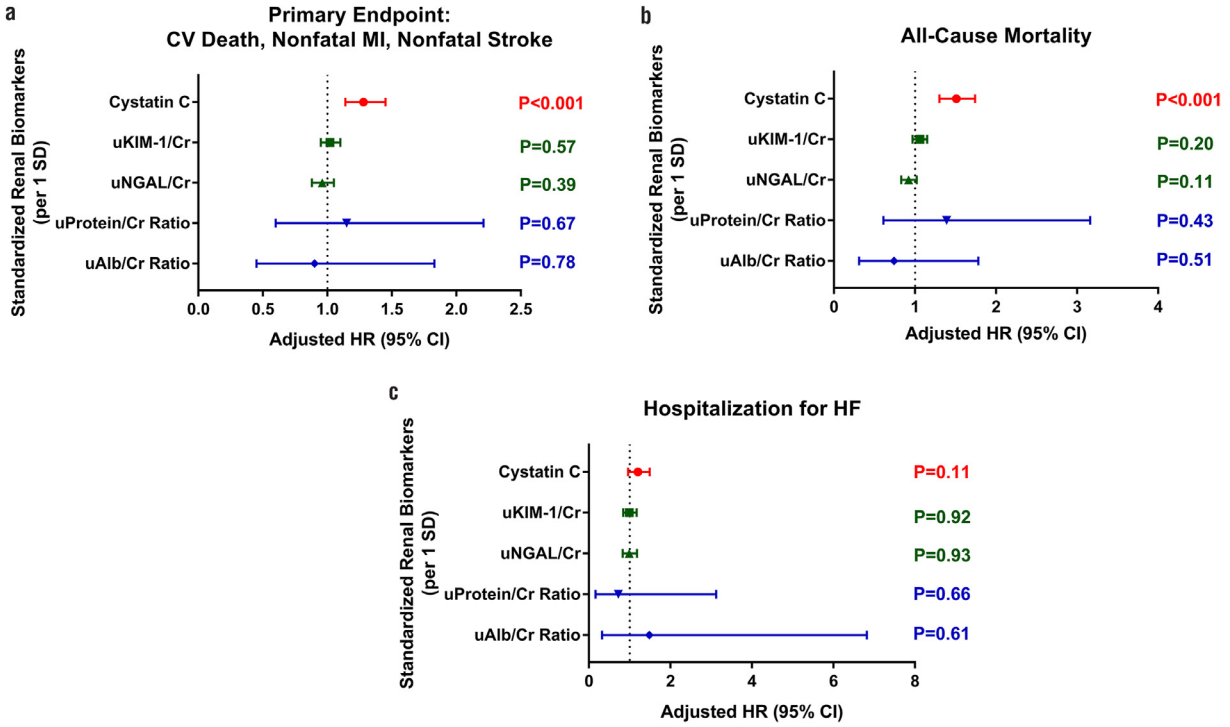

Figure 2. Multimarker prediction models for each clinical endpoint. Time from randomization to endpoint was fit using the Cox proportional hazards model, stratified by randomized treatment with alogliptin versus placebo, and adjusted for all 5 renal biomarkers and the following covariates: age, sex, history of hypertension, history of heart failure, qualifying acute coronary syndrome event, baseline glomerular filtration rate (estimated using the Chronic Kidney Disease Epidemiology Collaboration equation). Only subjects with all 5 renal biomarkers at baseline are included in this analysis. Given wide variability in the ranges and distribution, each renal biomarker was standardized and expressed per 1 SD increase in the biomarker concentration. aHR = adjusted hazard ratio; CV = cardiovascular; HF = heart failure; MI = myocardial infarction; SD = standard deviation; uKIM-1/Cr = urinary Kidney Injury Molecule-1/Creatinine; uNGAL/Cr = urinary Neutrophil Gelatinase Associated Lipocalin/Creatinine.

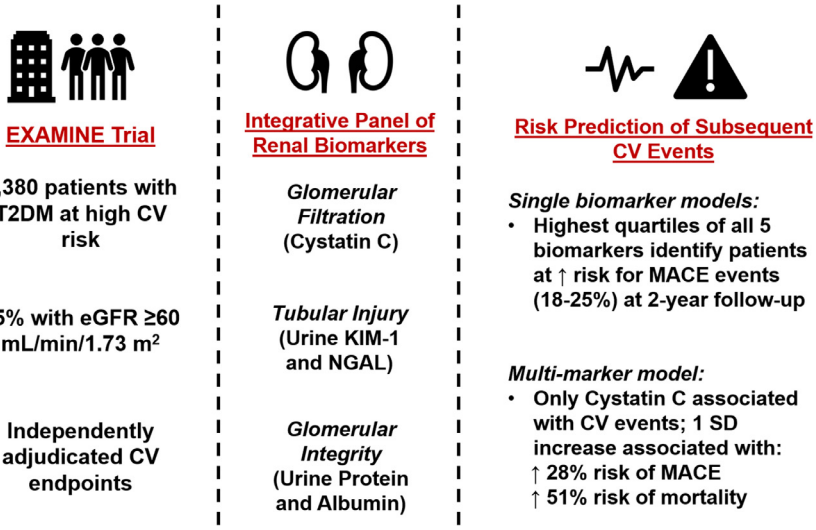

Figure 3. Exploring the interplay between diabetic kidney disease and cardiovascular disease with an integrative panel of renal biomarkers in the EXAMINE trial. CV = cardiovascular; MACE = major adverse cardiac events; SD = standard deviation; uKIM-1 = urinary Kidney Injury Molecule-1; uNGAL = urinary Neutrophil Gelatinase Associated Lipocalin.

and death.[24] Our study specifically examined these renal biomarkers in patients with T2DM. Although uKIM-1/Cr and uNGAL/Cr were each individually and independently associated with cardiovascular risk, this risk was attenuated when accounting for Cystatin C in multimarker models and these urinary kidney injury biomarkers failed to improve risk prediction. Since both uKIM-1 and uNGAL are markers of renal injury,

Table 2
Risk prediction with and without baseline renal biomarker concentrations

Primary endpoint: cardiovascular death, nonfatal myocardial infarction, nonfatal stroke	C-statistic	Goodness of fit chi-square	Continuous NRI	NRI (95% Bootstrap CI)	IDI	IDI (95% Bootstrap CI)
Base Model*	0.66	130.8				
Base Model + Cystatin C (mg/L)	0.68	66.5	−0.0387	(−0.235, 0.1246)	0.0063	(0.0026, 0.0106)
Base Model + Cystatin C (mg/L)+ Baseline Renal Biomarker						
uKIM-1/Cr (ng/mg)	0.68	61.3	0.0101	(−0.1877, 0.1744)	0.0070	(0.0032, 0.0112)
uNGAL/Cr (ng/mg)	0.68	67.5	−0.0289	(−0.226, 0.1334)	0.0069	(0.0032, 0.0114)
uProtein/Cr ratio (mg/mg Cr)	0.68	84.2	−0.0016	(−0.2033, 0.1712)	0.0055	(0.0016, 0.0098)
uAlbumin/Cr ratio (mg/g)	0.68	85.8	0.0104	(−0.193, 0.1861)	0.0055	(0.0017, 0.0099)
All-Cause Mortality	**C-statistic**	**Goodness of Fit Chi-Square**	**Continuous NRI**	**NRI (95% Bootstrap CI)**	**IDI**	**IDI (95% Bootstrap CI)**
Base Model*	0.72	87.4				
Base Model + Cystatin C (mg/L)	0.74	85.0	−0.0843	(−0.348, 0.1301)	0.0031	(−0.003, 0.0099)
Base Model + Cystatin C (mg/L)+ Baseline Renal Biomarker						
uKIM-1/Cr (ng/mg)	0.75	89.3	−0.0318	(−0.3167, 0.1926)	0.0042	(−0.0018, 0.011)
uNGAL/Cr (ng/mg)	0.74	82.8	−0.0551	(−0.3222, 0.1598)	0.0053	(−0.0011, 0.0126)
uProtein/Cr ratio (mg/mg Cr)	0.75	102.6	−0.0102	(−0.2743, 0.2017)	0.0011	(−0.005, 0.0077)
uAlbumin/Cr ratio (mg/g)	0.75	101.2	−0.0099	(−0.2752, 0.203)	0.0012	(−0.005, 0.0078)
Hospitalization for HF	**C-statistic**	**Goodness of Fit Chi-Square**	**Continuous NRI**	**NRI (95% Bootstrap CI)**	**IDI**	**IDI (95% Bootstrap CI)**
Base Model*	0.79	40.0				
Base Model + Cystatin C (mg/L)	0.79	22.2	−0.1925	(−0.3673, -0.011)	0.0074	(0.0005, 0.0158)
Base Model + Cystatin C (mg/L)+ Baseline Renal Biomarker						
uKIM-1/Cr (ng/mg)	0.79	45.0	−0.1335	(−0.314, 0.0499)	0.0084	(0.0014, 0.017)
uNGAL/Cr (ng/mg)	0.79	20.8	−0.1652	(−0.3393, 0.0174)	0.0083	(0.0012, 0.0164)
uProtein/Cr ratio (mg/mg Cr)	0.80	22.9	−0.1777	(−0.3662, 0.0074)	0.0068	(−0.0002, 0.0153)
uAlbumin/Cr ratio (mg/g)	0.80	24.2	−0.1508	(−0.3421, 0.0338)	0.0067	(−0.0002, 0.0155)

* The base model includes 6 predictors: age, sex, history of hypertension, history of heart failure, qualifying acute coronary syndrome event, baseline glomerular filtration rate (estimated using the Chronic Kidney Disease Epidemiology Collaboration equation).All 5 biomarkers variables Cystatin C, uKIM-1/Cr, uNGAL/Cr, uProtein/Cr ratio and uAlbumin/Cr ratio with missing values were estimated through Markov chain Monte Carlo methods with imputation over 5 times. Index ACS Event missing values were estimated through logistic regression with monotone missingness method with imputation over 5 times. Time from randomization to endpoint was fit using the Cox proportional hazards model with adjustment on the baseline variables listed above.IDI = integrated discrimination improvement; NRI = net reclassification index; uKIM-1/Cr = urinary Kidney Injury Molecule-1/Creatinine; uNGAL/Cr = urinary Neutrophil Gelatinase Associated Lipocalin/Creatinine.

specifically tubular damage, the lack of incremental discriminative utility of these urine renal biomarkers may be related to the large proportion of patients with preserved baseline eGFR in EXAMINE. Distinct renal biomarker profiles may characterize CV risk trajectories in patients with stable CKD and those experiencing acute kidney injury.

Another large study recently evaluated the utility of 1 of the 5 markers we evaluated, urine albumin/Cr ratio.[25] Over median follow-up ~2 years, urine albumin/Cr ratio showed a step-wise association with risk of CV events, independent of eGFR, but did not demonstrate incremental value when added to traditional CV biomarkers.[25] Taken together, spot measurement of protein excretion independently identifies high-risk patients for CV events, but this risk prediction potential is attenuated after addition of other discriminative renal and CV biomarkers.

Multiple classes of glucose-lowering therapies, including the DPP-4 inhibitors,[9,26,27] the sodium-glucose co-transporter-2 inhibitors,[4,5] and the glucagon-like-peptide-1 receptor agonists[3] are known to influence rates of renal progression. These renal biomarkers may inform mechanisms of renal and CV benefit with these therapies. Future studies should also assess the incremental value of serial renal biomarker assessments with relation to risk of CV events.

This analysis is subject to several limitations. The generalizability of our findings is limited to specific inclusion/exclusion criteria of the clinical trial. There were missing data, especially related to protein excretion. The association between renal biomarkers and subsequent CV risk may be subject to residual confounding. Our models did not account for time-updated covariates, including longitudinal changes in eGFR, medication use, or glycemic control.

Patients with T2DM and recent ACS face a broad spectrum of fatal and nonfatal CV events,[28] highlighting the ongoing need for timely risk stratification to guide resource allocation, follow-up care, and potentially, treatment intensification. This study does not support a role for urine-based renal biomarkers in the assessment of renal function to improve CV risk stratification in patients with T2DM early

after ACS. Although Cystatin C showed promise in this high-risk cohort, its role in reclassifying risk and use in clinical practice require further study in broader cohorts of varying CV risk. Renal biomarkers informing various aspects of kidney function may further our understanding of the complex interplay between diabetic kidney disease and CV disease.

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Supplementary materials

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